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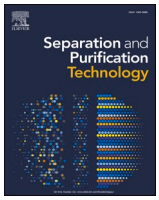

# Recovery of essential oils, polyphenols, fermentable sugars, and pectin from orange residues: Evaluation of extraction methodologies and characterization of value-added bioactive compounds

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ABSTRACT

Residues from orange processing are being continuously generated in vast amounts due to the increasing demand for this fruit and its byproducts worldwide. The valorization of Orange Residues is challenging in contrast to conventional "lignocellulosic residues" since this fruit-derived biomass contains high amounts of pectin and an extractive fraction rich in sugars, essential oils, and polyphenols. The relative amounts of these fractions are highly influenced by the juice/pulp extraction process. Even though several studies have explored how to produce added value from this biomass, it is necessary to compare how different techniques and operating conditions influence the bioactive compounds that can be recovered and the remnant biomass after processing. This study compares essential oil extraction, solvent extraction, and acid hydrolysis for fermentable sugar and pectin production to elucidate a feasible sequence for a biorefinery from Orange Residues. From our results, it was proposed a technically feasible sequence that maximizes the yields of i) essential oils (0.70 ± 0.05 g/ 100 g DM) from steam distillation (4 h, 1500 W), ii) naringin (0.19 g/100 g DM), hesperidin (1.27 g/100 g DM), and glucose (3.9 g/100 g DM) from solid-liquid extraction (Ethanol 61.6 % (w/v), 45.8 °C, 155.5 min, and 5 % (w/v) biomass load), iii) pectin (25.24 g/100 g DM) from citric acid hydrolysis (pH 1.5, 90 °C, 82.1 min, and 5 % (w/v) biomass load), and iv) glucose (12.41 g/100 g DM) and xylose (10.13 g/100 g DM) from sulfuric acid hydrolysis (Sulfuric acid 0.68 % (w/v), 121 °C, 24.1 min, and 7.32 % (w/v) biomass load), in a biorefinery scheme.

## 1. Introduction

Food products derived from oranges, mainly beverages, have become a fundamental part of people's diets in most parts of the world. Its flavor has been reported to be the favorite choice by consumers of juices, nectars, and drinks [1]. Currently, the major producers of oranges are Brazil (~16.8 million tons), India (~10 million tons), China (~7.5 million tons), the United States (~4.9 million tons), Mexico (~4.8 million tons), and Spain (~3 million tons), which dedicated most of their production to the processing industry, responding to the increasing demand on natural and orange-flavored products throughout the year [1,2]. However, orange production and processing leaves behind almost 50 % of the fruit as residue (i.e., peels, seeds, and remnant pulp), usually used for animal feed, composting, or left behind in landfills [2]. There is a clear opportunity for farmers and juice production companies worldwide to recover the valuable substances (e.g., essential oils, phenolic

*Abbreviations:* ANOVA, Analysis of Variance; BM, Biomass Load; BBD, Box-Behnken experimental design; DAD, Diode Array Detector; DM, Dry Matter; FID, Flame Ionization Detector; GC, Gas Chromatography; HD, Hydrodistillation; HPLC, High-Performance Liquid Chromatography; HMF, Hydroxymethyl furfural; MS, Mass Spectrometry; MAE, Microwave Assisted Extraction; OR, Orange Residues; RID, Refractive Index Detector; TPC, Total Phenolic Content; SLE, Solid-Liquid Extraction; SXE, Soxhlet Extraction; SD, Steam Distillation; UHPLC, Ultra-High-Performance Liquid Chromatography.

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compounds, sugars, and polysaccharides) present in Orange Residues (OR) that are currently discarded causing environmental damage.

OR are fruit-derived biomass with a different composition compared to conventional "lignocellulosic" residues (like sugarcane bagasse) [3] due to the high presence of extractives and pectin [2,4]. They also differ from biomasses derived from seeds (like Brewer's Spent Grains or Spent Coffee Grounds) or ash-rich biomasses (like rice husk) by not having high contents of either lipids, protein, or ash [5,6]. On top of that, the way OR are processed leaves behind different amounts of remnant pulp, peels, and seeds, affecting the amount of the individual fractions present in the biomass. The composition of OR makes its valorization challenging since it directly affects the yields of the different substances that can be obtained [2].

Several studies have evaluated the valorization of orange peels and OR through direct methods to recover bioactive compounds (essential oils, polyphenols, and pectin) [7–9], produce substances either by fermentation (biogas, enzymes, and fungal biomass) [10–12] or thermochemical conversion (Syngas, and activated charcoal), or approach their integral valorization under the biorefinery concept [4,13–15]. However, comparisons among these studies are challenging due to variations in biomass sources and sample preparation procedures, that affect directly product yields. For instance, procedures like the manual removal of orange flavedo are sometimes used to enhance essential oil and pectin yields [15], making standardized comparisons more challenging. As a result, identifying processing conditions that maximize yields of bioactive compounds from OR suitable for industrial-scale applications remains mostly unexplored.

Moreover, most studies have prioritized the extraction of single products, focusing on individual biomass fractions and limiting their applicability in an integrated biorefinery approach. For example, while the cellulosic fraction (via hydrolysis) is often used for applications such as ensiling [16], alcohol and organic acid production [17–19], or anaerobic digestion [9,20,21], other valuable fractions are frequently not considered and may undergo through undesired changes during processing (e.g., thermal degradation or oxidation). Additionally, studies focused on pectin recovery or extraction of essential oils and phenolic compounds [8,22–26] generally overlook the potential use of the remaining biomass and the potential impacts of the extraction techniques applied to it, which could hinder further processing steps due to the presence of fermentation inhibitors or traces of harmful solvents.

This study addresses these gaps by evaluating multiple methods and operating conditions for producing essential oils, polyphenols, sugars, and pectin from a single source of OR. The main purpose was to identify those conditions that could yield high-value products while facilitating integration within a biorefinery under an industrial-oriented approach. First, a thorough compositional analysis was performed to accurately identify the substances present in the biomass. Then, Steam Distillation (SD) and Hydrodistillation (HD) were compared to determine essential oil yields and the type of terpenes recovered. Solvent extraction methods (including Soxhlet Extraction (SXE), Microwave Assisted Extraction (MAE), and Solid-Liquid Extraction (SLE) with water, ethanol, ethyl acetate, and acetone) were also evaluated to identify bioactive compound yields. Furthermore, the recovery of fermentable sugars and pectin was also evaluated using acid hydrolysis with sulfuric acid at different operating conditions to identify which ones were favorable to enhance the breakdown of cellulose and hemicellulose into C5 and C6 sugars, as well as for improving the hydrolysis of pectin into galacturonic acid, a necessary step for its subsequent precipitation, purification, and recovery. In each case, the goal was to assess how each process and its conditions impact both product yields and the characteristics of the resulting product streams and residual fractions. Based on our findings, we discuss how these valorization methods could be integrated to propose a feasible OR-based biorefinery sequence that extracts effectively value from all biomass fractions.

## 2. Materials and methods

### 2.1. Sample collection and preparation

Orange residues (OR) (i.e., peels, seeds, and remnant pulp) were collected at an orange juice shop at Universidad de Los Andes. For the extractions, residues were milled (<5mm) (Food Processor WRP-FP-408, Würden) and dried at 45 °C in a convection oven (FD 115, Binder) until a moisture content <10 % was achieved (Fig. S1). For compositional analysis, samples were further milled (<1mm) in a universal cutting mill (Pulverisette 19, Fritsch), following the NREL procedures [27].

### 2.2. Compositional analysis

For the proximate analysis, moisture content, total solids (ASTM E17656), volatile matter (ASTM 1755), and ash (ASTM E872) were determined. The ultimate analysis (i.e., contents of C, N, H, S, and O by difference) was determined according to the ASTM D5373-16 method.

Chemical composition was determined for the previously prepared samples according to the NREL protocols. Ash content was determined using a muffle ramp up to 575 °C (Type F62700 Furnace, Barnstead International) [28]. Protein content, using the NREL protocol based on the Kjeldahl method [29] with a Nitrogen to protein conversion factor of 6.25 [30]. For extractives, a modification to the NREL procedure [31] was made by including an additional step to the water and ethanol Soxhlet extractions by extracting with hexane at the end to recover non-polar extractives. All the extracts were measured gravimetrically after solvent removal using a rotary evaporator at 40 °C (R-114 Rotary Vap System, Buchi) connected to a vacuum source. Extractive-free samples were used to determine structural carbohydrates through acid hydrolysis and HPLC quantification of sugars [32]. Lignin was determined gravimetrically from the solid fraction recovered using a muffle furnace (Type F62700 Furnace, Barnstead International) [32]. Besides that, starch was determined following the NREL protocol to determine non-cellulosic glucan [33].

Pectin was measured after a chemical and enzymatic treatment of the sample. First, the extractives-free sample was mixed into a 0.5 % EDTA solution. Its pH was modified first to 11.5 with 1 M sodium hydroxide and then to a pH between 5 and 5.5 with 0.25 M acetic acid. The pH modification in this step allows first pectin de-esterification under alkaline conditions, then the subsequent shift to acidic conditions (pH 5–5.5) weakens the glycosidic bonds, making it more susceptible to hydrolysis by disrupting the cell wall [34]. After this treatment, pectinase (*Aspergillus niger* 1.0 U/mg) was added and stirred for 1 h [7]. The mixture was filtered, and galacturonic acid was measured in the liquid phase with a colorimetric reaction using carbazole (0.15 % w/v) at 90 °C in the presence of 98 % sulfuric acid. After 25 min of reaction, measurements were taken in a UV–VIS spectrophotometer (T80+, PG instruments) at 530 nm [7]. Besides that, galacturonic acid content was also determined by HPLC [35], as described in 2.7.2.

### 2.3. Extraction of essential oils

#### 2.3.1. Steam distillation (SD)

A steam distillatory system (Laboratory Scale Essential Oil Extractor, Figmay) was loaded with 200 g of OR. The extraction was performed for 4 h by heating water (kept at a constant level of ~2L) at 1500 W to produce the steam that flowed through the sample [4]. The equipment (Fig. S2) included a condenser and collector system that separated essential oils from the hydrolate by decantation. The essential oil was recovered, and its yield was determined gravimetrically. Samples were then diluted in 1.0 mL of n-hexane and stored at −20 °C for further analysis.

#### 2.3.2. Hydrodistillation

A hydrodistillation setup (Fig. S3) was loaded with 50 g of OR, mixed with water, and kept at a constant level of ~0.5L. The mixture was heated for 4 h at boiling point in continuous stirring at 300 rpm with the help of a heating mantle (Hei-Connect, Heidolph) [8]. The essential oil was recovered, and its yield was determined gravimetrically. Samples were then diluted in 1.0 mL of n-hexane and stored at −20 °C for further analysis.

### 2.4. Solvent extraction of free sugars, polyphenols, and other bioactive compounds

Solvent extractions were evaluated to compare the yields of extracts and determine the bioactive compounds that can be recovered using water, ethanol (99 %), ethyl acetate, and acetone. Soxhlet Extraction (SXE) was used as a baseline to compare the results from Microwave Assisted Extraction (MAE) and Solid-Liquid Extraction (SLE). The extraction yields for each assay described below were determined gravimetrically after removal of the solvent in a vacuum rotary evaporator system (R-114 Rotary Vap System, Buchi). All experiments were run in triplicate.

#### 2.4.1. Soxhlet extraction (SXE)

OR (10 g) were loaded into a thimble and placed into a Soxhlet extraction setup (Fig. S4), where 200 mL of each solvent was used for the extractions. Hexane was included only for SXE. The process was performed for 12 h with a heating mantle adjusted to ensure at least six siphon cycles per hour (72 extraction cycles) [36]. Each sample was filtered, and an aliquot of 2 mL of the liquid phase was recovered and stored at −20 °C for further analysis.

#### 2.4.2. Microwave-assisted extraction (MAE)

Extractions were carried out in a focused microwave reactor (300 W CEM Discover® SP, $\nu = 2.45$ GHz) (Fig. S5) by placing in a sealed reaction vessel (30.0 mL) 1 g of OR, 10 mL of each solvent, and a Teflon-coated stir bar [37]. Extractions were done for 5 min at 100 W using temperatures 5 °C below the boiling point of each solvent. Each sample was filtered, and an aliquot of 2 mL of the liquid phase was recovered and stored at −20 °C for further analysis.

#### 2.4.3. Solid-liquid extraction (SLE)

A Box-Behnken experimental design (BBD) design (Table S1) was performed to study the effect of varying temperature (20 °C – 70 °C), time (30–120 min), and biomass load (5 – 15 g of orange residues / 100 mL of solvent) in the case of water, ethyl acetate, and acetone [38,39]. For ethanol, the effect of its concentration (50 – 96 % v/v) was also included [38]. OR (50 g) were added to a Schott flask (100 mL), mixed with 50 mL of each solvent, and placed in a Shaker (Orbital Shaker HD-3000, Dimaq) kept at 100 rpm using the conditions established in the experimental design. Each sample was filtered, and an aliquot of 2 mL of the liquid phase was recovered and stored for further analysis.

### 2.5. Extraction of fermentable sugars through acid hydrolysis

A Box-Behnken experimental design (BBD) (Table S2) was performed to study the effect of reaction time (10–30 min), acid concentration, and biomass load (5 – 15 g of OR / 100 mL of solvent) in the yield of sugars obtained from acid hydrolysis at 121 °C, using again the starting OR (after sample preparation). The range of concentrations evaluated was between 0.5–2 % (w/v) of sulfuric acid (Table S3) and 0.5–25 % (w/v) of citric acid (Table S4). These experiments were performed using previously reported conditions to obtain high yields of fermentable sugars [12,40]. After hydrolysis, the mixture was filtered using a cheesecloth, and an aliquot of 2 mL from the liquid phase was recovered and stored at −20 °C for further analysis. The extraction yield was determined gravimetrically after evaporating water from the extract using a rotary evaporator (R-114 Rotary Vap System, Buchi) connected to a vacuum source.

### 2.6. Extraction of pectin

#### 2.6.1. Recovery of pectin after hydrodistillation (HD)

In HD, hot water is in direct contact with OR throughout the process, which results in the release of pectin. The remaining biomass obtained after recovering essential oils from HD was filtered, and the liquid residue was mixed with 96 % ethanol in a ratio of 2:1 (v/v) for 18 h at 6 °C to precipitate pectin [41]. After that, pectin was recovered using a spatula and filtered with a cheesecloth. An additional washing with 96 % ethanol was included to remove the remaining impurities. Yields of pectin were determined gravimetrically after vacuum-drying the samples at 40 °C in a convection oven (FD 115, Binder) for 24 h [23].

#### 2.6.2. Recovery of pectin from acid hydrolysis

A Box-Behnken experimental design (BBD) (Table S5) was performed to study the effect of varying reaction time (10–30 min), pH (1.5–2.5), and biomass load (5 – 15 g of orange residues / 100 mL of solvent) in the yield of pectin obtained from acid hydrolysis using citric acid and sulfuric acid. These experiments were performed in a hot bath according to the experimental design, which was based in reported conditions to obtain high yields of galacturonic acid, which can be further precipitated into pectin [13,40]. The recovered hydrolysates were treated as described above for pectin precipitation.

### 2.7. Analytical methods

#### 2.7.1. Total phenolic content (TPC)

TPC was determined using the microscale version of the Folin-Ciocalteu method [42]. A Gallic acid calibration curve between 25 and 500 mg/L was used. Samples of 20 uL were mixed with 1.58 mL of distilled water and 100 uL of the Folin-Ciocalteús (Sigma-Aldrich) reactive (2 N). After mixing the samples for 8 min, 300 uL of sodium carbonate at 20 % (w/v) (PanReac-AppliChem ITW) was added. Samples were incubated at room temperature for 2 h in the dark. Absorbance was measured using a UV–Vis spectrophotometer (T80+, PG instruments) at 765 nm. Distilled water was used as the blank.

#### 2.7.2. HPLC quantification of sugars, organic acids, and furans

Samples were measured using High-Performance Liquid Chromatographer (HPLC) (Agilent Series 1200 HPLC system, Agilent Technologies, Santa Clara, CA, USA) equipped with a Refractive Index Detector (RID) used for sugar detection and a Diode Array Detector (DAD) used for the detection of organic acids (210 nm) and furans (276 nm). For the compositional analysis, the content of sugars (Glucose, xylose, arabinose, mannose, and galactose) was measured using 20 μL of injection volume on a Biorad Aminex HPX-87P column (300 × 7.8 mm, 9 μm particle size, and 8 % cross-linkage) (Bio-Rad, USA) operated at 85 °C, with HPLC grade water as the mobile phase, a flow rate of 0.6 mL/min during a total running time of 25 min [32]. For the quantification of sugars (Glucose, xylose, arabinose) and organic acids (acetic acid, formic acid, and galacturonic acid), samples obtained from solvent extractions and acid hydrolysis were measured using a Biorrad Aminex HPX-87 H column (1300 × 7.8 mm, 9 μm particle size, and 8 % cross-linkage) (Bio-Rad, USA) operated at 60 °C, with 0.005 M Sulfuric acid as the mobile phase, a flow rate of 0.6 mL/min, an injection volume of 20 μL, and a run time of 30 min [35]. The content of furfural and hydroxymethylfurfural (HMF) in the hydrolysates was measured using a Zorbax Eclipse Plus C18 column (4.6 mm × 150 mm, 5 μm particle size) (Agilent Technologies, Santa Clara, CA, USA) operated at 25 °C, with a mixture of 88 % solution A (water/acetic acid 1 %) and the balance with solution B (acetonitrile/acetic acid 1 %) in isocratic flux as the mobile phase, a flow rate of 0.8 mL/min, an injection volume of 20 μL, and a run time of 15 min [3]. Calibration curves were constructed at

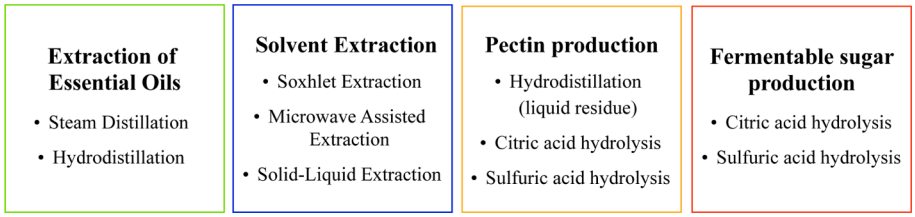


**Fig. 1.** Techniques tested for the recovery of bioactive compounds from Orange Residues.

0.05–5 mg/mL for sugars, 0.025–5 mg/mL for organic acids, and 0.0025 to 0.2 mg/L for furfural and HMF (Fig. S6). The assays were performed in triplicate.

### 2.7.3. Identification and quantitative analyses by GC–MS and GC-FID

Terpenes were initially identified in OR using a previously reported extraction procedure where 1 g of sample was added to 9 ml of n-Hexane and mixed for 24 h at 200 rpm in an orbital shaker [43]. The samples obtained from this extraction were stored at −20 °C for further analysis. Separation and identification were made using a Trace 1300 gas chromatograph (GC) coupled with a ISQ-LT single quadrupole mass spectrometer (MS) (Both equipment from Thermo Scientific, Waltham, MA, USA) equipped with a Zebron ZB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm) (Phenomenex, Torrance, CA, USA). Helium was used as carrier gas at 0,8 mL/min. Manual injection of 1 µl of the sample was done in split mode (1:10), maintaining the injector's temperature at 250 °C. The samples were diluted at 1:100 in n-hexane (Suitable for GC–MS analysis). The oven's initial temperature was 40 °C, increased to 325 °C at 10 °C/min, and kept at 325 °C for 10 min with a total run time of 39.0 min. An Electronic Ionization (EI) system was operated at 70 eV and the MS detector in full-scan acquisition mode at an *m/z* mass range from 40 to 450 Da with an initial scan time of 5.5 min. The temperatures of the ion source and transfer line were 250 °C and 290 °C, respectively [43].

Quantification was performed in a Trace 1300 GC using a flame ionization detector (FID) with an automatic injector serie AI 1310 (Thermo Scientific, Waltham, MA, USA) on a Zebron ZB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm) (Phenomenex, Torrance, CA, USA). Analytical conditions: 1 µL of injected volume, split ratio 1:10; helium as carrier gas at 1.00 mL/min; injector temperature and detector temperature were both set at 250 °C; air flow of 350 mL/min; nitrogen gas flow of 40 mL/min; and hydrogen flow of 35 mL/min. The initial temperature of the oven was 80 °C, then it was increased to 140 °C at a rate of 7 °C/min and kept at 140 °C for 4 min with a total run time of 12.56 min. For the quantification of limonene present in the samples, a calibration curve was constructed with (R)-(+)-limonene (~ 90 % sum of enantiomers, GC Sigma Aldrich) containing 5-Nonanol (GC Sigma Aldrich) as internal standard. Standards were diluted in n-Hexane to obtain calibration curves between 50 and 500 ppm (Fig. S7). The individual volatile compound concentration was expressed as a percent peak relative to the total peak area from the GC-FID analysis of each sample.

### 2.7.4. UHPLC-MS/MS identification and quantification

Extracts were analyzed by UHPLC-MS using an Ultra-High-Performance Liquid Chromatographer Dionex UltiMate 3000 equipped with a binary pump, online degasser, autosampler, a thermostated column compartment, and a diode-array detector (DAD). The DAD signals were recorded at 280, 320, 370, and 520 nm, although spectra from 220 to 800 nm were also obtained (peak width 0.1 min (2 s), slit 4 nm). The UHPLC system was coupled with an LCQ Fleet™ Ion Trap Mass Spectrometer (MS) through an ESI source operated in positive and negative modes (Thermo Scientific, San Jose, CA, USA). The RP-UHPLC separation was performed at 50 °C with an injection volume of 10 µL (samples were maintained at 5 °C) on a C18 column Kinetex C18 (1.7 µm, 100 Å, 2.1 × 50 mm, Phenomenex) using gradient separation with solvent A 0.1 % formic acid in water and solvent B 0.1 % formic acid in Methanol: Acetonitrile (1:1; v/v). The gradient was B 2 %–40 % in 18 min → B 40 %–100 % in 3 min → B 100 % for 3 min → B 100 %–2% in 2 min → B 2 % for 4 min at 0.4 mL/min (total running time: 30 min/sample) [44]. Optimized parameters after tuning for the ESI source with a flow injection of Hesperidin standard (0.1 % formic acid in /ACN: MeOH 1:1) were as follows in negative mode: sheath gas flow rate: 10 (arbitrary units); aux gas flow rate: 5 (arbitrary units); spray voltage: 4.50 kV; capillary temp: 360 ∘C; capillary voltage: −45 V; and tube lens: −125 V. Hesperidin standard ≥80 % (Sigma-Aldrich) was used for method development, qualitative evaluation, and quantification. Mass spectra were acquired as total ion current measurements with full ion scanning (*m/z* 50–1400) in Ion Tree analysis (MS3). In positive mode, the ESI source was operated with the following parameters: sheath gas flow rate: 10 (arbitrary units); aux gas flow rate: 5 (arbitrary units); spray voltage: 4.50 kV; capillary temp: 360 ∘C; capillary voltage: 39 V; and tube lens: 75 V. Mass spectra were acquired as total ion current measurements with full ion scanning (*m/z* 50–1400). For tandem MS analysis (30 % collision energy), the corresponding fragment ions were obtained with an isolation width of 2 *m/z*. Raw metabolite data were acquired and processed using the Xcalibur 4.5 software (Thermo Scientific, San Jose, CA, USA). For quantification, standards of naringenin and hesperidin were diluted in absolute ethanol to obtain calibration curves in a range between 50 and 500 ppm (Fig. S7). The contents of naringenin and hesperidin were not measured in acetone extracts due to solubility problems of the sample in the mobile phase and ionic suppression in ESI. Each calibration curve was recorded in triplicate.

**Table 1**
Characterization of Orange Residues (OR).

| **Proximate analysis** | | **Chemical composition** | |
|---|---|---|---|
| Substance | % Dry weight | Substance | % Dry weight |
| Ash (950 °C) | 3.33 ± 0.04 | Ash (575 °C) | 3.33 ± 0.04 |
| Volatile Matter | 78.61 ± 0.06 | Protein | 4.31 ± 0.05 |
| Fixed Carbon | 18.06 ± 0.15 | Extractives (Water) | 51.00 ± 0.55 |
| Calorific value (kJ/kg) | 16,675 | Extractives (Ethanol) | 4.64 ± 0.15 |
| **Ultimate analysis*** | | Extractives (Hexane) | 0.13 ± 0.001 |
| Substance | % Dry weight | Total extractives | 55.78 ± 0.71 |
| Carbon | 40.62 ± 0.05 | Lignin | 3.94 ± 0.22 |
| Hydrogen | 5.10 ± 0.05 | Hemicellulose | 5.73 ± 0.53 |
| Nitrogen | 0.86 ± 0.05 | Cellulose (Glucan) | 7.18 ± 0.52 |
| Sulfur | 0.14 ± 0.05 | Starch (Non-cellulosic glucan) | 1.48 ± 0.01 |
| Oxygen | 53.28 ± 0.05 | Pectin | 22.36 ± 0.84 |

* Ash-free basis.

### 2.8. Statistical analysis

Data analysis was performed in Minitab 19, and its visualization was done in Statistica 12. A One-way Analysis of Variance (ANOVA) was used to compare the yields obtained using different techniques evaluated for the recovery of bioactive compounds, as shown in Fig. 1. In the case of solvent extractions, a Two-Way Analysis of Variance (ANOVA) was performed by including an additional factor considering the type of solvent used. The significance level of the differences between means was determined using a Tukey test ($p < 0.05$). The assumptions of equal variances (Bartlett's Test) and normal distribution (Anderson-Darling test) were validated. Besides that, the software Statistica 12 was also used to evaluate the BBD constructed to evaluate the SLE of bioactive compounds, and the hydrolysis of OR to produce fermentable sugars and pectin, as shown in Tables S1–S5 (Supplementary information).

## 3. Results and discussion

### 3.1. Compositional analysis of orange residues (OR)

The results for the proximate and ultimate composition of OR (Table 1) reveal that this biomass has a high content of volatile matter, a higher proportion of Oxygen in contrast to its Carbon content, and a low ash content. Because of that, OR could be ideal for producing energy through gasification by obtaining syngas or anaerobic digestion by obtaining methane-rich biogas [9]. The low contents of Nitrogen and Sulfur of OR would result in lower emissions than other biomasses of environmentally harmful gases like $NO_x$ and $H_sS$. Similar values for the PA and UA have been reported in the literature for orange peels and OR [45].

Moreover, the chemical composition of OR is characterized by a high proportion of extractives and pectin. The amount of extractives found in this work (55.78 ± 0.71 % d.w.) for OR is similar to the one reported by [15] but higher than those reported by other authors (~20–40 %) [10,46]. This could be explained by differences in the juice extraction process, which could leave behind different amounts of remnant pulp. It was found that the extracts obtained from OR are predominantly polar. The polar fraction contains sugars, organic acids, and polyphenols, while the remnant non-polar fraction would consist of lipids and essential oils [9,10]. The substances recovered in OR extracts could be easily fermented or used to formulate multiple products [9,10]. The second most abundant fraction found in OR was pectin (22.36 ± 0.84 % d..w.), which had similar values to those reported in other reports (~18–22 %) [10,11]. Pectin has been extensively used in food products, and citrus residues have been conventionally used as a source of this valuable bioactive compound [47]. Its recovery is fundamental for a complete valorization of OR. Furthermore, since OR are fruit-derived biomass, their low lignin content facilitates access to the remnant cellulose, hemicellulose, and starch that can be directly hydrolyzed without requiring additional chemical alkaline pretreatments to remove lignin. Since structural carbohydrates are the third most abundant fraction in OR, producing C5 and C6 obtained from their hydrolysis could be attractive since these sugars can be used for fermentation to produce substances like ethanol or xylitol [3]. The values observed for structural carbohydrates, lignin, starch, protein, and ash are the same magnitude as those reported by other authors [4,10,15].

### 3.2. Extraction of essential oils

GC–MS analysis confirmed the presence of D-Limonene (97.13 %) and α-Myrcene (2.87 %) in the samples (Fig. S8). The yields of essential oils obtained with HD (1.04 ± 0.05 g/ 100 g DM) were higher than those obtained from SD (0.70 ± 0.05 g/ 100 g DM). These values are in the same magnitude as the yields (0.7–0.85 % for SD and ~1 % HD) reported by other authors using those processes [8,13,15]. The essential oils recovered from SD and HD contained only D-limonene, with the peak of α-Myrcene not being detected in those samples. Besides that, it is important to mention that during SD, the sample interacts only with steam, leaving the remaining biomass intact and with a moisture content of <10 %. On the other hand, during HD, the sample is in direct contact with water at boiling temperature, which could cause the release of soluble substances (e.g., sugars and galacturonic acid) from the sample and could cause the oxidation of other bioactive compounds (e.g., phenolic compounds) that remain in the mixture. For that reason, even though the yields from HD were slightly higher than those of SD, if the residual biomass were to be used for other valorization processes, it would be better to use SD since the integrity of the sample would be better preserved. Removing D-Limonene from OR is fundamental if the remnant biomass is used in fermentation processes (e.g., anaerobic digestion, ethanol production). D-Limonene has been reported to inhibit some bacteria and fungi, affecting the growth and the yields of desired products [9,48].

**Table 2**
Yields of extraction and individual compound yields for SXE and MAE.

| **SXE** | | | | | |
|---|---|---|---|---|---|
| Yield (g/100 g DM) | Ethanol | Water | Acetone | Ethyl acetate | Hexane |
| Overall | 44.07 ± 0.78 | 30.45 ± 0.51 | 9.75 ± 0.13 | 5.09 ± 0.5 | 1.98 ± 0.05 |
| TPC | 1.04 ± 0.02 | 0.55 ± 0.01 | 0.27 ± 0.01 | 0.40 ± 0.01 | 0.06 ± 0.003 |
| Glucose | n.d. | 13.4 ± 0.74 | n.q. | n.q. | n.q. |
| Fructose | n.d. | 11.4 ± 0.27 | n.q. | n.q. | n.q. |
| Acetic acid | n.d. | n.d. | n.q. | n.q. | n.q. |
| Furfural | n.d. | n.d. | n.q. | n.q. | n.q. |
| HMF | n.d. | n.d. | n.q. | n.q. | n.q. |
| Naringin | 0.22 ± 0.03 | 0.12 ± 0.01 | n.q. | 0.037 ± 0.004 | n.q. |
| Hesperidin | 4.58 ± 0.10 | 1.35 ± 0.016 | n.q. | 0.46 ± 0.04 | n.q. |
| D-Limonene | n.d. | n.d. | n.d. | n.d. | 1.51 ± 0.02 |

| **MAE** | | | | |
|---|---|---|---|---|
| Yield (g/100 g DM) | Ethanol | Water | Acetone | Ethyl acetate |
| Overall | 18.32 ± 0.68 | 27.30 ± 1.59 | 1.04 ± 0.30 | 0.97 ± 0.21 |
| TPC | 0.39 ± 0.07 | 0.39 ± 0.02 | 0.06 ± 0.003 | 0.13 ± 0.02 |
| Glucose | 2.32 ± 0.22 | 4.10 ± 0.25 | n.q. | n.q. |
| Fructose | 1.84 ± 0.14 | 3.11 ± 0.21 | n.q. | n.q. |
| Acetic acid | n.d. | n.d. | n.q. | n.q. |
| Formic acid | n.d. | n.d. | n.q. | n.q. |
| Furfural | <1 × 10–4 g/100 g | <1 × 10–4 g/100 g | n.q. | n.q. |
| HMF | 7 × 10–3 g/ 100 g | 7 × 10–3 g/ 100 g | n.q. | n.q. |
| Naringin | ~0.0005 g/ 100 g | ~0.0005 g/ 100 g | n.q. | ~0.004 g/100 g |
| Hesperidin | 1.90 ± 0.006 | 0.39 ± 0.04 | n.q. | 0.076 ± 0.002 |
| D-Limonene | 0.296 ± 0.001 | n.d. | 0.047 ± 0.001 | 0.296 ± 0.001 |

SXE: Soxhlet Extraction, MAE: Microwave Assisted Extraction, n.d.: Not detected, n.q.: Not quantified, HMF: Hydroxymethyl furfural, TPC: Total Phenolic Content.

### 3.3. Solvent extractions

#### 3.3.1. Soxhlet extraction (SXE)

The results obtained for SXE are summarized in Table 2. Ethanol resulted in the highest yields of extraction (44.07 ± 0.78 g/100 g DM) for SXE, followed by water (30.45 ± 0.51 g/100 g DM), acetone (9.75 ± 0.13 g/100 g DM), ethyl acetate (5.09 ± 0.51 g/100 g DM), and hexane (1.98 ± 0.05 g/100 g DM). The values obtained with SXE could be

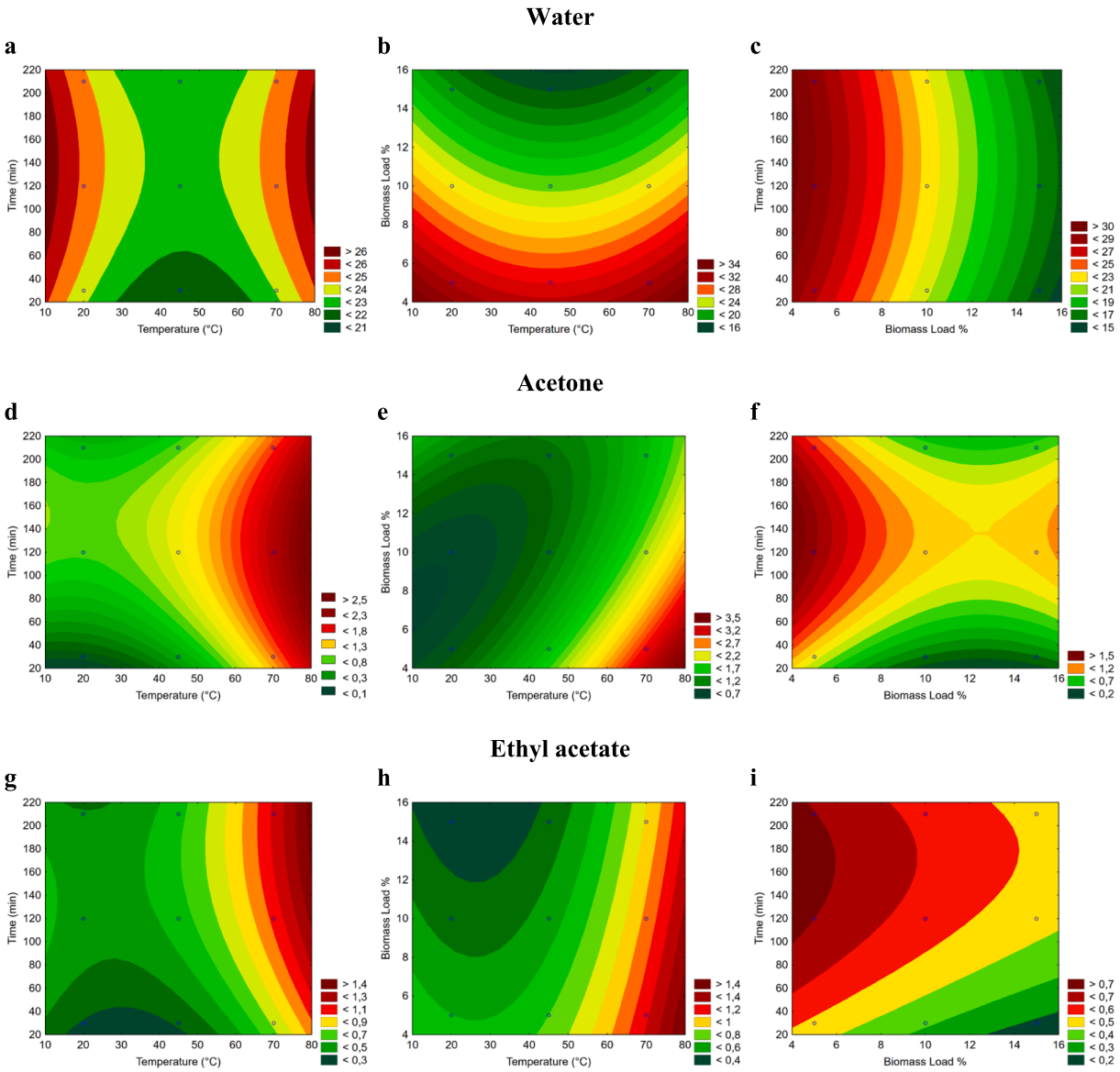


**Fig. 2.** Contour plots for the yields of extraction (expressed in g Extract per 100 g of Dry Matter (DM)) obtained from the Solid Liquid Extraction (SLE) of Orange Residues (OR) using water (a, b, c), acetone (d, e, f), and ethyl acetate (g, h, i).

considered the highest yields that can be obtained from OR using those solvents. The TPC of the samples was the highest for ethanol (1.04 ± 0.02 g/100 g DM), followed by water (0.55 ± 0.01 g/100 g DM), ethyl acetate (0.40 ± 0.01 g/100 g DM), acetone (0.27 ± 0.01 g/100 g DM), and hexane (0.06 ± 0.003 g/100 g DM). The results obtained for the yields of extraction and the yields of TPC are in line with the contents of extractives measured in the compositional analysis, indicating an abundance of polar compounds in the samples. The recovery of these polar compounds had more affinity with ethanol than water. Moreover, it was observed that ethyl acetate and acetone can selectively recover the phenolic compounds present in OR.

HPLC quantification of SXE extracts revealed that the water extract resulted in 13.4 ± 0.74 g/100 g DM of glucose and 11.4 ± 0.27 g/100 g DM of fructose. Glucose and fructose were not recovered in the SXE with absolute ethanol in which they have poor solubility [49], even more at the low temperature to which the solvent is condensed during the process. The presence of acetic acid, formic acid, furfural, and hydroxymethyl furfural (HMF) was not detected, revealing that during SXE, the sugars and the structural carbohydrates present in OR were not degraded. The phenolic compounds identified in solvent extractions are listed in Table S9, from which naringin and hesperidin were the most abundant. Naringin yields were 0.22 ± 0.03 g/100 g DM for ethanol, 0.12 ± 0.01 g/100 g DM for water, and 0.037 ± 0.004 g/100 g DM for ethyl acetate. For hesperidin, the yields were 4.58 ± 0.10 g/100 g DM for ethanol, 1.35 ± 0.016 g/100 g DM for water, and 0.46 ± 0.04 g/100 g DM for ethyl acetate. In the literature, it has been reported how both naringin and hesperidin are more soluble in ethanol and have poor solubilities in water [50,51], which was also confirmed from the results obtained in this work. Even though ethyl acetate is highly selective for the recovery of these two phenolic compounds, the yields are considerably lower compared to those of ethanol and water. Furthermore, GC-FID analysis of the samples revealed that D-Limonene was not present in the solvents tested for SXE, except for hexane (1.51 ± 0.02 g/100 g) which was able to retrieve this essential oil due to its non-polar nature. The total amount of D-Limonene recovered in OR was at least 50 % more than that recovered with SD and HD, revealing the maximum amount that can be recovered from this substance using those methods.

### 3.3.2. *Microwave assisted extraction (MAE)*

The results obtained for MAE are summarized in Table 2. The highest

**Table 3**
Maximum and minimum yields of bioactive compounds measured in SLE extractions.

**SLE with water**

| | Maximum Yield | | | | Minimum Yield | | | |
|---|---|---|---|---|---|---|---|---|
| Substance | T (°C) | t (min) | BM% | Yield(g/100 g) | T (°C) | t (min) | BM% | Yield(g/100 g) |
| Glucose | **20** | 30 | **5** | 4.21 | **20** | 30 | **15** | 2.34 |
| Fructose | **20** | **90** | **5** | 14.26 | **50** | **30** | **15** | <1.0 |
| TPC | 70 | 30 | 5 | 1.31 | 28.6 | 30 | 15 | <0.2 |

**SLE with acetone**

| | Maximum Yield | | | | Minimum Yield | | | |
|---|---|---|---|---|---|---|---|---|
| Substance | T (°C) | t (min) | BM% | Yield(g/100 g) | T (°C) | t (min) | BM% | Yield(g/100 g) |
| TPC | 70 | 151.8 | **5** | 0.21 | 20 | 30 | **11.57** | 0.0075 |

**SLE with ethyl acetate**

| | Maximum Yield | | | | Minimum Yield | | | |
|---|---|---|---|---|---|---|---|---|
| Substance | T (°C) | t (min) | BM% | Yield(g/100 g) | T (°C) | t (min) | BM% | Yield(g/100 g) |
| TPC | 70 | 210 | **5** | 0.35 | 20 | 30 | **11.46** | 0 |

**SLE with ethanol**

| | Maximum Yield | | | | | Minimum Yield | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Substance | T (°C) | t (min) | BM% | % EtOH (v/v) | Yield(g/100 g) | T (°C) | t (min) | BM% | % EtOH (v/v) | Yield(g/100 g) |
| Glucose | **20** | 210 | **5** | **50** | 9.13 | **70** | 30 | **15** | **50** | 3.28 |
| Fructose | **43.2** | **210** | **5** | **96** | 4.38 | **20** | **30** | **15** | **96** | <2.0 |
| TPC | 20 | 133 | **5** | **50** | 0.9 | 20 | 30 | **15** | **96** | <0.2 |

BM: Biomass Load, TPC: Total Phenolic Content, EtOH: Ethanol.
*Values in bold font indicate which factors were significative for the measured compound.

yields of extraction were observed using water (27.30 ± 1.59 g/100 g DM), followed by ethanol (18.32 ± 0.68 g/100 g DM), acetone (1.04 ± 0.30 g/100 g DM), and ethyl acetate (0.97 ± 0.21 g/100 g DM). The yield of TPC observed in the extracts was the highest for ethanol (0.48 ± 0.07 g/100 g DM), followed by water (0.39 ± 0.02 g/100 g DM), ethyl acetate (0.13 ± 0.02 g/100 g DM), and acetone (0.06 ± 0.003 g/100 g DM). The yields of extraction found for MAE were lower than those achieved with SXE, with reductions of 10.3 %, 58.4 %, 80.9 %, and 89.3 % in the amount of extract recovered using water, ethanol, ethyl acetate, and acetone, respectively. In the same way, the yields of TPC decreased by 28.8 %, 54.0 %, 77.2 %, and 66.8 % using water, water, ethanol, ethyl acetate, and acetone, respectively. Nonetheless, the recovery of polar substances using MAE was also favored for the water and ethanol extracts, similarly to SXE.

HPLC quantification of MAE extracts revealed that water extracts resulted in 4.10 ± 0.25 g/100 g DM of glucose and 3.11 ± 0.21 g/100 g DM of fructose, and ethanol extracts resulted in 2.32 ± 0.22 g/100 g DM of glucose and 1.84 ± 0.14 g/100 g DM of fructose. In this case, MAE could have helped to release sugars into the solvent. Acetic acid and formic acid were not detected. However, the presence of furfural ($<1 \times 10^{-4}$ g/100 g DM) and hydroxymethyl furfural (HMF) ($<7 \times 10^{-3}$ g/100 g DM) was observed water and ethanol extracts. The naringin yields were ~0.004 g/100 g DM for ethyl acetate, and ~0.0005 g/100 g DM for water and ethanol. For hesperidin, the yields were 1.90 ± 0.006 g/100 g DM for ethanol, 0.39 ± 0.04 g/100 g DM for water, and 0.076 ± 0.002 g/100 g DM for ethyl acetate. The use of MAE resulted in a higher recovery of hesperidin in comparison to naringin, but their yields are considerably lower than those obtained in SXE. This indicates that both polyphenols could have been degraded during MAE. It was again seen how naringin and hesperidin are more soluble in ethanol than in the other solvents. D-Limonene was detected in the extracts with yields of 0.296 ± 0.001 g/100 g DM for ethanol, 0.047 ± 0.001 g/100 g DM for acetone, 0.296 ± 0.001 g/100 g DM for ethyl acetate, and it was not detected for water. The yield of D-Limonene recovered with MAE was considerably lower than that obtained with SD, HD, and SXE with hexane. The conditions used for MAE caused the release of D-Limonene and some phenolic compounds, in a mixture that would require further separation if those substances were to be valorized independently.

Further studies using MAE could be performed in future works to evaluate conditions where the yields using this method could be increased even more. However, the use of MAE in an industrial application would be limited even though extraction times are lower than other methods. MAE requires specialized equipment (operating at high temperatures and pressures) with limitations in its dimensionality that restrict the amount of OR that can be processed per single batch. Moreover, MAE has been reported as a method for pectin production since it modifies the biomass structure causing cell breakdown and allowing the solvent to interact directly [52]. It was observed how phenolic compounds were degraded during MAE, resulting in the release of oxidized forms of these substances. As a result, the integrity of OR could have also been compromised from the process altering the remnant biomass and compromising the possibility of using it for other purposes.

### 3.3.3. Solid-liquid extraction (SLE)

*3.3.3.1. SLE with water.* The results for the yields obtained with SLE extractions using water can be seen in the contour plots of Fig. 2a–c. SLE water extractions were affected by biomass load and temperature, as shown in the Pareto charts of Fig. S9. It is possible to see in Fig. 2a how the yields increase either when the temperature is ~20 °C or when it is ~70 °C reaching yields of extraction higher than 30 g/100 g DM, with a saddle point around 50 °C. The high yields obtained at 70 °C could have been caused not only by the release of sugars but also due to the release of starch and certain phenolic compounds in water at higher temperatures [53]. It is also evident that low biomass loads (~5% (w/v)) considerably increase the yields of extraction high up to 30 g/100 g DM.

The yields of sugars and total phenolics for the BBD (Table S6) are shown in Figs. S10–S13, from which Table 3 was constructed to summarize the conditions that maximize or minimize the yields of these compounds. From Table 3, it is evident that yields of glucose (~4.2 g/100 g DM) and fructose (~14.3 g/100 g DM) increased when water

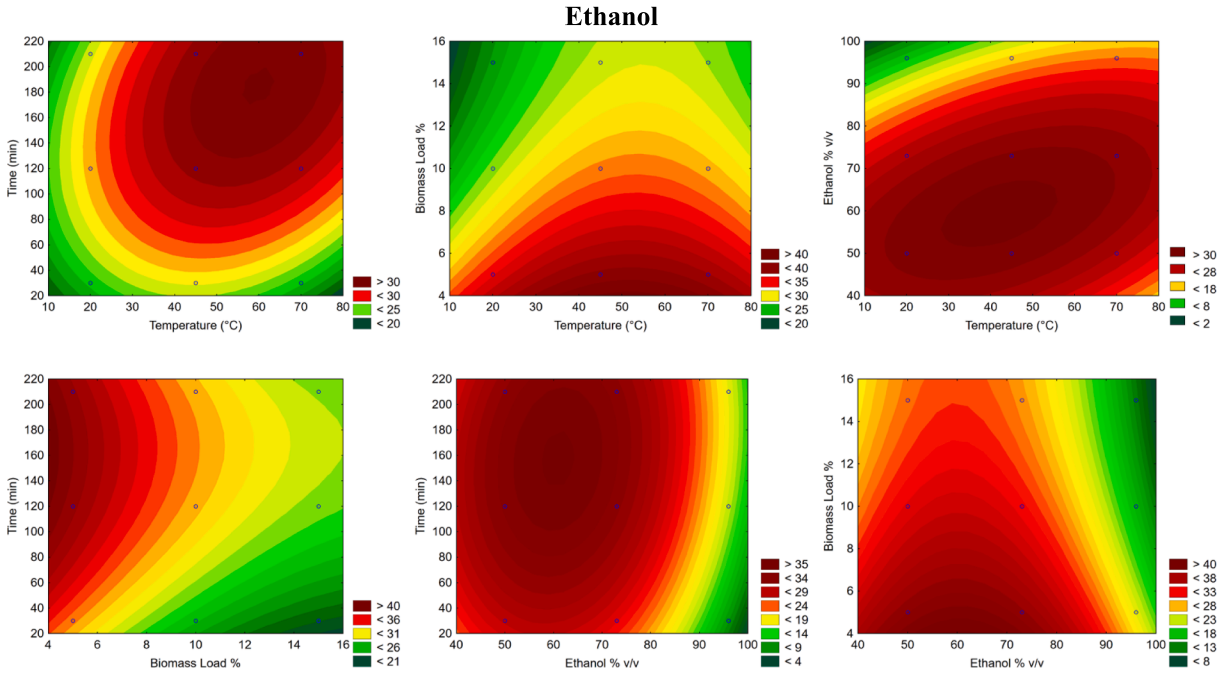


**Fig. 3.** Contour plots for the yields of extraction (expressed in g Extract per 100 g of Dry Matter (DM)) obtained from the Solid-Liquid Extraction (SLE) of Orange Residues (OR) using ethanol.

extractions were performed at 20 °C and a biomass load of 5 % w/v. Moreover, the yield of TPC (~1.3 g/100 g DM) is the highest when the extractions were performed at 70 °C and a biomass load of 5 % w/v, recovering around 2.4-fold the amount measured in SXE water and demonstrating that the conditions used enhanced recovery of phenolic compounds. All yields decrease with higher biomass loads, indicating that the solid matrix restricts the mass transfer of bioactive compounds to the solvent. Since OR contains pectin, this hydrophilic substance may be retaining water avoiding proper extraction at high biomass loads. The optimal conditions obtained for the SLE with water were found at 20 °C, 30 min, and 5 % (m/v) of OR, with a yield of extraction of 29.39 % g/100 g DM (~53 % of the total extractives and ~96.5 % of the SXE water extract), with 4.22 g/100 g DM of glucose, 14.0 g/100 g DM of fructose, and 1.18 g/100 g DM of TPC. Compared to SXE, the naringin content (~0.13 g/100 g DM) found at the optimal conditions was almost the same but the hesperidin content (~0.16 g/100 g DM) was ~90 % lower. These results show again the low solubility of both polyphenols in water, affecting the most the hesperidin yield. Acetic acid, formic acid, furfural, HMF, and D-Limonene were not detected.

*3.3.3.2. SLE with acetone and ethyl acetate.* The extraction yields obtained using acetone (Table S7) can be seen in Fig. 2d–f. SLE acetone extractions were affected by biomass load and time, as shown in the Pareto charts of Fig. S9. The extractions were favored when low biomass loads were used and times near the central point (120 min). A maximum yield of 2.96 g/100 g DM was achieved with 134 min, 5 % (w/v) of biomass load, and 70 °C. The TPC was also maximized using these conditions with 0.21 g/100 g DM (Table 2). In this case, acetone could only recover 10 % of the extract yield measured in SXE extractions, but almost three times more of the extract and phenolic content recovered with MAE.

Ethyl acetate extraction yields in Fig. 2g and h were affected by biomass load, temperature, and time, as shown in the Pareto charts of Fig. S9. The extractions were also favored when low biomass loads, high temperatures, and longer times were used. A maximum yield of 1.26 g/100 g DM was obtained with 195 min, 5 % of biomass load, and 70 °C, achieving a yield of TPC of 0.33 g/100 g DM. A yield ~0.002 g/100 g DM of Naringin and ~0.002 g/100 g DM of hesperidin was only recovered using this solvent, which is considerably low. Ethyl acetate recovered the less extract among all the evaluated solvents tested for SLE. This solvent recovered 25 % of the amount measured for SXE and at least twice the amount recovered with MAE.

Regarding D-Limonene, only 0.048 g/100 g DM was recovered using ethyl acetate and 0.004 g/100 g DM using acetone. These values are considerably lower than those obtained with SXE using hexane, HD, and SD, showing that using these solvents at an industrial level to recover essential oils would be impractical. Even though acetone and ethyl acetate behave similarly [54] and have been reported to extract carotenoids, terpenes, phenolic acids, flavonoids, and sugars in MAE [53,55], the yields obtained in the present work do not favor the use of any of these solvents to recover bioactive compounds at a large scale.

*3.3.3.3. SLE with ethanol.* The extraction yields obtained using ethanol (Table S8), shown in Fig. 3, were influenced by ethanol concentration, biomass load, temperature, and time, as shown in the Pareto charts of Fig S9. The extraction yield increases with an ethanol concentration of 60 % (v/v), a temperature of 50 °C, longer times, and low biomass loads. A maximum yield of 41.95 g/100 g DM was obtained using 61.6 % (w/v) of ethanol, 45.75 °C, 155 min, and a biomass load of 5 % (w/v). Using a water–ethanol solution for SLE at the optimal experimental conditions allowed the recovery of almost the same amount of extract as the one obtained using absolute ethanol in SXE and two times that of MAE. Similar behavior has been reported by other authors who demonstrated that using water–ethanol mixtures expands the range of polarity of the solution resulting in an increment in the yields of bioactive compounds [56,57].

Table 2 shows that the yields of glucose increase at 20 °C and 50 % (v/v) of ethanol, but the yields of fructose increase when using 70 °C and 96 % (v/v) of ethanol, showing the affinity of glucose for water and the affinity of fructose for ethanol. At optimal conditions, a yield of 3.9 g/100 g DM of glucose, 6.7 g/100 g DM of fructose, and 0.78 g/100 g DM of TPC was obtained. A yield of 0.19 g/100 g DM of naringin and 1.27 g/100 g DM of hesperidin was obtained, recovering ~30 % and ~90 % of the yields measured in SXE, respectively. Similar results have been reported in the literature mentioning how ethanol concentration and temperature affect the yields of citrus polyphenols [58,59].

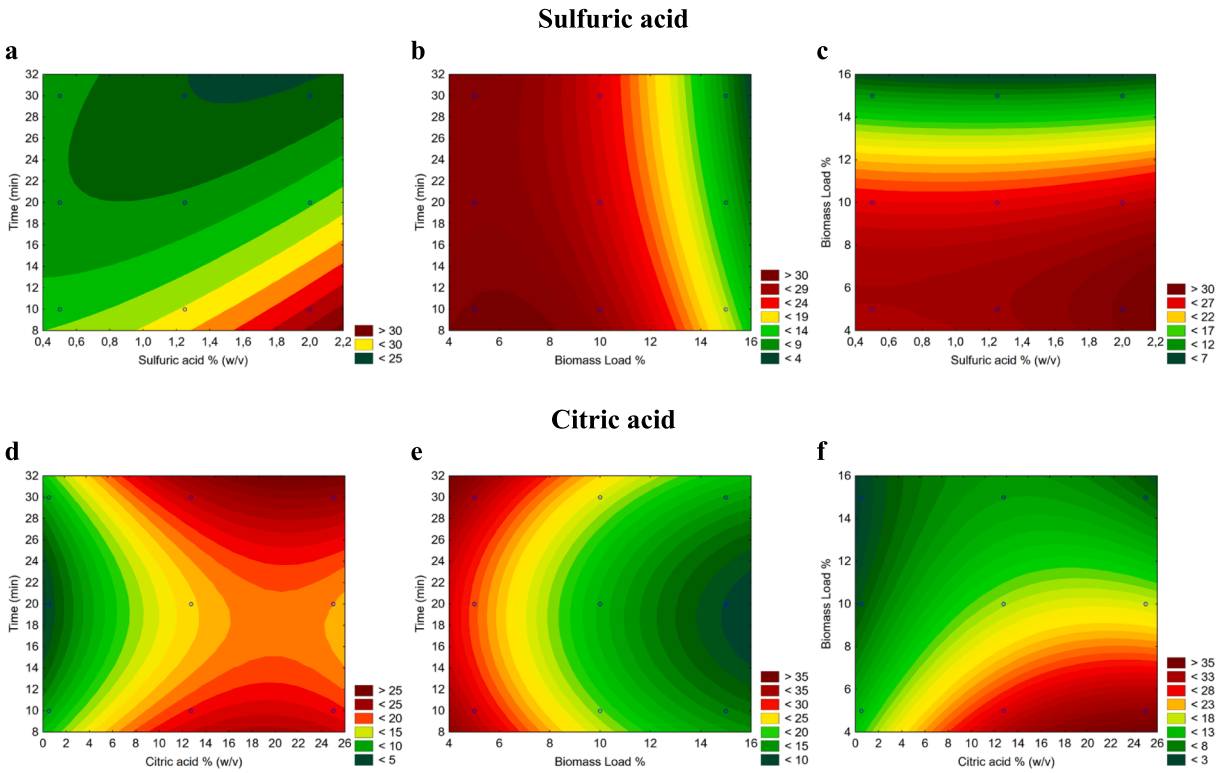


**Fig. 4.** Contour plots for the yields of hydrolysate (expressed in g Extract per 100 g of Dry Matter (DM)) obtained from the dilute acid hydrolysis of Orange Residues (OR) using sulfuric acid (a, b, c) and citric acid (d, e, f).

**Table 4**
Maximum and minimum yields of sugars, organic acids, furans, and total phenolics measured the dilute acid hydrolysis of Orange Residues (OR) using sulfuric acid and citric acid to produce fermentable sugars.

**Sulfuric acid hydrolysis**

| | Maximum Yield | | | | Minimum Yield | | | |
|---|---|---|---|---|---|---|---|---|
| | t (min) | Sulfuric acid % | BM% | Yield(g/100 g) | t (min) | Sulfuric acid % | BM% | Yield(g/100 g) |
| Glucose | 30 | **1.09** | **5.6** | 17.00 | 30 | **0.67** | **15** | 3.65 |
| Xylose | 30 | 0.5 | **5** | 14.31 | 30 | 2 | **15** | 2.64 |
| Arabinose | 30 | 1.33 | **5** | 2.87 | 30 | 0.5 | **15** | 0.51 |
| Gal. acid | 30 | 2 | 5 | 1.22 | 30 | 1.68 | 15 | 0.15 |
| Acetic acid | **10** | 0.5 | **5** | 1.80 | **20** | 1.55 | **14.55** | 0.21 |
| Formic acid | **10** | 2 | **5** | 1.05 | **20.1** | 1.25 | **14.17** | 0.17 |
| Furfural | 30 | **1.74** | **5** | 1.08 | 10 | **0.5** | **15** | 0.036 |
| HMF | 30 | **2** | 9 | 0.061 | 10 | **0.5** | 15 | 0.009 |
| TPC | **30** | **1.88** | **5** | 2.09 | **15.98** | **0.5** | **15** | 0.40 |

**Citric acid hydrolysis**

| | Maximum Yield | | | | Minimum Yield | | | |
|---|---|---|---|---|---|---|---|---|
| | t (min) | Citric acid % | BM% | Yield(g/100 g) | t (min) | Citric acid % | BM% | Yield(g/100 g) |
| Glucose | **30** | 14.1 | **5** | 7.42 | **19.5** | 0.5 | **15** | 1.70 |
| Xylose | **10** | 5.7 | **5** | 5.33 | **19.7** | 0.5 | **15** | 1.40 |
| Arabinose | **10** | **14.6** | **5** | 2.08 | **19.9** | **0.5** | **15** | 0.18 |
| Gal. acid | **30** | **25** | **5** | 27.92 | **19.3** | **0.5** | **11.16** | 0 |
| Acetic acid | 30 | 25 | **5** | 5.62 | 23.1 | 25 | **15** | 0.06 |
| Formic acid | 30 | 5.2 | **5** | 3.79 | 23.7 | 0.5 | **15** | 0 |
| Furfural | **30** | **25** | **5** | 0.66 | **10** | **0.5** | **15** | 0 |
| HMF | **30** | **25** | **15** | 0.06 | **10** | **0.5** | **15** | 0 |
| TPC | **10** | 0.5 | **5** | 1.09 | **23.9** | 0.5 | **15** | 0.27 |

BM: Biomass Load, HMF: Hydroxymethyl furfural, TPC: Total Phenolic Content.
*Values in bold font indicate which factors were significative for the measured compound.

Consequently, using SLE with water–ethanol mixtures appears to be the best option for the recovery of sugars and phenolic compounds at high yields. The content of D-Limonene was 0.296 g/100 g DM, which is five times lower than the total amount of D-Limonene in OR (SXE with hexane) and between half and a third of the one obtained with SD and HD. SLE appears to be not ideal for essential oil recovery. Acetic acid,

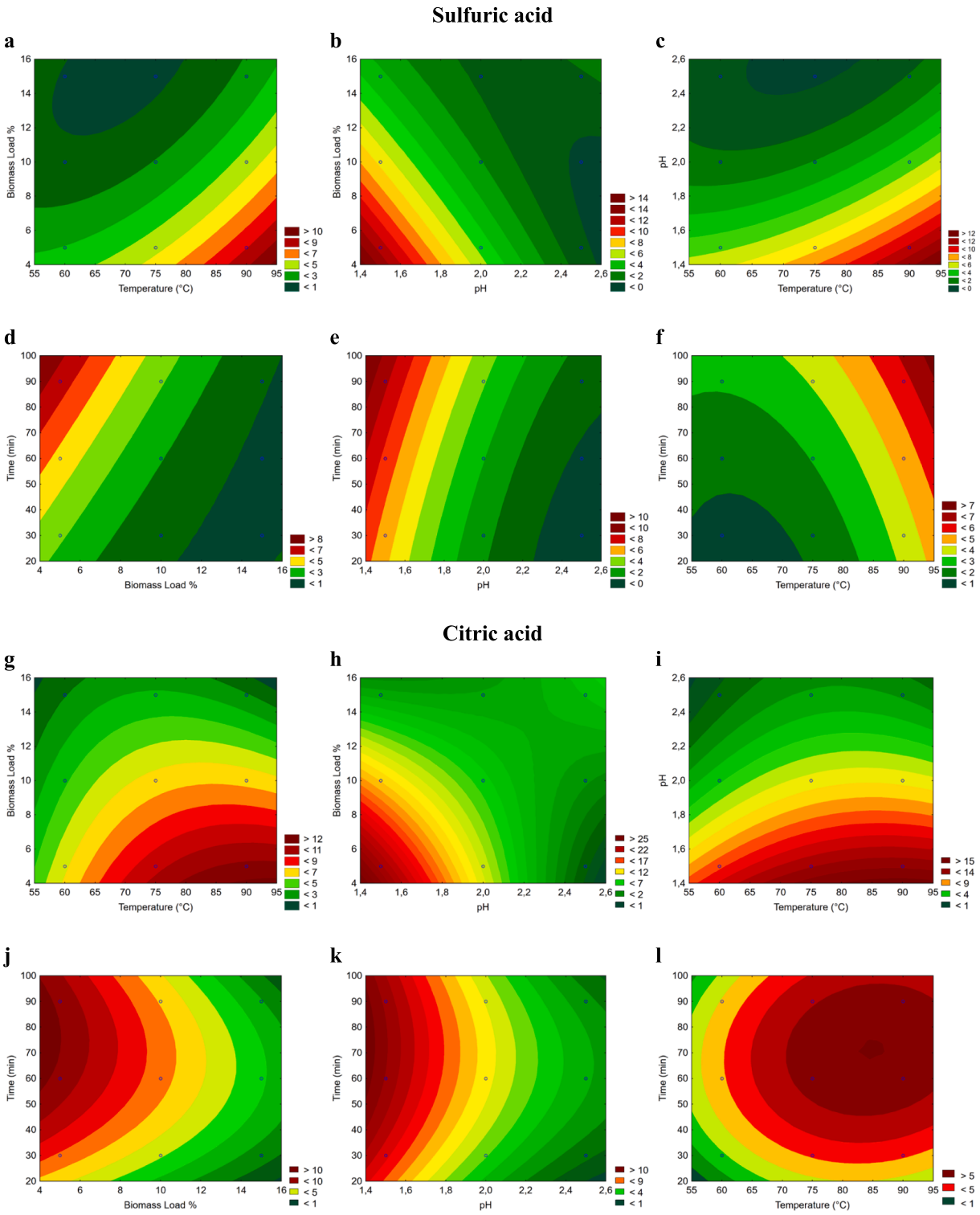


**Fig. 5.** Contour plots for the yields of pectin (expressed in g pectin per 100 g of Dry Matter (DM)) obtained from the dilute acid hydrolysis of Orange Residues (OR) using sulfuric acid (a, b, c) and citric acid (d, e, f).

formic acid, furfural, and HMF were not detected.

From the results, it is evident that SLE using the aqueous ethanol solution could be the best option for processing OR since it has lower times than those required for SXE and uses a simple setup that only requires agitation at moderate temperatures. What is more, SLE not only removes bioactive compounds that could interfere with further processing (e.g., soluble sugars) but also could help to maintain the integrity of the remnant biomass by maintaining it with low moisture, keeping the pectin in the solid matrix (since it is not soluble in ethanol), and cleaning the sample from decomposing microorganisms.

### 3.4. Production of fermentable sugars through acid hydrolysis

The yields obtained from the dilute acid hydrolysis (at 121 °C) of OR residues using sulfuric acid (Fig. 4a–c) were only affected by the biomass load, as shown in the Pareto charts of Fig. S14. A maximum hydrolysis

**Table 5**
Maximum and minimum yields of sugars, organic acids, furans, and total phenolics measured the dilute acid hydrolysis of Orange Residues (OR) using sulfuric acid and citric acid to produce pectin.

**Sulfuric acid hydrolysis**

| | Maximum Yield | | | | | Minimum Yield | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pH | T (°C) | t (min) | BM% | Yield(g/100 g) | pH | T (°C) | t (min) | BM% | Yield(g/100 g) |
| Glucose | 1.5 | 90 | 30 | 5 | 10.3 | 1.5 | 64.8 | 90 | 15 | 0.46 |
| Xylose | **1.5** | **90** | **30** | **5** | 11.05 | **1.5** | **79.1** | **90** | **15** | 1.51 |
| Arabinose | **2.5** | 60 | 90 | **15** | 1.65 | **1.5** | 64.2 | 90 | **15** | 0.44 |
| Gal. acid | **1.9** | 90 | **48.5** | 5 | 0.25 | **2.5** | 60 | **30** | 5 | 0.054 |
| Acetic acid | 1.5 | 60 | 30 | **5** | 11.01 | 1.5 | 84.2 | 90 | **15** | 0.41 |
| Formic acid | 1.5 | 60 | 30 | **5** | 6.10 | 1.5 | 81.5 | 90 | **14.8** | 0.32 |
| HMF | **2.5** | 60 | 90 | **15** | 0.0187 | **1.5** | 89.7 | 68.2 | **15** | 0.0039 |
| TPC | **1.8** | 90 | 30 | **5** | 1.04 | **1.5** | 90 | 90 | **15** | 0.08 |

**Citric acid hydrolysis**

| | Maximum Yield | | | | | Minimum Yield | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pH | T (°C) | t (min) | BM% | Yield(g/100 g) | pH | T (°C) | t (min) | BM% | Yield(g/100 g) |
| Glucose | 1.89 | 90 | 90 | **5** | 10.3 | 1.5 | 90 | 90 | **15** | 0.37 |
| Xylose | 1.5 | 60 | 90 | **5** | 2.17 | 1.5 | 90 | 90 | **15** | 0.39 |
| Arabinose | 1.5 | 90 | 90 | **5** | 0.10 | 1.5 | 90 | 90 | **15** | 0 |
| Gal. acid | **1.5** | 85.2 | 30 | **5** | 21.6 | **1.75** | 90 | 88.2 | **15** | 0.2 |
| Acetic acid | 2.17 | 90 | 30 | **5** | 5.24 | 1.5 | 90 | 62.5 | **15** | 0.36 |
| Formic acid | 2.05 | 90 | 30 | 5 | 3.26 | 2.5 | 90 | 57.9 | 15 | 0.04 |
| HMF | **1.92** | 90 | 30 | **5** | 0.02 | **1.5** | 60 | 30 | **12.78** | 0.0004 |
| TPC | **2.04** | 90 | **45.2** | **5** | 0.92 | **1.5** | 90 | **90** | **15** | 0.04 |

BM: Biomass Load, HMF: Hydroxymethyl furfural, TPC: Total Phenolic Content.
*Values in bold font indicate which factors were significative for the measured compound.
**Furfural was not detected in these experiments.

yield of 36.5 g/100 g DM was obtained using a biomass load of 6.71 % (w/v), 2 % (w/v) of sulfuric acid, and 10 min. Higher yields were obtained when the amount of OR in the hydrolysis experiments was minimized (Fig. 4a–c and Table S10). However, during acid hydrolysis, substances like organic acids and furans are produced as degradation products from sugars and other reactions, which could interfere negatively with fermentation processes. Therefore, even though the highest yields can be achieved with the aforementioned conditions, these are not necessarily ideal for maximizing the yields of sugars and minimizing the yields of potential fermentation inhibitors. Table 4 summarizes the maximum and minimum yields obtained for sugars, organic acids, and TPC (Contour plots for these substances can be found in Figs. S15–S20). The yields of sugars (glucose, xylose, and arabinose), organic acids (galacturonic acid, acetic acid, and formic acid), furfural, and TPC, increase simultaneously at low biomass loads (~%5 (m/v)).

Table 4 shows that the production of acetic acid and formic acid is favored at short reaction times (10 min), and the production of furans increases at high sulfuric acid concentrations (~2% (m/v)). TPC increased also at high sulfuric acid concentrations and long reaction times (30 min). For that, process conditions were optimized to enhance the overall yield and sugar recovery, and reduce acetic acid, formic acid, furans, and TPC in the hydrolysates. The optimal conditions that fulfilled those requirements were 7.32 % (m/v) of biomass load, 24.1 min, and a sulfuric acid concentration of 0.68 % (m/v) achieving a maximum hydrolysis yield of 30.1 g/100 g DM with 12.41 g/100 g DM of glucose, 10.13 g/100 g DM of xylose, 2.11 g/100 g DM of arabinose, 0.6 g/100 g DM of galacturonic acid, 0.617 g/100 g DM of acetic acid, 0.389 g/100 g DM of formic acid, 0.175 g/100 g DM of furfural, 0.013 g/100 g DM of HMF, and 1.08 g/100 g DM of TPC.

Regarding the yields obtained for the dilute acid hydrolysis (at 121 °C) of OR (Fig. 4d–f, and Table S11) using citric acid, these were affected not only by the biomass load but also by the citric acid concentration and the reaction time, as shown in the Pareto charts of Fig. S14. A maximum yield of hydrolysis of 36.76 g/100 g DM was achieved with a biomass load of 5 % (m/v), 15.6 % (m/v) of citric acid, and 30 min. In this case, even though the yields of sugars, organic acids, furans, and TPC increased at low biomass loads, the yields of glucose and galacturonic acid incremented at long reaction times (30 min), the yields of xylose and arabinose increased at short times (10 min), and the yields of furans and galacturonic acid increased both at high citric acid concentrations (25 % (m/v)). The conditions that maximize hydrolysis and sugar yield, and minimize yields of possible fermentation inhibitors were obtained at 8.73 % (w/v) of biomass load, 12.1 % (w/v) of citric acid, and 10 min achieving an overall yield of 22.4 g/100 g DM with 5.89 g/100 g DM of glucose, 4.73 g/100 g DM of xylose, 1.43 g/100 g DM of arabinose, 6.4 g/100 g DM of galacturonic acid, 1.72 g/100 g DM of acetic acid, 1.28 g/100 g DM of formic acid, 0.15 g/100 g DM of furfural, 0.02 g/100 g DM of HMF, and 0.77 g/100 g DM of TPC.

The use of sulfuric acid for hydrolysis would be the best option to obtain fermentable sugars since it produced almost two times the sugars compared to citric acid. In both cases, samples would require further detoxification to reduce the amount of acetic acid, formic acid, furans, and phenolic content to avoid complications when fermenting [60]. Nonetheless, the most important processing parameter to assure proper acid hydrolysis of OR is the amount of biomass that is processed. The interaction of the solid matrix with the acids is negatively affected at high biomass loads. The hydrophilic properties of cellulose and pectin and the low content of hydrophobic lignin [61,62] in OR could have propitiated the retention of liquid in the solid matrix forming a viscous mixture that does not allow the acid to fully interact with the whole sample.

It is worth noticing how the galacturonic acid contents in the sulfuric acid hydrolysates do not reach more than 1.22 g/100 g DM, which is extremely low considering the high content of pectin in OR. It has been reported that galacturonic acid (a uronic acid) is easily degraded at high temperatures [63,64]. That would explain why these experimental conditions have been used mostly for the obtention of fermentable sugars and not for pectin production [12,40]. What is more, the use of a strong acid in the process could have also contributed to further degradation [65]. In contrast, Table 4 shows that citric acid could be used to obtain high yields of galacturonic acid (~27 g/100 g DM) with citric acid concentrations of 25 % (w/v), 30 min, and 5 % (w/v) of

**Table 6**
Advantages and disadvantages of the evaluated methods, yields, operating conditions, possible products and characteristics of the remnant fractions after processing.

| Method | Overall yield | Product yields | Operating conditions | Possible products | Advantages | Disadvantages | Characteristics of the remnant fractions |
|---|---|---|---|---|---|---|---|
| **Steam Distillation** | 1.04 g/ 100 g DM | 1.04 g/100 g DM of Limonene | 4 h, 1500 W | D-Limonene. | Recovers of essential oils without altering sample integrity. | Slightly lower yields compared to hydrodistillation. | Moisture-free biomass that can be used for further processing. |
| **Hydrodistillation** | 0.70 g/ 100 g DM | 0.70 g/100 g DM of Limonene 5.48 g/100 g of Pectin | 4 h, 400 W, 300 rpm | D-Limonene, pectin | Slightly higher yields of essential oils compared to hydrodistillation. | Alters sample integrity since the biomass in in direct contact with boiling water for 4 h. Low yields of pectin from the process compared to hydrolysis. | Mixture rich in sugars, phenolics, galacturonic acid, and insoluble fibers. |
| **MAE with water** | 27.30 g/ 100 g DM | 4.10 g/100 g DM of Glucose 3.11 g/100 g DM of Fructose 0.39 g/100 g DM of TPC 0.39 g/100 g DM of Hesperidin | 5 min, 100 W, 95 °C | Glucose, Fructose, phenolic compounds. | High yields of extraction at short processing times. | The sample is altered by the microwave process due to the high temperature and pressure used. A low amount of biomass processed per batch and requires specialized equipment. | Humid biomass that could be altered from the process that adsorbs part of the sugars, phenolics, and furans present. |
| **MAE with ethanol** | 18.32 g/ 100 g DM | 2.32 g/100 g DM of Glucose 1.84 g/100 g DM of Fructose 0.48 g/100 g DM of TPC 1.90 g/100 g DM of Hesperidin 0.30 g/100 g DM of Limonene | 5 min, 100 W, 73 °C | Glucose, Fructose, phenolic compounds, D-Limonene. | High yields of extraction at short processing times. | The sample is altered by the microwave process due to the high temperature and pressure used. A low amount of biomass processed per batch and requires specialized equipment. | Moisture-free biomass that can be used for further processing. |
| **MAE with acetone** | 1.04 g/ 100 g DM | 0.06 g/100 g DM of TPC 0.05 g/100 g DM of Limonene | 5 min, 100 W, 51 | Phenolic compounds | High yields of extraction at short processing times. | The sample is altered by the microwave process due to the high temperature and pressure used. A low amount of biomass processed per batch and requires specialized equipment. | Moisture-free biomass possibly contaminated with acetone. |
| **MAE with ethyl acetate** | 0.97 g/ 100 g DM | 0.076 g/100 g DM of Hesperidin 0.13 g/100 g DM of TPC 0.004 g/100 g DM of Limonene | 5 min, 100 W, 72 °C | D-Limonene, Phenolic compounds. | Short processing times and selective extraction that recovers phenolic compounds and terpenes. | A low amount of biomass processed per batch and requires specialized equipment. The remaining solid could contain traces of ethyl acetate. | Moisture-free biomass possibly contaminated with ethyl acetate. |
| **SLE with water** | 29.39 g/ 100 g DM | 4.22 g/100 g DM of Glucose 14.0 g/100 g DM of Fructose 1.18 g/100 g DM of TPC 0.13 g/100 g DM of Naringin 0.16 g/100 g DM of Hesperidin | 20 °C, 30 min, 5 % BM | Glucose, Fructose, phenolic compounds. | High yields of extraction using a simple processing configuration. The sample is not degraded during the process. | Only recovers water-soluble sugars and phenolics. | Humid biomass (Still containing D-Limonene) that can be used for further processing. |
| **SLE with ethanol** | 41.95 g/ 100 g DM | 3.9 g/100 g DM of glucose 6.7 g/100 g DM of Fructose 0.78 g/100 g DM of TPC 0.19 g/100 g DM of Naringin 1.27 g/100 g | 45.75 °C, 155 min, 5 % BM, 61.6 % (w/v) EtOH | Glucose, Fructose, phenolic compounds, D-Limonene. | Obtains, with a simple processing configuration, the highest yields of extraction compared to other solvent extraction methods by recovering both water and ethanol soluble compounds without degrading the sample. | The extraction process removes partially D-Limonene | Moisture-free biomass that can be used for further processing. It could still contain some amount of D-Limonene. |

(*continued on next page*)

**Table 6** (*continued*)

| Method | Overall yield | Product yields | Operating conditions | Possible products | Advantages | Disadvantages | Characteristics of the remnant fractions |
|---|---|---|---|---|---|---|---|
| | | DM of Hesperidin | | | | | |
| **SLE with acetone** | 2.96 g/100 g DM | 0.21 g/100 g DM of TPC | 70 °C, 134 min, 5 % BM | D-Limonene, Phenolic compounds. | Selective extraction that recovers phenolic compounds and terpenes using a simple processing configuration. | Low yields and the remaining solid could contain traces of ethyl acetone. | Moisture-free biomass possibly contaminated with acetone. |
| **SLE with ethyl acetate** | 1.26 g/100 g DM | 0.33 g/100 g DM of TPC | 70 °C, 195 min, 5 % BM | D-Limonene, Phenolic compounds. | Selective extraction that recovers phenolic compounds and terpenes using a simple processing configuration. | Low yields and the remaining solid could contain traces of ethyl acetate. | Moisture-free biomass possibly contaminated with ethyl acetate. |
| **Production of sugars through acid hydrolysis with sulfuric acid** | 30.1 g/100 g DM | 12.41 g/100 g DM of glucose 10.13 g/100 g DM of xylose 2.11 g/100 g DM of arabinose 0.6 g/100 g DM of galacturonic acid | 121°C, 24.1 min, 7.32 % BM, sulfuric acid 0.68 % (m/v) | Glucose, Xylose, and Arabinose. | Obtains the highest yields of fermentable sugars compared to other alternatives using citric acid or lower temperatures. | Galacturonic acid is degraded. Organic acids, furans, and phenolics are produced, but their yields are lower than when using citric acid at 121 °C. Traces of unreacted sulfuric acid could remain both in the hydrolysate and the residual biomass. Terpenes and phenolic compounds could interact with sulfuric acid. | Humid biomass rich in fibers that have been altered due to processing at 121 °C. It can be used for further processing, but it contains traces of sulfuric acid and adsorbed organic acids, furans, and phenolic compound in the solid matrix. |
| **Production of sugars through acid hydrolysis with citric acid** | 22.4 g/100 g DM | 5.89 g/100 g DM of glucose 4.73 g/100 g DM of xylose 1.43 g/100 g DM of arabinose 6.4 g/100 g DM of galacturonic acid | 121 °C, 10 min, 8.73 % BM, citric acid, 12.1 % (m/v) | Glucose, Xylose, Arabinose, and Galacturonic acid. | Produces fermentable sugars at considerable yields, but lower than those obtained with sulfuric acid. The use of citric acid reduces galacturonic acid degradation. | The yields of fermentable sugars are almost half of those obtained with sulfuric acid. Organic acids, furans, and phenolics are produced, and their yields are higher than when sulfuric acid is used at 121 °C. Traces of unreacted citric acid could remain both in the hydrolysate and the residual biomass. Terpenes and phenolic compounds could interact with citric acid. | Humid biomass rich in fibers that have been altered due to processing at 121 °C. It that can be used for further processing, but it contains traces of citric acid and adsorbed organic acids, furans, and phenolic compound in the solid matrix. |
| **Pectin production through acid hydrolysis with sulfuric acid** | 18.49 g/100 g DM* | 5.74 g/100 g DM of glucose 1.15 g/100 g DM of xylose 1.15 g/100 g DM of arabinose 0.098 g/100 g DM of galacturonic acid | 90 °C, 90 min, 5 % BM, pH 1.5 | Low-quality pectin, Glucose, Xylose, Arabinose. | Uses a simple processing configuration and HMF is not produced at these conditions. | Galacturonic acid is degraded, causing lower pectin yields. Organic acids, furans, and phenolics are produced, and their yields are higher than when citric acid is used for pectin production. Terpenes and phenolic compounds could interact with sulfuric acid. | Humid biomass rich in fibers that can be used for further processing that could have adsorbed organic acids, furans, and phenolic compounds and contain traces of citric acid. The remaining hydrolysate contains sugars, organic acids, furans, phenolics, and unreacted acids. |
| **Pectin production through acid hydrolysis with citric acid** | 25.24 g/100 g DM* | 10.12 g/100 g DM of glucose 1.9 g/100 g DM of xylose 0.1 g/100 g DM of arabinose 20.26 g/100 g DM of galacturonic acid | 90 °C, 82 min, 5 % BM, pH 1.5 | High-quality Pectin, Glucose, Xylose, Arabinose, and Galacturonic acid. | Obtains, with a simple processing configuration, the highest yields of high-quality pectin compared to other alternatives with a selective hydrolysis due to the use of citric acid. At these conditions, HMF is not produced. | Organic acids, furans, and phenolics are produced with yields lower than those obtained sulfuric acid is used for pectin production. Terpenes and phenolic compounds could interact with sulfuric acid. | Humid biomass rich in fibers that can be used for further processing that could have adsorbed organic acids, furans, and phenolic compounds and contain traces of citric acid. The remaining hydrolysate contains sugars, organic acids, furans, phenolics, and unreacted acid. |

DM: Dry Matter, BM: Biomass Load, TPC: Total Phenolic Content, EtOH: Ethanol.
* Pectin yield.

biomass load, opening the possibility of for recovering pectin. However, it must be considered that the hydrolysate at those conditions would also contain high amounts of acetic acid (5.62 g/100 g DM), formic acid (3.38 g/100 g DM), furfural (0.66 g/100 g DM), HMF (0.039 g/100 g DM), and TPC (0.91 g/100 g DM), non-desired compounds that were possibly produced due to the high temperature and acid concentration employed.

### 3.5. Extraction of pectin

Regarding the extraction of pectin using sulfuric acid (Fig. 5a–f, and Table S12), it is significatively affected by pH, biomass load, temperature, and time, as shown in the Pareto charts of Fig. S21. The yield of pectin is increased at low pH (~1.5) and biomass loads values (~5% (m/v)), high temperatures (80 °C-90 °C), and long processing times (>70 min). A maximum yield of pectin of 18.49 g/100 g DM was obtained (after precipitation and drying) using a pH of 1.5, 90 °C, 90 min, and 5 % (w/v) of biomass load. At these conditions, the hydrolysate contained 8.66 g/100 g DM of glucose, 5.74 g/100 g DM of xylose, 1.15 g/100 g DM of arabinose, 0.098 g/100 g DM of galacturonic acid, 3.82 g/100 g DM of acetic acid, 2.82 g/100 g DM of formic acid, 0.007 g/100 g DM of HMF (Furfural was not detected), and 0.64 g/100 g DM of TPC. The overall low amount of galacturonic acid (<0.25 g/100 g DM) in the sulfuric acid hydrolysis experiments (Table 5) suggests that the recovered pectin is of low quality, even after precipitation and purification, and that the dried samples would consist mainly of sugars, organic acids, furans, and phenolic compounds. It is also evident that the use of sulfuric acid could have caused the degradation of galacturonic acid in the samples, as also seen in section 3.4.

The use of citric acid for extracting pectin (Fig. 5g–l, and Table S13) is affected by pH and biomass load, as shown in the Pareto charts of Fig. S21. The yield of pectin is increased at low pH values (~1.5) and biomass loads. A maximum yield of pectin of 25.24 g/100 g DM was obtained (after precipitation and drying) using a pH of 1.5, 90 °C, 82.07 min, and 5 % (w/v) of biomass load. At these conditions, the hydrolysate contained 10.12 g/100 g DM of glucose, 1.9 g/100 g DM of xylose, 0.1 g/100 g DM of arabinose, 20.76 g/100 g DM of galacturonic acid, 2.09 g/100 g DM of acetic acid, 1.60 g/100 g DM of formic acid, 0.013 g/100 g DM of HMF (Furfural was not detected), and 0.63 g/100 g DM of TPC. In this case, the hydrolysis using citric acid appeared to be more selective to pectin and favored high yields of galacturonic acid, as seen in Table 5. It is worth remembering that the weight of the pectin recovered consists not only of the galacturonic acid that was polymerized using ethanol, but also of the remnant sugars, organic acids, furans, and phenolic compounds that are present. The use of low biomass loads favors hydrolysis, causing a simultaneous increase in the yields of all the compounds. The pectin recovered at optimal conditions contained ~82.3 % of galacturonic acid, which is a good indicator of its quality. This also means that pectin could be further cleaned to increase its purity. The results obtained demonstrate that the use of citric acid is better for pectin recovery in contrast to sulfuric acid, producing a high-quality product using temperatures not higher than 90 °C in a simple processing configuration.

Moreover, the liquid phase recovered from HD contained 0.106 ± 0.005 g/100 g DM of glucose, 0.085 ± 0.004 g/100 g DM of xylose, and <0.002 g/100 g DM of galacturonic acid. After precipitation, purification, and drying the yield of pectin recovered from this fraction was 5.48 ± 0.15 g/100 g DM, which is around five times less than the yield obtained using citric acid at the optimal conditions.

### 3.6. Results summary and perspectives on the integral valorization of orange residues (OR) from the evaluated valorization alternatives

A summary of the advantages, disadvantages, yields, operating conditions, possible products, and characteristics of the remaining biomass for each one of the evaluated methods is presented in Table 6. The essential oil yields obtained for SD and HD in this work, ranging between 0.7 – 1.0 g/ 100 g DM, align with values reported in previous studies [8,13,15], supporting the suitability of these techniques as initial recovery steps for OR valorization. SD is preferable for D-Limonene recovery, as it does not alter the sample structure, unlike HD, which maintains the sample at boiling temperature for an extended period. SD maintains the biomass at a low moisture level, facilitating downstream recovery of phenolic compounds and free sugars, while removing D-Limonene to prevent interference in further processes. Although HD yields a slightly higher essential oil output and enables some pectin precipitation from the residual fraction, the process compromises the remaining fraction by subjecting it to prolonged heat exposure.

Regarding MAE, prior studies using domestic microwave ovens (120 s, 400–500 W) reported phenolic compound yields from orange peel with acetone (0.01–0.012 g/100 g TPC) and from lemon peel with ethanol (0.013–0.015 g/100 g TPC) [66,67]. These values are considerably lower than those achieved in this study for acetone (0.06 g/ 100 g of TPC), ethanol (0.48 g/ 100 g of TPC), and other solvents (Table 6), primarily due to the use of a focused microwave reactor. This indicates that while MAE can significantly enhance yields, its industrial application may be restricted by specialized equipment requirements and limited processing capacity for large biomass volumes. In our work, MAE effectively recovered sugars (~4 – 7 g/ 100 g DM) and hesperidin (~0.4 – 1.9 g/100 g DM) from OR using water and ethanol, with small amounts of 5-HMF and furfural. Other works have reported how MAE can be useful in the breakdown of OR polysaccharides to enhance pectin recovery [68–70]. Although MAE offers rapid processing and high yields using water or ethanol, potential sample degradation could occur. Nevertheless, the insights gained here offer a valuable reference point for future studies on OR valorization using non-conventional extraction methods, particularly for optimizing hesperidin recovery and purification.

Moreover, SLE resulted in higher extraction yields compared to MAE using a simple processing setup avoiding possible biomass degradation. SLE gave the best yields using water–ethanol mixtures with 41.95 g/100 g DM of extract at optimal conditions (45.75 °C, 155 min, 5 % BM, 61.6 % (w/v) EtOH), including 6.7 g/ 100 g DM of fructose and 3.9 g/ 100 g DM of glucose. Another study obtained 7.94 g/ 100 g of sugars from orange peel at optimal SLE conditions (45.75 °C, 60 min, 10 % BM, 65 % (w/v) EtOH) [71], which is lower than our yields. Although high sugar yields could also be obtained using water alone, this option increases the moisture content in OR which could be problematic for further processing. The enhanced sugar yields observed here could be attributed to differences on the biomass source (origin, variety, and sample preparation) or from the experimental design boundaries evaluated, where lower biomass loads (<10 % BM) and longer extraction times (>120 min) were used than those considered by Alonzo-Vásquez et al [71].

Regarding phenolic content, optimal SLE conditions yielded 1.18 g/100 g DM of TPC with water (20 °C, 30 min, 5 % BM), 0.78 g/100 g DM of TPC with ethanol 61.6 % (w/v) (45.75 °C, 155 min, 5 % BM), and 0.21 g/100 g DM of TPC. Other studies using SLE with ethanol have recovered 1.40–1.59 g/100 g DM of TPC [71–73], and 1.0–1.8 g/100 g DM of TPC [46,72], which could be attributed to the use of longer extraction times (>300 min) and differences on the OR source. However, the use water–ethanol mixtures for SLE enhanced the recovery of hesperidin (1.27 g/100 g DM) and naringin (0.19 g/100 g DM). Hesperidin yield with ethanol 61.6 % (w/v) was around 10-fold the one obtained with water alone, and 3.5 times higher than the one reported by Alonzo-Vásquez et al. at optimal SLE conditions (0.37 g/100 g of hesperidin) [71]. It is worth noting that the use of acetone and ethyl acetate may not be advisable due to potential residual traces in the biomass of those harmful substances. Overall, our optimized SLE conditions appear effective for maximizing compound extraction from OR, yielding high concentrations of sugars and hesperidin. Consequently, a water–ethanol mixture for SLE is the most favorable option, recovering the highest yield of bioactive compounds and leaving the remaining biomass ready for further processing.

Furthermore, during hydrolysis, the acid interacts with the structural carbohydrates from OR causing their breakdown into mixture of fermentable sugars, organic acids, furans, and phenolic compounds [74]. The results in Table 6 show that when hydrolysis occurs at a temperature of 121 °C, sugars (glucose, xylose, and arabinose) are released in higher yields than at lower temperatures (<90 °C). It is worth noting that galacturonic acid is easily degraded when hydrolysis occurs

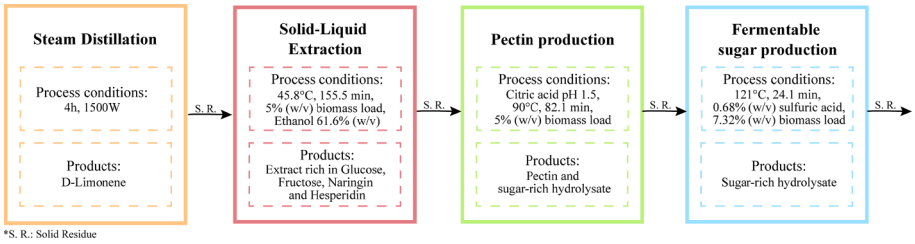


**Fig. 6.** Proposed sequence for a biorefinery from Orange Residues.

at 121 °C in the presence of sulfuric acid, resulting in low-quality pectin yields. In contrast, using citric acid at lower temperatures (<90 °C) produces the highest pectin yields with a high galacturonic acid content. For that reason, mild temperature conditions with citric acid would be preferable for pectin production and high temperatures with sulfuric acid would be best suited for fermentable sugar production.

Under optimal conditions (90 °C, 82 min, 5 % BM, pH 1.5) a maximum pectin yield of 25.24 g/100 g DM was achieved containing ~82.3 % of galacturonic acid. Other studies have reported pectin yields from orange peels of 19.62 g/100 g DM using HCl (80 °C, 5.8 % BM, pH 1.5, 50 min) and 17.95 g/100 g DM using aqueous extraction (95 °C, 5 % BM, 90 min) [73,75]. These variations could result from differences in the types of acid, process conditions, and biomass sources utilized. To our knowledge, the highest pectin yield with citric acid reported in the literature is 32.6 g/100 g DM (90 °C, 160 min, 2 % BM, pH 2.0) [15]. While pectin recovery is 28 % higher than in the present study, it requires manual removal of the flavedo, double the processing time (i.e. greater energy expenditure), and a considerably low biomass load (60 % less). Thus, the optimized conditions reported here appear to be better suited for processing larger biomass loads in less time, compared to the results of Tsouko et al.

Moreover, a maximum fermentable sugar yield of 30.1 g/100 g DM (including 12.41 g/100 g DM of glucose and 10.13 g/100 g DM of xylose) was obtained at optimal hydrolysis conditions (121 °C, 24.1 min, 7.32 % BM, sulfuric acid 0.68 % (m/v)) in the present work. Vaez et al. reported a higher hydrolysis yield (36.6 g/100 g DM of sugars, including 20.7 g/100 g DM of glucose and 2.57 g/100 g DM of xylose) from orange peels at optimal conditions (140 °C, 30 min, 7.14 % BM, sulfuric acid 1 % (m/v)) [22], as a result of using a higher temperature and sulfuric acid concentration. However, it is unlikely that these conditions could also assure low concentrations of organic acids and furans, like the ones optimized in our work and presented in Section 3.4.

Finally, an effective biorefinery sequence can be proposed from the evaluated techniques where the sample is first submitted to SD where essential oils are recovered, then used for SLE with ethanol to recover phenolic compounds and sugars, after that used for pectin production through citric acid hydrolysis, and finally used for fermentable sugar production trough dilute acid hydrolysis with sulfuric acid, all at optimal conditions established in this study (see Fig. 6). This approach would allow the recovery of solid and liquid fractions after each step for further processing by guaranteeing that (i) essential oils are removed to avoid interference with microbial processing, (ii) SLE extracts are sufficiently retrieved from OR to produce high-value molecules by fermentation (e.g. ethanol, phenolics, or single-cell protein [76–78]), (iii) pectin is extracted at high yields and preserves its quality, and (iv) the obtained hydrolysates contain the lowest possible amount of toxic substances and can be easily detoxified and fermented. After the recovery of all valuable substances, the residual biomass from the last step of the biorefinery could be then used for energy production. The proposed valorization cascade offers a technically feasible strategy for an OR-based biorefinery, efficiently utilizing its key compositional fractions. Further research must be done to assess additional valorization options and to compare their processing efficiencies and yields with those achieved here. In addition, future work could evaluate the proposed sequence here as an integrated system, providing essential data for techno-economic and sustainability assessments to determine its potential implementation within the orange processing industry.

## 4. Conclusions

The evaluation of different valorization alternatives for OR enabled us to identify strategies that support their integration into a sequential biorefinery, advancing beyond single-product valorization to a holistic, industrially viable approach. By examining how OR composition and process conditions impact both the yield and characteristics of extracted products, we gained insights into how each process affects the accessibility and suitability of the remaining biomass for further processing. The use of the same biomass source allowed for direct comparison of techniques based on product yields, a key metric for assessing the feasibility and scalability of the valorization alternatives investigated. The proposed sequence – Initial SD for essential oil recovery, followed by SLE with water–ethanol for extracting sugars and polyphenols, citric acid hydrolysis for pectin production, and concluding with sulfuric acid hydrolysis for fermentable sugars – optimizes the yield of valuable compounds while offering opportunities to valorize side streams further. This systematic approach demonstrates the novelty of integrating multiple valorization stages within a single biorefinery framework, paving the way for more sustainable and economically feasible utilization of OR.

## CRediT authorship contribution statement

**D.D. Durán-Aranguren:** Writing – original draft, Conceptualization, Methodology, Investigation, Validation, Visualization, Formal analysis, Data curation. **L.C. Villabona:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **G.D. López:** Methodology, Investigation, Writing – review & editing. **C. Carazzone:** Resources, Methodology, Writing – review & editing. **R. Sierra:** Writing – review & editing, Supervision. **J.A. Posada:** Writing – review & editing, Supervision. **S.I. Mussatto:** Resources, Writing – review & editing, Supervision.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mussatto, S. I. reports financial support was provided by Novo Nordisk Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2024.130590.

## Data availability

Data will be made available on request.

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