Bitter Orange Biorefinery: Development of a Process for Juice Production with Isolation of Valuable Molecules from Fruit Wastes

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"The greatest threat to our planet is the belief that someone else will save it."

Robert Swan

I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

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Do fundo do coração, muito obrigada a todos vós.

Abstract

Bitter orange (BO), unlike most citrus fruits, is not an edible fruit, due to its sour and bitter taste. Thus, its production is not intended for consumption, but for application in other industrial areas. Its tree is also used as a decorative element in cities, but when the fruit falls, it generates a waste very rich in added-value compounds. The main objective of this study is to avoid this waste by developing a process for BO juice production, with the valorization of the BO processing waste (BOPW) generated from it, to produce *p*-synephrine, polyphenols (flavonoids), and pectin.

The experimental work was focused on optimizing the extraction and isolation conditions of the compounds present in BO peel. The simultaneous extraction of *p*-synephrine and flavonoids was carried out by solvent extraction (SE) using aqueous and ethanolic solvents at room temperature (RT) and 90 ºC. It was concluded that RT increases the extraction yields of compounds, by around 38%, and the yields do not significantly depend on the solvent used. *p*-Synephrine was subsequently separated from the flavonoids by adsorption or ion exchange, where the selectivity, binding (%B), and *p*-synephrine recovery (%R) efficiencies of various resins were studied. It was concluded that the PD-206 resin is the most suitable, for BO peel extract obtained with 80% EtOH (aq) (14.54 selectivity, 100% %B, 51% %R), leading to higher process yields for flavonoids (93%), and higher revenues (35.38 million EUR/t BO peel). The pectin extraction was assessed with hydrochloric and citric acids, at pH 1.5 and 95 ºC, being concluded that citric acid leads to higher yields of pectin (57%) from BO peel. The nutritional value of BO juice was also assessed and showed to be similar to that of sweet orange juice with higher content of antioxidants, revealing that BO juice can be possibly considered for consumption.

Keywords

Bitter Orange; *p*-Synephrine; Flavonoids; Pectin; Solvent Extraction; Adsorption and Ion Exchange.

Resumo

A laranja amarga (LA), ao contrário da maior parte dos citrinos, não é uma fruta comestível, devido ao seu sabor azedo e amargo. Assim, a sua produção não tem como finalidade o consumo direto, mas sim a aplicação noutras áreas industriais. A sua árvore também é usada como elemento decorativo nas cidades, mas quando a fruta cai, gera-se um desperdício muito rico em compostos de valor acrescentado. O objetivo principal deste estudo é evitar este desperdício, desenvolvendo um processo de produção de sumo de LA, que inclui a valorização dos resíduos orgânicos que dele são gerados para produzir *p*-sinefrina, polifenóis (flavonoides) e pectina.

O trabalho experimental centrou-se na otimização das condições de extração e isolamento dos compostos extraídos dos resíduos. A extração conjunta de *p*-sinefrina e flavonoides foi feita por extração por solvente (ES) à temperatura ambiente (T_{amb}) e a 90 ºC, tendo-se concluído que à T_{amb} os rendimentos de extração aumentam em cerca de 38%, e que o rendimento não depende significativamente do solvente usado. A *p*-sinefrina foi posteriormente separada dos flavonoides por adsorção ou permuta iónica, onde foram estudadas a seletividade, e as eficiências de ligação (%B) e de recuperação da *p*-sinefrina (%R) de várias resinas. Concluiu-se que a resina PD-206 é a mais indicada, usando a solução de 80% EtOH (aq) na ES (14,54 seletividade, 100% %B, 51% %R), conduzindo a maiores rendimentos de processo, para os flavonoides (93%), e receitas mais elevadas (35.38 milhões EUR/t casca LA). A extração de pectina foi testada com os ácidos clorídrico e cítrico, a pH 1,5 e 95 ºC, tendo-se concluído que o ácido cítrico leva a maiores rendimentos de pectina (57%). O valor nutricional do sumo de LA foi também avaliado, mostrando-se semelhante ao do sumo de laranja doce, com maior teor em antioxidantes, o que revela que o sumo de LA poderá ser considerado para consumo.

Palavras-Chave

Laranja Amarga; *p*-Sinefrina; Flavonoides; Pectina; Extração por Solvente; Adsorção e Permuta Iónica.

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Acronyms List

AcOH	Glacial Acetic Acid
BO	Bitter Orange
BOPW	Bitter Orange Processing Waste
BP	Blood Pressure
BW	Body Weight
BWG	Body Weight Gain
CE	Conventional Extraction
CPW	Citrus Processing Waste
DW	Dry Weight
DBP	Diastole Blood Pressure
EAE	Enzyme-Assisted Extraction
EtOH	Ethanol
FW	Fresh Weight
GHG	Greenhouse Gases
HCl	Hydrochloric Acid
HPLC	High-Performance Liquid Chromatography
HR	Heart Rate
LA	Laranja Amarga
MAE	Microwave-Assisted Extraction
MeOH	Methanol
NaCl	Sodium Chloride
NERT	Neoeriocitrin
NHPD	Neohesperidin
NPs	Nanoparticles
NRG	Naringin
***p*-SYN**	*p*-Synephrine
SBP	Systolic Blood Pressure
SE	Solvent Extraction
SO	Sweet Orange
SWE	Subcritical Water Extraction
T	Temperature
UAE	Ultrasound-Assisted Extraction
VA	Ventricular Arrhythmias

Chapter 1

Aim of Studies: Motivation, Objectives, and Methodology

Bitter orange (BO) is not an edible fruit, owing to its bitter and sour taste that does not please consumers. As such, its production is not intended for fresh fruit consumption or juice production. BO is mainly used to make marmalade and as an ingredient in weight loss and muscle enhancer supplements, as well as in some cosmetic products (1). In addition, the unique and well appreciated aroma of BO flowers and its beautiful tree make the BO tree a widely used adorning item for European streets. They can be seen, for example, in Portugal, mainly in the Alentejo region, adorning the convents' cloisters, village's plazas, and schools' courtyards, but their existence is by far more prominent in Seville city, Spain.

In Seville, there are around 48 000 BO trees, producing the equivalent to 5.7 tons of oranges (2). Most of these oranges are exported to Great Britain for marmalade production, but there is still a large portion that is not used, ending up falling on the streets and becoming an inconvenience for pedestrians and the city's cleaning department. As a solution, Seville's municipal water company, Emasesa, has been piloting a scheme to use the unwanted oranges to produce clean electricity to run one of the water purification plants (2). The electricity is produced through the fermentation of the juice extracted from those oranges, producing a gas rich in methane that drives an electricity generator. The oranges leftovers, such as peel, pulp, and seeds, are used as fertilizer (3).

The citizens of Seville found a purpose for the falling BOs, but, in most cities of Europe, these oranges end up falling on the streets with no application assigned to them, wasting a resource that is rich in added-value compounds with several unique health benefits and industrial applications. Those compounds include pectin, alkaloids (*p*-synephrine *(p*-SYN*),* octopamine, tyramine, N-methyltyramine, and hordenine), phenolic acids (mainly *p*-coumaric and ferulic acids), flavonoids (rutin, epicatechin, naringin (NRG), hesperidin, neohesperidin (NHPD), and neoeriocitrin (NERT)), vitamin C, and essential oils, among others. In addition, the nutritional composition of BO juice is comparable to other citrus juices, like sweet orange (SO) juice, so BO juice must be explored, despite its bitter and sour taste.

Hence, in the scope of the circular bioeconomy, the global objective of this thesis is to develop a process for BO juice production, in which the fruit wastes (peels, seeds, rags, and damaged/unsuitable fruit) are valorized through the extraction of added-value compounds (pectin, *p*-SYN, and flavonoids), instead of being simply used as fertilizer or animal feed. The juice production section of the process was approached in this thesis in a conceptual manner, while the waste valorization was experimentally developed.

To accomplish this objective the following methodology was taken:

- Understand the nutritional value of BO juice and compare it to the nutritional value of the citrus juices that are produced on a large scale for consumption.
- Identify which compounds are responsible for the bitter and sour taste of BO juice, and search for methods that could make this juice suitable for consumption.
- Analyze the unitary operations involved in the citrus processing plants for juice production to identify in which operations fruit processing waste is generated and gather information about the organic content of such waste streams.
- Explore which extraction methods are already in use or under study to extract added-value compounds from citrus processing waste (CPW).
- Experimentally develop and optimize the waste valorization process.

Chapter 2

Introduction

2.1 Citrus Fruits

Citrus fruits grow in flowering trees and shrubs, being the greatest genus of the Rutaceae family (*Citrus L., Rutaceae*) (4,5). The genus *Citrus* is constituted of approximately 1300 species and each species has several varieties (6). Citrus fruits are divided into 9 categories: 1) sweet oranges (*Citrus sinensis*); 2) mandarins (satsuma (*C. unshiu*), tangerines (*C. tangerina*), *C. reticulata*, clementines (*C. clementina*)); 3) bitter oranges (*Citrus aurantium*); 4) lemons (*Citrus limon*); 5) limes (*Citrus aurantifolia* and *Citrus latifolia*); 6) grapefruits (*Citrus paradisi*); 7) pomelos (*Citrus grandis*); 8) hybrids; 9) citrons (*Citrus medica*) (4).

The origin of these fruits is uncertain and full of controversies, but it is believed that they emerged from North India, in the foothills of the Himalayas, North Myanmar, Southeast Asia, and South China (Yunnan province) (6). Due to their sensorial attributes and health-promoting nutrients and compounds (vitamin C, β-carotene, minerals, dietary fibers, polyphenols, limonoids, etc.), citrus fruits please consumers, making them one of the major horticultural crops in the world (4,6,7). They are mostly produced in tropical and subtropical regions, with Brazil, China, the USA, India, Mexico, and Spain ensuring 2/3 of the global production of citrus (6).

Oranges account for half of the global production of citrus fruits (53%), followed by tangerines (26%), lemons and limes (14%), and grapefruits (7%) (8). These fruits are mostly produced to be consumed fresh (75% of total production) and the remaining (25%) are processed into commercial products like juice, jams, marmalade, jellies, and candies, among others (9).

Concerning their structure, they are composed of a peel (also known as the outer rind or skin) and the edible part of the fruit (pulp). The peel comprises three tissues (figure 1): an outer colored tissue called exocarp or flavedo, an inner and thicker white tissue called mesocarp, and finally a thin tissue called endocarp that separates the mesocarp from the pulp. The mesocarp and the endocarp form together the albedo. It is in the flavedo that the oil glands are found. The edible part of the fruit is divided into sections by a continuous endocarp membrane that starts at the peel and passes through the pulp to the central core. Each section (or segment) is closely filled with juice sacs (or vesicles) and seeds. In addition to the flavedo, each juice sac also contains a small oil gland at the center (4).

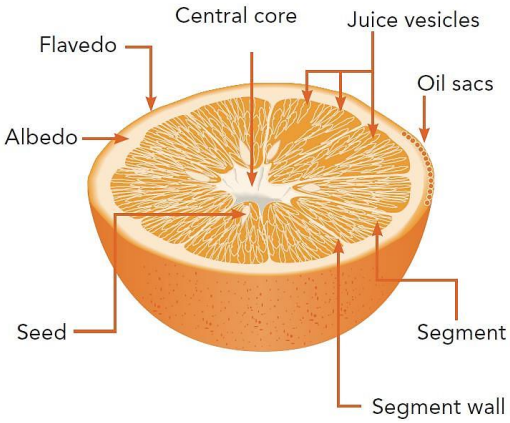

Figure 1. The structure of citrus fruits (10).

2.2 Orange Processing for Juice Production

Orange processing for juice production comprises several steps, which are schematized in figure 2 and will be briefly described (10).

I. Fruit Reception

Trucks loaded with oranges, from the orchards, arrive at the reception area, where they are discharged, allowing the fruit to roll off onto a conveyor. The fruit is then prewashed to remove superficial dirt and pesticide residues.

After prewashing phase, follows destemming and pregrading. At this stage, the leaves and twigs attached to the fruit fall through the conveyor bed, and the rotten and visibly damaged oranges are manually removed. The rejected fruit is headed to a feed mill, where it is dried and pelletized for animal feed, and the sound fruit moves to a bin with inclined multilevel internal baffles, allowing the fruit to be evenly distributed to avoid too much weight pressing on it. Meanwhile, a sample of fruit from each truck is analyzed in terms of juice yield, ºBrix[1], acidity, and color, so that each load can be identified when moving to bin storage. After bin storage, the fruit is drawn to another bin, the surge bin, where different fruit loads are combined.

Finally, the oranges are subjected to a last washing and grading. The final washing is more rigorous than the first one since the washing water contains a mild disinfectant to remove microbial organisms from the peel. The final grading consists of a final visual inspection, and it proceeds the same way as pregrading. The rejected fruit from grading also goes to a feed mill. If the facility does not own a feed mill, the solid waste is sent to another facility with a feed mill or it is disposed of, in a landfill, for example.

[1] It measures the dissolved solids in a liquid and is largely used to measure the dissolved sugar content in an aqueous solution, such as citrus juice. 1 ºBrix = 1 g of sucrose / 100 g of solution.

It should be mentioned that the prewashing step is optional: many processors opt to suppress it because the wet fruit inside the bins can make downstream sanitation more difficult.

II. Juice Extraction

Once the fruit is washed and graded, it is ready for the next orange processing stage: juice extraction. At this point, the fruit is squeezed in such a way, using extractors, to obtain as much juice from it as possible, preventing the peels, rag, seeds, and oil from entering the juice.

Because extractors are designed to handle fruit of only a particular size range, the fruit is firstly size graded. The size grading takes place on a sizing table with a set of rotating rollers, in series. Between each roller, there's a gap, whose size is predefined according to fruit size, and increases as the fruit pass over the table. Thus, the smallest fruit falls through the first gap, and the largest fruit falls through the last gap. A set of conveyors carry the fallen fruit to the extractor set for their size range. It should be noted that if the fruit is too big or too small for the extractor size range, it will be excessively squeezed, allowing the rags and peels to enter the juice and increase its bitterness. On the other side, if the fruit is poorly squeezed, the juice yield will be insufficient. Hence, fruit sizing is crucial to obtain a juice of high quality and/or yield.

After fruit sizing, the actual juice extraction follows. There are two main types of extractors: the squeezer type and the reamer type. They are especially designed for citrus fruit, being the most dominant in orange processing plants. Of these two types, the squeezer type is the most used, at a global level.

The head of a squeezer-type extractor contains a lower static cup, where the fruit settles, and an upper moving cup. Each pair of cups processes one orange. Both cups have metal fingers that mesh when the upper cup moves to the lower cup. A cutter emerges from the center of the lower cup, making a hole through the peel and allowing the inner parts of the fruit to flow out into a tube attached to the cutter – the strainer tube. When the cutter moves down, the peel breaks up, disintegrates, and passes through the metal fingers, releasing an oil. The fragments of the peel are water washed, creating an aqueous oil emulsion. At the same time, the extracted juice passes through the perforated walls of the strainer tube, moving into the juice manifold, while the core material held in the strainer tube is discharged.

Summing up, four main product streams result from juice extraction: the peel, the oil emulsion, the core material, and the juice. The solid part, composed of peel and fruit core material, is usually sent to the feed mill, while the oil emulsion flows to the peel oil recovery section, and the juice is subjected to a treatment to produce Frozen Concentrated Orange Juice (FCOJ) and/or Not-From-Concentrate Juice (NFC).

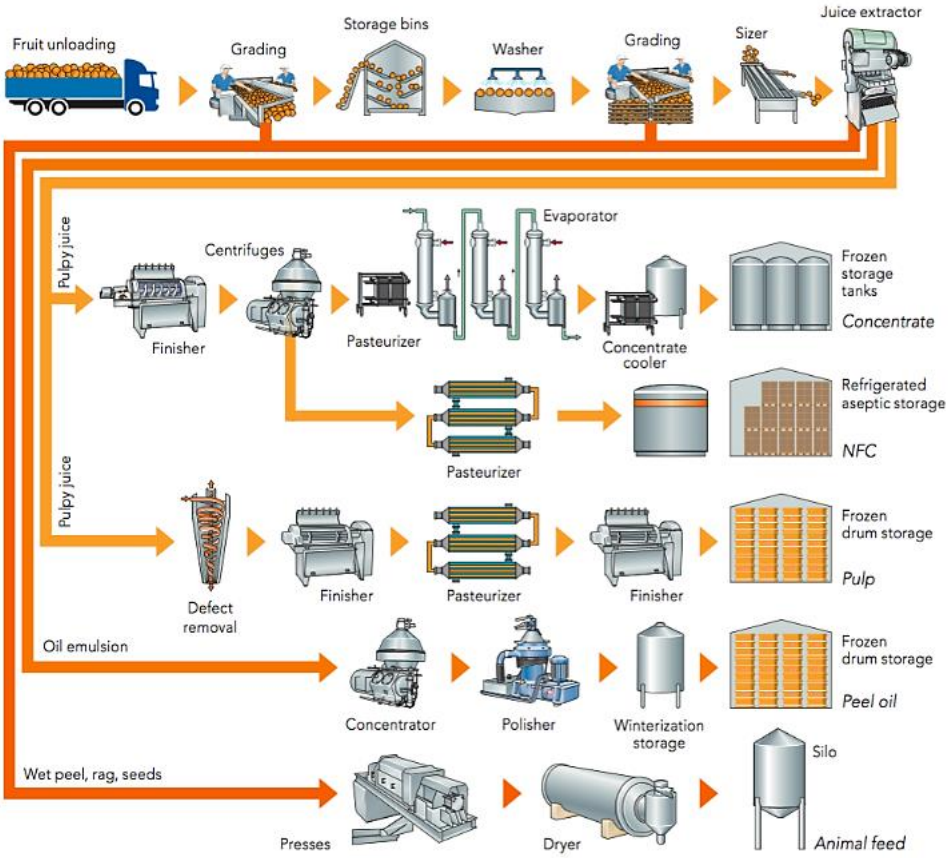

Figure 2. Flow chart showing typical processing steps found in an orange processing plant (10).

III. FCOJ and NFC Production

During juice extraction, the core material of the fruit is held inside the strainer tube, before being discharged. Nevertheless, some of it is carried by the juice when pouring out of the tube, conferring it a pulpy texture. Thus, to separate the pulp from the juice, a clarification operation is needed. The clarification is generally held by two finishers, often placed in series at the end of the extraction line, and a clarifying centrifuge.

After clarification, the juice is blended with juice from other batches to balance its flavor, color, acidity, and ºBrix levels. The pulp, on the other hand, is addressed to the pulp wash production section, if the intent is to sell the pulp, or to the feed mill, or even to a landfill.

Once the juice is clarified and blended, it moves to the actual FCOJ and/or NFC production lines.

a. FCOJ production

Following clarification and blending, the juice is pre-heated and kept at pasteurization temperature. Then, it moves to a set of evaporators to be concentrated till 66 ºBrix. At last, the juice is cooled, blended with other production batches, and stored in tanks or drums, sometimes for several years. It is important to mention that during juice

concentration, volatile flavor components evaporate, so a recovery essence unit can be installed in the facility.

b. NFC production

For NFC production, the clarified juice should be cooled to 4 ºC, before blending, to avoid the growth of microbial organisms. Contrary to FCOJ, the juice for NFC should get only the necessary thermal treatment to ensure the final product is physically and microbiologically stable. So, after blending, the juice is pasteurized and stored. The storage can be held under frozen or aseptic conditions, for some months up to one year. Deoiling and deaeration may be required within the process to minimize oil levels in the juice and to remove oxygen, respectively.

IV. Peel Oil Recovery

As mentioned above, the oil emulsion stream from the juice extraction line is sent to the peel oil recovery section for oil purification. The process is held firstly by a two-step clarification, using centrifugation, and secondly by winterization (refrigeration), which is an oil refinement technique to separate the waxes from the other desired oil compounds, based on the difference in their melting points, solubility, and volatility. Finally, the purified oil is stored in drums, under frozen conditions.

2.3 Citrus Processing Waste and its Valorization

The citrus fruit processing facilities generate enormous amounts of organic waste that is usually disposed of into the environment or used as animal feed. However, this waste teems on valuable compounds with a vast range of applications in industry and presenting health benefits. Therefore, several institutions, like the European Commission and the United Nations, have been establishing some policies to encourage the valorization of CPW by abandoning the linear economy (take, make, dispose of) and implementing other economies that privilege the re-usability and/or the valorization (6).

2.3.1 CPW Characterization

The CPW inhere mentioned refers to all the organic matter generated throughout the juice production process, excluding the waste from packaging and off-specification materials. This CPW is mainly composed of peels, rags, seeds, damaged fruit, leaf residues, and wastewater from fruit grading, juice extraction, juice clarification steps, and cleaning activities. It is characterized by having a low pH (3 – 4), high organic matter (95% of total solids), and high moisture (about 80 – 90%), with several added-value compounds, including fats, free sugars, vitamins, organic acids, carbohydrate polymers, enzymes, flavonoids, essential oils, and pigments (7). Although citrus fruits are very similar to each other in terms of nature and general composition, CPW's composition depends massively on the fruit variety, climate, harvesting season, and juice extraction technique (6).

2.3.2 Why Does CPW Must Be Valorized? Economic and Environmental Aspects

One of the biggest challenges humankind has faced, and still faces, is the capability to meet market demands, while avoiding environmental collapse. It is a hard challenge to overcome, yet a crucial path we must go through.

2.3.2.1 CPW Towards Bioeconomy and Circular Economy

In 2012, the European Commission created a new concept: bioeconomy (7). The bioeconomy, also known as the bio-based economy, is an economic system that uses only renewable biological resources, from land and sea, to produce everything humankind needs, such as food, fuel, materials, fibers, and energy (11). However, this concept should not be confused with the circular economy.

The circular economy, an older concept popularized in the 90s, relies on sharing, leasing, reusing, repairing, refurbishing, and recycling all the existent materials and products, for as long as possible. Although they are different, the bioeconomy and the circular economy concepts complement each other and aim at a more sustainable and resource-efficient world, avoiding the use of additional fossil materials (6,12–15).

As mentioned before, CPW is a rich source of vitamins, dietary fibers, pectin, polyphenols, essential oils, etc. These compounds have a wide variety of applications, especially in the food, health, pharmaceutical, cosmetic, and textile industries, making CPW a powerful tool to develop a circular bioeconomy (6).

Another example of the CPW potential is the study developed by Paggiola et al. (16), which assessed the replacement of toluene with limonene as an industrial cleaning agent. Toluene is a widely used petrochemical solvent and limonene, on the contrary, is a citrus-peel extract, i.e., fossil fuels free. The study concluded that the substitution of toluene by limonene, at a global level, is currently unreachable. However, in specific regional cases, both citrus-producing and citrus-importing countries, the results showed that limonene is a potential solvent to substitute toluene within and beyond the cleaning sector.

Moreover, the composition of CPW, particularly the high content of soluble and insoluble carbohydrate compounds and low lignin content, makes it an important feedstock for biofuels production, such as ethanol, methanol, methane, biochar, and so on (7). Recently, Selvarajoo et al. (17) studied the conversion of citrus peel biomass, from lemon and orange fruits, into biochar by slow pyrolysis. The highest biochar yield was 53.62 wt%, at a pyrolysis temperature of 300 ºC. Yet, among all biochar samples, the one produced at 500 ºC demonstrated the best fuel properties (higher carbon content and higher heating value), and, therefore, the most comparable to fossil coal, from an energy perspective. The CPW's potential to produce biofuels was also studied by Taghizadeh-Alisaraei et al. (18), by presenting Iran's case. In Iran, 4.3 million tons of citrus per year are grown, 20 – 30% of which is used for juice production and the remaining is discarded due to a lack of storage conditions and processing industries. This 20 – 30% portion is equivalent to approximately 1.3 million tons of fruit, whose

processing leads to over 0.6 million tons of CPW. Considering a large biorefinery (capacity for 39.5 L of ethanol or 45 – 116 m^3 of methane, per ton of CPW), this amount of waste can yield about 27 million liters of ethanol and 79 million m^3 of methane, through fermentation.

CPW utilization as nanoparticles (NPs) and biosorbents is also under study. Mesoporous cellulose and nanocellulose are examples of NPs obtained from CPW. Regarding biosorbents, agro-industrial waste, including CPW, has been used to remove contaminants like arsenic and Cu(II) from water supplies and wastewater (7).

2.3.2.2 Environmental Burdens

The environmental damages caused by citrus processing industries start at their very first stage, i.e., fruit production. Growing fruit consumes enormous quantities of water, and requires a bunch of chemicals, namely pesticides to prevent diseases, herbicides for soil management, and fertilizers to supply the nutrients needed by the trees. Considerable amounts of energy and fuel are also needed for irrigation systems, and machinery, as well as for soil management and weed control (19).

Nonetheless, the main environmental challenge of citrus processing industries is the management of citrus solid waste. Although packaging and off-specification materials also generate solid residues, the problematic solid waste is essentially the organic waste produced after juice extraction, also including some leaf residues (7). In citrus processing plants, this solid waste is commonly used for cattle feeding (wet or dried) or disposed of in landfills, as mentioned in section 2.2.

It is worth mentioning that, sometimes, organic waste is disposed of in the soil as compost to improve its fertility and water retention capacity, as well as to reduce soil erosion by enhancing its resistance to rainfall. It is also used as a soil conditioner when applied on cultivable land and loamy soil, in their dormancy years (6). However, despite these benefits, laying citrus solid waste on the ground, either for landfilling or as compost, has some drawbacks, mainly the emission of greenhouse gases (GHG) and odor generation, due to its high content of carbohydrates that leads to a fast fermentability and, consequently, to a fast degradation (7). To mitigate landfilling, the EU directive 2008/98/EC established that citrus waste, and any other kind of organic waste, may only be disposed of in landfills if it has been previously subjected to valorization for resource recovery (7). Incineration is also a traditional disposal practice to get rid of wastes, but, regarding citrus organic waste, this method is not so common because of the great amount of energy required to evaporate the water content, not to forget the air pollution it causes (6).

Using citrus solid waste as animal feed is a traditional disposal method for this kind of waste, being the cheapest, considering the savings obtained from the reduction of cattle dependency on their conventional cereal feed (6). In addition, citrus feed has been reported to promote animals' weight gain, while improving their digestive system (20). In fact, the citrus feed has high carbohydrate, fiber, and low lignin content, i.e., an adequate profile to supplement the cattle diet.

Nevertheless, the direct use of citrus waste as animal feed has several disadvantages, such as the high cost of its transportation, storage, and processing due to high moisture content. These disadvantages are also associated to the direct use of CPW as compost (6).

In addition to citrus processing organic waste, the wastewater originated from the citrus juice production facilities should also be valued, because of its richness in fibers, pectin, phenolic compounds, essential oils, sugars, and organic acids. Furthermore, wastewater incorrect disposal is a major concern, mostly when it is directly discharged into water bodies (wells, land, or sewer systems). Its high content of organic matter, suspended solids, essential oils, and low pH makes wastewater a threat to the ecosystems. Fortunately, there are, currently, several laws that restrain companies from discharging their industrial wastewater into nature, unless it is treated previously and fulfills certain discharging requirements. However, the citrus processing wastewater's richness in valuable compounds can be both an advantage and a disadvantage, because its complex nature limits the range of suitable techniques to recover the referred compounds, in addition to high processing costs (6,7).

2.4 The Bitter Orange Fruit

2.4.1 Plant and Fruit Characteristics

BO, also known as sour orange, Seville orange, marmalade orange, and bigarade, belongs to the Rutaceae family. Botanically, it is designated as *Citrus aurantium* and results from the cross between the pomelo (*Citrus maxima*) and the mandarin orange (*Citrus reticulata*) (21).

The BO tree foliage remains green and functional through more than one growing season – it is an evergreen tree. The trunk is more erect, and the crown is more compact than the SO tree. The tree can measure less than 3 meters up to 9 meters tall. The dark-green leaves are commonly oval-shaped, minutely toothed, with a short sharp point at the apex (tip of the leaf), measuring 6.5 – 13.75 cm long, and 3.75 – 10 cm wide. The highly fragrant flowers are about 3.75 cm wide. They are composed of 5 white, slender, straplike, recurved, widely separated petals, and 24 yellow stamens. The fruit is small-sized (7 – 8 cm in diameter) and is usually round, oblate, or oblong-oval. The peel is considerably thick, with a rough, dimpled, yellow-orange surface and bitter taste. The BO interior contains 10 – 12 segments, in which few to numerous oblong, white seeds are found. The pulp is remarkably bitter and acidic. As the fruit is growing, the central core becomes wider and wider, till it's completely hollow when the fruit is full-grown (22,23).

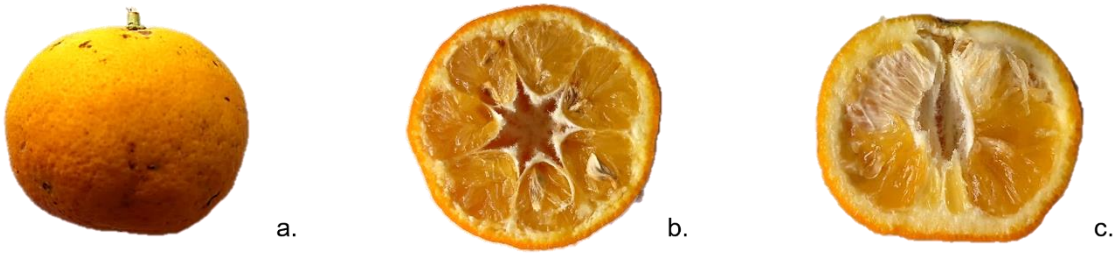

Figure 3 - Ripe BO fruit. a) whole fruit; b) fruit interior – transversal cut; c) fruit interior – longitudinal cut.

Figure 4. BO tree.

2.4.2 Origin and Global Distribution

BO historical events are a little uncertain. The fruit is native to Southeast Asia, like most citrus fruits, and natives of the South Sea Islands believe it was brought to their shores in prehistoric times. BO firstly appeared in Europe when the fruit was cultivated in Seville, Spain, by the Moors (also designated as Arabs), during the 10th century (way before SO conquered European lands). For 500 years, BO was the only kind of orange Europe had seen. It reached onwards Florida and the Bahamas when European colonialists moved into the New World (22,24).

Nowadays, BO fruit is extensively cultivated in the Mediterranean, China, India, Africa, the Middle East, Caribbean islands (the West Indies), and Brazil, but also in Argentina, Peru, Ecuador, Venezuela, Central America, and Europe (mainly in Portugal, Spain, and Italy) (25,26).

2.4.3 Varieties

In addition to the Seville orange variety of BO, there are other four main varieties (21,22):

- ***Citrus aurantium var. amara:*** native to Vietnam; spiny evergreen tree; used in marmalade and liquor making, in essential oil, neroli oil, and orange flower water (distilled from its flowers) production, and as graftage stock for citrus trees.
- ***Citrus aurantium var. daidai*****:** large fruits, very thick peel, very acidic pulp, and a lot of seeds; used in Chinese medicine and Japanese New Year Celebrations; its flowers are dried and added to tea.
- ***Citrus aurantium var. currassuviencis*** **(lahara or Curaçao orange):** grown in the Caribbean archipelago, Curaçao island, to make Curaçao liqueur.
- ***Citrus aurantium var. myrtifolia*** **(myrtle-leaved orange or chinotto):** sometimes considered a separate species (BO mutation); small fruits, compact shrub or tree, small leaves, and no thorns; mainly cultivated in French and Italian Riviera; its juice is used to make the Italian soda beverage Chinotto and the fruit is exported for candying.
- ***Citrus aurantium var. bergamia*** **(Bergamot Orange or bouquet orange):** probably a BO and limetta hybrid; round or pear-shaped fruits, aromatic peel, acidic pulp, small and sweetly fragrant flowers; cultivated in Italy to produce bergamot oil which is used by many brands of perfume and tea, like Earl Grey tea.

2.4.4 Applications

BO is not an edible fruit, owing to its bitter and sour taste that does not please most consumers. As such, its production is not intended for fresh fruit consumption or juice production, contrary to SO, lemon, mandarin, and other edible citrus fruits. BO fruit is used in the food industry, cosmetic, and medical applications (1). In Turkey, however, BO fresh juice is used as an alternative to lemon juice in salads (27).

It is in Great Britain that BOs find their main application within the food industry: BO marmalade. This fruit is ideal for making marmalade due to its high pectin content when compared to other citrus fruits, particularly SOs (28). BO marmalade is well appreciated by British consumers since early times. One of the oldest handwritten recipes for marmalade was found in the recipe book of Eliza Cholmondeley dating from 1677, and another recipe, dated from 1683, was found in the Dunrobin castle in Sutherland in the Scottish Highlands (2,24). Later, in 1797, the first commercial brand of BO marmalade in Great Britain – Keiller's Marmalade – was founded in Scotland (24). Nevertheless, the fruit for BO marmalade is not grown on British soil; it is brought from Seville and processed in Britain.

BO fruit, including its leaves, flowers, and essential oils, is used in many medicinal folk traditions. In South America and Mexico, the leaves are used as a tonic, a laxative, and a sedative for insomnia and anxiety, and the peel is used for stomach aches and to treat high blood pressure. In Europe, the

Basque people also use the leaves for relaxing purposes, as well as for stomach aches, whereas the peel is an anti-spasmodic. In traditional Chinese medicine, the peel of unripe fruit is used to treat digestive system-related problems, such as indigestion, abdominal pain, constipation, and dysenteric diarrhea. Other medical uses can also be considered: the strong sourness is capable of stimulating digestion and relieving flatulence; the essential oils are recognized for their sedative properties, in Western medicine, and are widely used in aromatherapy (e.g., neroli oil, which is also applied as relaxing massage oil); the distilled flower water has also sedative properties and is antispasmodic. Characteristic health benefits of citrus fruits, because of their vitamin C, bioflavonoids, phenolic acids and limonene high contents, should also be mentioned, including antiviral, anticancer, antioxidant, and anti-inflammatory, among others (29,30).

However, BO sees its main medical application in dietary supplements, as an appetite suppressant to improve weight loss and a muscle enhancer. These supplements contain *p*-SYN, an alkaloid mostly obtained from the peel extracts of unripe BO with sympathomimetic activity (1). Of all compounds, *p*-SYN contributes the most to the anti-obesity effect of BO, having a remarkable lipolytic action in adipose tissues, at elevated doses (1). In addition to *p*-SYN, these supplements also contain other compounds such as caffeine to enhance the weight loss effect (31). Until 2014, ephedrine was widely used as a fat burner (31). However, adverse effects on human health were attributed to ephedrine, resulting in its ban from herbal preparations and, therefore, its substitution by *p*-SYN (31). Even so, dietary supplements of BO extracts are controversial and generate discussion within the scientific community about their health benefits. This issue is wider discussed, in section 2.5.2.3.

In the cosmetic industry, *amara* variety of BO (C*itrus aurantium var. amara*) is used in several products such as sunless tanning, shampoo, conditioner, bar soap, makeup removal, exfoliant, blush, mask, facial cleanser, also including perfumery-making and other fragrance products (30,32). As an example, C*itrus aurantium var. amara* extracts are in the composition of some Clarins® products: petitgrain, flower water, and blossom wax (32). Petitgrain is an essential oil that is recognized for its calming and cleansing properties, whereas organic BO flower water is commonly used in skincare products for sensitive eye areas, and the blossom wax has protective and filmogenic properties (32).

2.5 Bitter Orange Added-value Compounds

2.5.1 Pectin

The growing concerns about climate change and pollution, have been forcing science and engineering towards the development of eco-friendly industrial processes and products, that are also financially sustainable. In this regard, and due to its easy conversion into added-value products, natural polymers' use has been gaining interest compared to synthetic polymers, which are derived from petroleum oil (33,34).

Pectin, a natural polymer present in the primary region and middle lamella of the plant cell wall, occupies a notable place among all natural polymers due to its vast applications in several fields, such as pharmaceutical, food, and beverage industry, as well as in textiles (33).

CPW is a valuable source of dietary fiber, including pectin. In general, citrus fruits present a high pectin content, especially BO fruit which is, therefore, prized for making marmalade (1). Hosseini et al. (35) obtained 18.35% of pectin from BO peel using distilled water, whereas Fakayode et al. (36) obtained a 30% lower yield from SO peel by using hydrochloric acid (HCl (aq)), pH 3, by applying similar extraction conditions. This shows that BO yields more pectin than SO, consequently being preferable for making marmalade.

2.5.1.1 *Molecular Structure and Properties*

Being composed of 17 different monosaccharides containing 20 different linkages, pectin is the most complex macromolecule in nature. Its molecular structure is mainly constituted by galacturonic acid monomers (70%), which can be in acetylated or methyl esterified forms (33,37). Galacturonic acid forms the backbone of five domains (figure 5): homogalacturonan (HG), xylogalacturonan (XG), apiogalacturonan (AG), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II).

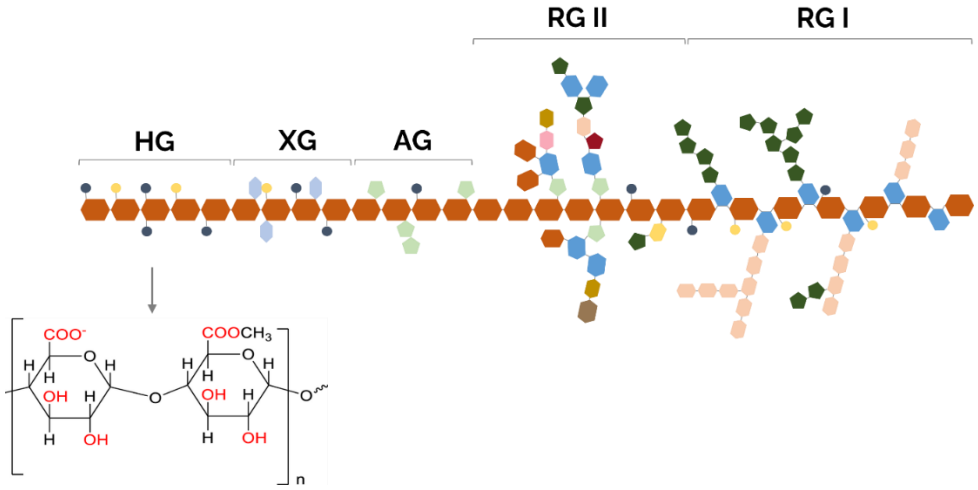

Figure 5. Structure of pectin showing the galacturonic acid backbone (dark orange) and the five domains. Adapted from (37)

Pectin is responsible for conferring strength and flexibility to the cell wall, being also liable for several biological functions, such as signaling, cell proliferation, differentiation, cell adhesion, and maintaining the turgor pressure of the cell (33).

In terms of solubility, there are two types of pectin, i.e., water-soluble, and water-insoluble, depending on pH, temperature, nature and concentration of solute, and pectin composition (monovalent cation of pectin is soluble, but di or trivalent are insoluble) (33).

Pectin has also gelation properties, making it suitable for a wide range of applications. This biopolymer can form a gel in the presence of acid, calcium, sugar, and dehydration agents, like alcohol (33). The formation of gel when contacting alcohol is particularly useful in pectin isolation from other compounds in a liquid mixture.

2.5.1.2 Low-Methoxyl Pectin (LMP) and High-Methoxyl Pectin (HMP)

Depending on the percentage of carbonyl groups esterified with methanol (degree of methylation or degree of esterification), pectin can be classified as Low-Methoxyl Pectin (LMP) and High-Methoxyl Pectin (HMP). A degree of methylation (DM) below 50% corresponds to LMP, whereas a DM above 50% corresponds to HMP. While HMP is considered the native pectin (it is mostly found in nature), LMP is obtained from HMP through demethylation by enzymatic or alkaline treatments. Due to their differences in the degree of methylation, LMP and HMP have distinguished physicochemical properties, for example, their ability to form hydrogels (37). The differences in their gelation properties will be further discussed in the next section.

2.5.1.3 Applications

Pectin is mostly used in the food and beverages industry, followed by the pharmaceutical industry, bakery, and dairy products (38).

Regarding the food industry (37), pectin was traditionally used only as a gelling agent for jams, jellies, and marmalades. Nowadays, pectin application in the food sector has been extended to fruit beverages, soft drinks, dairy products, confectionery, bakery fillings, and glazing. It should be highlighted that pectin physicochemical characteristics must comply with a set of requirements, depending on which food system pectin is addressed. For instance, LMP gels in the presence of divalent cations, like calcium ions (Ca^{2+}), being more suitable for stabilizing dairy products. On the contrary, HMP is used for jam and jelly preparation, because this kind of pectin can gel at relatively low pH (2 – 3,5) in the presence of soluble solids, such as sucrose. However, for low-calorie jam preparation, LMP is chosen instead of HMP due to the ability of LMP to gel in the absence of sugar. In soft drinks and beverages, pectin is used as a substitute for gums, since pectin, as a viscosifier, imparts a clean mouthfeel, whereas gums give a slimy mouthfeel. Beyond food products, pectin has also been gaining interest in food packaging with innovative pectin-based edible coatings. These eco-friendly coatings are enhanced and reinforced using other biopolymers, like cellulose and natural materials with antioxidant and antimicrobial properties.

In the pharmaceutical industry (33,37), pectin is used in the formulation of drugs for oral or nasal delivery, the preparation of scaffolds, and wound healing. From a biomedical point of view, the utilization of biopolymers in drug delivery systems is preferred over synthetic polymers because they are inert and biocompatible. Hence, pectin has been increasingly used in the formulation of drug delivery systems (e.g., tablets, gels, hydrogels, aerogels, beads, coated, and compression-coated dosage) due to its ability in forming a gel in acidic media and in dissolving in basic media, not forgetting its mucoadhesiveness. As an example, pectin is used in colon drug delivery because, in basic conditions, it is hydrolyzed by pectinolytic enzymes in the colon, releasing the bioactive molecule. On the other

hand, in gastric conditions (acidic medium), pectin forms a gel, therefore extending the contact time of the drug. Regarding nasal drug delivery, Sriamornsak et al. (39) showed that LMP has notable mucoadhesive properties making it able to bind to the mucin through hydrogen bonding. Pectin properties also make it a valuable biopolymer to be incorporated in the preparation of scaffolds for tissue engineering. Tentor et al. (40) reported adherence and proliferation of human osteoblast cells on the surface of a scaffold prepared from pectin and chitosan, showing that pectin is biocompatible and non-cytotoxic. In wound healing, pectin is used to remove exudates by forming a gel over the wound, to deliver drugs such as antibiotics, pain killers and tissue repair agents, and to create an acidic environment to protect the wound from bacterial and viral attacks.

2.5.1.4 Extraction and Isolation Methods

Currently, commercial pectin is mostly extracted from citrus peel (85.5%), apple pomace (14%), and sugar beet pulp from the sugar industry (0.5%). Other promising alternative sources have been studied, like banana peel, watermelon rinds, blueberry wine pomace, eggplant peel, and green tea leaf (37,41).

The conventional production process of pectin is schematically represented in figure 6. The biomass is pretreated (drying and maceration) before being subjected to an acid extraction, after which a filtration step follows to separate the biomass residues from the extract. Subsequently, the solubilized pectin in the extract is precipitated with ethanol and dried. The acid extraction occurs in an acidic aqueous medium (pH 1.5 – 3), using mineral acids (sulfuric, hydrochloric, or nitric), at elevated temperatures (75 – 100 ºC) for 1 to 3 hours. The acid is responsible for hydrolyzing pectin to turn the insoluble pectin into soluble. This reaction is enhanced by applying heat, as the plant cell wall is disrupted, facilitating the acidic solvent diffusion and, thus, the extraction of pectin. On the other hand, if the extraction is held at very high temperatures, pectin is hydrolyzed to short-chain molecules which will not precipitate in ethanol, negatively affecting the yield (37). Even so, the conventional method, as it is, is highly dependent on the heat, since without it the pectin yield would be reduced to 0.83%, according to Pancerz et al.

Although the conventional process is the mostly used at the industrial level, it has some inherent problems, including prolonged extraction time, high energy input due to demanding heat requirements, and environmental problems related to the discharge of wastewaters containing mineral acids, leading to increased costs. As a result, alternatives to conventional extraction have been emerging. These alternatives use organic acids (e.g., citric, and acetic acids) instead of mineral acids and are reported to be cleaner (green or natural), and more effective (37). Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Enzyme-Assisted Extraction (EAE), and Subcritical Water Extraction (SWE) are examples of non-conventional methods under study. Table 1 gathers some studies about pectin extraction from citrus fruits.

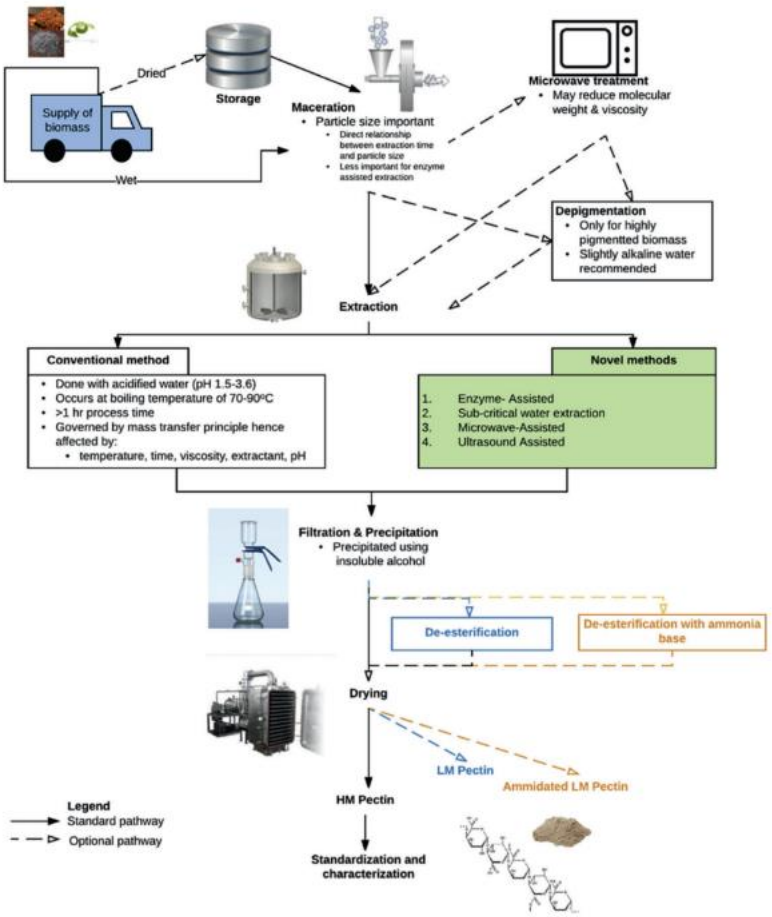

Figure 6. The industrial production process of pectin.

Table 1. Pectin extraction methods from citrus fruits.

Raw Material	Extraction Solvent/Solution	Precipitation Solvent/Solution	Extracting Conditions	Yield*	Reference
Conventional Extraction (CE)					
BO peel	Distilled water	98% EtOH	S/L ratio of 1:25, 95 ºC, 1.5h	18.35%	(42)
Lime peel	HCl 0.05 M	Absolute EtOH	S/L ratio of 1:20, 95 ºC, 1h	23.59%	(43)
Lime peel	Citric acid 0.05 M	Absolute EtOH	S/L ratio of 1:40, 95 ºC, 1h	19.63%	(43)
Ultrasound-Assisted Extraction (UAE)					
BO peel	Citric acid	96% EtOH	20 kHz, 150 W, pH 1.5, < 30 ºC, 20 min	26.60%	(44)
Grapefruit peel	HCl 0.5 M	95% EtOH	20 kHz, 12.22 W/cm^2, pH 1.5, 70 ºC, 30 min	27.51%	(45)
Microwave-Assisted Extraction (MAE)					
BO peel	Citric acid	96% EtOH	700 W, pH 1.5, 2 min	26.40%	(46)
Mandarin peel	H_2SO_4	95% EtOH	320 W, pH 1.5, 2 min	18.59%	(47)
Enzyme-Assisted Extraction (EAE)					
Lime peel	Citrate buffer 0.05 M + Laminex C2K	Propan-2-ol	S/L ratio of 1:30, pH 3.5, 50 ºC, 4h	23.00%	(48)
Lime peel	Citrate buffer 0.05 M + Validase TRL	Propan-2-ol	S/L ratio of 1:30, pH 3.5, 50 ºC, 4h	26.30%	(48)
Subcritical Water Extraction (SWE)					
Mandarin peel	Distilled water	Alcohol (NS)	S/L ratio of 1:30, 120 ºC, 5 min	21.95%	(49)

* Calculated on the dry weight of the sample (dry weight of extracted pectin ÷ dry weight of sample × 100)
S/L = solid mass to liquid volume ratio
NS = Not Specified

UAE and MAE techniques are reported to require less energy, less solvent, shorter extraction time, and lower temperature to obtain similar or higher yields than conventional extraction (37). As can be seen in table 1, the conventional extraction of pectin from BO peel with plain distilled water at 95 ºC took 1.5h to achieve a yield of 18.35%, whereas the application of ultrasound for only 20 min at a temperature lower than 30 ºC, in an acidic medium with citric acid (pH 1.5), gave a higher yield (26.60%). Similar yield results were achieved in an MAE study, in which analogous conditions to this UAE study were used. However, MAE only took 2 min to achieve a yield of 26.40%.

Contrarily to CE, UAE, MAE, and SWE, Enzyme-Assisted Extraction does not depend on heat to disrupt the plant cell wall, as it relies mainly on the ability of enzymes to do that (37). This is one of the main advantages of EAE, i.e., it requires low processing temperatures (sometimes even lower than UAE and MAE), and therefore low energy requirements. Other advantages are no corrosion of equipment by acids, increased quality of pectin, and improved or similar extraction yields as compared to CE (37). In general, the extraction time is also shorter than CE. Although both EAE studies shown in

table 1 do not prove the time reduction (they took 4h), Vasco-Correa et al. (50) studied the pectin extraction from passion fruit peel, in which 45 min at 37 ºC were enough to achieve a yield of 26.00%, using protopectinase. The major drawbacks of EAE are the cost of enzymes and the fact that the activity of enzymes might be affected by other extractable compounds such as alkaloids, flavonoids, and phenolics, thereby reducing pectin yield (37). It should be highlighted that, in EAE, buffers are used instead of acids because the enzymatic activity depends strongly on pH.

Instead of acids or organic solvents, SWE uses water at very high temperatures (100 – 374 ºC), remaining in the liquid state by applying pressure. Water is a non-toxic, cheap, readily available (for now), and easily disposed solvent, making SWE a cost-effective and environmentally friendly technique (51). Nevertheless, the demanding heat requirements lead to high energy costs, which is the major disadvantage of this operation (51).

Despite the disadvantages of the non-conventional methods, several investigators have been proving that they are promising alternative technologies to the conventional methods for pectin extraction, being in line with the development of “clean label” compounds. There are, however, some important obstacles to the implementation of such technologies at the industrial scale, namely the high equipment costs and the lack of scaling-up studies, at least until now.

2.5.2 Synephrine

Several alkaloids are found in BO fruit, such as synephrine, octopamine, tyramine, N-methyltyramine, and hordenine. However, synephrine is present in higher amounts (52), making this citrus the main commercial source of synephrine (25).

The content of synephrine is higher in the peel of unripe fruits and decreases with their maturation state (53) (Table 2).

Table 2. p-SYN concentrations in the peel, pulp, and juice of BO fruit.

Part of BO Fruit	*p*-SYN Content	Reference
Peel	0.56 mg/g peel	(54)
Pulp	0.19 mg/g peel	
Juice	56.9 mg/L (≈ 0.056 mg/g juice*)	(1)

* Calculated on the density of fresh BO juice obtained experimentally (ρ_{juice} = 1025 kg/m^3)

2.5.2.1 Molecular Structure and Properties

Synephrine is a phenethylamine alkaloid, i.e., it is a phenol with a 2-aminoethyl group (-$CH_2CH_2NH_2$) in which the H linked to C1 is substituted by a hydroxyl group (-OH) and one of the H linked to the amino nitrogen is substituted by a methyl group (-CH_3) (figure 7).

Depending on the hydroxyl group position in the benzene ring, three different positional isomeric forms exist: *p*-SYN, *m*-synephrine, and *o*-synephrine (figure 7). For several years, there was uncertainty about which synephrine isomers were present in BO fruit, but now it is known that it contains the *para* isomer. The *ortho* isomer content is unknown, while *m*-synephrine, also known as neosynephrine or phenylephrine, does not exist in any citrus fruit or other vegetal genera, being chemically synthesized (25).

Synephrine has a chiral center on the carbon atom linked to the -OH of the 2-aminoethyl group, so, besides the positional isomers, synephrine also exists in the form of two enantiomers – d- and l-synephrine. The d- isomer presents the (S) configuration and the l- isomer presents the (R) configuration (55).

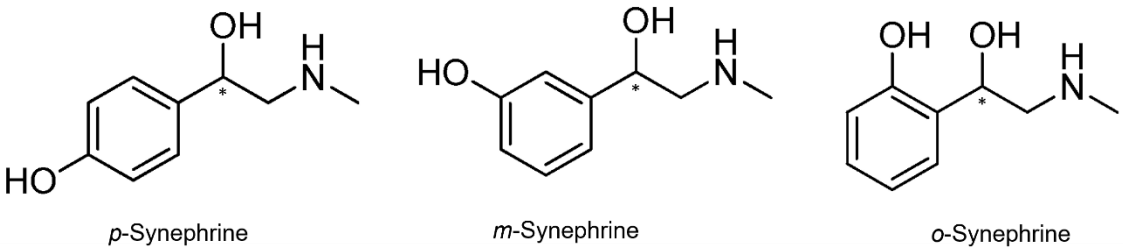

Figure 7. Structures of p-SYN, m-synephrine, and o-synephrine.

Due to its sympathomimetic properties (it mimics the effects of endogenous agonists of the sympathetic nervous system), *p*-SYN can reduce fat mass by stimulating lipolysis, raising the metabolic rate, and promoting fat oxidation through an increase in thermogenesis. In addition, *p*-SYN has been reported to have antidepressant effects, as shown by several studies (56,57) on rats.

Regarding its physical properties, *p*-SYN is a crystalline and white solid, is water-soluble and soluble in alcohols (55).

2.5.2.2 Applications

Synephrine is mostly used in the pharmaceutical sector.

The applications of *p*-SYN partially match the applications of BO extracts, mainly in weight loss management, sports performance, mental focus, appetite control, energy, and cognition (58), in the form of supplements. These applications are related to the sympathomimetic activity of synephrine, particularly the binding to β_3-adrenergic receptors that are responsible to regulate lipid and carbohydrate metabolism (58).

m-Synephrine is used in the treatment of shock, priapism, and bronchial problems related to asthma and hay fever, as well as in the preparation of intranasal decongestants, in surgical procedures as a vasopressor, and as a mydriatic agent (53,55,59).

2.5.2.3 Toxicity

Ephedrine utilization in herbal preparations or dietary supplements was banned from the market, in April 2004, by the Food and Drug Administration (FDA), due to reported adverse effects on human health. Since then, synephrine utilization in those products arose, as a way to replace ephedrine (60). Rapidly, the substitution of ephedrine for synephrine created some uncertainty about the safety and efficacy of BO extracts and synephrine itself, which was emphasized by the structural similarities between synephrine and ephedrine (figure 8).

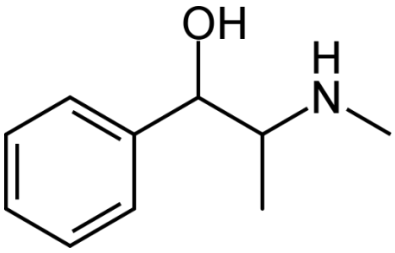

Figure 8. Structure of ephedrine.

Four studies about BO extracts and synephrine safety are summarized in table 3 and common daily doses of synephrine by weight loss supplements intake are found in table 4.

Hansen et al. (61) studied the cardiovascular effects of the daily intake, by gavage, of 10 and 50 mg synephrine (SYN)/kg of body weight (BW) in rats, for 28 days. The administrated synephrine was either in pure form (95% SYN BO extract) or in a 6% SYN BO extract. Caffeine addition (25 mg/kg BW) to both extracts was also assessed. They concluded that minimal effects were registered for pure synephrine, in any administrated dose. On the contrary, the animals treated with 6% SYN BO extract without caffeine showed more significant results than the ones treated with pure synephrine. This implies that other compounds present in the BO extract than synephrine may be responsible for the effects observed. However, the most prominent results were observed when caffeine was added; systolic and diastolic blood pressures and heart rate registered the highest increase compared to the control group values.

Body weight gain (BWG), blood pressure (BP), electrocardiogram, and mortality (M) were evaluated by Calapai et al. (62) when a population of rats was treated orally with 0.1 – 1.2 mg SYN/kg BW each day, for 15 days. Synephrine was also in the form of BO extracts. They observed that BWG decreased, and BP was maintained for the groups treated with BO extracts when compared to the control group. However, ventricular arrhythmias were also detected. In addition, mortality was exclusively verified in the groups treated with the BO extracts, with a maximum of mortality (50%) for

the 1.2 mg SYN/kg BW intake. However, this phenomenon was statistically insignificant and the authors state that it might be due to an insufficient number of animals, although the number was not specified.

Table 3. Studies concerning synephrine and BO extracts safety.

Product	Treated subjects	No. of Subjects	Duration	SYN Dose	Other Compounds	Main Results	Reference
6% SYN BO extract	Rats	27	28 days	10 and 50 mg/kg BW/day	-----	HR ↑ SBP ↑↑	(61)
95% SYN BO extract		27			-----	HR ↑ SBP ↑	
6% SYN BO extract		27			Caffeine (25 mg/kg BW)	HR ↑↑ SBP ↑↑ DBP ↑↑	
95% SYN BO extract		28			Caffeine (25 mg/kg BW)	HR ↑↑ SBP ↑↑ DBP ↑↑	
BO extracts	Rats	NS	15 days	0.1 – 1.2 mg/kg BW/day	NS	BWG↓ VA M_{max} = 50%	(62)
p-SYN supplement (S)	Humans	16	6 weeks	103 mg/week	-----	DBP_S<$DBP_{S+C, C}$ BP_S<$BP_{S+C, C}$	(63)
p-SYN + Caffeine supplement (S+C)				46 and 104 mg/week	Caffeine (233 and 337 mg/week)	SBP↑↑	
Caffeine supplement (C)				-----	Caffeine (240 and 325 mg/week)	SBP↑↑	
BO fresh juice	Humans	12	13h	13 – 14 mg	Other juice compounds	No significant effects	(64)

NS = not specified; BW = body weight; ↑ = increase; ↑↑ = significant increase; ↓ = decrease; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; BP = blood pressure; BWG = body weight gain; VA = ventricular arrhythmias; M_{max} = maximum mortality

Table 4. Common daily doses of p-SYN by weight loss supplements intake.

SYN Dose (mg/day)	Treatment Duration (days)	Reference
10	60	(65,66)
15	7	(67)
20	21	(68)
30	60 – 90	(69,70)

Similar experiments were done by Ratamess et al. (71) and Penzak et al. (64) in humans.

Ratamess et al. (71) gave various doses of *p*-SYN and caffeine present in *p*-SYN (S), *p*-SYN + caffeine (S+C), or caffeine (C) supplements to 16 subjects. This treatment was done in a single intake, one day a week, for 6 weeks. The most significant cardiovascular responses, particularly the highest increase in systolic blood pressure (SBP), were detected for S+C and C supplements, at the highest doses of caffeine. In addition, the intake of S supplements resulted in lower diastolic blood pressure (DBP) and mean BP compared to S+C and C supplements. Regarding heart rate (HR), no differences were observed.

Penzak et al. (64) studied the BO juice intake by 12 normotensive adults and its effects on SBP, DBP, BP, and HR. Each subject drank 240 mL of juice and 240 mL of water, on different occasions. The results showed that BO juice had no relevant effects on each cardiovascular parameter compared with water.

In fact, cardiovascular effects were observed in most of the presented studies, but the results should be taken carefully. Firstly, it is possible to conclude that caffein-added supplements or BO extracts tend to have more significant effects on DBP, SBP, BP, and HR increase, than synephrine or extracts alone. In many studies, products and BO extracts composition is not fully known, assigning erroneously the negative effects to synephrine or other BO fruit compounds. Furthermore, the doses given to rats by Hansen et al. (61) exceed the daily doses of *p*-SYN and caffeine humans usually consume, considering the standard metabolic equivalency factor. Giving 50 mg SYN/kg BW/day and 25 mg caffeine/kg BW/day to a rat is the same as giving 667 mg SYN/day and 333 mg caffeine/day to an 80 kg human. Considering the daily dose values of synephrine in the table 4, 667 mg of synephrine/day is way above those values, and the 333 mg/day of caffeine is like drinking 3 to 4 cups of 240 mL of brewed coffee, in a single shot (72). As can be seen, by Penzak et al. (64), the intake of BO juice at common doses of synephrine (13 – 14 mg) does not produce hazardous effects.

As mentioned before, the similarities between synephrine and ephedrine structures help the misconception about synephrine consumption. Nevertheless, this is meaningless, because even though their structures are similar, the pharmacokinetics and weight loss mechanisms are different due to important structural and stereochemical differences (25,73). Another aspect that clouds the safety and efficacy of synephrine and BO extracts is the fact that *p*-SYN is often confused with *m*-synephrine, a compound that may cause cardiovascular effects (25,73). Atkinson et al. (74) reported increases in systolic blood pressure along with decreases in heart rate for *m*-synephrine oral doses above 15 mg and an increase of 20 mmHg in SBP for an oral dose of 45 mg. However, it is important to remember that *m*-synephrine is not present in BO.

In conclusion, *p*-SYN, and BO extracts are safe at commonly consumed doses, such as in dietary and weight loss supplements, citrus juices, jams, teas, etc. However, many studies state that more long-term efficacy experiments in humans are needed.

2.5.2.4 Extraction and Isolation Methods

Table 5 gathers some patented processes (75–78) and studies (79–82) on the extraction of *p-SYN* from citrus materials.

Table 5. p-SYN extraction and isolation methods from citrus materials.

Raw Material	Extraction Solvent/Solution	Extracting Conditions	Isolation Method	Yield	Purity	Reference
Solvent Extraction (SE)						
Unripe BO peel	35 – 50% EtOH	40 – 45 ºC	Resin adsorption and ion exchange	2.5%	90%	(75)
Unripe BO peel	Sherwood oil or EtOH aqueous solution	70 ºC and 45 ºC	Resin adsorption	60%	97%	(76)
Mandarin peel	60 – 95% EtOH	50 – 70 ºC	Resin adsorption	83 – 91%	95%	(77)
Pericarp pomace	Boiling water	NS	Ion exchange	5%	98%	(78)
BO fruit, dry BO extracts, and herbal medicines	Water	RT, 30 min	---------	NS	NS	(79)
Ultrasound-Assisted Extraction (UAE)						
Stir-baked BO with bran	80% MeOH	420 W, 16 min	Membrane (MIM) permeation	NS	NS	(80)
Stir-baked BO with bran	65% EtOH	420 W, 16 min	Molecularly imprinted SPE	NS	NS	(81)
Unripe BO	68% EtOH	420 W, 16 min	Molecularly imprinted SPE	NS	NS	(82)

NS = not specified; RT = room temperature; MIM = molecularly imprinted membrane; SPE = solid phase extraction

p-SYN is mostly extracted from the peel of unripe citrus fruits, especially BOs (25). However, no concrete information about how the extraction is done at an industrial level was found, whereby research on the patented *p*-SYN extraction processes was attended. SE is often included in those processes at temperatures ranging from 40 ºC to 70 ºC, with ethanol aqueous solutions and water as the main used solvents/solutions. Resin Adsorption and Ion Exchange are the elected methods to isolate *p*-SYN, both carried out in adsorption columns. Ion exchange is held by strong-acid cation-exchange resins (75–78) and adsorption is held by polymeric resins, such as D101 (75), HP20 (77), and XAD-18 (77).

Alternative extraction and isolation methods are under study. J.-P. Fan et al. (80–82) studied the extraction and isolation of *p*-SYN from stir-baked BO with bran and unripe BO fruits using Ultrasound-Assisted Extraction (UAE) as the extraction method and Membrane Permeation or Molecularly Imprinted Solid-phase Extraction as isolation methods. UAE was held at 420 W with ethanol and methanol aqueous solutions for 16 minutes. In the Membrane Permeation study, a molecularly imprinted membrane (MIM) was prepared via polymerization using *p*-SYN as the template, a methacrylic acid/2-hydroxyethyl acrylate mixture as the co-functional monomers, ethylene glycol dimethacrylate (EGDMA) as the cross-linker and 2,2'-azoisobutyronitrile (AIBN) as the initiator. Regarding Molecularly Imprinted Solid-phase Extraction studies, molecularly imprinted polymers (MIP) were prepared using *p*-SYN as the template, EGDMA as the cross-linker, and AIBN as the initiator. For the *p*-SYN extracted from stir-baked BO, the MIP was synthesized via precipitation polymerization using an ionic liquid as the functional monomer and 80% (v/v) MeOH in water as the porogenic solvent, whereas, for the *p*-SYN extracted from unripe BO fruit, the MIP was synthesized via bulk polymerization, in which methacrylic acid and acetonitrile were used as functional monomer and porogen, respectively.

2.5.3 Polyphenols

Polyphenols, or phenolic compounds, are of great interest to several industrial sectors, especially the pharmaceutical one, owing to their important health-promoting properties such as their remarkable antioxidant activity. Daflon® is an example of medicine indicated in the treatment of venous circulation disorders and hemorrhoidal crises that is composed of phenolic compounds in the form of bioflavonoids (diosmin and hesperidin) (83).

Phenolic compounds are found in plants as secondary metabolites, i.e., substances manufactured by plants to guarantee their survival and competitiveness (1). Their function is to protect plants against UV light, parasites, and insects, being also responsible for their pigmentation and astringency (84).

Among plant genera, vegetables and fruits are the ones that stand out the most regarding polyphenols content (84). Citrus fruits are not the exception, being very popular for their anti-inflammatory, antioxidant, antitumor, antifungal, and blood clot inhibition properties, owing to the presence of bioactive compounds such as polyphenols, vitamin C, and carotenoids (85). Importantly, phenolic compounds are mostly found in the peel and inner white pulp of oranges and not so much in the juice, so this phytonutrient is often extracted from the processing waste of orange juice production, contributing to the implementation of a circular bioeconomy (86).

Regarding BO, phenolics are mostly found in its peel, seeds, and juice (87). The main phenolic compounds found in the BO peel are phenolic acids (74%), with emphasis on *p*-coumaric and ferulic acids, and flavonoids (23%), with rutin and NRG as the principal components (85). The seeds also teem in phenolics with flavonoids being reported as their major components at the ripe stage (56%) and phenolic acids in fewer concentrations (22%) (88). Phenolic acids represent 71% of the total phenolic

content of BO juice, again with the supremacy of *p*-coumaric (18%) and ferulic acids (19%), followed by flavonoids (23%), mainly rutin, NRG, and epicatechin (85). Vanillic and gallic acids may also be present in appreciable amounts in BO juices (85,89). Moreover, hesperidin, NHPD, and NERT are important flavonoids that can be found in BO fruits (84).

2.5.3.1 Molecular Structure and Properties

Polyphenols are aromatic compounds composed of one or more benzene rings bound to one or more hydroxyl groups. They are divided into five groups: phenolic acids, flavonoids, tannins, stilbenes, and lignans (84). Because the main phenolic compounds found in BO are phenolic acids and flavonoids, only these two groups are going to be approached.

Phenolic acids are the simplest phenolic compounds, in terms of molecular structure. They are composed of a single benzene ring and a carboxylic acid function. Two classes of phenolic acids exist: hydroxycinnamic acids (derived from cinnamic acid) and hydroxybenzoic acids (derived from benzoic acid). *p*-Coumaric and ferulic acids are hydroxycinnamic acids, whereas vanillic and gallic acids are hydroxybenzoic acids (84). Their molecular structures are shown in figure 9.

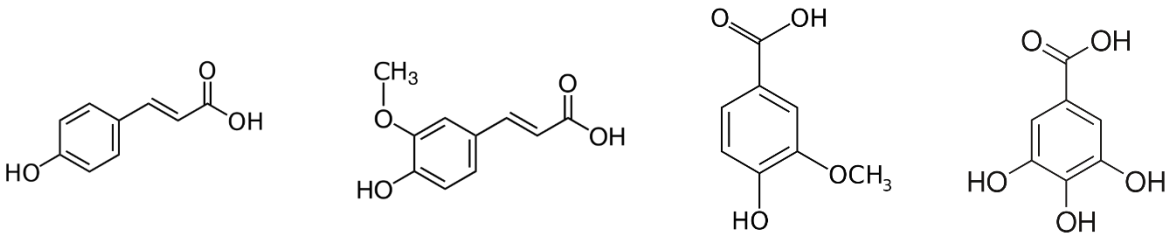

Figure 9. Structures of p-coumaric, ferulic, vanillic, and gallic acids.

Flavonoids have a more complex structure. They present two benzene rings bound to a heterocyclic pyran ring, resulting in different structural configurations and therefore different flavonoid families: flavonols, flavones, flavanones, flavanols, isoflavonoids, and anthocyanins (84). The structures of the most common flavonoids in BO fruit are shown in figure 10. Rutin is a flavonol, epicatechin is a flavanol and NRG, hesperidin, NHPD and NERT are flavanones.

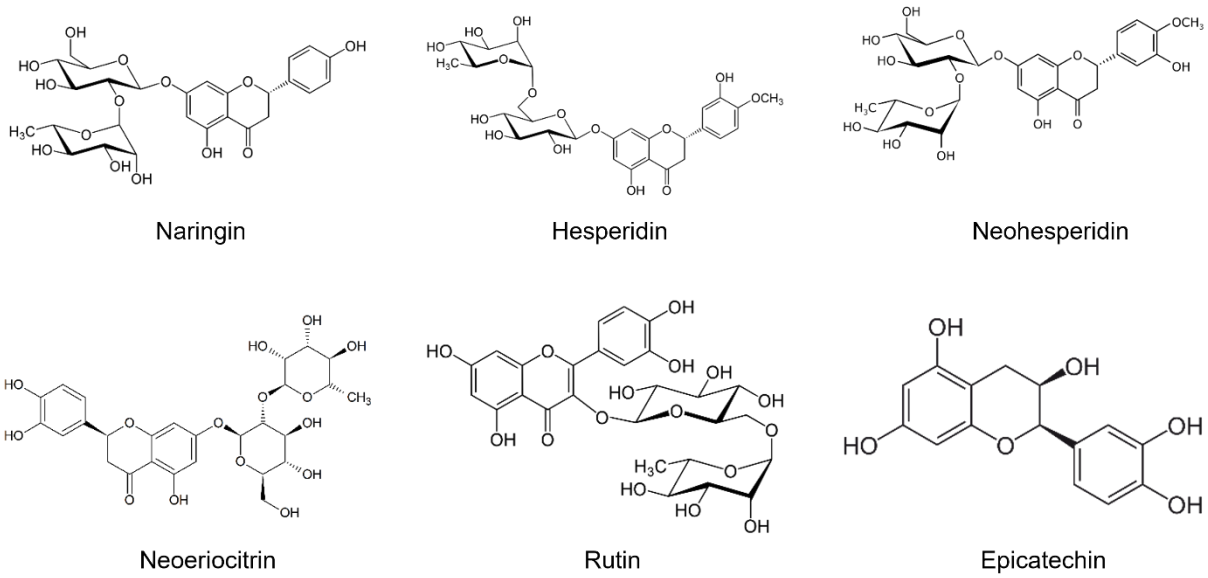

Figure 10. Structures of NRG, hesperidin, NHPD, NERT, rutin, and epicatechin.

One of the most important chemical properties of polyphenols is their antioxidant activity. Its importance relies on the scavenging of free radicals produced by oxidative processes that damage the organism cells, leading to diseases, including cancer (84). The antioxidant mechanism is based on the donation of a hydrogen atom and/or an electron to those radicals, resulting in the cease of the chain reaction of oxidation (84). Different numbers and positions of -OH groups in polyphenols' structures equals different antioxidation performances (84).

In addition to the antioxidant property, polyphenols have other important properties that accrue from their antioxidant activity, such as anti-inflammatory, antiviral, antimicrobial, anticancer, etc (86). Along with limonin, phenolic compounds are also responsible for the bitterness of citrus fruits, with special attention to NRG (90).

2.5.3.2 Applications

Antioxidant, anti-inflammatory, antiviral, antimicrobial, and coloring properties, among others, make polyphenols interesting compounds to be used in several industries, namely the pharmaceutical, food, packaging, cosmetic, and textile industries.

Flavonoids, phenolic acids, and anthocyanins obtained from plant extracts are very useful in the treatment and/or prevention of various diseases and health problems including cancer, diabetes, obesity, cardiovascular diseases, Alzheimer's, metabolic syndrome, etc (85). This relies on the ability of phenolic compounds to prevent or retard the oxidation of nucleic acids (DNA), proteins, and lipids, and to eliminate radicals, therefore, softening the severity or avoiding the evolution of a certain disease (91). In the case of cancer, phenolics, especially flavonoids, are reported to prevent the initiation of cancer, inhibit cell and tumor growth, block cell proliferation, and so on (92). Including polyphenol-rich food in our diet (vegetables, fruits, cereals) can prevent cardiovascular diseases due to the vasodilating properties and ability to reduce BP and Low Density Lipoprotein Cholesterol of phenolics (84).

Antioxidants have been added to foodstuffs to avoid their oxidative degradation and, thus, increase their shelf-life. However, the harmful effects of synthetic antioxidants on human health (liver damage, carcinogenesis, etc.) have been triggering the look for alternative antioxidants with less or no harmful effects (85). In this regard, natural antioxidants from plant extracts had and have been conquering their place in the food industry. It is the case of phenolic compounds which, due to their antioxidant and antimicrobial activities, meet all the requirements to be used as preservatives (84). As an example, a phenolic extract obtained from the pericarp of litchi, a fruit largely produced in China and India, showed a higher ability in retarding lipidic oxidation in sheep meat nuggets than the synthetic antioxidant butylated hydroxyl toluene (93). Beyond food preservative functions, phenolics are also added to food products just to enhance their nutritional value and health benefits (84). Moreover, the coloring properties of some phenolics are being explored to employ these compounds as coloring additives. It is the case of anthocyanins which are responsible for the red-purple color of some plant tissues (84). Though, low stability and photodegradation have been obstacles to their industrial use (84).

Polyphenols are also incorporated into packaging materials to preserve the products inside (84). In this regard, the inhibitory effect of chitosan-based biofilms with gallic and caffeic acids on *Bacillus subtilis* and *Staphylococcus aureus* was studied, showing higher antioxidant activity and bacterial growth inhibition than the simple chitosan films (94).

In the cosmetic industry, polyphenols are used as ingredients in the formulation of creams, such as skin wrinkle reduction and skin whitening creams, to replace synthetic for natural additives (95). When adding these compounds to cosmetic products, their anti-inflammatory, antimicrobial and anti-tyrosinase properties are not changed, as shown by a study on the addition of hydroxycinnamic acids to a semi-solid base cream (95). The use of phenolics in cosmetic products is reinforced by the fact that some phenolic compounds, like quercetin, resveratrol, and hydroxycinnamic acids, absorb UV radiation, thus functioning as a barrier to its penetration into our skin (84).

The coloring properties of some phenolic compounds are also being explored for textile applications, in order to substitute the synthetic dyes and, therefore, mitigate the chemicals-caused water contamination for which the textile industries are responsible. In addition, allergic reactions caused by synthetic dyes are avoided (84).

2.5.3.3 Extraction and Isolation Methods

Solvent extraction (SE) is the most common method to extract phenolic compounds (96). Several authors have reported methanol as the solvent that leads to higher extraction yields. For example, Goulas and Manganaris (97) studied the effect of solvent nature on the phenolics yield from mandora pulp, resulting in 70.2%, 46.1%, and 9.6% yields by using methanol, ethanol, and ethyl acetate, respectively. Similarly, Ma et al. (98) obtained higher yields of hesperidin by using methanol (52.5 mg/g DW) followed by ethanol (19 mg/g DW) and isopropanol (9 mg/g DW). However, methanol is often discarded in industrial applications due to its toxicity and consequent environmental problems, whereby it is substituted by non-toxic solvents with similar yields like ethanol, *n*-butanol, isopropanol, or petroleum

ether. Of the substitute solvents, ethanol is the most preferred to extract polyphenols from citrus fruits because of its efficiency and safety (96). Besides the solvent, temperature and time also have an important impact on phenolics yield. Their relation is normally inversely proportional (high temperatures with short extraction times and vice-versa) to avoid the thermal degradation of compounds since they are highly sensitive to heat (96).

Alternative methods to SE have been studied with the advantages of being more environmentally friendly and requiring less energy and solvent while leading to higher yields (96). The nature, principles, benefits, and drawbacks of the main alternatives match the alternatives to conventional extraction of pectin, already discussed in section 2.5.1.4. They are UAE, MAE, EAE, and SWE. Table 6 presents some studies on these extraction methods in comparison to SE.

The superiority of the alternative methods in terms of extraction yield and conditions in comparison with SE is clear. For example, Khan et al. (99) compared the UAE of flavanones from SO peels with SE under the same conditions, concluding that UAE has a positive effect on the extraction yield. A similar conclusion was obtained by Inoue et al. (100) who compared MAE with SE. An 8.9 mg flavonoids/g DW yield was achieved by using SE at room temperature for 2h, whereas 71.7 mg flavonoids/g DW was obtained by using MAE, under the same S/L ratio but at a higher temperature (140 ºC) and shorter time (7 min).

Regarding isolation and purification methods, adsorption onto resins or other adsorbent materials, such as MIPs, is regularly explored. For example, the resins Amberlite XAD-7HP, Amberlite XAD-16, Biotage RENSA PX, and RENSA PY were assessed towards *p*-coumaric acid, *trans*-resveratrol, and naringenin selectivity, while NRG adsorption was explored using XAD-4 resin, activated carbon, modified clay, and MIPs (101–104).

Table 6. Polyphenols extraction methods from citrus fruits.

Compounds	Raw Material	Extraction Solvent/Solution	Extracting Conditions	Yield	Reference
Solvent Extraction (SE)					
NRG and NHPD	BO peel	Absolute MeOH	S/L ratio of 1:50, RT, 2h, magnetic stirring	NRG: 5.1 ± 0.2 mg/g DW NHPD: 7.9 ± 0.2 mg/g DW	(105)
Hesperidin and NRG	SO peel	80% EtOH	S/L ratio of 1:4, 40 ºC, 60 min, magnetic stirring	HPD: 1.45 mg/g FW NRG: 0.51 mg/g FW	(99)
Flavonoids	*Citrus unshiu* peel	70% EtOH	S/L ratio of 1:10, RT, 2h, magnetic stirring	8.9 mg/g DW	(100)
Ultrasound-Assisted Extraction (UAE)					
Hesperidin	Mandarin peel	Absolute MeOH, EtOH, isopropanol	60 kHz, 3.2 – 56 W, S/L ratio of 1:40, 40 ºC, 60 min	MeOH: ≈ 52.5 mg/g DW EtOH: ≈ 19 mg/g DW Isopropanol: ≈ 9 mg/g DW	(98)
Flavonoids	Citrus peel	Distilled water	60 kHz, S/L ratio of 1:10, 40 ºC, 30 min	40.25 ± 12.09 mg/g DW	(106)
Hesperidin and NRG	SO peel	80% EtOH	25 kHz, 150 W, S/L ratio of 1:4, 40 ºC, 60 min	HPD: 2.05 mg/g FW NRG: 0.70 mg/g FW	(99)
Microwave-Assisted Extraction (UAE)					
Flavonoids	*Citrus unshiu* peel	70% EtOH	2.45 GHz, S/L ratio of 1:10, 140 ºC, 7 min	71.7 mg/g DW	(100)
Phenolic acids	Mandarin peel	Distilled water	400 W, S/L ratio of 1:2, 135 ºC, 3 min	23.2 mg GAE/g FW	(107)
Polyphenols	SO peel	51% Acetone	500 W, S/L ratio of 1:25, 2 min	12.2 mg GAE/g DW	(108)
Enzyme-Assisted Extraction (EAE)					
Polyphenols	Grapefruit peel	Aqueous Celluzyme MX solution	S/L ratio of 1:8, 50 ºC for 3h	0.9 – 1.62 mg GAE/g FW	(109)
Subcritical Water Extraction (SWE)					
Hesperidin and Narirutin	*Citrus unshiu* peel	Distilled water	160 ºC for 10 min	HPD = 72 ± 5 mg/g DW NRT = 11.7 ± 0.8 mg/g DW	(110)

RT = room temperature; HPD = hesperidin; GAE = gallic acid equivalent; DW = dry weight; FW = fresh weight

2.6 The Biorefining Concept

Contrary to what one might think, biorefineries are not a development of today. They are, actually, an innovation of the 19th century when steam-driven paper machines were introduced. Moreover, the production of vegetable oils, beer, and wine requiring pretreatment, separation, and conversion techniques developed thousands of years ago (111). An example of a primordial biorefining process is the production of bioethanol from lignocellulosic biomass, which was already in full operation 100 years ago (112). Current biorefineries are, nevertheless, more advanced than those of that time

with main innovations in food production: crystalline sugar, potato starch (early and mid-19th century), wheat, and corn starch (early 20th century), and recently soy oil, proteins, and vitamins (111).

Today, there is a multitude of different biorefining definitions. IEA Bioenergy Task 42 defines biorefining as “the sustainable processing of biomass into a spectrum of marketable products and energy” (113). Recently, a more specific official definition for biorefining came up in the “EU Biorefinery Outlook to 2030” study, also developed by IEA Bioenergy: “Biorefining is the processing of biomass into a portfolio of marketable bio-based products, which include co-production of food and feed, chemicals and materials and bioenergy (power, heat/cold, fuels)” (113). These definitions match the concept of waste valorization in the developed process, as it is centered in the processing of BOPW into a range of marketable compounds. Therefore, the waste valorization section of the process can also be designated as “BO biorefinery”.

The processing of biomass in a biorefinery begins with the feedstock selection, after which follows its treatment. The treated biomass is then fractionated into its components (sugars, proteins, oils, fibers/lignin, etc.), being further transformed into marketable bio-based products and/or bioenergy, through biochemical and/or thermochemical (catalytically supported) processes (114,115). Figure 11 shows a general schematic representation of a biorefinery. Biorefineries that are built with the purpose of producing added-value bio-based products, normally in small amounts, are called “product-driven” biorefineries. On the contrary, biorefineries whose main goal is to produce large amounts of low-cost energy are known as “energy-driven” biorefineries.

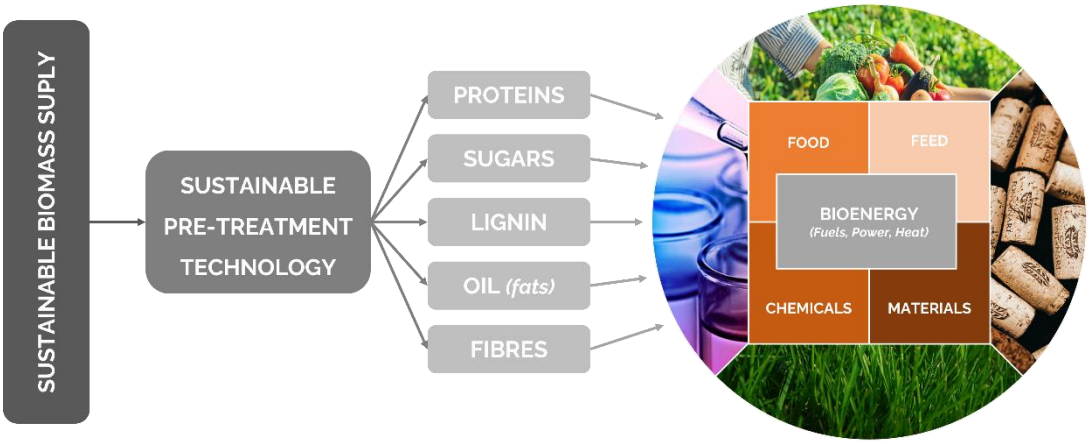

Figure 11. General schematic representation of a biorefinery. Adapted from (113).

Biorefineries are often compared to current oil refineries because their basic concepts are similar, i.e., both facilities have the purpose to fractionate a certain feedstock into a family of products. However, the feedstock and the range of products are, inevitably, different. The feedstock used in biorefineries is organic matter obtained from living or recently living organisms, in other words, biomass (115). On the contrary, the raw material used in oil refineries is the result of the biodegradation of crude oil over millions of years (115). Regarding the range of products obtained from the two refineries, a

biorefinery can produce all the products that an oil refinery is capable of and products that cannot be obtained from crude oil, like food products for instance (115). Despite of the differences between the two types of refineries, biorefineries attempt to mimic the energy efficiency of modern oil refining, which is something it is believed that future biorefineries will be able to do through heat integration and the development of co-products. In addition, biorefineries aim at (115):

1) Maximizing the value of the products obtained from the biomass by using the raw materials to the fullest (increasing the number of products, if possible) and minimizing the production of waste.
2) Reducing the dependency of many countries on fossil fuels.
3) Reducing the emission of GHG.
4) Stimulating regional and rural development.

Regarding goal 1), essential oils extraction processes, for example, with extraction yields of 1 – 2%, and 98 – 99% in biomass residues cannot be considered biorefineries if the biomass is not explored as maximum as possible to obtain other products or energy for the self-demand (115).

Because biorefineries have a strong relationship with sustainability, their whole value chain must meet certain parameters on the scope of environmental, economic, and social sustainability. It means that all stages of their life cycle must meet those parameters, including their operation, but also their construction, and dismantling. The parameters are related to resource demand, GHG emission, primary energy demand, the impact on water use and quality, changes in land use, soil carbon stock balance and fertility, impact on biodiversity, production costs, added-value, social impacts on the population/employees/stakeholders, as well as international and regional dynamics, end-users, and investment feasibility (114).

The biorefining concept is of great importance to the implementation of the circular bioeconomy, due to its ability to produce a vast and diverse range of products from biomass, in a friendly environmental way.

An analysis of the status of biorefineries, the main barriers to their deployment, and potential solutions is found in appendixes A and B.

2.7 Resin Adsorption and Ion Exchange

Sorption processes are widely used in the industry for purification purposes, such as gas purification in industrial exhausts and liquids purification in food and non-food sectors, among others (116). The principles of adsorption are already used since 3750 B.C. when Egyptians and Sumerians used charcoal to reduce the content of metals in bronze production (116). Clay, zeolites, and charcoal are well-known adsorbers, but nowadays more sophisticated adsorbers have been used and/or

explored, such as high-performance activated carbon, synthetic zeolites, and synthetic resins with polystyrene, polyacrylic esters, and phenolic matrices (116).

Ion exchange processes have been as well largely used, mainly in water and effluent treatment, but also in other areas such as chemical synthesis, food processing, mining, agriculture, and medical research (116). Like adsorbent materials, modern ion exchangers have also emerged to replace the archaic ones (e.g., clay, glauconite, humic acid, zeolites, etc.) and nowadays they have almost completely displaced them (116).

In addition to the already mentioned applications of adsorption and ion exchange, it is also important to mention the enrichment and purification of added-value compounds like polyphenols in which these techniques are commonly used (116). In the food industry, for example, resins are used in the stabilization, de-coloration, or debittering of fruit juices by decreasing the polyphenols content (116). Therefore, adsorption and ion exchange techniques are in agreement with the context of the present work.

Adsorption and ion exchange phenomena are similar, but some significant differences should be pointed out. Adsorption is the adhesion of ions and/or molecules from a fluid phase (liquid or gaseous) to the surface of a solid, through physical, chemical, or ionic interactions between the adsorbate and the adsorbent (116). Adsorption by physical or chemical interactions is, however, the most common. If the interactions are physical, the adsorptive phenomenon is called physisorption, in which Van-der-Waals interactions prevail. On the other hand, If the interactions are chemical, it is chemisorption, wherein a chemical reaction occurs between the surface of the adsorbent and the adsorbate in specific active sites. Figure 12 schematizes physisorption and chemisorption.

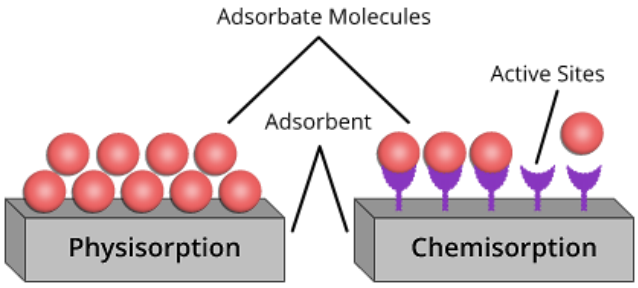

Figure 12. Physisorption and chemisorption schematic representations (143).

Ion exchange phenomena rely on the capture of dissolved compounds from a solution. However, instead of working with both molecular and ionic species, ion exchange only works with ionic species (116). The conventional ion exchange resins have a cross-linked polymer matrix to which charged functional groups are attached through covalent bonds (117). Those functional groups are linked ionically to the counterion of resins, i.e., the ion that exchanges with the ion from the solution,

therefore forming two ionic fluxes: one into the resin and the other in the opposite direction. The exchange occurs till electroneutrality is achieved. Equation (1) (117) represents how ion exchange occurs. The resin R has the ion Na^+ as the counterion. When a solution containing Ca^{2+} ions is put in contact with R, Ca^{2+} binds to functional groups of R, forcing an equal amount of Na^+ ions to flow out of the resin to the solution. Because ion exchange is reversible, to regenerate R back to Na^+ form, it must be treated with a sodium chloride solution, to be further used in another cycle of operation.

$$2RNa^+ + Ca^{2+} \leftrightarrow R_2Ca^{2+} + 2Na^+ \quad (1)$$

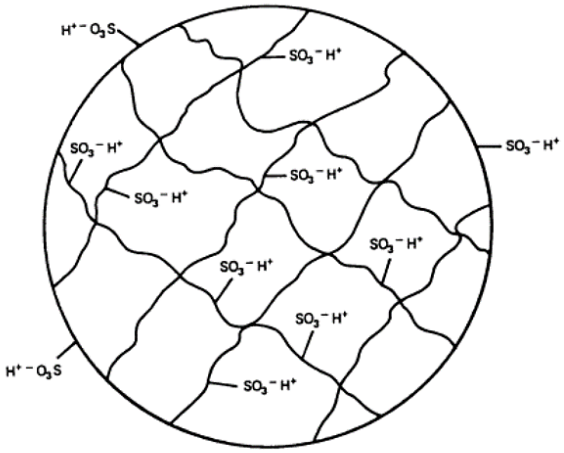

Figure 13. Schematic representation of a cation-exchange resin (117).

Four types of functional groups, and so four types of resins, exist (117,118):

- **Strong-acid cation-exchange:** sulfonic functional groups ($-SO_3^-$); the counterion of this type of resin can be H^+ or Na^+, so the resin is said to be in the H^+ form or in the Na^+ form, respectively.
- **Weak-acid cation-exchange:** carboxylic functional groups ($-COO^-$); the counterion of this type of resin is H^+.
- **Strong-base anion-exchange (type 1):** quaternary amine functional groups ($-N^+(CH_3)_3$); the counterion of this type of resin can be OH^- or Cl^-, so the resin is said to be in the OH^- form or in the Cl^- form, respectively.
- **Weak-base anion-exchange (type 2):** tertiary amine functional groups ($-N(CH_3)_2$).

To assess *p*-SYN isolation in this thesis, the binding and selectivity for this compound were explored for nine different resins: three cation-exchange resins (AG 50W-X8, PD-206, and MAC-3

Hydrogen Form), three anion-exchange resins (IRA-458, IRA-68, and CG-400), and three polymeric adsorbers (XAD-16, XAD-7, and XAD-4).

The characteristics of the selected resins are presented in table 7.

Table 7. Characteristics of the resins assessed in this thesis.

Resin	Type	Matrix	Functional Group	Ionic From
AG 50W-X8	Strong-acid cation-exchange	ST-DVB	Sulfonic	Na^+
PD206	Strong-acid cation-exchange	ST-DVB	Sulfonic	H^+
MAC-3 H Form	Weak-acid cation-exchange	Acrylic polymer	Carboxylic	H^+
IRA-458	Strong-base anion-exchange	Cross-linked acrylic gel	Quaternary amine	Cl^-
IRA-68	Weak-base anion-exchange	Cross-linked acrylic gel	Tertiary amine	Free base
CG-400	Strong-base anion-exchange	NS	Quaternary amine	Cl^-
XAD-16	Polymeric adsorber	Macroreticular aliphatic cross-linked polymer	NA	NA
XAD-7	Polymeric adsorber	Macroreticular aliphatic cross-linked polymer	NA	NA
XAD-4	Polymeric adsorber	Macroreticular aromatic cross-linked polymer	NA	NA

NS = not specified; NA = not applicable; ST-DVB = styrene divinylbenzene

Chapter 3

Materials and Methods

3.1 Plant Material

Ripe BOs were harvested from the Alameda campus of Instituto Superior Técnico, Lisbon, Portugal, in December 2020.

3.2 Reagents

HPLC-grade ethanol (EtOH), methanol (MeOH), glacial acetic acid (AcOH), hydrochloric acid (HCl (aq)) 37%, sodium hydroxide (NaOH) pellets, citric acid powder 99+%, naringin 97%, and Amberlite® XAD-16 were purchased from Thermo Fischer Scientific. Sodium chloride (NaCl) was purchased from PanReac AppliChem ITW Reagents. *p*-Synephrine >98%, neohesperidin >97%, naringenin >93%, and hesperetin >97% were purchased from TCI Chemicals Europe N.V. Neoeriocitrin 95%, narirutin >98%, and Dowex® MAC-3 Hydrogen Form were purchased from Sigma-Aldrich. Bio-Rad® AG 50W-X8 was purchased from Calbiochem. Purolite® PD-206 was kindly provided by Neoquímica, S.A. Amberlite® resin CG-400 was purchased from BDH Chemicals Ltd. Amberlite® IRA-458, Amberlite® IRA-68, Amberlite® XAD-4, and Amberlite® XAD-7 were purchased from Rohm and Haas S.A.

3.3 Instrumentation

A Sartorius analytical balance (CPA64, d = 0.1 mg) was used to weigh reagents and materials. Samples were centrifuged in a Hermle centrifuge (Z 233 M-2). *p*-SYN, flavonoids, and pectin extraction assays, as well as binding, recovery, and resin reusability experiments were carried on an Argo Lab heating plate with magnetic stirring (M3-D). The pH of samples was measured with a Metrohm pH meter (744). A Thermo Scientific vacuum drying oven (Vacutherm VT 6025) was used for samples and BO peels drying. BO peels were ground in a Moulinex grinder. Samples were shaken till homogeneity in a Janke & Kunkel IKA®-Labortechnik vortex (VF2). Water was purified using a Millipore milli-Q system, for HPLC analysis.

3.4 Methods

Although the developed process counts on the valorization of all the BOPW (peels, rags, seeds, and damaged/unsuitable fruit), only the BO peel was considered for the extraction experiments, with the intent to simplify the procedures.

After being washed with water, the fruit was cut into pieces and frozen at -19 ºC. In January 2022, the fruit was defrosted, and the peel was separated from the rest of the fruit, being dried in a vacuum oven (100 mbar) at 40 ºC, till constant weight. Once dried, the peel was ground.

3.4.1 Extraction of *p*-Synephrine and Flavonoids from Bitter Orange Peel

p-SYN and flavonoids extraction procedures were based on Pellati et al. (119) with some modifications. 0.4 g of ground peel were extracted with 25 mL of solvent for 2h at room temperature or at 90 ºC, using magnetic agitation (200 rpm). Six solvents/solutions were considered: distilled water, acidified water (pH 1.5), 50% (v/v) EtOH (aq), 80% (v/v) EtOH (aq), 96% (v/v) EtOH (aq), and 99.8% MeOH. The acidified water was prepared by adding HCl (aq) 4 M drop wisely till the pH of 1.5 was achieved. The 50%, 80%, and 96% EtOH (aq) solutions were obtained by diluting absolute ethanol.

After extraction, the extract was filtered under vacuum. For the extractions obtained at 90 ºC, the extract was cooled prior to filtration using an ice bath. 1 mL of the resulting filtrate was concentrated to dryness under vacuum (100 mbar) at 40 ºC in a vacuum oven and redissolved in MeOH to be quantified by HPLC, and the remaining volume moved to the next stage: *p*-SYN and flavonoids isolation. The cake obtained from the filtration was extracted two more times, using the same procedures. The extraction yields were reported as mg of compound per g of dried peel.

3.4.2 *p*-Synephrine and Flavonoids Binding Experiments

The separation of *p*-SYN from the flavonoid fraction present in the extract was performed by resin adsorption and ion exchange. 50 mg of each resin were put in a 2 mL Eppendorf tube, along with 1 mL of the filtrate from the first extraction (S/L ratio = 50 kg/m^3). Because the filtrate from the first extraction was the most concentrated, it was considered for the binding experiments, instead of the filtrates from the second and third extractions. Nine resins were tested: AG 50W-X8, PD-206, MAC-3 Hydrogen Form, IRA-458, IRA-68, CG-400, XAD-16, XAD-7, and XAD-4.

The filtrate was left in contact with the resin overnight (≈15h) at room temperature and under magnetic agitation (200 rpm). Each resin was tested in duplicate. Thereafter, the Eppendorf tubes were centrifuged at 10 000 rpm for 5 min and the supernatant was removed, using a syringe and a needle. The supernatant was then concentrated to dryness under vacuum (100 mbar) at 40 ºC, in a vacuum oven, and redissolved in MeOH to be analyzed by HPLC.

Before binding experiments, the anion-exchange resins were washed with NaOH (aq) 1 M, and the polymeric adsorbents were washed with distilled water, four times for 15 min, under magnetic agitation (200 rpm).

The percentage of *p*-SYN and flavonoids bound to each resin ($B\%$) was calculated using equation (2), where C_0 (ppm) is the initial compound concentration and C_f (ppm) is the final compound concentration in solution. The selectivity for *p*-SYN (S_{p-SYN}) was calculated by the ratio between the $B\%$ of *p*-SYN ($B\%_{p-SYN}$) and the $B\%$ of flavonoids ($B\%_f$) (equation (3)).

$$B\% = \frac{C_0 - C_f}{C_0} \times 100 \quad (2)$$

$$S_{p-SYN} = \frac{B\%_{p-SYN}}{B\%_f} \quad (3)$$

3.4.3 *p*-Synephrine and Flavonoids Recovery Experiments

To recover the *p*-SYN bound to the resins, 500 µL of eluant (10%, 15%, 25% NaCl (aq) for AG 50W-X8 and HCl (aq) 1 M, 5 M, and 8 M, for PD-206) were added to the Eppendorf tubes containing the resin and put under magnetic agitation (200 rpm) for 24h at room temperature. Then, the Eppendorf tubes were centrifuged at 10 000 rpm for 15 min and the supernatant was separated from the pellet, concentrated to dryness under vacuum (100 mbar) at 40 ºC, in a vacuum oven, and redissolved in MeOH to be analyzed by HPLC. These procedures were repeated two times (two steps of recovery for each resin duplicate). The HCl (aq) solutions were prepared by diluting a 37% HCl (aq) solution to achieve 1 M, 5 M, and 8 M concentrations.

The percentage of recovered compounds ($R\%$) was calculated using equation (4), where M_r is the recovered mass of each compound (mg), and M_b is the bound mass of each compound (mg). M_r and M_b were obtained from equations (5) and (6), respectively, where $C_{f'}$ (ppm) is the concentration of recovered compound in the supernatant, V_r (L) is the volume of eluant, and V_a (L) is the volume of solution used in the binding assay.

$$R\% = \frac{M_r}{M_b} \times 100 \quad (4)$$

$$M_r = C_{f'} \times V_r \quad (5)$$

$$M_a = (C_0 - C_f) \times V_a \quad (6)$$

3.4.4 Extraction of Pectin

Pectin extraction and isolation procedures were based on Fakayode et al. (36), with little modifications and using the reported optimal extraction conditions.

1.5 g of ground peel were extracted with 60 mL of acidified water (pH 1.5) or citric acid aqueous solution (pH 1.5) for 105 min at 95 ºC, under magnetic agitation (200 rpm). The acidified water was prepared by adding HCl (aq) 4 M drop wisely to get a pH of 1.5, and citric acid was prepared by dissolving 13.05 g of citric acid into 100 mL of water to achieve a pH of 1.5. Afterward, the obtained extract was cooled in an ice bath and filtered under vacuum. The filter cake was discarded, and the filtrate was transferred to a beaker.

An equal amount of 96% (v/v) EtOH (aq) was added to the filtrate to enable pectin precipitation. To accelerate this step, the mixture was left at 5 ºC. After 2h, the gelatinous pectin floating on the surface was cleared off using a spatula and transferred to a Petri dish. The remaining mixture was filtered through cheesecloth to prevent wasting pectin. Once all the pectin was removed, it was filtered by gravity through cheesecloth, washed two times with 96% (v/v) EtOH (aq), and weighed. Finally, pectin was dried at 65 ºC in a vacuum oven (100 mbar), till constant weight, and weighed again.

Dried pectin yield ($DPY\%$) was calculated using equation (7), where W_d is the obtained weight of dried pectin (g), and W_p is the initial weight of ground peel used for extraction (g).

$$DPY\% = \frac{W_d}{W_p} \times 100 \quad (7)$$

3.4.5 *p*-Synephrine and Flavonoids quantification

The amount of *p*-SYN and flavonoids found in the solutions obtained through all the experimental work was determined using HPLC with UV-vis detection. The quantified flavonoids were NRG, NHPD, and NERT.

Before being analyzed, each sample was centrifuged at 10 000 rpm for 5 min and the obtained supernatant was filtered through a 0.22 µm PTFE filter to a 2 mL vial. For the samples with a smaller volume (< 1 mL) (e.g., recovery samples), inserts of 500 µL were introduced in the vials. Between each sample, the PTFE filter, as well as the needle used to remove the supernatant, were washed with MeOH and air dried.

BO juice was characterized by quantifying *p*-SYN and flavonoid contents. Juice pH was also measured. BOs harvested in December 2020 were squeezed manually with the help of a domestic juice squeezer. The extracted juice was then transferred to plastic bottles and frozen at -19 ºC. In October 2021, the juice was defrosted, centrifuged at 10 000 rpm for 10 minutes, and filtered under vacuum. At this point, the pH was measured. For HPLC quantification, 1 mL of the filtrate juice was concentrated to dryness under vacuum (100 mbar) at 40 ºC, in a vacuum oven, and redissolved in MeOH. The sample

was diluted four times in order to allow compound concentrations to fall into the concentration range of the calibration curves.

The HPLC analysis were carried out on a VWR HITACHI Chromaster High-Performance Liquid Chromatography (HPLC) system equipped with a UV-vis detector (5420), a column oven (5310), an autosampler (5260), and a pump (5160), using a Luna 10 µm C18(2) 100 Å (250 x 4.6 mm) LC column with a mobile phase composed of 0.6% (v/v) acetic acid (aq) and MeOH, at 0.6 mL/min, a volume of injection of 10 µL and detection at 225 nm for *p*-SYN and 283 nm for NRG, NHPD, and NERT.

3.4.5.1 Calibration Curves

Standard solutions for each compound were prepared. The most concentrated solution (stock solution) was prepared by dissolving a known amount of pure compound in MeOH, and the less concentrated solutions were prepared by diluting the stock solution. A total of 12 to 20 solutions of each compound were prepared over the following ranges: *p*-SYN 0.5 – 254 ppm, NRG 0.5 - 1008 ppm, NHPD 0.5 - 620 ppm, and NERT 5 – 1000 ppm.

Each solution was analyzed by HPLC using the isocratic method described in table 8. The calibration curves were obtained by plotting the area of each peak (mAU.s) as a function of the concentration (ppm). They can be found in figures 36 - 40 in appendix C.

Table 8. Isocratic method for HPLC analysis.

Time (min)	A (%)	B (%)	Flow (mL/min)
Initial	60	40	0.6
31	60	40	0.6

A = 0.6% (v/v) acetic acid (aq)
B = MeOH

3.4.5.2 Quantification

Given the peak areas obtained by HPLC analysis, it was possible to determine the corresponding concentrations of *p*-SYN and flavonoids, by linear interpolation, using the calibration curves.

The gradient profile of the HPLC method used is described in table 9.

Table 9. Gradient profile of the HPLC method.

Time (min)	A (%)	B (%)	Flow (mL/min)
Initial	60	40	0.6
27	60	40	0.6
32	10	90	0.6
42	10	90	0.6
47	60	40	0.6
60	60	40	0.6

A = 0.6% (v/v) acetic acid (aq)
B = MeOH

Chapter 4

Results and Discussion

4.1 Overall Process Concept

The process for BO juice production was designed based on the concept of orange processing plants (10) and the unitary operations that underlie them. A simplified schematization of the process is shown in figure 14.

The envisioned process starts with the arrival of BO fruit at the processing plant (Fruit Unloading). The fruit is then conveyed to a prewash station, where it is washed to remove the superficial dirt and pesticide residue (Washing). After washing, the damaged fruit is manually separated from the sound fruit, along with some leaves or twigs that may still be attached to them (Destemming and Pregrading). The sound fruit is then stored (Fruit Storage) and, before being processed for juice extraction, it is subjected to a final washing (with disinfectant) and a final inspection to remove the damaged fruit that remained after the first grading (Final Washing and Grading).

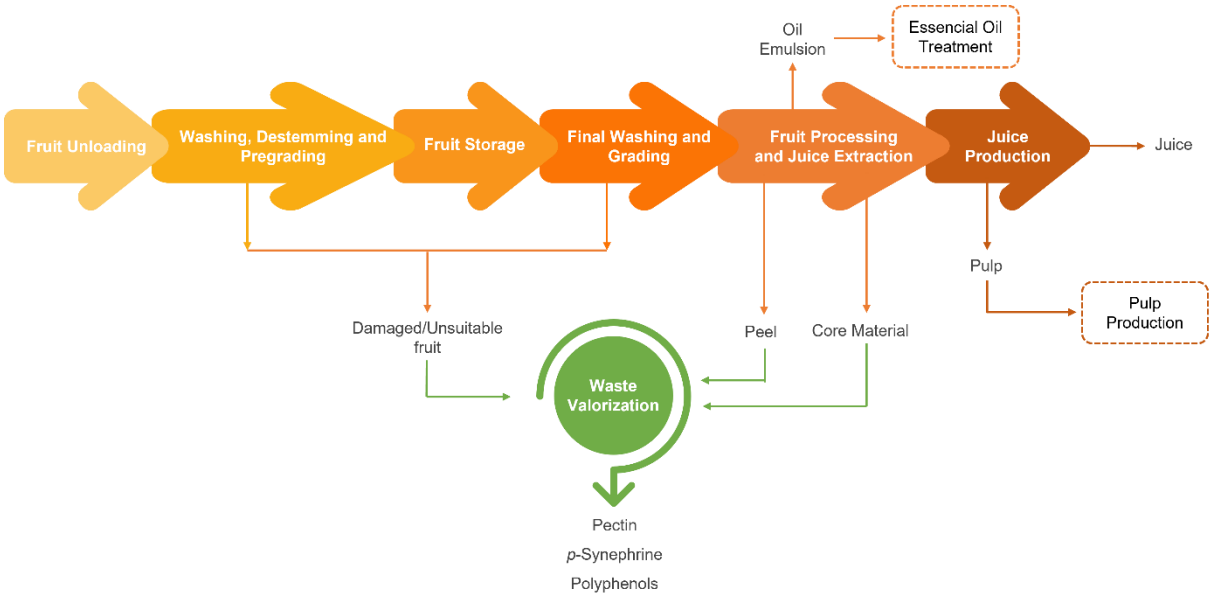

Figure 14. Simplified diagram of the overall process for BO juice production.

Once the fruit is washed and graded, juice extraction begins (Fruit Processing and Juice Extraction). In this operation, the fruit is squeezed to obtain a pulpy juice, and the peels and core material of the fruit are left aside. Because of the peel pressing by the juice extractor, an oil emulsion is produced, being afterward subjected to a treatment that is out of the scope of this thesis. Juice Production follows, wherein the pulp is separated from the juice, through clarification, and headed to a Pulp Production station, whose unitary operations are also out of the scope of this thesis.

In a conventional orange processing plant, the damaged/unsuitable fruit from the Grading steps, and the peel and core material from Juice Extraction, are usually conveyed to a feed mill where they are processed into animal feed. In the designed process, the BOPW is, instead, explored and valorized in order to produce added-value compounds, such as pectin, *p*-SYN, and polyphenols (flavonoids, in particular). A simplified diagram of the proposed waste valorization process is presented in figure 15.

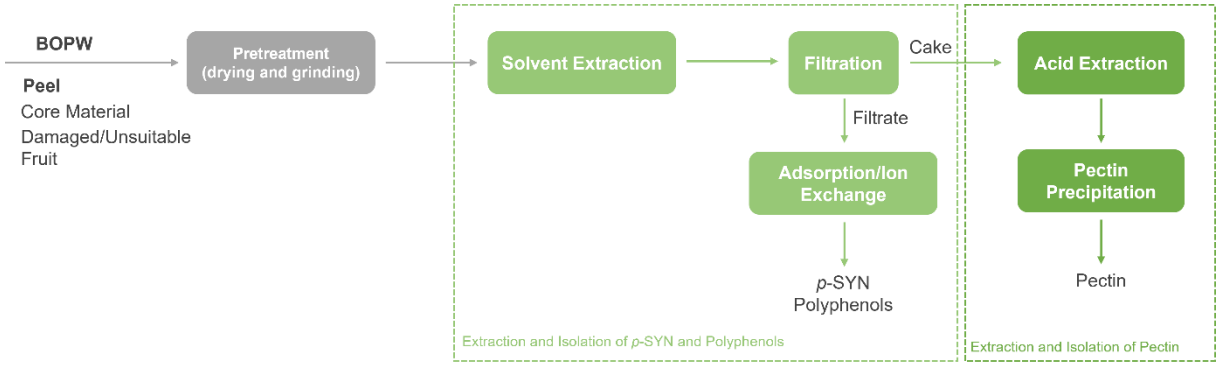

Figure 15. Simplified diagram of the proposed waste valorization process.

Before BOPW is subjected to any valorization process, it is dried and ground. Then, a solvent is used to extract *p*-SYN and polyphenols, followed by a filtration unit to separate the liquid phase, rich in *p*-SYN and polyphenols, from the solid phase (cake). This obtained cake continues to the section of pectin extraction and isolation, whereas the filtrate (liquid phase) is conveyed to the adsorption/ion exchange unit, in which a resin will be used to isolate *p*-SYN and polyphenols. An acid is used to extract pectin from the cake, after which follows a pectin precipitation unit, where alcohol is the precipitation agent. Hence, the experimental work was centered on the selection of the most appropriate *p*-SYN and polyphenols extraction solvent, resin, and pectin extraction acid, but also on finding the most suitable extraction conditions.

The following sections approach the Juice Production step, including the envisioned concept for BO juice, its nutritional value, and its characterization (section 4.2), and the Waste Valorization (biorefining) process, by making an immersion into the experimental development of its unitary operations (section 4.3).

4.2 Juice Production

4.2.1 Bitter Orange Juice Concept

As mentioned before, BO production is not intended for fresh fruit consumption or juice production, due to its unpleasant bitter and sour taste. However, this problem can be overcome by debittering technics, some already used and others under study, like syrup or florisil addition, lye treatment, hot water treatment and filtration, and enzymatic methods, among others (120).

Some studies have also been done to overcome the bitterness of citrus juices by removing bitterness-causing compounds. As an example, Ribeiro et al. (121) studied the removal of limonin and naringin from SO juice, by adsorption to different materials, including synthetic neutral resins such as Amberlite® XAD-4, XAD-7, and XAD-16. The experiment was successful, as the compounds were efficiently adsorbed onto XAD-7, with no adsorption of vitamin C and little adsorption of sugars and pigments.

The application of debittering techniques to BO fresh juice could open its range of applications. For instance, BO juice could be produced to be consumed as a conventional citrus juice or as a juice for weight loss, like a tasty liquid weight loss supplement, since it contains *p*-SYN. Aside from the food industry, BO juice could also be used to produce clean electricity, similar to what happens with the juice of the BOs from Seville city.

4.2.2 Comparing Bitter Orange Juice to Sweet Orange Juice

The chemical compositions of BO and SO juices were compared in order to understand to what extent the nutritional value of BO juice is similar to that of SO juice, a widely produced citrus juice for consumption. The values, under which this comparison was made, were not experimentally obtained by the author of this thesis, but by compiling the data available in the literature (1,89,122). The results are shown in figure 16.

The total soluble solids (TSS) content (which is the same as sugar content) is similar for both juices, as well as vitamin C and fatty acids contents. Among the fatty acids, palmitic acid, γ-linolenic acid, stearic acid, caprylic acid, oleic acid, and α-linolenic acid stand out the most, but the content of the last one is higher in SO juice than BO juice (8.63% and 0.32%, respectively). The titrable acidity content (TA), expressed in citric acid content, is higher in BO juice than in SO juice (61 g/L and 11 g/L, respectively).

The aroma content is, however, slightly higher in SO juice (23 mg/L) compared to BO juice (6 mg/L), with limonene being the most important aroma compound in both juices, followed by carvacrol (30.05% in BO juice, 11.74% in SO juice), and α-terpenyl acetate, which is higher in SO juice (13.15%) than in BO juice (1.46%).

BO juice has the highest content of flavonoids, expressed as mg of catechin equivalents (CE) per L of juice (67.80 mg EC/L versus 34.68 mg EC/L SO juice), and the highest content of phenolic acids, expressed as mg of gallic acid equivalents (GAE) per L of juice (677.00 mg GAE/L versus 214.00 mg GAE/L for SO juice) (89). The highest content of flavonoids and phenolic acids allowed us to conclude that BO juice is a valuable source of phenolic compounds and has a higher antioxidant activity than SO juice. BO juice is also richer in NRG, NERT, and NHPD, than SO juice (18.83, 14.01, and 11.09 mg/100 g BO juice, respectively, and 0.17, 0.04, and 0.00 mg/100 g SO juice, respectively) (122). Didymin, narirutin, and eriocitrin can also be found in both juices, but in fewer amounts (122). Regarding

phenolic acids, vanillic and *p*-coumaric acids are mostly present in BO juice (19.76% and 15.10%, respectively, versus 0.65% and 2.77%, respectively, in SO juice), whereas gallic, syringic, and chlorogenic acids are mostly present in SO juice (6.79%, 14.99%, 19.08%, respectively, and 2.60%, 0.83%, 0.44%, respectively, in BO juice). The content of ferulic acid is similar in both juices.

Finally, the content of *p*-SYN in BO juice is higher (56.90 mg/L (1)) than in SO juice (20.75 mg/L (123)).

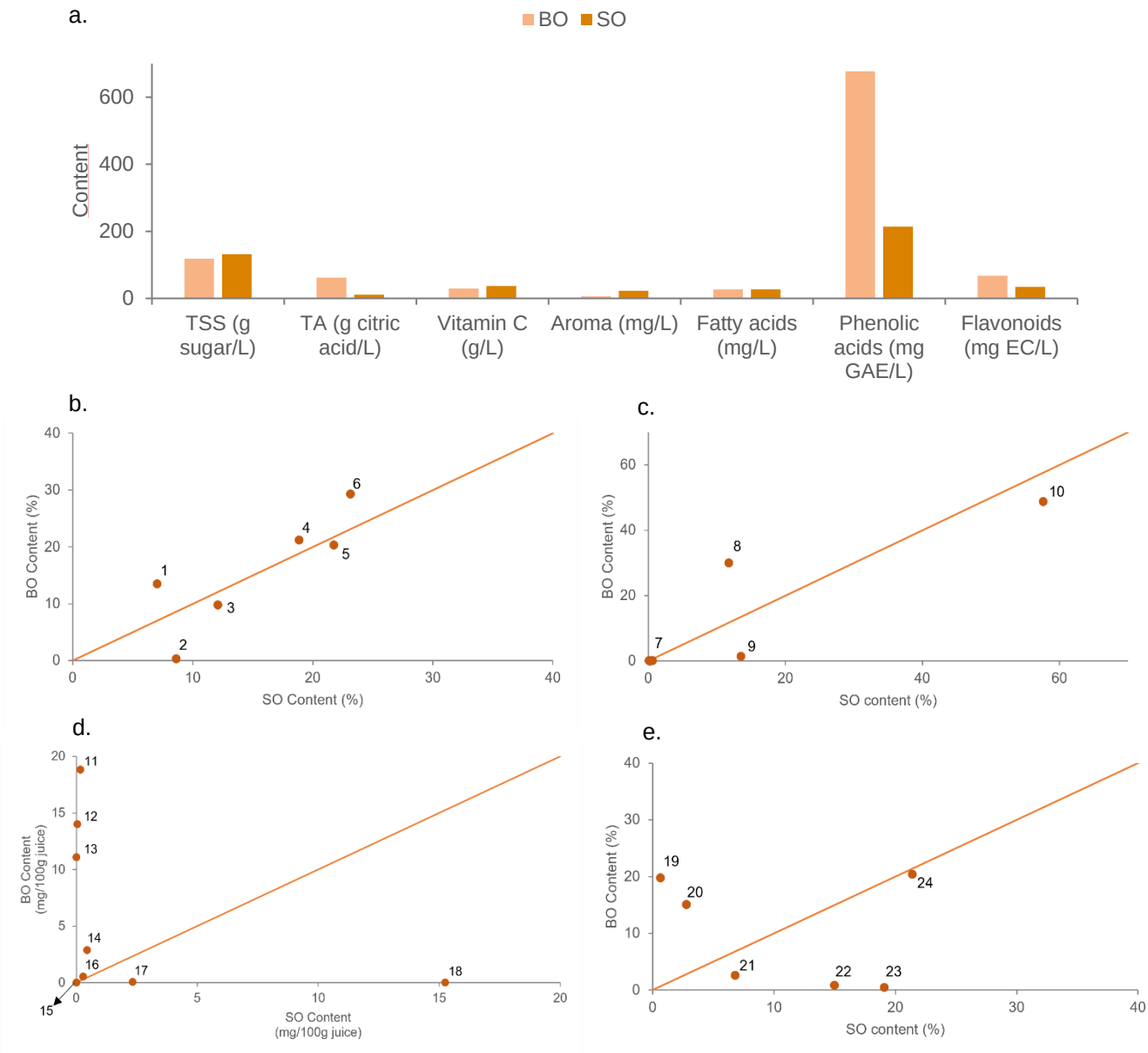

Figure 16. a) Content of TSS, TA, vitamin C, aroma, fatty acids, phenolic acids, and flavonoids in BO juice and SO juice. b) Content of fatty acids: 1 – palmitic acid, 2 – α-linoleic acid, 3 - γ-linoleic acid, 4 – stearic acid, 5 – caprylic acid, 6 – oleic acid. c) Content of aroma: 7 – (α-pinene, γ-terpinene, nerol), 8 – carvacrol, 10 – limonene. d) Content of flavanones: 11 – naringin, 12 – neoeriocitrin, 13 – neohesperidin, 14 – didymin, 15 – poncirin, 16 – eriocitrin, 17 – narirutin, 18 – hesperidin. e) Content of phenolic acids: 19 – vanillic acid, 20 – p-coumaric acid, 21 – gallic acid, 22 – syringic acid, 23 – chlorogenic acid, 24 – ferulic acid.

In conclusion, the nutritional value of BO juice is comparable to that of SO juice, with the advantage of having a higher content of antioxidants. Therefore, if it were not for the bitter and sour taste of BO juice, it could be considered for consumption, regarding its nutritional value. However, the juice yield of BOs (30.93%) is lower than that of SOs (51.23%) (89), so BO juice would be 1,66 times more expensive than SO juice, which is an important disadvantage of producing BO juice at the industrial scale.

4.2.3 Bitter Orange Juice Characterization: Identification and Quantification of Constituents

The HPLC chromatogram of BO juice is shown in figure 17. The peaks were identified by injecting standard solutions of pure *p*-SYN and pure flavonoids available in the laboratory (naringin, hesperidin, neohesperidin, neoeriocitrin, naringenin, hesperetin, and narirutin). *p*-SYN was detected with a retention time of 3.50 min, as well as NERT (13.50 min), NRG (21.50 min), and NHPD (26.60 min). The pH of the analyzed BO juice was around 3.

The concentrations of the detected compounds are in table 10. NERT is the most abundant compound, followed by NRG, NHPD, and *p*-SYN. The concentration of *p*-SYN is in accordance with the literature (1), unlike the flavonoids' concentrations, which are one order of magnitude above the concentrations obtained in the literature (122). This difference might be explained by the strong dependency of fruits and vegetables' chemical composition on the crop field's geographical area, growing season, and the period of harvest (87).

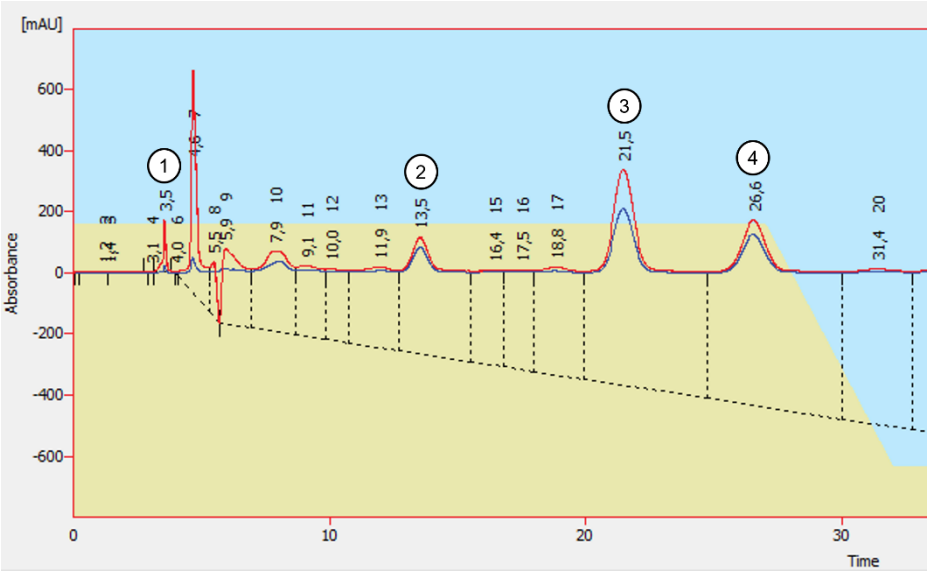

Figure 17. HPLC chromatogram of BO juice. Runtime of 35 min from a total runtime of 60 min. Red line: detection at 225. Blue line: detection at 283 nm. Peaks: 1 – p-SYN, 2 – NERT, 3 – NRG, 4 – NHPD.

Table 10. Experimental concentrations of p-SYN, NERT, NRG, and NHPD, in the BO juice analyzed.

Compound	Concentration (ppm)
p-SYN	54.17±1.86
NERT	3420.42±15.45
NRG	1931.62±88.77
NHPD	963.57±3.27

4.3 Waste Valorization (Bitter Orange Biorefinery)

4.3.1 *p*-Synephrine and Flavonoids Extraction

4.3.1.1 Extracts Characterization: Identification of Extracted Compounds

p-SYN and flavonoids were extracted from ripe BO peel, by using several solvents and solutions at room temperature or at 90 ºC, as mentioned in section 3.4.1. In figure 18 is represented a typical HPLC chromatogram obtained for these extracts. Like the characterization of BO juice, the peaks of the chromatograms were identified by injecting standard solutions of pure *p*-SYN and pure flavonoids. *p*-SYN was detected with a retention time of 3.30 min, as well as NERT (13.30 min), NRG (21.00 min), and NHPD (25.90 min).

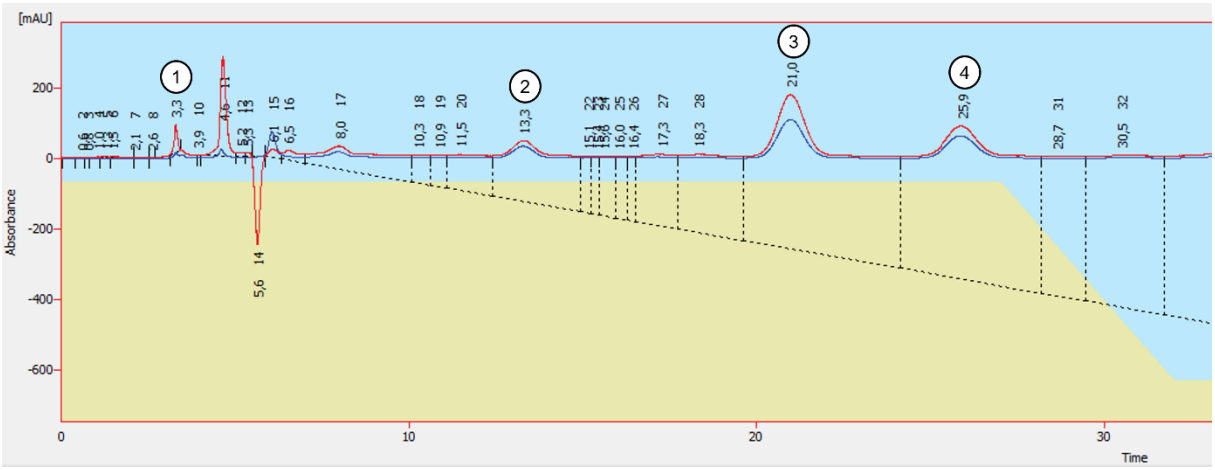

Figure 18. HPLC chromatogram of BO peel extracted with 80% EtOH (aq), at room temperature. Runtime of 32 min from a total runtime of 60 min. Red line: detection at 225. Blue line: detection at 283 nm. Peaks: 1 – p-SYN, 2 – NERT, 3 – NRG, 4 – NHPD.

The content of adrenergic amines and flavonoids in BO fruits (*Citrus aurantium var. amara*) was also studied by Pellati et al (79). They were able to detect *p*-SYN and all the seven flavonoids available in the laboratory (naringin, hesperidin, neohesperidin, neoeriocitrin, naringenin, hesperetin, and narirutin), with NRG, NHPD, and NERT being the most abundant ones. The reason why hesperidin, naringenin, hesperetin, and narirutin were not detected in the present work might be the differences, in the content of compounds, between the *amara* variety, studied by Pellati et al. (79), and the BO variety studied in the present work (*seville*). The degree of maturation of the fruits may also be considered, as the concentration of flavonoids varies with the maturation stage. For example, hesperidin concentration tends to decrease, which might explain why this flavonoid was not detected in the peel extracts of the matured BOs (124).

Although three flavonoids were detected in the BO extract, the following sections of results and the respective discussion will be focused on NRG, since, at the time the experimental work was carried out, only NRG was available in the laboratory and so it was the first flavonoid to be quantified. Only later the remaining two flavonoids (NHPD and NERT) were identified and quantified. However, in a final presentation of the overall waste valorization process, a total flavonoid fraction will be considered for discussion.

4.3.1.2 Impact of Different Solvents on Extraction Yield

It was decided to convey the extraction experiments with dried peel instead of fresh peel because, industrially, drying the peel is advised to reduce the volume of bulk material, which helps to reduce the equipment size and facilitates the handling. Furthermore, the extraction becomes more efficient, since the dry material is more porous, facilitating the diffusion of solvent, and the raw material is stabilized (its degradation is avoided) (96,106,125).

According to the literature, EtOH aqueous solutions are commonly used for the extraction of either *p*-SYN or flavonoids. Therefore, EtOH was one of the selected extraction solvents to carry BO peel extractions. Different volume percentages of EtOH in water (50%, 80%, and 96%) were considered to evaluate how this parameter would affect the extraction yield.

Water was also assessed as an extraction solvent because some authors often use water to extract and quantify *p*-SYN from citrus materials and related products. As an example, Pellati et al. (79) used water instead of EtOH to extract adrenergic amines, including *p*-SYN, in BO fruit, extracts, and herbal medicines, whereas EtOH was used to extract flavonoids, in particular flavanones. To assess the effect of pH on the extraction yield, acidified water with HCl (aq) was also considered.

MeOH was included in this study, since many authors report MeOH as the best phenolic compound extraction solvent, in terms of extraction yield (96), due to the high solubility of phenolics in MeOH (126). However, it was only used as a reference because MeOH is toxic, causing environmental problems, and is considered unsafe to be used in food applications, so it should be avoided as an extraction solvent, in this context (96).

Two different temperatures were also considered for each solvent/solution, at room temperature, and at 90 ºC, to evaluate compound degradation at elevated temperatures and the effect of temperature on extraction yield.

The results on extraction yield expressed in mg of extracted compound per g of peel are found in figure 19. In general, all solvents and solutions gave similar results on extraction yield, either for *p*-SYN or NRG, with none standing out. The yield of *p*-SYN in the aqueous extracts, obtained at room temperature, agrees with the literature (79), but the yield of NRG in the ethanolic extracts, obtained at 90 ºC, is slightly higher (23.77 mg NRG/g peel for 80% EtOH (aq), in the present work, versus 15.95 mg NRG/g peel for 80% EtOH (aq), in the literature (79)).

The solvent/solution selection must consider additional criteria, since, according to the envisioned process, the extract obtained from the BOPW is directly put in contact with a resin to separate *p*-SYN from the remaining extracted compounds. Those criteria include *p*-SYN binding onto resins, selectivity, and recovery efficiencies, considering that different solvents imply different resin behaviors. For this reason, no solvent was selected based only on its extraction performance.

Another important observation is that higher yields were generally attained at room temperature, except for 96% EtOH (aq) and 99.8% MeOH. Although temperature increases compound solubility accelerating the extraction, too high temperatures along with long extraction times may result in compound degradation (96). This might explain why room temperature led to higher yields. However, it should be noted that the prevalence of room temperature over the application of heat is questionable in acidified water experiments, for *p*-SYN's case. The mean extraction yields are different, but the respective standard deviations fall within each other. Hence, a Student's *t*-test was applied between the two temperature situations to verify if the difference in extraction yields was statistically significant. The resultant p-value was higher than 0.05 ($p = 0.43$), concluding that the results are not statistically significant and, therefore, using acidified water at room temperature or at 90 ºC is barely the same from this point of view.

By comparing water (pH 4) to acidified water (pH 1.5), at room temperature, it was concluded that lowering the pH had a slightly favorable impact on extraction yield, increasing 8% for *p*-SYN and 14% for NRG.

The results also showed similar extraction yields attained by using 50% EtOH (aq) or 80% EtOH (aq), at room temperature, regarding *p*-SYN (1.30 mg *p*-SYN/g peel and 1.33 mg *p*-SYN/g peel, respectively) and NRG (30.38 mg NRG/g peel and 30.12 mg NRG/g peel, respectively). Nonetheless, the yields decreased by approximately 30% and 26%, for *p*-SYN and NRG, respectively, when using 96% EtOH (aq) at room temperature. This phenomenon might be explained by the lower concentration of water in the 96% ethanolic solution, considering that some authors have shown that mixing water with EtOH or MeOH was more suitable to extract phenolic compounds (96). Even though, it should be mentioned that, in the present case, this tendency was inversed when heat was applied, with higher yields for 96% EtOH (aq)).

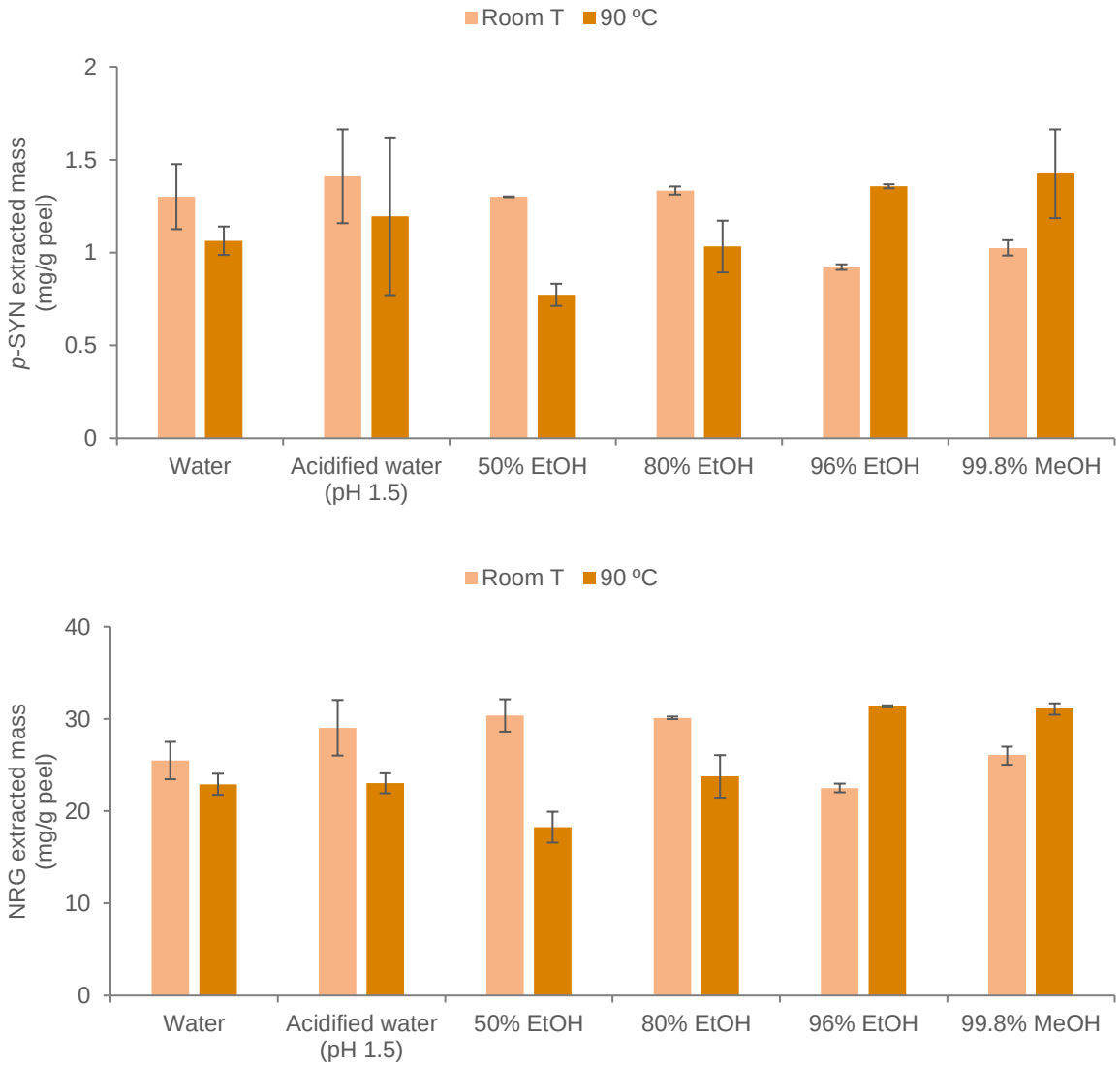

Figure 19. Top: impact of different solvents and solutions on p-SYN extraction yield, at room temperature and 90 ºC, with a S/L ratio of 1:62.5. Bottom: impact of different solvents and solutions on NRG extraction yield, at room temperature and 90 ºC, with a S/L ratio of 1:62.5.

Although many authors (97,127) report that, in terms of extraction yield, MeOH is a better solvent than EtOH to extract phenolic compounds from citrus peel, this is not observed in this case. Li et al. (128) compared the total phenolic content extracted from the peel of various citrus fruits with 95% EtOH (aq) and with 95% MeOH (aq) under the same conditions, concluding that the extraction yields were very similar. Our results show that using 99.8% MeOH or 96% EtOH (aq) led to similar extraction yields, confirming the results obtained by Li et al. (128). The extraction performance of 50% EtOH (aq) and 80% EtOH (aq) was also comparable to the above-mentioned solvent and solution.

The extraction profiles of NHPD and NERT (figures 41 and 42, appendix D) were very similar to the profile of NRG. Therefore, similar conclusions were taken for those two flavonoids, regarding the influence of the various solvents/solutions on their extraction yields.

Because, in general, room temperature experiments led to higher *p*-SYN and flavonoid yields than 90 ºC experiments, only the aqueous and ethanolic extracts, obtained at room temperature, were

subjected to resin adsorption and ion exchange. It should also be mentioned that these extracts have the advantage of requiring lower energy inputs (lower energy operational costs) when compared to the extracts obtained at 90 ºC.

4.3.2 Resin Adsorption and Ion Exchange

The objective of resin adsorption and ion exchange experiments was to selectively bind *p*-SYN onto a resin and obtain a flavonoid-rich stream, therefore simplifying the process to obtain both fractions. The tested resins are usually manufactured for aqueous media, so assessing their behavior on different extracts, with various solvent matrices, is crucial to realize which is the most suitable resin and respective extraction solvent/solution, regarding *p*-SYN binding and selectivity. The results are presented in figure 20. The detailed binding percentages of *p*-SYN, NRG, NHPD, and NERT can be found in figures 43 - 47, appendix E.

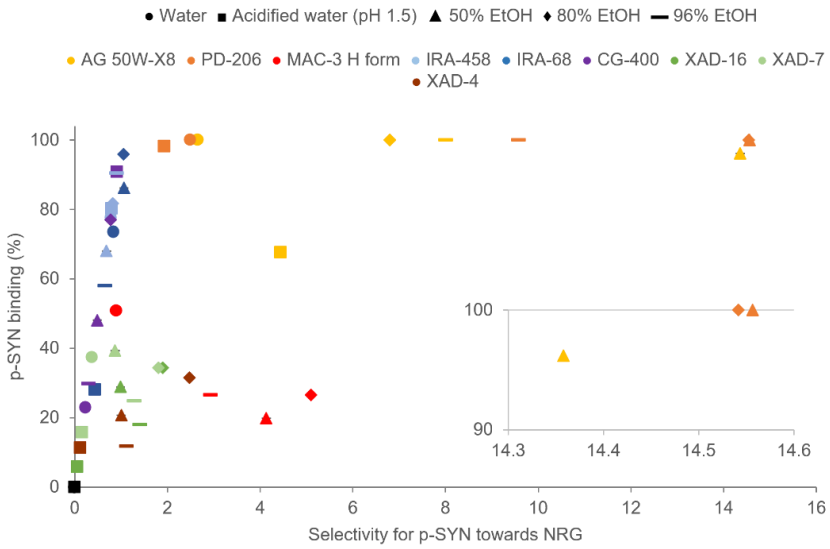

Figure 20. Binding and selectivity for p-SYN towards NRG of various resins, with a S/L ratio of 50 kg resin/m^3, for BO peel extracted with aqueous and ethanolic solvents/solutions at room temperature.

In general, the highest binding percentages of *p*-SYN (68% - 100%) were mainly achieved for the strong-acid cation-exchange resins (AG 50W-X8 and PD-206) and for the anion-exchange resins (IRA-458, IRA-68, and CG-400). The affinity of the strong-acid cation-exchange resins for *p*-SYN might be explained by the ionic state of *p*-SYN present in solution depending on its pH, when in contact with the resins. For the strong-acid cation-exchange resins, the BO extracts present a pH ranging from 1.5 to 5; *p*-SYN has a pKa of around 9.6 (129), which means that, in this case, it was present as a cation with a positive charge. Therefore, *p*-SYN was able to exchange with the counterions of AG 50W-X8 and PD-206 resins (Na^+ and H^+, respectively). On the other hand, the high binding percentages observed

for the anion-exchange resins might be explained by Van-der-Waals interactions and/or hydrogen bonding between *p*-SYN and the matrices of IRA-458, IRA-68, and CG-400 resins.

Similar binding results were obtained by Esteves et al. (130), however for another alkaloid, lupanine (pKa 9.1 (131)). The binding percentages reached 100% for strong-acid cation-exchange resins, including AG 50W-X8 and PD-206, in an aqueous pH 4 media (lupin beans wastewater). However, for the anion-exchange resins in the same media, the binding percentages were considerably lower (below 20%), contrasting with the results obtained in the present work for IRA-458, IRA-68, and CG-400. It is believed that this disparity is explained by the fact that pretreatment was not performed on any of the tested resins by Esteves et al. (130), including the resins in regard.

The highest values of selectivity for *p*-SYN (figure 20) were attained for AG 50W-X8 and PD-206 resins, together with 50%, 80%, and 96% ethanolic extracts (6.79 – 14.56 selectivity). The same conclusions were attained when the percentage of *p*-SYN binding was plotted as a function of the selectivity for *p*-SYN towards the total fraction of flavonoids (NRG + NHPD + NERT), after NHPD and NERT quantification in the BO extracts (figure 48, appendix E).

In conclusion, owing to their high binding and selectivity for *p*-SYN, AG 50W-X8 and PD-206 were the selected resins to engage in the recovery assays for *p*-SYN, along with the ethanolic extracts obtained at room temperature. However, 96% EtOH (aq) extracts were not included in the recovery study since, at room temperature, 50% and 80% EtOH (aq) were superior in terms of extraction yield (figure 19).

Summing up, the following sets of resins and extracts were addressed for *p*-SYN recovery:

- PD-206/50% EtOH (aq), room T extract.
- PD-206/80% EtOH (aq), room T extract.
- AG 50W-X8/50% EtOH (aq), room T extract.
- AG 50W-X8/80% EtOH (aq), room T extract.

4.3.3 *p*-Synephrine Recovery

To recover *p*-SYN from AG 50W-X8 and PD-206 resins, the alkaloid must be eluted by using a solution (known as eluant) containing a cation for which the resin matrix has high affinity, or a high concentration of an ion with equivalent or lower affinity, to force *p*-SYN to leave the resin and flow to the eluant. On the other hand, the regenerating solution must contain the resin's counterion, in a suitable concentration, so the resin is regenerated back to its original form, where the counterion moves from the regenerating solution to the matrix of the resin (117). In an attempt to simultaneously recover *p*-SYN and regenerate the resin, the eluant was chosen based on the regenerating solution of each resin.

Since AG 50W-X8 has Na^+ as the counterion, the regenerating solution must contain Na^+. According to the literature (118), the regenerating solution of a strong-acid cation-exchange resin, in Na^+ form, is NaCl (aq) with a concentration between 5% and 25%. Therefore, 10%, 15%, and 25% NaCl solutions were prepared to recover *p*-SYN from AG 50W-X8.

PD-206 is similar to AG 50W-X8 but the counterion is H^+, so the regenerating solution must contain H^+ ions. According to the literature (118), the regenerating solution of a strong-acid cation-exchange resin, in H^+ form, is HCl (aq) 5.5 M. Three HCl solutions were prepared with 1 M, 5 M, and 8 M concentrations in order to test *p*-SYN recovery from PD-206.

From figure 21 it is observed that *p*-SYN was efficiently recovered from AG 50W-X8 resin (85% - 96%) using 10% and 25% NaCl (aq) for BO extracts obtained with 80% EtOH (aq), while for PD-206 resin lower *p*-SYN recoveries were obtained (27% - 51%) with HCl (aq) solutions. In comparison, for the BO extracts obtained with 50% EtOH (aq), *p*-SYN was not efficiently recovered from both resins (53% - 60% for AG 50W-X8 and 19% - 44% for PD-206). Because 10% and 25% NaCl (aq) led to similar *p*-SYN recovery results for the 80% EtOH (aq) extract (figure 21), a *t*-test was applied, resulting in a p-value of 0.48, i.e., using 10% NaCl (aq) or 25% NaCl (aq) is statistically similar. However, from an industrial perspective, opting for the lower NaCl concentration implies a reduction of 150% in raw material costs (NaCl bulk price = 39.99 €/kg (132); obtained from the price of the technical grade NaCl, affected by an average correction factor of 0.86, according to (133)). For this reason, the concentration of 10% NaCl is the most appropriate to recover *p*-SYN from AG 50W-X8.

In these experiments, NRG was also recovered from AG 50W-X8 resin (10% - 34%). The binding of NRG was considerably lower (6.70% - 14.72%) when compared to *p*-SYN (≥ 96%) (figure 22). Although NRG was present in higher concentrations in the extracts of BO peel when compared to *p*-SYN, the recovered mass for both compounds is similar (0.20 – 1.49 mg of NRG per g of peel versus 0.66 – 1.28 mg of *p*-SYN per g of peel). Therefore, the recovery percentage of NRG from AG 50W-X8 should not be neglected. Concluding, the *p*-SYN recovered from AG 50W-X8 resin is contaminated with NRG in 23% - 54%.

Regarding the other two flavonoids, NERT could not be recovered from the resins and NHPD only showed a low recovery from AG 50W-X8 (21% - 25%, corresponding to 0.31 – 0.37 mg NHPD per g of peel), for BO peel extracted with 50% EtOH (aq) (figure 49, appendix F).

Adding to the fact that PD-206 resin showed higher selectivity for *p*-SYN, during binding, it was concluded that PD-206 is more selective for *p*-SYN than AG 50W-X8 resin, in terms of binding and recovery. However, *p*-SYN is more easily recovered from AG 50W-X8 than from PD-206. Therefore, two ion exchange scenarios (using AG 50W-X8 or PD-206) will be analyzed, in section 4.3.6, to decide which is the most appropriate to be implemented in the waste valorization process.

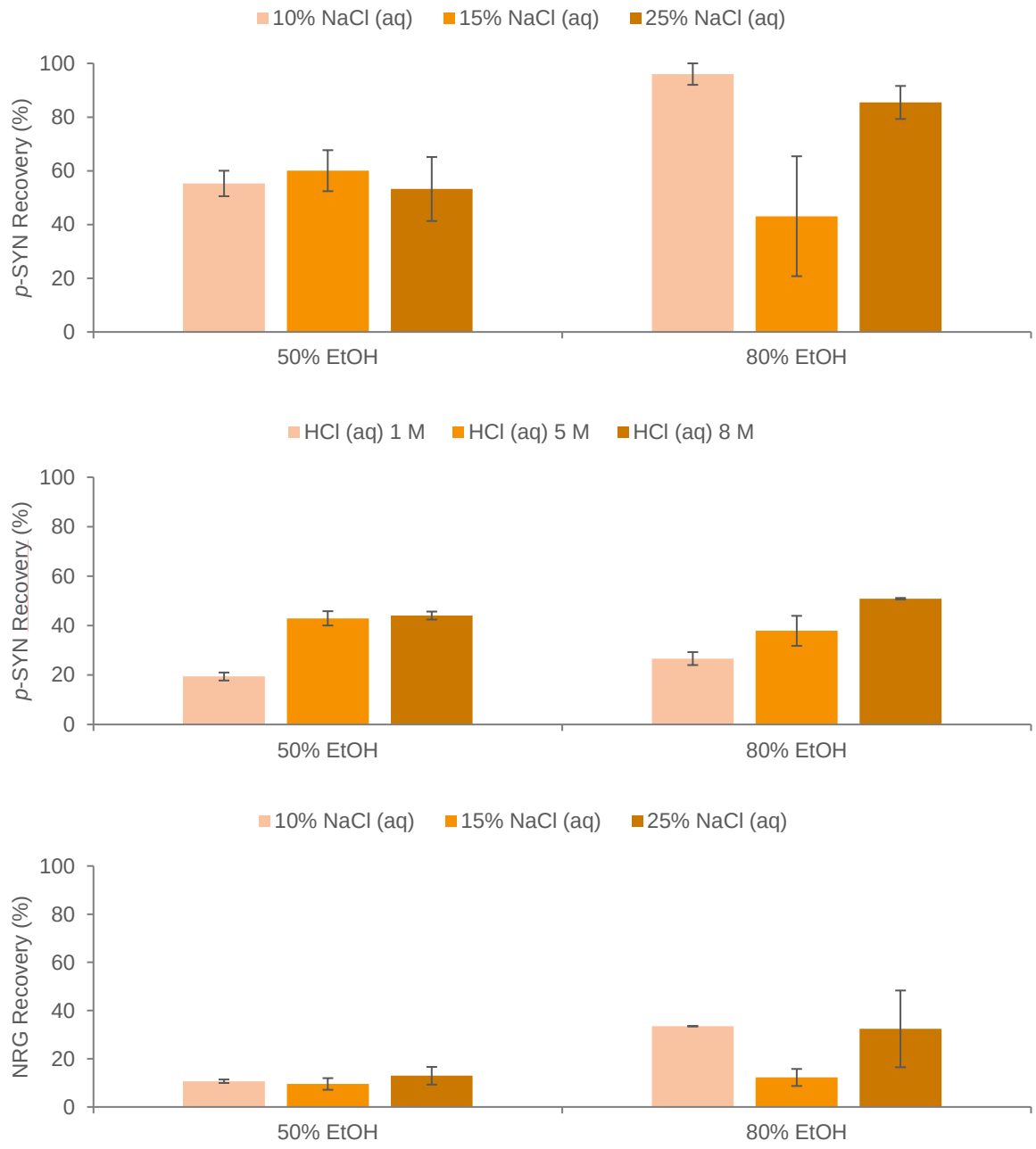

Figure 21. Top: p-SYN recovery from AG 50W-X8 resin, with a S/L ratio of 100 kg resin/m^3. Middle: p-SYN recovery from PD-206 resin, with a S/L ratio of 100 kg resin/m^3. Bottom: NRG recovery from AG 50W-X8 resin, with a S/L ratio of 100 kg resin/m^3.

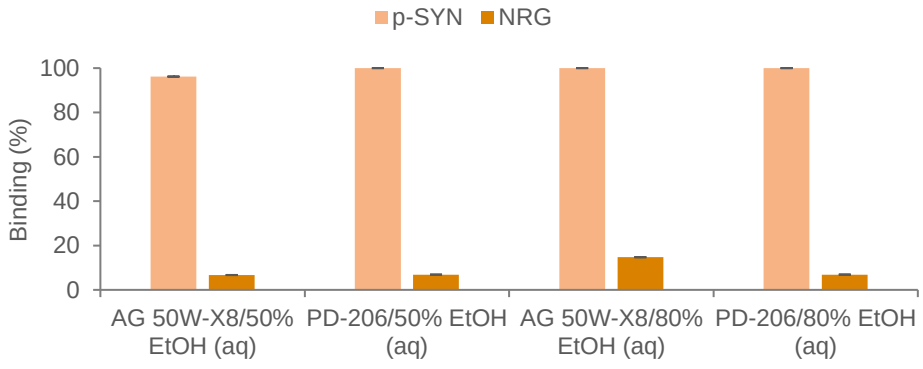

Figure 22. Binding of p-SYN and NRG to AG 50W-X8 and PD-206 resins, with a S/L ratio of 50 kg resin/m^3, for BO peel extracted with 50% EtOH (aq) and 80% EtOH (aq), at room temperature.

Furthermore, 80% EtOH (aq) was chosen to be implemented in the waste valorization process as *p*-SYN and flavonoids extraction solution because it contains less water than 50% EtOH (aq), i.e., after the ion exchange operation, the 80% ethanolic extract is conveyed to a distillation column, where EtOH is recovered at the top of the column and a residue, containing essentially water and flavonoids, is obtained at the bottom; the less water this residue contains, the easier it will be to dry and obtain a dried flavonoid-rich product. The volume of the residue obtained from 80% EtOH (aq) extract will be lower (≈ 0.2 m^3/m^3 extract) than the residue obtained from 50% EtOH (aq) extract (≈ 0.5 m^3/m^3 extract), so the drying operation is facilitated by using 80% EtOH (aq) as extraction solution.

4.3.4 AG 50W-X8 Resin Reusability

Since, according to the literature (118), a NaCl (aq) solution is suggested to regenerate AG 50W-X8 resin, and since it was observed that, for the 80% EtOH (aq) extract, the concentration of 10% NaCl allowed the recovery of 96% of *p*-SYN bound to the resin, we assessed if the recovery step would be enough to regenerate the AG 50W-X8 resin at the same time, so that it could be reused in further binding/recovery cycles for *p*-syn isolation.

In the first cycle of binding/recovery/regeneration, 100% binding and 96.0% recovery for *p*-SYN were obtained. However, after the 2nd binding, the chromatogram obtained for this step showed a prominent peak at the retention time of *p*-SYN (figure 23). After the injection of pure *p*-SYN, it was confirmed that the peak was, in fact, assigned to *p*-SYN, corresponding to a binding of <4%. Thus, it was concluded that the regeneration of the resin with 10% NaCl (aq) was not efficient. No alternative regeneration procedures were performed due to time scheduling limitations.

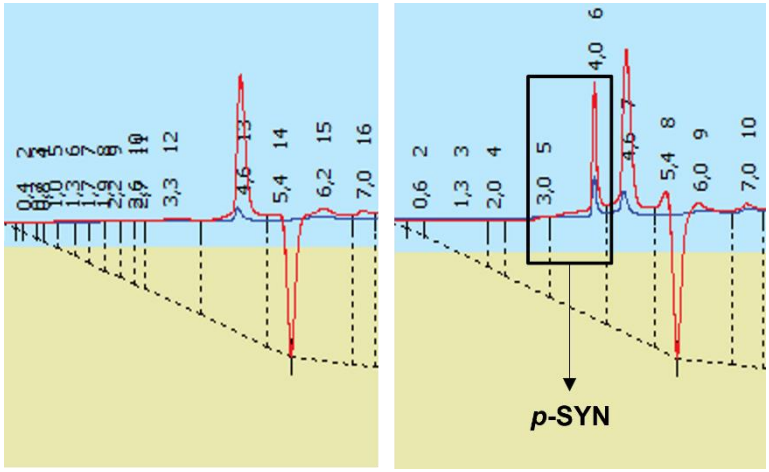

Figure 23. Left: HPLC chromatogram of the 1st binding of 80% EtOH (aq) extracts. Righ: HPLC chromatogram of the 2nd binding of 80% EtOH (aq) extracts. Runtime of 7 min from a total runtime of 60 min. Red line: detection at 225. Blue line: detection at 283 nm.

4.3.5 Pectin Extraction

4.3.5.1 Comparing Mineral Acids with Organic Acids

Pectin was extracted from BO peel using HCl and citric acid aqueous solutions, under the same conditions, to select the best extraction solvent, but also to establish a comparison, in terms of extraction yield, between commonly used conventional extraction solvents (mineral acids), and green extraction solvents (less polluting acids, such as citric acid). The results on dried pectin yield (DPY) are shown in figure 24 and pictures of extracted pectin before and after drying are presented in figure 25.

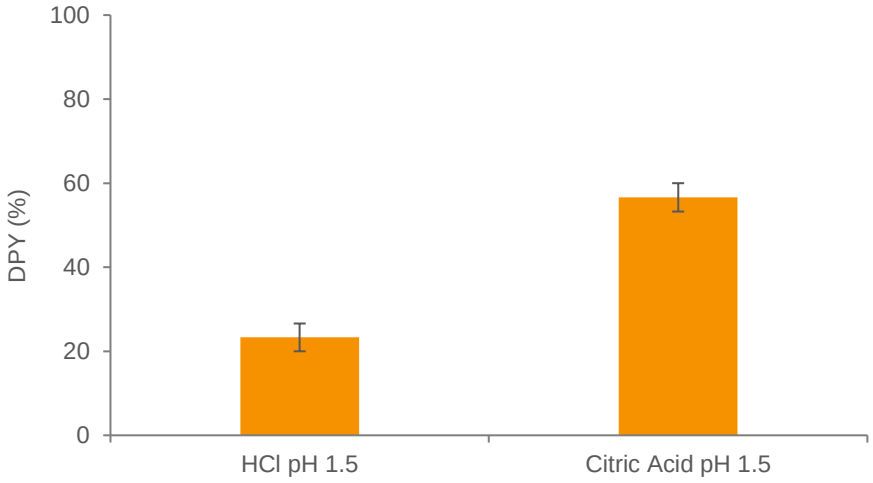

Figure 24. DPY obtained by using HCl (aq) and 13% citric acid (aq), pH 1.5, at 95 ºC, with a S/L ratio of 1:40.

Figure 25. Left: extracted pectin before drying. Middle: extracted pectin with HCl (aq), after drying. Right: extracted pectin with 13% citric acid (aq), after drying.

Citric acid led to a higher pectin yield (56.60%) than HCl (23.30%). Similar results were obtained by Fakayode et al. (36) and Devi et al. (134), however for other citrus fruits.

Fakayode et al. (36) studied the effects of extraction temperature, extraction time, and extraction pH on pectin yield from *Citrus sinensis* (SO) dried peel, using HCl (aq) as extraction solution and 95% EtOH (aq) as precipitating agent. The highest yield they obtained was 29.05%, at 95 ºC and pH 1.5 for 105 minutes. The lowest extraction yield was 12.93%, obtained at 90 ºC and at a higher pH (3.0), for 90 minutes.

Similarly, Devi et al (134). compared the effects of temperature, time, and pH on pectin yield from sweet lemon peel powder, using nitric acid and citric acid aqueous solutions, and absolute EtOH for pectin precipitation. Pectin yield ranged from 21% to 76% for citric acid, and from 17% to 46% for nitric acid, i.e., once again citric acid reveals better yield results than mineral acids. The extraction yields of 76% and 46.4% were both obtained under the same operating conditions of pH 1.5, 80 ºC, and 60 min. In the present work, a higher temperature (95 ºC) and extraction time (105 min) led to a lower yield in pectin from BO peel (56.60%), regarding the extraction with citric acid. This result might be due to the different pectin contents in sweet lemon and BO.

Because HCl is a stronger acid than citric acid it was expected that HCl would lead to a higher pectin yield. However, this was not observed. Although HCl (aq) has a higher acid dissociation constant than citric acid, releasing more hydrogen ions to hydrolyze pectin, the extracted pectin particles are so small (low molecular size) that it increases pectin solubility to a point that they can not be precipitated afterward (37,134). On the contrary, citric acid produces bigger pectin particles, i.e., less soluble, facilitating pectin precipitation and resulting in a higher mass obtained (37,134). This might be a reason why citric acid leads to higher yields.

In conclusion, citric acid (aq) led to higher pectin yields than HCl (aq), so it was the chosen solution to extract pectin from the BOPW, being used in all subsequent experiments on pectin. It should be mentioned that because the processes for pectin extraction from citrus peel are well established in the industry (citrus peel is the main source of commercial pectin) and the main objective here was merely to compare mineral acids with organic acids to select the best scenario, no further optimization on this matter was done. In addition, there was no available equipment in the laboratory to try innovative extraction techniques, like UAE, MAE, SWE, etc.

4.3.5.2 Impact of p-Synephrine and Flavonoids Extraction Solvents on Pectin Yield

To study the influence of the solvents/solutions used to extract *p*-SYN and flavonoids on pectin yield, *p*-SYN and flavonoid extraction was done in series with pectin extraction. *p*-SYN and flavonoid extraction was performed at room temperature, following the procedures described in section 3.4.1. The obtained wet peel was separated from the liquid extract by filtration and the resulting cake was stirred with citric acid at 95 ºC to extract pectin, as described in section 3.4.4.

As it was expected, the lowest yield of pectin (100 mg/ g peel) was obtained when *p*-SYN and flavonoids were extracted with acidified water (pH 1.5), at 90 ºC: because pectin is efficiently extracted from BO peel in acidic media at elevated temperatures, this polymer was extracted simultaneously with the alkaloid and flavonoids, therefore negatively affecting the pectin yield obtained in the subsequent extraction with citric acid, at 95 ºC (figure 26). On the other hand, the yield of pectin was potentiated by using distilled water (765.00 mg pectin/g peel) or 96% EtOH (aq) (832.00 mg pectin/g peel) in the extraction of *p*-SYN and flavonoids. However, the amount of flavonoids extracted with distilled water or 96% EtOH (aq) (25.48 mg NRG/g peel and 22.52 mg NRG/g peel, respectively) was lower when compared with other extraction solutions, such as 50% EtOH (aq) and 80% EtOH (aq) (30.37 mg NRG/g peel and 30.12 mg NRG/g peel, respectively) (figure 27). The extracted amount of *p*-SYN was approximately constant and the extraction yields of NHPD and NERT followed the same tendency as NRG.

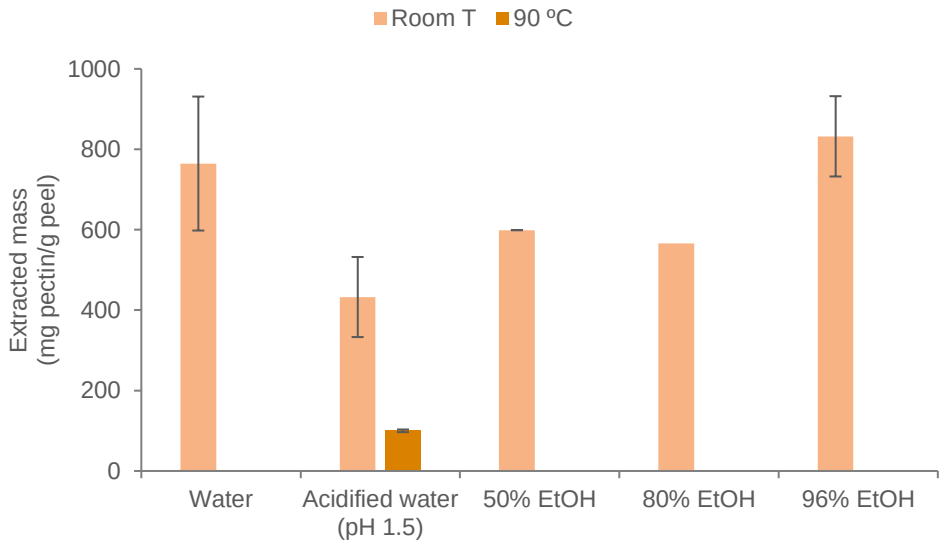

Figure 26. Impact of p-SYN and flavonoids extraction solvents/solutions on pectin yield.

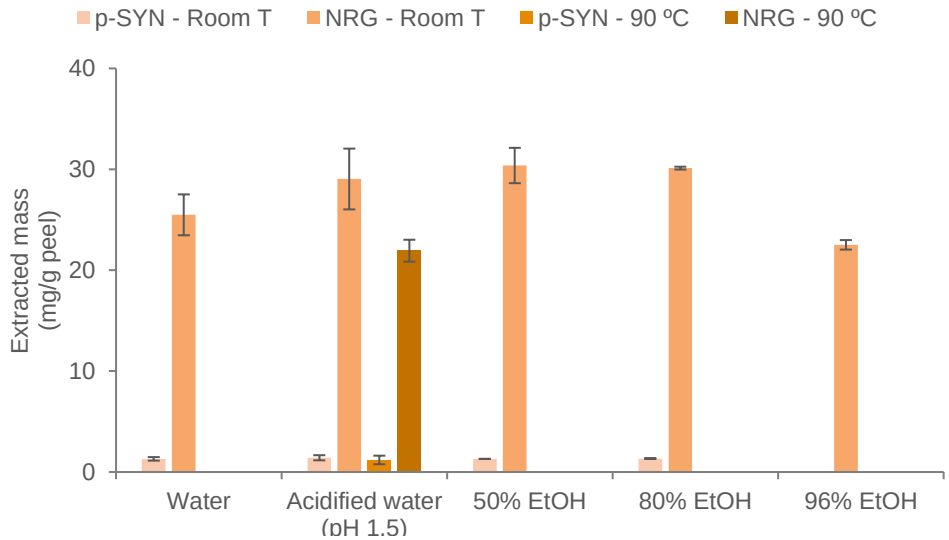

Figure 27. Impact of different solvents/solutions on the extraction yields of p-SYN and NRG, at room temperature and 90 ºC.

Table 11 presents two economic scenarios: one in which water or 96% EtOH (aq) is used to extract *p*-SYN and flavonoids (scenario (1)), and another in which 50% EtOH (aq) and 80% EtOH (aq) are considered (scenario (2)). It was concluded that the total revenue obtained from the extracted compounds (≈ $31.34x10^6$ - $39.56x10^6$ EUR/t peel) is mainly driven by the revenue of NERT (≈ $31.23x10^6$ - $39.43x10^6$ EUR/t peel). Consequently, the choice of the solvent/solution to carry BO peel extractions must be guided by the extraction yield of NERT and not by the extraction yield of pectin. Therefore, it was decided to opt for scenario (2), using 80% EtOH (aq), a solution that leads to higher NERT extraction yields than distilled water or 96% EtOH (aq), and, consequently, to a higher total revenue (≈ $39.56x10^6$ EUR/t peel). Moreover, by using 80% EtOH (aq), the drying of the flavonoid-rich final product is facilitated, in comparison with 50% EtOH (aq), as explained in section 4.3.3.

Table 11. Scenario 1: water or 96% EtOH as p-SYN and flavonoids extraction solvent/Solution. Scenario 2: water or 96% EtOH as p-SYN and flavonoids extraction solution.

Compound	Average Yield (kg/t peel)	Bulk Price (EUR/kg)	Revenue (EUR/t peel)	Total Revenue* (EUR/t peel)
Scenario 1 - Water or 96% EtOH as *p*-SYN and Flavonoids Extraction Solvent/Solution				
Pectin	798.50	12.36 (135)	9,869.46	$31.34x10^6$
p-SYN	1.11	68.17**	75.67	
NRG	24.00	16.41**	393.84	
NHPD	12.36	7,774**	96,086.64	
NERT	37.93	823,400**	$31.23x10^6$	
Scenario 2 – 50% EtOH (aq) or 80% EtOH (aq) as *p*-SYN and Flavonoids Extraction Solution				
Pectin	582.50	12.36 (135)	7199.70	$39.56x10^6$
p-SYN	1.32	68.17**	89.98	
NRG	30.30	16.41**	497.22	
NHPD	15.89	7,774**	123,528.86	
NERT	47.89	823,400**	$39.43x10^6$	

* The real total revenue will be lower because the calculated value did not consider the binding and selectivity for flavonoids. However, the difference is negligible since the extracted amount of flavonoids is negatively affected by just one order of magnitude.
** Obtained from the price of the technical grade product, affected by an average correction factor of 0.0046, according to (133)

4.3.5.3 Simultaneous Extraction of p-Synephrine, Flavonoids, and Pectin

As discussed in section 4.3.1.2, lowering the pH of the extracting media has a favorable impact on the yields of *p*-SYN and flavonoids. The effect of acidified water in *p*-SYN and flavonoids yields was assessed, contrarily to citric acid. Therefore, assays were done to evaluate the extraction yield of *p*-SYN and flavonoids, using citric acid and, thus, realize if it would be plausible to explore the simultaneous extraction of *p*-SYN, flavonoids, and pectin, as it would reduce the amount of equipment and occasionally its inherent costs, in the developed process.

The assays were carried out under the operating conditions of pectin extraction (described in section 3.4.4), i.e., using dried peel and a citric acid solution at pH 1.5 and 95ºC, for 105 minutes.

However, as the solid/liquid (S/L) ratio considered for *p*-SYN and flavonoid extraction was 1:62.5 and the S/L ratio of pectin extraction was 1:40, the volume of citric acid solution was ruled by the first S/L ratio, and so 94 mL of acid were used instead of 60 mL.

The results were compared to the extraction performed with 80% EtOH (aq) (figure 28), one of the most promising extraction solutions to be implemented in the process. Once again, NRG is representing the extracted flavonoid fraction.

This comparison showed that exploring this strategy is not plausible as the *p*-SYN and NRG extraction yields using citric acid, at 95 ºC (0.53 mg *p*-SYN/g peel, 9.22 mg NRG/g peel), were much lower than the ones obtained using 80% EtOH (aq) at room temperature (1.33 mg *p*-SYN/g peel, 30.12 mg NRG/g peel).

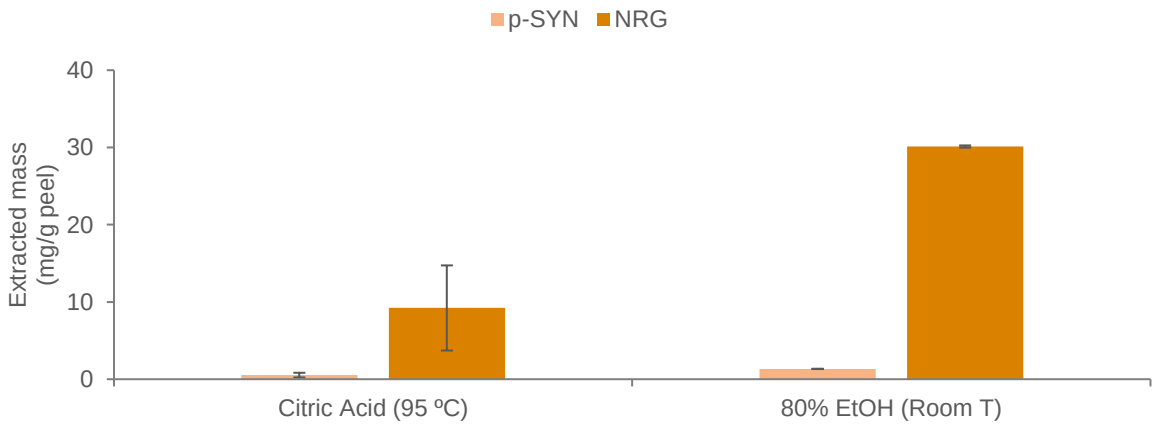

Figure 28. Yield of p-SYN and NRG extracted with 13% citric acid (aq) and 80% EtOH (aq).

4.3.6 Final Remarks

4.3.6.1 Selecting the Best Scenario for the Waste Valorization Process

As explained in section 4.3.3, two scenarios will be analyzed in the present section, to decide which is the most appropriate to be implemented in the waste valorization process. Schematic representations of both scenarios can be found in figure 29, concerning the extraction and isolation of *p*-SYN and flavonoids. The unitary operations of both scenarios are carried under the same conditions, except for the isolation of *p*-SYN and flavonoids, by ion exchange, in which AG 50W-X8 resin is used along with 10% NaCl (aq) as eluant in scenario (A), and PD-206 resin is used with HCl 8 M as eluant, in scenario (B).

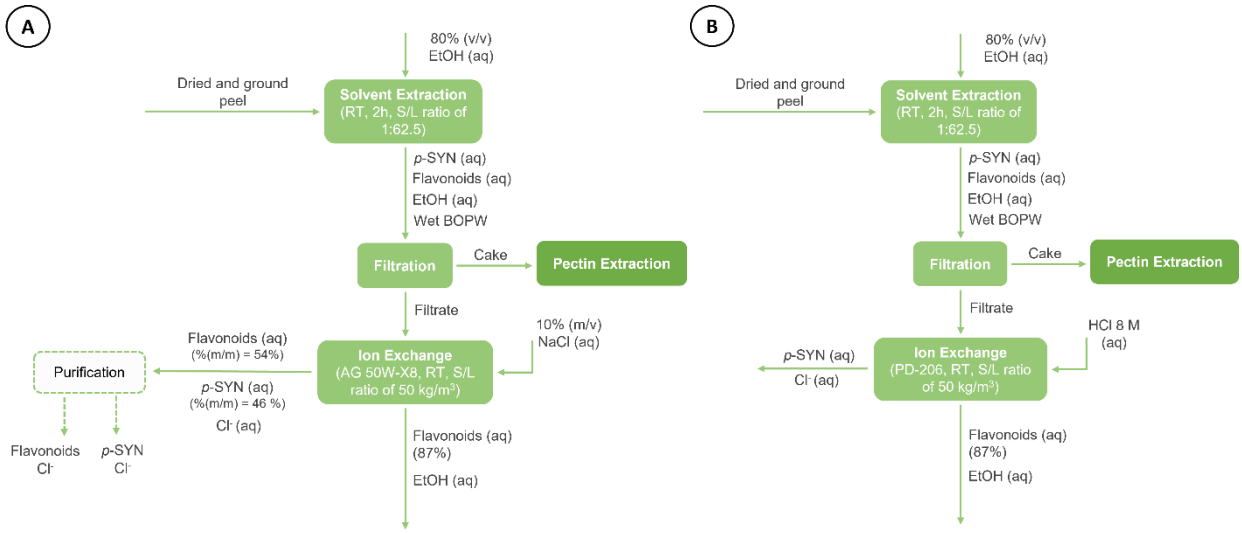

Figure 29. Schematic representations of scenario A and scenario B.

From table 12 and table 13 it is observed that, using PD-206 resin, around 0.68 kg *p*-SYN is produced from one ton of peel, whereas 600 g of *p*-SYN are additionally produced (1.28 kg *p*-SYN/t peel), by using AG 50W-X8, since *p*-SYN is more efficiently recovered from it. Either way, using one resin or the other, the amount of *p*-SYN is negligible, as well as its revenue (4.63×10^{-5} - 8.73×10^{-5} million EUR/t peel), when compared to the amount and revenue of the total flavonoid fraction (81.06 - 84.92 kg/t peel, 34.97 – 35.37 million EUR/t peel). Therefore, *p*-SYN should be seen not as a selling product, but as a contaminant of the flavonoid product.

Because AG 50W-X8 resin allows the recovery of NRG, along with *p*-SYN, the stream flowing out, after recovery, contains 54% (m/m) and 46% (m/m) of NRG and *p*-SYN, respectively (1.28 kg *p*-SYN/t peel, 1.49 kg NRG/t peel); this does not occur when PD-206 is used, since no flavonoids are recovered. Moreover, the binding percentages of NRG, NHPD, and NERT to AG 50W-X8 resin are higher than the binding percentages to PD-206 resin, which negatively affects the process yield of flavonoids, in scenario (A) (85% for NRG, 85% for NHPD, and 92% for NERT), when compared to the yields obtained in scenario (B) (93% for NRG, 92% for NHPD, and 93% for NERT). For all these reasons, scenario (B) was chosen to be implemented in the waste valorization process, with a total revenue of around 35.38 million EUR/t peel. It should be noted that the total revenue is mainly driven by the revenue of NERT (35.26 million EUR/t peel) due to its high bulk price (823,400 EUR/kg). However, these revenue values should be taken carefully and reviewed since they seem to be inflated.

Table 12. Mass balances and revenues of scenario A.

Calculation Basis: 1 t peel								
Compound	**Extracted mass (kg)**	**Bound mass to AG 50W-X8 (kg)**	**Recovered mass from AG 50W-X8 (kg)**	**Final (kg)**	**Process Yield (%)**	**Bulk Price (EUR/kg)**	**Revenue (million EUR)**	**Total Revenue (million EUR)**
Pectin	566.02	--------	--------	566.02	100	12.36 (135)	0.0070	34.98
p-SYN	1.33	1.33	1.28	1.28	96	68.17*	8.73×10^{-5}	
NRG	30.12	4.43	1.49	25.69	85	16.41*	0.00042	
NHPD	15.53	2.25	0.00	13.28	85	7,774*	0.10	
NERT	45.98	3.89	0.00	42.09	92	823,400*	34,87	

* Obtained from the price of the technical grade product, affected by an average correction factor of 0.0046, according to (133)

Table 13. Mass Balances and revenues of scenario B.

Calculation Basis: 1 t peel								
Compound	**Extracted mass (kg)**	**Bound mass to PD-206 (kg)**	**Recovered mass from PD-206 (kg)**	**Final (kg)**	**Process Yield (%)**	**Bulk Price (EUR/kg)**	**Revenue (million EUR)**	**Total Revenue (million EUR)**
Pectin	566.02	--------	--------	566.02	100	12.36 (135)	0.0070	35.38
p-SYN	1.33	1.33	0.68	0.68	38	68.17*	4.63×10^{-5}	
NRG	30.12	2.07	0.00	28.05	93	16.41*	0.00046	
NHPD	15.53	1.17	0.00	14.36	92	7,774*	0.11	
NERT	45.98	3.41	0.00	42.57	93	823,400*	35.26	

* Obtained from the price of the technical grade product, affected by an average correction factor of 0.0046, according to (133)

4.3.6.2 Process Inventory and Raw Material Costs

The process inventory and the raw materials costs are presented in table 14. PD-206 resin is the one that contributes the most (80%) to the total raw material costs (365.51 thousand EUR/t peel), followed by EtOH (58.34 thousand EUR/t peel), citric acid (11.97 thousand EUR/t peel), HCl (1.50 thousand EUR/t peel), and distilled water (0.11 thousand EUR/t peel). It should be mentioned that the bulk price of PD-206 resin was based on the bulk price of AG 50W-X8 resin, in H^+ form, since the price of PD-206 resin was not available for consultation.

Table 14. Required amounts of reagents and solvents, respective costs, and total raw material cost, regarding the waste valorization process.

Reagents	Required amount (t/t peel)	Bulk Price (EUR/kg)	Cost (Thousand EUR/t peel)	Total Cost (Thousand EUR/t peel)
HCl	5.78	0.26 (136)	1.50	365.51
PD-206	3.13	93.8* (137)	293.59	
Citric Acid	5.22	2.29 (138)	11.97	
Solvents	**Required amount (m^3/t peel)**	**Bulk Price (EUR/m^3)**	**Cost (Thousand EUR/t peel)**	
EtOH	88.40	659.91 (139)	58.34	
Distilled water	85.35	1.29 (135)	0.11	

* Obtained from the price of the technical grade raw material, affected by a correction factor of 10.

4.3.6.3 Process Description

The overall diagram of the waste valorization process is presented in figure 30. It is important to mention that, although the process envisions the valorization of all BOPW (peels, core material, and damaged/unsuitable fruit), the following described conditions of the unitary operations only consider the peel, which was the studied fraction of BOPW. The unitary operations in white blocks with dotted outlines are out of the scope of this thesis.

After being dried and ground, the BOPW is extracted with 80% (v/v) EtOH (aq) for 2h, at room temperature, with a solid/liquid ratio of 1:62.5. From this operation, a stream containing *p*-SYN, flavonoids, EtOH, water, and wet BOPW, is obtained. This stream is then filtered to separate the BOPW (cake) from the liquid phase (filtrate). The cake is conveyed to the section of pectin extraction and the filtrate is addressed to an adsorption column in which flavonoids are separated from *p*-SYN by ion exchange, using PD-206 resin, at room temperature, with a solid/liquid ratio of 50 kg resin/m^3. The *p*-SYN bound to PD-206 resin is then eluted with HCl (aq) 8 M. The stream that flows out of the adsorption column has 87% content of flavonoids. This stream is then conveyed to a distillation column in order to recover EtOH at the top of the column. 90% (arbitrary value) of the recovered EtOH (recov. EtOH) is recycled to the solvent extraction unit and the remaining is discarded (purge), to avoid the accumulation of solvent, cycle after cycle of operation. Before it gets to the solvent extraction unit, the recovered EtOH is mixed with water, to achieve a concentration of 80% EtOH. An aqueous residue, containing flavonoids, is obtained at the bottom of the column, being afterward dried to obtain a flavonoid-rich product.

The section of pectin extraction starts with an acid extraction, in which 13% (m/v) citric acid (aq) is used to extract pectin from BOPW, with a solid/liquid ratio of 1:40, at 95 ºC, for 105 min. A filtration unit follows to separate the BOPW from the remaining mixture (filtrate). The BOPW is discarded (waste). An equal amount of 96% (v/v) EtOH (aq) is added to the filtrate to trigger pectin precipitation (5 ºC for 2h), obtaining a stream rich in pectin, citric acid, EtOH, and water. This mixture is then centrifuged to

separate the solid phase (pectin) from the liquid phase (citric acid solution, EtOH, water). Pectin is washed with 96% (v/v) EtOH (aq) and dried. Downstream of the centrifugation unit, there is a distillation column to recover EtOH at the top and citric acid (aq) at the bottom. 90% (arbitrary value) of the recovered EtOH is recycled to the pectin precipitation and pectin washing units (the remaining 10% is discarded), and 90% (arbitrary value) of the recovered citric acid (aq) is recycled to the acid extraction unit (the remaining 10% is discarded). The concentration of the recovered citric acid (aq) is corrected to 13% (m/v), before it gets to the acid extraction unit.

The process E-Factor (equation (8)) was calculated in order to measure its environmental acceptability. An E-Factor of 13.96 t waste/t product was obtained. According to the literature (140), for a total mass of product in the order of magnitude of 10^{-1} tons, which is the case, the E-factor should range between 2.5 and 10. The calculated E-Factor is slightly above this range, so optimization should be performed to decrease the amount of produced waste per total mass of product and, so, increase the environmental acceptability of the process.

$$E - Factor = \frac{Total\ mass\ of\ waste\ from\ process}{Total\ mass\ of\ product} \quad (8)$$

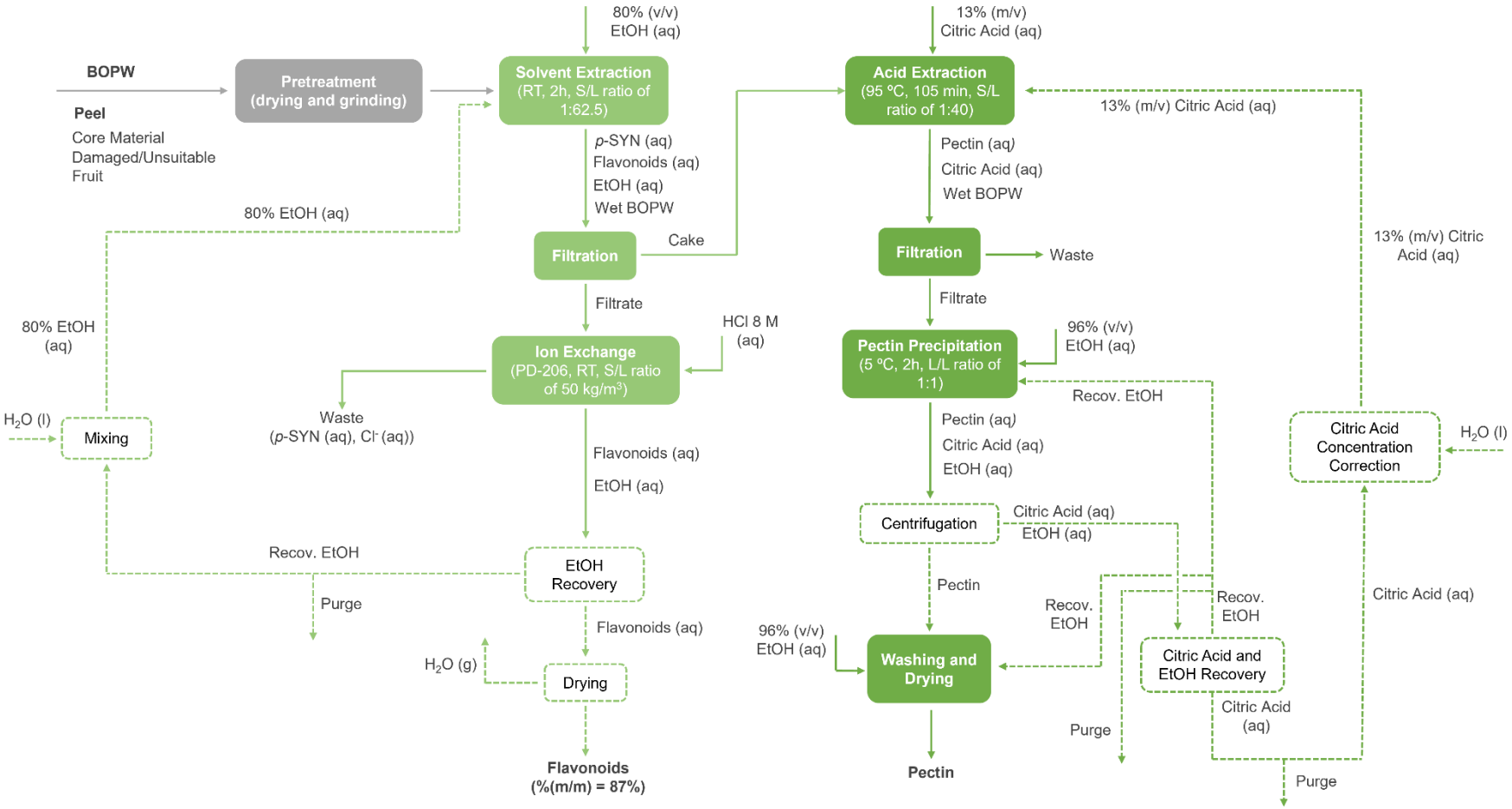

Figure 30. Detailed diagram of the waste valorization process.

Chapter 5

Conclusions and Perspectives for Future Work

Nowadays, the need to find eco-friendly products, industrial processes, and ways of living has been increasingly imperative, as climate change, global warming, and biodiversity reduction are becoming more and more evident. To be able to achieve this, while meeting market demands, is certainly one of the biggest challenges humankind has faced. The objective is to create an economic system that mixes bioeconomy and circular economy concepts. In this regard, CPW valorization is an important tool to achieve this objective, by disclosing the many added-value compounds that constitute CPW, being further used to produce bio-based products. The theme of this thesis falls into this scope, by studying BOPW.

BO production does not have fruit or juice consumption as an objective. However, this fruit is a rich source of several compounds with health benefits, so it deserves to be explored, including its juice, whose nutritional value was proved, in this thesis, to be comparable to that of SO juice. Even though, some treatments should be done to make it suitable and pleasant for consumption by mitigating the sour and bitter taste.

After extracting the juice, in a hypothetical BO juice production plant, tons of processing waste will be generated, including peels, core material, and damaged/unsuitable fruit. The experimental work focused on the valorization of this waste, using only peel as a sample to simplify the experimental procedures. The waste valorization process is composed of two sections: one in which *p*-SYN and flavonoids are extracted and isolated, and another in which pectin is extracted. The extraction of *p*-SYN and flavonoids was carried out by solvent extraction wherein different solvents and solutions were tested at room temperature and at 90 ºC, concerning the extraction yield of the compounds in regard. The solvents/solutions gave similar yields, with none standing out, but it was observed that, in general, room temperature led to higher yields, except for 96% EtOH (aq) and 99.8% MeOH. Therefore, only the BO extracts obtained at room temperature were addressed to binding experiments in order to separate *p*-SYN from the flavonoids.

The binding experiments were done by adsorption or ion exchange in which nine resins were studied. The strong-acid cation-exchange resins, AG 50W-X8 and PD-206, showed the highest binding percentages and selectivity for *p*-SYN in BO extracts obtained with 50% EtOH (aq) and 80% EtOH (aq). In the recovery experiments, *p*-SYN was more efficiently recovered from AG 50W-X8 resin, with 10% NaCl (aq) and 25% NaCl (aq), but PD-206, despite the lower recovery percentages, was more selective to *p*-SYN since the bound flavonoids were not recovered from this resin using HCl (aq). Therefore, two scenarios for the waste valorization process were assessed, concluding that PD-206 is the most suitable, leading to higher process yields for flavonoids and higher revenue. Furthermore, 80% EtOH

(aq) was chosen to carry solvent extraction because it eases the drying of the flavonoid-rich final product when compared to 50% EtOH (aq).

Regarding the extraction of pectin, 13% citric acid (aq) led to higher pectin yields than HCl (aq). The impact of the *p*-SYN and flavonoid extraction solvents/solutions on pectin yield was also assessed, concluding that distilled water and 96% EtOH (aq) gave higher yields of pectin. However, it was observed that the total revenue of the process is mainly driven by the revenue of NERT, so the choice of 80% EtOH (aq) to carry the initial solvent extraction of the BO peel was maintained, as it is one of the solutions that led to higher NERT yields. The simultaneous extraction of *p*-SYN, flavonoids, and pectin with 13% citric acid (aq) was also explored, but the yield of *p*-SYN and flavonoids was very low compared to other solvents/solutions, so it was concluded that this simultaneous extraction was not plausible to be implemented in the process.

Finally, additional investigation is advised to pursue the work developed in this thesis:

- Experimentally explore BO juice debittering.
- Perform a public sensory analysis of fresh BO juice and debittered BO juice, in order to assess the consumer appeal for the 2nd juice and realize if it would be plausible to produce BO juice for consumption or as a medical product for weight loss; an economic assessment should also be done to understand if BO juice production is worth from an economic point of view.
- Scale up and economically assess the waste valorization process.
- Explore the extraction of *p*-SYN, flavonoids, or pectin, by using non-conventional extraction methods, like UAE, MAE, EAE, and SWE.
- Try to regenerate the AG 50W-X8 resin by performing a backwashing step, followed by the addition of the regenerating solution, and a final washing, as advised in the literature (118), to realize if the regeneration is efficient.
- Test the regeneration of PD-206 resin.
- Extract *p*-SYN, flavonoids, and pectin from BO core material in order to complement the results obtained from BO peel.

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Appendix

A. Current Status of Biorefineries

In this section, an analysis of the global deployment status of biorefineries is presented. All data was collected from IEA Bioenergy Task 42 Database (141), available for public consultation. It is good to mention that this database does not include every available data on this subject; it should be complemented with other databases. Nonetheless, Task 42 database, by itself, is representative of the present situation of biorefineries.

According to Task 42 Database, there are currently 589 biorefineries, of which 537 are in operation, 46 are under construction, and 6 are under commissioning. In addition, 58 biorefineries are planned to be constructed (figure 31). “Canceled”, “Stopped”, “On Hold”, and “Idle” biorefineries, as well as “Other” and “Not Available” status, were not considered for this analysis. They are, however, available for consultation.

The United States of America (USA) is the region that possesses most operational biorefineries (365), followed by Europe (90). Regarding under-construction and planned biorefineries, the USA detains most under-construction biorefineries (28), having also 13 planned biorefineries. Europe has more planned biorefineries than the USA (39, in total), being the region with most planned biorefineries, but has just 4 biorefineries under construction (United Kingdom, Sweden, Finland, and Romania). The 6 biorefineries under commissioning can be found in Canada, France, Germany, and Finland.

Figure 31. Global distribution of operational, under-construction, in-commissioning, and planned biorefineries (141).

i. Operational Biorefineries

As shown in figure 32, primary biomass is the main feedstock type of operational biorefineries (49%), followed by secondary biomass (29%). Of all primary biomass types, starch crops and oil crops are the ones that stand out the most, with a percentage of 70% and 17%, respectively. Sugar crops and lignocellulosic biomass from forestry, croplands, and grasslands are also important primary biomass feedstocks. Regarding secondary biomass feedstocks, 40% of the operational biorefineries that process this type of raw material use residues from forestry and forest-based industry, and residues from agriculture. However, most secondary biomass operational biorefineries use other organic residues as feedstock (70%).

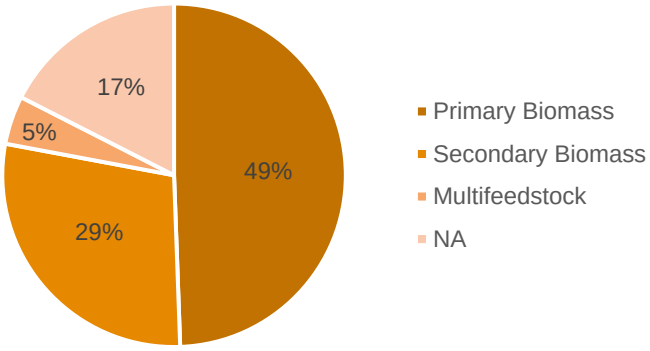

Figure 32. Feedstock distribution of operational biorefineries (141).

Concerning the products of operational biorefineries, energy products are ahead with a percentage of 87%, i.e., most existing biorefineries are energy-driven biorefineries. Following the energy products, there are chemicals and multiproduct categories, both with a percentage of 4%. Material products are the less produced (3%). Figure 33 shows a representation of the operational biorefineries based on the products they produce.

Within energy products, fuels and the joint production of fuels and solvents are assigned to 98% of the energy-driven biorefineries (49% each), and the remaining 2% owns to heat production. The main product type of chemicals-driven biorefineries is building blocks (56%), followed by additives (11%) and the joint production of nutraceuticals, pharmaceuticals, paints, and coatings (11%). Regarding materials, polymers are the main type (53%), followed by fibers (27%) and composites along with fibers (13%).

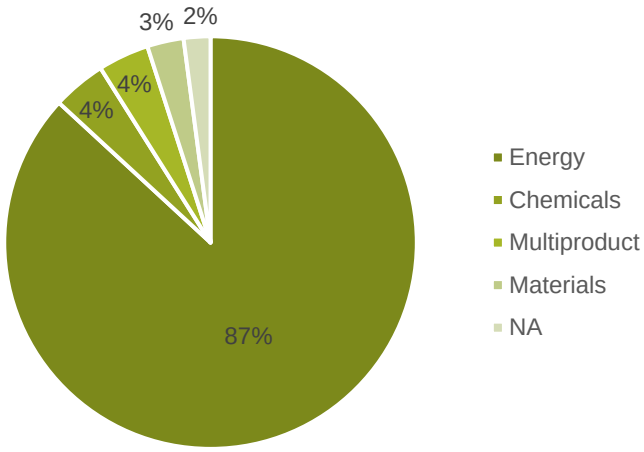

Figure 33. Product distribution of operational biorefineries (141).

ii. Under-construction, In-commissioning, and Planned Biorefineries

Contrarily to operational biorefineries, most of the under-construction, in-commissioning, and planned biorefineries (56%) will process secondary biomass, showing that companies are focusing on secondary biomass for future biorefineries (figure 34). Primary biomass takes 2nd place (24%).

The "Other Organic Residues" category is the most important secondary biomass type, with a percentage of 41% of all secondary biomass biorefineries in operation. This category is followed by residues from forestry and forest-based industry, and residues from agriculture (37% and 15%, respectively). Regarding primary biomass feedstocks, lignocellulosic biomass from forestry and starch crops are the main types (32% each), followed by oil crops, aquatic biomass, and sugar crops + starch crops (18%, 14%, and 5%, respectively).

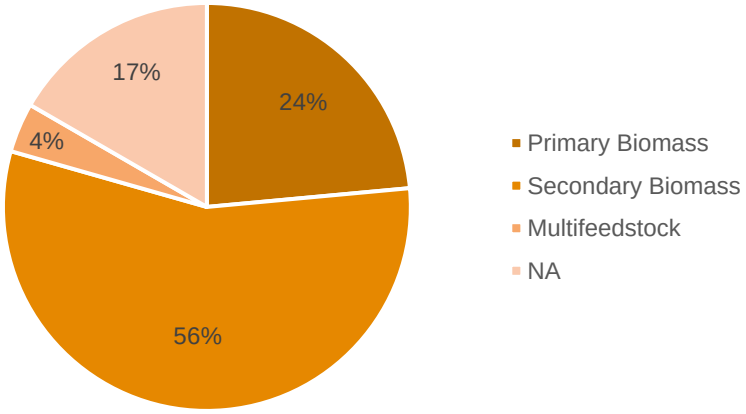

Figure 34. Feedstock distribution of under-construction, in-commissioning, and planned biorefineries (141).

As with operational biorefineries, most under-construction, in-commissioning, and planned biorefineries will produce energy products (89%) (figure 35), mainly fuels, as well as fuels + solvents (74% and 26% of all energy-driven biorefineries, respectively). Chemicals and materials will be produced on a much smaller scale (4% and 3%, respectively). The multiproduct category is also negligible when compared to energy products (4%). There is not a single product that stands out the most, among chemical products; building blocks, building blocks + additives, and building blocks + flavors and fragrances are distributed uniformly (33% each). The materials category only includes polymers.

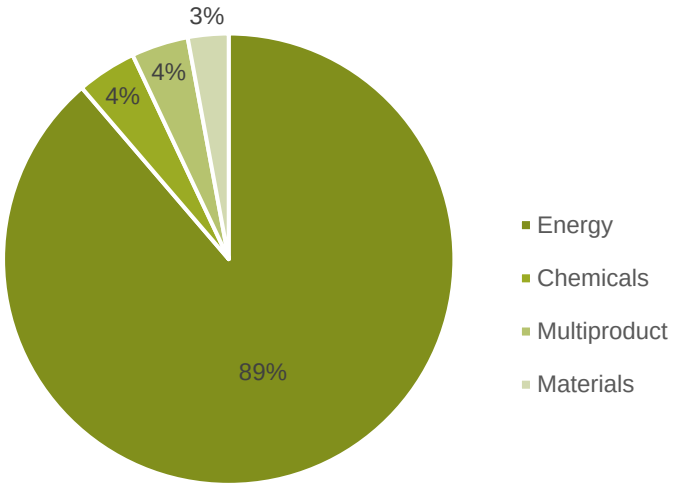

Figure 35. Product distribution of under-construction, in-commissioning, and planned biorefineries (141).

B. Barriers to Biorefinery Deployment and Potential Solutions

From a general perspective, the main barriers to biorefinery deployment are high production costs, the consumer willingness to pay more for bio-based products, lack of information and recognition of environmental benefits, the limited biomass cultivation due to restricted land availability for non-food uses (little availability of sustainable biomass), the complex nature of biomass feedstocks, requiring different pre-treatment and valorization processes, and poor financial support for RD&I, to develop innovative and more efficient processes, and for scale-up. Other important barriers should also be mentioned, such as (113):

- Limited biomass cultivation due to climate changes that lead to soil dryness.
- Poorly developed methods for feedstock harvesting, collection, and storage, leading to degradation and loss of biomass.
- Unstable biomass supply due to seasonality, poor infrastructure, low quality, and cost.
- Uncertainty regarding the assurance of lower environmental impact of feedstocks.
- Lower quality and lower performance of some bio-based products when compared to fossil-based products.
- Loss of functionalities or properties of intermediate products during their conversion into new products.
- Lack of recognition of bio-based content in products.

Because biorefineries are one of the main drivers of the circular bioeconomy, overcoming these barriers is a must.

Although there are currently many studies about biomass valorization, most of them are done at a lab scale, assuming the input materials are readily available and in sufficient quantity when scaled up. This is a wrong assumption since the availability of biomass for biorefinery use is limited, as stated before. So, to overcome this problem, it is imperative to encourage the RD&I activities to accelerate the development of efficient conversion, harvesting, and storage technologies, able to optimize to the fullest the valorization of biomass (zero-waste). Another solution is to bet on secondary biomass feedstocks (i.e., wastes) because, unlike primary biomass, secondary biomass availability is not affected by seasonality, being a steady and reliable stream of raw materials supply year-round. Emerging technologies should also be able to process more than one kind of feedstock as a way to address the seasonality of several feedstocks, reducing the downtime of biorefineries. Concerning the competitiveness between feed and food for land, the use of species that can grow on marginal land with little needs could be considered in order to use infertile soils as farmland (12).

Finding more efficient conversion, harvesting and storage technologies, along with process integration, can also help to decrease production costs by reducing capital costs and facility operating costs. In this regard, additional strategies could be considered: reducing feedstock costs (using waste and low-quality raw materials, for example), utilizing existing infrastructures (in the case of fuel

production, petroleum refining infrastructures and equipment can be used with little adjustments), and developing high-value products (142).

The pricy environmentally friendly products are a major obstacle to the deployment of a circular bioeconomy, as consumers tend to opt for conventional and cheaper products. Evidently, bio-based products are not the exception. To encourage consumers to pay more for these products some measures should be implemented, such as corporate social responsibility and favorable purchase policies, lowering the cost over the product lifecycle, and increasing the number of bio-based applications. In addition, certification and standardization for bio-based products, including the measure of the bio-based content in products, should be implemented, along with the development of instruments to communicate their environmental benefits (113,142).

At last, modifications to improve bio-based properties and to broaden the applications to other markets, along with the development of efficient conversion processes, should be considered to enhance the quality and performance of bio-based products when compared to conventional alternatives (mostly fossil-based) (113,142).

C. Calibration Curves

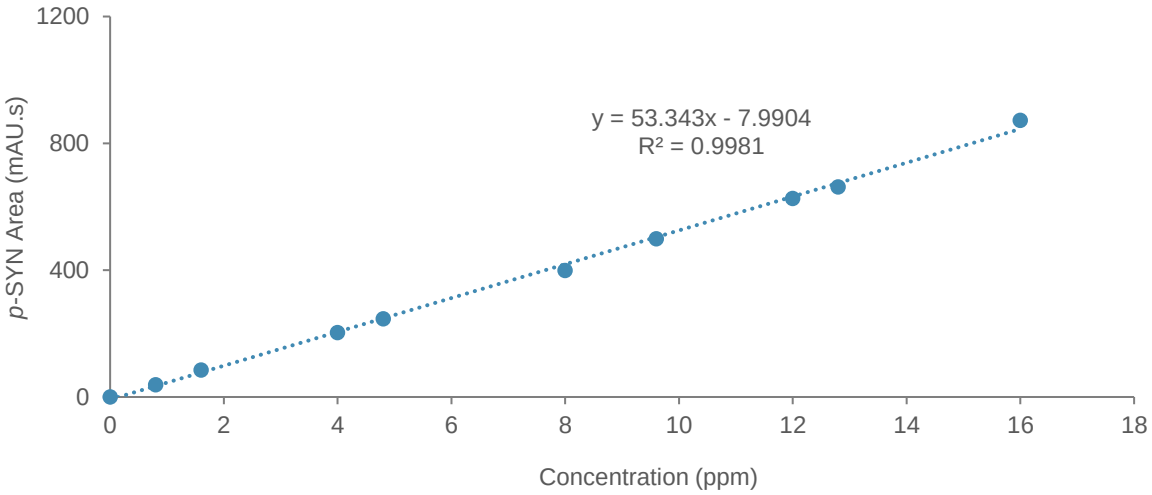

Figure 36. Calibration curve of p-SYN in concentrations ranging from 0 to 16 ppm.

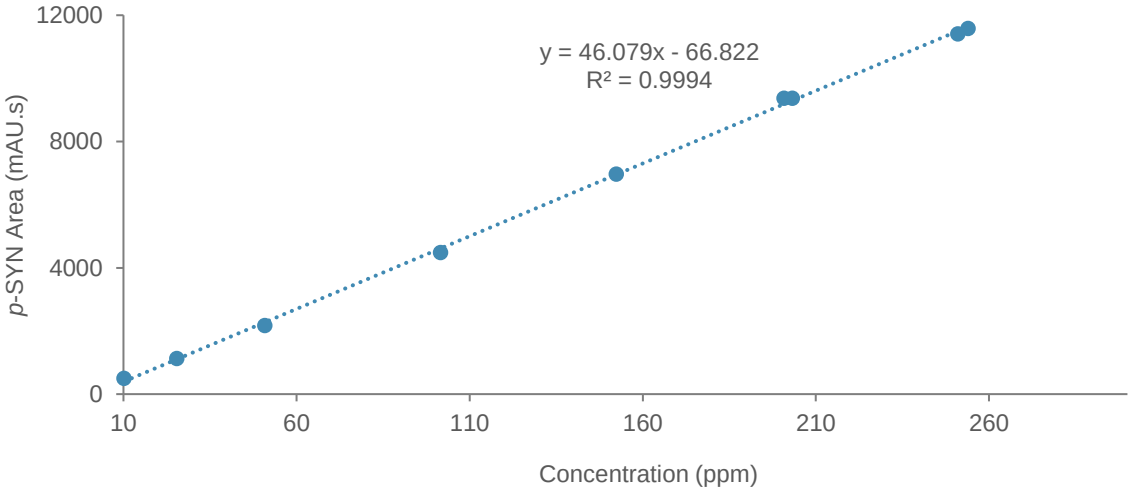

Figure 37. Calibration curve of p-SYN in concentrations ranging from 10 to 251 ppm.

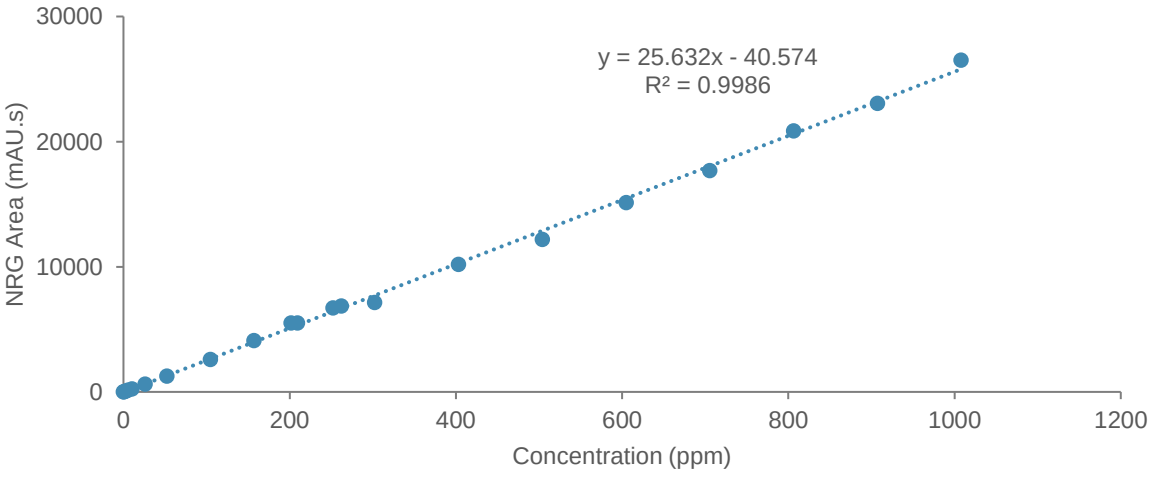

Figure 38. Calibration curve of NRG in concentrations ranging from 0 to 1008 ppm.

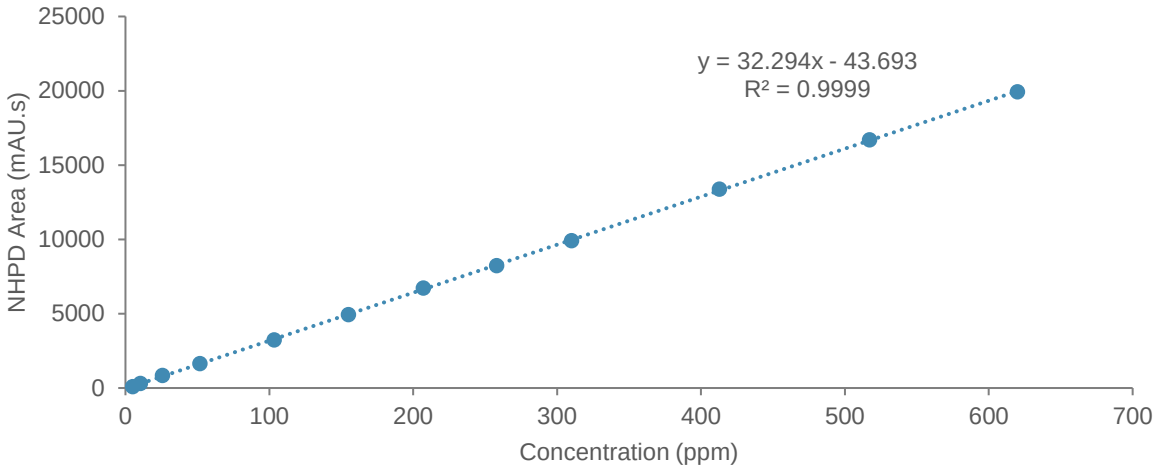

Figure 39. Calibration curve of NHPD in concentrations ranging from 0 to 620 ppm.

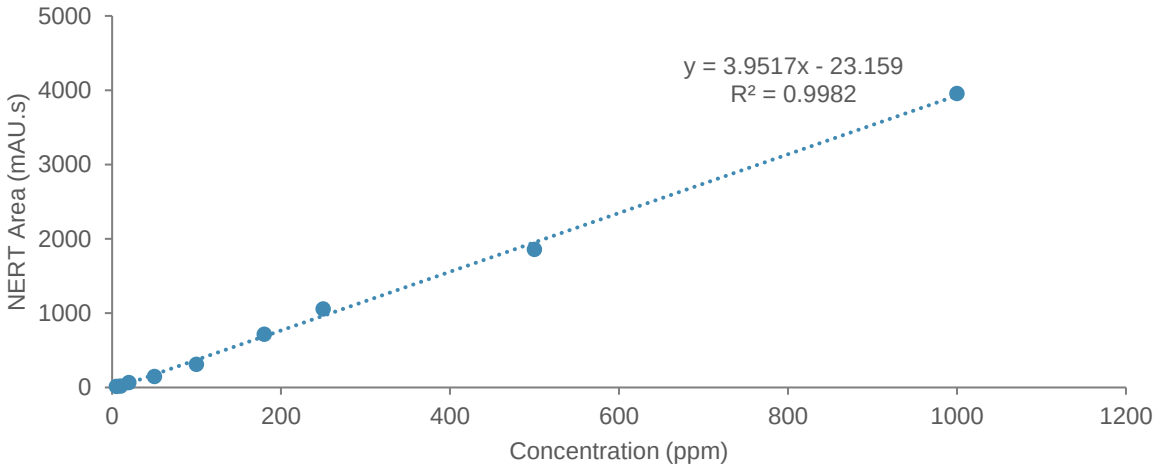

Figure 40. Calibration curve of NERT in concentrations ranging from 0 to 1000 ppm.

D. Impact of Different Solvents on the Extraction Yield of Neohesperidin and Neoeriocitrin

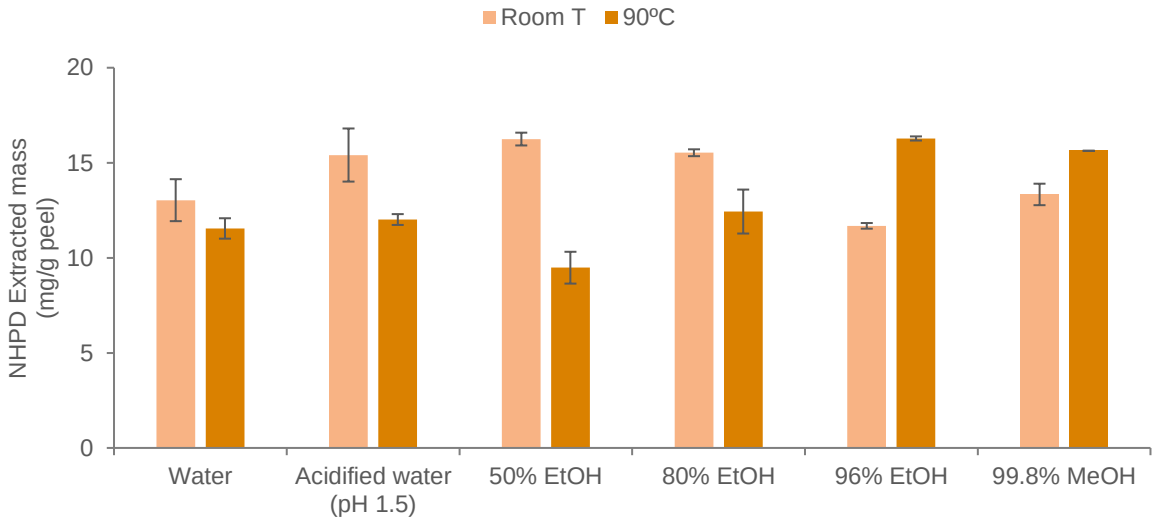

Figure 41. Impact of different solvents and solutions on NHPD extraction yield, at room temperature and 90 ºC, with a S/L ratio of 1:62.5.

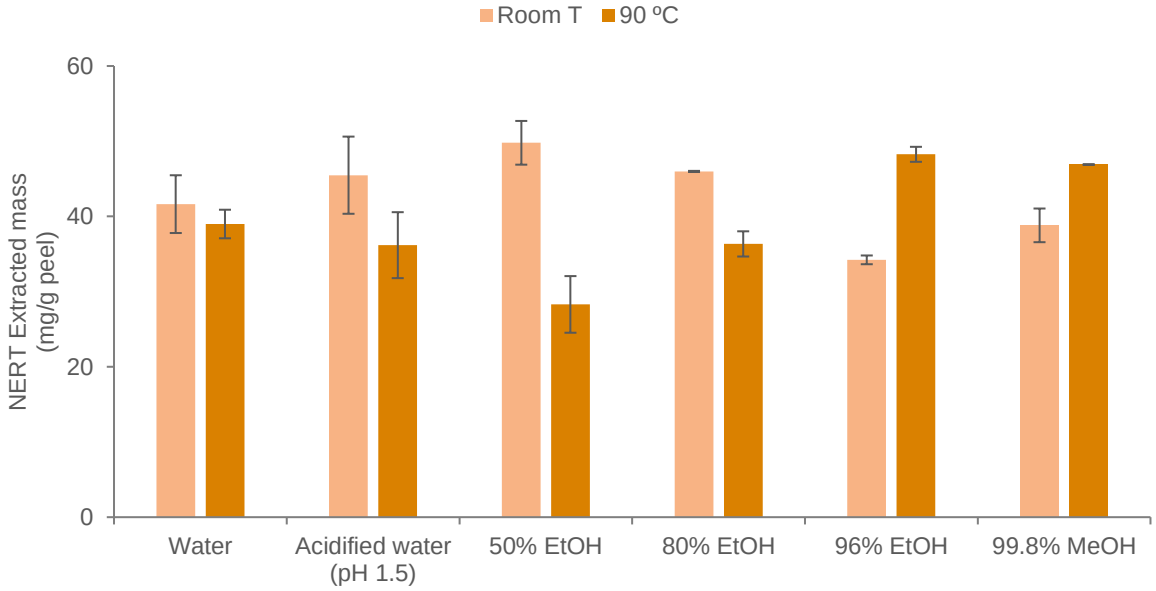

Figure 42. Impact of different solvents and solutions on NERT extraction yield, at room temperature and 90 ºC, with a S/L ratio of 1:62.5.

E. Resin Adsorption and Ion Exchange

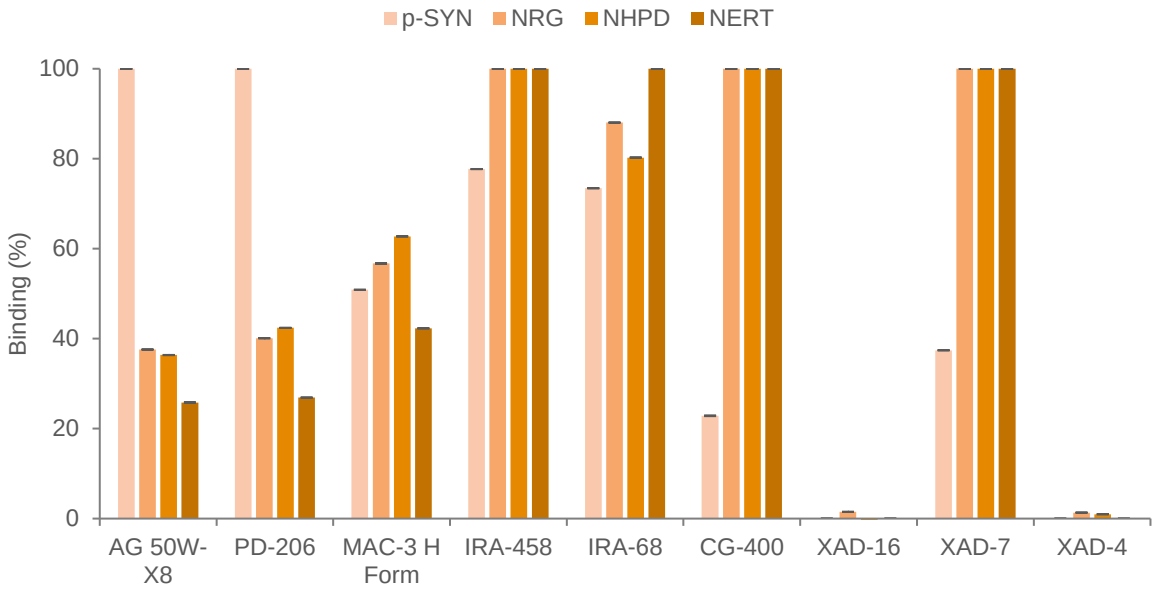

Figure 43. Binding of p-SYN, NRG, NHPD, and NERT to various resins for BO peel extracted with distilled water, at room temperature, with a S/L ratio of 50 kg resin/m^3. The null binding% correspond to inconclusive results for the respective compound.

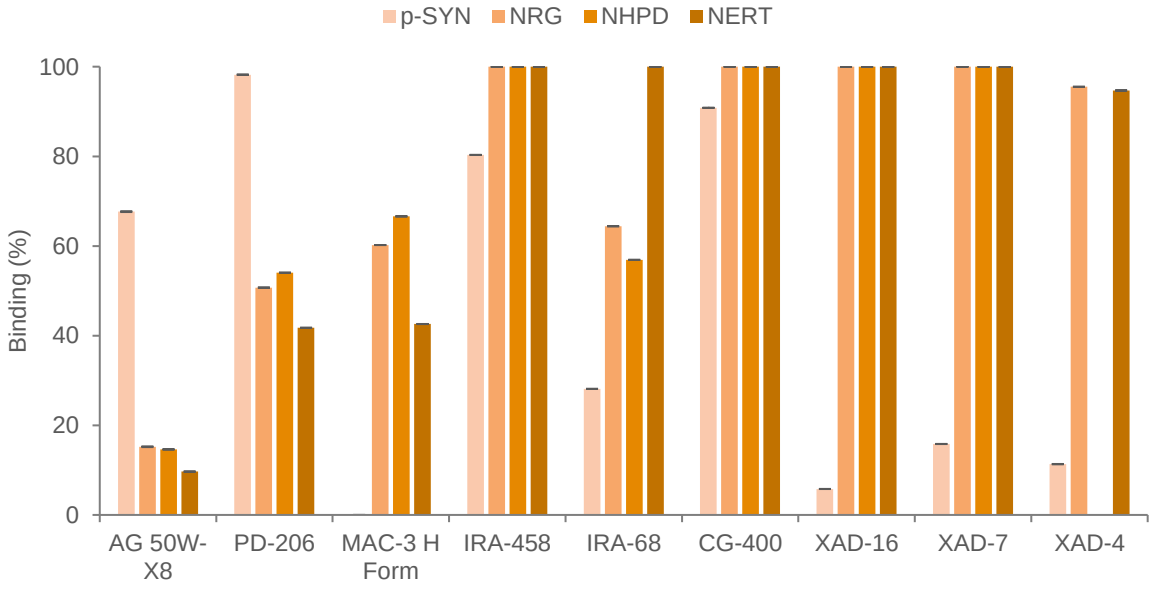

Figure 44. Binding of p-SYN, NRG, NHPD, and NERT to various resins for BO peel extracted with acidified water (pH 1.5), at room temperature, with a S/L ratio of 50 kg resin/m^3. The null binding% correspond to inconclusive results for the respective compound.

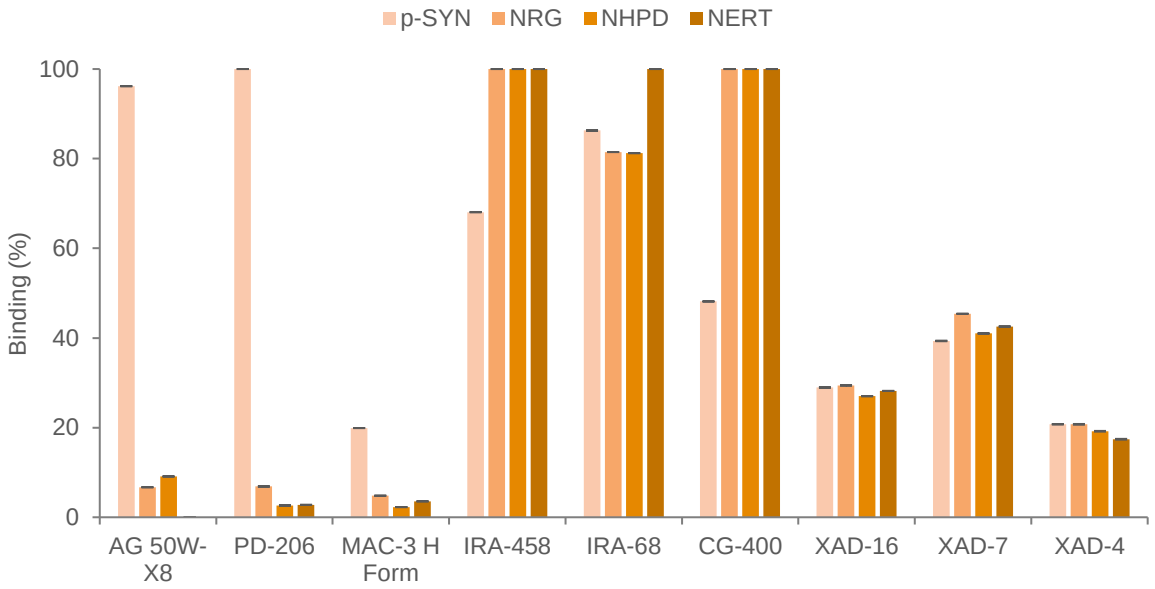

Figure 45. Binding of p-SYN, NRG, NHPD, and NERT to various resins for BO peel extracted with 50% EtOH (aq), at room temperature, with a S/L ratio of 50 kg resin/m^3.

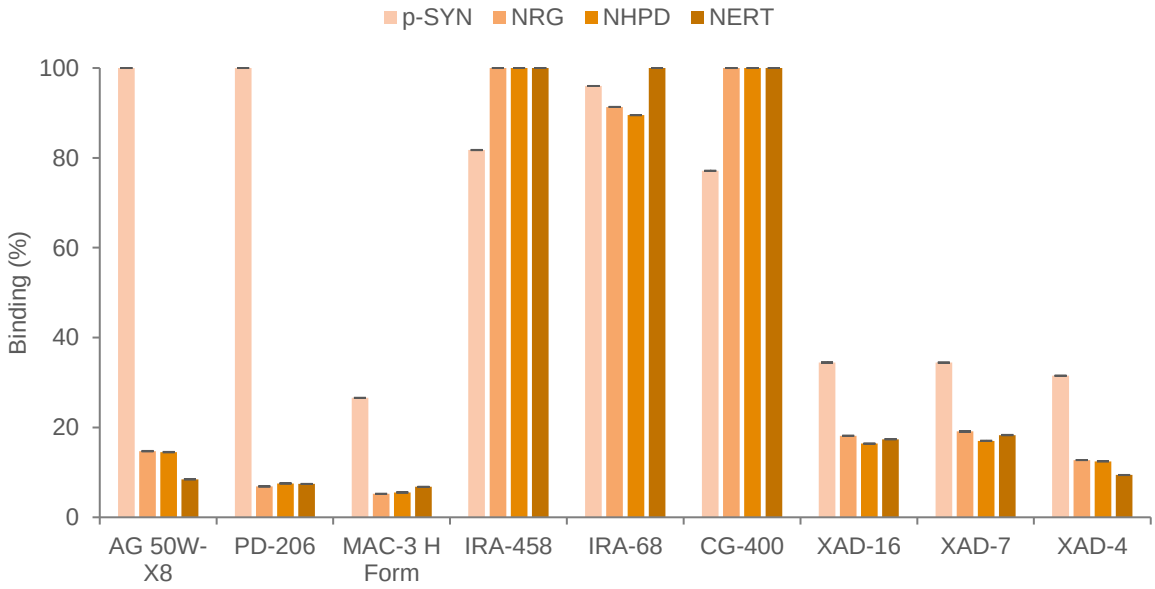

Figure 46. Binding of p-SYN, NRG, NHPD, and NERT to various resins for BO peel extracted with 80% EtOH (aq), at room temperature, with a S/L ratio of 50 kg resin/m^3.

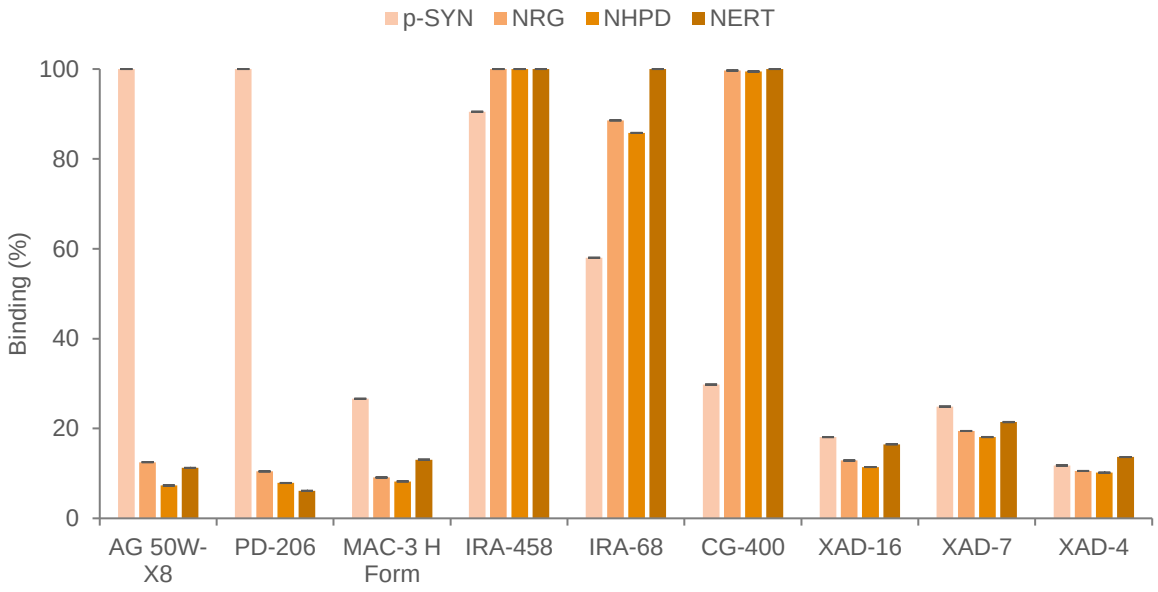

Figure 47. Binding of p-SYN, NRG, NHPD, and NERT to various resins for BO peel extracted with 96% EtOH (aq), at room temperature, with a S/L ratio of 50 kg resin/m^3.

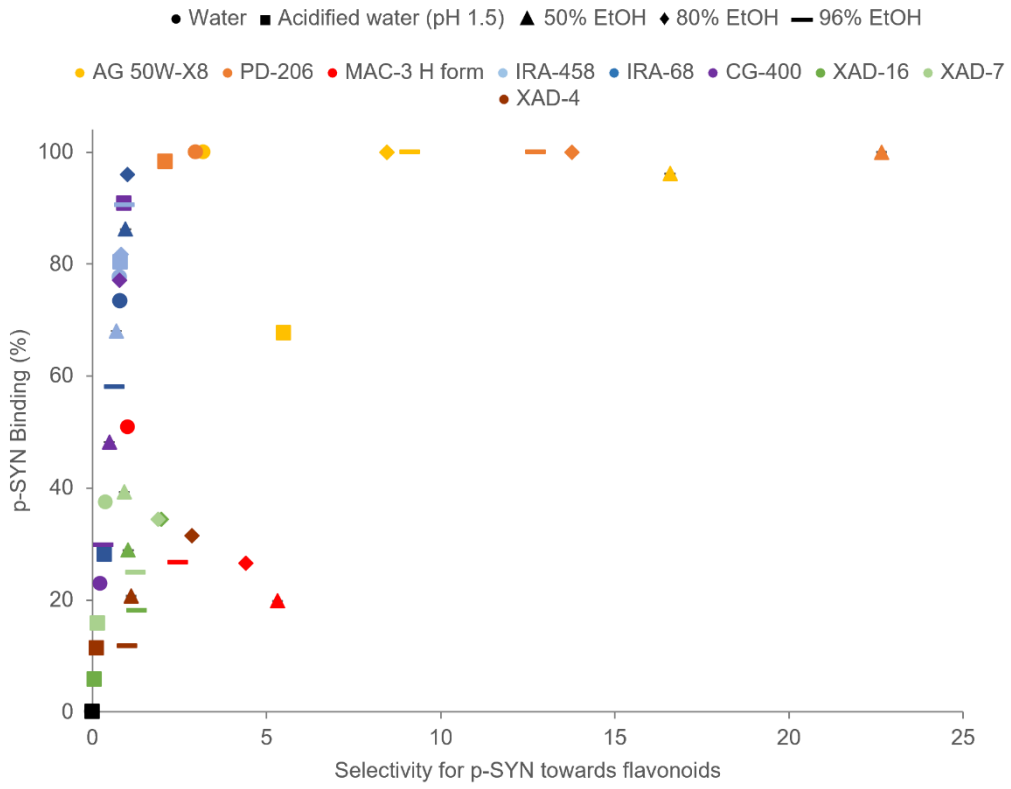

Figure 48. Binding and selectivity for p-SYN towards the fraction of flavonoids of various resins, with a S/L ratio of 50 kg resin/m^3, for BO peel extracted with aqueous and ethanolic solvents/solutions at room temperature.

F. Neohesperidin Recovery from AG 50W-X8 Resin

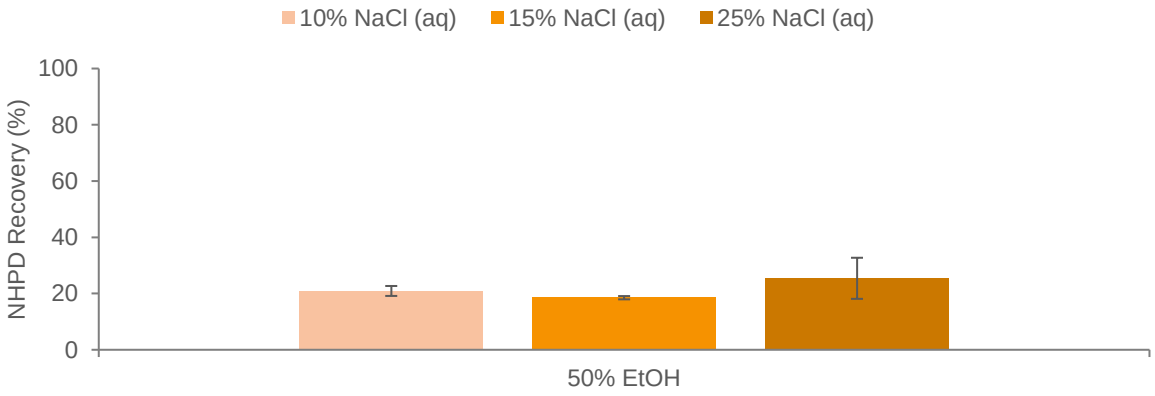

Figure 49. NHPD recovery from AG 50W-X8 resin, S/L ratio of 100 kg resin/m^3.