Title: Hydrothermal treatments enhance the solubility and antioxidant characteristics of dietary fiber from asparagus by-products

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ABSTRACT

Asparagus by-products were submitted to hydrothermal treatments to improve their solubility and antioxidant capacity. Four severity conditions were applied ($\log R_o$ 3.05, 3.64, 4.23, 4.82) and two fractions were isolated from each treatment. The solid fibrous residues were enriched in cellulose, and depleted of hemicelluloses and phenolics. Their antioxidant activities were higher than those presented for asparagus bioactive fibers (81-154 vs 21-26 mmol Trolox/Kg) and did not correlate with phenol content. The soluble liquors were rich in phenolics and also contained changeable amounts of mono-, oligo-, and polysaccharides as a function of severity. The majority of the released oligosaccharides (xylooligosaccharides) are considered as prebiotic and their percentage could be optimized by modifying hydrothermal treatment conditions. This fraction presented very high antioxidant activity (131-232 mmol Trolox/Kg), and had a moderate correlation with phenols. Melanoidins are suggested to be present in both fractions. A new fiber fraction with potential prebiotic activity and high antioxidant activity has been obtained from asparagus by-products. Its high solubility in water and a pleasant flavor broaden its applications in food formulations

Keywords: asparagus by-products; hydrothermal treatments; severity; phenolics; oligosaccharides; melanoidins

Abbreviations used: ARC: antiradical capacity; FA: ferulic acid; HCA: hydroxycinnamic acid; HTT: hydrothermal treatment; RM: raw material; TFA: trifluoroacetic acid.

1. Introduction

Asparagus is a vegetable which is widely consumed all over the world and is highly appreciated by its flavor and for its nutritional and functional characteristics. The total production of asparagus was about $8.7 \cdot 10^6$ Tm in 2016 and has been growing since the 90's, when the global crop reached $2 \cdot 10^6$ Tm in 1990 (FAO, 2018). Spain is the fifth producer after China, Peru, Mexico and Germany. Spain also has a prevailing position in the global trade, being the fourth exporting country in both fresh and canned asparagus, at around 87 million\$ in 2016 (OEC, 2018).

Almost half of the length of harvested asparagus spears is considered as waste by canning industries due to its fibrousness and stiffness. Looking at the global production, it is easy to evaluate the environmental impact that this by-product supposes for asparagus producers. In previous studies, insoluble dietary fiber extracts were obtained from asparagus wastage by water/ethanol treatments (Fuentes-Alventosa et al., 2009a; Fuentes-Alventosa et al., 2009b), and a process has been patented for obtaining insoluble bioactive fiber (Guillén-Bejarano et al., 2012).

Hydrothermal treatments (HTT) have been widely used for the extraction of oligosaccharides (Carvalho et al., 2009) and antioxidants (Conde et al., 2017) from agricultural by-products, and these processes are probably the best alternative for biomass management within the framework of bioeconomy and sustainable development (Ruiz et al, 2013, 2017). Among the HTT methods, steam explosion treatment is one of the most commonly used for fractionation of biomass components. This technique is very useful for the separation of cellulose, hemicellulose and lignin from raw materials, as wheat straw (Montane et al, 1998), bagasse (Kar et al, 1998), and olive stone (Fernandez-Bolaños et al., 2001), obtaining a wide range of added-value compounds: from the soluble fraction, oligosaccharides coming from hemicelluloses

(mainly xylooligosaccharides of different molecular weight), and antioxidants released from hemicellulose and/or from lignin structures. A cellulose-rich solid residue can be also isolated, which is more susceptible to enzymatic hydrolysis than the original raw material (Ruiz et al, 2013). The aim of this study was to apply this technology to asparagus waste for the first time in order to evaluate the release of oligosaccharides and phenols. With this approach, in addition to insoluble dietary fiber, a soluble product could be obtained and offered to the food industry for the formulation of a wider range of healthier foods which are fortified with soluble fiber and antioxidants.

2. Materials and methods

2.1. Asparagus by-products

Asparagus by-products were obtained from Centro Sur S.C.A. (Huétor-Tájar, Granada, Spain). Prior to canning, freshly harvested, green asparagus spears were cut to obtain the 15 cm length upper portion (edible part) and the rest of spear (15–18 cm) was considered as a by-product. This by-product was sent to our laboratory within 24 h and kept at 4° C until processing.

These basal portions were homogenized in water at a 2:1 solid:liquid ratio. They were then filtered and the slurry was oven-dried at 60° C during 18 hours. This dry solid was considered as the raw material (RM) for this study.

2.2. Hydrothermal treatments

The different HTTs were carried out using a 2-L capacity steam-explosion reactor (Fernández-Bolaños et al., 1999), designed by our research group at the Instituto de la Grasa (CSIC). 200 g RM were treated with saturated steam at a maximum operating pressure of 42 kg/cm². The reactor was equipped with a quick-opening ball valve and an electronic device programmed for the accurate control of steam time and temperature

for the final steam explosion. Four temperatures were assayed at 180°, 200°, 220°, and 240° C (180-HTT, 200-HTT, 220-HTT, and 240-HTT, respectively), with a 5-minute treatment duration. The severity of treatments was defined by a single factor called R_o which links the effects of time (t, min) and temperature (T, ° C) (Heitz et al., 1987). The index of severity was expressed as $\log R_o$

$$R_o = t \exp ((T-100)/14.75)$$

After opening the reactor, the samples were collected, vacuum filtered through filter paper (73 g/cm², Albet, Barcelona, Spain), and the fibrous solids were dried in an oven at 60° C. The liquors were stored at -20° C until analysis.

2.3. Ethanol-acetone (E/A) extraction

RM and the fibrous solid fractions after thermal treatments were extracted with ethanol at a final concentration of 80%. The samples were homogenized using an Ultraturrax (T-25) at top speed for 1 min. After filtration, a second similar extraction was performed using 70% acetone. Both fractions (ethanol and acetone) were combined to have the E/A extracts, which were used to determine soluble esterified phenols, sugars and antiradical capacity (ARC). The slurries were let to dry overnight at room temperature.

2.4. Determination of neutral sugars from solid residues

Saeman hydrolysis was applied to solid samples. Sulphuric acid 72% (0.5 mL) was added to 10 mg of sample (in triplicate) in screw-cap tubes. The tubes were placed in a heating block at 40° C for 2 hours. Afterwards, 5.5 mL water was added to each tube and the hydrolysis continued at 100° C for 2 hours. 0.5 mL of the hydrolysate was neutralized with NH₄OH and 50 µg myo-inositol were added as internal standard. After reduction and peracetylation, the released sugars were quantified as alditol acetates by gas chromatography (Dos-Santos et al., 2011). A HP 6890 Plus+ gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) fitted with a 30 m × 250 µm × 0.20 mm

capillary column (SP-2330, Supelco, Bellefonte, PA, USA) was used. The carrier gas was helium with a constant flow of 2.2 mL/min and pressure of 21.5 psi (148.24 kPa). The injection was performed in splitless mode. The oven temperature was held at 50° C for 2 min after injection, then programmed to 180° C at 35° C/min, held at 180° C for 5 min, and then immediately increased to 220° C at 5° C/min and held at 220° C for 22 min. The total run time was 40.7 min. The injector temperature was 250° C and the flame ionization detector was set at 300° C.

2.5. Extraction of ester-linked phenolics

Esterified phenolics were released by treatment of the solid residues or liquid samples with 2 M NaOH for 24 h, at room temperature, under nitrogen in the dark (Jaramillo et al., 2007). When the liquid samples were analyzed, an aliquot (in duplicate) was dried in a screw-cap tube under a nitrogen stream. After saponification, the samples were filtered through glass fiber paper, and trans-cinnamic acid (10 µg) was added to the filtrate as internal standard. The solutions were acidified to pH < 2 with concentrated HCl and then extracted three times with ethyl acetate (three volumes). The phenolic ethyl acetate extracts were evaporated to dryness under a stream of nitrogen, and then the samples were dissolved in 200 µL of 50% (v/v) aqueous methanol prior to analysis by HPLC.

2.6. Analysis of esterified phenols by HPLC and diode array detection (HPLC-DAD)

Esterified phenols were detected and quantified by HPLC as described by Waldron et al. (Waldron et al., 1996) with some modification (Jaramillo, et al., 2007). The separation of phenolics was made using a SYNERGI 4µHYDRORP80A and a reverse phase column (25 cm × 4.6 mm i.d., 4 µm; Phenomenex, Macclesfield, Cheshire, UK) with gradient elution employing progressively increasing methanol–acetonitrile levels

in 2 mL/L acetic acid. The gradient profile for the separation of wall-bound phenolic monomers and dimers was formed using solvent A (10% (v/v) aqueous acetonitrile plus 2 mL/L acetic acid) and solvent B (40% methanol, 40% acetonitrile, 20% water, plus 2 mL/L acetic acid) in the following program: initially A 90%, B 10%; linear gradient over 17 min to A 57.5%, B 42.5%; held isocratically at A 57.5%, B 42.5% for a further 6 min; linear gradient over 17 min to B 100%; linear gradient over 5 min to A 90%, B 10%; held isocratically at A 90%, B 10% for a further 5min. The flow rate was maintained at 1mL/min. Phenolics were detected using a Jasco-LC NetII ADC liquid chromatograph system equipped with DAD. Quantification was made by the integration of peak areas at 280 nm, with reference to calibrations made using known amounts of pure compounds.

2.7. Management of the treatment liquors for phenolic and sugar analysis

Liquid fractions after HTT and filtration were concentrated up to 1 L under vacuum. 100 mL aliquot (in duplicate) were extracted with ethyl acetate (refluxed at 77° C) for 6 h by a continuous liquid-liquid extractor. The organic phase was vacuum evaporated at 37° C to obtain the free phenolic extracts. The dry residue was redissolved in 50% ethanol and analyzed for free phenolics by HPLC-DAD as previously described (section 2.6). In the remaining aqueous fraction, soluble esterified phenolics were extracted as detailed in section 2.5.

250 mL (in duplicate) of the concentrated liquor were freeze-dried. The solid residue was suspended in 300 mL of 80% ethanol. The soluble portion was concentrated under vacuum up to 50 mL. For the determination of monosaccharides, aliquots were taken from these concentrates, placed in screw-cap tubes, dried under air flow, and directly reduced with sodium borohydride in 2M NH₄OH, and then peracetylated with acetic anhydride and methyl-imidazole. The obtained alditol acetates were quantified by GC as

previously described (2.4. heading). The presence of oligosaccharides was studied by TFA hydrolysis (2N, 121° C, 1 h), reduction and peracetylation from the same 50 mL concentrates. The amount of oligosaccharides was corrected by subtraction of the quantified monosaccharides. The insoluble fraction after 80% ethanol suspension was hydrolyzed with TFA under the same conditions to measure the amount of polysaccharides present in the liquors.

2.8. Determination of the antioxidant capacity: antiradical capacity

The method described by Rodríguez et al. (G. Rodríguez et al., 2007) was used. For the absorbance measurements a microplate reader (550 model from Bio-Rad, Hercules CA, USA) was used. Briefly, aliquots of 5 µL of each treatment liquor (and also the E/A extracts from fibrous residues) and 195 µL of the DPPH· solution (3.8 mg/50 mL) were placed in each microplate well in triplicate. For each sample, a blank with methanol instead of DPPH· solution was included. A delay of 30 min was programmed in the reader to reach a steady absorbance. The decrease in absorbance (expressed as percent of the initial absorbance) was plotted against the concentration of the antioxidant solution in the reaction mixture. The efficient concentration EC50 was calculated by regression analysis for each sample and ARC was expressed as mmol Trolox equivalent/Kg of lyophilized liquor by means of a dose response curve for Trolox.

2.9. Specific extinction coefficient of treatment liquors at 280, 325, and 405 nm

A 1 mg/mL solution was prepared from each lyophilized treatment liquor. The absorbance at 280, 325, and 405 nm was measured in a UV/Vis spectrophotometer Jasco V-530 model following the method described by Bekedam et al (Bekedam et al., 2006). The values of absorbance at every wavelength must be between 0.1-1.3, so the absorbance was measured at 405 nm from the solution without dilution, but for the other wavelengths, the samples had to be diluted eight times with distilled water. The

measurements were conducted in triplicate. The specific extinction coefficient (K_{mix}) was calculated according to the Lambert-Beer law.

The UV spectrum of each treatment liquor was also measured with the same equipment.

2.10. Statistical analysis

The results are expressed as the average value of at least two repetitions. To assess the differences among samples, a multiple sample comparison was performed using the Statgraphics® Plus program Version 2.1. A multivariate analysis of variance (ANOVA), followed by Duncan's multiple comparison test, was performed to differentiate the groups. The level of significance was $P < 0.05$. A regression analysis was made at the same confidence level.

3. Results and discussion

3.1. Characterization of the RM.

Prior to HTT, RM was extracted with ethanol/acetone to characterize both soluble and insoluble fractions. The results of their glycosidic and phenolic compositions are presented in Tables 1 and 2, respectively. Glucose was the major sugar quantified (Table 1) in the solid fraction, nearly 70%, indicating the presence of a high percentage of cellulose in this material, as was previously reported (Fuentes-Alventosa, et al., 2009a). Xylose and arabinose accounted for higher than 20%, as arabinoxylans were also postulated as the main hemicellulose in white and green asparagus cell walls (Jaramillo-Carmona et al., 2017; R. Rodríguez et al., 1999) together with xyloglucans. The esterified phenolics were hydroxycinnamic acids (HCA), mainly coumaric, ferulic (FA) and its derivatives, diFA. The quantified amount was in the range previously described for green asparagus (Fuentes-Alventosa, et al., 2009a; R. Rodríguez et al., 2005). FA and diFA were more abundant than coumaric acid, with diFA in a higher percentage than FA. Monomers and dimers are very important elements in the

asparagus cell wall structure as they are partly responsible for the distinctive and valued texture of fresh white and green asparagus (Rodríguez-Arcos et al., 2002b, 2004), for their hardening process during postharvest storage (Jaramillo, et al., 2007; Rodríguez-Arcos et al., 2002a), and also for the antioxidant activity that asparagus dietary fiber exhibits (Fuentes-Alventosa et al., 2013; Jaramillo-Carmona, et al., 2017). The dimer 8-O-4' was the most abundant, followed by 8-5'BF, 8-8' and 8-8'AT in similar percentages.

Table 1. Glycosidic and phenolic composition of ethanol/acetone-treated raw material and fibrous residue after hydrothermal treatment.

	log R_0	RM	180-HTT 3.05	200-HTT 3.64	220-HTT 4.23	240-HTT 4.82
<u>Total sugars¹</u>		271.80±10.52 a	330.22±26.40 ab	282.42±21.30a	363.54±26.25b	335.45±6.75 ab
Rha ²		0.89±0.08 c	0.21±0.01 b	0.14±0.00 ab	0.03±0.00 a	0.02±0.00 a
Fuc ²		0.18±0.01 b	n.d.	0.01±0.00 c	n.d.	n.d.
Ara ²		5.60±0.53 d	2.24±0.10 b	1.61±0.03 b	0.50±0.01 a	0.66±0.04 a
Xyl ²		16.82±0.89 d	9.77±0.82 bc	7.87±0.75 b	3.86±0.14 a	2.55±0.04 a
Man ²		3.48±0.35 bc	4.03±0.19 c	3.28±0.23 b	1.93±0.03 a	1.50±0.03 a
Gal ²		4.28±0.52 d	3.19±0.33 c	2.38±0.36 bc	1.90±0.17 ab	0.86±0.07 a
Glu ²		68.74±2.83 a	80.55±7.24 ab	84.72±6.35 ab	91.76±7.30 b	94.42±1.95 b
<u>Total esterified phenolics³</u>		1.78±0.10 bc	2.00±0.11 c	1.80±0.10 bc	1.53±0.08 b	0.97±0.05 a
Coumaric acid ²		44.19±4.02 a	45.54±4.14 a	48.68±4.43 a	47.60±4.33 a	47.50±4.32 a
Ferulic acid ²		21.53±1.96 a	22.93±2.09 a	25.66±2.33 a	27.68±2.52 a	27.50±2.50 a
8,8' AT diFA ²		4.25±0.39 ab	6.69±0.61 c	5.26±0.48 bc	6.64±0.60 c	3.00±0.27 a
8,8' diFA ²		5.95±0.54 b	5.41±0.49 b	0.66±0.06 a	0.37±0.03 a	1.00±0.09 a
8,5' diFA ²		1.98±0.18bc	2.23±0.20 c	1.64±0.15 b	1.48±0.13 ab	1.00±0.09 a
5,5' diFA ²		1.70±0.15 cd	1.27±0.12bc	0.99±0.09 ab	0.74±0.07 a	2.00±0.18 a
8-O-4' diFA ²		14.16±1.29 a	14.01±1.27 a	15.79±1.44 a	15.50±1.41 a	18.00±1.64 a
8,5' BF diFA ²		6.23±0.57 b	1.91±0.17a	1.32±0.12 a	n.d.	n.d.

¹ mg sugar/g material; ² % on total sugars/phenolics quantified; ³ mg hydroxycinnamic acids/g material.

RM: raw material; Rha: rhamnose; Fuc: fucose; Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glu: glucose; diFA: diferulic acid.

Means bearing the same letter in the same row are not significantly different at 5% level as determined by Duncan's multiple range test.

In the E/A soluble fraction (Table 2) no phenolics were detected and only a small amount of sugars was quantified (7 mg/g RM in soluble fraction *vs* 272 mg/g RM in the insoluble one). Again, glucose was the most abundant sugar, followed by galactose, arabinose and xylose. Soluble arabinogalactans type II, were very abundant in the asparagus cell wall (R. Rodríguez, et al., 1999), and could be the origin of this high percent of soluble galactose.

Table 2.-Glycosidic and phenolic composition of the ethanol-soluble fraction from raw material and fibrous residue after hydrothermal treatment

	RM	180-HTT 3.05	200-HTT 3.64	220-HTT 4.23	240-HTT 4.82
$\log R_0$					
Total sugars ¹	6.74±0.06 c	15.35±0.60 e	12.69±0.98 d	4.13±0.06 b	2.34±0.36 a
Rha ²	8.12±0.31 c	5.56±0.42 b	3.90±0.42 a	3.75±0.63 a	3.29±0.88 a
Fuc ²	n.d.	0.10±0.11 a	0.15±0.08a	n.d.	n.d.±
Ara ²	13.65±0.28 c	10.22±0.20 b	7.55±1.30 a	8.08±0.71 a	7.41±0.67 a
Xyl ²	11.63±0.18 a	15.75±0.61 b	35.93±6.11 e	22.56±1.80 d	18.98±1.21 c
Man ²	6.99±0.26 a	21.16±0.44 e	10.87±1.30 b	13.59±0.15 c	16.23±1.94 d
Gal ²	15.89±0.69 d	10.15±0.13 b	8.75±2.56 a	13.53±0.31 c	15.71±0.85 d
Glu ²	43.72±0.80 c	37.06±0.54 b	32.84±4.36 a	38.49±1.79 b	38.37±2.17 b
Total phenolics ¹	n.d.	1.32±0.09 b	1.31±0.08 b	0.91±0.08 a	0.89±0.01 a
Coumaric acid ²		25,24±2.30 ab	31,00±2.82 b	21,57±1.96 a	21,36±1.94 a
Ferulic acid ²		18,45±1.86 a	23,00±2.32 ab	25,49±2.57 ab	29,13±2.94 b
8,8' AT diFA ²		n.d.	n.d.	n.d.	n.d.
8,8' diFA ²		17,48±2.13 ab	13,00±1.58 a	24,51±2.99 b	23,30±2.84 b
8,5' diFA ²		8,74±1.42 b±	4,00±0.65 a	2,94±0.48 a	3,88±0.63 a
5,5' diFA ²		4,85±0.40 c	5,00±0.42 c	2,94±0.24 b	0,00 a
8-O-4' diFA ²		19,42±1.67 a	24,00±2.06 a	22,55±1.94 a	22,33±1.92 a
8,5' BF diFA ²		5,83±1.26	n.d.	n.d.	n.d.

¹ mg sugar/g fibrous residue. ² % on total sugars/phenolics quantified

RM: raw material; Rha: rhamnose; Fuc: fucose; Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glu: glucose; diFA: diferulic acid.

Means bearing the same letter in the same row are not significantly different at the 5% level as determined by Duncan's multiple range test.

3.2. Effect of HTT on fibrous solid composition.

Four different treatments were assayed with $\log R_o$ values of 3.05, 3.64, 4.23, and 4.82.

After HTT, the whole sample was collected and filtered to separate the solid fibrous fraction and the liquor. The oven-dried solid was E/A extracted as described for RM.

The general composition of the dried fibrous residues and their ARC against the severity index ($\log R_o$) are presented in Figure 1. The results are calculated adding the amount of sugar and phenols quantified in the E/A extracts together with those of the corresponding slurries.

Figure 1. General composition and antioxidant activity of recovered fibrous solids after HTT against severity index. Yield (%), total sugar content (mg/g recovered solid) and antiradical capacity (ARC, mmol Trolox/Kg recovered solid) are expressed in the primary axis, and the total phenol content (mg/g recovered solid) in the secondary one.

(Single column image)

The yield of HTTs decreased with severity. At severity 3.05, 64% of initial RM was recovered as a dried fibrous solid, but this percentage decreased to 54, 44, and 43% as the severity rose to 3.64, 4.23 and 4.82, respectively. Similar results were found when

olive cake (Felizón et al., 2000) and barley husks (Garrote et al., 2008) were treated at the same severity range. A significant increase in the sugar content was quantified only after 220° C HTT ($\log R_0$ 4.23), unlikely for phenolic content which went down linearly as severity increased. ARC of the different solids increased with severity, with significant differences only between treatments of low (80-87 mmol Trolox/Kg) and high severity indexes (125-154 mmol Trolox/Kg). The quantified ARC was much higher than those previously reported for asparagus by-products processed under different conditions, 21-26 mmol Trolox/Kg (Fuentes-Alventosa, et al., 2013; Fuentes-Alventosa, et al., 2009a), although the content in HCA was very similar in all the samples. In addition, in the present study, ARC did not correlate with phenols as in previous studies. New antioxidant compounds must be being generated during HTT, whose concentration may be related to treatment severity and also to the presented ARC. These fibrous solids (and liquors too) had a brown color and a pleasant smell which resembles roasted coffee or cocoa. Melanoidins and Maillard compounds are responsible for the brown color of these food products and have been demonstrated to have high antioxidant activity and multiple biological activities (Wang et al., 2011). The important concentration of sugar and proteins that asparagus by-products present (Fuentes-Alventosa, et al., 2009b) could support the idea of melanoidins formation during HTT.

In Table 1, the glycosidic composition of E/A-treated fibrous residues after HTT are presented. Although only 220-HTT showed a significant gain in the total content of sugar compared with RM, important changes were detected in the percent composition. The glucose proportion increased significantly in all the treatments, suggesting that the obtained fibrous solids were being enriched in cellulose and reached the maximum at 220-HTT and 240-HTT. Consequently, the rest of the sugars were reduced: rhamnose

and fucose almost disappeared; arabinose and xylose dropped to 14-19% of their initial content in RM, and galactose to 25%. The behavior of mannose was different since in 180-HTT and 200-HTT no significant differences were detected for this sugar, but decreases to 72% and 53% in 220-HTT and 240-HTT, respectively, were quantified. Mannans are also present in the asparagus cell wall and they tend to accumulate during post-harvest storage and are very closely linked to cellulose. It is known that mannans form more stable complexes with cellulose than xyloglucans and cellulose do (Whitney et al., 1998), so this steady association could protect mannose from being lost during the treatment at lower severities. This enrichment in cellulose and the solubilization of hemicelluloses was also observed while working with olive cake (Felizón, et al., 2000) and corncobs (Vázquez et al., 2006). In these cases the percent of cellulose did not reach such high values as for asparagus because their initial content in hemicellulose was much higher. This enrichment in cellulose in the solid residue is one of the objectives of HTT, so afterwards it could undergo enzymatic or microbiological treatments for obtaining saccharification and bioethanol, or for biocomposite manufacturing (Alonso et al., 2011).

The composition of phenolics present in the ethanol/acetone treated solid was also studied (Table 1). Only esterified HCA were detected. The total amount remained unchanged and only after the most intense treatment was there a decrease to 55% of the initial amount. The percent composition did not show important variations, which is unlikely for sugars. The total percentage of diFA decreased with HTT exclusively, from 34% in RM to 25% in 240-HTT. It is interesting to note that phenolics are more resistant to HTT than sugars because only in the most severe treatment losses in every HCA were they detected. Studying their amounts in mg/g fibrous residue (data not shown), they showed different lability: 8,5BF was the only dimer which decreased in all

the treatments; 8,8' and 5,5' were solubilized as of 200-HTT and 8,5' as of 220-HTT. Coumaric acid, FA, 8,8' AT diFA and 8-O-4' diFA were released only by the most severe treatment. Both dimers have demonstrated their relationship with texture characteristics in plant foods: the loss of 8,8' AT diFA correlates with a decrease in strength during the thermal processing of Chinese water chestnut (C. C. Parker et al., 2003), and it increases in asparagus tips during post-harvest storage (Rodríguez-Arcos, et al., 2002a; R. Rodríguez, et al., 2005); 8-O-4' diFA was postulated as a thermally stable dimer in chufa tubers (M. L. Parker et al., 2000) and it was the key dimer for asparagus toughening (Jaramillo, et al., 2007). Our results confirm the thermal resistance of these dimers and their strong cross-linking into the asparagus fiber structure.

In Table 2, the characterization of the E/A soluble fraction of treated asparagus fiber is presented. The less severe treatments led to higher amounts of soluble sugars, but they decreased as severity went up. Glucose was again the most abundant sugar, followed by galactose and arabinose. The changes with treatments differed depending on the studied sugar: glucose and arabinose diminished but mannose and galactose rose. Xylose also increased, but showed its maximum in 200-HTT, where it was present in a percent even higher than glucose.

After thermal treatments, esterified phenolics were detected in the E/A extracts but not free phenolics. Their amounts in the extracts (0.89-1.32 mg/g treated fiber) were similar to those found in the residue after extraction (0.97-2.00mg/g treated fiber), which was unlikely to happen for sugar as the amount quantified in the E/A extracts (2.34-15.35 mg sugar/g treated fiber) were much lower than those from the residue (271.80-363.54 mg sugar/g treated fiber). The total content of esterified phenolics decreased with severity. In these extracts, FA structures (monomer and dimers) were clearly the most

abundant, with the coumaric acid between 20-30%. The FA monomer, 8,8' and 8-O-4' diFA were the predominant components. Monomer percent increased with severity but both dimers showed minor changes.

3.3. Sugar content in HTT liquors and total sugar balance

Figure 2 represents the yield and sugar content and distribution of the lyophilized treatment liquors against severity index.

Figure 2. Yield (g lyophilized liquor/100 g RM) and sugar content and distribution (mg/g lyophilized liquor) of the treatment liquors after lyophilization against severity index.

(Single column image)

The yield, expressed as g lyophilized liquor/100 g RM increased with severity, showing significant differences only between the less and the most severe treatments. However, the content in sugar (mg/g lyophilized liquor) was higher in the mildest treatments, suggesting that the sugar degradation process occurred at higher temperatures. From the three fractions characterized, the oligosaccharides were the most abundant in all cases, reaching a maximum at 3.64 severity index and decreasing henceforth. Mono- and polysaccharides were also present in the treatment liquors. At lower severities, monosaccharides was the minor fraction but increased as severity went up, reaching

about 20% of the total sugar quantified at 4.82 severity index. The polysaccharidic fraction showed opposite changes, decreasing from 40 to 7% of total sugar.

Table 3.-Glycosidic composition of the different treatment liquors.

	180-HTT	200-HTT	220-HTT	240-HTT
$\log R_0$	3.05	3.64	4.23	4.82
Monosaccharides¹	10.66±0.59 c	3.99±0.13 a	5.69±0.25 b	11.52±0.44 d
Rha ²	3.62±0.51 a	16.64±0.79 b	5.76±3.63 a	3.62±2.41 a
Fuc ²	1.53±1.12 a	8.36±0.73 b	1.22±1.73 a	1.47±0.91 a
Ara ²	14.87±0.46 a	26.15±0.66 b	26.47±3.44 b	23.16±3.34 b
Xyl ²	4.02±0.93 a	19.95±1.22 b	33.30±0.67 c	31.70±0.44 c
Man ²	36.52±1.23 c	11.19±0.68 a	14.11±0.37 b	11.80±0.37 a
Gal ²	1.96±0.45 a	11.92±0.29 b	14.65±1.44 c	13.89±0.99 c
Glu ²	34.49±1.42 c	5.78±0.28 a	4.49±0.03 a	14.06±1.22 b
Oligosaccharides¹	53.01±2.16 b	92.54±5.85 c	50.16±0.42 ab	44.35±1.84 a
Rha ²	5.47±0.19 d	4.15±0.22 c	1.19±0.04 a	1.72±0.04 b
Fuc ²	0.90±0.02 c	0.70±0.08 b	n.d.	0.30±0.02 a
Ara ²	38.36±0.33 d	13.17±1.58 c	7.05±0.10 a	9.00±0.07 b
Xyl ²	26.92±0.16 a	53.12±2.73 b	58.90±0.09 c	58.37±0.55 c
Man ²	6.36±0.25 b	4.35±1.40 a	4.87±0.11 a	8.15±0.18 c
Gal ²	11.85±0.10 a	12.18±1.07 ab	12.33±0.35 ab	13.03±0.40 b
Glu ²	10.14±0.42 ab	12.33±2.90 b	15.66±0.23 c	9.43±0.30 a
Polysaccharides¹	39.61±1.20 c	13.83±0.82 b	5.22±0.27 a	4.27±0.38 a
Rha ²	13.32±0.38 d	9.06±0.67 c	6.72±0.69 a	7.72±0.23 b
Fuc ²	0.57±0.01 c	0.28±0.01 b	n.d.	0.07±0.06 a
Ara ²	14.14±0.44 c	8.63±0.06 b	6.17±0.33 a	8.84±0.24 b
Xyl ²	32.31±0.62 b	34.87±0.71 c	36.15±0.86 c	26.93±0.90 a
Man ²	4.63±0.36 b	3.42±0.35 a	6.95±0.21 d	5.40±0.39 c
Gal ²	28.56±0.80 a	37.24±0.52 c	31.08±0.36 b	36.55±1.11 c
Glu ²	6.47±0.24 a	6.50±0.20 a	12.93±0.05 b	14.48±0.20 c
Total balance ³ (%)	89.59	67.62	63.68	57.19

¹ mg/g lyophilized liquor. ²% on total sugars quantified. ³ including fibrous solid fraction and liquor.

Rha: rhamnose; Fuc: fucose; Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glu: glucose.

Means bearing the same letter in the same row are not significantly different at the 5% level as determined by Duncan's multiple range test.

The glycosidic composition of the three isolated fractions is presented in Table 3. In the oligosaccharidic fraction, arabino-xylooligosaccharides were the most abundant (xylose and arabinose accounted for more than 60% of sugars in all the liquors), although fragments of xyloglucans and mannans could also be present. These thermal treatments are widely applied for obtaining xylooligosaccharides (XOS) from agro-industrial wastes (Gullón et al., 2010; Parajó et al., 2004), so asparagus by-products could be considered as an interesting raw material for these purposes. Nowadays, XOS are in the focus of research in food technology due to their wide applications and health-related effects (Broekaert et al., 2011). In our study, with the treatment at 200° C (log R_o 3.64) the maximum quantity of XOSs was obtained, although deeper investigations have to be done to optimize sugar extraction with minimal sugar degradation.

For monosaccharides, mannose and glucose were the most abundant ones at 3.05 severity index. As a consequence of the severity increment, the liquors became richer in the xylose monomer thus showing an important degradation of solubilized xylans (xylose was also the main sugar quantified in the polysaccharidic fraction, with the exception of 240-HTT). This exchange between different molecular weight fractions was also observed during the autohydrolysis of corncobs (Vázquez et al., 2006). Working with wheat straw (Carvalho et al., 2009), similar results were found: due to HTT, a solubilization of xylans and an increase in XOS were described; but at severities higher than 4, furfural was detected in increasing amounts as a degradation compound of pentoses while XOS diminished massively. Furfural was also detected in the liquors of the present study and this will be discussed below.

The total balance of sugars (solid fibrous residue plus liquors) is also presented in Table 3. The percentages decreased with severity but they varied among sugars (data not shown). Glucose was the sugar with the highest retrieval, showing nearly 80% in the most intense treatments (220-HTT and 240-HTT). In 200-HTT, most of the sugars (rhamnose, xylose, mannose, and galactose) were between 60-70% and this percent shrank to 20% in the most severe treatment. Again, the treatment at 200 °C (severity 3.64) seemed to determine a limit for the HTT performance.

3.4. Phenolics content in HTT liquors and total phenol balance

Important amounts of phenolics were also found in the liquors, since hydrothermal pretreatments are also widely used for obtaining antioxidant extracts from agricultural by-products (Alonso, et al., 2011).

Figure 3. Total content and distribution of phenols (mg/g lyophilized liquor) in the treatment liquors after lyophilization and their antiradical activity (ARC, mmol Trolox/Kg lyophilized liquor) against severity index.

(Single column image)

Total phenolics increased with HTT (Figure 3) both as free and esterified phenolics. At 3.05 severity, the esterified fraction was more abundant than the free one, but at higher severity values, free phenolics increased at a greater rate and became the most abundant.

ARC increased with severity, presenting moderate correlations with the concentration of total phenolics ($R^2=84.75$). These ARC results were very high in comparison with others found in the bibliography: sugar cane molasses at around 100 (Valli et al., 2012), and okara at 19-29 (Stanojevic et al., 2014). Similar results to ours were described when working with citrus fruit wastes at 70-240 mmol Trolox/Kg (Marín et al., 2007), which were the highest found in the bibliography. The cited by-products are composed mainly of insoluble fiber, but these products obtained by lyophilization of the HTT liquors from asparagus wastes are water-soluble and have a very pleasant smell. These characteristics make these new products easily included in a wider range of food matrixes.

Good correlations between the content of phenols and antioxidant activity are not always obtained. Working with non-commercial date fruits (Mrabet et al., 2016) and some extracts from winemaking wastes (Cruz et al., 2004), similar correlations were reached, with R^2 87 and 71%, respectively. However, in studies with liquors from hydrothermally treated pineapple by-products (Sepúlveda et al., 2018), only R^2 22-33% was found. This could be due to antagonistic-synergistic effects of the different components present in the liquors or to the occurrence of newly synthesized compounds as an effect of HTT conditions. These results suggest again the presence of melanoidins and Maillard compounds, which could also be responsible in part for the antioxidant activity in both the fibrous solid and liquors obtained from asparagus by-products after HTT processes.

In Table 4, the phenolic composition (both free and esterified phenols) and the degradation compounds (furfural and methyl-furfural) in the liquors are presented. The FA monomer was the main phenol identified in the free fraction followed by coumaric acid and rutin. Rutin (quercetin-3-O-rhamnoglucoside) is the most abundant flavonol glycoside described in asparagus spears and is the one that is mainly responsible for its

antioxidant activity (Drinkwater et al., 2015). It is partially soluble in water and most of its content should have been extracted during the initial homogenization of asparagus by-products with water. FA and rutin increased the most due to treatment severity. At 240-HTT, both compounds were quantified at about four times higher than in 180-HTT. Among esterified phenolics, in general, the FA monomer and dimers were predominant, but in percentages similar to coumaric acid. Only two dimers were detected, 8,8' diFA, only in the mildest conditions and 8-O-4' in all of them, with their percentage being much lower than those of monomer.

Table4.-Phenolic contents of the different treatment liquors.

		180-HTT	200-HTT	220-HTT	240-HTT
	$\log R_0$	3.05	3.64	4.23	4.82
Free phenolics ¹		0.61±0.03a	1.04±0.05b	1.33±0.07c	1.74±0.10d
Coumaric acid ²		35.02±3.19 b	32.79±2.99 b	27.83±2.53 a	24.37±2.22 a
Ferulic acid ²		49.02±4.92 a	46.78±4.73 a	51.64±5.22 a	54.21±4.48 a
Rutin ²		16.11±0.69 a	20.43±0.87 b	20.51±0.88 b	21.41±0.92b
Esterified phenolics ¹		0.83±0.003b	0.90±0.002c	0.77±0.002a	1.01±0.003d
Coumaric acid ²		47.12±4.29 a	51.49±4.68 a	47.06±4.28 a	41.72±3.79 a
Ferulic acid ²		33.65±3.40 a	45.52±4.60 a	50.42±5.09 a	48.34±4.88 a
8,8' diFA ²		9.62±1.17	n.d.	n.d.	n.d.
8-O-4' diFA ²		9.62±0.83 b	2.99±0.26 a	2.52±0.22 a	9.93±0.55 b
Degradation products ¹		0.47±0.02 a	0.65±0.05 b	0.82±0.07 c	0.68±0.05 b
Hydroxymethyl-furfural ²		32.00±1.47 b	16.84±0.73 a	48.36±2.00 c	33.33±0.92 b
Furfural ²		67.00±2.85 b	83.15±3.28 c	51.63±1.94 a	66.67±1.67 b
Total balance ³ (%)		103.73	87.14	72.82	70.67

¹mg/g lyophilized liquor. ² % on total phenols quantified. ³ including fibrous solid fraction and liquors. diFA: diferulic acid.

Means bearing the same letter in the same row are not significantly different at the 5% level as determined by Duncan's multiple range test.

Degradation products, such as hydroxymethylfurfural and furfural, were also found in the liquors coming from the degradation at high temperature of hexoses and pentoses, respectively. The amounts found in this study were much lower than others published for wheat straw (Carvalho, et al., 2009) at similar severity ranges. Besides differences caused by the raw material characteristics, another reason for this low quantity, especially of furfural, is that the reactor used in the present study has an expansion step prior to opening which could cause furfural to volatilize, and be lost at the reactor opening (Mrabet, et al., 2016). Treatment conditions must be carefully designed in order to minimize furfural production at the expense of XOS degradation.

The balance of phenolics in the whole process is also presented in Table 4. These compounds are more resistant to HTT than sugars, and were recovered at the most severe treatment in a percentage higher than 70%. For individual compounds (data not shown), coumaric acid was retrieved between at 100-70% and FA between 100-85% at the mildest and the most severe conditions, respectively. It is clear that the major part of FA dimers that were lost during treatment were recovered as FA monomers. Again 8,8'-AT and 8-O-4' diFA showed the best balance, nearly 60% of their initial quantity in 240-HTT. The other dimers were between 20-40%. These results support the idea of the significant role of both dimers in maintaining asparagus fiber structure.

HTT is a technology very often applied to obtain antioxidants from classical phenol-rich by-products (olive cake, wheat straw, cereal bran, or wood) and thanks to which, other unconventional wastes could be valorized as sources of bioactive phenols such as winemaking solids (Cruz, et al., 2004), pineapple wastes (Sepúlveda, et al., 2018) or the asparagus by-products presented in this work.

3.5. Presence of melanoidins in HTT liquors

Melanoidins are brown pigments which are associated by consumers with high-grade products of desirable texture and flavor (Wang, et al., 2011). They can absorb light in the whole UV spectrum up to 420 nm and are responsible for the characteristic brown color of food such as coffee, cocoa, bread, and malt. A method for identifying their presence has been developed in coffee (Bekedam, et al., 2006). In this method, three different measurements of absorbance are done (280, 325, and 405 nm). At 280 nm, proteins and chlorogenic and hydroxycinnamic acids have their maximum absorbance, but at 325 nm, only the second group has a maximum. 405 nm was the wavelength selected for melanoidins, since other compounds do not absorb light at this wavelength. These three measurements provide useful information on the relative amount of melanoidins and other compounds in food samples. The absorption at a specific wavelength was expressed as K_{mix} value: $K_{\text{mix}, 280}$ for proteins, chlorogenic, and hydroxycinnamic acids, $K_{\text{mix}, 325}$ for chlorogenic and hydroxycinnamic acids, and $K_{\text{mix}, 405}$ for melanoidins. By comparing the values of these K_{mix} , the relative percentage of these compounds could be estimated. The UV spectra of the different HTT liquors are presented in Figure 4, and the values of the three K_{mix} and their ratios in Table 5.

Figure 4. UV spectra of the different HTT liquors

(Single column image)

Table 5.- K_{mix} values (L/cm/g) and K_{mix} ratios of the different treatment liquors

	180-HTT	200-HTT	220-HTT	240-HTT
$\log R_0$	3.05	3.64	4.23	4.82
$K_{\text{mix}, 405}$	0.4	0.5	0.7	0.8
$K_{\text{mix}, 325}$	3.0	3.8	5.0	3.7
$K_{\text{mix}, 280}$	4.5	5.7	7.1	9.0
$K_{\text{mix}, 325:405}$	7.28	7.22	7.43	4.65
$K_{\text{mix}, 280:405}$	10.90	10.74	10.51	11.25

The three K_{mix} increased with severity with the exception of $K_{\text{mix}, 325}$ at 240-HTT. This evolution followed the spectra presented in Figure 1 where continuous increases in absorbance at 280, 325 and 405 nm can be observed as well as the exception. The marked rise at 280 nm in the 240-HTT liquor was not accompanied by intensification in 325 nm absorbance; therefore these changes may be caused by a higher solubilization of proteins but not of hydroxycinnamic acids. The ratio $K_{\text{mix}, 325:405}$ was around 7 and went down to 4 in the 240-HTT liquor, suggesting a higher proportion of melanoidins in this fraction. The ratio $K_{\text{mix}, 280:405}$ remained almost stable in the four samples. These results could imply the presence of melanoidins in the liquors (and probably also in the fibrous solids) in increasing amounts as treatment severity grew, and that their proportion should be higher in the 240-HTT liquor. The existence of these compounds could also influence the antioxidant activity of all the samples assayed.

4. Conclusions

The application of HTT to asparagus by-products leads to autohydrolysis processes and to obtaining two new products. One of them is an insoluble fibrous solid, enriched in cellulose and with lower amounts of hemicelluloses and phenolics than the original material. Its antioxidant capacity increased with treatment severity. It is probable that

the existence of melanoidins in this fibrous solid produces an increase in its antioxidant capacity, regardless of the phenol content. Apart from its use as insoluble dietary fiber, this solid could be a suitable substrate for saccharification and bioethanol production. On the other hand, a liquid fraction is also obtained. After lyophilization, a water-soluble solid with a powdery appearance and odors of coffee and cocoa is collected. It is composed mainly of XOS, which are nowadays considered high in value due to their health-related effects. In addition, a changeable percent of mono- and polysaccharides can be isolated. The relative abundance of each fraction (mono-, oligo- and polysaccharides) could be defined by adjusting the treatment conditions, although in these experiences the maximum in oligosaccharides was achieved with treatments at 200° C (severity index 3.64). Anyway, the final conditions must be optimized in order to diminish sugar degradation. This soluble solid also has a great antioxidant activity which is related to its phenolic composition. Melanoidins could contribute to this action as well.

Both products, the insoluble and the soluble ones, could be easily added as ingredients to the formulation of functional foods, and enrich them in insoluble and/or soluble fibers, and in antioxidants at the same time. However, further investigations related to the elucidation of the presence of melanoidins and their implication in bioactivity must be broached.

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References

- Alonso, J. L., Domínguez, H., Garrote, G., González-Muñoz, M. J., Gullón, B., Moure, A., Santos, V., Vila, C., Yáñez, R. 2011. Biorefinery processes for the integral valorization of agroindustrial and forestal wastes. *CyTA-Journal of Food*, 9, 282-289. <https://doi.org/10.1080/19476337.2011.598949>
- Bekedam, E. K., Schols, H. A., Van Boekel, M. A. J. S., Smit, G. 2006. High molecular weight melanoidins from coffee brew. *Journal of Agricultural and Food Chemistry*, 54, 7658-7666. <http://doi.org/10.1021/jf0615449>
- Broekaert, W. F., Courtin, C. M., Verbeke, K., Van de Wiele, T., Verstraete, W., Delcour, J. A. 2011. Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylanoligosaccharides, and xylooligosaccharides. *Critical Reviews in Food Science and Nutrition*, 51, 178-194. <http://doi.org/10.1080/10408390903044768>
- Carvalho, F., Silva-Fernandes, T., Duarte, L. C., Gírio, F. M. 2009. Wheat straw autohydrolysis: process optimization and products characterization. *Applied Biochemistry and Biotechnology*, 153, 84-93. <http://doi.org/10.1007/s12010-008-8448-0>
- Conde, E., Moure, A., Domínguez, H. 2017. Recovery of phenols from autohydrolysis liquors of barley husks: kinetic and equilibrium studies. *Industrial Crops and Products*, 103, 175-184. <https://doi.org/10.1016/j.indcrop.2017.03.048>
- Cruz, J. M., Domínguez, H., Parajó, J. C. 2004. Assessment of the production of antioxidants from winemaking waste solids. *Journal of Agricultural and Food Chemistry*, 52, 5612-5620. <https://doi.org/10.1021/jf049376c>
- Dos-Santos, N., Jiménez-Araujo, A., Rodríguez-Arcos, R., Fernández-Trujillo, J. P. 2011. Cell wall polysaccharides of near-isogenic lines of melon (*Cucumis melo* L.) and their inbred parentals which show differential flesh firmness or

- physiological behavior. *Journal of Agricultural and Food Chemistry*, 59, 7773-7784. <https://doi.org/10.1021/jf201155a>
- Drinkwater, J. M., Tsao, R., Liu, R., Defelice, C., Wolyn, D. J. 2015. Effects of cooking on rutin and glutathione concentrations and antioxidant activity of green asparagus (*Asparagus officinalis*) spears. *Journal of Functional Foods*, 12, 342-353. <https://doi.org/10.1016/j.jff.2014.11.013>
- FAO. 2018. On-line statistical database of the food and agricultural organization of the United Nations. <http://faostat3.fao.org/> Last accessed: 31/08/2018
- Felizón, B., Fernández-Bolaños, J., Heredia, A., Guillén, R. 2000. Steam-explosion pretreatment of olive cake. *Journal of the American Oil Chemical Society*, 77(1), 15-22. <https://doi.org/10.1007/s11746-000-0003-y>
- Fernández-Bolaños, J., Felizón, B., Heredia, A., Guillén, R., Jiménez, A. 1999. Characterization of the lignin obtained by alkaline delignification and of the cellulose residue from steam-exploded stones. *Bioresource Technology*, 68, 121-132. [https://doi.org/10.1016/S0960-8524\(98\)00134-5](https://doi.org/10.1016/S0960-8524(98)00134-5)
- Fernandez-Bolaños, J., Felizón, B., Heredia, A., Rodríguez, R., Guillén, R., Jiménez, A. 2001. Steam-explosion of olive stones: hemicelluloses solubilization and enhancement of enzymatic hydrolysis of cellulose. *Bioresource Technology*, 79, 53-61. [https://doi.org/10.1016/S0960-8524\(01\)00015-3](https://doi.org/10.1016/S0960-8524(01)00015-3)
- Fuentes-Alventosa, J. M., Jaramillo-Carmona, S., Rodríguez-Gutiérrez, G., Guillén-Bejarano, R., Jiménez-Araujo, A., Fernández-Bolaños, J., Rodríguez-Arcos, R. 2013. Preparation of bioactive extracts from asparagus by-product. *Food and Bioproducts Processing*, 91, 74-82. <https://doi.org/10.1016/j.fbp.2012.12.004>
- Fuentes-Alventosa, J. M., Jaramillo-Carmona, S., Rodríguez-Gutiérrez, G., Rodríguez-Arcos, R., Fernández-Bolaños, J., Guillén-Bejarano, R., Espejo-Calvo, J. A.,

- Jiménez-Araujo, A. 2009a. Effect of extraction method on phytochemical composition and antioxidant activity of high dietary fibre powders obtained from asparagus by-products. *Food Chemistry*, 116, 484-490.
<https://doi.org/10.1016/j.foodchem.2009.02.074>
- Fuentes-Alventosa, J. M., Rodríguez-Gutiérrez, G., Jaramillo-Carmona, S., Espejo-Calvo, J. A., Rodríguez-Arcos, R., Fernandez-Bolaños, J., Guillén-Bejarano, R., Jiménez-Araujo, A. 2009b. Effect of extraction method on chemical composition and functional characteristics of high dietary fibre powders obtained from asparagus by-products. *Food Chemistry*, 113, 665-671.
<https://doi.org/10.1016/j.foodchem.2008.07.075>
- Garrote, G., Cruz, J. M., Domínguez, H., Parajó, J. C. 2008. Non-isothermal autohydrolysis of barley husks: product distribution and antioxidant activity of ethyl acetate soluble fractions. *Journal of Food Engineering*, 84, 544-552.
<https://doi.org/10.1016/j.jfoodeng.2007.06.021>
- Guillén-Bejarano, R., Rodríguez-Gutiérrez, G., Fuentes-Alventosa, J. M., Jaramillo-Carmona, S., Rodríguez-Arcos, R., Jiménez-Araujo, A., Fernández-Bolaños, J. 2012. Procedimiento de obtención de compuestos funcionales de origen vegetal. In CSIC (Ed.), vol. ES2387279A1). Spain. Last accessed.
- Gullón, B., Yáñez, R., Alonso, J. L., Parajó, J. C. 2010. Production of oligosaccharides and sugars from rye straw: a kinetic approach. *Bioresource Technology*, 101, 6676-6684. <https://doi.org/10.1016/j.biortech.2010.03.080>
- Heitz, M., Carrasco, F., Rubio, M., Brown, A., Chornet, E. 1987. Physicochemical characterization of lignocellulosis substrates pretreated via autohydrolysis: an application to tropicalwoods. *Biomass*, 13, 255-273.

- Jaramillo-Carmona, S., Guillén-Bejarano, R., Rodríguez-Arcos, R., Jiménez-Araujo, A. 2017. Feruloylated arabinoxylans: antioxidant polysaccharides from asparagus dietary fiber. *Journal of Natural Products Research Updates*, 3, 1-10.
- Jaramillo, S., Rodríguez, R., Jiménez, A., Guillén, R., Fernández-Bolaños, J., Heredia, A. 2007. Effects of storage conditions on the accumulation of ferulic acid derivatives in white asparagus cell walls. *Journal of the Science of Food and Agriculture*, 87, 286-296. <https://doi.org/10.1002/jsfa.2718>
- Kar, W. E., Gutiérrez, C.V., Kinoshita, C.M. 1998. Steam explosion of sugarcane bagasse as a pretreatment for conversion to ethanol. *Biomass and Bioenergy*, 14, 1-9. [https://doi.org/10.1016/S0961-9534\(97\)10038-1](https://doi.org/10.1016/S0961-9534(97)10038-1)
- Marín, F. R., Soler-Rivas, C., Benavente-García, O., Castillo, J., Pérez-Álvarez, J. A. 2007. By-products from different citrus processes as a source of customized functional fibres. *Food Chemistry*, 100(2), 736-741. <https://doi.org/10.1016/j.foodchem.2005.04.040>
- Montane, D., Farriol, X., Salvado, J. 1998. Fractionation of wheat straw by steam explosion pretreatment and alkali delignification, cellulose pulp and byproducts from hemicellulose and lignin. *Journal of Wood Chemistry and Technology*, 18, 171-191. <https://doi.org/10.1080/02773819809349575>
- Mrabet, A., Jiménez-Araujo, A., Fernández-Bolaños, J., Rubio-Senent, F., Lama-Muñoz, A., Sindic, M., Rodríguez-Gutiérrez, G. 2016. Antioxidant phenolic extracts obtained from secondary Tunisian date varieties (*Phoenix dactylifera* L.) by hydrothermal treatments. *Food Chemistry*, 196, 917-924. <https://doi.org/10.1016/j.foodchem.2015.10.026>
- OEC. 2018. The Observatory for Economic Complexity. <https://atlas.media.mit.edu/en/>
Last accessed: 31/08/2018

- Parajó, J. C., Garrote, G., Cruz, J. M., Dominguez, H. 2004. Production of xylooligosaccharides by autohydrolysis of lignocellulosic materials. *Trends in Food Science & Technology*, 15, 115-120.
<https://doi.org/10.1016/j.tifs.2003.09.009>
- Parker, C. C., Parker, M. L., Smith, A. C., Waldron, K. 2003. Thermal stability of texture in Chinese water chestnut may be dependent on 8,8'-diferulic acid (aryltetralyn form). *Journal of Agricultural and Food Chemistry*, 51, 2034-2039.
<https://doi.org/10.1021/jf020759p>
- Parker, M. L., Ng, A., Smith, A. C., Waldron, K. 2000. Esterified phenolics of the cell wall of chufa (*Cyperus esculentus* L.) tubers and their role in texture. *Journal of Agricultural and Food Chemistry*, 48, 6284-6291.
<https://doi.org/10.1021/jf0004199>
- Rodríguez-Arcos, R. C., Smith, A. C., Waldron, K. W. 2002a. Effect of storage on wall-bound phenolics in green asparagus. *Journal of Agricultural and Food Chemistry*, 50, 3197-3203. <https://doi.org/10.1021/jf011687p>
- Rodríguez-Arcos, R. C., Smith, A. C., Waldron, K. W. 2002b. Mechanical properties of green asparagus. *Journal of the Science of Food and Agriculture*, 82, 293-300.
<https://doi.org/10.1002/jsfa.1033>
- Rodríguez-Arcos, R. C., Smith, A. C., Waldron, K. W. 2004. Ferulic acid crosslinks in asparagus cell walls in relation to texture. *Journal of Agricultural and Food Chemistry*, 52, 4740-4750. <https://doi.org/10.1021/jf030610t>
- Rodríguez, G., Rodríguez, R., Fernández-Bolaños, J., Guillén, R., Jiménez, A. 2007. Antioxidant activity of effluents during the purification of hydroxytyrosol and 3,4-dihydroxyphenyl glycol from olive oil waste. *European Food Research and Technology*, 224, 733-741. <https://doi.org/10.1007/s00217-006-0366-1>

- Rodríguez, R., Jaramillo, S., Guillén, R., Jiménez, A., Fernández-Bolaños, J., Heredia, A. 2005. Cell wall phenolics of white and green asparagus. *Journal of the Science of Food and Agriculture*, 85, 971-978. <https://doi.org/10.1002/jsfa.2053>
- Rodríguez, R., Jiménez, A., Guillén, R., Heredia, A., Fernández-Bolaños, J. 1999. Turnover of white asparagus cell wall polysaccharides during postharvest storage. *Journal of Agricultural and Food Chemistry*, 47, 4525-4531. <https://doi.org/10.1021/jf981392k>
- Ruiz, H.A., Rodríguez-Jasso, M.A., Fernandes, B.D., Vicente, A.A., Teixeira, J.A. 2013. Hydrothermal processing, as an alternative for upgrading agriculture residues and marine biomass according to the biorefinery concept: A review. *Renewable and Sustainable Energy Reviews*, 21, 35-51. <https://doi.org/10.1016/j.rser.2012.11.069>
- Ruiz, H.A., Thomsen, H.T., Trajano, H.L. (Eds) 2017. *Hydrothermal processing in biorefineries: production of bioethanol and high added-value compounds of second and third generation biomass*. Springer. <https://doi.org/10.1007/978-3-319-56457-9>
- Sepúlveda, L., Romaní, A., Aguilar, C. N., Teixeira, J. 2018. Valorization of pineapple waste for the extraction of bioactive compounds and glycosides using autohydrolysis. *Innovative Food Science and Emerging Technologies*, 47, 38-45. <https://doi.org/10.1016/j.ifset.2018.01.012>
- Stanojevic, S. P., Barac, M. B., Pesic, M. B., Zilic, S. M., Kresovic, M. M., Vucelic-Radovic, B. V. 2014. Mineral elements, lipooxygenase activity, and antioxidant capacity of okara as a by-product in hydrothermal processing of soy milk. *Journal of Agricultural and Food Chemistry*, 62, 9017-9023. <https://doi.org/10.1021/jf501800s>

- Valli, V., Gómez-Caravaca, A. M., Di Nunzio, M., Danesi, F., Caboni, M. F., Bordon, A. 2012. Sugar cane and sugar beet molasses, antioxidant-rich alternatives to refined sugar. *Journal of Agricultural and Food Chemistry*, 60, 12508-12515.
<https://doi.org/10.1021/jf304416d>
- Vázquez, M. J., Alonso, J. L., Domínguez, H., Parajó, J. C. 2006. Enhancing the potential of oligosaccharides from corncob autohydrolysis as prebiotic food ingredient. *Industrial Crops and Products*, 24, 152-159.
<https://doi.org/10.1016/j.indcrop.2006.03.012>
- Waldron, K. W., Parr, A. J., Ng, A., Ralph, J. 1996. Cell wall esterified phenolic dimers: identification and quantification by reverse phase high performance liquid chromatography and diode array detection. *Phytochemical Analysis*, 7, 305-312. [https://doi.org/10.1002/\(SICI\)1099-1565\(199611\)7:6<305::AID-PCA320>3.0.CO;2-A](https://doi.org/10.1002/(SICI)1099-1565(199611)7:6<305::AID-PCA320>3.0.CO;2-A)
- Wang, H. Y., Qian, H., Yao, W. R. 2011. Melanoidins produced by the Maillard reaction: structure and biological activity. *Food Chemistry*, 128, 573-584.
<https://doi.org/10.1016/j.foodchem.2011.03.075>
- Whitney, S. E. C., Brigham, J. E., Darke, A. H., Reid, J. S. G., Gidley, M. J. 1998. Structural aspects of the interaction of mannan-based polysaccharides with bacterial cellulose. *Carbohydrate Research*, 307, 299-309.
[https://doi.org/10.1016/S0008-6215\(98\)00004-4](https://doi.org/10.1016/S0008-6215(98)00004-4)