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Semi-continuous hydrolysis of onion skin wastes with subcritical water: Pectin recovery and oligomers identification

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ABSTRACT

The semi-continuous hydrolysis of onion skin wastes (OSW) using subcritical water (SubW; working conditions: 105–180 °C; 5 MPa; 2.5 mL/min) has been studied in this work. Liquids after hydrolysis showed a sharp increase in total organic carbon (>30 mg/g OSW) at temperatures above 145 °C, when SubW began to promote the partial hydrolysis of the structural components of OSW. Among them, pectin was one of main components recovered (extraction yield up to 9% at 145 °C), whereas cellulose was barely hydrolyzed in the range of temperatures studied. The composition of pectin demonstrated that SubW promoted the recovery of the valuable RG-I (21.1 ± 1.1 mol%) regions compared to acid water extraction processes (10.8 ± 0.9 mol%). The control of the hydrolysis conditions was found to be critical, since high temperatures and long hydrolysis times led to the formation of organic acids (acetic, formic and levulinic) and degradation products such as furfural (up to 0.8 mg/g OSW) from the C5 sugars. Finally, the High Heating Value (HHV) calculated for the solid residue obtained at 180 °C (16.4 ± 0.2 MJ/kg) indicated the potential use of this residue as a fuel, once the valuable compounds (phenolics and pectins) have been recovered.

1. Introduction

Nowadays a significant number of by-products from the food industry end up in landfills despite their high content in bioactive molecules. As an example, onion skin wastes (OSW) represent a real problem for the food industry. The large amount of OSW generated every year, 10% of the 5.36 Mt of onion harvested in the European Union in 2018 [1], cannot be used as fertilizer or cattle feeding due to the presence of sulphur compounds. In order to find a solution for this sort of situations, the European Union has launched the circular economy plan *"Closing the loop — An EU action plan for the Circular Economy, COM (2015) 614"*, in the year 2015. One of the main goals of this ambitious plan is the valorization of these by-products through the recovery of bioactive compounds.

OSW is a source of carbohydrates and phenolic compounds (mainly quercetin and quercetin derivatives) [2]. The cell walls of OSW show a very complex structure, mainly formed by pectin (42.4%), followed by hemicelluloses (36.6%) and cellulose (21%) [3]. Pectin is a complex polysaccharide composed by uronic acids (66.4%) and neutral sugars (galactose, arabinose, xylose, mannose, glucose or rhamnose) in variable composition. Besides phenolic compounds, pectin arises as one of the most interesting compounds to be recovered from OSW due to its growing worldwide demand: it is estimated to be as high as 40,000 t per year, with an annual growth of about 5% [4]. This growing demand requires new sources of pectin and new extraction methods.

As summarized by Benito-Román et al. [5], the recovery of phenolic compounds from OSW has been extensively studied in the past, using processes either based on organic solvents or subcritical water (SubW). Those authors demonstrated that SubW is an excellent alternative to recover valuable compounds from vegetal matrices due to its ability to induce the acid hydrolysis of the bonds between the bioactive compounds and the solid matrix. SubW refers to water at temperatures ranging from 100 °C (boiling point) to 374 °C (critical point) that remains in a liquid state due to the application of pressure. Under these conditions of pressure and temperature, water physical properties change, presenting higher ionic product and lower dielectric constant than at ambient conditions [6]. At ambient temperature, water is a polar solvent with a dissociation constant, K_w, of 10^{-14} and a relatively high dielectric constant of 80 at 20 °C. This implies that the pH changes from 7.0 at room temperature to about 5.7 at 180 °C and 5 MPa resulting in

Abbreviations: OSW, Onion Skin Waste; DOSW, Dry Onion Skin Waste; SubW, Subcritical Water.

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higher ionic strength of hydronium and hydroxide ions than at ambient temperature [7].

Although SubW has been used to extract phenolic compounds from OSW [8–12], in none of these works the composition of the liquid extracts in terms of sugars or other structural components has been presented. These works are only focused on the quercetin (and derivatives) extraction yield. However, due to the ability of SubW to promote hydrolysis reactions, it is expected that structural components from OSW are partially hydrolyzed and co-extracted with the phenolic compounds. As far as the authors' knowledge, the hydrolysis of the OSW using SubW in a semi-continuous reactor has not been reported in the literature so far; therefore, the direct comparison of our results with other studies results complicated. The work done by Salak et al. [13] studied the hydrolysis of OSW using SubW in a batch reactor, quantifying some sugars (frutose, glucose and sucrose) and some free acids, but not analyzing the presence of pectin or any other sugars usually present in the OSW (such as rhamnose, galactose or arabinose). In general, the studies carried out to induce the hydrolysis of OSW structural components to obtain valuable compounds are based on enzymatic reactions [14,15] or acidic reactions [16]. The former are time consuming and, since a cocktail of enzymes is required, the overall cost of the process is increased, whereas the latter are effective to release the structural components from the solid matrix, but their major drawback is the lack of selectivity [6]. Alternatively, SubW arises as an excellent reaction medium for the fractionation of different valuable compounds from lignocellulosic biomass, as it is has been recently demonstrated [17,18]. More specifically, the discovery and design of efficient and greener pectin extraction processes is still necessary, mainly to reduce energy consumption, to avoid the use of toxic solvents that results in lack of selectivity, to ensure the production of safe and high-quality products [19], and to avoid the use of expensive and time consuming enzymes.

Therefore, in this work, the hydrolysis of OSW using SubW (at temperatures up to 180 °C) was studied in a semi-continuous reactor. The liquid after hydrolysis was analyzed: first to determine the amount of OSW dissolved, second to determine the sugars present and the degradation products formed as a consequence of the hydrolysis process, and third to determine the oligomers obtained (structural components partially hydrolyzed), which are not decomposed to yield the monomers). From the liquids after the hydrolysis process it was possible to isolate pectin by ethanol precipitation, which composition was determined and compared to pectin obtained by a conventional acid extraction process. Finally, the composition of the solid residue after SubW treatment was also evaluated, considering a potential further use of this waste as a fuel, once the main valuable components are recovered.

2. Materials and methods

2.1. Sample preparation

Onion (*Allium cepa* L. cv. Horcal) wastes were collected from a local factory (Embutidos Cardeña). Onion wastes were manually processed to separate the brown skins, which were dried at room temperature. Subsequently, they were milled using the cutting mill SM100 (Retsch GmbH, Germany), equipped with a 1 mm sieve. The raw material moisture content was determined by drying it at 105 °C for 24 h. It resulted to be 9.5 ± 0.2%. All the results obtained in this work were presented per gram of dry OSW (DOSW).

2.2. Subcritical water hydrolysis

The subcritical water (SubW) experiments were carried out in a semi-continuous laboratory scale plant previously described by Benito-Román et al. [5]. In the semi-continuous working procedure, fresh solvent is continuously pumped through the raw material. This has two important effects: first, the equilibrium is continuously displaced [20]; and second, the solute is pumped out of the extractor as soon as it is dissolved, which reduces the time it is exposed to the high temperatures and thus its thermal degradation and the formation of degradation products. The disadvantage of the semi-continuous operation is related to the fact that it requires higher volumes of solvent than batch methods, which results in the dilution of the target compounds.

In each experiment, approximately 3.5 g of onion skin wastes were loaded in the extractor (volume, 26.5 mL; internal diameter, ½"). In brief, experiments were carried out at five different temperatures ranging from 105 to 180 °C, at a constant flow rate of 2.5 mL/min and 5 MPa of pressure. In all the experiments done, the extraction time was 180 min, whereas samples were collected periodically: 0–10 min; 10–20 min; 20–30 min; 30–45 min; 45–60 min; 60–80 min; 80–110 min; 110–140 min and 140–180 min). All the liquors collected were completely characterized. From these results, the accumulated extraction curves could be calculated.

In the dynamic extraction mode, the temperature and the residence time (affected by the volume of the extractor and the flow rate) are key parameters [21]. In order to achieve the correct design and operation of fixed-bed extractors, a suitable selection of length to diameter ratio must be made in order to prevent channeling, an excessive pressure drop and clogging.

When using SubW as a reaction media, a common tool to evaluate the simultaneous effect of temperature and time is the severity factor [21], calculated according to Eq. (1):

$$\log R_0 = \log\left(t \cdot e^{\left(\frac{T-T_{ref}}{14.75}\right)}\right) \quad (1)$$

where t is the treatment time (min), T is the working temperature (°C) and T_{ref} is equal to 100 °C. In the specific case of a semicontinuous reactor, the treatment time has to be replaced by the residence time (τ) of the water in the reactor, which is calculated according to Eq. (2).

$$\tau = \frac{V}{F} = \frac{V}{F_0}\frac{\rho_r}{\rho_0} \quad (2)$$

Where V is the reactor volume (mL), F_0 is the flow rate measured in ambient conditions and expressed in mL/min, ρ_o is the water density at ambient conditions (g/L) and ρ_r the water density at the reaction conditions, expressed in g/L. In our experiments, the severity factor ranged from 1.15 (105 °C) to 3.33 (180 °C).

After each extraction experiment, the OSW solid residue was collected, dried at 45 °C for 24 h, accurately weighted and kept at 4 °C until a complete characterization was carried out.

2.3. Pectin isolation

In this work, pectin was isolated from OSW following two different techniques: conventional extraction based on acidified water and SubW extraction (as an alternative procedure). In the first procedure, 10 g of OSW were mixed with 100 mL of water (pH 2, using HCl) at 85–90 °C for 1 h. Later on, solid was separated from liquid by filtration and the filtrate was collected by alcohol precipitation (using ethanol 80%, v/v) under stirring. Alternatively, pectin was isolated from SubW extracts obtained at 125, 145 and 180 °C. 10 mL of the SubW extracts were first centrifuged (4500 rpm, 4 °C, 15 min) to remove insoluble solids. The supernatant was mixed with ethanol 80% (v/v) under stirring in order to precipitate pectin.

In both cases, the precipitate formed was separated by centrifugation (4500 rpm, 15 min, 4 °C) using the centrifuge Sorvall ST16R (Thermo Scientific, USA) and dried at 40 °C overnight. Then, the solid material was accurately weighted and stored at 4 °C until further analysis.

The pectin extraction yield was calculated according to the following equation:

$$Pectin\ \ yield\ \ (\%) = \frac{Pectin\ \ isolated\ \ (g)}{Raw\ \ material\ \ (g)} \cdot 100 \quad (3)$$

2.4. Samples characterization

2.4.1. OSW liquid samples

2.4.1.1. Carbohydrates identification. Identification and quantification of sugars and their degradation products in SubW extracts was carried out following protocol NREL/TP-510–42623. For that purpose, the HPLC system equipped with a Biorad Aminex HPX-87 H column (300 ×7.8 mm, Bio-Rad) coupled with the guard column Micro-Guards Cation H^+ (BioRad), with a variable wavelength detector (VWD) and a refractive index detector (RID), using 0.005 M sulfuric acid as mobile phase (0.6 mL/min) was used. The column and detectors were maintained at 40 °C and the total running time was 67 min per injection. The monomeric sugars and degradation products in the SubW extracts were first determined, after centrifugation (4500 rpm, 15 min, 4 °C) and filtration through a 0.22 μm pore size syringe filter (Scharlab, Spain). Total sugars were determined after hydrolysis of the sample according to the NREL/TP-510–42618 (*Determination of structural carbohydrates and lignin in biomass*) Analytical Procedure.

The following carbohydrates, cellobiose (Cel), glucose (Glu), galactose (Gal), xylose (Xyl), rhamnose (Rha), arabinose (Ara) and other compounds such as glucuronic acid (GluA) and galacturonic acid (GalA), pyruvaldehyde, glyceraldehyde, glycolaldehyde, and sugar degradation products, 5-hydroxymethyl furfural (HMF) and furfural (Fur), formed during the SubW hydrolysis process were determined by HPLC.

The extraction yield of the different sugars was calculated by dividing the sugar content in the liquid extract (as the sum of the monomer and oligomer) by the sugar content in the solid raw material, according to Eq. (4):

$$Total\ \ yield\ \ (\%) = \frac{monomeric + oligomeric\ \ sugar\ \ in\ \ the\ \ extract}{Total\ \ amount\ \ of\ \ sugar\ \ in\ \ the\ \ solid\ \ material} \cdot 100 \quad (4)$$

2.4.1.2. Total organic carbon (TOC) and total nitrogen (TN). TOC and TN were determined using the Shimadzu TOC-V CSN Analyzer (Shimadzu Co., Japan). Liquid extracts were diluted 10 times before analysis. TOC was calculated as the difference between total carbon (TC) and inorganic carbon (IC). The standard for the TC determination was potassium hydrogen phthalate ($C_8H_5KO_4$), in a concentration range from 0 ppm to 1000 ppm. For inorganic carbon (IC) determination, a solution of sodium hydrogen carbonate ($NaHCO_3$) up to 1000 ppm was used as standard, and for TN, a calibration curve obtained from a potassium nitrate (KNO_3) solution up to 200 ppm was used.

2.4.2. OSW solid samples

The composition of the onion skin wastes before and after the subcritical water treatment was determined following the protocol NREL/TP-510–42618. In brief, solid samples were subjected to acid hydrolysis and then the resulting sugar monomers were determined by HPLC, using the chromatographic system described in Section 2.4.1.1. Moisture, ash content and lignin (both soluble and insoluble) were also determined according to the NREL protocol.

The elemental analysis (C, H, N, S) of the solid samples was carried out using the FLASH 2000 elemental analyzer (Thermo Scientific, USA). The oxygen content in the samples was determined by difference, considering the ash content of the sample.

2.4.3. Pectin samples

2.4.3.1. Structural components of pectin. In general, there are two main structures that form pectin: homogalacturonan (HG), formed by α-1,4-linkage of GalA residue, and rhamnogalacturonan (RG, domains I and II), which is branched due to the presence of neutral sugars (Gal, Ara, Xyl, Glu or Rha) attached to the GalA chain [16]. The determination of the neutral sugars and uronic acids was carried out using the HPLC system described in Section 2.4.1.1. For that purpose, an aqueous solution of pectin (1 mg/mL) was prepared and hydrolyzed: 5 mL of this solution were transferred to a 15 mL test tube and 0.174 mL of H_2SO_4 (72%, v/v) were added. Then, the sample was kept for 1 h at 121 °C. Finally, calcium carbonate was used to neutralize the sample. From the results of composition in terms of GalA and neutral sugars, it was estimated the prevalence of HG and RG-I domains using the equations proposed by M'sakni et al. [22]:

$$HG\ (\%, mol) = GalA - Rha \quad (5)$$

$$RG - I(\%, mol) = [GalA - HG] + Rha + Gal + Ara \quad (6)$$

2.4.3.2. Pectin molecular weight (MW). The weight-average molecular weights (MW) and polydispersity of the samples were determined employing high-pressure size exclusion chromatography coupled to refraction index detector (HPSEC-RID). The 1260 HPLC system (Agilent Technologies, CA, USA) consisted of a PL Aquagel guard column linked in series with PL Aquagel-OH 30, PL Aquagel-OH 40 columns from Agilent Technologies (300 mm × 7.5 mm, particle size 8 μm). Characterization of pectin from OSW and subcritical water hydrolysates was performed at 40 °C. 60 μL of each sample were eluted in isocratic mode with 0.01 M NH_4Ac, at a flow rate of 0.7 mL/min. In addition, a PEO/PEG standard set (117.9 – 0.194 kDa) was used for calibration and data were analyzed with Agilent OpenLab Data Analysis 2.5 software. Standards and samples were filtered through 0.45 μm syringe filters.

2.5. Statistical analysis

All the statistical calculations were done using Statgraphics 18-X64. The significance of the differences was determined based on an analysis of the variance with the Fisher's least significant difference (LSD) procedure at p-value ≤ 0.05. The correlation between different responses used in this work, was carried out using the Pearson product moment correlations at a 95% confidence level.

3. Results and discussion

3.1. Raw material composition

The composition of the OSW used as raw material in this work is presented in Table 1. The extractives content was determined and resulted to be 9.1 ± 0.2%. The composition of the raw material used in this work is essentially similar to that reported in the literature (Table 2), although different onion cultivars are used. This is the first time, as far as the authors' knowledge, that a complete characterization of the onion skins from Horcal onions is reported. Glucans are the main carbohydrates found in the OSW used in this work, followed by others formed by xylose, galactose or arabinose. Uronic acids are also present in great extent in onion skins (between 17.1% as reported by Jaime et al. [23], and up to 37.9%, according to Ng et al. [24]). In the *Horcal* variety used in this work, galacturonic acid represents 18.9 ± 0.3% of the total weigh, but glucuronic acid was found in small amounts. According to the results presented in Tables 1 and 2, it is possible to conclude that protein is a minor component in onion skins ranging from 1.9% reported by Suutarinen et al. [25] to 6.9% reported by Vojvodić et al. [26]. 3.2% was found to be in the *Horcal* variety used in this work, considering a protein factor of 6.25. Ash content is close to 10%, which is in accordance with Jaime et al. [23], who reported that ash was mainly concentrated in external skins. Lignin was found in onion skin wastes used in this work, being acid insoluble lignin 4.02 ± 0.15% (Klason lignin) and acid soluble lignin 10.84 ± 0.13%. Choi et al. [15] reported that Klason lignin was 9.4 ± 0.1%; Vojvodić et al. [26] reported that total lignin (acid

Table 1
Composition of the raw material and solid residues after subcritical water hydrolysis experiments at different temperatures. Results are expressed in g/100 g.

	Raw Material	SubW temperature (°C)				
		105	125	145	160	180
Glucan	33.3 ± 0.2^{A}	41.8 ± 0.2^{B}	45.7 ± 0.4^{C}	63.5 ± 0.11^{D}	64.9 ± 0.7^{E}	72.2 ± 0.9^{F}
Arabinan	1.63 ± 0.11^{E}	1.24 ± 0.07^{D}	0.95 ± 0.05^{D}	0.69 ± 0.01^{B}	0.61 ± 0.04^{C}	0.43 ± 0.05^{A}
Galactan	2.01 ± 0.21^{E}	1.49 ± 0.03^{D}	1.16 ± 0.13^{D}	0.95 ± 0.02^{B}	0.89 ± 0.01^{C}	0.87 ± 0.04^{A}
Xylan	2.1 ± 0.1^{A}	2.13 ± 0.03^{A}	2.23 ± 0.15^{A}	2.49 ± 0.04^{B}	2.45 ± 0.02^{B}	2.71 ± 0.09^{C}
Rhamnan	0.9 ± 0.03^{B}	0.17 ± 0.02^{A}	–	–	–	–
Glucuronic Ac.	0.16 ± 0.02^{B}	0.12 ± 0.02^{A}	–	–	–	–
Galacturonic Ac.	18.9 ± 0.3^{D}	21.9 ± 0.6^{F}	19.71 ± 0.18^{E}	5.9 ± 0.3^{B}	6.41 ± 0.15^{C}	3.09 ± 0.06^{A}
Total Ash	8.53 ± 0.30^{D}	5.33 ± 0.03^{A}	7.94 ± 0.13^{C}	8.52 ± 0.14^{D}	7.85 ± 0.11^{C}	6.88 ± 0.04^{B}
Soluble Lignin	10.5 ± 0.4^{D}	8.7 ± 0.3^{C}	8.94 ± 0.15^{C}	4.4 ± 0.3^{B}	4.88 ± 0.02^{B}	3.5 ± 0.2^{A}
Insoluble Lignin	5.88 ± 0.22^{A}	5.91 ± 0.07^{A}	6.8 ± 0.2^{B}	8.8 ± 0.3^{D}	7.89 ± 0.15^{C}	9.9 ± 0.2^{E}
Total Lignin	16.3 ± 0.6^{D}	14.6 ± 0.1^{A}	15.7 ± 0.4^{D}	$13.2 \pm 0.6^{A,B}$	12.77 ± 0.17^{A}	13.4 ± 0.4^{B}
Protein	3.2 ± 0.4^{C}	$2.9 \pm 0.23^{B,C}$	2.63 ± 0.11^{B}	1.23 ± 0.04^{A}	1.22 ± 0.04^{A}	1.28 ± 0.07^{A}
C	40.3 ± 0.3^{B}	38.4 ± 0.5^{A}	38.0 ± 1.0^{A}	40.3 ± 0.1^{B}	40.2 ± 0.1^{B}	41.4 ± 0.5^{C}
H	5.6 ± 0.1^{A}	5.6 ± 0.1^{A}	5.8 ± 0.0^{B}	5.8 ± 0.0^{B}	5.9 ± 0.1^{C}	$5.9 \pm 0.1^{B,C}$
N	0.7 ± 0.1^{D}	0.5 ± 0.0^{C}	0.4 ± 0.0^{B}	0.2 ± 0.0^{A}	0.2 ± 0.0^{A}	0.2 ± 0.0^{A}
O	44.8 ± 0.7^{A}	50.2 ± 0.8^{C}	47.9 ± 1.4^{B}	45.2 ± 0.3^{A}	45.9 ± 0.2^{A}	45.6 ± 0.2^{A}
Ratio H/C	1.68 ± 0.04^{A}	$1.75 \pm 0.06^{A,B}$	1.82 ± 0.06^{B}	$1.72 \pm 0.01^{A,B}$	$1.77 \pm 0.03^{A,B}$	1.71 ± 0.04^{A}
Ratio O/C	0.80 ± 0.01^{D}	0.87 ± 0.02^{C}	$0.84 \pm 0.04^{B,C}$	$0.75 \pm 0.01^{A,B}$	$0.76 \pm 0.01^{A,B}$	0.74 ± 0.02^{A}
Solid Solubilized (%)	–	18.3	20.9	37.9	36.2	43.6
HHV (MJ/kg)	15.8 ± 0.1^{C}	14.5 ± 0.2^{A}	$14.8 \pm 0.3^{A,B}$	15.9 ± 0.0^{C}	16.0 ± 0.1^{C}	16.4 ± 0.2^{D}

**Different letters indicate statistically significant differences at a 95% confidence level, according to the LSD test.*

Table 2
Chemical composition of onion reported by other authors.

Source	Onion Variety	Component (g/100 g raw material)									
		Protein	Ash	Lignin (Klason)	Glu	Xyl	Gal	Ara	Rha	Man	GalA
Choi et al.[15]	OSW	NR	5.7 ± 0.3	9.4 ± 0.1	40.6 ± 1.7	4.2 ± 0.6	1.8 ± 0.4	0.4 ± 0.1	2.5 ± 0.4	3.7 ± 1.0	22.1 ± 0.1 (TUA)
Suutarinen et al. [25]	Brown skin	1.9	5.7	1.5	32.3	2.2	1.2	0.4	1.2	1.1	16.6
Ng et al.[24]	*Sturon* (papery scales)	NR	NR	NR	35.5	3.2	1.6	0.8	0.8	1.6	37.9 (TUA)
Babbar et al.[16]	Onion hulls	NR	NR	NR	26.1 ± 0.1	2.9 ± 0.2	7.0 ± 0.9	0.3 ± 0.1	0.4 ± 0.5		19.3 ± 0.9
Kim et al.[27]	Onion Waste	NR	NR	NR	35.1	0.5	4.0	0.4	NR	0.6	
Jaime et al.[23]	*Sturon* (skin)	3.1 ± 0.1	8.5 ± 0.3	4.2 ± 0.2	17.9	0.8	0.4	0.4	0.4	1.6	17.1 (TUA)
	Hysam (skin)	3.4 ± 0.2	6.9 ± 0.1	1.6 ± 0.1	20.3	1.1	0.25	0.5	0.25	1.6	30.8
	Grano de oro (skin)	1.6 ± 0.1	7.2 ± 0.2	1.6 ± 0.4	31.4	1.2	0.3	0.6	0.3	1.8	25.3
Vojvodić et al.[26]	Onion peel	6.9 ± 0.0	9.4 ± 0.1	6 (AIL+ASL)	26.7	1.27	2.74	0.45	0.61	1.14	20.8
Benítez et al.[28]	*Recas* (brown skin)	2.3 ± 0.1	10.6 ± 0.6	NR	NR						
	Figueres (brown skin)	2.4 ± 0.0	9.3 ± 0.1	NR	NR						

TUA: total uronic acids; NR: Not reported; ASL: Acid soluble lignin; AIL: acid insoluble lignin.

soluble and acid insoluble lignin) was around 6%. Suutarinen et al. [25] found that Klason lignin was only 1.5%, value extremely lower compared to other authors' results and this work. Jaime et al. [23] reported that Klason lignin was in the range from 1.6% to 4.2% in three different onion cultivars.

3.2. Liquid phase after subcritical water hydrolysis

3.2.1. TOC and TN in liquid extracts

Fig. S1 (supplementary material) presents the effect of the subcritical hydrolysis temperature on TOC and TN content in the liquid extracts. More than 30% of the carbon present in raw material was extracted at 180 °C, whereas at 105 °C only 16% of the initial carbon was extracted. These results indicated that the hydrolysis process of the structural components of OSW, was favored by the change in the properties of SubW with temperature: the higher the temperature the higher the ionic product of water (K_w). This results in higher ionic strength of hydronium and hydroxide ions than at ambient temperature [24], which may induce the acid hydrolysis of the bonds between the bioactive compounds and the solid matrix, promoting their release. Similar trend was observed for the TN, with a highest yield of 30% at 185 °C. This increase in the TOC content of the liquid extract was probably due to the higher quantity of sugars extracted and other degradation products formed. Therefore, in the following sections, the study of the composition of the liquid extracts in terms of carbohydrates hydrolysates (both in monomeric and oligomeric form), pectin and degradation products is presented.

3.2.2. Sugars (C6 and C5)

The results presented in Table 3 show that most carbohydrates were recovered as oligosaccharides and that their extraction was strongly dependent on the working temperature. A sharp increase in the

Table 3
Accumulated concentration of monomeric and oligomeric sugars in the liquid extract after 180 min of extraction at different temperatures.

		SubW temperature (°C)				
		105	125	145	160	180
Monomer concentration (mg/g DOSW)	**Glu**	0.79 ± 0.05^{A}	0.91 ± 0.03^{A}	2.56 ± 0.06^{B}	3.48 ± 0.11^{C}	3.2 ± 0.5^{C}
	Gal	0.11 ± 0.01^{A}	0.67 ± 0.02^{A}	4.6 ± 0.2^{B}	3.89 ± 0.09^{B}	4.3 ± 0.7^{B}
	Rha	ND	ND	ND	ND	ND
	Ara	0.34 ± 0.03^{A}	1.55 ± 0.12^{B}	1.64 ± 0.08^{B}	3.7 ± 0.2^{C}	4.3 ± 0.2^{D}
	Xyl	0.11 ± 0.01^{A}	0.27 ± 0.03^{A}	1.8 ± 0.2^{B}	1.81 ± 0.15^{B}	1.88 ± 0.11^{B}
Oligomer concentration (mg/g DOSW)	**Glu**	8.0 ± 0.3^{A}	11.0 ± 0.8^{B}	14.2 ± 1.0^{C}	11.7 ± 0.9^{B}	14.8 ± 1.1^{C}
	Gal	5.5 ± 0.4^{A}	8.8 ± 0.6^{B}	9.5 ± 0.7^{B}	9.1 ± 0.8^{B}	8.8 ± 0.6^{B}
	Rha	3.04 ± 0.11^{A}	5.5 ± 0.2^{B}	7.2 ± 0.2^{D}	6.4 ± 0.3^{C}	$6.7 \pm 0.2^{C,D}$
	Ara	2.98 ± 0.05^{A}	6.72 ± 0.14^{B}	10.9 ± 0.8^{C}	7.4 ± 0.5^{B}	7.3 ± 0.4^{B}
	Xyl	1.02 ± 0.15^{A}	1.9 ± 0.1^{B}	$2.3 \pm 0.2^{B,C}$	2.04 ± 0.16^{B}	2.5 ± 0.2^{C}
Extraction yield (%)	**Glu**	2.6 ± 0.1	3.6 ± 0.3	5.0 ± 0.3	4.6 ± 0.3	5.4 ± 0.5
	Gal	27.9 ± 2.7	47.1 ± 4.3	70.1 ± 5.2	64.6 ± 3.2	65.2 ± 2.9
	Rha	32.7 ± 2.2	59.1 ± 4.1	77.4 ± 4.6	68.8 ± 3.6	72.0 ± 3.5
	Ara	20.4 ± 1.1	50.7 ± 3.2	76.9 ± 2.8	68.1 ± 2.4	71.2 ± 3.1
	Xyl	5.4 ± 1.0	10.3 ± 1.1	19.5 ± 1.8	18.3 ± 2.3	20.9 ± 1.9

extraction yield was observed when temperature reached 145 °C; probably due to the release of the pectic substances that form part of the OSW cell walls. The analysis of the composition of the liquid extracts will be useful to shed light on it. The results observed are in agreement with Pedras et al. [29], who reported similar trend when extracting bioactive compounds from coffee spent grains. Those authors also reported that monomeric sugars only account for the 5% of the total saccharides extracted using SubW. According to Table 3, glucose and galactose were the most abundant sugars, but other sugars such as galactose, rhamnose and arabinose had great extraction yields.

In Fig. 1 the hydrolysis kinetics of both monomeric and oligomeric sugars glucose (a), galactose (b), xylose (c), arabinose (d) and rhamnose (e) is shown. It is possible to observe that the concentration increases within the first working hour, reaching then a plateau. Temperature influenced the extraction yield of glucose up to 145 °C. The presence of glucose in the extract can be due to free glucose present in the raw material or the hydrolysis of any compound, but it is unlikely to be due to the hydrolysis of cellulose, due to the overall low glucose monomer yield (about 5% at 180 °C). According to the literature, it is possible to conclude that cellulose is not easily hydrolyzed under SubW conditions, since temperatures above 327 °C are required to achieve the complete hydrolysis of cellulose [30]. Unlike glucose, galactose yield is above 60% at temperatures higher than 145 °C, which indicates that this sugar is being released in great extent from OSW because of the SubW treatment. According to Klinchongkon et al. [31], galacturonan is the main structural component of pectin, so, the presence of galactose in the liquid extract indicates the release and hydrolysis of this structural component. A plateau around 22% extraction yield of monomeric galactose was found at temperatures above 145 °C, after 80 min of extraction.

Arabinose and xylose were found in both monomeric and oligomeric forms. Arabinose was found at 145 °C mainly as oligomer, but further increases in temperature decreased the amount of this oligomer. The amount of arabinose as monomer, however, did increase with temperature (160 and 180 °C), indicating that part of the oligomer is hydrolyzed to monomer. Xylose release was affected by the working temperature: at temperatures above 145 °C, more than 43% of the xylose in the liquid extract was found in the monomeric form, whereas at temperatures below 145 °C, this free form represented less than 15%. Rhamnose was only detected as oligomer, indicating that it is attached to the pectin structural polymers or linked to the flavonoids, forming quercetin-3-rhamnoside, as proved Benito-Román et al. [5].

The solubilization curves presented in Fig. 1, respond to the typical shape obtained in a semi-continuous process: sugars solubilization is relatively slow in the first minutes of operation, since it usually takes a few minutes to reach the extraction temperature. In our case, the configuration of the reactor used in this work allowed us to minimize this lag time to only 15 min. Then, sugars solubilization increases and reaches the highest rate after about 45 min, yielding the fractions with the highest concentration of sugars, and finally the solubilization rate decreases, so a plateau is reached [32]. Not further increases in the extraction yield of sugars are observed, which can be due to the combination of two factors, first the hydrolysis conditions are not harsh enough to produce the hydrolysis of the lignocellulosic materials, and second, sugars released are degraded. It would be necessary to perform further kinetic studies to evaluate which reaction (hydrolysis or degradation) is controlling the process. In all cases, a precipitate was formed when the liquid extracts cooled down, a phenomenon that could be due to the precipitation of the oligomers formed, which are soluble at high temperatures but not at room temperature. Extracts obtained at higher temperatures produced higher amounts of precipitates.

3.2.3. *Uronic acids*

In the extracts obtained from OSW under SubW conditions, GalA was the main uronic acid extracted, which appeared mainly in oligomeric form, as it is shown in Fig. 2. This is indicating that it is very likely that pectin is being extracted, so it will be studied in detail. The highest concentration of GalA was obtained at 145 °C, followed by a plateau (total GalA, 92 mg/g DOSW). Under these extraction conditions, almost 50% of the GalA was extracted, according to Fig. 2. The monomeric fraction reached a maximum at 145 °C, and further increases in temperature do not increase the monomeric GalA content in the extract (around 7 mg GalA/g DOSW). Small amounts of GluA (below 1 mg/g DOSW) were also detected in the aqueous extracts.

Given the acidic nature of pectin [33], the correlation between GalA concentration and pH was studied. The Pearson product moment correlations between those two parameters provided strong correlation (p-value 0.0004, at a 95% confidence level). This correlation can be observed in Fig. S2, where the total GalA (as the sum of the monomeric and oligomeric fraction) concentration in the extracts obtained at different extraction times together with the pH of the extract is shown.

3.2.3.1. Pectin isolation: composition and molecular weight. Pectin present in the SubW extracts obtained at three temperatures (125, 145 and 180 °C) was precipitated by ethanol addition. The recovered solid precipitate was dried, analyzed and compared to the composition of pectin extracted from OSW using the conventional procedure based on acid water (pH 2, obtained with HCl) at lower temperature (90 °C). Results are presented in Table 4.

It can be observed that SubW extraction provided higher pectin extraction yields than conventional extraction. Pectin extracted from OSW using water acidified with HCl had a relatively high GalA content (45.4%), but low concentration of neutral sugars (ratio uronic acids: neutral sugars (UA:NS), 7.8) which indicates that the pectin extracted

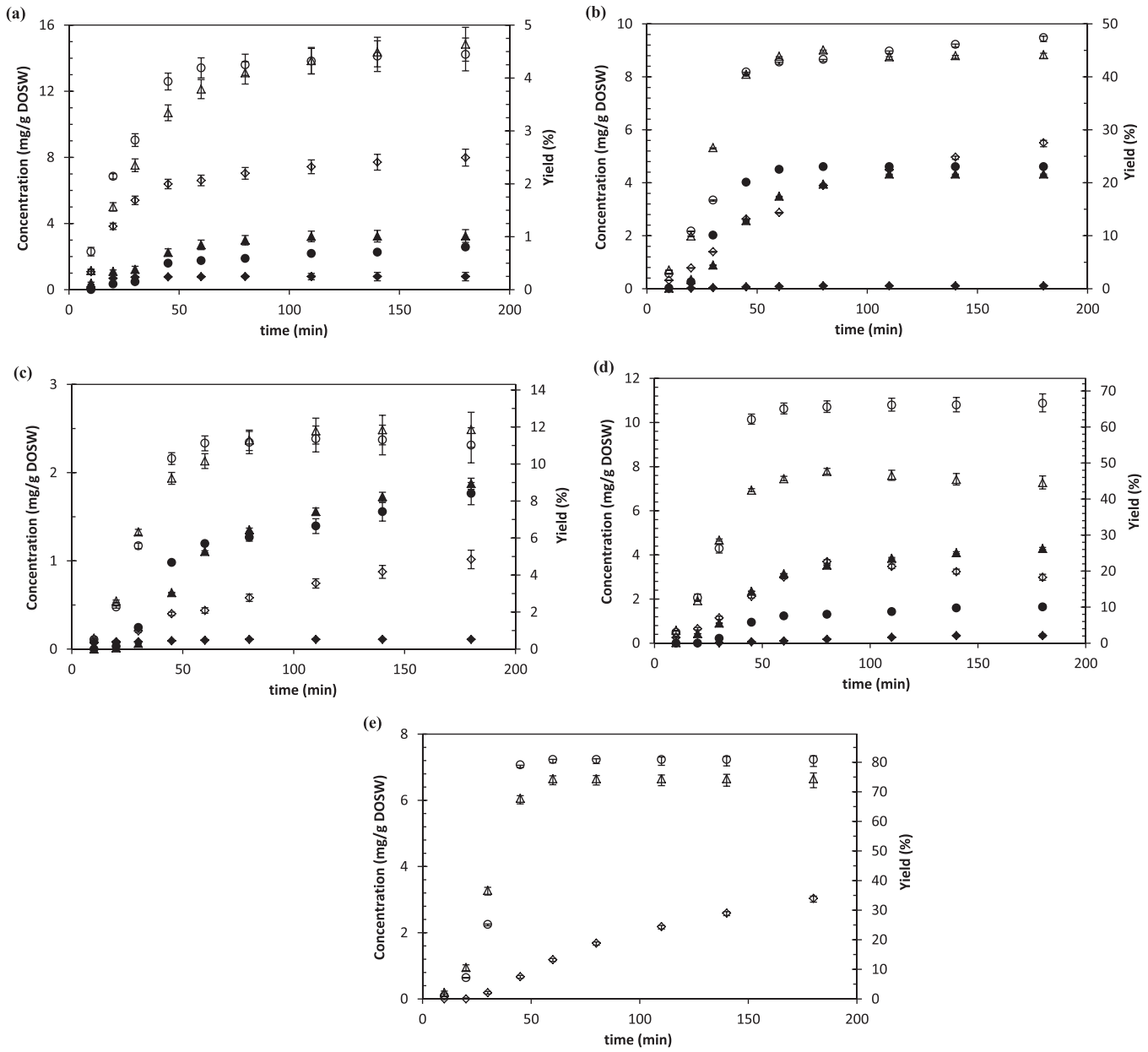

Fig. 1. Glucose (a), galactose (b), xylose (c), arabinose (d) and rhamnose (e) extraction kinetics as a function of the temperature (▲,△ 180 °C; ●, ○ 145 °C; ◆,◇ 105 °C). Full symbols refer to the monomeric sugar and empty symbols refer to oligomeric sugar.

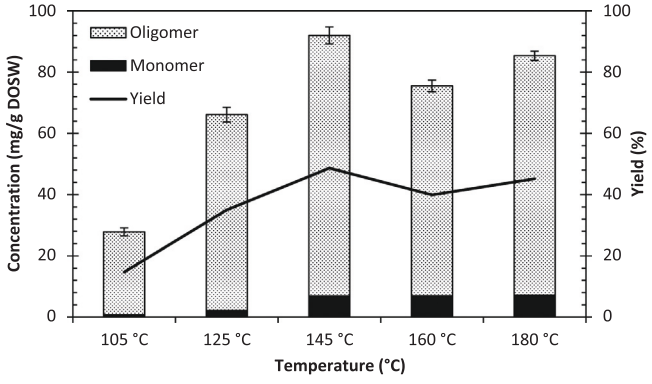

Fig. 2. Total GalA concentration as a function of temperature after 180 min of extraction. Both the oligomeric fraction and the monomeric fraction are presented, as well as the GalA extraction yield.

under acidic conditions was mainly composed by the HG domain. The calculations done by Eqs. (5) and (6) confirmed it: HG accounted for almost 81.6% of the total, with relatively small prevalence of RG-I domain. The calculation of the ratio Rha/GalA, which reflects the contribution of the RG-I domain in pectin, was 0.02. If this molar ratio ranges from 0.05 to 1, then the main constituent of the pectin is considered to be the RG-I region [34]; since this ratio is only 0.02, it can be concluded that in OSW pectin, HG is the predominant domain. It is known that acid extraction favors the recovery of the HG domain, as it promotes the degradation of the side chains of the RG-I domain [35]. The determination of the ratio (Gal + Ara)/Rha is helpful to determine whether or not the RG-I is highly branched: Ara and Gal are the major sugars in side chains and Rha is the branching point, so the molar ratio between them can reveal some RG-I domain properties [36]. In the case of OSW pectin extracted in acidic medium, the ratio (Gal+Ara)/Rha resulted to be 5.9, which is indicating that the RG-I domain is not highly branched, in contrats to potato pectins, which had a ratio in the range

Table 4
Composition of pectin extracted from OSW following a conventional extraction procedure and SubW treatment. Pectin yield was calculated according to Eq. (3).

	Conventional Extraction HCl	SubW Extraction		
		125 °C	145 °C	180 °C
Yield (%)	2.6 ± 0.1^{A}	5.2 ± 0.2^{B}	9.0 ± 0.4^{C}	8.6 ± 0.3^{C}
GluA (%)	0.9 ± 0.1^{A}	0.83 ± 0.07^{A}	1.67 ± 0.14^{C}	1.35 ± 0.06^{B}
GalA (%)	45.4 ± 0.4^{A}	72.6 ± 1.1^{C}	67.0 ± 0.1^{B}	67.7 ± 0.8^{B}
Ara (%)	0.32 ± 0.03^{A}	0.82 ± 0.06^{B}	0.41 ± 0.02^{A}	1.19 ± 0.03^{C}
Gal (%)	3.8 ± 0.3^{A}	9.3 ± 0.5^{C}	6.6 ± 0.4^{B}	9.2 ± 0.4^{C}
Rha (%)	0.65 ± 0.12^{A}	2.29 ± 0.11^{B}	4.5 ± 0.3^{C}	6.6 ± 0.3^{C}
Xyl (%)	0.56 ± 0.08^{A}	1.39 ± 0.02^{C}	1.07 ± 0.06^{B}	0.64 ± 0.02^{A}
HG (mol%)	80.3 ± 1.6^{C}	75.4 ± 2.7^{B}	71.7 ± 2.4^{B}	64.2 ± 2.2^{A}
RG-I (mol%)	10.8 ± 0.9^{A}	17.8 ± 0.9^{B}	21.1 ± 1.1^{C}	29.1 ± 1.8^{C}
Rha/GalA	0.02	0.04	0.08	0.13
(Gal+Ara)/ Rha	5.9	4.1	1.4	1.5
UA:NS	7.8 ± 0.5^{C}	4.7 ± 0.4^{B}	4.8 ± 0.6^{B}	3.4 ± 0.3^{A}

24–33 [34], but close to the that of pectins extracted from hawthorn berry (they were in the range from 8 to 10) [37].

Regarding the SubW extracted pectins composition, these showed much higher GalA content (around 67% at 145 and 180 °C) and even higher at 125 °C (72.6 ± 1.1%) than that extracted using acid extraction process. Galactose was the most abundant neutral sugar, which has also been detected in other raw materials, such as potato pulp [38], followed by rhamnose. The ratio Rha/GalA increased with the temperature, up to 0.13 at 180 °C, which is indicating the increased presence of the RG-I domain in the SubW extracted pectin. The calculation of the HG and RG-I domains confirmed the increased content in the RG-I domain, which opens a wide range of opportunities for the hairy pectins (RG-I) extracted from OSW using SubW. Recent studies have indicated that there is an increased interest in the RG-I-rich pectins (known as pectin-derived oligosaccharides –POS-) as they have prebiotic functions [35,39]. The bioactivity and ability to form gels of these molecules is related to their molecular weight, composition and structure. The decrease in the (Gal + Ara)/Rha molar ratio indicates that SubW pectin is not highly branched (it seems that these are linear chains formed by Gal) or at least the branches are short, probably due to hydrolysis reactions happening during the extraction process. Arabinose and xylose were also present in the structure of pectins, which might be indicating the presence of arabinogalactan and xylogalacturonan chains [40]. Jaime et al. [23] indicated that the Gal, Ara and Xyl content decreased in the onion brown skin, in comparison with inner layers. This was translated in a loss of the galactan and arabinan side chains, which makes the ratio UA:NS to increase in the outer layers of the onion. Pectins obtained in the present work using SubW presented a ratio UA:NS very low compared to other authors. Pectin extracted using the conventional procedure had a ratio UA:NS equal to 7.8, whereas this value was lower for pectins extracted using SubW. Therefore, various types of oligosaccharides can be released in the SubW hydrolysis processes: rhamnogalacturonan-oligosaccharides, galacturonan-oligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides and arabino-galactan oligosaccharides.

The molecular weight of the pectin samples was determined. Gel permeation chromatograms are shown in Fig. 3 and in Table S1 (supplementary material), where a complete description of the molecular weight distribution of each sample is presented. Important differences can be observed between pectin obtained using SubW and conventional acid extraction. Pectin after acid extraction only presented one peak at 16 min (corresponding to a MW > 200 kDa), whereas in the SubW pectins, besides this peak, up to 5 more peaks appeared, indicating the presence of lower molecular weight populations. Considering the composition of pectins reported in Table 4, in which acid extraction pectin was mainly composed by HG structures, it can be concluded that the peak that eluted at 16 min corresponds to HG domain, which is in agreement with the results reported by Dias et al. [41]. In SubW extracted pectins, HG domain represented from 6.7% at 145 °C to 10% at 125 °C. In these pectins, the main population had a MW in the range 52–56 kDa, with a polydispersity index from 1 (125 °C) to 1.2. Pectins obtained at 145 and 180 °C presented monodisperse populations with

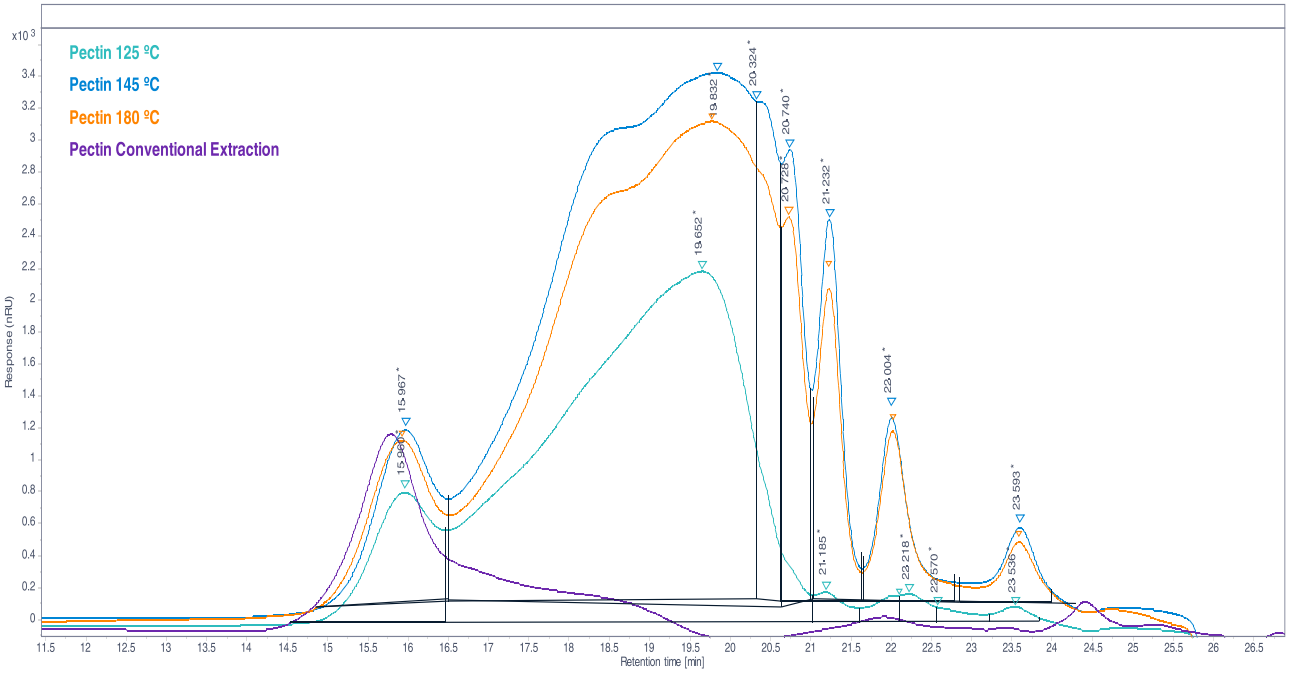

Fig. 3. Molecular weight distributions of OSW pectins extracted by different methods: conventional extraction (purple line) and SubW: 125, 145 and 180 °C (in green, blue and orangelines respectively).

smaller MW. This can be explained by hydrolysis reactions happening due to the high temperature. For instance, Klinchongkon et al. [42] studied the degradation of passion fruit pectin in SubW using a continuous reactor, in temperatures from 80 °C (0–25 min, residence time) to 160 °C (0–5 min residence time). MW was strongly affected by the working conditions being reduced from values higher than 200 kDa (initial pectin) to 7 kDa in the most severe conditions (severity factor, 2.47). At 80 °C the degradation was slower, and took 25 min to achieve a decrease in MW of 39% at 80 °C (severity factor, 0.81). The degradation reaction resulted to be temperature dependent and followed the Arrhenius equation. Dias et al. [41] and Pedraza-Guevara et al. [43], also reported multimodal distributions for pectins obtained from gabiroba fruit and unripe papaya, respectively. More specifically, the latter estimated that 10% of the populations had MW in the range from 0.34 to 1.6 kDa.

3.2.4. Organic acids

The main organic acids detected in the OSW extracts were acetic, formic and levulinic (Fig. 4). The conversion of sugars and uronic acids into organic acids and other products in subcritical/supercritical water is very complex, as has been presented in several research works [44–46]. Regarding the degradation of uronic acids, it has been demonstrated that they are more susceptible to degradation than pentoses and among the uronic acids, GluA suffers a faster degradation than GalA in SubW at temperatures in the range from 140 to 160 °C [47]. The comparison of the present results with those published in the literature is hard, because in most cases these reactions are carried out under supercritical conditions. Moreover, those research works used pure compounds (glucose, fructose, inulin) as raw material, whereas in the present study onion skin was used, which has a complex composition (as summarized in Table 1).

According to Fig. 4, it is possible to see that these acids were detected when temperature was above 145 °C. At lower temperatures, low amounts of formic and acetic acids were detected. Acetic acid increased with temperature to reach a maximum at 180 °C, as well as formic acid, whereas levulinic acid concentration decreased at temperatures above 160 °C. The presence of organic acids was only detected after 45 min of extraction, moment in which the maximum amount of GalA was extracted, which might be indicating the role of pectin degradation in the formation of organic acids and other degradation products.

Formic acid (HCOOH) can be formed by direct conversion of sugars or through a more complex pathway which involves first the conversion of fructose in HMF (by dehydration) and then its rehydration to yield levulinic acid plus formic acid. Salak et al. [13] found that formic was the main organic acid detected in the subcritical hydrolysis of onion skin (batch reactor, 5 min residence time, severity factor in the range from 0.7 to 7.2), and that its production began at 160 °C (severity factor, 2.47; formic acid concentration below 2 mg/g onion skin (db)) with a maximum at 240 °C (severity factor, 4.82) close to 21 mg/g onion skin (db). These authors [13] also found that the production of acetic acid showed a steady increase from 180 °C to 320 °C (maximum 10 mg/g onion skin (db)). These authors also detected lactic acid, only formed at temperatures above 220 °C, similarly to levulinic acid, although this one had a concentration 20 times lower than lactic acid [13]. In our results, in which the severity factor ranged from 1.15 (at 105 °C) to 3.33 (at 180 °C), longer residence times in the reactor compared to those reported by Salak et al. [13], favored the formation of formic acid in greater extent. Formic acid has been also reported to be one of the main degradation products from GalA in SubW [48]. These authors showed that high methyl ester citrus-apple pectin, at 150 °C after 40 min, yielded 63.7 mg/mg raw material of formic acid and 18.6 mg/mg raw material of furfurals. In our case, the acid hydrolysis of GalA (1 h, 121 °C, 4% H_2SO_4) yielded mainly furfural and formic acid (data not shown), being degraded nearly 40% of the initial GalA.

In our work, lactic acid was not detected. From C6 sugars, according to Aida et al. [44], lactic acid would be formed by retro aldol reactions that yield glyceraldehyde, which is further converted into pyruvaldehyde via dehydration, which in turn can form lactic acid via benzylic acid rearrangement and further dehydration. In our samples, according to Fig. S3, glyceraldehyde was hardly detected at temperatures below 145 °C, the maximum being reached after 80–110 min, whereas at higher temperatures it was found in larger concentrations (but less than 1 mg/g DOSW) after 30 min of extraction. Pyruvaldehyde was not detected, which might be indicating that the working conditions are not harsh enough to force to conversion of sugars into lactic acid. In the extracts obtained at temperatures higher than 145 °C, glycolaldehyde was also found, (total amounts accumulated after the extraction were 0.63 mg/g DOSW at 145 °C and 2.28 mg/g DOSW at 180 °C). This compound is formed from glucose that is first converted into erythrose and then via retro aldol reactions in glycolaldehyde [45]. This compound is mainly formed at high temperature (180 °C) and after long extraction time (>80 min).

3.2.5. Degradation products: HMF and furfural

Furfural and HMF were detected in the extracts obtained from OSW in the temperature range 105–180 °C, as presented in Fig. 5. The formation of furfural and HMF can be explained by two different mechanisms: via sugar dehydration reactions [49] or Maillard reactions [45]. Maillard reactions happen between the carbonyl group of the sugar and the amino group of an amino acid, at temperaures above 50 °C and slightly acid pH (4−7) [50].

In general, HMF is formed by the dehydration (−3 molecules of H_2O) of the six carbon sugars (glucose, fructose). The formation of HMF usually begins from fructose; which means that, if the raw material is glucose, first, the isomerization of glucose to fructose (via tautomerization) must happen [50]. The formation of furfural uses the C5 sugars present in biomass (xylan, arabian or others forming hemicelluloses) as precursors [51]. The formation of furfural and HMF as degradation products is influenced by the pH of the media, the initial sugar

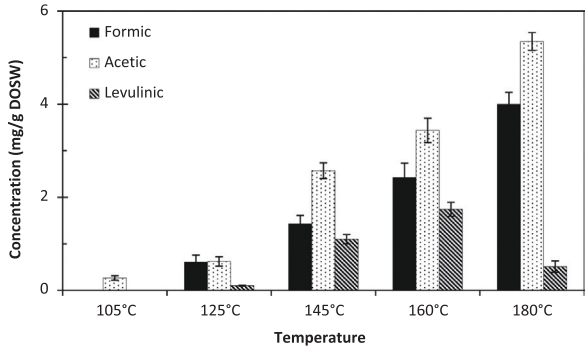

Fig. 4. Accumulated organic acids in the liquid samples obtained at different temperatures.

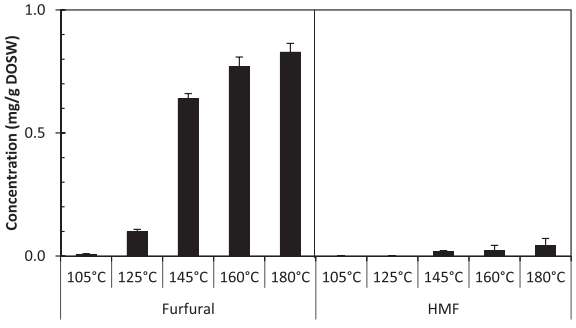

Fig. 5. Furfural and HMF concentration found in the SubW extracts (total accumulated).

concentration and the nature of the acid used to decrease the pH [52], in the case it is used.

According to Fig. 5, HMF was barely found in the liquid extracts. As explained before, HMF is formed from C6 sugars; but the amount of free C6 sugars (especially glucose) detected in the liquid extracts obtained in our work, is very low. In turn, furfural was the main degradation product, and it was also observed that the higher the temperature the more furfural detected. It was observed that the concentration of xylose (and the other C5 sugars) did not increase with temperature when it was increased above 145 °C (see Table 3), which might be indicating the degradation of this sugar and the formation of furfural. As previously indicated, furfural is formed because of the GalA degradation. It is also possible that furfural is formed from C6 sugars, but it is a complex process that only happens at high pressure and temperature (above 350 °C), according to Aida et al. [44]. Given the fact that in this work the highest temperaure achieved was 180 °C, it is very unlikely that furfural was formed from the hydrolysis of the C6 sugars. In Fig. S4 the furfural production kinetics at three different temperatures is shown.

3.3. Solid phase after SubW hydrolysis

The temperature used in the SubW hydrolysis experiments affected the amount of solid phase obtained after the experiment, as well as the final appearance of the OSW residue after extraction (Fig. S5). In general, the higher the temperature the more OSW was dissolved, as summarized in Table 1. It is possible to see that at 145 °C a sharp increase in the biomass dissolved was observed. At 180 °C, about 40% of the raw material was dissolved, whereas at 105 °C less than 20% was dissolved. Salak et al. [13] reported similar results, indicating that onion skins are composed by strong fibers that are barely dissolved in SubW at temperatures around 180 °C. However, those authors used a batch reactor to treat OSW for 5 min at different temperatures, whereas our hydrolysis experiments were carried out in a semicontinuous reactor, where fresh solvent was being continuously pumped through the OSW for 180 min, with a residence time of the solvent in the extractor in the range from 9.5 (at 180 °C) to 10.2 min (at 105 °C). The presence of high content of lignin and glucans may explain these results (Table 1). It has been observed that, in all cases, the fraction of insoluble lignin increases in the solid residue after the subcritical water extraction treatment. It seems that working conditions are not harsh enough to dissolve lignin. Nevertheless, soluble lignin decreases in the solid residue when increasing the hydrolysis temperature, indicating that the soluble fraction of lignin has been partially extracted under the working conditions. The higher the temperature the higher the glucans content with clear statistically significant differences attributed to the working temperature. This may be indicating that structural components such as cellulose are not being dissolved in subcritical water, whereas other structural components such as pectin (mainly formed by uronic acids) are being dissolved, according to the results presented in Table 1.

The elemental analysis (CHNS) revealed differences between the subcritical water experiments carried out at the lowest temperatures (105 and 125 °C) and the others. In none of the samples sulphur was detected. In Table 1, it is possible to see an increase in the carbon content with temperature, observing statistically significant differences in the carbon content of the residue when shifting temperature from 125 to 145 °C. Regarding the content of oxygen in the solid residue, a decrease in the solids treated at higher temperatures is observed, which might be due to the loss of water molecules associated to dehydration reactions that happen during subcritical water treatment. The conversion of sugars, such as glucose or xylose, to degradation products such as furfural or HMF, are linked to dehydration reactions [49].

From the results presented in Table 1, the molar ratio H/C was calculated, which indicates the relative proportion of double bonds in the chemical species present in the samples (the presence of aromatic rings compared to other kind of aliphatic molecules). In general, it is possible to observe that the ratio H/C tends to increase with temperature. This is indicating a lower share of aromatic rings or double bonds in the solid residue after subcritical water experiments compared to the raw material. The lowest values for the ratio H/C was observed at 145 and 180 °C, temperature at which, according to Benito-Román et al. [5], the highest amounts of flavonoids (such as quercetin and derivatives) had been extracted. The ratio O/C was also calculated. When temperature was above 145 °C, this ratio was statistically lower than that calculated for the raw material, according to the results presented in Table 1. This indicates a higher share of carbon in the structure, which makes it more suitable as raw material for thermochemical processes.

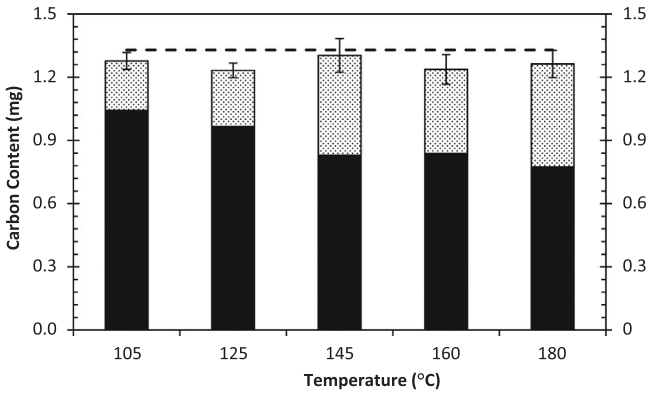

Fig. 6. Recovered carbon in liquid extract (dotted bar) and total carbon in solid residue (solid bar) after SubW hydrolysis. Dashed horizontal line represents the initial amount of carbon present in the OSW sample loaded in the reactor.

The High Heating Value (HHV) of the solid residue and the raw material, was evaluated using Eq. (7) [13]. The HHV is an indicator of the enthalpy of complete combustion of a fuel [53].

$$HHV \quad \left(\frac{MJ}{kg}\right) = [151.2C + 499.7H + 45.0S - 47.7O + 27.0N]\cdot 2.326\cdot 10^{-3} \tag{7}$$

Results of the HHV are presented in Table 1. HHV tends to increase in the onion residues obtained after extractions carried out at higher temperatures, with a maximum at 180 °C. Salak et al. [13] reported an increase of the HHV with temperature to reach a maximum at 270 °C (residence time, 5 min; HHV 16.99 MJ/kg)). Further increases in temperature decreased the HHV, probably due to the loss of the amount of carbon through gasification reactions. According to these results, it is possible to consider the solid residue as a fuel after the complete removal of extractives and other structural components such as sugars or pectin as it has been described in previous sections.

3.4. Carbon mass balance

Fig. 6 presents the carbon mass balance. It is possible to see how the higher the temperature the more carbon is found in the liquid extract, proving that SubW promotes hydrolysis reactions. It is also possible to see that the amount of carbon quantified in the solid extract and the amount of carbon present in the liquid does not completely match the initial carbon present in the sample loaded in the reactor. This can be due to solid residue losses after the extraction or to gasification reactions.

4. Conclusions

In this work, it has been demonstrated that SubW is an efficient reaction medium to promote the solubilization of high added value components from OSW, especially the RG-I pectin, compared to the conventional pectin isolation process that yields mainly the linear HG pectin. The temperatures studied in this work (105–180 °C) and residence time (10.5 min) were not high enough to produce monomers from

the hydrolysis of the structural components in onion skin wastes since most of the sugars are recovered in oligomeric form. The extraction of oligosaccharides was promoted by temperature: a sharp increase of the hydrolysis rate was found at temperatures above 145 °C. Besides sugars, the presence of organic acids (mainly formic, acetic and levulinic) was detected together with degradation products such as furfural and in lower extent HMF. The latter was barely found, which might be indicating that the hydrolysis of glucans to produce C6 sugars is very limited under the working conditions used in this work, whereas furfural formed from C5 sugars and GalA, indicates that these compounds are primarily released.

The solid residue obtained after the extraction process can be used as a fuel due to its low moisture and high HHV value, which is higher the higher the extraction temperature. This demonstrates that SubW is an efficient way of valorizing onion skin wastes, first promoting the extraction of flavonoids, then the recovery of sugars in oligomeric form, yielding an exhausted solid residue that could be used as a fuel.

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CRediT authorship contribution statement

Ó. Benito-Román: Investigation, Data curation, Writing – original draft, Funding acquisition. **P. Alonso-Riaño:** Investigation, Data curation. **E. Díaz de Cerio:** Investigation, Data curation. **M. T. Sanz:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **S. Beltrán:** Writing – review & editing, Conceptualization, Visualization, 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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.107439.

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