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Recovering rhamnogalacturonan-I pectin from sugar beet pulp using a sequential ultrasound and microwave-assisted extraction: study on extraction optimization and membrane purification

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Corresponding Author:	Susana Lucas, Ph. D. University of Valladolid Valladolid, Valladolid SPAIN
First Author:	Esther del Amo-Mateos
Order of Authors:	Esther del Amo-Mateos
	Berta Cáceres
	Mónica Coca
	M. Teresa García-Cubero
	Susana Lucas, Ph. D.
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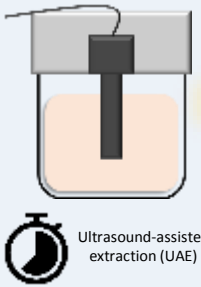
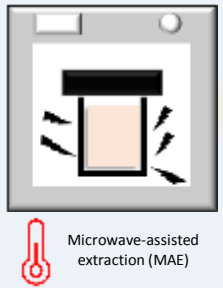
Graphical Abstract (for review)

Extraction process

Sugar beet pulp

100 g



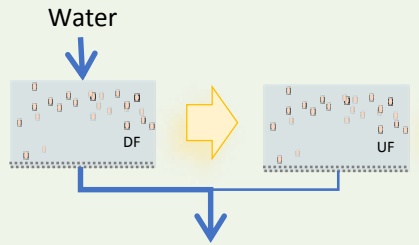


Sequential UAE – MAE
Box-Behnken Design:
Initial pH, UAE-time, MAE-temperature

POS* yield: 66.0 %

POS: pectooligosaccharides

Purification process



Continuous Diafiltration + Ultrafiltration
Membranes: **3** and 5 kDa

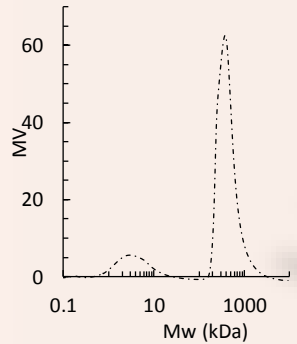
POS recovery: 90.0 %

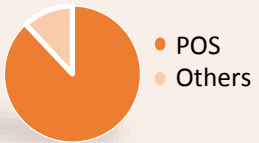
Freeze-drying

Characterization

RG-I rich pectin

39.7 g





- Efficient RG-I pectin recovery with sequential UAE/MAE and membrane purification.
- Use of short-time UAE decreased MAE time and temperature.
- Optimal recovery of 66.0 % of pectooligosaccharides from sugar beet pulp.
- The 90 % of POS were recovered using continuous DF and UF with 3 kDa MWCO membrane.
- High-purity RG-I pectin with high-methoxyl attributes similar to prebiotics.

Recovering rhamnogalacturonan-I pectin from sugar beet pulp using a sequential ultrasound and microwave-assisted extraction: study on extraction optimization and membrane purification

Esther del Amo-Mateos[a,b], Berta Cáceres[b], Mónica Coca[a,b], M. Teresa García-Cubero[a,b], Susana Lucas[a,b,*]

[a] Institute of Sustainable Processes. University of Valladolid, Spain

[b] Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina, s/n, Valladolid, Spain

*Corresponding author. E-mail address: susana.lucas.yague@uva.es (S. Lucas)

E-mail addresses: esther.amo@uva.es (E. Del Amo-Mateos), berta.caceres@estudiantes.uva.es (B. Cáceres), monica.coca@uva.es (M. Coca), mtgarcia@uva.es (M. T. García-Cubero), susana.lucas.yague@uva.es (S. Lucas)

Abstract

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Keywords

Pectooligosaccharides, Box-Behnken design, green extraction, ultrafiltration, diafiltration

1 Introduction

Pectin is a valuable polysaccharide that can be extracted from various plant sources. It has many applications in the food, pharmaceutical, and biomedical sectors due to its gelling, thickening, stabilizing, and emulsifying properties, as well as its health benefits as a dietary fiber and prebiotic (Chandel et al., 2022). However, pectin is not a homogeneous substance, but rather a complex mixture of different structural elements, such as

homogalacturonan (HG), rhamnogalacturonan-I (RG–I), and rhamnogalacturonan-II (RG–II) (del Amo-Mateos et al., 2023). Of these, the RG–I region is a branched pectic domain that consists of a backbone of alternating galacturonic acid (GalA) and rhamnose units, with various side chains of arabinose and galactose (Y. Mao et al., 2019). RG–I is particularly interesting for its potential applications in food and pharmaceutical products, as it can modulate the rheological properties of pectin gels, enhance the stability of emulsions, and exhibit biological activities such as anti-inflammatory, anti-cancer, and immunomodulatory effects (G. Mao et al., 2019). Therefore, recovering pectin from plant sources and optimizing the extraction methods to preserve the RG–I structure are important research topics that can lead to the development of novel and functional pectin-based products such as pectooligosaccharides (POS). POS are known for their potential prebiotic properties with a superior ability to regulate the human intestine microbiota (Y. Mao et al., 2019).

Pectin is extracted mainly from citrus and apple fruits due to the composition (Chandel et al., 2022). However, using waste or by-products can enhance the industry's sustainability and feasibility. Sugar beet pulp (SBP), a by-product generated after sugar extraction, has a global production of approximately 120 million tons (90 % humidity) (Bonnin et al., 2009). SBP is a fibrous material rich in cellulose, hemicellulose, and pectin, with a predominant presence of RG–I pectin (Y. Mao et al., 2019). Due to its availability and price, it has been used for low-value applications such as animal feed, while SBP stands as a valuable raw material for RG–I pectin extraction.

Extracting RG–I pectin is challenging, as conventional extraction of HG pectin using acidic hot solutions (pH 1.5 – 3, 60 – 100 °C) over several hours is ineffective (G. Mao et al., 2019). Under these conditions, the side chains present in the hairy region are degraded. Moreover, commercial extraction requires large amounts of solvent and high energy and water consumption (Chandel et al., 2022). Emerging technologies such as ultrasound and

51 microwave-assisted extraction have received research attention to address the
52 conventional extraction limitations.
53 Ultrasound-assisted extraction (UAE), a non-thermal extraction method, utilizes high-
54 frequency sound waves to create cavitation bubbles that disrupt plant tissues. Thus, the
55 solvent can easily penetrate the cells and facilitate pectin release. Previous research
56 employed UAE for pectin recovery from waste grapefruit peel using HCl (pH 1.5) as solvent
57 obtaining a yield of 23.49 % with an RG-I content of 38.31 % (Wang et al., 2017).
58 Microwave-assisted extraction (MAE), a thermal extraction technique, employs
59 electromagnetic waves to heat the sample rapidly, promoting the breakdown of cell walls
60 and the release of pectin (Marić et al., 2018). Some advantages of MAE over conventional
61 heating are homogeneous energy dispersion and fast heating. MAE has been studied for
62 pectin extraction from SBP under alkaline conditions (pH 13) at 90 °C for 120 min achieving
63 a yield of 23.4 % rich in sugars (60.09 %) (Y. Mao et al., 2019).
64 A growing research interest is integrating ultrasound and microwave-assisted extraction
65 (UMAE) to combine their advantages. This hybrid approach can accelerate extraction and
66 minimize energy consumption, resulting in higher pectin yields and enhanced quality
67 (Gharibzahedi et al., 2019). Based on their mechanism extraction, the sequential use of
68 UAE followed by MAE may reduce MAE operation times and temperatures, thereby
69 minimizing RG-I pectin degradation and energy consumption (Liew et al., 2016).
70 Consequently, the combination of these emerging technologies offers a promising path
71 forward for the efficient and sustainable production of RG-I pectin. Gharibzahedi et al.
72 (2019) compared the acidic hot water and sequential UMAE extraction method for pectin
73 recovery from fig skin increasing the yield from 6.05 % to 13.97 %. Liew et al. (2016)
74 compared MAE, UAE, sequential UMAE and microwave-ultrasound extraction of pectin
75 from pomelo peels achieving the highest yield using UMAE (36.33 %) and the lowest with

UAE (14.25 %). However, to the best of the author’s knowledge, RG-I region extraction has not previously been reported using the combination of these emerging technologies.

After pectin extraction, a purification method is essential to remove impurities that may be present in the extract. Diafiltration (DF) and ultrafiltration (UF) are promising options due to their cost-effectiveness, scalability, and low energy and solvent requirements (Ramos-Andrés et al., 2021). However, pectin extracts are complex and can lead to membrane fouling, reducing the permeate flux (Jin et al., 2022). Some research studies have applied membrane processes to purify pectin by discontinuous DF (Gómez et al., 2013; Ramos-Andrés et al., 2021). Continuous DF can mitigate membrane fouling by reducing the feed solution's viscosity, while subsequent UF processes can be employed to concentrate the extract.

This study develops an integrated process for RG-I pectin extraction from SBP through sequential UMAE and a purification process of the extract obtained by continuous DF and UF. The extraction process of RG-I pectin employing sequential UAE an MAE was optimized using a Box-Behnken design. The purification of the optimized extract based on a continuous DF and UF process was assessed and, to that end, the comparison of the performance of membranes with different MWCO, according to compound recovery and membrane fouling, was carried out. The characterization of the RG-I pectin-rich solids obtained was studied in terms of composition, molecular weight, degree of esterification and the chemical structure of the surface. To the best of author’s knowledge, this is the first work proposing an integrated process using sequential UMAE and subsequent purification by a continuous DF and UF membrane process, with promising results for the recovery of RG-I pectin, with potential prebiotic applications, from SBP. This research proposes a significant advance in the development of efficient technologies for POS recovery from a

byproduct, which is in line with the sustainable development goal 12: Ensure sustainable consumption and production patterns and goal 7: Affordable and clean energy.

2 Materials and methods

2.1 Raw material

AB Azucarera Iberia kindly supplied SBP, which was washed, dried at 60 °C and ground (particle size < 1 mm) before use.

2.2 Sequential UMAE for RG–I pectin extraction

Pectin from SBP was extracted using a two-step approach involving UAE followed by MAE. UAE was conducted in ultrasound equipment with direct sonification (20 kHz) (Hielscher Ultrasound Technology UIP1000hd transducer, Hielscher Ultrasonics GmbH, Germany). Based on previous studies, the amplitude was 90 %, and the operating temperature was 70 °C (Fernández-Delgado et al., 2023). Once the UAE was completed, the mixture was subjected to MAE, performed in a closed microwave-assisted reaction system (Multiwave PRO SOLV reactor 50 Hz with a Rotor type 16HF100, Anton Paar GmbH, Austria, Europe). The slurry from the UAE was placed into vessels made of PTFE-TFM (volume capacity of 100 mL) provided with magnetic stirrers. The temperature and pressure of each vessel were continuously recorded by an infrared sensor, and a temperature/pressure sensor controlled the microwave power (del Amo-Mateos et al., 2022). The MAE operation time was 9.4 min, corresponding to the optimum time extraction previously established for OGalA (del Amo-Mateos et al., 2022).

The SBP and the diluted acid solution, prepared by adjusting the pH using H_2SO_4, were mixed in a solid-to-liquid ratio of 10 % (w/V) (15 g dried SBP and 150 mL diluted acid solution). After the UMAE, the slurry was vacuum filtrated. The solid fraction was dried at 60 °C and weighed for solid recovery determination (g spent solid/g SBP). The liquid was

stored at 4 °C for further analysis (monomeric and oligomeric compounds and degradation products).

2.3 Experimental design for RG-I-pectin extraction

To optimize the conditions for RG-I pectin extraction by UMAE, a response surface method with a Box-Behnken design (RSM-BBD) was planned. The parameters selected were extraction pH (X_1, 1 – 4), UAE time (X_2, 5 –25 min), and MAE temperature (X_3, 120 – 160 °C).

The response variable was the concentration of POS, calculated as the sum of oligogalacturononoides (OGalA), galactooligosaccharides (GalOS), rhamnooligosaccharides (RhaOS) and arabinooligosaccharides (AraOS). Thus, 15 experiments (Table 1) were carried out, including three replicates at the center of the design. The experimental results were analyzed by the software Statgraphics Centurion XVIII. Lastly, three confirmatory runs were performed under the optimal conditions for POS extraction to verify the results obtained by the statistical software.

2.4 POS purification and concentration by DF and UF

The extract obtained under optimal UMAE conditions was first subjected to dead-end microfiltration through a polyethylene sulfone filter (0.45 μm) to remove larger particles. It was then refined in a two-step membrane process (continuous diafiltration and ultrafiltration) (Fig. 1). The purification process was compared using two membranes with different MWCO: 3 and 5 kDa. The extract was processed in a Labscale tangential flow filtration (TFF) system (Millipore Corporation, United States) using a membrane with a MWCO of 5 kDa (Pellicon XL 5 kDa Biomax membrane, Millipore Corporation) and a Minimate TFF system (Pall Corporation, USA) with a membrane of 3 kDa MWCO (Minimate TFF Capsule with Omega 3K membrane, Pall Corporation). Both membranes were made of polyethersulfone (PES) and had a filtration area of 50 cm^2. Feed pressure was provided by

a peristaltic pump, and retentate pressure was controlled by a regulator. Both feed and retentate pressures were measured by a gauge attached to the feed and retentate streams. The experimental runs were performed at room temperature, the transmembrane pressure (TMP) was set to $1.38 \cdot 10^5$ Pa, and the solution was continuously stirred with a magnetic stirrer. To reduce the viscosity and enhance the removal of impurities and small molecules in the DF step, the extract (150 mL) was diluted with water to a total volume of 500 mL before being subjected to continuous diafiltration. After obtaining a total permeate volume of 270 mL, the retentate was subjected to ultrafiltration until the retentate was concentrated up to 2.2 times.

The recovery yields of a component i (monosaccharides, oligosaccharides, degradation compounds) in the retentate were calculated using equation 1:

$$Y_i = \frac{C_{R_{UF}i} \cdot V_{R_{UF}}}{C_{0i} \cdot V_0} \cdot 100 \quad (1)$$

where C and V refer to concentration and volume, respectively, and the subscripts R_{UF} to the retentate after the ultrafiltration process, and 0 to the extract obtained under optimal conditions (Fig. 1).

The retentates obtained, R_{UF} – 3 and R_{UF} – 5 (Fig. 1), were analyzed for their monomeric, oligomeric, and degradation compounds content, freeze-dried (Telstar LyoQuest 55) and stored for further analysis.

2.5 Model for membrane fouling

The permeate flux of particle-free water across the clean membrane is described by Darcy's law as:

$$J_w = \frac{\Delta p}{\mu \cdot R_m} \quad (2)$$

where J_w is the water permeation flux ($m^3/(m^2 \cdot s)$), Δp is the TMP (Pa), μ is the viscosity of the water (Pa·s), and R_m is the clean membrane resistance (m^{-1}).

171 The permeate flux during the UF process can be expressed using equations 3 and 4.

$$J = \frac{V_P}{A \cdot t} \quad (3)$$

$$J = \frac{\Delta p}{\mu \cdot R_T} \quad (4)$$

172 where J is the permeate flux ($m^3/(m^2 \cdot s)$), V_P is the permeate volume (m^3), A is the filtration
173 membrane area (m^2), t is the filtration time (s), Δp is the TMP (Pa), μ is the viscosity of the
174 sample considered as water (Pa·s), and R_T is the total resistance (m^{-1}). The total resistance
175 (R_T) in a membrane system is the sum of the membrane (R_m) and fouling (R_F) resistances
176 (equation 5):

$$R_T = R_m + R_F \quad (5)$$

177 The total and membrane resistances can be calculated from the sample and water
178 permeation flux, respectively.
179 Hermia models were proposed to describe the flux decline in the tangential-flow UF
180 process. At the beginning of the UF process, the flux depends on membrane resistance,
181 and decreases over time due to membrane fouling. The mechanisms of membrane fouling
182 include pore blocking and cake formation. The linear models for these cases are given by
183 the following equations (Lim and Bai, 2003): membrane resistance-limited (equation 6),
184 pore blocking resistance-limited (equation 7) and cake resistance-limited (equation 8).

$$\frac{1}{J} = \frac{1}{J_0} + K_m \cdot t \quad (6)$$

$$\ln(J) = \ln(J_0) + K_P \cdot t \quad (7)$$

$$\frac{1}{J^2} = \frac{1}{J_0^2} + K_C \cdot t \quad (8)$$

185 where J is the permeate flux ($m^3/(m^2 \cdot s)$), J_0 is the initial permeate flux ($m^3/(m^2 \cdot s)$), K_m, K_P
186 and K_C are the membrane, pore blocking and cake constants, respectively, and t is the time

(s). After fitting the experimental data to the models, J_0 was calculated as the average of the J_0 fit parameter of each model.

2.6 Statistical analysis

Statistical analysis and RSM-BBD were carried out by the software Statgraphics Centurion XVIII. An ANOVA test was performed to conclude the significant difference or relationship at a confidence level of 95 % ($p < 0.05$).

2.7Analytical methods

2.7.1 SBP characterization

Structural carbohydrates, lignin, ash, and extractive content of SBP were analyzed according to the Laboratory Analytical Procedures of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008b, 2008a). Protein content was calculated as N×6.25, N being the Total Kjeldahl Nitrogen (TKN) measured by acid digestion with H_2SO_4 and distillation (KjelFlex K-360 distillatory, BUCHI, Mexico). The samples were analyzed in triplicate.

2.7.2 Sugar composition, GalA and degradation compounds analysis

High-Performance Liquid Chromatography (HPLC) with an Aminex column HPX-87H at 60 °C was used to analyze the concentration of sugars (glucose, galactose, rhamnose, and arabinose), galacturonic acid, and degradation compounds (acetic and formic acids, 5-(hydroxymethyl)furfural (HMF) and furfural). The system used was a refractive index detector (Waters 2414, USA). The operational conditions were 0.01 N H_2SO_4 as the mobile phase, at a flow rate of 0.6 mL/min, and an injection volume of 20 µL. Total sugars and GalA in the hydrolysates were determined after acid hydrolysis (121°C, 30 min and in a 3 % (v/v) H_2SO_4/ sample ratio). Oligomeric compounds were calculated as the difference between total free sugars or GalA after and before acid hydrolysis. The solids, Pectin – 3

and Pectin – 5 (Fig. 1), were dissolved in water (10 g/L) before analysis. All samples were filtered through 0.22 μm nylon filters before being analyzed.

2.7.3 Degree of esterification

The esterification degree of the Pectin – 3 and Pectin – 5 were measured according to Gharibzahedi et al. (2019) and calculated using Eq. 9:

$$\text{Esterification degree } (\%) = \frac{V_2}{V_1 + V_2} \cdot 100 \quad (9)$$

where V_1 and V_2 are the volume of 0.1 M NaOH used to titrate the sample for the first and second time, respectively. The analysis was performed in duplicate.

2.7.4 Molecular weight distribution of pectins

High-Performance Size Exclusion Chromatography (HPSEC) with an Ultrahydrogel 250 column (Waters, Japan) at 35 °C was used to analyze the molecular weight distribution of the dissolved pectins (10 g/L). The system used was a refractive index detector (Waters 2414, USA). The operational conditions were ultrapure water as the mobile phase at a flow rate of 0.7 mL/min and an injection volume of 50 μL. Dextran standards from 1 to 670 kDa were used. All samples were filtered through 0.22 μm nylon filters.

2.7.5 Surface structural characterization of pectins

Fourier Transform Infrared Spectroscopy (FTIR) analyzed the surface functional groups of pectins using an FTIR system (Alpha model, with a Platinum ATR single reflection diamond module, Bruker, USA). The absorbance was measured from 4000 to 400 cm^{-1}.

3 Results and discussion

3.1 Characterization of SBP

The characterization of the SBP determined its composition in dry basis (% wt.): galacturonan (17.7 ± 0.7), glucan (19.7 ± 0.9), galactan (9.1 ± 0.4), rhamnan (3.6 ± 0.1), arabinan (16.9 ± 0.4), protein (10.2 ± 0.1), extractives in ethanol (5.6 ± 0.8), extractives in water (3.5 ± 0.2), total lignin (9.22 ± 0.12, 58.6 % being the acid soluble lignin), and ash

(3.7 ± 0.0). The characterization was in accordance with previously reported data (Bellido et al., 2015; Martínez et al., 2009). The major polysaccharides present in SBP were glucan (19.7 %) and galacturonan (17.7 %), comprising 37.4 % of the total composition.

Galacturonan is a pectic polysaccharide found in plant cell walls. The presence of rhamnan moieties and the high content in arabinan and galactan might indicate that the pectin present in SBP corresponds to the RG-I region. This pectin structure in SBP was also observed by Y. Mao et al. (2019). The RG-I region is made up of rhamnogalacturonan polysaccharide with side chains of neutral sugars such as arabinose and galactose. Thus, the composition of SBP makes it a valuable source of pectin, which finds applications in various industries, such as food, pharmaceutical, and cosmetics (del Amo-Mateos et al., 2022). Additionally, there has been interest in the oligosaccharides derived from the RG-I pectin region, as they have shown potential as prebiotics (Prandi et al., 2018).

3.2 RG-I pectin extraction from SBP by sequential UMAE

The influence of the initial pH solution, UAE time and MAE temperature on POS extraction from SBP by sequential UMAE were appraised by an RSM-BBD (Table 1). Three experiments at the central point (pH 2.5, UAE-t: 15 min; MAE-T: 140 °C) were carried out to estimate the experimental error and evaluate the reproducibility of the extraction process.

The extract pH and solid recoveries can be found in Table 1. The MAE temperature was the most significant parameter for solid recovery. The highest solid recoveries were found when the MAE was carried out at 120 °C (Runs 1, 9 and 14). However, the solid recovery of Run 2 (55.4 %) suggests that a low initial pH (pH 1) could enhance biomass solubilization at lower temperatures. The results show that the UAE time did not impact the final extract pH or solid recovery. Degradation compound concentration (see supplementary material) ranges were: 0.0 – 1.3 g formic acid/L, 0.1 – 1.8 g acetic acid/L, 0.0 – 0.7 g furfural/L. HMF was not detected in any run. The highest concentrations were found in Run 7 (1.3 g formic

acid/L, 1.8 g acetic acid/L and 0.7 g furfural/L). Moreover, as can be seen from Fig. 2a-e, Run 7 also exhibited the highest monomer concentration accounting for 30.1 g/L (1.9 g galacturonic acid/L; 0.9 g glucose/L; 4.9 g galactose/L; 1.8 g rhamnose/L and 20.6 g arabinose/L). Based on this, the observed high concentration of degradation compounds may be attributed to the degradation of sugars and acetyl groups linked to the oligosaccharides due to the severity of the extraction conditions (pH 1, MAE temperature 160 °C). The same tendency was found in the study of oligosaccharides extraction from *Robinia pseudoacacia* wood by MAE carried out by Pérez-Pérez et al. (2023), where a higher concentration of degradation compounds was obtained under more severe extraction conditions. Furthermore, concentrations exceeding 1 g/L were found for the experimental runs performed at initial pH 1 or MAE temperature of 160 °C (Runs 3, 11, 13, 15), suggesting that the UAE time did not influence the concentration of these compounds.

POS composition in the extracts can be found in Fig. 2f. The average concentrations at the central point were: 10.3 ± 0.1 g GalA/L, 1.9 ± 0.1 g galactose/L, 1.9 ± 0.1 g rhamnose/L, 12.2 ± 0.6 g arabinose/L, 1.0 ± 0.0 g glucose/L, and 25.3 ± 0.9 g POS/L. GalA is the main component of pectin. The OGalA concentration ranged from 7.1 (Run 14) to 10.6 g/L (Run 10). On the other hand, the POS concentration ranged from 10.5 (Run 7) to 31.6 g/L (Run 3). OGalA and AraOS were the main components of the POS. Nevertheless, the composition of the POS was indeed influenced by the initial pH. The degradation of sugars to their monomeric form was observed in the runs conducted at pH 1 (Runs 2, 7, 10 and 13). Consequently, under acidic conditions, the POS composition mainly comprised OGalA. The highest concentration of POS (31.6 g/L) was observed in Run 3, where AraOS comprised 60.1 % of the composition, while OGalA accounted for 24.3 %. The observed composition distribution aligns with previous research findings (del Amo-Mateos et al., 2023), where it was also pointed out that higher temperatures during the extraction process

result in more sugar extraction than GalA. The results suggest that the composition of the POS is influenced by the pH and MAE temperature. However, no clear relationship was found between the extraction of POS and the UAE time.

3.3 Optimization of POS extraction from SBP by UMAE

A second-order polynomial equation for the POS concentration was proposed to relate the response with the independent variables (Eq. 10)

$$POS\ (g/L) = -143.236 - 21.6033 \cdot X_1 + 0.425 \cdot X_2 + 2.423 \cdot X_3 + 0.105 \cdot X_1 \cdot X_2 + 0.221 \cdot X_1 \cdot X_3 + 1.40 \cdot 10^{-4} X_2 \cdot X_3 - 1.610 \cdot X_1^2 - 0.021 \cdot X_2^2 - 0.010 \cdot X_3^2 \quad (10)$$

$R^2 = 0.944$; $R^2_{adjusted} = 0.844$

where X_1 is the initial pH, X_2 is the UAE time (min) and X_3 the MAE temperature (°C).

The quadratic model exhibited a significant fit to the POS concentration ($p < 0.05$), indicating that the model significantly impacts the variability of POS extraction. Additionally, the lack of fit test yielded non-significant results ($p > 0.05$), suggesting that the model adequately captures the observed data. Among the parameters investigated, initial pH and MAE temperature significantly influenced POS extraction at a confidence level of 95 %. However, UAE time did not significantly affect POS extraction ($p > 0.05$). The response surface graphs are shown in Fig. 3a and 3b.

The initial pH had a significant effect on the extraction of POS. Previous studies have demonstrated that extracting pectin under acidic conditions raises HG pectin yields. For instance, Liew et al., (2019) observed increased pectin yield from 3.68 to 36.33 g pectin/100 g pomelo powder when the extraction was conducted at pH 1.8 using UMAE. This increase is attributed to the breakdown of protopectin, an insoluble pectin precursor found in plants, into soluble pectin molecules. Acidic solutions facilitate the hydrolysis of protopectin, resulting in water-soluble pectin molecules. However, as mentioned in section 3.2, the use of acid solutions can degrade sugars and consequently reduce the yield of

POS extraction. Fig. 3a illustrates the relationship between pH, MAE temperature, and POS concentration. Lower pH values enhance POS extraction at low temperatures. Conversely, at higher temperatures, a pH of 1 resulted in the lowest POS concentration due to the pronounced degradation of sugars caused by the severity of the extraction conditions.

The UAE time was assessed to determine its impact on the extraction process. The statistical analysis concluded that the UAE time was insignificant for the model ($p > 0.05$) in the range tested. Liew et al. (2016) conducted a study on the extraction of pectin using a sequential UMAE approach. As in the current study, their findings indicated that the duration of UAE (12 – 28 min) did not significantly impact the yield of pectin. Fig. 3b depicts the combined effect of the MAE temperature and UAE time on the POS concentration. The graph illustrates that the application of UAE for around 10 – 15 min results in a slight increase in POS concentration when compared to 5 min of application. This suggests that increasing the UAE time from 5 to 10 – 15 min has a modest positive impact on the efficiency extraction of POS. The mechanism of UAE is based on high frequency sound waves that disrupt plant materials through acoustic cavitation. This process generates cavitation bubbles that implode, causing fragmentation, erosion, pore formation, shear forces, and increased absorption. These mechanisms reduce particle size, boost surface area, and enhance solubilization of bioactive compounds in the solvent. UAE also improves water absorption, diffusivity, and swelling index in plant tissues. All these mechanisms collectively led to increase the extraction yield (Kumar et al., 2021).

Finally, the MAE temperature plays a crucial role on POS extraction, with higher temperatures resulting in enhanced extraction of POS as can be observed in Fig. 3. The MAE temperature is evolved in two of MAE mechanisms, the penetration of solvent into the plant matrix and the elution and dissolution of the bioactive compounds. Higher extraction temperature leads to better solvent penetration and diffusivity of the pectin during elution

and dissolution into the solvent (Chan et al., 2017). Moreover, particle size is also related to the two mechanisms mentioned above (Chan et al., 2017). The reducing of particle size during UAE due to cavitation, increased the surface area which could enhance the pectin extraction. However, it is important to note that excessively high temperatures can lead to the degradation of pectin and oligosaccharides into smaller molecules, resulting in a loss of their structural integrity and functional properties. Additionally, using a short MAE time (9.4 min) could mitigate pectin degradation by reducing exposure to high temperatures, as was found in the study of Liew et al. (2016).

Based on the model proposed, the optimal conditions to maximize POS extraction were initial pH 4, 10 min and 157 °C. Three confirmatory runs were carried out under these conditions to validate the model. The composition of the extract obtained under optimal extraction conditions is summarized in Table 2. The t-test concluded that there were no significant differences between the experimental result for POS concentration (32.5 ± 0.5 g/L) and the predicted value of 31.6 g/L ($p > 0.05$). Thus, a good agreement was established between the model and the experimental results. Under the given optimal conditions, there was a solid recovery of 59.7 ± 2.5 % and the extract pH was 3.9 ± 0.1. The recovery of POS in the extract from SBP was 66.0 ± 1.0 %. This yield exceeded the results of previous studies conducted with either MAE or UAE. The POS extraction yield from SBP obtained by hydrothermal MAE (165 °C, 12 min) reached 59.7 % (del Amo-Mateos et al., 2022). Additionally, the extract pH was similar in both studies (extract pH ≈ 4), suggesting that the higher yield achieved in this study was attributed to the use of sequential UMAE. Furthermore, the results of Fernández-Delgado et al. (2023) focused on GalA extraction from SBP by UAE showed a recovery of around 20 % of total GalA (pH 4, UAE amplitude 90 % and 90 min) compared to the 46.9 ± 2.3 % of OGalA recovered in the current research. The results obtained reveal that the combination of UMAE may be a

suitable technology to enhance POS recovery from SBP. Moreover, energy consumption is a key factor in the development of new technologies. It seems that the use of UAE for a short duration before MAE can effectively disrupt the cell walls, leading to higher yields at lower temperatures and shorter MAE times. Consequently, this approach may have the potential to decrease energy consumption during the extraction process.

3.4 POS purification and concentration

During the extraction of RG-I pectin from SBP, other non-targeted compounds, such as monomers, organic acids, or furfural and HMF from pentose and hexose dehydration, can be found in the extracts (Moure et al., 2006). Thus, these small molecules should be removed to increase the purity of the extract. Among the techniques available for pectin purification and concentration, the use of membranes (DF and UF) avoids the use of harmful organic substances, requires little space and is energy efficient (Gómez et al., 2013). However, the MWCO is a key parameter to consider when selecting a membrane. The process yield and filtration flux are affected by the MWCO. Higher MWCO leads to higher filtration flux, which means shorter operation times, but a lower process yield, since more targeted molecules can pass through the membrane.

This study compared the recovery yield of POS and the filtration flux using two membranes with different MWCO (3 and 5 kDa). Table 2 summarizes the composition of the extract obtained under UMAE optimal conditions, the composition of the retentates, and the yield of the membrane processes calculated using Eq. (1).

In the continuous DF process, the objective was to remove small molecules and refine the extract; while the UF process aimed to clean the small molecules remaining after DF and to concentrate the extract. As shown in Table 2, after the membrane process, the concentration of POS increased in both retentates (R_{UF} – 3: 61.2 g/L and R_{UF} – 5: 53.5 g/L), indicating a successful concentration. Simultaneously, the monomers and degradation

compounds passed through the membranes and were removed from the retentate. The POS recovery yield was significantly higher in the R_{UF} – 3 retentate than in the R_{UF} – 5. This difference may be attributed to the presence of some small OGalA and AraOS molecules, the major components of POS, which could pass through the membrane of 5 kDa. In both cases, monomers and degradation compounds were effectively removed, as their highest concentration was only 0.1 g/L from the 2.0 g monomers/L and 1.4 g/L of degradation compounds present in the extract.

The recovery yields (Table 2) obtained with the membrane process were higher when compared to previous studies. Gómez et al. (2013) refined a pectin extract from lemon peel using a cellulose membrane with an MWCO of 1 kDa through discontinuous DF and UF, reporting recovery yields of 96.1, 59.3 and 79.8 % for OGalA, AraOS and GalOS, respectively; whereas, up to the 26.1 % of the monomers remained in the retentate after the membrane process. In contrast, the recovery yields of the oligomers obtained in the current study and the removal of monomers, using an MWCO membrane of 3 kDa, were higher (Gómez et al., 2013). This suggests that using a higher MWCO membrane, such as the 3 kDa membrane used in this study, does not result in a loss of targeted compounds and can increase the elimination of small molecules when employing a continuous DF process. The reason for the improved results in the current study could be the viscosity of the feed. In the case of the discontinuous DF process, the viscosity increased due to concentration. A higher viscosity solution could reduce the efficiency of the process (Field and Wu, 2022). Furthermore, the highest recovery yield for OGalA reported by Jin et al. (2022) was approximately 75 % during UF using a PES membrane with an MWCO of 3 kDa. This yield is significantly lower than the value obtained in this research using the same MWCO membrane (3 kDa). The inclusion of the previous DF step in the current study may have contributed to the higher recovery yield of OGalA.

Additionally, the change in the permeate flux over time is depicted in Fig. 4a. The trend observed for both membranes is similar, with the permeate flux reaching a steady state after approximately 1.5 hours of operation. However, there was a notable difference in the permeate flux. The permeate flux using the MWCO membrane of 5 kDa was $6.7 \cdot 10^{-6}$ $m^3/(m^2 \cdot s)$; whereas, for the 3 kDa MWCO membrane, it only reached $1.7 \cdot 10^{-6}$ $m^3/(m^2 \cdot s)$. This discrepancy is an important factor to consider, since the operation time required to refine pectin using the smaller MWCO membrane would be much longer. Similar results were reported in the study of Jin et al. (2022).

Fouling is another important parameter that must be considered. The fitting models for membrane fouling have been used (see supplementary material). An R^2 higher than 0.91 was found for the three models for both membranes, confirming the suitability of the fouling models proposed and suggesting that the membrane, pore blocking, and cake formation resistances were limited in the first 1.5 h of operation. During the initial 1.5 hours of UF (Fig. 4b), there is a significant contribution of total resistance. The total resistance increased to 60.6 % during the UF process using the 3 kDa MWCO membrane, but only 23.7 % using the 5 kDa MWCO membrane. Thus, the fouling resistance was much more pronounced in the case of the 3 kDa MWCO membrane. This trend is consistent with the decline in the permeate flux (Fig. 4a), which was significantly higher, as mentioned above. Based on the calculated constants of the filtration resistance models (equations 6 – 8) (see supplementary material), there is a statistical difference between the constant parameters for the two membranes used. Nevertheless, the difference is notably higher in the case of K_C followed by K_m. Thus, the much lower permeate flow observed during operation with the 3 kDa MWCO membrane could be due to the resistance of the membrane and the cake formation, and somewhat less to the pore blocking.

The findings suggest that the membrane process employed in this study yields better results than previous research, particularly in terms of the recovery yields of the targeted compounds. The inclusion of a continuous DF step in the process likely contributed to achieving higher recovery yields, while efficiently removing the undesired compounds. Additionally, the selection of the appropriate MWCO membrane is crucial, as recovery yields may be compromised due to the lower permeate flux when using a smaller MWCO membrane.

3.5 Refined POS characterization

After the membrane process, the refined extracts (R_{UF} – 3 and R_{UF} – 5) were freeze-dried. Although alcoholic precipitation is a widely used method for HG pectin recovery from extracts, it is not efficient in recovering RG-I pectin, particularly arabinose (del Amo-Mateos et al., 2022). In contrast, freeze-drying is a commercial technology used for the dehydration of food-grade products (García-Velásquez and van der Meer, 2023) and allows the recovery of all the components present in the extract.

The global yield (g pectin/100 g SBP), composition and degree of esterification can be found in Table 3. The global yields were 39.7 and 39.0 % for Pectin – 3 and Pectin – 5, respectively, indicating that the MWCO of the membrane used did not influence the global yield. Both yields were higher than those reported for conventional pectin extraction and/or ethanol precipitation. For instance, Adiletta et al. (2020) reported a pectin yield from SBP of 25 % using hot acidic extraction (pH 1.5, 90 °C, 4 h, solid-to-liquid ratio 1:30 g/mL) followed by ethanol precipitation and the yield obtained by Y. Mao et al. (2019) after MAE (90 °C, 120 min) and alcohol precipitation was 23.4 %.

The highest performance in the membrane process was achieved with the 3 kDa MWCO membrane, resulting in a 90 % recovery of POS. This has resulted in a slightly higher purity of the final pectin solid from R_{UF} – 3 (80.2 % POS) as compared to 72.9 %

457 POS in Pectin–5. It is worth noting that the purity of both pectins were in the range of
458 commercial prebiotics. The POS content in pectins was much higher than those reported in
459 previous research (60.9 %), where the extract was not purified before being subjected to
460 freeze-drying (del Amo-Mateos et al., 2023). This result allowed to conclude the suitability
461 of a membrane purification process to increase the content of POS. As in the extract,
462 AraOS was the major component in both pectins, while OGalA accounted for the 22.4 and
463 17.0 % in Pectin – 3 and Pectin – 5, respectively. In both pectins, a small amount of protein
464 was detected, comprising 3.7 % (Pectin – 3) and 4.7 % (Pectin – 5) of the composition.
465 Based on the degree of esterification, pectin can be classified as high methoxyl (HM) and
466 low methoxyl (LM). The degree of esterification was similar in both pectins and can be
467 considered as high-methoxyl pectin (degree of esterification > 50 %), indicating that pectins
468 obtained in this study can form gels at low pH and in the presence of sugars. High-methoxyl
469 pectins are applied as stabilizers, as a rheology modifier, and in sugary products (Abboud
470 et al., 2020).
471 The molecular weight distribution curves of the two pectins (see supplementary material)
472 showed a similar pattern, indicating a comparable distribution. Both pectins exhibited a
473 major peak at a molecular weight of 377.4 kDa. Additionally, a secondary peak was
474 observed, with a relatively lower intensity, at a molecular weight of 3.0 kDa in Pectin – 3
475 and at 4.3 kDa in Pectin – 5, suggesting the presence of smaller molecules in the pectins.
476 The surface structure of pectins was analyzed by FTIR for their functional groups in the
477 range of 4000 – 400 cm^{-1} (see supplementary material). The patterns obtained showed the
478 typical pectin chemical composition (Concha Olmos and Zúñiga Hansen, 2012). The peaks
479 found at 1740 and 1650 – 1680 cm^{-1} were attributed to the C=O stretching vibration of
480 esterified and ionic carboxyl groups, respectively (Jiang et al., 2012). The higher

481 absorbance intensity of the ester carboxyl group corroborated that both pectins belong to
482 the high-methoxyl category.

483 *3.6 Future perspectives of the integrated process for RG-I pectin recovery: technical and*
484 *economic considerations*

485 A preliminary study of the energy consumption of the sequential UMAE was carried out.
486 The ultrasound and microwave equipment continuously monitored the power consumption.
487 Thus, the total amount of energy required for each extraction step was calculated according
488 to equation 11:

$$Q = \int P(t) \cdot dt \quad (11)$$

489 where Q is the energy required (kW·h), P is the power dissipated (kW) and t is the
490 extraction time (h).

491 The total energy consumption to produce 1 g of pectin was 0.02 kW·h for both pectin – 3
492 and pectin – 5 (see supplementary material). This value was significantly lower compared
493 to the one obtained by Liew et al. (2019) for conventional extraction (1.05 kW·h). This lower
494 value may be explained by the much shorter operation time required during sequential
495 UMAE (10 min UAE and 9.4 min MAE) compared to the one used for conventional
496 extraction (141.4 min).

497 Previous research has shown the potential prebiotic properties of POS obtained from
498 SBP (Prandi et al., 2018). Traditional pectin extraction methods typically target GalA
499 recovery from the HG region, which has market prices around $21/kg (Moslemi, 2021). In
500 contrast, the market price range for prebiotics has been reported to be significantly higher,
501 ranging from 120 to 850 €/kg (www.consumerlab.com). Furthermore, the market of
502 prebiotics and pectin were estimated at $6.0 billion and $1.5 billion, respectively with an
503 annual growth rate for 2030 of 11 % for prebiotics and 5 % for pectin
504 (www.rearchandmarkets.com). Although a deep economic analysis should be done to

address the viability of the process proposed in this study and consider all the costs involved, the higher value-added of the products obtained compared to conventional extracted pectin, the higher yields, shorter operation times, the less amount of solvent required may lead to a feasible and economically viable process.

4 Conclusions

This study provides valuable insights into RG-I pectin extraction from SBP, optimizing conditions through sequential UMAE (pH 4, 10 min UAE, 157°C MAE). UAE step for a brief duration reduces the time and temperature required for MAE, resulting in higher POS recoveries (66.0%), surpassing prior studies. Continuous DF reduced extract viscosity, and enhanced yields with higher MWCO membranes. This purification approach yields highly refined POS, with the 3 kDa MWCO membrane resulting in the highest POS concentration and slightly improved global yield. These refined RG-I pectins are suitable for various applications due to their high-methoxyl content, molecular weight, and purity.

E-supplementary data for this work can be found in e-version of this paper online.

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Figure captions

Figure 1. Scheme of the sequential UMAE extraction and membrane purification

Figure 2. RSM-BBD results: composition of the extracts and POS. POS: pectooligosaccharides; OGalA: Oligogalacturonoides; GalOS: galactooligosaccharides; RhaOS: rhamnooligosaccharides; AraOS: arabinooligosaccharides

Figure 3. Response surface of RSM-BBD: effect of the independent variables on the concentration of POS in the extracts

Figure 4. Time courses of permeate flux (J) (a) and total resistance (R_T) (b) during the ultrafiltration process

660 **Tables**

661 **Table 1.** RSM-BBD: experimental conditions, pH of extracts and solid recovery

Run	Independent variables						Extract	Solid recovery
		X_1		X_2		X_3	pH	(%)
	x_1	(Initial pH)	x_2	(UAE-t [1], min)	x_3	(MAE-T [2], °C)		
1	0	2.5	1	25	-1	120	4.0 ± 0.1	74.0 ± 1.1
2	-1	1	0	15	-1	120	1.5 ± 0.3	55.4 ± 0.9
3	1	4	0	15	1	160	3.8 ± 0.1	46.5 ± 1.5
4	0	2.5	0	15	0	140	3.8 ± 0.2	64.9 ± 1.7
5	1	4	1	25	0	140	4.0 ± 0.2	58.7 ± 1.2
6	0	2.5	0	15	0	140	3.8 ± 0.3	62.2 ± 0.9
7	-1	1	0	15	1	160	1.6 ± 0.2	42.1 ± 0.8
8	0	2.5	0	15	0	140	3.9 ± 0.1	66.8 ± 0.9
9	1	4	0	15	-1	120	4.2 ± 0.2	73.4 ± 1.1
10	-1	1	-1	5	0	140	1.5 ± 0.2	42.3 ± 1.4
11	0	2.5	1	25	1	160	3.8 ± 0.3	44.9 ± 0.8
12	1	4	-1	5	0	140	4.0 ± 0.1	62.0 ± 1.2
13	-1	1	1	25	0	140	1.5 ± 0.2	61.7 ± 1.3
14	0	2.5	-1	5	-1	120	4.0 ± 0.3	74.1 ± 0.9
15	0	2.5	-1	5	1	160	4.0 ± 0.2	53.1 ± 1.5

662 [1]UAE-t: Ultrasound-assisted extraction time; [2]MAE-T: Microwave-assisted extraction
663 temperature

664 **Table 2.** Composition of the extract obtained under optimal UAE and MAE conditions and
665 the composition of the final retentates obtained after the purification membrane processes
666 (R_{UF} – 3, R_{UF} – 5). Recovery yields after the DF/UF membrane processes

	Concentration (g/L)			Recovery yield (%)	
	Extract	R_{UF} – 3	R_{UF} – 5	R_{UF} – 3	R_{UF} – 5
Monomers	2.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.9	2.8
Degradation compounds[1]	1.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	2.4	3.7
OGalA[2]	8.8 ± 0.4	18.0 ± 0.7	12.4 ± 0.1	97.5	64.0
GalOS[3]	3.9 ± 0.1	7.9 ± 0.4	8.7 ± 0.1	95.7	~100
RhaOS[4]	1.6 ± 0.1	2.9 ± 0.1	3.9 ± 0.0	82.3	~100
AraOS[5]	18.3 ± 0.3	32.5 ± 1.5	28.6 ± 1.4	84.9	71.2
GlcOS[6]	0.9 ± 0.0	0.0 ± 0.0	1.4 ± 0.0	0.0	84.4
POS[7]	32.5 ± 0.4	61.2 ± 2.7	53.5 ± 1.6	90.0	74.6

667 [1]Degradation compounds: formic acid, acetic acid, HMF and furfural. [2]OGalA:
668 Oligogalacturonoides; [3]GalOS: galactooligosaccharides; [4]RhaOS: rhamnooligosaccharides;
669 [5]AraOS: arabinooligosaccharides; [6]GlcOS: glucooligosaccharides; [7]POS:
670 pectooligosaccharides

671 **Table 3.** Pectin yield, composition, and structural characteristics of final RG-I pectin-rich

672 solids: Pectin – 3 and Pectin – 5

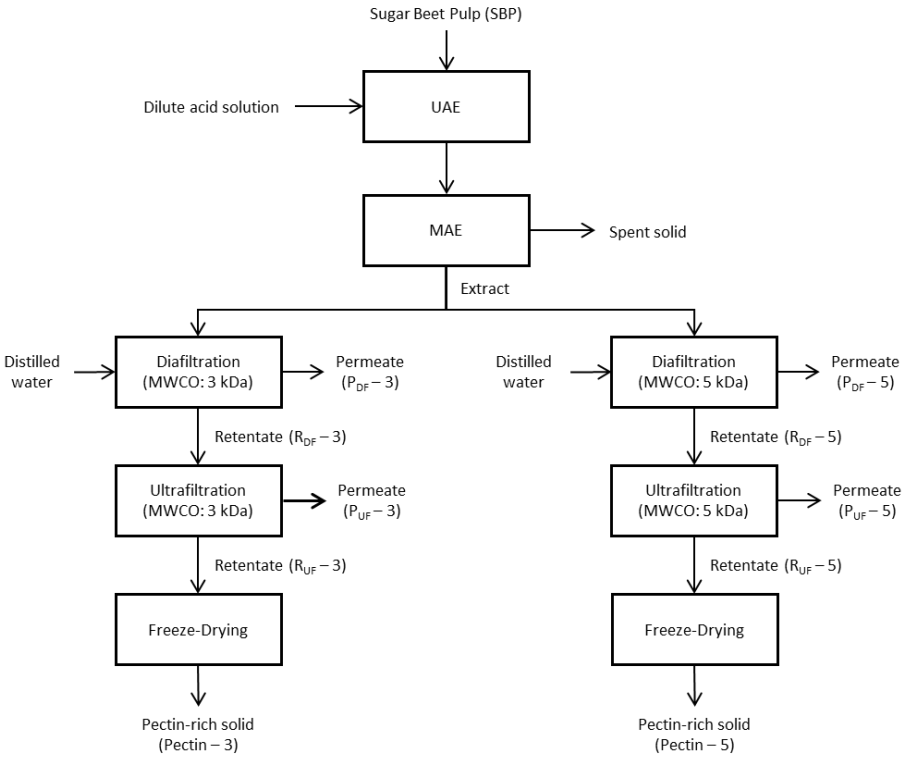
	Pectin – 3	Pectin – 5
Yield (%, g pectin/g SBP)	39.7 ± 0.5	39.0 ± 0.3
Degree of esterification (%)	73.4 ± 1.0	69.6 ± 0.9
Composition (%)		
OGalA[1]	22.4 ± 0.1	17.0 ± 0.3
GalOS[2]	11.1 ± 0.0	12.0 ± 0.5
RhaOS[3]	3.7 ± 0.1	4.4 ± 0.2
AraOS[4]	43.1 ± 0.0	39.6 ± 1.3
GlcOS[5]	0.1 ± 0.0	1.2 ± 0.0
POS[6]	80.2 ± 0.0	72.9 ± 1.9
Protein	3.7 ± 0.2	4.7 ± 0.2

673 [1]OGalA: Oligogalacturonoides; [2]GalOS: galactooligosaccharides; [3]RhaOS:

674 rhamnooligosaccharides; [4]AraOS: arabinooligosaccharides; [5]GlcOS:

675 glucooligosaccharides; [6]POS: pectooligosaccharides

676 **Figures**



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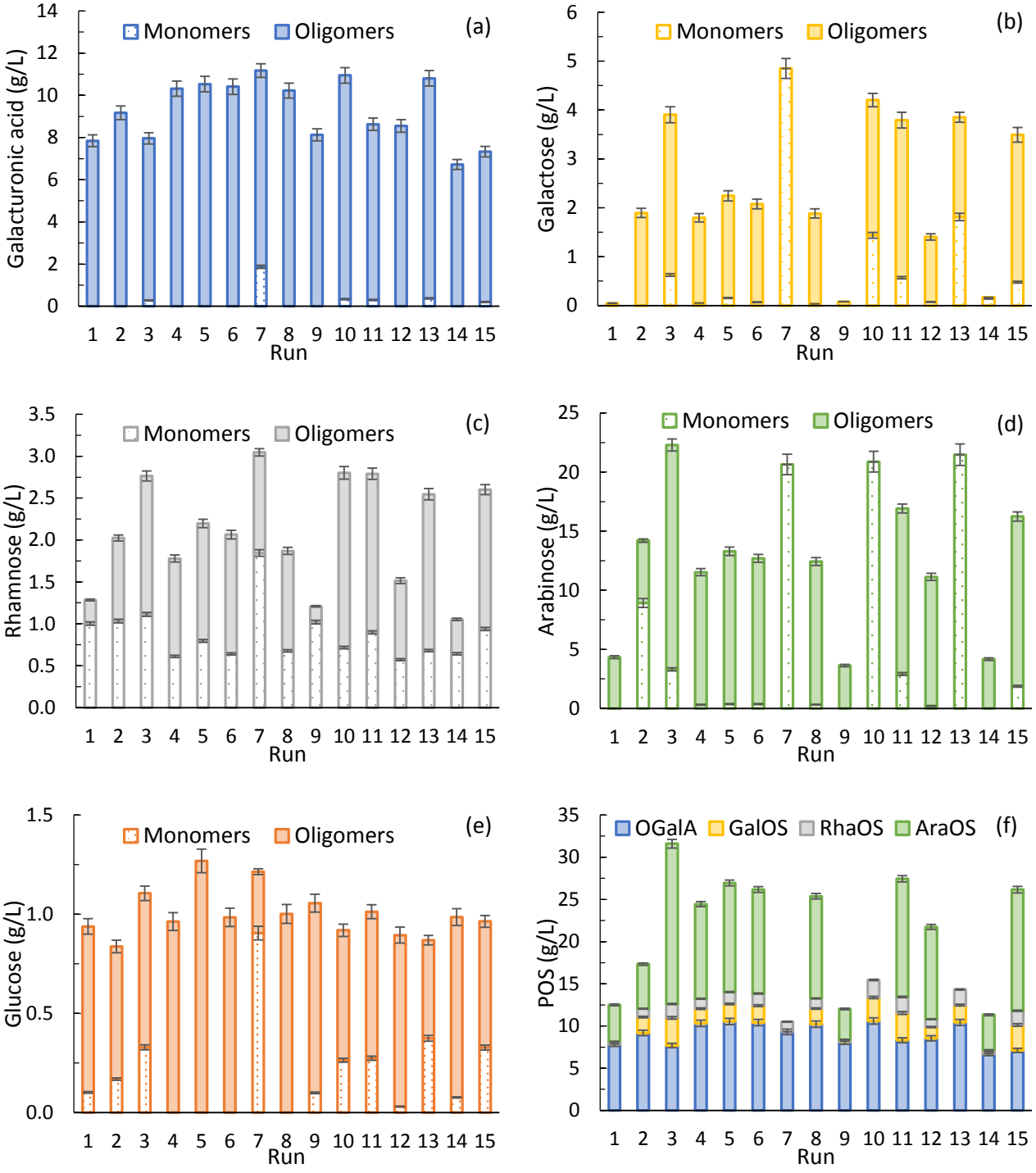
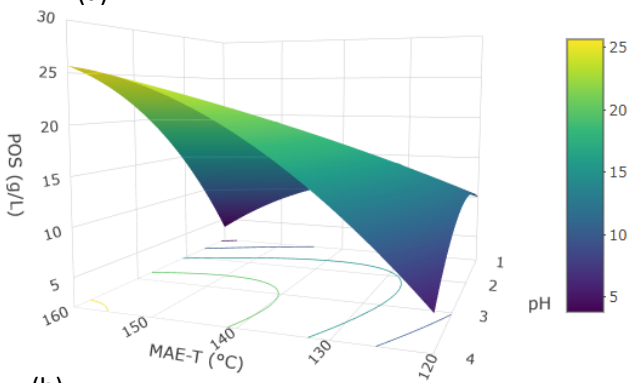

(a)
Monomers
Oligomers
Galacturonic acid (g/L)
Run
(b)
Monomers
Oligomers
Galactose (g/L)
Run
(c)
Monomers
Oligomers
Rhamnose (g/L)
Run
(d)
Monomers
Oligomers
Arabinose (g/L)
Run
(e)
Monomers
Oligomers
Glucose (g/L)
Run
(f)
OGalA
GalOS
RhaOS
AraOS
POS (g/L)
Run

678

(a)



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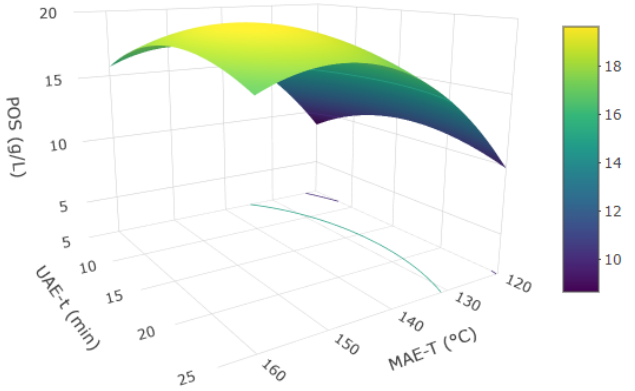
(b)



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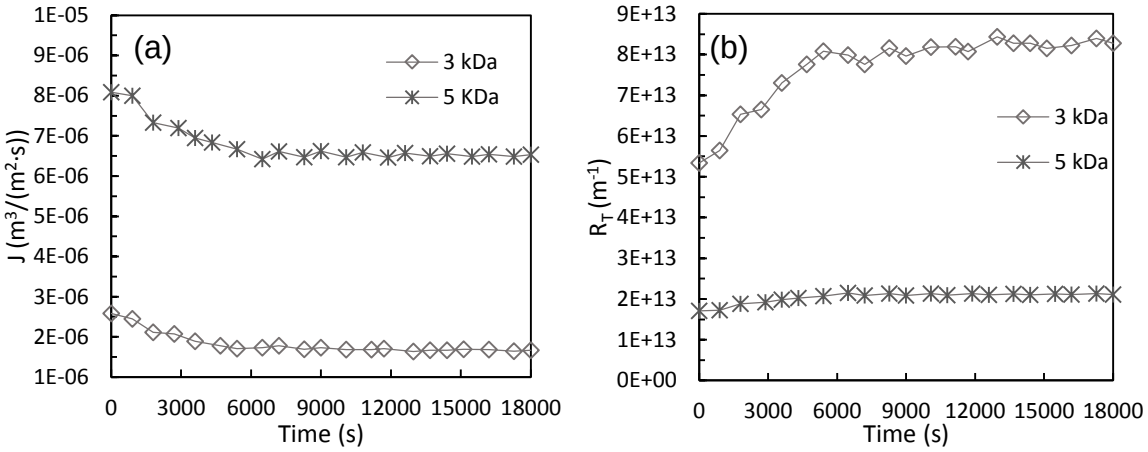
(a)
3 kDa
5 KDa
J (m³/(m²·s))
1E-05
9E-06
8E-06
7E-06
6E-06
5E-06
4E-06
3E-06
2E-06
1E-06
0
3000
6000
9000
12000
15000
18000
Time (s)
(b)
3 kDa
5 kDa
R_T (m⁻¹)
9E+13
8E+13
7E+13
6E+13
5E+13
4E+13
3E+13
2E+13
1E+13
0E+00
0
3000
6000
9000
12000
15000
18000
Time (s)

682

Credit Author Statements

Esther del Amo Mateos → Investigation, methodology, supervision, writing-original draft

Berta Cáceres → Investigation, methodology, visualization

Mónica Coca → Conceptualization, formal analysis, supervision

María Teresa García-Cubero → Conceptualization, formal analysis, supervision

Susana Lucas → Conceptualization, writing -review & editing, project administration