Journal of Cleaner Production 389 (2023) 135995

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Valorization of discarded red beetroot through the recovery of bioactive compounds and the production of pectin by surfactant-assisted microwave extraction

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ARTICLE INFO

Handling Editor: Maria Teresa Moreira

Keywords:
Microwave-assisted extraction
Galacturonic acid
Pectooligosaccharides
Polyethylene glycol
Antioxidants
Betalains

ABSTRACT

Discarded red beetroot (DRB) is an organic waste generated in the food industry. This study is focused on the valorization of DRB through the recovery of bioactive compounds. The characterization of the DRB juice confirmed a higher content of antioxidants (DPPH 504 ± 24 μmol TE/L, FRAP 10920 ± 440 μmol TE/L, ABTS 22012 ± 592 μmol TE/L), phenolic compounds (1789 ± 56 mg GAE/L), flavonoids (471 ± 17 mg CE/L) and betalains (1426 ± 24 mg/L) than commercial juices. DRB pomace contains pectin that was recovered by microwave extraction aided by a surfactant (polyethylene glycol, PEG4000). Conditions (temperature, time and surfactant concentration) for the extraction of galacturonic acid (GalA) or pectooligosaccharides (POS) were optimized by a central composite experimental design. POS were extracted at high temperatures (160 °C, 5.3 min, 8.4 g PEG4000/L, yield of 271.2 g POS/kg dry pomace). In comparison, galacturonic acid extraction was favored at moderate conditions (137 °C, 5 min, 2.5 g PEG4000/L, yield of 120.1 g GalA/kg dry pomace). The characterization of the freeze-dried hydrolysates revealed that the solid obtained under moderate temperature conditions (137 °C) showed a higher GalA content (49.5%) and lower neutral sugars (11.4%), as GalA degrades at lower temperatures than pentoses. The recovered pectin can be considered high-methoxyl pectin, as the degree of esterification was higher than 50%. FTIR spectra of the freeze-dried hydrolysates showed functional groups consistent with pectin. MALDI-TOF-MS analysis revealed the presence of oligosaccharides of hexoses and pentoses with different structures and degrees of polymerization. Thus, DRB, a low-value vegetable waste, can be converted into high-value-added bioproducts in a biorefinery framework.

1. Introduction

Every year, 1.3 billion tons of food waste is generated worldwide (United Nations Development Programme, 2022), representing a third of the total food industry production. Waste poses a risk to achieving the 12th SDG of the United Nations: responsible consumption and production. Among food waste, FVW constitutes the main part (42%) (Ganesh et al., 2022). Due to the huge amount produced, effective management of FVW is needed. Conventionally, FVW has been used for energy production, animal feed, and composting or it is disposed of in landfills (Ganesh et al., 2022). Conventional applications may result in a loss of valuable biomass, as FVW is a precious source of bioactive compounds for recovery in the context of a circular economy (Esparza et al., 2020).

Red beetroot is well known for its large amount of bioactive and nutritional compounds such as phenolics, carbohydrates, fiber, vitamins, and betalains, among others (Dhiman et al., 2021). Red beetroot has shown pharmacological applications due to its anti-anemic, lipid and blood pressure-lowering effects and for its diuretic, antioxidant, expectorant, anti-diabetic, and antifungal properties (Dhiman et al., 2021). Red beetroot can be used as a source of natural pigments, rich in polyphenols and antioxidants (Kusznierewicz et al., 2021; Trishitman et al., 2021). Natural pigments are mainly found in the juice, while the pomace fraction is rich in carbohydrates. However, few studies have so far attempted to valorize these carbohydrates (Fissore et al., 2011; Hotchkiss et al., 2022).

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https://doi.org/10.1016/j.jclepro.2023.135995
Received 10 October 2022; Received in revised form 20 December 2022; Accepted 9 January 2023
Available online 10 January 2023

Abbreviations

BC	Betacyanin
BX	Betaxanthin
CE	Catechin equivalents
DRB	Discarded red beetroot
FRAP	Ferric reducing antioxidant power
FTIR	Fourier transform infrared
FVW	Fruit and vegetable waste
GAE	Gallic acid equivalents
GalA	Galacturonic acid
GalA-S	Freeze-dried solid obtained under optimal conditions for GalA recovery
HG	Homogalacturonan
HMF	Hydroxymethylfurfural
HPLC	High-performance liquid chromatography
HPSEC	High-performance size exclusion chromatography
MAE	Microwave-assisted extraction
MALDI-TOF MS	Matrix-assisted laser desorption/ionization-time of flight mass spectrometry
Mw	Molecular weight
PEG	Polyethylene glycol
POS	Pectooligosaccharides
POS-S	Freeze-dried solid obtained under optimal conditions for POS recovery
RG-I	Rhamnogalacturonan I
RG-II	Rhamnogalacturonan II
SDG	Sustainable development goal
TE	Trolox equivalents
TFC	Total flavonoid content
TPC	Total phenolic content

Carbohydrates are a source of valuable compounds such as pectin, which is a heteropolysaccharide made up of different domains, HG, RG-I, and the much less usual RG-II. The HG region, known as "smooth" pectin, contains linear chains of α-1,4-linked-D-galacturonic acid. However, the RG-I region, known as "hairy" pectin, is composed of 1,2-α-L-rhamnose-1,4-α-D-galacturonic acid. Rhamnose molecules have side chains, highly branched by neutral sugars such as arabinose and galactose (Abou-Elseoud et al., 2021). Pectin can be used as a gelling, stabilizing, and thickening agent in food systems. On the other hand, the RG-I region is a source of the new class of prebiotics known as POS. Driven by growing health awareness, the prebiotics market size was valued at US $6.05 billion in 2021 and is predicted to grow from 2022 to 2030 at a rate of 14.9% (Market Analysis Report, 2020).

Pectin extraction from agro-food waste is conventionally carried out through a solid-liquid extraction that requires long operation times. Nevertheless, greener extraction procedures, in terms of energy consumption and yield, such as enzymatic hydrolysis (Abou-Elseoud et al., 2021), dynamic high-pressure microfluidization (Chen et al., 2013), ultrasound-assisted extraction (Hu et al., 2022) and MAE (Mao et al., 2019), among others, have been developed in recent years. The advantages of MAE are a reduced processing time, reduced use of solvents (Sharma and Dash, 2022), and higher extraction yields (Cardoso-Ugarte et al., 2014). The microwaves penetrate uniformly in the material, leading to a fast temperature increase inside the biomass cells, which produces the breakdown of the cell walls and the extraction of the targeted compounds (Li et al., 2013). Moreover, microwaves show selective heating because of its electromagnetic nature, which facilitates the extraction through temperature-induced diffusion (Arrutia et al., 2020).

Khodaei et al. (2016) reported an extraction yield of 21.9 g galactan-rich RG I/100 g of potato pulp using 1.5M KOH, 2.9% (solid/liquid ratio), 2 min operation time, and 36 W power. Hotchkiss et al. (2021) achieved a yield of 2.2 g pectin/100 g of blueberry pomace under the following conditions: HCl (pH 1) as solvent, 10 min, 120 °C. Regarding beetroot pomace, Fissore et al. (2011) reported the need for pretreatment with 2M NaOH, followed by pectin extraction in a citrate buffer with or without enzyme addition. However, using acid and alkali solutions to extract pectin presents many drawbacks, such as the need to neutralize the extract as well as equipment corrosion and environmental issues. Additionally, to extract the RG-I domain, high temperatures are needed for galactose and arabinose extraction, while galacturonic acid is extracted at lower temperatures (del Amo-Mateos et al., 2022b).

On the other hand, surfactants are promising solvents to extract biocompounds that prevent the disadvantages of harsh solvents, allowing higher yields to be reached. Surfactants, also known as surface-active agents, are amphiphilic molecules able to form micelles, which interact with hydro- and lipophilic substances. Owing to the wide range of pectin polarity, surfactants can achieve a higher pectin extraction yield. Su et al. (2019) observed a higher pectin yield, with higher GalA content by MAE aided by surfactants in comparison to solvent extraction. The lower operating time of MAE and the use of surfactants avoid the biomass being exposed to hot acidic solutions. Thus, the degradation of pectin is minimized (Cui et al., 2020) and higher methyl-esterified pectins are obtained (Dranca et al., 2021).

This work aims to address the valorization of DRB by recovering pectin from the pomace fraction and obtaining a juice rich in bioactive compounds such as natural pigments, antioxidants, and phenols. Pectin extraction was carried out by a greener extraction procedure, such as MAE, using a surfactant (PEG4000) as solvent to increase the extraction yield. A surface area experimental design evaluated the influence of temperature, time, and surfactant concentration. Extraction conditions were optimized to maximize the extraction of GalA or POS (oligosaccharides of arabinose, galactose, and GalA). The hydrolysates obtained under optimal conditions were freeze-dried, and the characteristics of the solids were compared in terms of global yield, degree of esterification, sugar composition, and chemical structure (FTIR, MALDI-TOF MS). Thus, DRB, a low-value FVW, can be converted into high-value-added bioactive products, thus contributing to the achievement of SDG 12: Ensure sustainable consumption and production patterns.

2. Materials and methods

2.1. Raw material

The DRB was supplied by Huercasa (Sanchonuño, Spain) and stored frozen at −20 °C until use. The DRB was washed to remove the remains of dirt and processed by a juice extractor (Kenwood PureJuice One JMP400WH) to separate the pomace from the juice. From 1 kg of fresh DRB, 433 g of pomace were obtained. The pomace was washed and dried at 60 °C, while the juice was frozen for further analysis.

2.2. Microwave-assisted extraction

MAE was conducted in a closed microwave-assisted reaction system (Multiwave PRO SOLV reactor 50 Hz with a Rotor type 16HF100, Anton Paar GmbH, Austria, Europe). The DRB pomace and PEG4000 solution were mixed in vessels made of PTFE-TFM (volume capacity of 100 mL) fitted with magnetic stirrers. An infrared sensor continuously recorded the pressure and temperature of each vessel. The temperature/pressure sensor controlled the microwave power applied to the reactor. For more details, see del Amo-Mateos et al. (2022).

The DRB pomace and PEG4000 solution were mixed in a solid to liquid ratio of 10% (w/v) (5 g of dry DRB pomace and 50 mL of

solution). After pretreatment, a vacuum filter was used to separate the solid and liquid fractions. The solid was dried at 60 °C and weighed to calculate the solid recovery (g pretreated solid/g DRB pomace). The liquid was stored at 4 °C for further analysis (monomeric and oligomeric sugar content and degradation compounds).

2.3. Experimental design for pectin extraction

A surface area central composite experimental design was proposed to optimize the MAE conditions for POS and GalA extraction. The central composite design is based on a 2^3 factorial design (−1, 0, 1) with the additional star points at the axial distance $\alpha = 1.68179$. The experimental factors were temperature (100–160 °C), time (5–15 min), and PEG4000 concentration (2–10 g/L). Thus, 17 experiments were planned: 8 in the experimental factor range, 6 at the star points, and 3 at the central point. The ANOVA test was used to conclude the statistical differences at a confidence level of 95% ($p < 0.05$). The ANOVA test and the central composite experimental design were carried out using Statgraphics Centurion XVIII.

A confirmatory run was carried out in triplicate under the optimal conditions obtained for POS or GalA extraction to verify the results predicted by the statistical software. These hydrolysates were concentrated in a rotavapor system (Heidolph VV2000, Germany) under reduced pressure before being freeze-dried (Telstar LyoQuest 55). The freeze-dried solids were stored for analysis (sugar composition, galacturonic acid content, degree of esterification, Mw distribution, and structural characterization).

2.4. Analytical methods for DRB juice characterization

The DRB juice samples were centrifuged at 13000 rpm for 10 min (MiniSpin Eppendorf, Germany), and the supernatant was diluted and used for analysis. All analytical determinations were carried out in triplicate.

2.4.1. Total phenolic content

The TPC was analyzed using the Folin-Ciocalteu method with gallic acid as a standard (Ozturk et al., 2018). Briefly, 3 mL ddH_2O was mixed with 40 μL of the sample supernatant and 200 μL of Folin-Ciocalteu reagent. The mixture was allowed to stand for 5 min at room temperature, and then 600 μL of sodium carbonate solution (20%) was added. After 30 min at 40 °C, the samples were allowed to cool, and absorbance was measured at 765 nm in a UV-VIS spectrophotometer (Shimadzu, Japan). The TPC was expressed as GAE in mg/L.

2.4.2. Total flavonoid content

The TFC was analyzed using catechin as the standard by a colorimetric method (Zhishen et al., 1999). A 1 mL sample supernatant was mixed with 300 μL of sodium nitrite (5%). After 5 min, 500 μL of aluminum chloride (2%) was added. After 6 min, the samples were mixed with 500 μL of sodium hydroxide (1M). The samples were allowed to stand for 8 min, 10 mL of ddH2O was added, and the absorbance was measured at 510 nm in a UV-VIS spectrophotometer (Shimadzu, Japan). The TFC was expressed as CE in mg/L.

2.4.3. Antioxidant capacity

The antioxidant capacity of the DRB juice was mined using three methods: DPPH radical scavenging, FRAP, and ABTS radical scavenging. A Trolox solution in methanol was used as standard. The absorbance was measured in a UV-VIS spectrophotometer (Shimadzu, Japan). The results were expressed as TE in mg/L.

The DPPH assay was done according to Brand-Williams et al. (1995), though slightly modified. In brief, a 200 μL sample supernatant was added to 2000 μL of $6{\cdot}10^{-5}$ M DPPH (2,2-diphenyl-1-picrylhydrazyl) methanol solution and mixed. After 15 min in darkness, the decrease in absorbance was measured at 517 nm. FRAP tests were done as follows: a 100 μL sample supernatant was mixed with 3 mL of FRAP reagent (Benzie and Strain, 1996). The absorbance was measured after 6 min in darkness at 593 nm. 3 mL of diluted ABTS solution (Re et al., 1999) was added to the 30 μL sample supernatant for ABTS analysis. The absorbance was analyzed after 6 min in darkness at 734 nm.

2.4.4. Betalain content

The betalain content was calculated as the sum of the BC and BX concentrations, in accordance with Elbe (2001). The absorbances corresponding to betacyanin, betaxanthin, and impurities were measured at 538, 476, and 600 nm, respectively. The BC and BX concentrations were calculated using Eq. (1):

$$C\ (mg\ /\ L) = \frac{A{\cdot}DF{\cdot}1000}{\varepsilon{\cdot}i} \quad (1)$$

where C is the BC or BX concentration, DF is the dilution factor, ε is the extinction coefficient, and i is the path length (cm). For the BC concentration, A is the absorbance of BC corrected by the absorbance of the colored impurities: $A = 1.095{\cdot}(A_{538nm} - A_{600nm})$, and the extinction coefficient $\varepsilon = 112$ L/(g·cm). Similarly, for the BX concentration, A is the absorbance of BX corrected with the contribution of BC and the colored impurities: $A = A_{476nm} - 0.258{\cdot}A_{538nm} - 0.742{\cdot}A_{600nm}$, and the extinction coefficient $\varepsilon = 75$ L/(g·cm).

2.4.5. Sugar composition by HPLC

The sugar composition (sucrose, glucose, and fructose) was analyzed by an HPLC system using an Aminex HPX-87N column (Waters, USA) with a refractive index detector (Waters 2414, USA). Operational conditions were 0.01 M Na_2HPO_4 as the mobile phase, 0.6 mL/min flow, 80 °C and an injection volume of 20 μL.

2.5. Analytical methods for DRB pomace, hydrolysates, and freeze-dried solids

2.5.1. DRB pomace characterization

The structural carbohydrates and lignin content of the DRB pomace were analyzed following the methods described by the Laboratory Analytical Procedures of the Renewable Energy Laboratory (NREL) (Sluiter et al., 2008a). The water and ethanol extractives and water extractive composition were also determined according to the NREL procedures (Sluiter et al., 2008b). The samples were analyzed in triplicate.

2.5.2. Sugar composition, GalA and degradation compounds by HPLC

The sugar composition (glucose, arabinose, and galactose), GalA, and degradation compounds (formic and acetic acids, HMF, and furfural) in the MAE hydrolysates were analyzed by HPLC. The monomeric and oligomeric compounds were determined. To determine the total sugar content, the samples were subjected to acid hydrolysis (del Amo-Mateos et al., 2022b). The oligomers were calculated as the total content minus the monomer content. The HPLC analysis was performed in a refractive index detector (Waters 2414, USA) using an Aminex HPX-87 H analytical column (Waters, USA). The operational conditions were: 0.01 N H_2SO_4 as the mobile phase, 0.6 mL/min flow, 60 °C and 20 μL of injection volume. The samples were analyzed in duplicate. The sugar composition of the freeze-dried solids was analyzed following the same procedure.

2.5.3. Galacturonan content

The galacturonan content of the DRB pomace and freeze-dried solids was determined according to the colorimetric method described by Melton and Smith (2001), using galacturonic acid as the standard. Before analysis, the solids were dissolved in ddH_2O (1 g/L). The absorbance was measured at 525 nm in a UV-VIS spectrophotometer (Shimadzu, Japan). The samples were analyzed in triplicate.

2.5.4. Determination of the degree of esterification

The esterification degree of the freeze-dried solids was determined according to Gharibzahedi et al. (2019) and calculated using Eq. (2):

$$\text{Degree of esterification}\ (\%) = \frac{V_2}{V_1 + V_2} \cdot 100 \quad (2)$$

where V_1 is the 0.1 M NaOH volume used to titrate the sample for the first time, and V_2 is the 0.1 M NaOH volume used to titrate the sample for the second time. The samples were analyzed in duplicate.

2.5.5. Molecular weight distribution

The Mw distribution of the freeze-dried solids was evaluated by HPSEC using a refractive index detector (Waters 2414, USA) and an Ultrahydrogel 250 column (Waters, Japan). Ultrapure water at a flow rate of 0.7 mL/min was used as the mobile phase at 35 °C. Dextran standards were used as Mw standards from 1 to 670 kDa. Before analysis, the solids were dissolved in ddH_2O (1 g/L) and passed through 0.22 μm nylon filters. The samples were analyzed in triplicate.

2.5.6. Structural characterization

FTIR and MALDI-TOF MS analyzed the structural characterization of the freeze-dried solids. The surface functional groups were analyzed by FTIR (Alpha model, with a Platinum ATR single reflection diamond module, Bruker, USA). The absorbance spectra were obtained in the wavenumber range from 4000 to 400 cm^{-1}.

The MALDI-TOF MS was carried out in the Laboratory of Instrumental Techniques (LTI) following the slightly modified method described by Gómez et al. (2014). The solids were dissolved in ddH_2O (10 g/L), and the samples were treated with an ion-exchange resin (Lewatit MonoPlus S 108 H), provided by Lanxess AG (Germany), for 30 min at room temperature. After centrifugation, 1 μL of the sample was mixed with 1 μL of the matrix solution (25 mg/mL of 2,5-dihydroxybenzoic acid in acetonitrile 50% and NaCl 2 mM) on a MALDI plate. A MALDI-TOF MS analysis was performed using an Autoflex speed workstation (Bruker Daltonics, Bremen, Germany).

3. Results and discussion

3.1. DRB juice characterization

A complete characterization of the DRB juice was addressed to quantify its bioactive components and its composition can be found in Table 1. Bioactive compounds, such as phenolics and flavonoids, possess functional properties and are used in the food, cosmetic and pharmaceutical industries (Yusoff et al., 2022). The TPC and TFC were 1789 mg GAE/L and 471 mg CE/L, respectively (Table 1). The TPC was slightly higher than that reported for a commercial fruit and vegetable juice (70% apple, 9% strawberry, 12% carrot and beet 9%) (1052 mg/L) by Paredes et al. (2022). Both the TPC and TFC were higher than those determined for orange juice and two varieties of oranges: 'Washington Navel' (25.03 mg GAE/100 mL and 44.31 mg TFC/100 mL) and 'Cara Cara' (21.00 mg GAE/100 mL and 45.60 mg TFC/100 mL) by De Ancos et al. (2020).

The antioxidant capacity was evaluated using three assays: DPPH, FRAP, and ABTS. Compared to 22 other commercial fruit and vegetable juices (tomato, carrot, tropical, apple and vegetable juice), red beetroot juice showed the highest antioxidant capacity (Wootton-Beard et al., 2011). Only lychee juice presents an antioxidant activity, measured by DPPH method of 920 μmol TE/L (Visuthiwan and Assatarakul (2021), higher than that obtained in this research for DRB juice (504 μmol TE/L). The antioxidant capacity of DRB juice evaluated by FRAP and ABTS showed higher values than those reported for other fruit and vegetable juices. In the study of Wang et al. (2022), the antioxidant capacity of kiwifruit juice, determined by FRAP, was around 6000 μmol TE/L as compared to the 10920 μmol TE/L obtained in this work. Paredes et al. (2022) evaluated the antioxidant activity of a commercial juice (70% apple, 9% strawberry, 12% carrot, and 9% beet) by ABTS and achieved 684.33 μmol TE/L, while the ABTS for the DRB juice was 22012 μmol TE/L.

Red beetroot is well known for containing large amounts of betalains (BC and BX), used as natural colorants. Moreover, these pigments possess high amounts of antioxidant potential and are beneficial for human health (Wruss et al., 2015). The total betalain content measured by spectrophotometry was 1426 mg/L (Table 1). The BC and BX concentrations were much higher than those reported by Li et al. (2022) for red dragon fruit peel: around 30 mg BC/L and 20 mg BX/L. The high betalain content of DRB juice compared to other fruit and vegetable juices could explain the high antioxidant activity mentioned above. The DRB juice was rich in total sugars (94.7 g/L). 91.7% of the total sugar content was sucrose. Higher sucrose than fructose content is preferable, since fructose is related to gastrointestinal distress, limits the physiological response, and reduces human exercise capacity (Murray et al., 1989).

Hence, DRB juice is rich in bioactive compounds (phenols and flavonoids), betalains, and sucrose and shows a high antioxidant activity. Moreover, its complete characterization has allowed the DRB juice to be compared with other commercial juices, and its greater bioactive properties have been verified.

3.2. Characterization of DRB pomace

The characterization of the DRB pomace according to NREL methods is shown in Table 2. Galacturonan (32.2%) was the major component, followed by glucan (24.2%) and arabinan (19.5%). The DRB composition was consistent with RG-I pectin. The composition agrees with the data reported by Hotchkiss et al. (2022). The total lignin (5.2%) comprised 96.2% acid-soluble lignin and 3.8% acid-insoluble lignin.

3.3. POS and GalA extraction from DRB pomace by MAE

A central composite experimental design was appraised to study the influence of temperature, time, and PEG4000 concentration on pectin extraction (Table 3). Three experiments (Runs 1, 2, and 6) were carried out at the central point: 130 °C, 10 min, and 6 g PEG4000/L. It should be noted that the surfactant PEG4000 was selected from previous experiments (del Amo-Mateos et al., 2022a) carried out by MAE at 120 °C for

Table 1
Discarded red beetroot juice characterization. Total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity (DPPH, FRAP and ABTS), pigments and sugar concentration.

TPC (mg GAE/L) [a]	TFC (mg CE/L) [b]	Antioxidant capacity (μmolTE/L) [c]			Pigments (mg/L)			Sugar content (g/L)			
		DPPH	FRAP	ABTS	Betacyanins	Betaxanthins	Betalain total	Sucrose	Glucose	Fructose	Total
1789 ± 56	471 ± 17	504 ± 24	10920 ± 440	22012 ± 592	901 ± 9	525 ± 11	1426 ± 24	86.8 ± 0.3	3.8 ± 0.0	4.1 ± 0.1	94.7

[a] GAE: gallic acid equivalent.
[b] CE: catechin equivalent.
[c] TE: trolox equivalent.

Table 2
Discarded red beetroot pomace composition (%, dry weight).

Glucan		24.2 ± 0.2
Arabinan		19.5 ± 0.4
Galactan		7.3 ± 0.1
Galacturonan		32.2 ± 1.5
Extractives in ethanol		10.6 ± 0.0
Extractives in water		15.4 ± 0.8
Composition of extractives in water	Glucose	0.2 ± 0.0
	Arabinose	0.3 ± 0.0
	Galactose	0.6 ± 0.0
	Galacturonic acid	1.2 ± 0.0
Lignin	Acid-soluble lignin	5.0 ± 0.1
	Acid-insoluble lignin	0.2 ± 0.0

Table 3
Conditions of experimental design for galacturonic acid and pectooligosaccharides recovery by microwave-assisted extraction.

Run	Temperature (°C)	Time (min)	PEG 4000 (g/L)	pH	Solid recovery (%)
1	130	10	6	4.9	74.7
2	130	10	6	4.9	75.5
3	160	15	10	4.0	46.2
4	130	18.4	6	4.8	74.8
5	180	10	6	4.1	44.1
6	130	10	6	4.9	76.6
7	79	10	6	6.0	85.0
8	160	5	2	4.4	60.0
9	160	15	2	4.0	48.2
10	130	10	12.72	4.8	79.0
11	130	10	0	5.1	77.2
12	160	5	10	4.2	55.7
13	100	5	2	5.8	85.0
14	100	15	2	5.5	83.2
15	130	1.6	6	5.2	81.3
16	100	5	10	5.8	86.9
17	100	15	10	5.6	85.1

10 min, comparing the efficiency of different surfactants (PEG4000, PEG8000, Tween-80 and tea saponin at a concentration of 8 g/L). Under these conditions, POS recovery was higher when PEG4000 was used.

The pH of the hydrolysates and solid recoveries are shown in Table 3. The mean pH values and solid recovery at the central point were 4.9 ± 0.0 and 75.6 ± 1.0%, respectively. Temperature affected both the pH of the hydrolysates and solid recovery. Lower solid recoveries and pH were obtained under higher temperatures (Runs 3, 5, and 9). The pH of the hydrolysate was 4.1, and 44.1% of the solid was recovered at 180 °C (Run 5); while at 79 °C (Run 7), the hydrolysate pH increased to 6.0 and the solid recovery to 85.0%. On the other hand, pH values and solid recoveries varied slightly when time (central point and Runs 4 and 15) or PEG4000 concentration (central point and Runs 10 and 11) were modified. Statistical analysis ($p < 0.05$) concluded that pH and solid recovery were significantly affected by temperature, but not by time and PEG4000 concentration. Shafie et al. (2019) also showed that temperature had a much greater effect on pectin extraction than the type of solvent used or the time. Regarding degradation compounds (Table S1), HMF and furfural were not detected in any experimental runs. Formic acid concentrations were lower than 1 g/L in all the experiments; whereas acetic acid was detected in concentrations higher than 1 g/L in the experiments carried out at high temperatures (160 and 180 °C). Acetic acid was also the most abundant degradation compound found in the research developed by Chadni et al. (2019), who extracted pectin from spruce wood by MAE with an alkali solvent. However, the degradation compounds reached a concentration of 8 g/L (Chadni et al., 2019), considerably higher than the highest found in this study (3.5 g/L, Run 5). This fact shows that the use of non-alkali or -acid solutions minimizes the concentration of the degradation compounds.

Regarding the composition of the hydrolysates (Fig. 1), the POS was calculated as the sum of GalA, arabinose, and galactose in oligomeric form. The average yield in the central point was: 2.0 ± 0.1 g glucose, 36.4 ± 1.5 g arabinose, 15.8 ± 0.7 g galactose, 123.2 ± 3.2 g GalA, and 173.6 ± 4.8 g POS per kg of dry DRB pomace. The POS (Fig. 1e) and GalA (Fig. 1d) production ranged from 79.2 g/kg (Run 7) and 29.2 g/kg (Run 5) to 270.9 g/kg (Run 12) and 126.0 g/kg (Run 2), respectively. The use of PEG4000 significantly increased both the POS and GalA extraction. 172.9 g POS/kg and 123.1 g GalA/kg were recovered using 12.72 g PEG4000/L as solvent (Run 10), which is an increment of 22% and 18%, respectively, compared to that obtained when water was used as the solvent (Run 11). The higher extraction of pectin when the concentration of surfactant is higher may be due to the ability of PEG4000 to form micelles, which interact chemically and physically with the wide polarity range of pectin, increasing the extraction yield (Su et al., 2019). The POS composition mainly depended on temperature. Arabinose was the main component in runs carried out at 160 °C (Runs 3, 8, 9, and 12) and 180 °C (Run 5), while GalA was the most predominant component at lower temperatures. This is in agreement with previous results. Chadni et al. (2019) studied the effect of microwave power (125–573 W) on POS extraction from spruce wood. The composition of the POS extract varied depending on the microwave power. The extraction results using water as solvent showed that the sugar content increased when the power rose from 125 to 573 W, while the highest GalA content was found at 125 W.

3.4. MAE conditions optimization for POS and GalA extraction

In order to obtain the conditions for POS or GalA recovery, these two responses were maximized. Two second-order polynomial equations for POS (Eq. (3)) and GalA (Eq. (4)) concentrations were proposed to predict the response from the experimental data.

$$\text{POS (g/kg DRBpomace)} = 114.16 - 2.25\cdot T + 7.67\cdot t - 6.90\cdot C - 53.63\cdot 10^{-3}\cdot T\cdot t + 6.49\cdot 10^{-2}\cdot T\cdot C + 19.28\cdot T^2 \quad (3)$$

$$R^2 = 0.984;\ R^2_{adjusted} = 0.973$$

$$\text{GalA (g/kg DRBpomace)} = -696.14 + 11.51\cdot T + 10.87\cdot t - 85.98\cdot 10^{-3}\cdot T\cdot t - 4.03\cdot 10^{-2}\cdot T^2 \quad (4)$$

$$R^2 = 0.944;\ R^2_{adjusted} = 0.923$$

where T is the temperature (°C), t is the time (min), and C is the PEG4000 concentration (g/L). Both POS and GalA concentrations were predictive with a confidence level of 95% ($p < 0.05$).

Fig. 2 represents the surface area of the experimental design. Fig. 2a and b shows the effect of temperature, time, and PEG4000 concentration on POS extraction. As can be seen, a higher POS extraction was obtained under higher temperatures, while the time and PEG4000 concentration barely affected it. Based on Eq. (3), the quadratic temperature, time, and the combined effect of the temperature and PEG4000 concentration have a positive impact on POS extraction, the temperature being the most significant. On the contrary, Fig. 2c represents the effect of temperature and time on the GalA extraction. The temperature effect was different for GalA than for POS extraction. The surface graph shows a maximum GalA concentration at around 130 °C; after that, the GalA concentration decreases. This might be because GalA degradation occurs at lower temperatures than pentoses (Usuki et al., 2008) and hexoses (Khajavi et al., 2005). Eq. (4) revealed that the GalA extraction was positively affected by temperature and time. The combined effect of temperature and time and quadratic temperature showed a negative effect. The PEG4000 concentration was not significant for GalA extraction. Based on these models, the optimal conditions were: 160 °C, 5.27 min, and 8.44 g PEG4000/L for maximizing POS extraction and 137 °C,

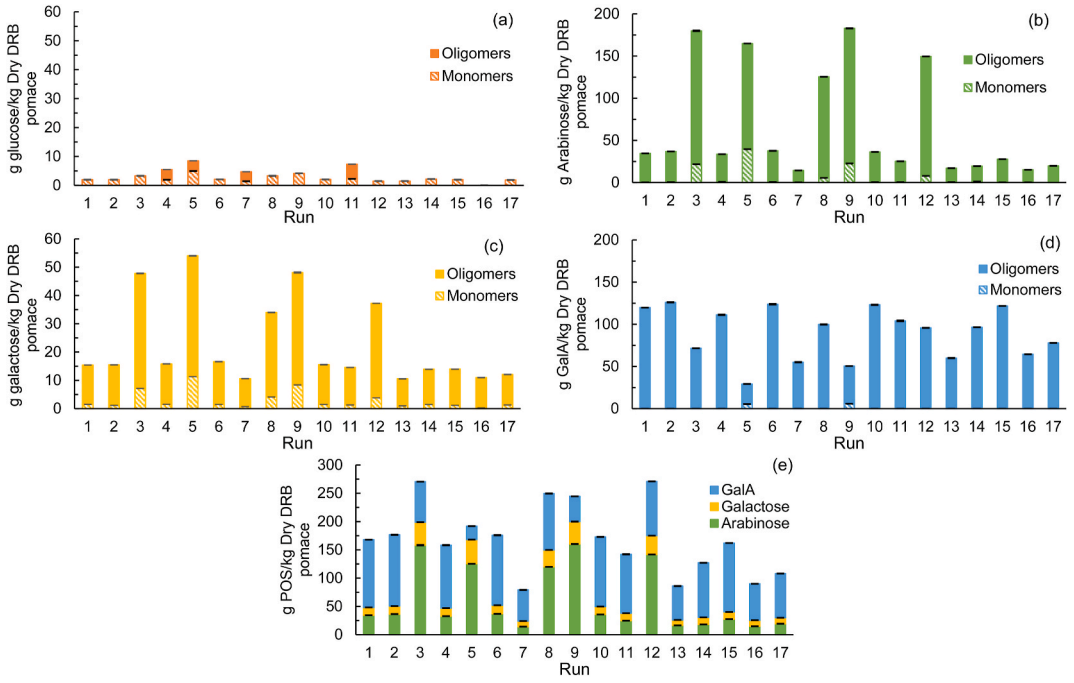


Fig. 1. Galacturonic acid and pecto-oligosaccharides recovery by microwave-assisted extraction from discarded red beetroot pomace: hydrolysates composition (a-Glucose, b-Arabinose, c-Galactose, d-Galacturonic acid, e-pectooligosaccharides, POS).

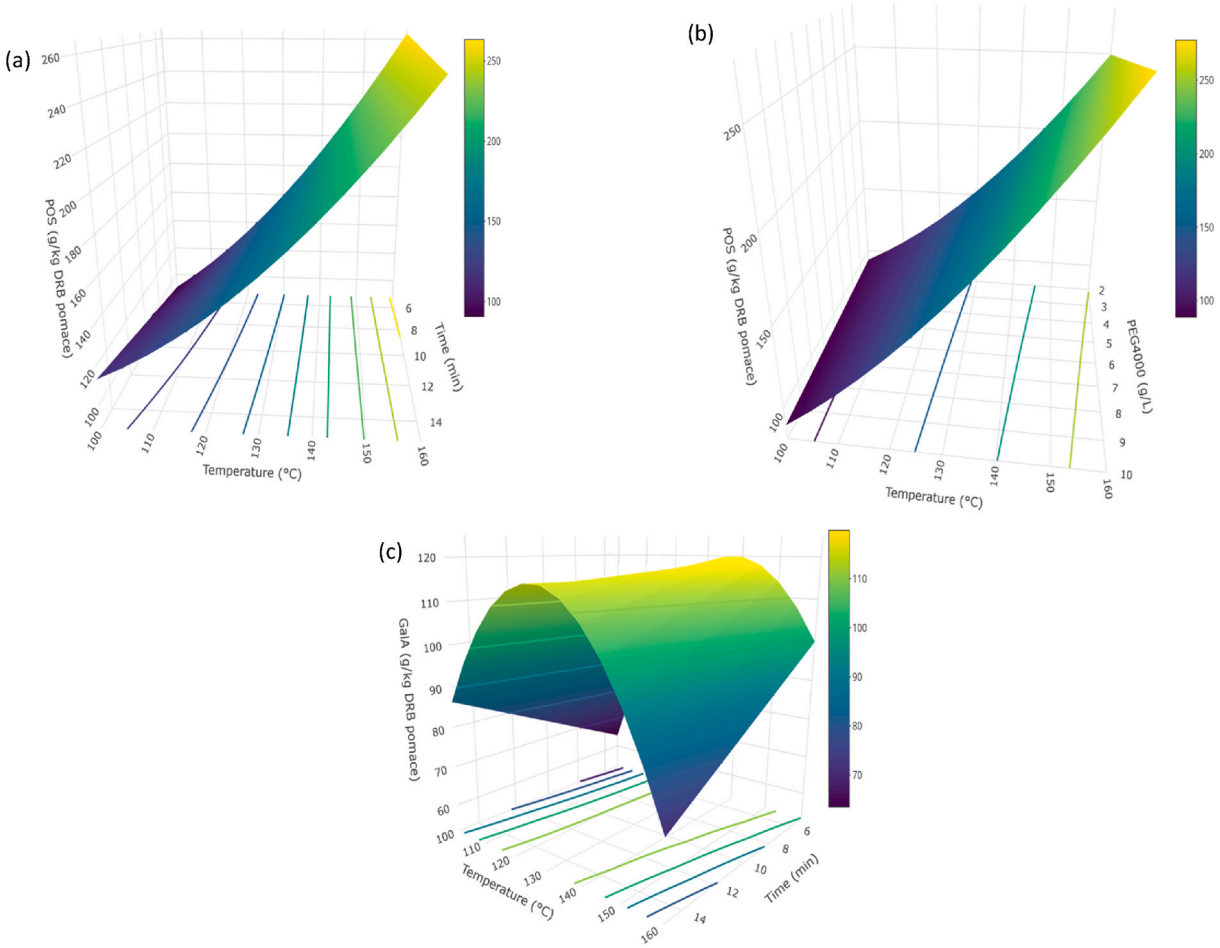


Fig. 2. Response surface of experimental design: influence of experimental parameters on POS (a,b) and GalA (c) concentration in the hydrolysates.

5 min, and 2.55 g PEG4000/L for maximizing GalA recovery.

In order to validate the models, experimental runs under optimal conditions were carried out in triplicate. Table 4 shows the pH of the hydrolysate, solid recoveries, and hydrolysate compositions under optimal conditions. The POS and GalA concentrations predicted by the model under their respective optimal conditions were 271.2 g POS and 120.1 g per kg of dry DRB pomace. The ANOVA concluded that there were no significant differences ($p < 0.05$) between the experimental and predicted values. Thus, a good agreement was established between the proposed models and the experimental results. The concentrations of degradation compounds were lower than 1 g/L (Table 4).

There is a gap in the literature on the composition of hydrolysates obtained after pectin extraction from DRB pomace. Other research works studied the extraction of pectin and oligosaccharides from different biomasses, such as Ramos-Andrés et al. (2021), who studied pectin extraction from carrot pulp by hydrothermal extraction. The highest arabinogalactan and pectin extraction yields were 72.48 g (160 °C) and 29.13 g (180 °C) per kg of dry pulp. del Castillo-Llamosas et al. (2021) obtained 14.3 g oligosaccharides and 6.51 g GalA per 100 g of avocado peel by autohydrolysis at 150 °C. Chadni et al. (2019) evaluated conventional and microwave extraction of hemicelluloses in neutral and basic mediums from spruce wood. The highest yield of hemicellulose was 32.3 mg/g dry spruce wood (MAE in basic medium at 573 W), and of GalA, around 1.5 mg/g dry spruce wood (conventional extraction in basic medium). Both POS (267.4 g/kg dry DRB pomace) and GalA (120.1 g/kg dry DRB pomace) extraction yields found in this study were much higher than those reported in the literature. Thus, DRB is a suitable raw material for pectin production by MAE combined with PEG4000.

3.5. Characteristics of the freeze-dried solids

The hydrolysates obtained under optimal conditions were vacuum concentrated and freeze-dried. The solid obtained from the POS hydrolysate was noted as POS-S; whereas that from the GalA hydrolysate was denominated GalA-S.

Both solids were analyzed for their composition, esterification degree, Mw distribution, and structural characterization. Yield, composition, and esterification degree are all shown in Table 5. Yields were 616 g of POS-S and 413 g of GalA-S per kg of dry DRB pomace. Galacturonan was the major component in both solids. However, the galacturonan concentration in GalA-S was 49.5%, while it reached 27.2% in POS-S. Hotchkiss et al. (2022) also found galacturonic acid to be the major component in red beet pectin. Arabinan was the next main component, followed by galactan. Based on the composition, highly branched pectin from the RG-I region was obtained in POS-S; while GalA-S was mainly made up of slightly branched HG pectin. The degree of esterification of both pectins was higher than 50%, so it can be considered a high-methoxyl pectin. However, the esterification of POS-S was lower than that of GalA-S, which may be due to their composition. The degree of esterification depends on the GalA residues that have formed methyl esters. As there is more content in GalA, there is a greater possibility of forming methyl esters and, thus, a higher degree of esterification in GalA-S than in POS-S. HPSEC confirmed the presence of high Mw compounds in both solids. The main Mw of the compounds found in POS-S were 561 ± 2 and 239 ± 11 kDa. GalA-S showed similar Mw distributions: 502 ± 2 and 264 ± 5 kDa. These values were lower than those reported by Hotchkiss et al. (2022), 581 kDa for pectin recovered from red beetroot by isopropanol precipitation after MAE (6 min, 120 °C). The differences could be related to the recovery method. Only high Mw particles precipitate in alcohol, while the small molecules remain in the supernatant. After freeze-drying, molecules with high and small Mw remain in the solids.

The surface chemical structure of the solids was revealed by FTIR analysis. Fig. 3 shows the FTIR spectrum of POS-S (Fig. 3a) and GalA-S (Fig. 3b) in the range of 4000–400 cm^{-1}. The patterns obtained show the typical pectin functional groups. Both patterns showed a broad band at around 3400 cm^{-1} that can be attributed to hydroxyl groups. Peaks at around 2900 cm^{-1} corresponded to the C–H stretching of the CH_2 groups (Jiang et al., 2012). Esterified carboxyl groups and non-esterified carboxyl groups were identified at 1730 and 1600 cm^{-1}, respectively

Table 4
Hydrolysates after microwave-assisted extraction under optimal conditions for pectooligosaccharides (POS) and galacturonic acid (GalA) recovery: pH, solid recovery, and composition.

	POS hydrolysate [a]	GalA hydrolysate [b]
pH	4.1 ± 0.1	4.7 ± 0.0
Solid recovery (%)	58.0 ± 2.3	73.5 ± 0.4
Glucose (g/kg Dry DRB pomace)	2.4 ± 0.0	1.9 ± 0.0
Arabinose (g/kg Dry DRB pomace)	140.4 ± 0.3	41.2 ± 0.5
Galactose (g/kg Dry DRB pomace)	37.0 ± 0.7	17.0 ± 0.0
Galacturonic acid (g/kg Dry DRB pomace)	100.7 ± 0.2	120.1 ± 0.2
POS (g/kg Dry DRB pomace)	267.4 ± 1.0	175.8 ± 0.6
Formic acid (g/L)	0.4 ± 0.0	0.1 ± 0.0
Acetic acid (g/L)	0.9 ± 0.0	0.3 ± 0.0
HMF (g/L)	0.0 ± 0.0	0.0 ± 0.0
Furfural (g/L)	0.0 ± 0.0	0.0 ± 0.0

[a] Optimal conditions:160 °C, 5.27 min, 8.44 g PEG 4000/L.
[b] Optimal conditions: 137 °C, 5 min, 2.55 g PEG 4000/L.

Table 5
Solids obtained from hydrolysates under optimal conditions for pectooligosaccharides (POS-S) and galacturonic acid (GalA-S) recovery: yield, composition, and esterification degree.

	POS-S	GalA-S
Yield (g solid/kg dry DRB)	616 ± 30	413 ± 2
Arabinan (g/100 g solid)	19.4 ± 0.6	7.5 ± 0.4
Galactan (g/100 g solid)	5.8 ± 0.3	3.9 ± 0.0
Galacturonan (g/100 g solid)	27.2 ± 0.2	49.5 ± 2.3
Esterification Degree (%)	57.8 ± 1.8	78.3 ± 0.7

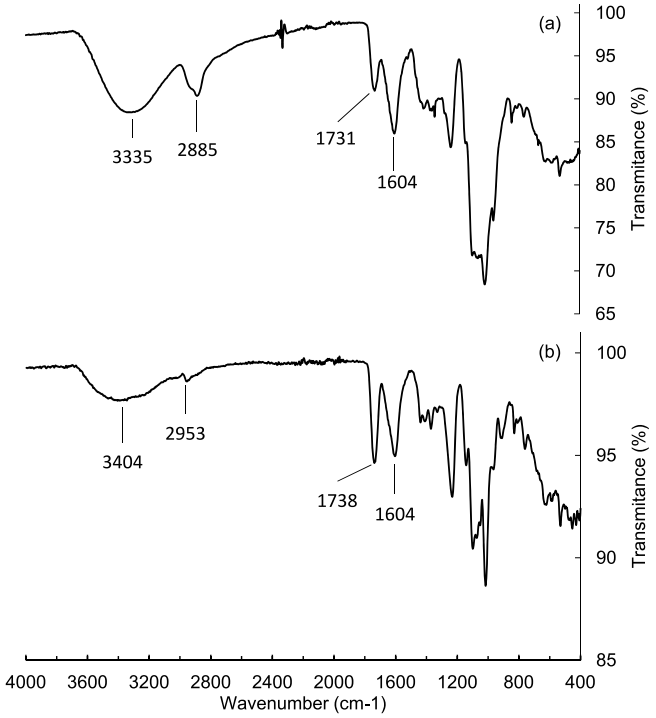
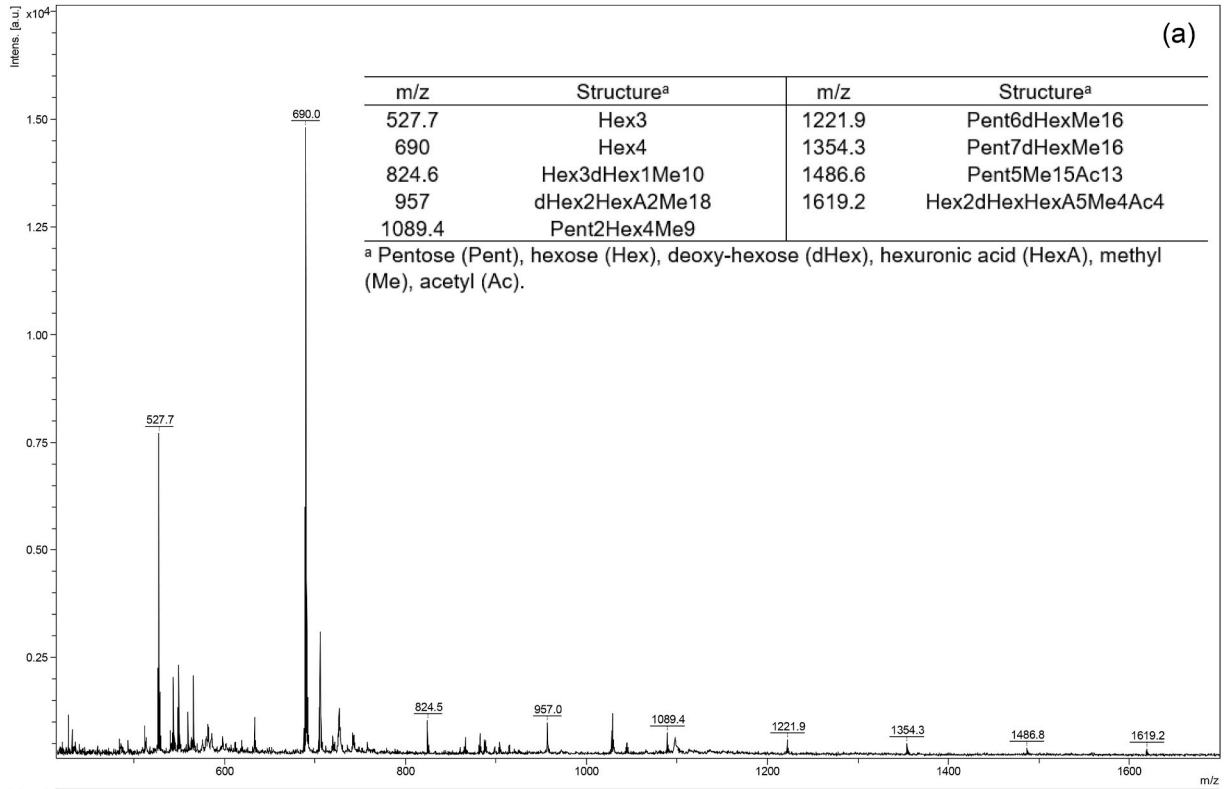


Fig. 3. FTIR spectra of the POS-S (a) and GalA-S (b).

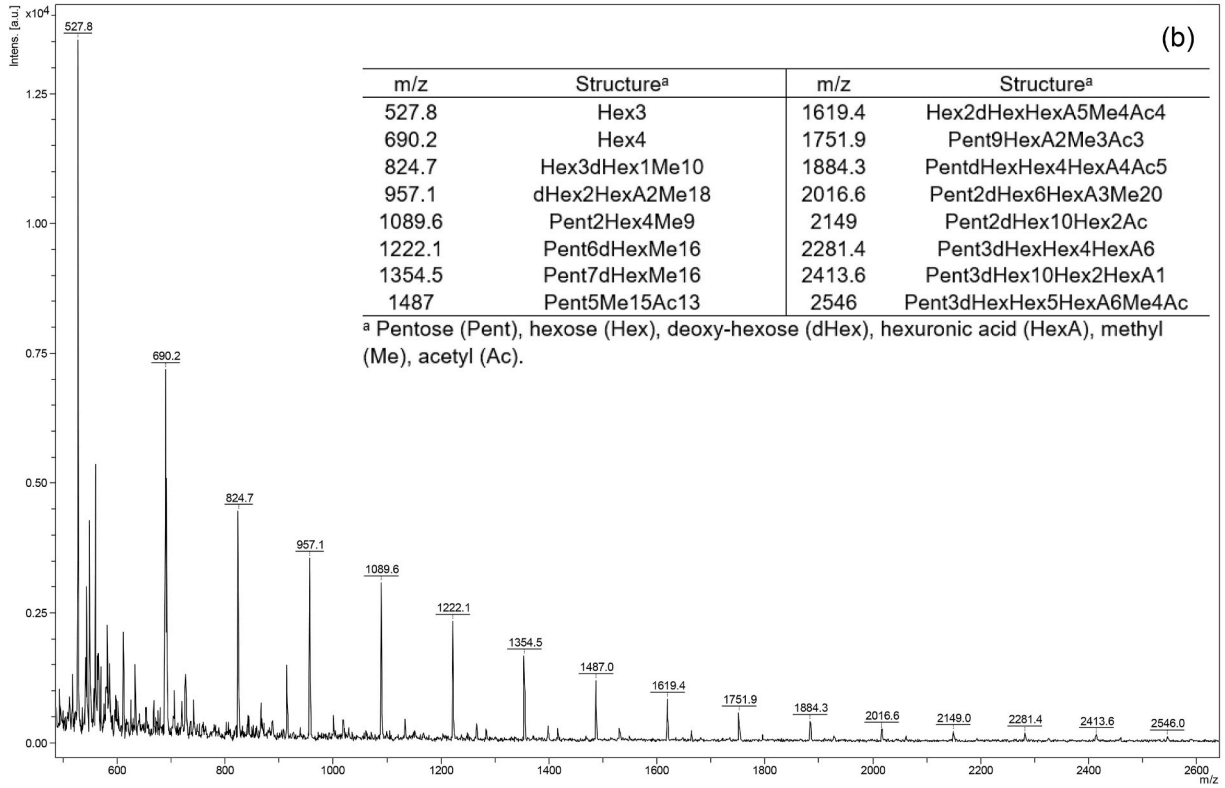
(Lim et al., 2012). The ratio between the two peaks was different depending on the sample. A higher esterification degree (GalA-S) pattern showed a greater intensity ratio of ester to non-ester carboxyl peaks. In addition, absorption peaks found at 1300–800 cm^{-1} corresponded to the functional groups C–O–C, OH, and CH_3 (Jiang et al., 2012). Bands at 800–400 cm^{-1} were regarded as O–C–O, C–O=O, O–C=O, and CH deformation (Jiang et al., 2012).

The MALDI-TOF mass spectrums and the suggested structures of compounds in the freeze-dried hydrolysates are shown in Fig. 4. Hexose oligosaccharides were observed in both solids. Based on the composition of the solids (Table 5), these oligosaccharides can be attributed to galactooligosaccharides, galacto-tetraose being the most abundant in POS-



m/z	Structure[a]	m/z	Structure[a]
527.7	Hex3	1221.9	Pent6dHexMe16
690	Hex4	1354.3	Pent7dHexMe16
824.6	Hex3dHex1Me10	1486.6	Pent5Me15Ac13
957	dHex2HexA2Me18	1619.2	Hex2dHexHexA5Me4Ac4
1089.4	Pent2Hex4Me9		

[a] Pentose (Pent), hexose (Hex), deoxy-hexose (dHex), hexuronic acid (HexA), methyl (Me), acetyl (Ac).



m/z	Structure[a]	m/z	Structure[a]
527.8	Hex3	1619.4	Hex2dHexHexA5Me4Ac4
690.2	Hex4	1751.9	Pent9HexA2Me3Ac3
824.7	Hex3dHex1Me10	1884.3	PentdHexHex4HexA4Ac5
957.1	dHex2HexA2Me18	2016.6	Pent2dHex6HexA3Me20
1089.6	Pent2Hex4Me9	2149	Pent2dHex10Hex2Ac
1222.1	Pent6dHexMe16	2281.4	Pent3dHexHex4HexA6
1354.5	Pent7dHexMe16	2413.6	Pent3dHex10Hex2HexA1
1487	Pent5Me15Ac13	2546	Pent3dHexHex5HexA6Me4Ac

[a] Pentose (Pent), hexose (Hex), deoxy-hexose (dHex), hexuronic acid (HexA), methyl (Me), acetyl (Ac).

Fig. 4. MALDI-TOF mass spectrum of POS-S (a) and GalA-S (b).

S and galacto-triose in GalA-S. Other oligosaccharide structures were identified, and based on the composition, the hexuronic acid can be GalA. The oligosaccharide structures were consistent with galacturonic acid, with arabinogalactooligosaccharide side chains or free neutral sugar oligosaccharides. Galacturonan was the major component of the solids (Table 5). However, a low number of molecules of hexuronic acid were detected in oligosaccharides structures compared to neutral sugars. This might be due to the fact that GalA is part of the polysaccharide structure of pectin. Methyl and acetyl groups were identified in some oligosaccharide structures. The studies of Gómez et al. (2016, 2019) confirmed the prebiotic properties of oligosaccharides derived from pectin. Thus, the oligosaccharide structures found in this research might indicate the potential of the freeze-dried solids as prebiotics, which will be addressed in future research.

4. Conclusions

This study assesses the valorization of DRB through the recovery of high-value bioactive compounds in a biorefinery approach. The DRB juice contains higher levels of antioxidants, phenolics and flavonoids than other commercial juices reported in the literature. Betalains in the DRB juice (1426 ± 24 mg/L) are rich in antioxidants beneficial for human health. The DRB pomace can be valorized through pectin extraction by microwave in the presence of a surfactant (PEG4000). Moderate extraction conditions (137 °C, 5 min, 2.5 g PEG4000/L) are required to maximize the galacturonic acid recovery (120.1 g GalA/kg dry pomace), as it degrades at a lower temperature than pentoses. The structural characterization of the freeze-dried hydrolysates by FTIR and MALDI-TOF-MS confirmed the extraction of high-methoxyl pectin and the presence of oligosaccharides of different structures and degree of polymerization, depending on the extraction conditions. Future work needs to analyze the functional properties of the freeze-dried hydrolysates.

CRediT authorship contribution statement

Esther del Amo-Mateos: Methodology, Investigation, Data curation, Writing – original draft. **Marina Fernández-Delgado:** Methodology, Investigation. **Susana Lucas:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Juan Carlos López-Linares:** Methodology, Formal analysis. **M. Teresa García-Cubero:** Methodology, Funding acquisition, Project administration. **Mónica Coca:** Conceptualization, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors acknowledge the financial support from the Spanish Ministry of Science and Innovation (project PID2020-115110RB-I00/AEI/10.13039/501100011033) and the Junta de Castilla y León (UIC 320, VAG028G19, CLU-2017-09). Esther del Amo-Mateos would also like to thank the Junta de Castilla y León for providing her PhD grant (REF EDU/875/2021). The authors thank the company HUERCASA for providing the discarded red beetroot. The authors also acknowledge Nora Carrera, a researcher at the Laboratory of Instrumental Techniques (University of Valladolid), for her assistance on MALDI-TOF-MS analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2023.135995.

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