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

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ABSTRACT

A process for bitter orange (BO) juice production with fruit processing waste valorization was developed. 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, 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%).

1 Introduction

Citrus fruits are one of the major horticultural crops in the world (1–3), due to their sensorial attributes and health-promoting nutrients and compounds (vitamin C, β-carotene, minerals, dietary fibers, polyphenols, limonoids, etc.) that please consumers. Among the citrus, oranges account for half of the global production of citrus fruits (53%), followed by tangerines (26%), lemons and limes (14%), and grapefruits (7%) (4). 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 (5). To produce these products, the citrus processing facilities generate enormous amounts of organic waste that are usually used as animal feed or disposed of into the environment, leading to the emission of greenhouse gases and odor generation (3). Therefore, several institutions, like the European Commission and the United Nations, have been establishing some policies to encourage the valorization of citrus processing waste by abandoning the linear economy (take, make, dispose of) and implementing other economies that privilege the re-usability and/or the valorization, like bioeconomy and circular economy (1).

Bitter orange (BO), also known as sour orange, Seville orange, marmalade orange, and bigarade, belongs to the Rutaceae family, like all citrus fruits. Botanically, it is designated as *Citrus aurantium* and results from the cross between the pomelo (*Citrus maxima*) and the mandarin orange (*Citrus reticulata*) (7). 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 sweet orange (SO), lemon, mandarin, and other edible citrus fruits. BO fruit is used in the food industry to make marmalade (8,9), in many medicinal folk traditions (laxative, sedative for insomnia and anxiety, and to treat stomach aches, high blood pressure, indigestion, abdominal pain, constipation, and dysenteric diarrhea, among others (10,11)), in dietary supplements as an appetite suppressant, to improve weight loss, and a muscle enhancer (12), as well as in cosmetic and hygiene products (11,13). 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 of 5.7 tons of oranges (9). 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 (9). 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 (14).

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 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 the present study 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, however, approached only in a conceptual manner, contrarily to the waste valorization that was experimentally developed.

2 Materials and Methods

2.1 Plant Material

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

2.2 Reagents

HPLC-grade ethanol (EtOH), methanol (MeOH), glacial acetic acid, 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.

2.3 Extraction of p-SYN and Flavonoids from BO peel

p-SYN and flavonoids were extracted from ripe BO peel, using solvent extraction. The procedures were based on Pellati et al. (15) 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. 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 *p*-SYN and flavonoids isolation by resin adsorption and ion exchange. The cake obtained from the filtration was extracted two more times, using the same procedures.

2.3.1 Extraction Solutions Preparation

Acidified water was prepared by adding HCl (aq) 4 M drop wisely till the pH of 1.5 was achieved. 50%, 80%, and 96% EtOH (aq) solutions were obtained by diluting absolute ethanol.

2.4 p-SYN and Flavonoids Binding Experiments

50 mg of each resin were put in a 2 mL Eppendorf tube, along with 1 mL of the most concentrated filtrate, i.e., the filtrate from the first extraction (S/L ratio = 50 kg/m^3). Nine resins were tested: two strong-acid cation-exchange resins (AG 50W-X8 and PD-206), one weak-acid cation-exchange resin (MAC-3 Hydrogen Form), two strong-base anion-exchange resins (IRA-458 and CG-400), one weak-base anion-exchange resin (IRA-68), and three polymeric adsorbents (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). Thereafter, the Eppendorf tubes were centrifuged at 10 000 rpm for 5 min and the supernatant was removed and concentrated to dryness under vacuum (100 mbar) at 40 ºC, in a vacuum oven. Once dried it was redissolved in MeOH to be analyzed by HPLC. The percentage of *p*-SYN and flavonoids bound to each resin ($B\%$) was calculated using equation (1), 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 (2)).

$$B\% = \frac{C_0 - C_f}{C_0} \times 100 \quad (1)$$

$$S_{p-SYN} = \frac{B\%_{p-SYN}}{B\%_f} \quad (2)$$

2.4.1 Anion-exchange Resins and Polymeric Adsorbents Pretreatments

Before the 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).

2.5 p-SYN 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). The HCl solutions were prepared by diluting a 37% HCl 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.

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

$$M_r = C_f, \times V_r \quad (5)$$

$$M_r = C_f, \times V_r \quad (6)$$

2.6 Extraction of Pectin

Pectin extraction and isolation procedures were based on Fakayode et al. (16), with little modifications and using the reported optimal extraction conditions. 1.5 g of ground peel were extracted with 60 mL of acidified water and citric acid aqueous solution 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. The 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).

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

2.7 p-SYN and Flavonoids Identification in the BO Peel Extracts

The peaks in the High-Performance Liquid Chromatography (HPLC) chromatograms of BO peel extracts were identified by comparing them to the chromatograms of standard solutions of pure *p*-SYN and pure flavonoids available in the laboratory (naringin, hesperidin, neohesperidin, neoeriocitrin, naringenin, hesperetin, and narirutin).

2.8 p-SYN 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. 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. The HPLC analysis were carried out on a VWR HITACHI Chromaster HPLC system equipped with a UV-vis detector (5420), a column oven (5310), an autosampler (5260), and a pump (5160). A Luna 10 µm C18(2) 100 Å (250 x 4.6 mm) LC column was used with a mobile phase composed of 0.6% (v/v) acetic acid (aq) (solvent A) and MeOH (solvent B), at 0.6 mL/min. The volume of injection was 10 µL and detection was set at 225 nm for *p*-SYN and at 283 nm for flavonoids. The gradient program was as follows: 60% A/40% B 0 – 27 min, 10% A/90% B 27 – 32 min, 10% A/90% B 32 – 42 min, 60% A/40% B 42 – 47 min, and 60% A/40% B 47 – 60 min. Given the peak areas obtained by HPLC, it was possible to determine the corresponding concentrations of *p*-SYN and flavonoids, by linear interpolation, using the calibration curves.

2.8.1 Calibration Curves

Standard solutions for *p*-SYN, NRG, NHPD, and NERT were prepared. The most concentrated solution (stock solution) was prepared by dissolving a known amount of pure compound in MeOH, and the lower concentration 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.

3 Results and discussion

3.1 Overall Process Concept

When the fruit arrives at a conventional orange processing plant for juice production, it is washed, destemmed, and graded to separate the damaged fruit from the sound fruit. The sound fruit is then stored 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. The damaged fruit, on the other hand, is usually conveyed to a feed mill where it is processed into animal feed. During juice extraction, the fruit is squeezed to obtain a pulpy juice, and the peels and core material of the fruit join the damaged fruit to be processed into animal

feed (17). Juice Production follows, wherein the pulp is separated from the juice, through clarification, and headed to a Pulp Production unit (17), whose unitary operations are out of the scope of this thesis. The process for BO juice production was designed based on these unitary operations but, instead of being simply used for animal feed, the BOPW is 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 1.

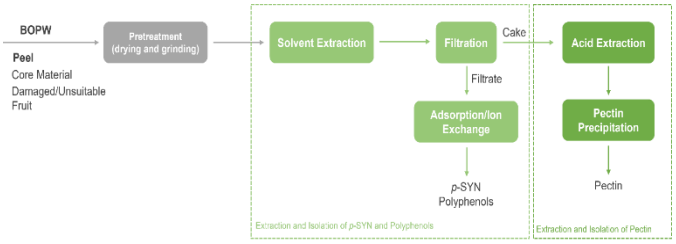

Figure 1. Simplified diagram of the proposed waste valorization process.

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 precipitating 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.

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.

3.2 BOPW Valorization

3.2.1 p-SYN and Flavonoids Extraction

p-SYN was detected in the BO peel extracts with a retention time inside the Luna column of 3.30 min, as well as NERT (13.30 min), NRG (21.00 min), and NHPD (25.90 min). The content of adrenergic amines and flavonoids in BO fruits (*Citrus aurantium var. amara*) was also studied by Pellati et al. (15) They were able to detect *p*-SYN, NRG, hesperidin, NHPD, NERT, 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. (15), 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 (18).

Although three flavonoids were detected in the BO extracts, the presentation 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.

The results on extraction yield are found in figure 2. 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 (15), 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 (15)).

MeOH was included in this study, since many authors report MeOH as the best phenolic compound extraction solvent, in terms of extraction yield (19), due to the high solubility of phenolics in MeOH (20). 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 (19).

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 (19), which might explain why room temperature led to higher 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.

The results also showed similar extraction yields 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 (19). 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)). After quantifying NHPD and NERT in the BO peel extracts, it was concluded that the extraction profiles of NHPD and NERT were very

similar to the profile of NRG. Therefore, similar conclusions were taken for those two flavonoids, regarding the influence of the various solvents and solutions on their extraction yields.

In addition to the extraction performance, the solvent selection must consider *p*-SYN binding onto resins, selectivity, and recovery efficiencies, 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, considering that different solvents/solutions imply different resin behaviors.

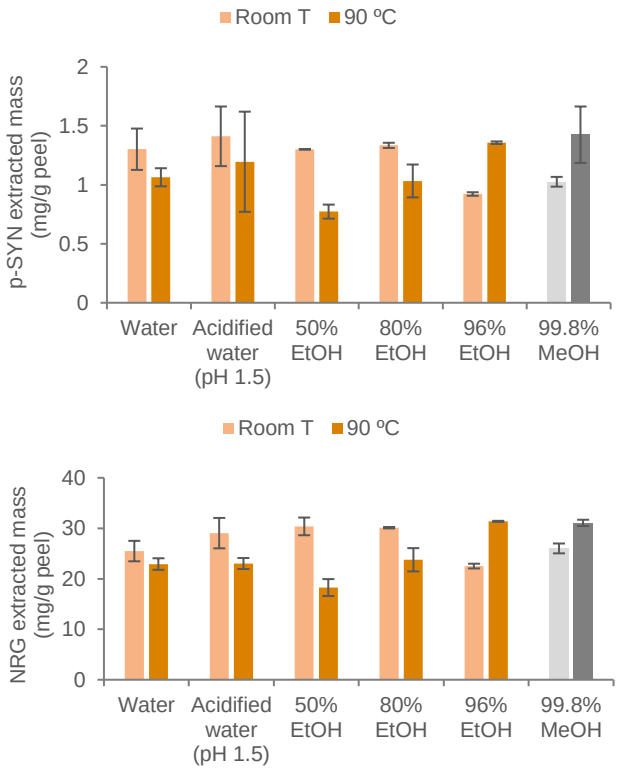

Figure 2. Top: impact of different solvents and solutions on *p*-SYN extraction yield, at room temperature and 90 ºC, with a S/L ratio of 1:62.5. Bottom: impact of different solvents and solutions on NRG extraction yield, at room temperature and 90 ºC, with a S/L ratio of 1:62.5.

3.2.2 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 results are presented in figure 3.

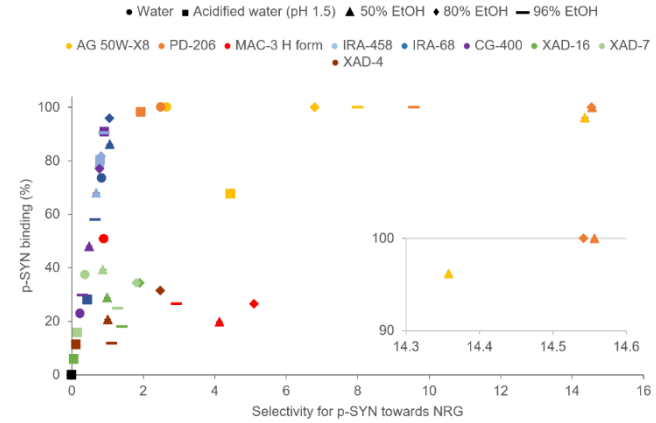

Figure 3. Binding and selectivity for *p*-SYN towards NRG of various resins for BO peel extracted with aqueous and ethanolic solvents/solutions at room temperature, with a S/L ratio of 50 kg resin/m^3.

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 (21), 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. (22), however for another alkaloid, lupanine (pKa 9.1 (23)). 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. (22), including the resins in regard.

The highest values of selectivity for *p*-SYN (figure 3) 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.

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 2).

3.2.3 p-SYN Recovery

The solutions to recover *p*-SYN from AG 50W-X8 and PD-206 resins were chosen based on the regenerating solution of each resin. According to the literature (24), the regenerating solution of a strong-acid cation-exchange resin, in Na^+ form, is NaCl (aq) with a concentration between 5% and 25%, and the regenerating solution of a strong-acid cation-exchange resin, in H^+ form, is HCl (aq) 5.5 M.

From figure 4 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). In these experiments, NRG and NHPD were also recovered from AG 50W-X8 resin (10% - 34% NRG, and 21% - 25% NHPD using 50% EtOH (aq)). NERT could not be recovered from both resins.

The recovered mass of *p*-SYN and NRG (figure 5) from AG 50W-X8 was similar (0.20 – 1.49 mg NRG/g peel, 0.66 – 1.28 mg *p*-SYN/g peel), whereas the recovered mass of NHPD was lower (0.31 – 0.37 mg/g peel). Then, it was concluded that the *p*-SYN recovered from AG 50W-X8 resin is contaminated with NRG in 23% - 54% and NHPD in 12% - 26%.

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, so two ion exchange scenarios (using AG 50W-X8 or PD-206) will be analyzed, in section 3.2.5, to decide which is the most appropriate to be implemented in the waste valorization process.

Furthermore, 80% EtOH (aq) was chosen to extract *p*-SYN and flavonoids from BO peel, in the waste valorization process 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.

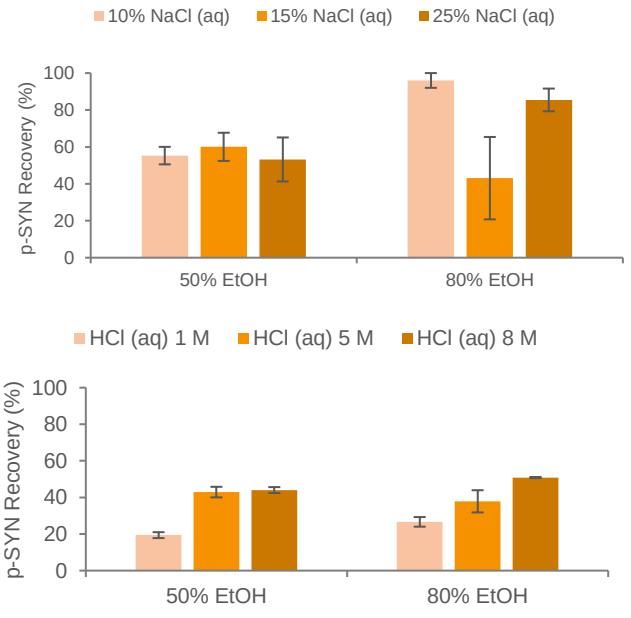

Figure 4. Top: p-SYN recovery from AG 50W-X8 resin, with a S/L ratio of 100 kg resin/m^3. Bottom: p-SYN recovery from PD-206 resin, with a S/L ratio of 100 kg resin/m^3.

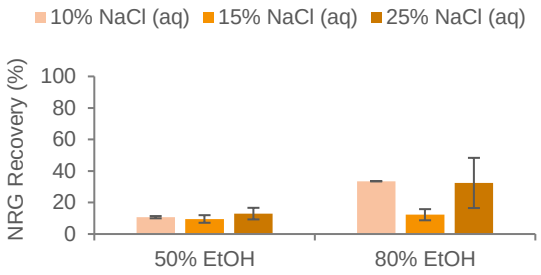

Figure 5. NRG recovery from AG 50W-X8 resin, with a S/L ratio of 100 kg resin/m^3.

3.2.4 Pectin Extraction

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 6.

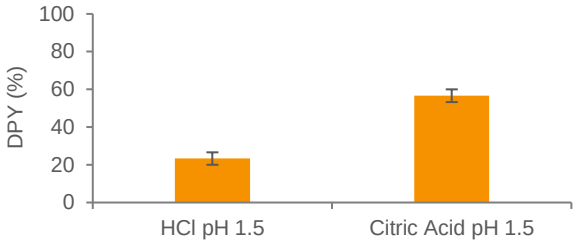

Figure 6. DPY obtained by using HCl (aq) and 13% citric acid (aq), pH 1.5, at 95 ºC, with a S/L ratio of 1:40.

Citric acid led to a higher pectin yield (56.60%) than HCl (23.30%), so it was the chosen solution to extract pectin from the BOPW.

Similar results were obtained by Fakayode et al. (16) and Devi et al. (25), however for other citrus fruits. Fakayode et al. (16) 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 (25). 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.

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 cannot be precipitated afterward (25,26). On the contrary, citric acid produces bigger pectin particles, i.e., less soluble, facilitating pectin precipitation and resulting in a higher mass obtained (25,26). This might be a reason why citric acid leads to higher yields.

3.2.5 Final Remarks

3.2.5.1 Selecting the Best Scenario for the Waste Valorization Process

As explained in section 3.2.3, two scenarios will be analyzed in the present section, to decide which is the most appropriate to be implemented in the waste valorization process. 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). Schematic representations of both scenarios can be found in figure 7.

From tables 1 and 2, 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. However, this value should be taken carefully and reviewed since it seems to be inflated.

3.2.5.2 Process Inventory and Raw Material Costs

The process inventory and the raw materials costs are presented in table 3. 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.

3.2.5.3 E-Factor

The E-Factor of the process (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 (27), 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.

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

3.2.5.4 Process Description

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 overall diagram of the waste valorization process is presented in figure 8, in which the unitary operations in white blocks with dotted outlines were not studied.

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.

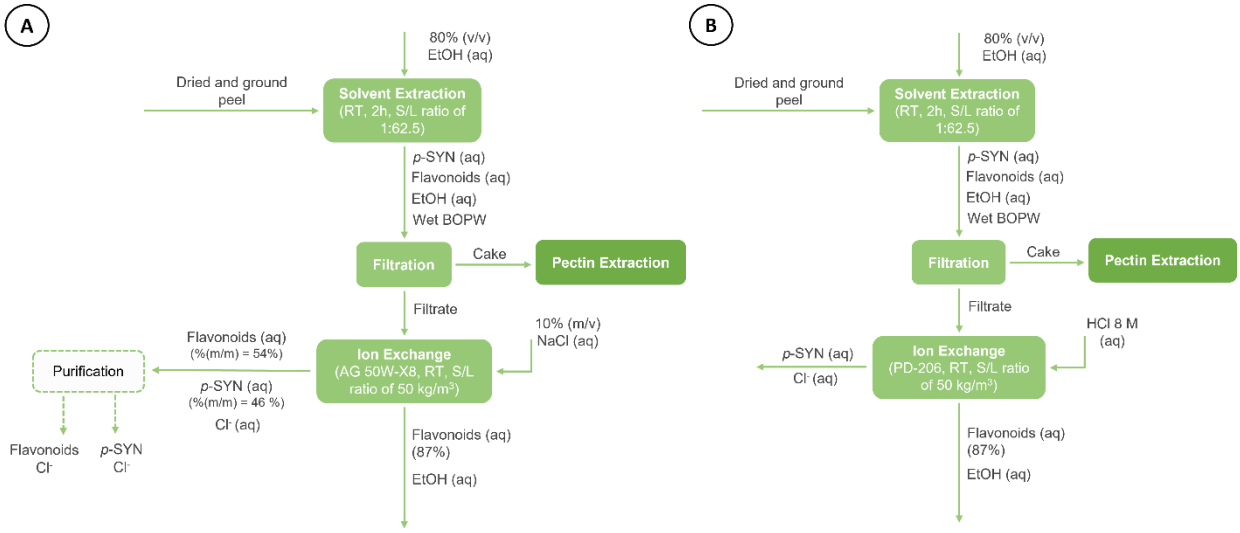

Figure 7. Schematic representations of scenario A and scenario B.

Table 1. 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 (32)	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 (33)

Table 2. 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 (32)	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 (33)

Table 3. 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 (28)	1.50	365.51
PD-206	3.13	93.8* (29)	293.59	
Citric Acid	5.22	2.29 (30)	11.97	
Solvents	**Required amount (m^3/t peel)**	**Bulk Price (EUR/m^3)**	**Cost (Thousand EUR/t peel)**	
EtOH	88.40	659.91 (31)	58.34	
Distilled water	85.35	1.29 (32)	0.11	

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

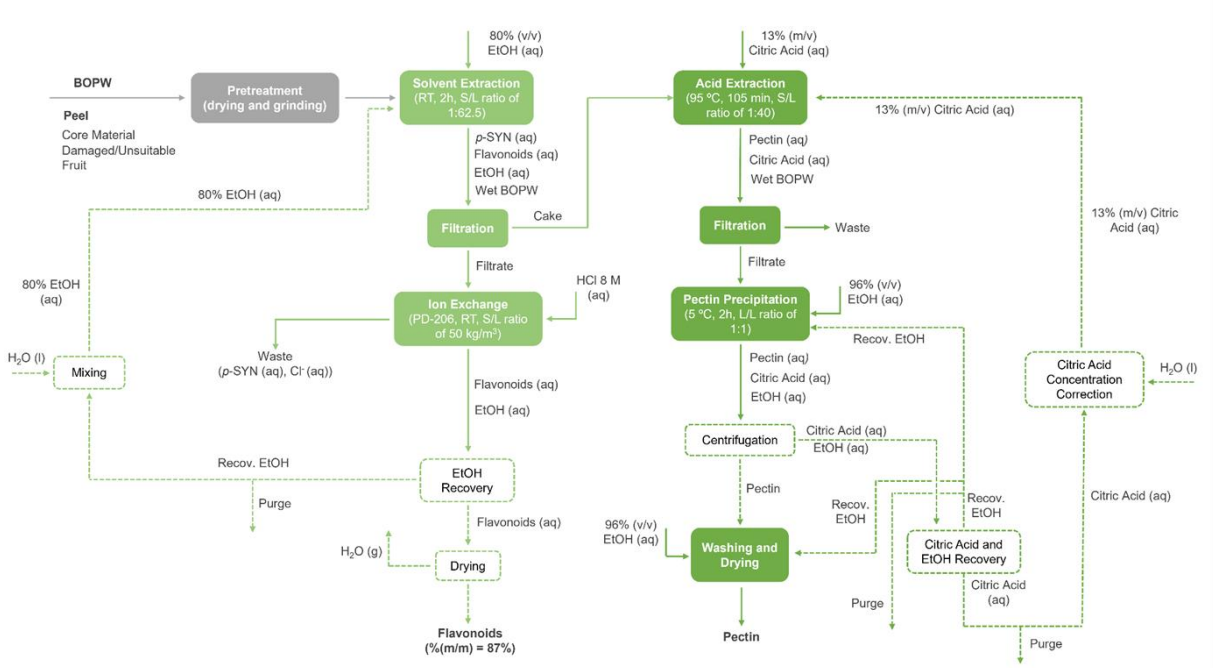

Figure 8. Detailed diagram of the waste valorization process.

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.

4 Conclusion

p-SYN and flavonoids were extracted from BO peel by solvent extraction wherein different solvents were tested at room temperature and at 90 ºC. The solvents 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.

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), concluding that citric acid is more suitable to extract pectin from BO peel than HCl (aq). In addition, because citric acid is not a mineral acid, the discharge of wastewaters containing citric acid is not so associated to environmental problems as HCl acid, which is a mineral acid. Therefore, using citric acid instead of HCl has the advantage of requiring less costs related to the discharge of wastewaters.

Although the experimental results appeared to be very promising to the implementation of a process for BOPW valorization, the final proposed process lacks a complete economic assessment that should be done in future work. The regeneration of PD-206 resin is also an important parameter that was not assessed during the present study, due to time scheduling limitations, but demands investigation in the future. In addition, the extraction of *p*-SYN, flavonoids, and pectin from BO core material should be performed in order to complement the results obtained from BO peel.

Furthermore, the production of BO juice must be deeply investigated and explored, by performing, for example, a public sensory analysis of fresh BO juice and treated BO juice, using debittering techniques, 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 it from an economic point of view.

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