

# Manufacture of Nutraceutical Compounds from Chestnut Shells by Hydrothermal Processing

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The depletion of the fossil resources entails that the modern society must face two important challenges: the substitution of fossil-based products by biomass-based ones and the change from a linear economy towards a circular model, which could allow establishing a global sustainable bio-economy. To accomplish these great challenges, in the last decades the employment of by-products and residues from the agro-food industry to manufacture added-value products has been explored. This would constitute an important step towards economically sustainable and environmentally friendly industrial processes. In this scenario, waste products of lignocellulosic nature represent an attractive, inexpensive and renewable feedstock for the manufacture of biofuels, energy, chemicals and polymers. Chestnut shells are rejected lignocellulosic residues from the agro-food industry with promising possibilities as a suitable feedstock to be used under a biorefinery perspective. These by-products have been employed as a source of antioxidants, mono- and oligosaccharides and to prepare adsorbents for pollutants removal. The development of alternative applications for the chestnut shells could contribute to the integral valorisation of their main constituents. In this work, the hydrothermal treatment of chestnut shells was proposed with a double purpose: to assess its suitability for the solubilisation of the hemicellulosic fraction and phenolic compounds and to determine the optimal conditions that allow the obtaining of the maximum concentration of hemicellulosic oligosaccharides with the minimum amount of monosaccharides and degradation products in the liquid phase. The yield in hemicellulosic oligosaccharides and phenolic compounds is a key parameter for the economic evaluation of the hydrothermal process. This process would permit a selective fractionation of the feedstock as it is designed to solubilise a high percentage of hemicelluloses and phenolic compounds leaving a solid enriched in cellulose and lignin. The obtained results showed that the temperature that enabled the obtaining of the highest concentration of total oligosaccharides and the maximum total phenolic compounds was 180 °C.

## 1. Introduction

The current trend in the development of eco-sustainable processes is based on the valorization of biomass feedstock according to the biorefinery concept (Niglio et al., 2017). Throughout the last two decades, the manufacture of bioactive compounds from agro-industrial by-products has received great attention. This interest is due to their high content of phytochemicals which could contribute to the maintenance of the human health and to the reduction of chronic diseases (Grajek et al., 2005).

Functional food ingredients are winning an outstanding position in the food industry as apart from their nutritive value they could reduce the risk of chronic disease and benefit the host's health. Among the currently known functional ingredients, non-digestible oligosaccharides (NDOS's) hold a prominent place due to their prebiotic activity (Ayyappan et al., 2016).

The oligosaccharides obtained during the hydrolysis of the hemicelluloses could be used as functional ingredients in the food industry as they present a potential prebiotic effect as the NDOS's. These types of

compounds tend to present antioxidant properties as they are frequently bound to phenolic compounds (Gullón et al., 2017), conferring them several effects that promote the consumer's health. Apart from their prebiotic and antioxidant effects, the hemicellulosic oligosaccharides also present advantages over other NDOS's such as water solubility, lower sweetness and heat stability among others (Ayyappan et al., 2016).

The interesting properties of the hemicellulosic oligosaccharides have increased the researchers' interest in the obtaining of these bio-compounds from hemicellulose-containing agro-industrial by-products (Ayyappan et al., 2016). From the different agro-industrial by-products that could be used, chestnut shells have not been exploited yet. These residues are produced in large quantities by the chestnut industries as they represent approximately 10 – 15 wt. % of the chestnut. The chestnut shells obtained during the peeling process of the marron-glaçé and the chestnut flour production could be an inexpensive and renewable source of bio-compounds (Vella et al., 2017). Chestnut shells are lignocellulosic biomass composed mainly by cellulose, hemicelluloses and lignin (González-López et al., 2012).

Until now, the main applications of these undervalued residues have been the production of bio-fuels and antioxidants, but their suitability for the production of hemicellulosic oligosaccharides has not been addressed so far. Among the different technologies that could be used to obtain bioactive oligosaccharides, the hydrothermal processing has gained interest since it is an environmentally friendly process that allows the solubilisation of hemicelluloses yielding prebiotic oligosaccharides, leaving the structure of the remaining compounds unaltered (Rico et al., 2018).

The main goal of this work was to carry out the solubilisation of the hemicellulosic fraction from the chestnut shells by a hydrothermal treatment. The hydrothermal treatment was carried out in a non-isothermal regimen at different temperatures to evaluate its effect on the yield and on the composition of the liquid phase in oligosaccharides and phenolic compounds. These assays permitted the determination of the temperature that maximized the simultaneous production of oligosaccharides and phenolic compounds and minimized the generation of monosaccharides and degradation products. The experimental scheme followed throughout this work is shown in Figure 1.

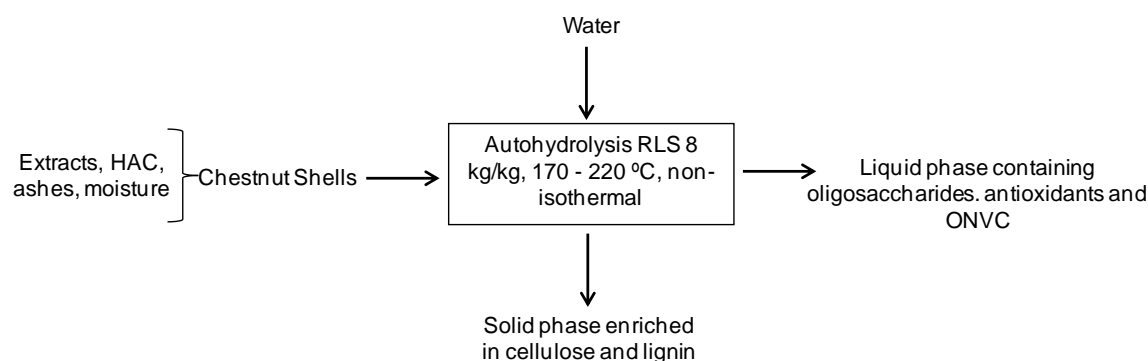


Figure 1: Scheme of the experimental procedure followed in this work

## 2. Materials and Methods

### 2.1 Raw material

The chestnut shells were supplied by a local chestnut processing plant (Cuevas & Cia S.A., San Cibrao das Viñas, Spain) after being obtained by a slow steam peeling process. The collected shells were air dried, milled and sieved to achieve a particle size smaller than 0.4 mm. The milled shells were homogenized in a single lot to avoid differences in the composition among aliquots and they were stored at room temperature until their use. An aliquot of the chestnut shells was subjected to a solvent extraction (TAPPI T204 cm-97) and then the chestnut shells without extractives were subjected to a quantitative acid hydrolysis with 72 % H<sub>2</sub>SO<sub>4</sub> for the determination of its chemical composition following standard methods (T-249-em-85) (more detailed information is found in Dávila et al., 2016). The ash content of the solid was determined by calcinating the solid at 525 °C (TAPPI T 211 om 02).

### 2.2 Hydrothermal treatment

Chestnut shells and water were mixed at the desired proportions in a 1.5 L stainless steel Parr reactor with a liquid to solid ratio of 8 kg/kg (oven dry basis). The hydrothermal treatments were carried out in a non-isothermal

regimen, so once reached the desired temperature (in the range 170 - 220 °C) the reactor was cooled down and the liquid and solid phases were separated by filtration.

The solid residues were washed with water, air-dried, quantified and subjected to moisture determination (method ISO 638) in order to calculate the amount of substrate dissolved during each treatment. The chemical composition of the solids was analyzed by subjecting them to the methodology described for the determination of the chemical composition of the raw material (see section 2.1). The obtained liquid phases were analyzed by High Performance Liquid Chromatography (HPLC) to determine their content in monosaccharides (glucose, xylose and arabinose), acetic and galacturonic acids and degradation products (hydroxymethylfurfural and furfural). The concentration of oligosaccharides and acetyl groups linked to them were determined by HPLC from the concentrations of monosaccharides and acetic acid present in liquors previously subjected to a quantitative post-hydrolysis (treatment with 4 % H<sub>2</sub>SO<sub>4</sub> at 121 °C for 30 min). The content of non-volatile compounds (NVC) of the liquid phases was determined by oven-drying them at 105 °C until constant weight. The content of other non-volatile compounds (ONVC) was calculated by difference between the NVC and the saccharides present in the liquors. All the determinations were made in triplicate. More detailed information about these analytical methods is found in Gullón et al. (2010).

### 2.3 Total phenolic content (TPC)

The liquid phases were evaluated for TPC using the Folin-Ciocalteu method (Singleton and Rossi, 1965). The obtained results were expressed as g of gallic acid equivalents (GAE)/L of liquors. The analysis was made in triplicate.

## 3. Results and discussion

### 3.1 Composition of raw material

The chemical composition of the chestnut shells used as raw material in this work is presented in Figure 2. As it can be observed, chestnut shells are mainly composed by lignin (44.6 %), followed by hemicelluloses (21.45 %) and glucan (20.6 %). The hemicellulose content was calculated considering the joint contribution of xylan (10.5 %), arabinosyl substituents (3 %), acetyl groups (2 %) and uronic acids (6 %). The hemicellulose content is in the same range as those reported for other agro industrial by-products such as sunflower seed shells (Gullón et al., 2009) or peanut shells (Rico et al., 2018) but it is lower than the one reported for rye straw (Gullón et al., 2010). The lignin content of the chestnut shells is remarkably higher than those observed for other raw materials such as vine shoots (Dávila et al., 2017), rice husk (Vegas et al., 2004) or corn stover (Buruiana et al., 2014), but it is similar to the one found in peanut shells (Rico et al., 2018) or hazelnut shells (Surek and Buyukkileci, 2017).

The high hemicellulosic content of the chestnut shells makes them a potential source for the production of hemicellulosic derived oligosaccharides.

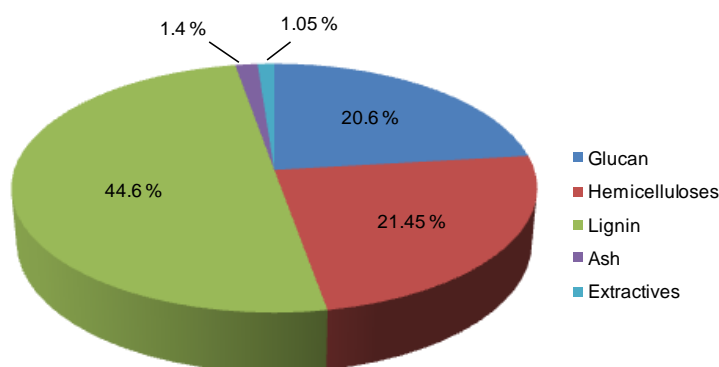


Figure 2: Composition of chestnut shells (g component/100 g chestnut shells in dry basis)

### 3.2 Effects of the temperature on the composition of liquid phases

A set of hydrothermal treatments was carried out in order to determine the optimal conditions to obtain the maximum oligosaccharide content with the minimum generation of monosaccharides and degradation products.

Hemicellulosic oligosaccharides from chestnut shells were obtained by subjecting them to a hydrothermal treatment performed at several temperatures in the interval of 170 - 220 °C under non-isothermal conditions. The hydrothermal treatment promotes reactions in the media that cause the solubilization of hemicelluloses and other fractions generating both non-volatile (NVC) and volatile compounds (VC) (Dávila et al., 2016). The variation of the operational conditions used in the non-isothermal hydrothermal treatment of xylan containing feedstock allows reaching maximal concentration of solubilized hemicelluloses (Vegas et al., 2004). The composition of the liquid phases is summarized in the Table 1. The monosaccharide and oligosaccharide content of the liquid phases is expressed as mass fraction (g compound/g NVC). The most abundant types of compounds present in liquid phases were hemicellulose-derived saccharides. As it can be seen from the Table 1, the temperature of the treatment influenced both the oligosaccharide and monosaccharide content of the liquid phase. The concentration of total oligosaccharides (considered as the joint contribution of xylooligosaccharides (XOS), arabinosyl substituents (AOS), acetyl groups (AcOS) and glucooligosaccharides (GlcOS)) first increased with the temperature until it reached its maximum at 180 °C (0.611 g/g NVC) then it started to decrease, due to decomposition reactions. From the different type of oligosaccharides, XOS were the main fraction, being them target compounds of the hydrothermal processing. The XOS content accounted its maximum mass fraction at 180 °C (0.263 g/g NVC) showing a similar behavior to the one observed for the total oligosaccharides. The second most abundant type of oligosaccharides was GlcOS, which reached a mass fraction of 0.252 g/g NVC at 180 °C, without a defined trend with the temperature. The saccharide substituents, which included arabinosyl units in oligomers (at mass fraction 0.062 g/g NVC), and the acetyl groups linked to the oligosaccharides (at mass fraction 0.034 g/g NVC) decreased with the increase of the temperature. These trends were also observed by Dávila et al. (2016) when they subjected vine shoots to hydrothermal treatments at different temperatures working in a non-isothermal regimen.

With regard to the monosaccharides present in the liquors the glucose and xylose content increased with the temperature (from 0.007 - 0.077 g/g NVC and 0.034 - 0.141 g/g NVC, respectively) whereas the arabinose content tended to decrease until it disappeared. But not only the monosaccharide content increased with the temperature, the same occurred with acetic acid generated by the cleavage of acetyl groups linked to hemicelluloses and oligomers, which increased from 0.011 g/g NVC at 170 °C to 0.289 g/g NVC at 220 °C.

It can be noted that at 180 °C (optimal temperature to reach the maximum concentration of oligosaccharides) decomposition products such as hydroxymethylfurfural and furfural were not detected. The other non-volatile compounds (ONVC) increased from 0.264 g ONVC/g NVC at 170 °C to 0.440 g ONVC/g NVC at 220 °C, with no defined dependence on temperature. Dávila et al. (2016) also reported high ONVC values of 0.45 g/g NVC in experiments performed with vine shoots at 215 °C.

Table 1: Effects caused by the hydrothermal treatment on the liquor composition

Component (g/g NVC) <sup>a</sup>	Temperature (°C)								
	170	175	180	185	190	200	210	215	220
Glucose	0.007	0.003	0.005	0.012	0.015	0.027	0.007	0.053	0.077
Xylose	0.034	0.013	0.018	0.052	0.066	0.129	0.133	0.119	0.141
Arabinose	0.035	0.054	0.061	0.090	0.089	0.067	0.000	0.000	0.000
Acetic acid	0.011	0.014	0.019	0.023	0.032	0.064	0.111	0.117	0.289
Total									
monosaccharides	0.087	0.084	0.103	0.177	0.337	0.287	0.251	0.289	0.507
GlcOS <sup>b</sup>	0.230	0.239	0.252	0.251	0.233	0.246	0.183	0.121	0.172
XOS <sup>c</sup>	0.163	0.226	0.263	0.249	0.237	0.212	0.065	0.043	0.078
AOS <sup>d</sup>	0.114	0.087	0.062	0.022	0.014	0.014	0.000	0.000	0.000
AcOS <sup>e</sup>	0.040	0.048	0.034	0.033	0.033	0.036	0.034	0.029	0.071
Total oligomers	0.547	0.600	0.611	0.555	0.517	0.505	0.282	0.193	0.243

<sup>a</sup>NVC: Non-Volatile Compounds

<sup>b</sup>GlcOS: glucooligosaccharides

<sup>c</sup>XOS: xylooligosaccharides

<sup>d</sup>AOS: arabinosyl substituents

<sup>e</sup>AcOS: acetyl groups

These results are in agreement with the trend observed by Buruiana et al. (2014) during the non-isothermal autohydrolysis treatment of corn stover; for this raw material, the maximum concentration of XOS was obtained at 205 °C. They also observed that the increase of the temperature resulted in a decrease of this concentration and an increase in the concentration of xylose. Several works have been carried out using different lignocellulosic feedstock achieving results that are in agreement with the ones shown here: e.g., Carvalheiro et al. (2004) used brewery's spent grain to obtain oligosaccharides and Yáñez et al. (2009) employed *Acacia dealbata* with the same aim.

### 3.3 Total phenolic content (TPC)

Another important aspect evaluated was the concentration of phenolic compounds present in the hydrothermal liquors obtained from the chestnut shells, as they could confer the liquors with antioxidant activity. The concentration of phenolic compounds was measured in terms of their TPC. This parameter achieved its maximum value at 180 °C (0.175 g GAE/g NVC or 4.69 g GAE/L liquors) coinciding with the maximum amount of hemicellulosic oligosaccharides. With the increase of the temperature the TPC decreased, showing the same trend that the one described for the total oligosaccharides. This demonstrated that the hydrothermal liquors of the chestnut shells could be a good source of phenolic compounds.

Several works have been reported about the antioxidant content of the liquors obtained by hydrothermal treatments. Conde et al. (2009) studied the effect of temperature during the liquid hot water processing of olive tree pruning residues (wood and leaves) on the total phenolic content. They reported a maximum of TPC of 2.29 g GAE/100 g pruning dry basis at 230 °C, a value considerably lower than the one obtained for the liquors from chestnut shells (3.88 g GAE/100 g chestnut shells dry basis). Conde et al. (2011) evaluated the TPC of chestnut burs liquors obtaining a higher TPC (4.4 g GAE/100 g chestnut burs dry basis at 240 °C) than the one obtained in this work. The difference with these two studies can be explained because at high temperatures (> 215 °C) the depolymerization of the lignin could take place (Erdocia et al., 2017) yielding phenolic compounds, and therefore, increasing the TPC value. On the other hand, under severe conditions of hydrothermal treatment also occurs the release of phenolic compounds linked to the solubilized oligosaccharides (Leschinsky et al., 2008). More recently, Rico et al. (2018) evaluated the TPC of liquors obtained in the hydrothermal treatment of peanut shells and found values close to 1.63 g GAE/100 g peanut shells. Vázquez et al. (2008) carried out conventional extractions of chestnut shells using several solvents to obtain antioxidant compounds; the higher TPC was obtained with methanol/water (80/20) at boiling point (15.3 g GAE/100 g chestnut shells dry basis). This difference in the results with those obtained in the present study could be attributed to the higher susceptibility of some of the phenolic compounds from the chestnut shells at the temperatures used for the hydrothermal treatments that could result in its degradation. Moreover, based on the results reported by other authors and the obtained in this work, it is clear that the technology employed to extract the phenolic compounds influences the TPC.

## 4. Conclusions

The chestnut shell, a natural and renewable undervalued by-product, is a potential feedstock to obtain hemicellulosic oligosaccharides and phenolic compounds by environmentally friendly technologies. In this work, the suitability of hydrothermal treatments to solubilize hemicelluloses and phenolic compounds from chestnut shells was demonstrated. The optimal temperature for the achievement of the goal of this study was 180 °C, since the maximum concentration of oligosaccharides and phenolic compounds was obtained. At this temperature, 52 % of the hemicelluloses (xylan and arabinosyl and acetyl substituents) present in the raw material were solubilized as oligosaccharides (this corresponded to a mass fraction of 0.372 g/g NVC) and the 15 % as monosaccharides. Therefore, chestnut shell could be a suitable feedstock to manufacture nutraceutical compounds with rising applications as functional ingredients in the food industry that can cover the consumers' demands.

### Acknowledgement

This work was funded by the Spanish Ministry of Economy and Competitiveness (MINECO, CTQ2014-58879-JIN and CTQ2016-78689-R) and by the "Xunta de Galicia" (project ref. ED431F 2016/001). The authors B. Gullón, G. Eibes M. Moreira belong to the Galician Competitive Research Group GRC 2013-032 and to the CRETUS Strategic Partnership (AGRUP2015/02). All these programmes are co-funded by FEDER (EU). The authors I. Davila, J. Labidi and P. Gullón belong to the Basque Competitive Research Group IT1008-16 funded by the Department of Education of the Basque Government. B. Gullón and P. Gullón would like to express her gratitude to MINECO for financial support (Grant references IJCI-2015-25305 and IJCI-2015-25304, respectively). I. Dávila would like to thank the Agriculture, Fishing and Food Department of the Basque Government (scholarship of young researchers training).

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