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Title: Production of molecular weight fractionated hemicelluloses hydrolyzates from spent coffee grounds combining hydrothermal extraction and a multistep ultrafiltration/diafiltration

Article Type: Original research paper

Keywords: coffee; polysaccharides; autohydrolysis; membrane; supercritical CO2

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Research Data Related to this Submission

Title: Data for: Production of molecular weight fractionated hemicelluloses hydrolysates from spent coffee grounds combining hydrothermal extraction and a multistep ultrafiltration/diafiltration
Repository: Mendeley Data
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***Detailed Response to Reviewers**

Manuscript Number: BITE-D-19-03822R1

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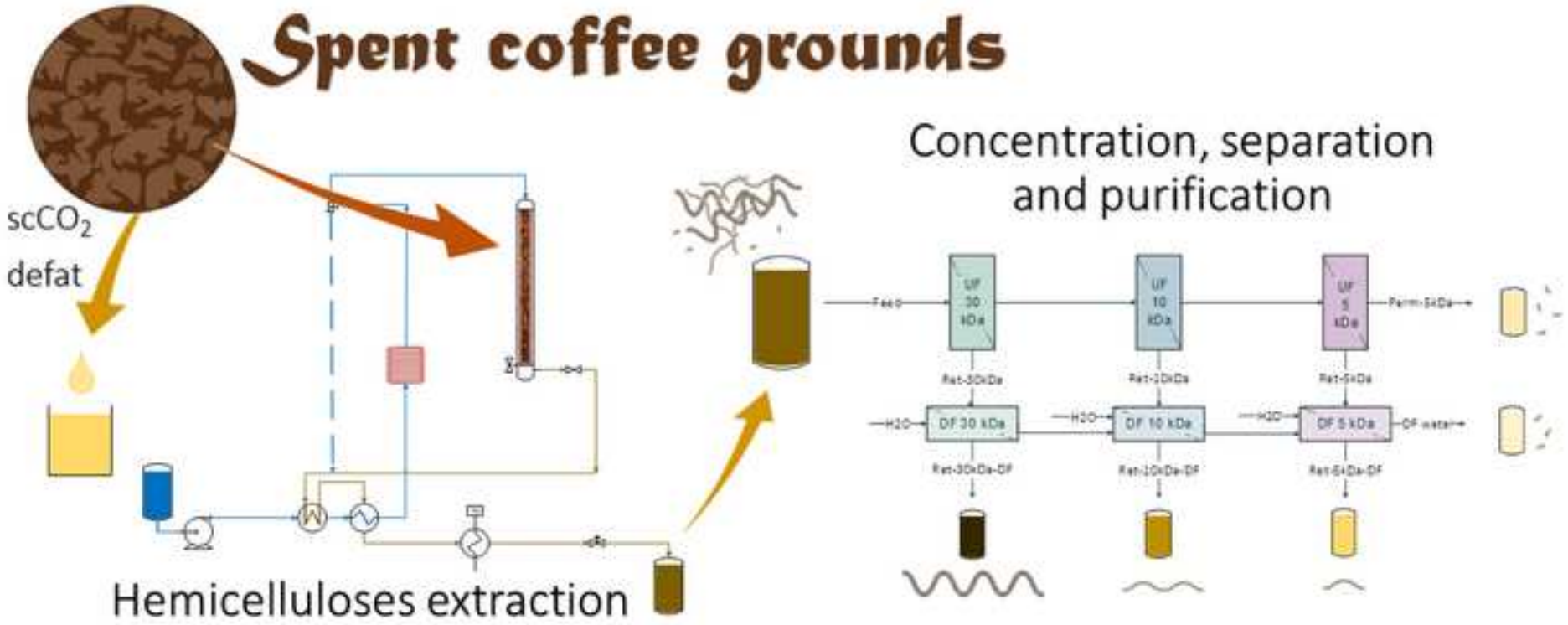
Comments:

We appreciate the editor's helpful comments. The latest modifications have been marked in green in the manuscript.

According to instructions on supplementary material, a sentence like "The ATR-FTIR spectra of spent coffee grounds oil and spent coffee grounds before and after extractions were determined (E-supplementary data of this work can be found in online version of the paper)." cannot be accepted. The part between brackets must simply be deleted.

It was deleted as indicated.

Graphical Abstract (for review)



HIGHLIGHTS

- Spent Coffee Grounds supercritical CO2 defat and hydrothermal hydrolysis
- Concentration of target groups of hemicelluloses up to 5-fold by ultrafiltration
- Separation of hemicelluloses in three fractions by a cascade ultrafiltration system
- Purification of fractionated hemicelluloses by diafiltration reusing water
- Reduction of the concentration of not target hemicelluloses by diafiltration

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Production of molecular weight fractionated hemicelluloses hydrolyzates from spent coffee grounds combining hydrothermal extraction and a multistep ultrafiltration/diafiltration

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Abstract

Spent coffee grounds are a huge residual stream from instant coffee makers. The production of spent coffee oil and molecular weight fractionated hemicellulose hydrolysates via supercritical CO_2 and a hydrothermal treatment followed by concentration, separation, and purification through cascade ultrafiltration/diafiltration (30-10-5 kDa) was studied. Hemicelluloses extraction yield reached 3.49 g/100 g of dry defatted spent coffee after 40 min at 160 ºC. The ultrafiltration system allowed concentrating up to 5-fold certain groups of hemicellulose, being most of them retained in the first membrane. Hemicellulose concentration and molecular weight of the feed exerted a great influence on the mass transfer through the membrane due to the formation of aggregates. However, purification through diafiltration allowed both to decrease by-products retentions from 45.6% to 8.7%, increasing the molecular weight of each fraction. Six hemicellulose products were obtained with purities between 83.7 – 97.8 wt% and weight-average molecular weights between 1641 and 49733 Da.

Keywords: coffee; polysaccharides; autohydrolysis; membrane; supercritical CO_2

1. Introduction

Spent coffee grounds are one of the main organic wastes from the food industry the opportunity of revalorization is huge. In both the preparation of a coffee beverage and in the production of soluble coffee, the coffee is subjected to extraction with hot water or steam for the release of several substances that are transferred to the liquid phase. The solid residue of this process is known as spent coffee grounds. Worldwide, coffee is the second-largest commodity, being a 50 % of its worldwide production destined for the manufacture of soluble coffee which generates around 6 million tons of spent coffee grounds per year (Ballesteros et al., 2014). This waste is currently burned to produce energy (e.g. in cement production) or dumped in the landfills but these options have disadvantages. Thus, incineration only recovers part of the energy and emit polluting gases into the atmosphere, and the deposition in landfills means (1) occupying a space with waste instead of using it for other purposes such as cultivation, (2) emission of odors associated with fermentation processes, and (3) release into the environment of substances of certain toxicity such as caffeine, tannins and polyphenols (Melo et al., 2014). In this sense, due to environmental problems that affect our society, it is necessary to look for alternative resources for the production of high value-added products being these resources, renewable and non-polluting. In addition, it is desirable that their use as biomass raw material (RM) does not compete with any other possible application such as the food industry.

In recent years several studies have been focused on the valorization of spent coffee grounds looking for alternative uses. Due to this, several compounds of interest have been extracted, mainly coffee oil (Jin et al., 2018; Loyao et al., 2018), carbohydrates (Getachew et al., 2018; Nguyen et al., 2019), and bioactive compounds (Pettinato et al., 2019; Shang et al., 2017). Coffee oil has applications as additive in cosmetic formulations, in the manufacture of biodiesel and in the bioproduction of polyhydroyalkanoates; coffee carbohydrates can be used as both, food and pharmaceutical additives, and also as carbon source in biotechnology; bioactive compounds have applications as supplements and in pharmacy due to their antioxidant, antibacterial, antiviral, anti-inflammatory and anticarcinogenic properties (Campos-Vega et al., 2015; Zabaniotou and Kamaterou, 2018). The solid residue after the extraction of these valuable compounds has also applications such as filler for polymers, adsorbent, biochar, bio-oil, fuel, and compost (Kovalcik et al., 2018).

Among the mentioned compounds, the extraction of carbohydrates from spent coffee grounds is of great interest as cellulose and hemicellulose constitute approximately 45% of the weight of the RM (Campos-Vega et al., 2015). In spent coffee grounds, hemicelluloses are heterogeneous carbohydrates generally formed by hexoses (mannose, galactose, and glucose) and pentoses (arabinose) (Campos-Vega et al.,

2015). Consequently, the types of hemicelluloses that may be mainly present are glucomannan and galactoglucomannan. Galactomannans are high molecular weight polysaccharides with a low level of branching, composed of a backbone of mannose containing single galactose side groups. Depolymerization and debranching of galactomannans occur during the roasting process increasing their solubility in water (Ballesteros et al., 2017). It should be noted that about 70 wt% of the hemicelluloses glucomannan and galactoglucomannan remain in the spent coffee grounds matrix after the coffee brewing process or during soluble coffee production (Zabaniotou and Kamaterou, 2018).

After the extraction, hemicelluloses can exhibit different configurations such as monomeric, oligomeric, and polymeric with different degrees of polymerization. The properties and, therefore, the industrial applications will be different depending on their configuration and molecular weight distribution. In monomeric form, hemicelluloses are known for their applications in the production of biochemicals, biosugars, and energy (Baptista et al., 2018; Dutta and Chakraborty, 2018; Luo et al., 2019); in their oligomeric form, hemicelluloses are used as feed and pharmaceutical additives (Samanta et al., 2015); and in their polymeric form, hemicelluloses have applications in food packaging, hydrogels, and thermoplastics (Farhat et al., 2018; García-Uriostegui et al., 2018; Liu et al., 2019). With regards to hemicelluloses extracted from spent coffee, films formed from glucomannan have proven to have interesting properties such as good gas barrier, flexibility, and mechanical strength, making them suitable for food packaging (Fortunati et al., 2016). Hydrogels with drug-delivery applications have also been developed from galactoglucomannan (Voepel et al., 2009). In their oligomeric form, mannooligosaccharides from spent coffee have been produced by hydrothermal treatment at 220 ℃, with further monosaccharides removal by carbon chromatography (Takao et al., 2006). The major mannooligosaccharides were mannobiose (19 %), mannotriose (27 %), mannotetraose (21 %) and mannopentose (17 %). These oligosaccharides had good effects reducing abdominal and subcutaneous fat accumulation in humans when administered daily. The bioactivity of these hemicelluloses, associated with their oligomeric form, was also studied by Getachew and Chun (2017) through a reduction of molecular weight by hydrothermal treatment in a semi-continuous reactor. Hemicelluloses from coffee were extracted by alkaline treatment, being less than 5% of the total hemicelluloses in monomeric form. The modified hemicelluloses obtained by hydrothermal treatment at 180 and 220 ℃ showed a much higher antioxidant capacity.

The high valuable hemicelluloses can be extracted by different extraction methods: dilute acid extraction, hot water extraction, steam explosion extraction, alkali extraction, dilute acid steam explosion extraction, organic solvents extraction, and microwave-assisted extraction. Both dilute acid extraction and alkaline extraction are

among the most commonly used, as hemicelluloses can be extracted in both alkaline and acid solutions (Fortunati et al., 2016). These extractions give rise to quite defined hemicelluloses whose molecular weight range is not very wide, with polydispersity values usually lower than 2. On the contrary, the extraction of hemicelluloses by hydrothermal treatment can produce a wide variety of molecular weights in hemicelluloses depending on the severity of the treatment.

The variety of molecular weights in the hemicelluloses that can be extracted by hydrothermal treatment makes necessary a downstreaming process that allows both to obtain hemicelluloses of adequate purity and to separate them depending on the molecular weight. The most common downstream processes are precipitation with ethanol and chromatography. However, following the philosophy of respect for the environment, it is worth noting the use of ultrafiltration (UF) membranes. Although the fractions of hemicelluloses obtained by membranes are more heterogeneous (Peng et al., 2012), this separation process has multiple advantages, as low energy requirements, mild operating conditions, no chemicals are required except for cleaning, and the equipment has a simple and easily scalable design (Nayak and Bhushan, 2019).

Ultrafiltration/Diafiltration (UF/DF) has been used by several authors in different types of biomass to obtain different hemicellulose fractions. Thuvander and Jönsson (2016) used downstream processing of galactoglucomannan-type hemicelluloses from the process water from a thermomechanical pulp mill with spruce as primary RM. The process was carried out using a cascade system of prefiltration, microfiltration and UF. The concentration was increased thanks to UF from 0.85-1.5 g/L to 25-52 g/L, using a high feed volume reduction of up to 98%. Likewise, in a later study of the same authors, they used diafiltration (DF) to purify the previously ultrafiltered hemicelluloses, but the desired effect was not achieved since the percentage of retention of hemicelluloses and lignin in the membrane was very similar, not achieving the separation between them (Thuvander et al., 2016). Laine et al. (2015) carried out extraction by alkaline hydrolysis with NaOH from both wood pulp and brewer's spent grain. It was demonstrated that their UF/DF system allows not only to separate and purify the hemicelluloses but also to recover and recirculate part of the NaOH used in the extraction.

González-Muñoz et al. (2013) used a hemicelluloses concentration and DF system composed of 5 membranes in cascade with molecular weight cut-off (MWCO) of 10, 5, 3, 1 and 0.3 kDa. With this system, they were able to separate and purify the hemicelluloses obtained by hydrothermal treatment from *Pinus pinaster* wood. The initial concentration of hemicelluloses in the autohydrolysis liquor was 17.92 g/L, meanwhile, the concentration of the hemicellulose fractions obtained after the downstream process was in the range 1.08-6.01 g/L. This decrease in concentration is normal due to obtaining several fractions from a single feed. The purity was increased from 69.4 wt% in the feed liquor to values between 80.5-90.3 wt% in the diafiltered fractions, which also had different molecular weight distributions.

To the best of our knowledge, very few UF experiments have been published on carbohydrates obtained from spent coffee grounds as biomass. Moreover, environmental concerns make it necessary not only to use residual and renewable raw materials but also to apply environmentally friendly both extraction and downstream processes, using as few auxiliary chemicals as possible. The raw material was conditioned by extracting the spent coffee oil using supercritical CO_2 ($scCO_2$). In the hemicellulose extraction process, hydrothermal treatment requires only biomass and water at certain pressure and temperature. During the downstream stage by UF/DF, only a certain transmembrane pressure is required and water as the only agent in the purification step. Accordingly, the aim of this work was to investigate the technical feasibility of the hydrothermal extraction of hemicelluloses from spent coffee grounds, and the subsequent concentration, separation, and purification of hemicelluloses through prefiltration plus cascade UF/DF. The target of the downstream processing was the production of fractions of purified hemicelluloses with different properties and potential applications. The results from this work will make it possible to plan the production of hemicelluloses of different composition and molecular weight distribution on a larger scale, for the subsequent direct application or transformation into high value-added products.

2. Materials and methods

2.1. Raw material and sample preparation

Spent coffee grounds were supplied by PROSOL Productos Solubles S.A. (Palencia, Spain). The RM was dried and stored in a cool, dry and dark place so that its composition and physical properties would not be affected by atmospheric conditions. When necessary, the sample was sieved to obtain different fractions.

2.2. Analysis of the raw material

The composition of the dry RM was determined according to the standard methods published by National Renewable Energy Laboratory (NREL), as in previous works of the research group (Gallina et al., 2018; Yedro et al., 2015).

The humidity content of the biomass was determined in order to express the results on a dry weight basis, being the weight contribution of moisture mathematically removed. A 105 ºC convection oven-drying procedure was applied to the biomass for this determination.

The sample was subjected to three consecutive Soxhlet extractions using hexane, water, and ethanol as solvents, respectively for 24 hours/each. Hexane, a non-polar solvent, was used for extracting lipophilic compounds, such as terpenoids and fats. Water

extractives may include non-structural sugars and sugar acids, nitrogenous materials, and inorganic materials. Ethanol soluble material includes waxes, chlorophyll, and other minor compounds.

The amount of inorganic material in the biomass was measured as ashes. Structural ash is mainly inorganic material bonded in the physical structure of biomass, while extractable ash is an inorganic material that can be the result of soil remaining in the material. To quantify the total ashes, a certain amount of the sample was subjected to dry oxidation at 575 ºC until constant weight. The unburnt part corresponded to the ashes.

The protein content was evaluated indirectly by a standardized Kjeldahl method. Measurement of the nitrogen content was corrected by nitrogen-to-protein conversion multiplier to determine the protein content in the biomass.

Structural carbohydrates and lignin were determined using a two-step acid hydrolysis. In the first step, 3 mL of sulfuric acid (72%) were added to 300 mg of a biomass sample while stirring. The mixture was incubated at 30 ºC for 1 hour in a thermostatic bath. In the second step, 84 mL of Milli-Q water were added and the sample was autoclaved for 1 hour at 121 ºC. A liquid aliquot of 50 mL was used to determine the acid-soluble lignin and carbohydrates. The hydrolysis process fractionated the biomass into acid-insoluble material and acid-soluble material. The product after autoclaving was vacuum filtered and rinsed. An aliquot of 50 mL was used to determine structural carbohydrates and acid-soluble lignin. Structural carbohydrates were hydrolyzed into monomeric sugars, which are soluble in the hydrolysis solution and quantified by HPLC following the method described in section 2.6.1.2. Acid-soluble lignin was quantified by UV-Vis spectroscopy. The acid-insoluble lignin was measured gravimetrically. The solid residue from the vacuum filtration was dried at 105 ºC for 24 hours and weighed. The dried solid was placed in a furnace at 575 ºC and weighed again. The final solid after the weight is constant was considered as ash, and the difference between the solid weight before and after combustion at 575 ºC corresponds with the amount of acid-insoluble lignin.

2.3. Supercritical fluid extraction

The spent coffee grounds oil was recovered through supercritical CO_2 ($scCO_2$) extraction at 300 bar, 45 ºC and a flow rate of 5 kg/h of $scCO_2$ in recirculation, during 2 h. The details of the experimental supercritical extraction pilot plant were described recently (Mustapa et al., 2015).

2.4. Hydrothermal treatment

Extraction of hemicelluloses from the RM was performed using a flow-through pilot reactor, schematized in Fig. 1 and previously described by (Gallina et al., 2018). Biomass is placed inside the reactor of 2 L volume (R-01) with the help of a cartridge, which facilitates biomass handling. A constant flow of fresh water is pumped (P-01) from a feed tank (D-01) through two concentric tube heat exchangers (12 m total length, ¼'' internal tube- 3/8'' external tube) and an electric heater with a maximum power of 5 kW (E-01, E-02 and H-01, respectively). After heating, water passes through a three-way valve (V-T1), which allows the water to be introduced into the reactor from the top or to be bypassed to the outlet of the reactor. The biomass-loaded reactor is initially filled with water pumped (P-02) from a second feed tank (D-02). After filling, the reactor is closed, and heated by the electric clamp resistors located on the wall of the reactor. When both water inside the reactor and water bypassed have reached the temperature setpoint, the three-way valve (V-T1) allows fresh water to enter the reactor, starting the operation. The outlet valve (V-01) is also opened at the same time, and thus the extraction process begins. Autohydrolysis liquor passes through the two previous heat exchangers (E-01 and E-02) again, thus heating the feed stream and achieving significant energy savings. Finally, the output stream passes through a third concentric tubes heat exchanger (E-03) operating with cooling water, and through a go-back pressure valve (V-GO) to maintain the plant pressurized. The cold product is finally collected in a tank (D-03). A sample-taking port (V-D1) placed at the outlet of the reactor allows sampling at different times, in these experiments 0, 7, 15, 30 and 40 minutes from the starting of the operation. The reaction temperatures were 140 and 160 ºC so that the severity factor ($\log R_o$) was in the range 2.02 to 2.78 (140 ºC) and 2.61 to 3.37 (160 ºC).

The extraction experiments were carried out at constant water flow rate (10 L/h). The pressure was sufficient to keep the water in liquid phase (almost 10 barg). A pressure test with cold water was done before each experiment, in order to check the presence of leaks in the plant.

The spent solid after autohydrolysis was collected from the cartridge and dried for 24 at 60 ºC for further structural characterization.

The autohydrolysis liquor corresponding to the first 10 minutes of extraction was taken and transferred to the downstream stage, composed of UF and DF. The reasons for the selection of this initial volume were: first, a larger volume of liquor is not required (1 L is sufficient) for the lab-scale membranes; second, the average molecular weight of the hemicelluloses is higher at this short time; and third, the hemicelluloses are more concentrated.

2.5. Ultrafiltration and Diafiltration

Autohydrolysis liquors corresponding to the first 10 minutes of extraction were pre-filtered to remove particles and extractives by means of dead-end filtration. A high pressure filter with a pore size of 10 µm was used. Multi-step UF was selected to separate and concentrate hemicelluloses. Three Pellicon XL Biomax polymeric membranes (Millipore, Bedford, MA) were employed in cascade, with molecular weight cut-off (MWCO) of 30, 10 and 5 kDa, and a filtration area of 50 cm^2. Hemicellulose concentration was achieved by collecting permeate in a separate container and recirculating continuously retentate to the feed container. UF was carried out until a feed volume reduction of 80 % was achieved.

Autohydrolysis liquors were ultrafiltrated using a cascade configuration: (1) feed liquor passed through 30 kDa membrane, (2) 30 kDa permeate passed through 10 kDa membrane, and (3) 10 kDa permeate passed through 5 kDa membrane. Multiple-step UF allowed obtaining three concentrate product streams with different molecular weight distribution. To purify the retained hemicelluloses, the retentates were subjected to a discontinuous DF process (Fig. 2). During DF, a known volume of water was added to the retentate and it was collected in the permeate, carrying small substances that were not able to pass through the membrane. In this study, the volume of water added at each DF step was equal to the retentate volume (one diavolume was added). Retentate of 30 kDa membrane underwent two DF steps (two diavolumes) with Milli-Q water. Three-diavolume DF was performed on the retentates of 10 kDa and 5 kDa membranes, using different waters: (1) Milli-Q water, (2) water from the DF of the previous membrane in the cascade system, and (3) Milli-Q water. Following this strategy, hemicelluloses removed in the previous DFs can be partially or even totally recovered in the next membrane.

Transmembrane pressure (TMP) was maintained in the range 1.5 to 2.0 bar by a manually adjusted valve on the retentate side, and feed flow was selected in the range 0.8 to 1.0 mL/min/cm^2.

After cascade experiments were finished, samples were taken from feed liquor (Feed), retentate of 30 kDa membrane (Ret-30 kDa), retentate of 10 kDa membrane (Ret-10 kDa), retentate of 5 kDa membrane (Ret-5 kDa), permeate of 5 kDa membrane (Perm-5 kDa), and retentates after DF process (Ret-30 kDa-DF, Ret-10 kDa-DF, Ret-5 kDa-DF) as shown in Fig. 2. Hemicelluloses and by-products contents in the samples were verified by mass balance after analysis of composition by HPLC.

After each experiment, the UF membranes underwent a cleaning and flushing stage as recommended by the manufacturer.

2.6. Analysis

2.6.1. Chemical characterization

2.6.1.1. Supercritical Fluid Extraction extract

Determination of the fatty acid profile was carried out through sample derivatization and Gas Chromatography (GC), following the official method of the AOAC (Official Methods and Recommended Practices of the American Oil Chemists' Society, 1995) (Society., 1995).

Neutral lipid analysis was performed by High-Performance Liquid Chromatography (HPLC) using an Agilent 1200 series HPLC equipment with an evaporative light scattering (ELS) detector. The column was Lichrosphere DIOL-5, 250 mm x 4 mm, 5 mm. A gradient analysis method was used, with a flow of 1 mL/min of solvent A (isooctane) and solvent B (methyl tert-butyl ether with 0.1% (v/v) acetic acid). The detector was maintained at 35 ºC.

2.6.1.2. Autohydrolysis and UF/DF liquors

Composition analysis of the liquid samples was done by High-Performance Liquid Chromatography (HPLC) as previously described by Gallina et al. (2018). The column SUGAR SH-1011 Shodex was used at a temperature of 50 ºC for the identification and quantification of sugars, aldehydes, acids, and degradation products, using as mobile phase flow of 0.8 ml/min of 0.01 N sulfuric acid in Milli-Q water. Degradation products (5-HMF and furfural) were determined with a Waters dual λ absorbance detector 2487 (210 nm and 254 nm). Sugars, aldehydes, and acids were identified with a Waters IR detector 2414. To determine the amount of polysaccharides, samples were subjected to a standardized post-hydrolysis step to break all the polymers and oligomers into monomers. Briefly, 0.8 ml of sulfuric acid (72%) and 15 ml of Milli-Q water were mixed with 5 ml of the liquid sample. The solution was autoclaved for 1 h at 121 ºC. Post-hydrolysis samples were neutralized with $CaCO_3$ and filtered (Pore size 0.22 µm, diameter 25 mm, Nylon; FILTER-LAB) before HPLC analysis. The standards employed for the analysis were: cellobiose (98%), glucuronic acid (98%), galacturonic acid (97%), glucose (99%), mannose (99%), xylose (99%), galactose (99%), fructose (99%), arabinose (99%), glyceraldehyde (90%), glycolaldehyde (99%), lactic acid (85%), formic acid (98%), acetic acid (98%), levulinic acid (98%), acrylic acid (99%), 5-hydroxymethylfurfural (99%), and furfural (99%), all of them from Sigma-Aldrich. The concentration of hemicelluloses was calculated using anhydrous corrections of 0.9 and 0.88 for hexoses and pentoses, respectively.

2.6.2. Molecular Size Distribution

Molecular size characterization was carried out by Size Exclusion Chromatography (HPLC-SEC) using a GPC column (SB-803 HQ; Shodex), protected by a guard column (SB-

G; Shodex). Temperature was maintained at 35 °C with a mobile phase flow rate of 0.5 ml/min ($NaNO_3$ 0.1 M + NaN_3 0.02% in Milli-Q water). The molecular weight of the hemicelluloses was determined using a Waters IR detector 2414. The calibration curve was obtained with a set of 5 pullulan standards (STANDARD P-82; Shodex) dissolved in Milli-Q water, ranged between 6.1 and 113 kDa of weight-average molecular weight.

Chromatographic data provided information on the molecular weight distribution but does not identify different groups of compounds depending on the molecular weight. Assuming that the concentration is proportional to the signal of the refraction index (RI) detector, chromatographic curves were subjected to deconvolution in multiple narrow Gaussian curves. Following this strategy, hemicelluloses can be classified depending on their molecular weight in different groups, whose percentage is known from the intensity of the RI detector. The Gaussian curves are grouped to provide the following division of hemicelluloses: (1) mono (2) di, (3) tri, (4) tetra, (5) penta, (6) hexa, (7) hepta, (8) octa, (9) nona, (10) deca, (11) 1.6-5 kDa, (12) 5-10 kDa, (13) 10-30 kDa, and (14) >30 kDa, where mono stands for monosaccharide, di for diose, tri for triose, etc.

2.6.3. Structural characterization

The extraction of coffee oil and hemicelluloses from spent coffee grounds were analyzed by changes in the molecular structure of different samples: spent coffee grounds, defatted spent coffee grounds, coffee oil, and spent coffee grounds after hemicellulose extraction. The structural characterizations were carried out by Attenuated total reflectance (ATR)-Fourier transform infrared spectroscopy (FT-IR) (Bruker, Alpha model, with a Platinum ATR single reflection diamond module). Absorbance spectra were obtained in the wavenumber range from 4000 to 400 cm^{-1}, acquiring 64 scans per sample at a resolution of 2 cm^{-1}.

3. Results and Discussion

3.1. Raw material characterization

The RM characterization enabled to determine the composition in dry basis: lignin (26.90 wt% ± 0.10), ash (0.60 wt% ± 0.04), gluco- (11.97 wt% ± 0.05), manno- (17.46 wt% ± 0.09), galacto- (0.58 wt% ± 0.03), proteins (11.11 wt% ± 0.77), water extractives (9.84 wt% ± 0.41), ethanol extractives (2.40 wt% ± 0.24), and hexane extractives (20.00 wt% ± 0.04). The sugars that form the polysaccharides of the spent coffee grounds were glucose, mannose, and a small percentage of galactose. Hemicelluloses are therefore glucomannan or galactoglucomannan. The percentage of hexane extractives was considerably decreased after $scCO_2$ extraction.

3.2. Spent coffee grounds oil characterization

The yield of coffee oil reached by $scCO_2$ was 14.04 % of the dry RM, which represents a 70.2 % of the extraction yield obtained by Soxhlet hexane extraction. Similar results were obtained by Andrade et al. (2012) whose yield was 10.5 % (dry RM) using $scCO_2$ extraction. On the other hand, their extractions with other methods such as ultrasound and Soxhlet resulted in yields ranging between 9 and 15 %. Also, Couto et al. (2009) performed a $scCO_2$ extraction of coffee oil during 3 h that resulted in a yield up to 85% compared to the yield reached through Soxhlet hexane extraction. The lower extraction yield in the present work compared to Couto et al. could be due to a lower extraction time (2 h in present work vs. 3 h). In addition, it should be noted that the amount of oil present in the spent coffee grounds can vary depending on the type of coffee, roasting conditions, and the brewing process (Andrade et al., 2012).

The main lipids in spent coffee grounds of this research were free fatty acids (58.00 wt% ± 1.00) and triglycerides (32.00 wt% ± 2.00). In addition, the minor components detected were: short-chain esters (0.50 wt% ± 0.10), long-chain esters (6.60 wt% ± 0.70), diglycerides (1.71 wt% ± 0.03) and monoglycerides (1.26 wt% ± 0.05).

Transformation of spent coffee oil into biodiesel has been studied through direct transesterification into fatty acid methyl esters with short-chain alcohols such as methanol by many research groups. However, this oil can contain high levels of free fatty acids, which is a disadvantage in the transesterification process. The free fatty acids can give rise to soap by-products during the alkali-catalyzed process because they neutralize the catalyst, resulting in the formation of unwanted emulsions. In addition, the presence of free fatty acids is responsible for the high degree of viscosity of the spent coffee oil, which hinders the mixing during the reaction as well as reduces the separation yield between the biodiesel and the glycerol formed. To overcome the problem of free fatty acid, Al-Hamamre et al. (2012) implemented a two-stage transesterification process including a first stage catalyzed by acid prior to the alkali-catalyzed stage. In certain cases, the biodiesel obtained through this double transesterification process did not meet the NP EN 14214:2009 standards, so it has to be cut either with other vegetable oils or with other biodiesel (Caetano et al., 2014). Tuntiwiwattanapun et al. (2017) developed a process of direct transesterification of the spent coffee, without the need to extract the oil, resulting in a biodiesel yield of 77 %. In this process, methanol wash was carried out to reduce the content of free fatty acids, and the influence of temperature, reaction time and particle size on the biodiesel yield were studied. Methanol was separated by flash distillation, and biodiesel was recovered from glycerol by adding hexane, which was later also removed by flash distillation.

Determination of fatty acids profile showed a content of 45.1 ± 0.3 % in saturated fatty acids, 11.40 ± 0.04 % in monounsaturated fatty acids and 43.4 ± 0.3% in polyunsaturated fatty acids. The major fatty acids were linoleic acid C18:2n-6 (42.1 ±

0.3%), palmitic acid C16:0 (33.6 ± 0.2%), oleic acid C18:1n-9 (10.47 ± 0.02%), stearic acid C18:0 (7.27 ± 0.08%) and arachidic acid C20:0 (3.23 ± 0.02%), among others. Campos-Vega et al. explained that spent coffee grounds can be divided into two types of oil depending on the ratio between saturated and polyunsaturated fatty acids. Since in this case, the unsaturated/saturated ratio is 1.11, this oil could be considered as less atherogenic and thrombogenic than those with ratio <1 (Campos-Vega et al., 2015). Furthermore, spent coffee grounds oil with a very similar composition to the one obtained in this study (also extracted by $scCO_2$) was used in the synthesis of biodegradable polymers with high yield (0.77 g polyhydroxyalkanoates/kg spent coffee grounds oil) (Obruca et al., 2014).

3.3. Extraction of hemicelluloses and by-products

The extraction yield was determined by dividing the mass of hemicelluloses extracted by the total amount of carbohydrates in the RM (holocellulose). The accumulated yield evolves slightly asymptotically over time, reaching a maximum value 9.96 wt% when the extraction was carried out at 160°C ($\log R_o = 3.37$), and a maximum of 8.22 wt% at 140 °C ($\log R_o = 2.78$). This relatively low yield may be due to several factors: (1) the repellent effect of the oil, which was not completely removed and may suppose an obstacle in the extraction of hemicelluloses; and/or (2) spent coffee grounds could require a higher temperature than the one used in the production of soluble coffee or in the coffee brewing process so that the previously unextracted polysaccharides were extracted now, as recently demonstrated by Pedras et al. (2019). L F Ballesteros et al. (2017) extracted the hemicelluloses from spent coffee grounds through autohydrolysis without previous extraction of oil, and their maximum accumulated yield was 5.72 wt% ($\log R_o = 3.83$). The hemicelluloses recovered at these conditions were arabinogalactans and galactomannans.

Although the hemicelluloses extraction yield could have been higher if more lipids had been removed, the extraction with $scCO_2$ is the most appropriate option. The yield of oil extraction was high in relation to the time (70 % in 2 h). In addition, as already mentioned, some of the most important potential application of hemicelluloses are in the food, cosmetics and pharmaceutical industries, so it is very important to avoid any toxicity or smell of the product. Due to this, $scCO_2$ has the great advantage of having low toxicity and no-smell in relation to other solvents such as hexane. Furthermore, it allows operating at low temperatures which reduce the risk of thermal degradation of components present in the spent coffee grounds and has a low cost compared to other solvents.

The cumulative extraction of hemicelluloses and by-products, taking the RM in the dry base as reference, is shown in Fig 3a. The extraction of by-products was low and remained approximately constant over time. The extracted hemicelluloses, therefore,

had a relatively high purity due to the low presence of sugars, aldehydes and degradation products, which were probably mostly extracted in the soluble coffee production process. Within the temperature range of this study, a higher extraction temperature in spent coffee grounds results in slightly higher extraction of both hemicelluloses and by-products. Mayanga-Torres et al. (2017) obtained an accumulated yield of 8.8 g/100 g RM through autohydrolysis (175 ºC, 36 min, 22.5 MPa, $\log R_o$ = 3.76), also using a flow-through reactor system with a residence time of 9 min. In our study, the residence time was 12 min and the maximum cumulative yield obtained was 3.49 g/100 g RM. This lower yield value can be attributed to milder conditions of both pressure and temperature during extraction, as well as, lower presence of carbohydrates in the spent coffee grounds.

Regarding the extracted by-products, the main ones were acids, followed by sugars and aldehydes, as can be seen in Fig. 3b. Also, degradation products (furfural and 5-HMF) are released during extraction with very low yields (up to 0.02 g/100 g). The higher temperature (160 °C) led to a higher content of organic acids due to the hydrolysis of high molecular weight hemicelluloses already extracted into lower molecular weight hemicelluloses. The rest of the by-products were also in greater proportion when the temperature was higher.

Previous studies showed that hydrothermal treatment could be tailored towards the selective recovery of hemicelluloses and the reorganization of the remaining cellulose and lignin fractions with improved morphological characteristics (Nitsos et al., 2016, 2013). In these studies the severity factor was in the range $\log R_O$ = 1.90 – 4.69, where a good correlation between hemicelluloses yield extraction and $\log R_O$ was obtained. However, it was demonstrated that at the same $\log R_O$ values, hydrolyzates with different composition and molecular weight can be obtained. In the present study, low severity values were used: in the extraction at 140 ºC $\log R_O$ = 2.02, 2.35, 2.65 and 2.78 were analyzed, while at 160 ºC $\log R_O$ = 2.61, 2.94, 3.24 and 3.37 were analyzed. According to these values and to the evolution in the extraction of hemicelluloses and by-products (Fig. 3a), purity was always higher in the points studied at 140 ºC than those at 160 ºC, even though the $\log R_O$ was of a very similar order. At both temperatures, the highest purity was obtained for the highest $\log R_O$ (2.78 for 140 ºC and 3.37 for 160 ºC). This higher purity could be attributed to the higher extraction time, where the extraction by-products increased in much smaller magnitude than the extraction of hemicelluloses.

3.4. Molecular weight distribution of extracted hemicelluloses

Fig. 4 shows the evolution of the molecular weight distribution of the hemicelluloses at the outlet of the flow-through reactor. At the beginning of extraction (Fig. 4a) the presence of hemicelluloses was higher in the 160 °C extract than in the 140 °C extract,

except for those hemicelluloses > 30 kDa. After 15 minutes of extraction (Fig. 4b), hemicelluloses > 30kDa obtained at 160 °C (logR_o = 2.94) were fully hydrolyzed to hemicelluloses of lower molecular weights. On the other hand, the 140 °C extract (logR_o = 2.35) still maintained hemicelluloses > 30 kDa. At this time, the total concentration of hemicelluloses decreased considerably in the 160 °C extract, meanwhile, it is still almost constant in the 140° C extract comparing to time 0. After 40 min (Fig. 4c), hemicelluloses > 30 kDa were also lost in the 140 °C extract (logR_o = 2.78). Hemicelluloses obtained at 140 °C suffered at this final time a more accentuated decrease in their total concentration than those obtained at 160 °C (logR_o = 3.37), which may be explained by the fact that at 140 °C it is not possible to extract much more hemicelluloses from the RM.

The distribution of oligosaccharides was similar in both experiments at 140 and 160 ℃, being the diose and triose configuration the most abundant, followed in decreasing order by tetraose, pentose, etc. The concentrations of monomers and oligosaccharides, both decreasing over time, were always higher in extraction at 160 ℃ than at 140 ℃. At 160 ℃ the greatest decrease occurred after the first 15 min of extraction, while at 140 ℃ the most abrupt decrease occurred at the end of extraction, after 40 min. This difference may be due to a faster extraction/production of oligosaccharides at 160 ℃ than at 140 ℃.

Relating the molecular weight distribution (Fig. 4) with the logR_O used, the evolution can be studied depending on the extraction temperature. At 140 ℃, moving from 15 min (logR_O = 2.65) to 40 min (logR_O = 2.78), the ratio monomers/total hemicelluloses decreased, while at 160 ℃ the ratio increased from 15 min (logR_O = 3.24) to 40 min (logR_O = 3.37). This may be due to the more accentuated autohydrolysis process at a higher temperature. The proportion of the oligomeric groups from diose to heptaose increased with the increase of logR_O at both temperatures, especially at 140 ℃, possibly due to less rupture towards monomers or to a more accentuated decrease in the concentration of hemicelluloses in polymeric form. Conversely, molecular weight groups higher than heptaose decreased their proportion with the increase of logR_O at both temperatures, especially at 160 ℃.

3.5. Concentration, purification and separation by ultrafiltration/diafiltration

The three objectives of the downstream process were concentration, purification, and separation of hemicelluloses.

3.5.1. Concentration

The increase in the concentration of certain groups of hemicelluloses was mainly carried out at the UF stage. Feed volume reduction of 80 % was fixed in each membrane, resulting in a maximum increase of the concentration of 5-fold respect to

the normalized concentration in the Feed stream. This increase in the concentration respect to the normalized Feed concentration was called concentration factor, and it is represented in Fig. 5. This factor was defined as the ratio of the RI detector intensity of each group of hemicelluloses (mono, di, tri, etc.) in the UF and UF/DF streams comparing with the Feed stream. At 140 ºC the weight-average molecular weight of the Feed stream was 12763 Da (hemicellulose concentration: 358.1 mg/L), while at 160°C was 6720 Da (hemicellulose concentration: 556.9 mg/L). This difference occurred due to the fact that at a lower temperature (140 ºC) hemicelluloses were less autohydrolyzed.

Regarding the first membrane (30 kDa), in the 140 ºC extract hemicelluloses were more retained than in the 160 ºC extract, as the concentration factor of all the groups suffered a greater increase. This higher retention could be attributed to a higher weight-average molecular weight of the Feed stream, which makes more difficult mass transfer across the membrane. Hemicelluloses from the > 30 kDa group were almost completely retained in the first membrane in both cases, as the concentration factor in Ret-30 kDa-DF was almost 5, being 5 the maximum that can be reached with a VR = 80 %.

In the last two membranes (10 and 5 kDa), there was no considerable increase in concentration compared with the Feed. This is due to the high retention in the first membrane (30 kDa). In the case of 160°C due to the lower molecular weight of hemicelluloses in this extract, the concentration in these retentates was higher than in 140°C extract, as more hemicelluloses were able to cross the first membrane.

3.5.2. Purification

After UF, it is usually necessary to purify the retentates due to the retention of some hemicelluloses whose MW < MWCO. In the present work, the used of DF purified the hemicelluloses retained not just by reducing the concentration of by-products but also by enrichment in hemicelluloses of higher molecular weight, thus a higher weight-average molecular weight of the product. Fig. 5 shows the changes in the concentration factor of the products after purification by DF, called UF/DF products.

In the first membrane (30 kDa), DF reduced the presence of all the hemicellulose groups in the 140 ºC extract, being the more reduced the ones of lower molecular weight (from mono to 1.6-5 kDa). In the 160 ºC extract, DF was able to reduce the presence of an only certain group of hemicelluloses (from mono to 1.6-5 kDa), but not of the others. This could be due to a higher hemicelluloses concentration of the 160 ºC Feed stream (556.9 mg/L) compared to 140 ºC Feed stream (358.1 mg/L). Monomers, dimers, and trimers may have been trapped on the feed side by other hemicelluloses. Regarding intermediate size hemicelluloses (5-30 kDa), problems for crossing the UF membranes agree with previous studies. In this sense, Strand et al. (2015) reported that the

substances more harmful for the UF capacity were intermediate size hemicelluloses. Otherwise, the highest molecular weight hemicelluloses (>30 kDa) cannot cross the membrane being retained easily, and the lowest molecular weight substances (by-products) can generally pass through it causing no problems of concentration polarization and/or membrane fouling.

Regarding the last two membranes (10 and 5 kDa), in the 140 ºC extract part of the hemicelluloses of 10-30 kDa group dragged in the DF of the first membrane (30 kDa) were recovered in the DF of the second membrane (10 kDa), thanks to the reuse of the DF water. However, in the DF of 160 ºC extract, there was no recovery of hemicelluloses in the second membrane as hemicelluloses >10 kDa were not dragged during the DF of the 30 kDa membrane. DF of the 10 kDa and 5 kDa retentates also allowed to decrease the presence of hemicelluloses whose MW < MWCO. If a higher decrease were desired, a higher number of diavolumes would be necessary.

One of the main goals of DF is to reduce the presence of by-products in the UF retentates. This effect can be seen in Table 1. By-products were more retained in the membrane when hemicelluloses had the highest weight-average molecular weight, and the DF was more crucial to purify the retentates. This higher retention of by-products may be due to the formation of certain aggregated by high molecular weight hemicelluloses, which trap the by-products. The percentage of by-products removal after DF was 56.3 % for 140 ºC extract, compared to 31.6 % for the 160 ºC extract, whose molecular weight was lower (6720 Da vs. 12763 Da). Similarly, in the case of lower molecular weight hemicelluloses, a higher percentage of by-products was recovered directly in the permeate 49.0 % (160 ºC extract) against 31.2 % (140 ºC extract). The maximum retention of by-products in the purified retentates was 8.7 %, so the DF can be considered as a good method for purification. It should be noted that the application of a greater number of diavolumes would further reduce this retention. The increase in the purity of the products through the removal of by-products is shown in section 3.6.

3.5.3. Separation

The third objective of this work was the hemicellulose separation depending on the molecular weight. This purpose was achieved partially in the UF process and also in the UF/DF process above mentioned. After DF results showed that the majority of the target hemicelluloses (> 5 kDa) were recovered in the purified retentate. At 160 ºC, hemicelluloses from the groups 5-10 kDa, 10-30 kDa and >30 kDa were recovered with percentages of 78.9, 99.5 and 100%, respectively. In the case of 140 ºC, the recoveries were lower with values of 57.4, 85.7 and 90.6 % of the groups 5-10 kDa, 10-30 kDa and > 30 kDa, respectively. These lower recoveries could be attributed to mass transfer difficulties associated with the high molecular weight of the Feed stream, and

therefore, these hemicelluloses trapped into the membrane were dragged to the DF water.

On the contrary, certain hemicelluloses were not the target in this particular process, thus the objective was to separate them from the purified retentates. These substances were monomers, dimers and trimers, oligomers from tetra to penta, and hemicelluloses between 1.6-5 kDa. At 160 ºC, the total removal percentage of these compounds was 57.2 % compared to 66.2 % in the case of 140 ºC. All these substances were eliminated by the 5 kDa permeate and the DF waters.

3.6. Characterization of the products

Table 2 shows some parameters of the Feed streams, UF products, and UF/DF products: weight-average molecular weight (MW), hemicelluloses concentration, by-products concentration, and purity. Feed volume reduction of 80% allowed that most of the hemicellulose concentrations were similar to or higher than the hemicellulose concentration in Feed steam, despite the fact that hemicelluloses are only partially retained by each of the membranes and are distributed in three retentates, one permeate, and the DF water.

Cascade UF system allowed obtaining three differentiated fractions of hemicelluloses: Ret-30 kDa, Ret-10 kDa, and Ret-5 kDa. These products differ considerably in their weight-average molecular weight. In the 140 ºC extract, the MWs were 34965 Da (Ret-30 kDa), 2029 Da (Ret-10 kDa), and 1564 kDa (Ret-5 kDa). The UF products from the 160 ºC extract had MWs of 15265 Da (Ret-30 kDa), 3618 Da (Ret-10 kDa), and 1388 Da (Ret-5 kDa).

Purity has been determined as the relation between the concentration of hemicelluloses in the solution and the total concentration of compounds detected by HPLC (hemicelluloses, sugars, aldehydes, acids, furfural, and 5-HMF). The assumption that the lignin that may be present did not contribute significantly to purity is justified by the low severity used in the autohydrolysis and the very low percentage of acid-soluble lignin quantified in the raw material characterization. The purity of the three products after UF was in the range 67.0 to 89.5 wt%, being the average purity of the Feed liquors 79.8 wt%.

According to Table 2 and previous results, purification by DF allowed (1) to increase the MW of the products, (2) to recover hemicelluloses dragged in the DF of the previous membrane in the cascade, and (3) to diminish the presence of by-products increasing the purity of the retentates. The purity of the products after UF/DF was in the range 83.7 to 97.8 wt%.

The reported data allow the formulation of UF material balances with Feed, Ret-30 kDa, Ret-10 kDa, Ret-5 kDa, and Perm-5 kDa, taking into account that feed volume reduction was 80 % (v/v) in each of the UF steps. Discrepancies can be attributed to experimental/analytical errors and small retention of the compounds on the membrane surface.

Molecular weight characterization of the feed liquors and the UF/DF products (Fig. 6) shows the different molecular weight distributions obtained. Feed streams had a variety of hemicelluloses, thus having a considerable polydispersion. Hemicelluloses between 1.6-5 kDa were the more common in both feed of 140 and 160 ℃ extract with percentages of 28.9 wt% and 31.9 wt%, respectively. The most abundant oligomer was mannotriose with percentages of 8.9 wt% (140 ℃ extract) and 9.3 wt% (160 ℃).

Comparing each of the products individually, the UF/DF of 140 ℃ extract resulted in a Ret-30 kDa-DF containing mainly > 30 kDa hemicelluloses (28.2 wt%), with a lower proportion of monomers, dimers, trimers, oligomers and also 1.6-5 kDa hemicelluloses than the Feed. This was thanks to the improved mass transfer across the membrane during DF. The other two retentates (Ret-10 kDa-DF and Ret-5 kDa-DF) contained mainly hemicelluloses between 1.6-5 kDa (25.8 wt% in both cases), with a similar or higher proportion of oligomeric hemicelluloses than the Feed. The most abundant oligomer of these two retentates was again mannotriose with percentages of 9.8 wt% (Ret-10 kDa-DF) and 17.0 wt% (Ret-5 kDa-DF). In the case of 160 ℃ extract, Ret-30 kDa-DF was rich in 1.6-5 kDa hemicelluloses (30.4 wt%). It is also worth noting: 1) the increase in the proportion of hemicellulose of molecular weight higher than the group 1.6-5 kDa and 2) the decrease in the proportion of hemicelluloses of molecular weight lower than 1.6-5 kDa, comparing both with the Feed. Ret-10 kDa-DF and Ret-5 kDa-DF had their higher proportion in hemicelluloses between 1.6-5 kDa (30.2 and 20.6 wt%, respectively), with mannotriose as the most abundant oligomer (9.3 wt% and 14.7 wt%, respectively).

3.7. Structural characterization of the spent solids

The ATR-FTIR spectra of spent coffee grounds oil and spent coffee grounds before and after extractions were determined. The broad bands in 3600-3000 cm^{-1} region are attributed to the hydroxyl group of O-H stretching vibrations related to cellulosic materials (Lazzari et al., 2018). The absorbance in this region increased after the extraction of oil from spent coffee grounds. This increase shows that oil removal is a good pre-treatment, as the polysaccharides appeared to be more concentrated and accessible in the matrix of the raw material. The decrease in absorbance after hemicellulose extraction was greater at a higher extraction temperature, indicating a greater breakage of bonds due to hemicellulose extraction. The region between 3000-2800 cm^{-1} is related to C-H stretching vibration (Ballesteros et al., 2015). Two peaks

have high absorption in this range: 2920 cm^{-1} and 2845 cm^{-1}. These bands are due to the CH_2 symmetrical and asymmetrical stretching, respectively (Lazzari et al., 2018). Some authors have related these stretching with aliphatic bounds attributed to the presence of caffeine and lipids (Li et al., 2014). This can be confirmed by the presence of the same peaks in the spectrum of spent coffee grounds oil. Other authors have related the peak at 2920 cm-1 with the presence of hydrogen bonds in cellulose. The peaks between 1750 cm^{-1} and 1730 cm^{-1} represent ester moieties in the hemicellulose fraction related to the bonds between lignin and polysaccharides (Ravindran et al., 2017). This peak appears after extraction of oil from spent coffee grounds, indicating that the oil release made the ester groups more available in the matrix. These ester groups are part of both the hemicellulose-lignin complexes and the oil. The region 1700-1500 cm^{-1} is related with carbonyl groups (C=O) asymmetrical and symmetrical stretching vibrations, and the peaks at 1700 and 1650 cm^{-1} are highly associated to the presence of caffeine and chlorogenic acid (Ballesteros et al., 2015). The presence of caffeine in the spent coffee oil is again confirmed by the peak 1700 cm^{-1}. Regarding the second peak (1650 cm^{-1}), the absorbance was constant after oil extraction by $scCO_2$, which could indicate that the chlorogenic acid remained in the defatted spent coffee matrix. The band at 1369 cm^{-1} is attributed to the C-H deformation in cellulose and hemicellulose (Traoré et al., 2016). The intensity of this band increased after oil extraction, and decreased after hemicellulose extraction more markedly at a higher extraction temperature. This decrease was due to the breakage of C-H bonds in the hemicelluloses and between hemicelluloses and cellulose during extraction. The broad region between 1200-920 cm^{-1} is related to the stretching vibration of C-O in C-O-H bonds such as glycosidic bonds, attributed to polysaccharide sugars (Ballesteros et al., 2015). After oil extraction, the intensity of this band increased considerably, which can be attributed to a greater presence of this type of bond without unions linkages with the lipid phase in the matrix. There was a large decrease in absorbance in this region after the extraction of the hemicelluloses so that the higher the extraction temperature the higher the absorbance decrease. This decrease in intensity suggests the rupture of hydrogen bonds between cellulose and hemicelluloses, as well as the hydrolysis of polysaccharides. Within this region, the peak at 1035 cm^{-1} is related to the C-O, C=C, and C-C-O stretching between polysaccharides and lignin (Ravindran et al., 2017). The peaks at 935 cm^{-1}, 869 cm^{-1} and 801 cm^{-1} can be attributed with glycosidic linkage in cellulose and hemicellulose (Ballesteros et al., 2017; Feng Xu et al., 2013). The intensity of these peaks decreased by a very similar degree at both temperatures, as the ruptures of glycosidic bonds in hemicelluloses are of greater importance when they occur in the liquid phase (autohydrolysis).

4. Conclusions

Spent coffee grounds where defatted using supercritical CO_2 and hydrolyzed in subcritical water at 140 and 160 ºC in a pilot plant. The hemicellulose hydrolyzates were concentrated, purified and separated by means of multiple-step ultrafiltration/diafiltration. The concentration of hemicelluloses occurred mainly in the first membrane due to difficulties in the mass transfer by the formation of aggregates. The separation and purification were improved by diafiltration, which considerably reduced the retention of both by-products and not target hemicelluloses. Obtaining purified hemicellulose fractions at pilot scale allows the production on a larger scale for potential applications in food, pharmaceutical, and biopolymers industries.

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References

1. Al-Hamamre, Z., Foerster, S., Hartmann, F., Kröger, M., Kaltschmitt, M., 2012. Oil extracted from spent coffee grounds as a renewable source for fatty acid methyl ester manufacturing. Fuel 96, 70–76. . Fuel

2. Andrade, K.S., Gonalvez, R.T., Maraschin, M., Ribeiro-Do-Valle, R.M., Martínez, J., Ferreira, S.R.S., 2012. Supercritical fluid extraction from spent coffee grounds and coffee husks: Antioxidant activity and effect of operational variables on extract composition. Talanta 88, 544–552. . Talanta

3. Ballesteros, L.F., Cerqueira, M.A., Teixeira, J.A., Mussatto, S.I., 2015. Characterization of polysaccharides extracted from spent coffee grounds by alkali pretreatment. Carbohydr. Polym. 127, 347–354. . Carbohydr. Polym.

4. Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I., 2017. Extraction of polysaccharides by autohydrolysis of spent coffee grounds and evaluation of their antioxidant activity. Carbohydr. Polym. 157, 258–266. . Carbohydr. Polym.

5. Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I., 2014. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. Food Bioprocess Technol. 7, 3493–3503. . Food Bioprocess Technol.

6. Baptista, S.L., Cunha, J.T., Romaní, A., Domingues, L., 2018. Xylitol production from lignocellulosic whole slurry corn cob by engineered industrial Saccharomyces cerevisiae PE-2. Bioresour. Technol. 267, 481–491. . Bioresour. Technol.

7. Caetano, N.S., Silva, V.F.M., Melo, A.C., Martins, A.A., Mata, T.M., 2014. Spent coffee grounds for biodiesel production and other applications. Clean Technol. Environ. Policy 16, 1423–1430. . Clean Technol. Environ. Policy

8. Campos-Vega, R., Loarca-Piña, G., Vergara-Castañeda, H.A., Oomah, B.D., 2015. Spent coffee grounds: A review on current research and future prospects. Trends Food Sci. Technol. 45, 24–36. . Trends Food Sci. Technol.

9. Couto, R.M., Fernandes, J., da Silva, M.D.R.G., Simões, P.C., 2009. Supercritical fluid extraction of lipids from spent coffee grounds. J. Supercrit. Fluids 51, 159–166. . J. Supercrit. Fluids

10. Dutta, S.K., Chakraborty, S., 2018. Mixing effects on the kinetics and the dynamics of two-phase enzymatic hydrolysis of hemicellulose for biofuel production. Bioresour. Technol. 259, 276–285. . Bioresour. Technol.

11. Farhat, W., Venditti, R., Ayoub, A., Prochazka, F., Fernández-de-Alba, C., Mignard, N., Taha, M., Becquart, F., 2018. Towards thermoplastic hemicellulose: Chemistry and characteristics of poly-(ε-caprolactone) grafting onto hemicellulose backbones. Mater. Des. 153, 298–307. . Mater. Des.

12. Feng Xu, Jianming Yu, Tesfaye Tesso, Floyd Dowell, Donghai Wang, 2013. Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques : A mini-review. Appl. Energy 104, 801–809. . Appl. Energy

13. Fortunati, E., Luzi, F., Puglia, D., Torre, L., 2016. Chapter 1 – Extraction of Lignocellulosic Materials From Waste Products, in: Multifunctional Polymeric Nanocomposites Based on Cellulosic Reinforcements. Elsevier Inc., pp. 1–38. , in: Multifunctional Polymeric Nanocomposites Based on Cellulosic Reinforcements

14. Gallina, G., Alfageme, E.R., Biasi, P., García-Serna, J., 2018. Hydrothermal extraction of hemicellulose: from lab to pilot scale. Bioresour. Technol. 247, 980–991. . Bioresour. Technol.

15. García-Uriostegui, L., Delgado, E., Meléndez-Ortiz, H.I., Camacho-Villegas, T.A., Esquivel-Solís, H., Gatenholm, P., Toriz, G., 2018. Spruce xylan/HEMA-SBA15 hybrid hydrogels as a potential scaffold for fibroblast growth and attachment. Carbohydr. Polym. 201, 490–499. . Carbohydr. Polym.

16. Getachew, A.T., Cho, Y.J., Chun, B.S., 2018. Effect of pretreatments on isolation of bioactive polysaccharides from spent coffee grounds using subcritical water. Int. J. Biol. Macromol. 109, 711–719. . Int. J. Biol. Macromol.

17. Getachew, A.T., Chun, B.S., 2017. Molecular modification of native coffee polysaccharide using subcritical water treatment: Structural characterization, antioxidant, and DNA protecting activities. Int. J. Biol. Macromol. 99, 555–562. . Int. J. Biol. Macromol.

18. González-Muñoz, M.J., Rivas, S., Santos, V., Parajó, J.C., 2013. Fractionation of extracted hemicellulosic saccharides from Pinus pinaster wood by multistep

membrane processing. J. Memb. Sci. 428, 281–289. . J. Memb. Sci.

19. Jin, L., Zhang, H., Ma, Z., 2018. Study on capacity of coffee grounds to be extracted oil, produce biodiesel and combust. Energy Procedia 152, 1296–1301. . Energy Procedia

20. Kovalcik, A., Obruca, S., Marova, I., 2018. Valorization of spent coffee grounds: A review. Food Bioprod. Process. 110, 104–119. . Food Bioprod. Process.

21. Laine, C., Kemppainen, K., Kuutti, L., Varhimo, A., Asikainen, S., Grönroos, A., Määttänen, M., Buchert, J., Harlin, A., 2015. Extraction of xylan from wood pulp and brewer's spent grain. Ind. Crops Prod. 70, 231–237. . Ind. Crops Prod.

22. Lazzari, E., Schena, T., Marcelo, M.C.A., Primaz, C.T., Silva, A.N., Ferrão, M.F., Bjerk, T., Caramão, E.B., 2018. Classification of biomass through their pyrolytic bio-oil composition using FTIR and PCA analysis. Ind. Crops Prod. 111, 856–864. . Ind. Crops Prod.

23. Li, X., Strezov, V., Kan, T., 2014. Energy recovery potential analysis of spent coffee grounds pyrolysis products. J. Anal. Appl. Pyrolysis 110, 79–87. . J. Anal. Appl. Pyrolysis

24. Liu, R., Du, J., Zhang, Z., Li, H., Lu, J., Cheng, Y., Lv, Y., Wang, H., 2019. Preparation of polyacrylic acid-grafted-acryloyl/hemicellulose (PAA-g-AH) hybrid films with high oxygen barrier performance. Carbohydr. Polym. 205, 83–88. . Carbohydr. Polym.

25. Loyao, A.S., Villasica, S.L.G., Dela Peña, P.L.L., Go, A.W., 2018. Extraction of lipids from spent coffee grounds with non-polar renewable solvents as alternative. Ind. Crops Prod. 119, 152–161. . Ind. Crops Prod.

26. Luo, Y., Li, Z., Li, X., Liu, X., Fan, J., Clark, J.H., Hu, C., 2019. The production of furfural directly from hemicellulose in lignocellulosic biomass: A review. Catal. Today 319, 14–24. . Catal. Today

27. Mayanga-Torres, P.C., Lachos-Perez, D., Rezende, C.A., Prado, J.M., Ma, Z., Tompsett, G.T., Timko, M.T., Forster-Carneiro, T., 2017. Valorization of coffee industry residues by subcritical water hydrolysis: Recovery of sugars and phenolic compounds. J. Supercrit. Fluids 120, 75–85. . J. Supercrit. Fluids

28. Melo, M.M.R. De, Silvestre, A.J.D., Silva, C.M., 2014. Supercritical fluid extraction of vegetable matrices : Applications , trends and future perspectives of a convincing green technology. J. Supercrit. Fluids 92, 115–176. . J. Supercrit. Fluids

29. Mustapa, A.N., Martin, Á., Mato, R.B., Cocero, M.J., 2015. Extraction of phytocompounds from the medicinal plant Clinacanthus nutans Lindau by microwave-assisted extraction and supercritical carbon dioxide extraction. Ind. Crops Prod. 74, 83–94. . Ind. Crops Prod.

30. Nayak, A., Bhushan, B., 2019. An overview of the recent trends on the waste valorization techniques for food wastes. J. Environ. Manage. 233, 352–370. . J. Environ. Manage.

31. Nguyen, Q.A., Cho, E.J., Lee, D.S., Bae, H.J., 2019. Development of an advanced integrative process to create valuable biosugars including manno-oligosaccharides and mannose from spent coffee grounds. Bioresour. Technol. 272, 209–216. . Bioresour. Technol.

32. Nitsos, C.K., Choli-Papadopoulou, T., Matis, K.A., Triantafyllidis, K.S., 2016. Optimization of hydrothermal pretreatment of hardwood and softwood lignocellulosic residues for selective hemicellulose recovery and improved cellulose enzymatic hydrolysis. ACS Sustain. Chem. Eng. 4, 4529–4544. . ACS Sustain. Chem. Eng.

33. Nitsos, C.K., Matis, K.A., Triantafyllidis, K.S., 2013. Optimization of hydrothermal pretreatment of lignocellulosic biomass in the bioethanol production process. ChemSusChem 6, 110–122. . ChemSusChem

34. Obruca, S., Petrik, S., Benesova, P., Svoboda, Z., Eremka, L., Marova, I., 2014. Utilization of oil extracted from spent coffee grounds for sustainable production of polyhydroxyalkanoates. Appl. Microbiol. Biotechnol. 98, 5883–5890. . Appl. Microbiol. Biotechnol.

35. Pedras, B.M., Nascimento, M., Sá-Nogueira, I., Simões, P., Paiva, A., Barreiros, S., 2019. Semi-continuous extraction/hydrolysis of spent coffee grounds with subcritical water. J. Ind. Eng. Chem. 72, 453–456. . J. Ind. Eng. Chem.

36. Peng, F., Peng, P., Xu, F., Sun, R.C., 2012. Fractional purification and bioconversion of hemicelluloses. Biotechnol. Adv. 30, 879–903. . Biotechnol. Adv.

37. Pettinato, M., Casazza, A.A., Ferrari, P.F., Palombo, D., Perego, P., 2019. Eco-sustainable recovery of antioxidants from spent coffee grounds by microwave-assisted extraction: Process optimization, kinetic modeling and biological validation. Food Bioprod. Process. 114, 31–42. . Food Bioprod. Process.

38. Ravindran, R., Jaiswal, S., Abu-Ghannam, N., Jaiswal, A.K., 2017. Evaluation of ultrasound assisted potassium permanganate pre-treatment of spent coffee waste. Bioresour. Technol. 224, 680–687. . Bioresour. Technol.

39. Samanta, A.K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A.P., Senani, S., Sridhar, M., 2015. Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. Bioact. Carbohydrates Diet. Fibre 5, 62–71. . Bioact. Carbohydrates Diet. Fibre

40. Shang, Y.F., Xu, J.L., Lee, W.J., Um, B.H., 2017. Antioxidative polyphenolics obtained from spent coffee grounds by pressurized liquid extraction. South African J. Bot. 109, 75–80. . South African J. Bot.

41. Society., A.O.C., 1995. Official methods and recommended practices of the American oil chemists' Society. American Oil Chemists' Society.

42. Strand, E., Kallioinen, M., Reinikainen, S.P., Arkell, A., Mänttäri, M., 2015. Multivariate data examination in evaluation of the effect of the molecular mass of

lignin and hemicelluloses on ultrafiltration efficiency. Sep. Purif. Technol. 144, 146–152. . Sep. Purif. Technol.

43. Takao, I., Fujii, S., Ishii, A., Han, L.-K., Kumao, T., Ozaki, K., Asakawa, A., 2006. Effects of Mannooligosaccharides from Coffee Mannan on Fat Storage in Mice Fed a High Fat Diet. J. Heal. Sci. 52, 333–337. . J. Heal. Sci.

44. Thuvander, J., Jönsson, A.S., 2016. Extraction of galactoglucomannan from thermomechanical pulp mill process water by microfiltration and ultrafiltration-Influence of microfiltration membrane pore size on ultrafiltration performance. Chem. Eng. Res. Des. 105, 171–176. . Chem. Eng. Res. Des.

45. Thuvander, J., Oinonen, P., Jönsson, A.S., 2016. Enzymatic treatment of hemicelluloses and lignin isolated from thermomechanical pulp mill process water. Chem. Eng. J. 296, 141–145. . Chem. Eng. J.

46. Traoré, M., Kaal, J., Martínez Cortizas, A., 2016. Application of FTIR spectroscopy to the characterization of archeological wood. Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 153, 63–70. . Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.

47. Tuntiwiwattanapun, N., Monono, E., Wiesenborn, D., Tongcumpou, C., 2017. In-situ transesterification process for biodiesel production using spent coffee grounds from the instant coffee industry. Ind. Crops Prod. 102, 23–31. . Ind. Crops Prod.

48. Voepel, J., Sjöberg, J., Reif, M., Albertsson, A.-C., Hultin, U.-K., Gasslander, U., 2009. Drug diffusion in neutral and ionic hydrogels assembled from acetylated galactoglucomannan. J. Appl. Polym. Sci. 112, 2401–2412. . J. Appl. Polym. Sci.

49. Yedro, F.M., Cantero, D.A., Pascual, M., García-Serna, J., Cocero, M.J., 2015. Hydrothermal fractionation of woody biomass: Lignin effect on sugars recovery. Bioresour. Technol. 191, 124–132. . Bioresour. Technol.

50. Zabaniotou, A., Kamaterou, P., 2018. Food Waste valorization advocating Circular Bioeconomy -A critical review of potentialities and perspectives of Spent Coffee Grounds Biorefinery. J. Clean. Prod. 211, 1553–1566. . J. Clean. Prod.

Figure Captions

Figure 1. Diagram of the flow-through pilot reactor used for hemicellulose extraction

Figure 2. Ultrafiltration and diafiltration cascade process

Figure 3. Extraction yield (g/100g of dry raw material) of hemicelluloses and by-products at 140 and 160 °C

Figure 4. Evolution of the molecular weight distribution of hemicelluloses during extraction at 140 and 160 °C

Figure 5. Increase in the concentration of the different groups of hemicelluloses in each of the UF and UF/DF products compared to the Feed stream

Figure 6. Molecular weight distribution of the Feed and the UF/DF products

Figures

Fig. 1

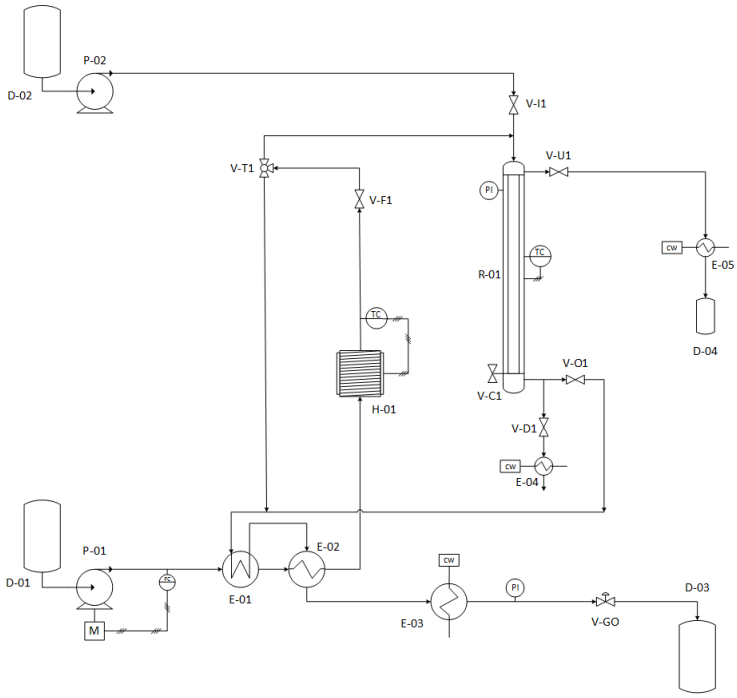
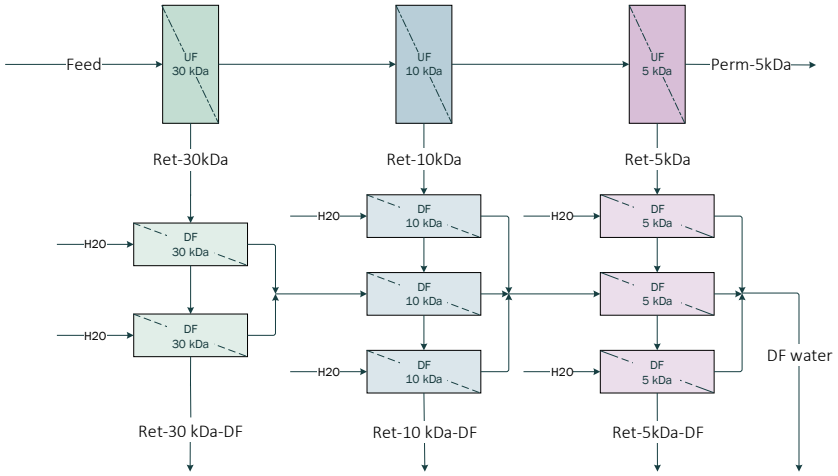


Fig. 2


Feed
UF
30 kDa
UF
10 kDa
UF
5 kDa
Perm-5kDa
Ret-30kDa
Ret-10kDa
Ret-5kDa
H2O
DF
30 kDa
H2O
DF
30 kDa
H2O
DF
10 kDa
DF
10 kDa
H2O
DF
10 kDa
H2O
DF
5 kDa
DF
5 kDa
H2O
DF
5 kDa
DF water
Ret-30 kDa-DF
Ret-10 kDa-DF
Ret-5kDa-DF

1003 Fig. 3

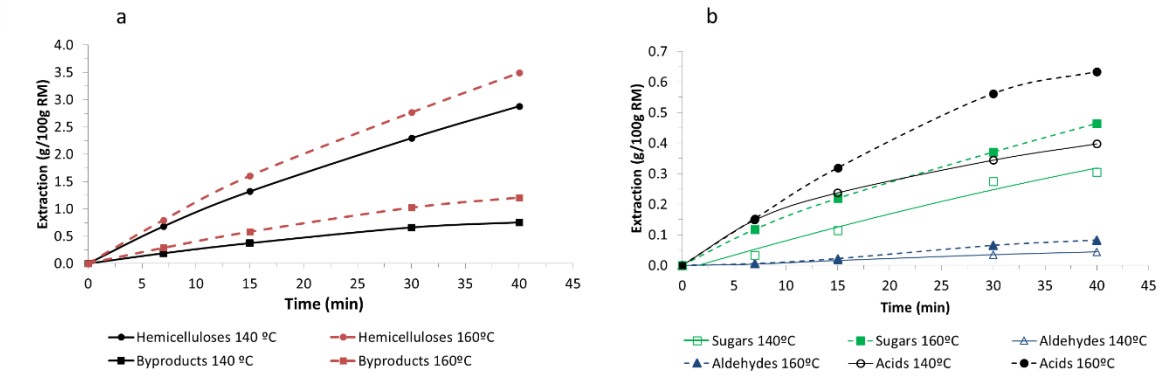


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Fig. 4

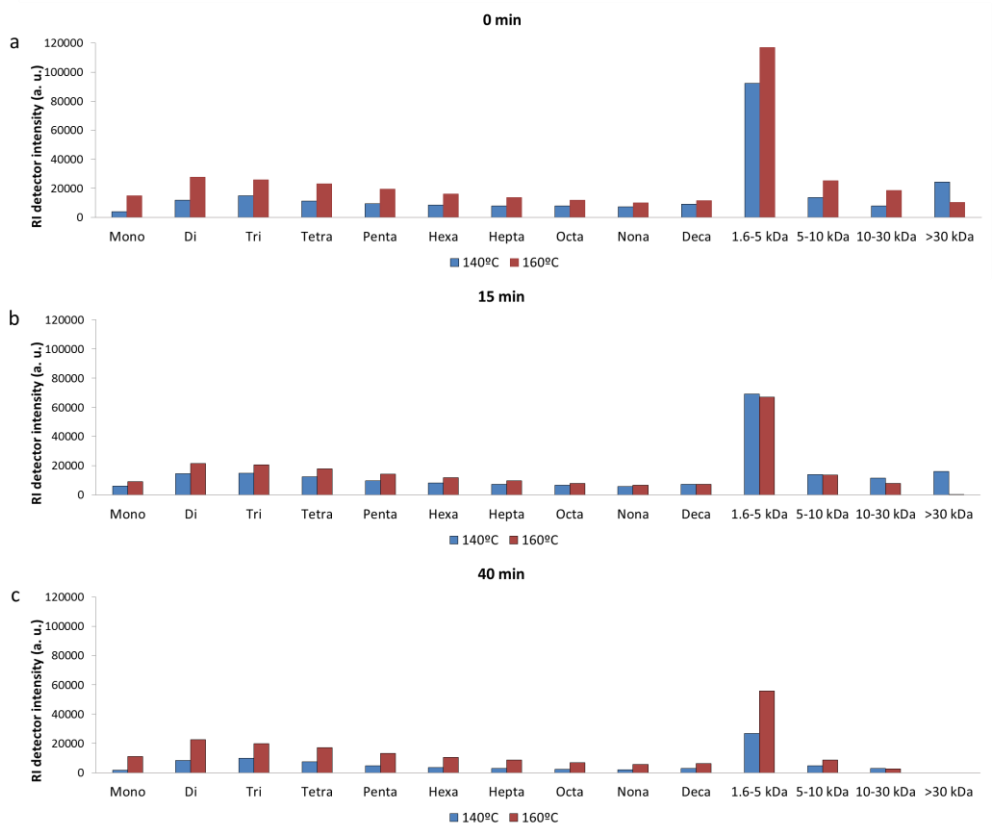

a
0 min
RI detector intensity (a. u.)
120000
100000
80000
60000
40000
20000
0
Mono
Di
Tri
Tetra
Penta
Hexa
Hepta
Octa
Nona
Deca
1.6-5 kDa
5-10 kDa
10-30 kDa
>30 kDa
140ºC
160ºC
b
15 min
RI detector intensity (a. u.)
c
40 min
RI detector intensity (a. u.)

Fig. 5

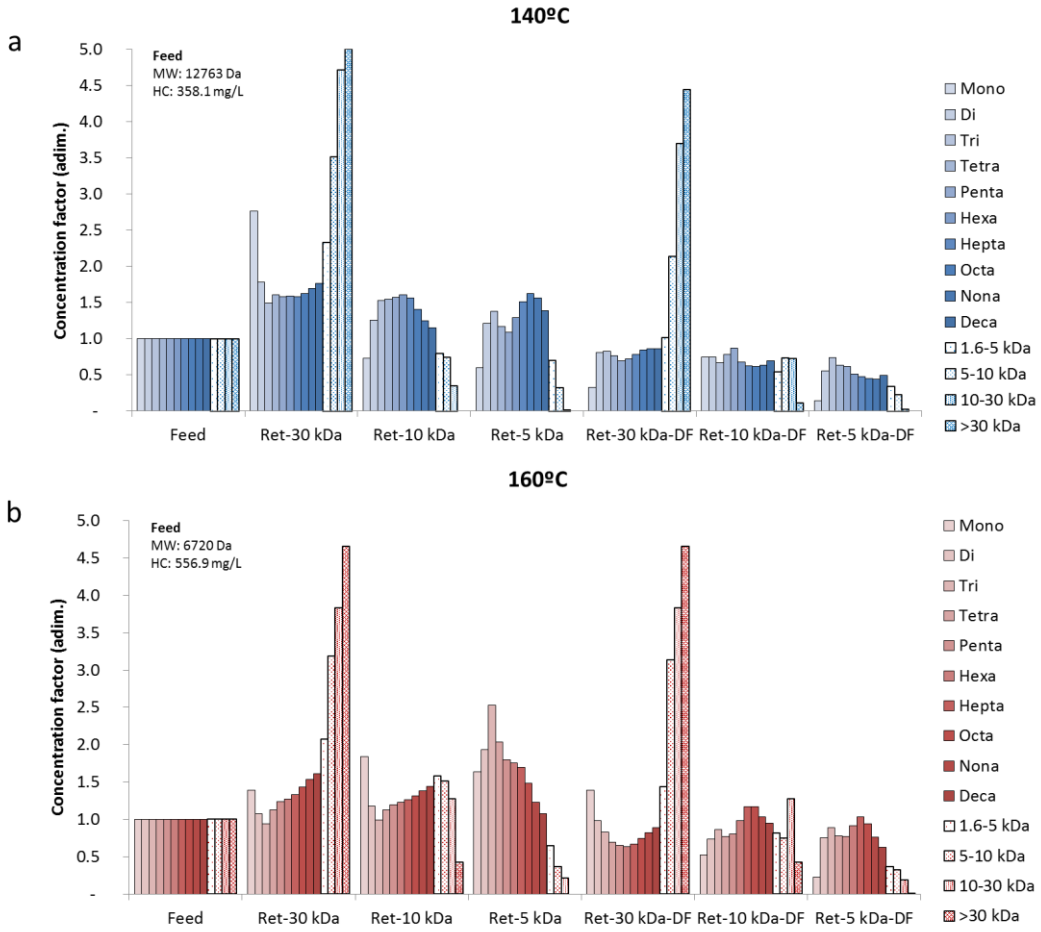
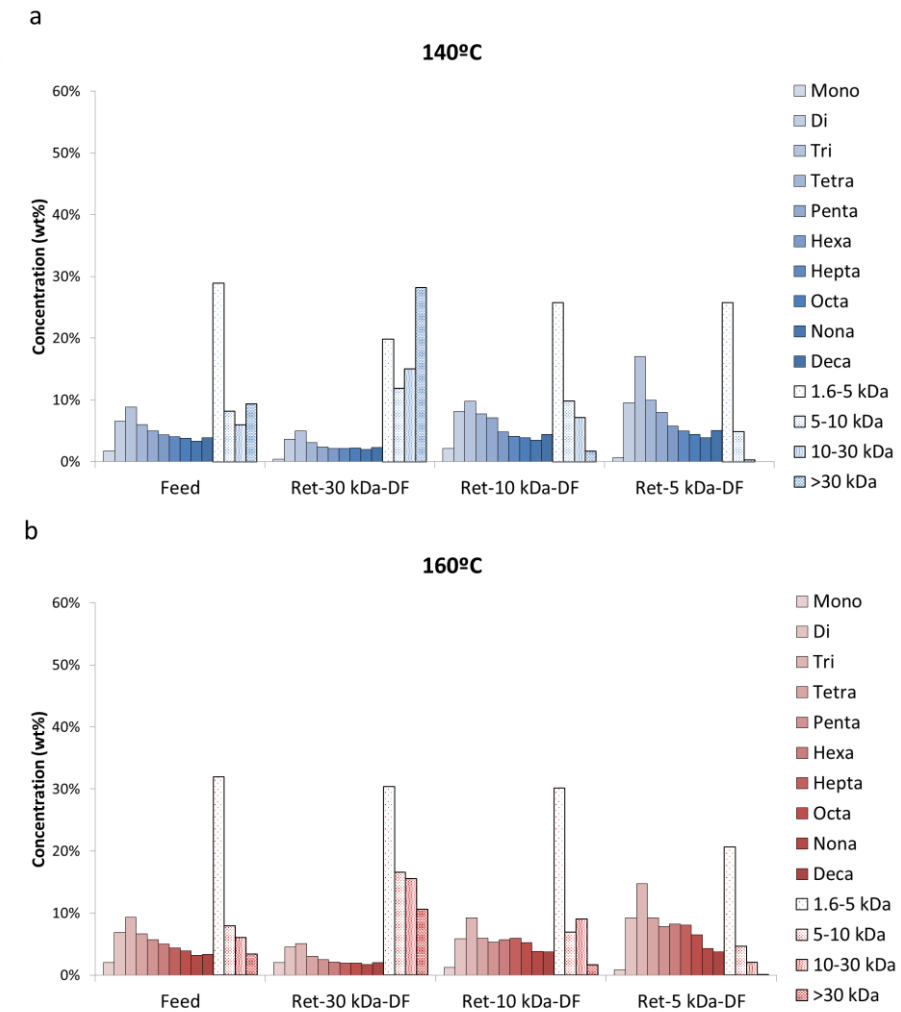

a
140ºC
Feed
MW: 12763 Da
HC: 358.1 mg/L
Concentration factor (adim.)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
-
Feed
Ret-30 kDa
Ret-10 kDa
Ret-5 kDa
Ret-30 kDa-DF
Ret-10 kDa-DF
Ret-5 kDa-DF
Mono
Di
Tri
Tetra
Penta
Hexa
Hepta
Octa
Nona
Deca
1.6-5 kDa
5-10 kDa
10-30 kDa
>30 kDa
b
160ºC
Feed
MW: 6720 Da
HC: 556.9 mg/L
Concentration factor (adim.)
Feed
Ret-30 kDa
Ret-10 kDa
Ret-5 kDa
Ret-30 kDa-DF
Ret-10 kDa-DF
Ret-5 kDa-DF
Mono
Di
Tri
Tetra
Penta
Hexa
Hepta
Octa
Nona
Deca
1.6-5 kDa
5-10 kDa
10-30 kDa
>30 kDa

Fig. 6


a
140ºC
Concentration (wt%)
60%
50%
40%
30%
20%
10%
0%
Feed
Ret-30 kDa-DF
Ret-10 kDa-DF
Ret-5 kDa-DF
Mono
Di
Tri
Tetra
Penta
Hexa
Hepta
Octa
Nona
Deca
1.6-5 kDa
5-10 kDa
10-30 kDa
>30 kDa
b
160ºC
Concentration (wt%)
60%
50%
40%
30%
20%
10%
0%
Feed
Ret-30 kDa-DF
Ret-10 kDa-DF
Ret-5 kDa-DF
Mono
Di
Tri
Tetra
Penta
Hexa
Hepta
Octa
Nona
Deca
1.6-5 kDa
5-10 kDa
10-30 kDa
>30 kDa

Tables

Table 1

Recovery of by-products after UF and UF/DF

		UF by-products recoveries				UF/DF by-products recoveries			
	MW (Da)	Perm-5 kDa	Ret-30 kDa	Ret-10 kDa	Ret-5 kDa	Ret-30 kDa-DF	Ret-10 kDa-DF	Ret-5 kDa-DF	DF water*
140 °C	12763	31.2 %	45.6 %	9.3 %	13.9 %	2.7 %	3.4 %	6.5 %	56.3 %
160 °C	6720	49.0 %	19.1 %	16.5 %	15.4 %	3.1 %	7.5 %	8.7 %	31.6 %

* recovery of by-products in the different DF waters was determined by material balance

Table 2

Characterization of the UF and UF/DF products

	140 °C				160 °C			
	MW (Da)	Hemicelluloses (mg/L)	By-products (mg/L)	Purity (wt%)	MW (Da)	Hemicelluloses (mg/L)	By-products (mg/L)	Purity (wt%)
Feed	12785	358.1	118.0	75.2 %	6720	556.9*	103.9	84.3 %
Ret-30 kDa	34965	457.4	225.7	67.0 %	15265	770.7	90.3	89.5 %
Ret-30 kDa-DF	49733	382.7	13.3	96.6 %	23236	656.9	14.8	97.8 %
Ret-10 kDa	2029	390.2	57.5	87.2 %	3618	561.4	97.5	85.2 %
Ret-10 kDa-DF	4158	341.7	21.0	94.2 %	4043	494.4	44.6	91.7 %
Ret-5 kDa	1564	368.1	107.5	77.4 %	1388	642.1	113.6	85.0 %
Ret-5 kDa-DF	1641	256.7	49.9	83.7 %	1803	442.1	64.2	87.3 %
Perm-5 kDa	1941	323.5	60.3	84.3 %	1596	439.1	90.6	82.9 %

*determined by an average of the extract concentrations corresponding to the first 10 minutes of extraction

Electronic Annex

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