DOI: https://doi.org/10.1016/j.indcrop.2011.08.017

ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS OBTAINED BY AUTOHYDROLYSIS OF CORN RESIDUES

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Abstract

This study aimed to evaluate the effect of autohydrolysis temperature of corn residues in the antioxidant activity of the phenolic compounds extracted from the liquid phase. The treatments were carried out at 160, 180, 190 and 200 ºC for 30 min in a pressurized batch reactor. In order to exploit all extracted compounds in the autohydrolysis liquor and improve the purity of solubilized hemicelluloses, two different methods for phenolic compounds extraction from the liquid phase were investigated. For that purpose, solvent extraction with ethyl acetate and acidic precipitation were performed for phenolic compounds recovery. These methods have been compared in terms of extraction yield, physicochemical properties of obtained polyphenols (characterization by Fourier Transform Infrared Spectroscopy, Thermogravimetric Analysis and Gel Permeation Chromatography), total phenolic content, total antioxidant capacity and Trolox equivalent antioxidant capacity (TEAC) values, measured in DPPH (2,2-diphenyl-1-picrylhydrazyl) test system. The maximum phenolic contents ranged from 6.04 mg GAE/100 mg extract in acidic precipitated samples to 16.45 mg GAE/100 mg extract in ethyl acetate soluble fractions. The results indicated that the ethyl acetate fractions possessed the highest antioxidant

activity, reaching after 30-60 minutes the same capacity reported for the reference synthetic antioxidants (Trolox).

Keywords: Corn residues, hydrolysis, antioxidant capacity; precipitation, extraction

1. Introduction

The lignocellulosic waste materials represent an abundant source of chemicals and polymeric materials, their renewable origin and qualities found in their components convert them in a very promising alternative resource. Applying adequate treatments to fractionate these materials, such as hydrothermal treatments (Díaz et al., 2010; Sasaki et al., 2003), soda, organosolv (García et al., 2011), among others, can be obtained a variety of high value added products with important applications in industrial scale. Nowadays, there are many studies focused on the extraction of antioxidant compounds from agricultural wastes.

Reactive oxygen, nitrogen, and sulphide species are understood to likely cause molecular deterioration in food, synthetic materials and living cells. These reactive agents, influenced by the oxygen and ultraviolet light, provide high amount of free radicals and therefore they can cause damages in the systems (Parejo et al., 2003). The use of antiradical species, called antioxidants, could prevent these reactions. The antioxidants inhibited the initiation or propagation of oxidative chain reactions, donating hydrogen atoms and neutralizing free radicals. They act by one or more of the following mechanisms: reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen (Tachakittirungrod et al., 2007).

The antioxidant capacity of lignin depends on many factors such as the type of raw material, extraction method, the treatments conditions used during its isolation, its concentrations and the oxidation conditions used. García et al. (2010) studied the

characterization of lignin obtained from black liquor resulting from different fractionation processes (soda, organosolv and autohydrolysis treatments) of *Miscanthus Sinensis*. In that work, the lignin obtained by organosolv fractionation process presented the highest antiradical activity followed by autohydrolysis and alkaline lignins. The use of ultrafiltration membranes also were studied to improve the antioxidant capacity due to narrow molecular weight distribution and lower carbohydrate contamination obtained in lignin samples. Vazquez et al. (2008) have obtained extracts from chestnut shell and eucalyptus bark using organic solvents of different polarity. They concluded that the extraction yield, the antioxidant activity and the total phenols content were greater in chestnut shell than in eucalyptus bark, maybe due to the differences in their chemical composition and amount of phenolic compounds found in the extracts.

In this study, corn waste, used as raw material was subject to autohydrolysis treatment, an environmental-friendly process which uses water as only reagent, as first step of a biomass fractionation. Many experiments have focused on the use of corn stover due to the high content of hemicelluloses, specially used for ethanol production mainly by dilute acid, hot water, steam explosion and enzymes pretreatments (Kazi et al., 2010). Only a small part of this raw material is used for producing chemicals such as furfural, additives for pulp and paper and for composite elaboration (Kadam and McMillan, 2003). In this study, different autohydrolysis liquors were obtained from corn waste using different hydrolysis temperatures: 160 ºC, 180 ºC, 190 ºC and 200 ºC. The obtained liquors, rich in soluble sugars, could be used in fermentation media to obtain high added value product such as xylitol, bioethanol etc. Hydrolysis is fast and easy to perform, however, is hampered by non-selectivity and by-product formation, decreasing the yield of fermentability of enzymes by weak acids, furan and phenolic inhibitory products

(Palmqvist and Hagerdal, 2000). For that purpose, it is necessary to remove the acid soluble phenolic compounds from the liquid phase (the main principal inhibitors of microorganism) to improve the fermentability of the sugars.

In this context, many studies have been developed for phenolic compounds recovery from a wide range of raw material hydrolysates (Palmqvist and Hagerdal, 2000). For that purpose, many extraction method were used, such as activate charcoal adsorption, ion-exchange resin (Villarreal et al., 2006), being the solvent extraction by ethyl acetate extensively used in previous works (Singh et al., 2007; Martha-Estrella et al., 2008; Li et al., 2007; Garrote et al., 2008). In this study, an alternative process for phenolic compound recovery, by acidic precipitation, was performed and compared with ethyl acetate liquid extraction. The antioxidant property of the precipitated lignin by this method has not been extensively discussed.

2. Materials and methods

2.1 Raw material conditioning and characterization

The corn stalks used in the experiments was kindly supplied by the company Straw Pulping Engineering (SPE), S.L. (Zaragoza, Spain). The residue was conditioned up to constant moisture, and then it was ground in a hammer and sieved to obtain the 4–6 mm size fraction.

Its chemical composition was analyzed following standard methods and procedures found in the literature: ash (TAPPI T211 om-93); ethanol–toluene extractives (TAPPI T204 cm-97); lignin (TAPPI T222 om-98); α-holocellulose (Wise et al., 1946), α-cellulose (Rowell, 1983) and hemicelluloses content.

2.2 Autohydrolysis processes

Autohydrolysis treatments were carried out in 4 L batch reactor (Autoclave Engineers EL0723Iberfluid) equipped with electronic control unit for pressure and temperature control, under the following conditions: 160, 180, 190 and 200 ºC with a solid: liquid ratio 1:20 (w/w) for 30 min after reaching the desired temperature. Once the reaction is ended, the reactor is cooled and then the hydrolysis liquors were separated from the solid phase by filtration.

2.3 Liquors characterization

In order the determine the purity of obtained autohydrolysis liquors and sugars content, the liquors have been characterized in terms of total dissolved solids (TDS), inorganic matter content (IM), organic content (OM), concentration of monomeric sugars and acetic acid content. TDS were measured after keeping a weighed sample at 100 ºC until constant weight (NREL LAP-012). IM was determined after combustion of the liquor at 525 ºC (TAPPI T211 om 93) and OM was defined as the difference between TDS and IM.

2.4 Phenolic compounds extraction

To recover phenolic compounds, two extraction processes from autohydrolysis liquors have been studied, liquid-liquid extraction by ethyl acetate and acidic precipitation, respectively. These methods were compared in terms of extraction yield, purity of the obtained samples, total phenols content and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of obtained samples. The synthetic antioxidant (Trolox) was tested at same conditions.

Fig.1 represents the different steps followed in this study to extract the phenolic components from the autohydrolysis liquors. The solvent extraction with ethyl acetate has been applied to autohydrolysis liquor using a liquid: solvent ratio 1:3 (v/v), at room temperature for 24 h. The organic phase was vacuum evaporated to remove the solvent and the extract was oven dried at 50 ºC. In the case of acidic precipitation, the pH of the liquor was adjusted to pH = 2 with 96% sulphuric acid and the precipitated lignin was filtrated and oven dried at same conditions.

2.5 Sugars content

The hemicelluloses sugars concentration in the liquid phase before and after different phenolic compound extraction was quantitatively determined. The characterization of total sugars monomers was carried out in a high performance liquid chromatography (HPLC) Jasco LC Net II /ADC (with column oven and quaternary gradient pump) equipped with a refractive index detector and a photodiode array detector. A Phenomenex Rezex ROA HPLC column (300mm x 7.8mm) with precolumn (Phenomenex Security Guard holder with carbo-H+ cartridges) was used for the experiments. Hydrolysis liquors were subjected to post-hydrolysis process for oligosaccharide monomeric sugars quantification. Therefore, the total sugars monomers, were constituted by the monomers contained in the autohydrolysis liquor's and those obtained from the depolimerisation of olygomers in post-hydrolysis process. The selection of the post-hydrolysis operating conditions was based on the maximum depolymerisation of hemicelluloses with minimum loss of sugars, being the optimum parameters obtained for this study the utilization of sulphuric acid (5% w/w) at 100 ºC for 60 min with a ratio of liquor: acid of 4:1 (v/v). 0.005 N H_2SO_4 prepared with 100% deionised and degassed water was used as mobile phase (0.35 mL/min flow, 40 ºC

and injection volume 20 μL). High purity D(+) glucose, D(+) xylose, D(-) arabinose and acetic acid were used for calibration curve.

2.6 Physicochemical characterization of extracts

Fourier Transform Infrared Spectroscopy (FTIR) measurements were performed in a Perkin-Elmer 16PC instrument by direct transmittance using KBr pellet technique. Each spectrum was recorded over 20 scans, in the range from 4000 cm^{-1} to 600 cm^{-1} with a resolution of 4 cm^{-1}. KBr was previously oven-dried to avoid interferences due to the presence of water, and background spectra were collected before every sampling.

Thermal degradation of the samples was studied by Thermogravimetric Analysis (TGA) which was carried out in a TGA/SDTA RSI analyzer of Mettler Toledo. The samples of ~ 5 mg were heated of 25 ºC up to 800 ºC at a rate of 10 ºC/min, using a constant nitrogen flow as inert atmosphere during the experiment.

Gel Permeation Chromatography (GPC) was used to determinate the average molecular weight of the compounds. Polyphenols samples were examined through THF-eluted GPC technique, using a Perkin-Elmer with an interface (PE Series 900). For that purpose, all samples were acetylated (García et al., 2009) in order to enhance their solubility in THF. Three Waters Styragel columns (HR 1, HR 2 and HR 3) ranging from 100 to 5×10^5 and a refractive index detector (Series 200) were employed, with a flow rate of 1 mL/min. Polystyrene standards were used for the elaboration of the calibration curve.

2.7 Total phenols content

Total phenolic content was analyzed using the Folin–Ciocalteu method described by Singleton and Rossi (1965). A volume of 2.5 mL Folin–Ciocalteu reactive was diluted with

water (1:10, v/v), and mixed with 2 mL of 75 g/L aqueous solution of sodium carbonate. The resultant solution was added to 0.5 mL of an aqueous solution of sample. The mixture was kept for 5 min at 50 ºC before measuring the absorbance at 760 nm. A standard curve with gallic acid solutions was used to express the concentrations of phenolics as mg Gallic acid equivalents (GAE)/100mg of samples (on a dry basis). Moreover, the molar concentration in GAE of the samples was estimated.

2.8 DPPH antioxidant capacity

The antioxidant activity was determined by spectrophotometric method based on the use of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) using a spectrophotometer Jasco V-630. This test was developed by Brand-Williams et al. (1995) and modified by Dizhbite et al. (2004). Extracted samples, dissolved in dioxane/water (90:10, v/v) at a concentration of 1 g/L, the 0.1 mL were mixed with 3.9 mL of a 6×10^{-5} mol/L DPPH solution, and the absorbance at 518 nm of the mixture was measured at different times, 0 min, 30 min, 60 min and 90 min. The absorbance could not be measured a longer time due to the peak displacement at lower wavelengths. A reference commercial synthetic antioxidant (Trolox) was tested under the same conditions. The results were expressed as percentage reduction absorbance shown by the samples respect to the DPPH solution. Similarly, the antioxidant capacity of the samples was calculated in equivalents of Trolox (TEAC). A calibration curve of different concentration of Trolox, ranged from 0.1 g/L to 1.5 g/L was performed.

3. Results and discussion

3.1 Composition of corn residues

Raw material presented the following chemical composition (% on an oven-dry weight basis): ash content: 9.27 ± 0.20; ethanol-benzene extractives: 1.08 ± 0.10; Klason lignin: 17.18 ± 2.40; holocellulose: 70.79 ± 3.50; α-cellulose: 49.22 ± 2.70 and hemicelluloses: 25.57 ± 0.70. Comparing the hemicellulose percentages found in other lignocellulosic residues such as barley straw (27.00%) (Reddy and Yang, 2005), rice straw (25.10%), wheat straw (33.30%) and corn stover (29.60%) (Wartelle and Marshall, 2006), the percentage found suggested the suitability of this material as source for sugars extraction. In relation to the lignin and cellulose content, these values were in the range found in other agricultural wastes.

3.2 Autohydrolysis liquors characterization

Table 1 represents the content in IM, OM and TDS for studied autohydrolysis liquors. The liquor with higher content of OM was obtained at 180 ºC and 30 min with 1.30% OM followed by the treatment at 190 ºC and 30 min with 1.29% OM. The minimum OM was reached under mild conditions, at 160 ºC and 30 min about 0.74% OM, respectively. On the other hand, the maximum solubilisation of IM (considered as impurities) was achieved at 190 ºC with 0.64%, whereas the minimum value was obtained at 160 ºC with 0.31% of IM.

3.3 Phenolic compounds extraction yield and sugars content

As observed in Table 1, the higher extraction yield was obtained by liquid-liquid extraction process; approximately the yield obtained by ethyl acetate extraction was three times higher than obtained by acid precipitation. These high yields are influenced by the preferential solubilization in this solvent of small molecules constituted by phenolic and non-phenolic compounds (such as monomeric sugars etc.). In addition, the autohydrolysis

temperature affects hardly the extraction yield obtained by acidic precipitation. The precipitation yield decreased from values around 1.38 g/100 g dry matter at 160 ºC to a value of 0.21 g/100 g dry matter at 200 ºC. The precipitation process depends partially on the molecular weight of the samples. The high temperatures may cause compounds fractionation, providing small molecules in the liquor (being difficult its precipitation). Therefore, it is expected to obtain lower amount of precipitate at high temperature. Following the same argument, in the case of ethyl acetate extraction, the maximum extraction yield should be obtained at 200 ºC. Although it was one of the highest yield values, the maximum yield was obtained at 160 ºC, with 3.56 g/100g dry matter. The high temperatures can promote the depolimerisation of molecules but also their degradation as in the case of monomeric sugars present in the hydrolysate.

The total amount of monomeric sugars obtained in aqueous phases, is presented in Fig.2, before and after each phenolic extraction. As can be observed, the monomeric sugars were increased after each phenolic extractions process. These experimental results could be ~~explained by~~ due to the better conditions formed to fractionate the oligomeric sugars in absence of phenolic compounds in post-hydrolysis process. The reagents used for phenolic compounds extraction could help to improve the depolymerisation medium of hemicelluloses. The predominant monomer present in all liquors was xylose, with small quantities of glucose and arabinose.

3.4 Physicochemical characterization

The Fig.3a and b (the original and expanded spectra) shows FTIR bands of precipitated lignins from autohydrolysis liquors 160 ºC, 180 ºC, 190 ºC and 200 ºC, named

as L_{160} , L_{180}, L_{190}, and L_{200}, respectively. The Fig.4a and b shows FTIR spectra of ethyl acetate extracts from autohydrolysis liquors 160 ºC, 180 ºC, 190 ºC and 200 ºC, named as E_{160} , E_{180}, E_{190}, and E_{200}, respectively.

As it can be observed, the bands found at 3400, 2920-2850 and 1458 cm^{-1}, which indicate the OH stretching and CH bond deformation of methyl and methylene groups, were presented in all samples. The band found at 1704 cm^{-1} is referred to C=O stretching of carboxylic group, whereas 1370 cm^{-1} is corresponded to bending vibration of OH of phenolic groups. The higher intensity of bands found in ethyl acetate extracts at 1600 cm^{-1} and 1512 cm^{-1} referred to phenylpropane aromatic skeletal vibration, indicates higher content of this functional group in these samples. The characteristic band of C=O stretching of syringyl ring and guaicyl ring was also observed at 1330 cm^{-1} and 1220 cm^{-1}. On the other hand, the band found at 1165 cm^{-1} was represented the OH stretching of primary alcohol. Finally, the bands found at 1040 cm^{-1} and 835 cm^{-1} indicated the presence of xylans and the domain of β-glycosidic bonds between sugars, being more intense in precipitated lignins. It was concluded that all samples showed the most characteristics bands of lignin, being more intense in ethyl acetate extracts.

The TGA results of precipitated lignins and ethyl acetate extracts are summarized in Fig.5 and Fig.6, respectively. The thermogravimetric analysis showed different steps during samples degradation. In the case of L_{160}, L_{180} and L_{190} precipitated lignins (Fig.5a and its derivative in Fig.5b) appeared two maximum weight loss rates; slightly defined peak at 250 ºC, maybe to carbohydrate presence and second peak at 350 ºC, referred to presence of lignin molecule fraction. Especially in the lignin obtained at 200 ºC, the fist peak

disappeared to become a broad peak around 400 ºC. In the case of extracts, showed also two maximum loss rate but a lower temperatures, the first principal peak was at 180-200 ºC could be to carbohydrate presence and the second at 325 ºC, due to the presence of lower molecule fraction of lignin.

Moreover, the GPC chromatograms obtained for all samples showed two main peaks corresponding to two different molecular weight components. The Table.2 shows only the main component molecular weight and total phenolic content of the samples.

As it could be observed, the lignin precipitates were characterized by higher molecular weight species than ethyl acetate extracts. For the precipitated lignin samples, the molecular weight of predominant component varies between 896 and 1142. This fraction in L_{160}, L_{180} and L_{190} lignins presented about 90% of total precipitated solids. In the case of lignin obtained at 200 ºC this portion was reduced to 53%. The use of high temperature in hydrolysis process causes more amounts of smaller molecular weight molecules. Nevertheless, in ethyl acetate extracts the main component molecular weight varies from 775 at 190 ºC to 850 at 160 ºC, constituting about 66% of total molecules present. The remaining percentages in precipitated lignins and ethyl acetate extracts were formed by 300-400 molecular weight species. It could be concluded that the ethyl acetate extracts were constituted mainly by small molecules. These results are in agreement with those obtained by TGA measurements due to the lower degradation temperature found in these samples. On the other hand, the GAE values obtained in ethyl acetate extraction compounds were much higher than obtained in precipitated lignins. The maximum phenolic contents ranged from 6.04 mg GAE/100mg extract in L_{190} to 16.45 mg GAE/100mg in E_{200}.

As it could be observed, the total phenolic compounds in the samples increased progressively with temperatures and decreased with higher molecular weight.

3.5 Antioxidant capacity

As shown in Fig.7a and b, the 50% of absorbance reduction capacity at t = 0 min was reached only with commercial antioxidant, whereas for the other samples (except for lignin obtained at 200 ºC) this capacity was achieved between 30 and 60 minutes. These results could be explained by the better reduction capacities demonstrated by samples with the time (except for L_{160} which reduction capacity decreases after 60 minutes).

As it can be observed in Fig.7, the autohydrolysis temperature affects more hardly the reduction capacity of the samples at t = 0 min, being L_{190} which has highest capacity (30%). This difference between reduction capacities decreases with the time showing a slight effect of the process temperature. On the other hand, maximum reduction capacity in ethyl acetate extracts, about 82%, was reached after 90 minutes from 200 ºC autohydrolysis liquor, while the lignin obtained from the same liquor at same condition, demonstrated only 47% of capacity. The E_{200} sample presents high content of phenols (shown by GAE values) and high antioxidant capacity, while L_{200} sample has completely different properties. These result showed clearly the influence of extraction method had in the antioxidant capacity of samples obtained from the same hydrolysate.

The reduction capacity observed by Cruz et al. (1999) of ethyl acetate extracted phenols from eucalyptus hydrolysate was 64% of the value found respect to the synthetic antioxidant BHT (at 0.4 g/L of concentration). The antioxidant activity of phenols obtained from corn tassels in DPPH system by Mohsen and Ammar (2009), ranged from 83.0% to

85.2%. It could be concluded that the reduction capacity of obtained samples in this study, were in the range found in many plant extracts (Kähkönen et al., 1999).

Respect to the obtained GAE values and the reduction capacity of the samples, it could be observed that L_{190} sample, although it presents lower values of GAE than E_{190} extract, 6.04-13.15 respectively, it achieves the same reduction capacity. This result could indicate that its phenolic structure allows it to reach a similar antioxidant capacity than E_{190} extract. This observation that the antioxidant capacity cannot be predicted only by total phenol content of the samples was observed in other studies (Kähkönen et al., 1999).

The molar Gallic acid equivalent values (GAE) obtained per mol of sample and the TEAC values (defined as the concentration of Trolox solution with equivalent antioxidant potential of 1g/L concentration of the sample and their molar concentration), have been summarized in Table 3.

As in previous case, the E_{200} sample (with 0.69 mol GAE/mol sample), demonstrated higher values of molar TEAC than L_{200} (with 0.27 mol GAE/mol sample). The obtained molar TEAC values range from 0.96 to 5.97 for E_{200} and from 0.08 to 3.86 for L_{200}, respectively. However, the molar TEAC values obtained from L_{180} after 60 min, corresponding to 6.14 value, was slightly higher than obtained by E_{180} at same time, E_{180} = 5.73. For these samples, the reduction capacity and therefore the mM Trolox equivalents shows an opposite tendency, corresponding to L_{180} = 58.90% reduction capacity and 5.00 mM Trolox equivalent versus E_{180} = 79.76% reduction capacity and 7.30 mM Trolox equivalents. This result is influenced by the molecular weight presented by L_{180}, providing higher values of TEAC in molar concentration. As can be observed in Table 3, the mM Trolox equivalent values vary from 0.09 to 7.60. The antioxidant activity obtained by Conde et al. (2009) from hydrothermal treatments of olive tree pruning phenols, ranged

from 2.05 to 2.17 mmol Trolox equivalents. These results indicate the high capacity of obtained samples in this study.

In general, it is observed that the ethyl acetate extracts had higher antioxidant capacity over time than lignins obtained by acidic precipitation, influenced mostly by the higher total phenols content of the sample.

It can be concluded that ethyl acetate extraction method has allowed obtaining phenols with smaller molecular weight molecules, less polydisperse, with high phenolic content and better antioxidant capacity. This fact could be due to the affinity demonstrated by this solvent to extract small molecular weight phenolic components and therefore to obtain higher extraction yield than acidic precipitation. Whereas, acidic precipitation allowed obtaining more polydisperse lignins due to the fact that the precipitation process is not as selective as liquid-liquid extraction, providing lower yield.

4. Conclusions

The study has shown that phenolic compound recovery from different autohydrolysis liquor by ethyl acetate method, have better antioxidant capacity than precipitated lignin. The preferential solubilisation of this solvent to small molecules, provides extracts with lower molecular weight than precipitated lignin, higher extraction yield, higher total phenol content and better radical scavenger capacity. In general, the antioxidant activity of samples was increased with the time and reached the same capacity obtained with the synthetic antioxidants after 30 or 60 min. It could be concluded that the autohydrolysis temperature used in this work, affected more strongly the antioxidant capacity of obtained polyphenols at t = 0 min, whereas over time, the difference between

capacities decreases. However, the used extraction method affects more significantly the antioxidant capacity of the samples.

Acknowledgements

Authors would like to thank the Department of Education, Universities and Investigation of the Basque Government (pre-doctoral scholarship programs of young researchers training) and the Spanish Ministry of the Environment and Rural and Marine Affairs for supporting financially this work.

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Table captions

Table.1 Inorganic matter IM (%), organic matter OM (%), total dissolve solids TDS (%) of autohydrolysis liquors and extraction yield respect to the 100 g oven-dry substrate.

Table.2 Average molecular weight of predominant species, polydispersity and total phenolic content of the samples.

Table.3 Molar Gallic acid equivalent and TEAC (Trolox equivalent antioxidant capacity) of the samples.

Table 1

	Autohydrolysis temperature (ºC)			
	160	180	190	200
% Total dissolve solids (TDS)	1.04 ± 0.10	1.65 ± 0.08	1.94 ± 0.12	1.29 ± 0.09
% Inorganic matter (IM)	0.31 ± 0.02	0.35 ± 0.01	0.64 ± 0.03	0.36 ± 0.02
% Organic matter (OM)	0.74 ± 0.05	1.30 ± 0.04	1.29 ± 0.07	0.92 ± 0.06
Precipitated lignins (g/100 g dry matter)	1.38 ± 0.20	0.98 ± 0.10	0.64 ± 0.05	0.21 ± 0.01
Ethyl acetate extracts (g/100 g dry matter)	3.56 ± 0.25	2.65 ± 0.30	1.89 ± 0.15	3.15 ± 0.10

Table 2

Hydrolysis liquor	160 ºC			180 ºC			190 ºC			200 ºC		
	M_w[a]	IP[b]	GAE[c]	M_w	IP	GAE	M_w	IP	GAE	M_w	IP	GAE
Lignin precipitates	946	1.10	5.52	1142	1.40	5.90	974	1.30	6.04	896	1.09	5.58
Ethyl acetate extracts	850	1.25	4.85	787	1.08	9.33	775	1.12	13.15	787	1.06	16.45

[a] Weight-average molecular weight.
[b] Polydispersity (M_w/M_n).
[c] mg Gallic acid equivalent (GAE)/100 mg of extracts.

Table 3

Hydrolysis liquor		GAE[a]	t = 0 min		t = 30 min		t = 60 min		t = 90 min	
			TEAC[b]	TEAC[c]	TEAC[b]	TEAC[c]	TEAC[b]	TEAC[c]	TEAC[b]	TEAC[c]
Lignin precipitates	160 ºC	0.28	3.00	2.74	5.00	4.68	6.00	6.02	4.00	3.44
	180 ºC	0.36	1.00	1.71	4.00	4.80	5.00	6.14	6.00	6.98
	190 ºC	0.31	0.60	0.61	4.80	4.70	6.20	6.00	6.80	6.58
	200 ºC	0.27	0.09	0.08	2.40	2.13	3.50	3.13	4.30	3.86
Ethyl acetate extract	160 ºC	0.22	0.90	0.77	4.20	3.56	5.80	4.90	6.50	5.51
	180 ºC	0.39	2.40	1.90	6.10	4.82	7.30	5.73	7.50	5.90
	190 ºC	0.54	0.90	0.69	4.60	3.58	5.90	4.57	6.50	5.07
	200 ºC	0.69	1.20	0.96	5.50	4.34	6.80	5.37	7.60	5.97

[a] mol Gallic acid equivalent/mol sample.
[b] mM Trolox equivalent obtained from 1g/L sample concentration
[c] mol Trolox equivalent /mol sample.

Figure captions:

Fig.1 Scheme of different extraction processes employed for phenolic compounds recovery.

Fig.2 Total sugars monomer quantifications before and after each phenolic extraction process.

Fig.3 Original Infrared spectrum (a) and its expanded spectra (b) of precipitated lignins from autohydrolysis liquors: lignin from 160 ºC liquor (L_{160}), lignin from 180 ºC liquor (L_{180}), lignin from 190 ºC liquor (L_{190}), and lignin from 200 ºC liquor (L_{200}).

Fig.4 Original Infrared spectrum (a) and expanded spectra (b) of ethyl acetate extracts from autohydrolysis liquors: extract from 160 ºC liquor (E_{160}), extract from 180 ºC liquor (E_{180}), extract from 190 ºC (E_{190}) and extract from 200 ºC liquor (E_{200}).

Fig.5 Thermogravimetric analysis TG (a) and its derivative DTG curves (b), of precipitated lignins from autohydrolysis liquors: lignin from 160 ºC liquor (L_{160}), lignin from 180 ºC liquor (L_{180}), lignin from 190 ºC liquor (L_{190}), and lignin from 200 ºC liquor (L_{200}).

Fig.6 Thermogravimetric analysis TG (a) and its derivative DTG curves (b), of ethyl acetate extracts from autohydrolysis liquors: extract from 160 ºC liquor (E_{160}), extract from 180 ºC liquor (E_{180}), extract from 190 ºC (E_{190}) and extract from 200 ºC liquor (E_{200}).

Fig.7 Antioxidant capacity expressed as % of reduction absorbance respect to DPPH solution of lignins (a) and ethyl acetate extracts (b).

Figure 1

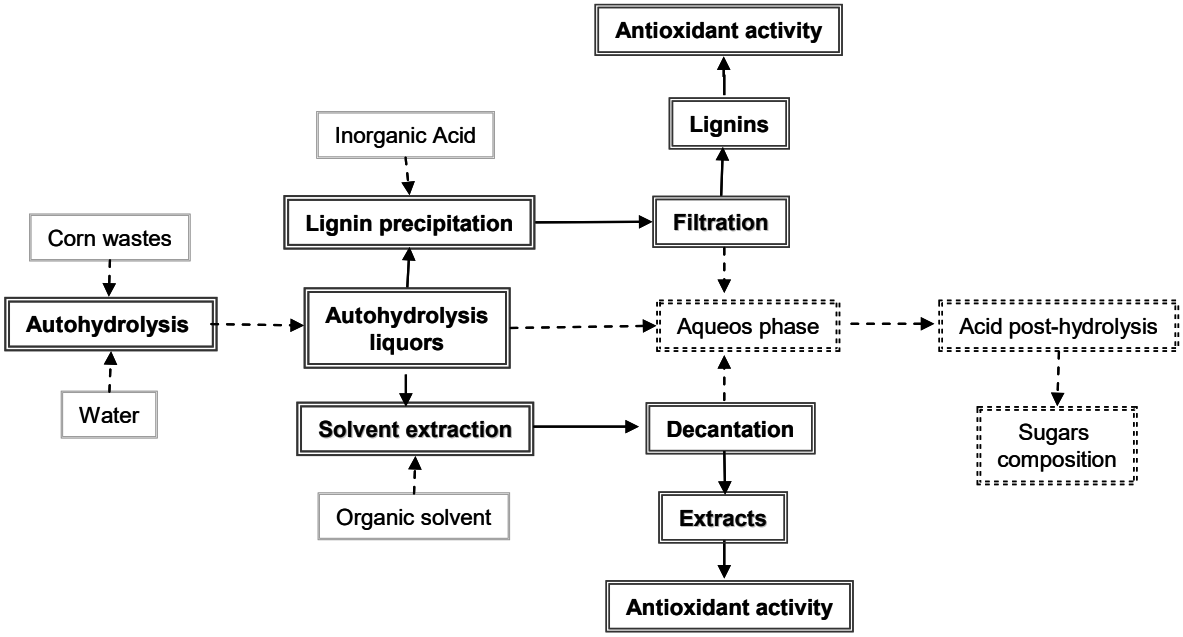
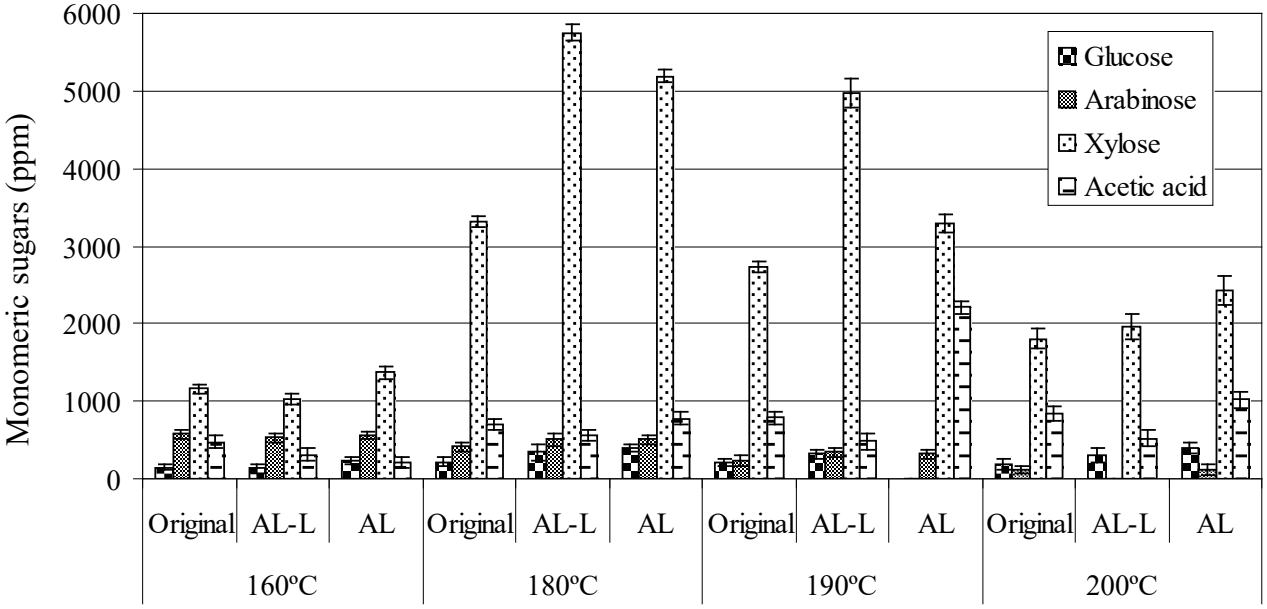
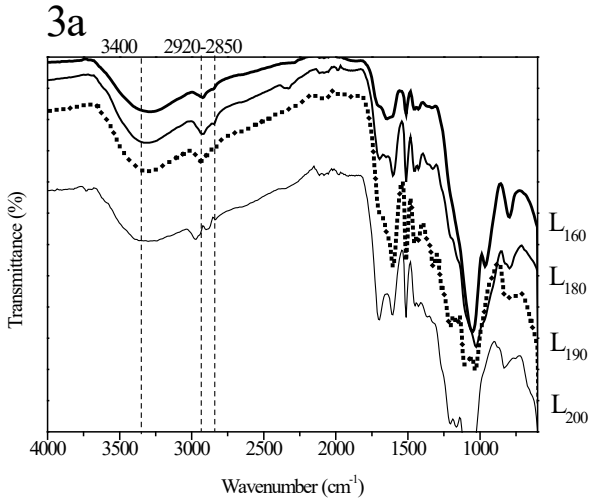

Antioxidant activity
Lignins
Inorganic Acid
Lignin precipitation
Filtration
Corn wastes
Autohydrolysis
Autohydrolysis liquors
Aqueos phase
Acid post-hydrolysis
Water
Solvent extraction
Decantation
Sugars composition
Organic solvent
Extracts
Antioxidant activity

Figure 2



Original: original hydrolysis liquor
AL-L: sugars after ethyl acetate extraction
AL: sugars after lignin precipitation

Figure 3


3a
3400
2920-2850
Transmittance (%)
L160
L180
L190
L200
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

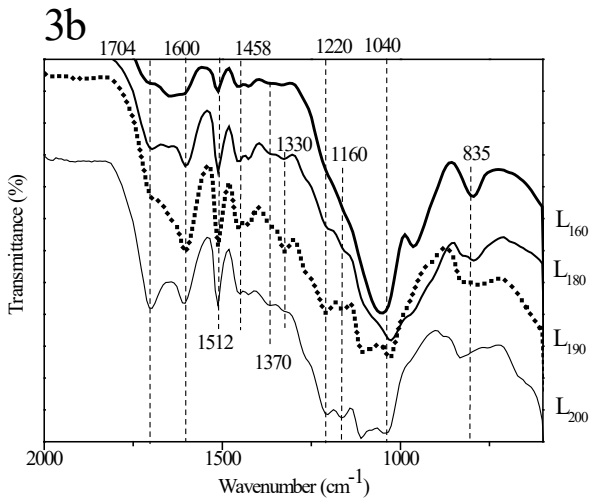

3b
1704
1600
1458
1220
1040
1330
1160
835
1512
1370
Transmittance (%)
L160
L180
L190
L200
2000
1500
1000
Wavenumber (cm-1)

Figure 4

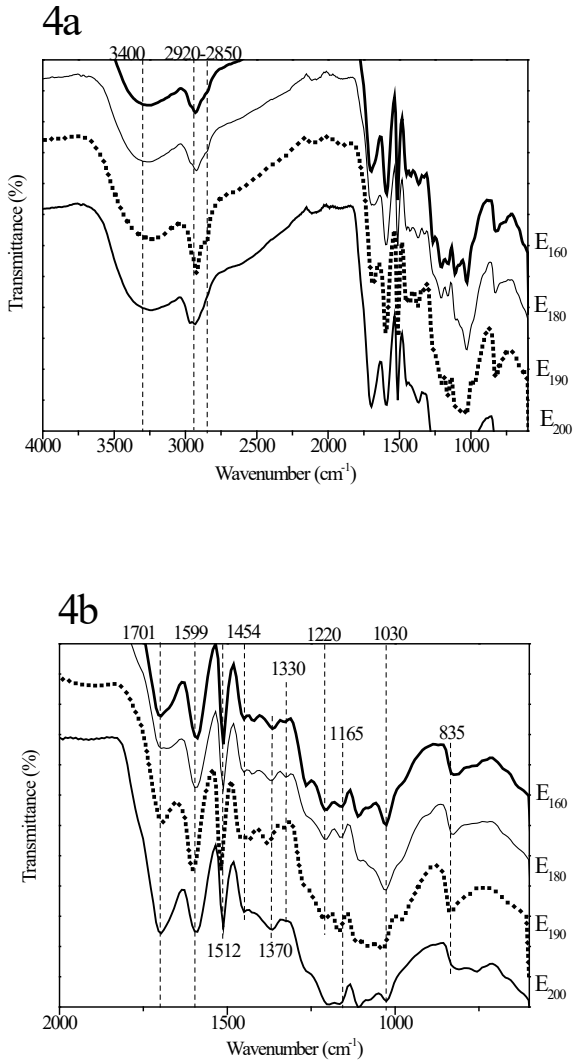
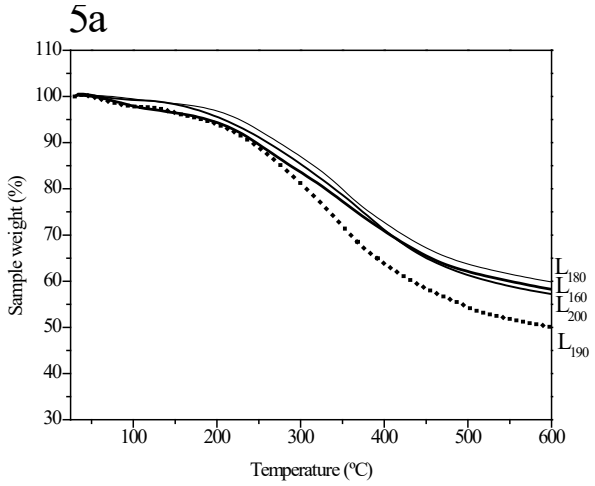

4a
3400
2920-2850
Transmittance (%)
E160
E180
E190
E200
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)
4b
1701
1599
1454
1220
1030
1330
1165
835
1512
1370
Transmittance (%)
E160
E180
E190
E200
2000
1500
1000
Wavenumber (cm-1)

Figure 5


5a
Sample weight (%)
Temperature (ºC)
L180
L160
L200
L190

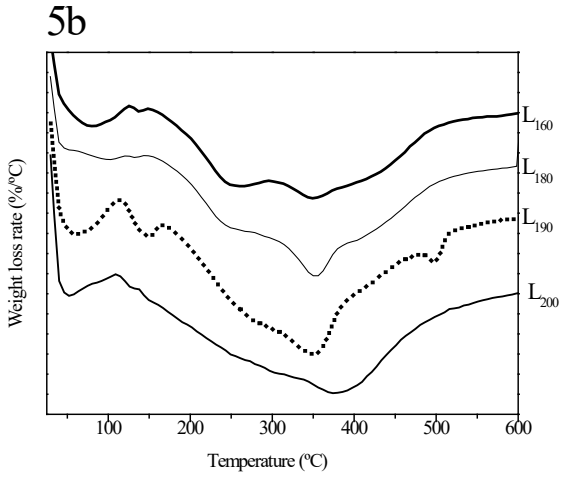
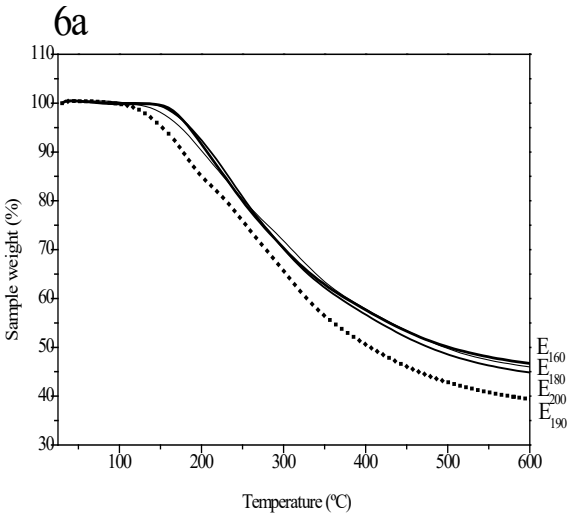

5b
Weight loss rate (%/ºC)
Temperature (ºC)
L160
L180
L190
L200

Figure 6


6a
Sample weight (%)
Temperature (ºC)
E160
E180
E200
E190

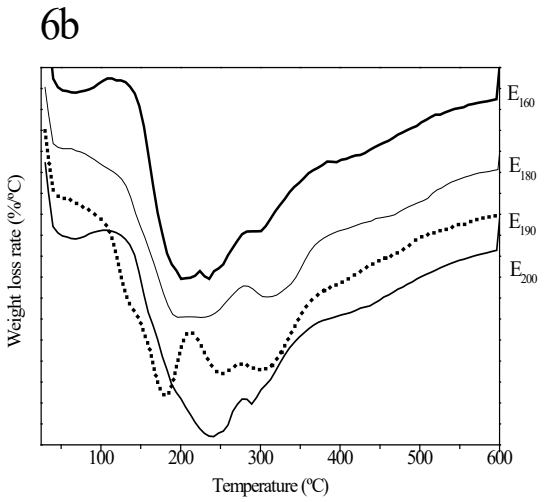
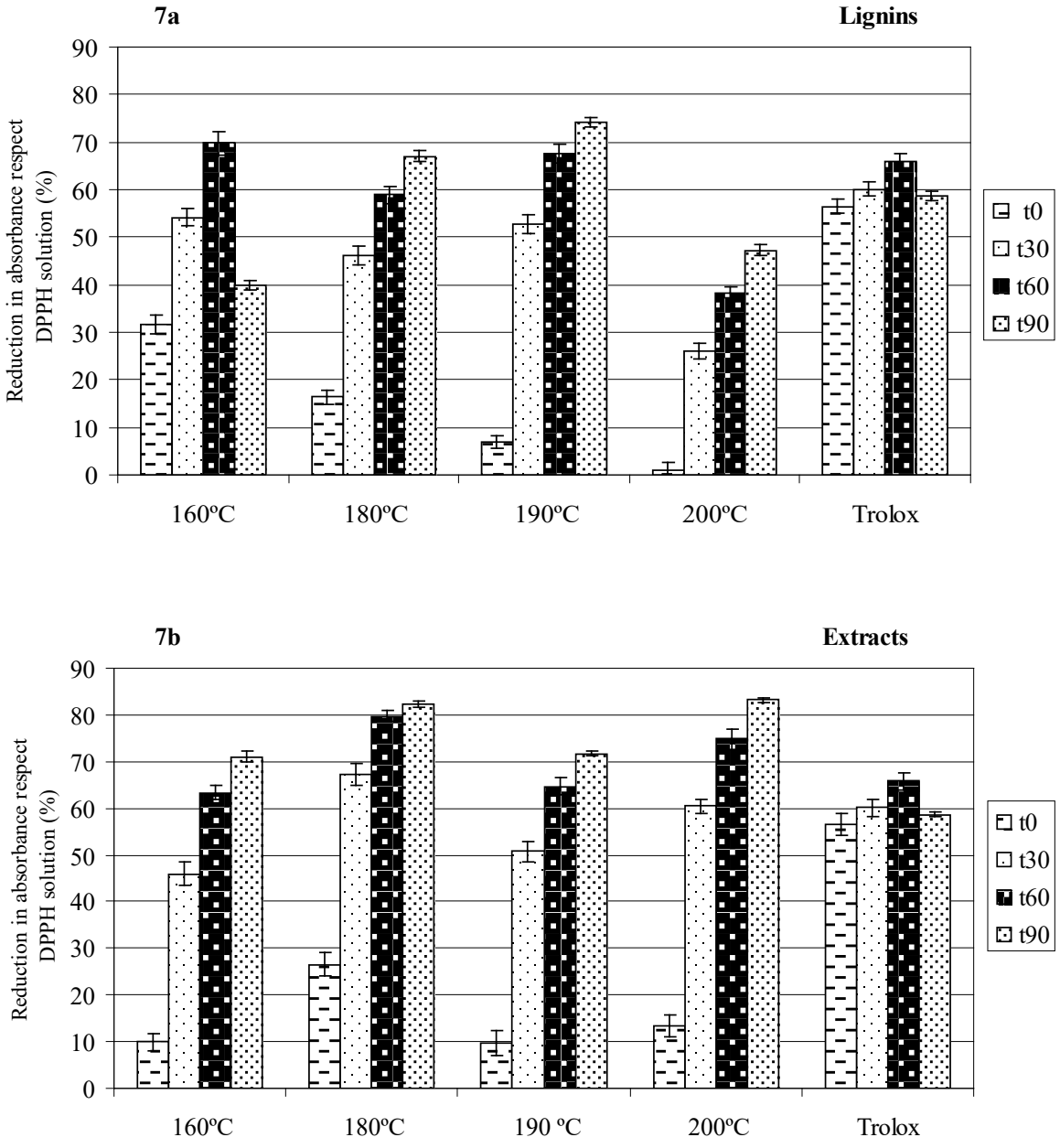

6b
Weight loss rate (%/ºC)
Temperature (ºC)
E160
E180
E190
E200

Figure 7


7a
Lignins
Reduction in absorbance respect DPPH solution (%)
90
80
70
60
50
40
30
20
10
0
160ºC
180ºC
190ºC
200ºC
Trolox
t0
t30
t60
t90
7b
Extracts
Reduction in absorbance respect DPPH solution (%)
90
80
70
60
50
40
30
20
10
0
160ºC
180ºC
190 ºC
200ºC
Trolox
t0
t30
t60
t90