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Implementation of Auto-Hydrolysis Process for the Recovery of Antioxidants and Cellulose from Wheat Straw

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Featured Application: The paper discusses the application of a lignocellulosic fractionation process to recover both antioxidant phenolic compounds and cellulose from wheat straw, where the first hydrolysis step is carried out as an auto-hydrolysis novel process instead of a conventional mild acid hydrolysis. The two different processes were compared and auto-hydrolysis resulted more effective in separating cell-wall bound phenolic compounds, antioxidants and cellulose from wheat straw. Therefore, it could be recommended for replacing the aggressive acid hydrolysis step in the isolation of natural antioxidants and fibers from wheat straw and other similar biomasses.

Abstract: Wheat straw is an easily affordable, cost-effective and natural source of antioxidants and cellulose, but its full potential is not yet utilized. In the present investigation, an auto-hydrolytic process was applied to recover both antioxidant phenolic compounds and cellulose from wheat straw. Two three-step acid/alkaline fractionation processes were applied differing for the first step: a conventional mild acid hydrolysis or an auto-hydrolysis. The liquors from the first step were analyzed for the recovery of antioxidants, while the final residues from the whole process were analyzed for cellulose yield and purity. The auto-hydrolysis process led to a higher yield in antioxidants but also in sugars (glucose and xylose) and sugar degradation products (5-HMF, 5-MF, furfural) than the acid hydrolysis process. The overall cellulose recovery (about 45% g/100 $g_{\text{cellulose wheat straw dm}}$) and purity was comparable in the two processes; therefore, the auto-hydrolysis-based process could be recommended as a potentially more environmentally friendly process to recover antioxidants and cellulose from wheat straw for different applications. Finally, a first study on the optimization of hydrolysis step was provided from the point of view of improving the cellulose yield, monitoring the sugars release during both the acid hydrolysis and the auto-hydrolysis process.

Keywords: wheat straw; auto-hydrolysis; cellulose; phenolic compounds; antioxidants

1. Introduction

Wheat straw (WS) is an agricultural residue from wheat grass (*Triticum aestivum* L.) that presents many interesting characteristics that facilitate its biotechnological upgrade in a bio-refinery framework [1]. Specifically, it is an herbaceous crop, soft material that can be transported in relatively high-density form and typically has a low water content (i.e., about 6–8%) [2] that enables its easy

storage [3]. Moreover, it is a very abundant material and it does not present an excessive commercial value (about 97 USD/ton) [4]. The average yield of WS is 1 kg per 1.3–1.4 kg of grain, which leads to a considerable surplus amount [5] even considering the fractions to be used for soil improvement and livestock use. Based on the data from FAO [6], world annual WS production was about 564 million tons in 2018. Despite the production showed a slightly decrease in 2018, these amounts are significant enough to consider WS as a complementary source in the production of fibers and antioxidants.

The main constituents of WS cell walls are cellulose (33.7–40%), hemicelluloses (21–26%), and lignin (11–22.9%) [7]. The cell walls also contain small amounts of p-hydroxy-cinnamic acids such as ferulic and p-coumaric acids, glycoproteins, pectins, wax, and ash [7]. Currently, WS is used for low value applications such as mulch [8], animal-feed [9], bedding [10], energy [11] and pulp production [12]. Despite this, it is considered to have high potential for the production of second-generation bioethanol in Europe [13] and different studies were conducted in the past regarding the bound phenolic compounds of WS and their antioxidant properties [14–18]. Plant cell walls are three-dimensional structures formed with a polysaccharide network of which cellulose, hemicelluloses and pectin are the most important components. It is well established that they contain hydroxyl cinnamic acids covalently linked to polysaccharides through ester linkages [19]. Phenolic compounds in plants are present in the free, esterified or bound forms. Phenolic acids may form both ester and ether linkages owing to their bifunctional nature through reactions involving their carboxylic and hydroxyl groups, respectively, which allows phenolic acids to form cross-links with cell wall macromolecules [20].

The antioxidant phenolics and cellulose can be isolated from WS during the same lignocellulosic fractionation process, such as an acid/alkaline hydrolysis process [7]. The literature review indicates the use of acid and alkali hydrolysis in wheat, rice, rye, barley and maize straws [21]; wheat straw, wheat bran, maize bran, rice bran and sugar cane bagasse [22]; wheat bran [23] and corn fiber [24]; barley bran and corn cobs [25]; corn bran [26]; corn cob [27]; triticale bran and straw [28]; maize bran [29]; rice bran [30,31]; barley bran, corn cobs, corn leaves and oat fiber [25,32]; barley husk [33]. From the environmental point of view, it is better to avoid the use of chemicals which are used in conventional fractionation process such as the sulfuric acid in the acid hydrolysis step. Even though a powerful agent for cellulose hydrolysis, it is toxic, corrosive and hazardous and the concentrated acid must be recovered after hydrolysis to make the process economically feasible [34]. For these reasons, auto-hydrolysis instead of acid hydrolysis process is gaining more attention nowadays and was already tested to recover phenolics from grape stalks [35,36]; corn cob [37,38]; almond shells and chestnut burs [37], barley husks [39]; olive tree branches [40] and corn stalks [41]. Hence, in the present study, a potential environmentally friendly auto-hydrolytic process was applied for the recovery of both antioxidants and cellulose from wheat straw in a three-step process consisting in a high pressure/temperature auto-hydrolysis step, followed by an alkaline hydrolysis and a final bleaching step. The auto-hydrolysis liquor was analyzed for antioxidant compounds content and activity, while the solid residue obtained at the end of the process was analyzed for cellulose content. A conventional process with initial mild sulfuric acid hydrolysis was applied as benchmarking for cellulose and antioxidant recovery. Finally, sugars formation and degradation over acid/auto-hydrolysis time were monitored and investigated. This can be the base for future process optimization in terms of both cellulose isolation and antioxidant compounds, in order to develop an economically sustainable process.

2. Materials and Methods

Wheat straw was kindly provided by a farmer in Piemonte (Northern Italy) and simply stored without additional drying. Sample (1 kg) was milled to 2 mm particle size using a lab hammer mill (THOMAS SCIENTIFIC, Model 4 Wiley® Mill, New York, NY, USA). Wheat straw composition, in terms of cellulose, hemicellulose, lignin, extractives and ashes, was assessed using the TAPPI analytical method [19].

2.1. Fractionation Process

The WS was submitted to the same process of lignocellulosic fractionation adapted from Vadivel et al. [7] and schematized in Figure 1, where the operation conditions for each step have been specified.

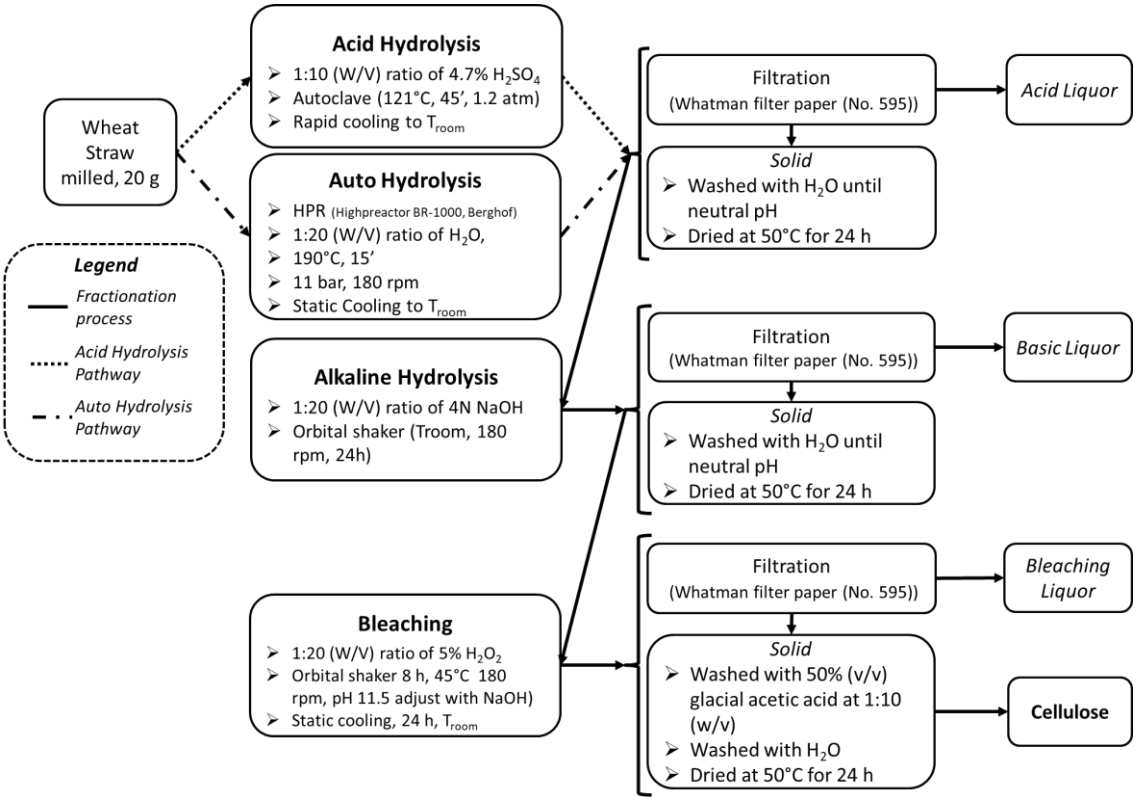

Figure 1. Block flow diagram of the wheat straw fractionation process with operating conditions.

Acid and Auto-Hydrolysis

Figure 1 shows a block flow diagram of the operating conditions of wheat straw fractionation process. Briefly, conventional acid hydrolysis, as reported in detail by Vadivel et al. [7], was carried out in a static autoclave, while in the alkaline hydrolysis and bleaching steps, the samples were kept agitated in an orbital shaker (HT Infors AG CH-4103, Switzerland) at specified speed. After the acid hydrolysis, the samples were taken out from the autoclave at 80 °C and rapidly cooled under water to room temperature (20 ± 2 °C) before the filtration step.

In the auto-hydrolysis process, wheat straw and distilled water were taken in a Teflon container and placed inside a high-pressure reactor (HPR, Highpreactor BR-1000, Berghof, Germany). The reactor was operated manually at 190 °C temperature and 11 bar pressure (i.e., saturation pressure of water) for 15 min (as holding time). The reactor's stirrer was set at 200 rpm to better homogenize the sample. After the reaction, the reactor was let cooling down exchanging at ambient temperature (20 ± 2 °C). Then, the gas valve was opened to release residual internal pressure and the hydrolyzed sample was taken out. The acid liquors were analyzed for sugar composition, acetic acid content, total phenol content, cinnamic acids content, antioxidant capacity (FRAP, ABTS and Superoxide assays), phenolic profile and sugar degradation products. The cellulose residues were analyzed for the structural carbohydrates, acid soluble and insoluble lignin, glucose, xylose and acetic acid. It is important to point out that, as reported by Carvalheiro et al. [42], the auto-hydrolysis process, similar to acid hydrolysis, is catalyzed by H+ and H_3O+ ions. The latter are generated in situ by both water auto-ionization (i.e., auto-hydrolysis) and acetic acid, which results from acetyl substituents of

hemicelluloses. This aspect was also confirmed by Perez et al. [43] reporting that, with an increase in temperature, the hydrolytic effect of the water is intensified. Indeed, temperature affects the pKa of the water resulting in pH of pure water at 200 °C equal to 5.0. In this way, hot water, in combination with the high pressure—which makes the biomass polymers more accessible [44]—breaks the hemiacetal linkages of hemicellulose leading to the formation of acetic and uronic acids. The release of these acids helps to catalyze removal of oligosaccharides from hemicellulose facilitating in this way its hydrolysis. On the other hand, in the acid hydrolysis, H+ and H_3O+ ions are directly generated by the dissociation of sulfuric acid in water. For this reason, it is necessary to use a higher amount of water in the auto-hydrolysis process, in order to promote the dissociation reaction of water, since it is a thermodynamically disadvantaged reaction.

2.2. Kinetics of the Acid Hydrolysis and Auto-Hydrolysis Step

A kinetics study of acid hydrolysis and auto-hydrolysis processes was carried out to monitor the release of sugars and of their degradation products during these processes. For the conventional acid hydrolysis, different samples are prepared, and the hydrolysis time is varied to investigate the process kinetics. For each trial, 30 min were waited for equipment and samples cooling before opening the autoclave, and then the samples were cooled under sink water for 15 min before filtration. For the auto-hydrolysis process, a stainless-steel body reactor, instead of the Teflon one previously used, was used to speed up both the heating and cooling step and reduce the severity of the process. Working conditions were set to reach a final temperature of 190 °C with a pressure of 11 bar, and this condition was maintained for 15 min. Liquor samples were taken during the process through a specific sampling tube, but only few samples could be taken due to clogging problems of the sampling tube.

All the collected liquor samples were analyzed for phenolic profile and sugar degradation products were analyzed by HPLC, while free monosaccharides (glucose and xylose) were quantified by specific enzymatic kits (Megazyme kit, GOPOD-format, K-Glucose and K-Xylose).

2.3. Structural Carbohydrates

The cellulose residue was analyzed as reported by Vadivel et al. [7] according to the method proposed by Sluiter et al. [45] for structural carbohydrates and lignin. Briefly, the sample (600 mg) was hydrolyzed in a screw-capped bottle with 3 mL of 72% (*w*/*w*) sulfuric acid at 30 °C for 60 min, mixing well the slurry with a glass rod every 10 min. After this hydrolysis, the obtained acid sample was diluted until a final sulfuric acid concentration of 4% (*w*/*w*) by adding distilled water (84 mL), and then the sample was autoclaved at 121 °C for 60 min. The sample is let cooling down at room temperature (20 ± 2 °C) and filtered (ash-free Whatman filter paper, No. 589/3). The liquid was analyzed for glucose (Megazyme kit, GOPOD-format, K-Glucose), xylose (Megazyme kit, K-Xylose), acetic acid (Megazyme kit, AK/PTA format, K-Acetrm) and acid soluble lignin (ASL) (absorbance reading at 320 nm multiplied by the absorbance coefficient of 30 L/g/cm).

The content of xylose multiplied by the correction factor of 0.88 for pentoses was used to estimate the hemicellulose content, while the glucose content multiplied by the correction factor of 0.90 for hexoses gave the estimated cellulose (glucan) content.

Acid insoluble lignin (AIL) was calculated from the solid residue after moisture and ash content determination. Total lignin content was calculated as the sum of ASL and AIL.

2.4. Total Phenolic Content (Folin's Assay)

The total phenolic content was analyzed using a micro-volume version of the Folin's assay [46]. The sample (50 µL opportunely diluted) was added to 250 µL of Folin–Ciocalteu reagent (undiluted) in a test tube and vortexed. Then, 4.7 mL of 2.2% sodium carbonate solution was added and the mixture was vortexed again. A blank was prepared with 50 µL of the sample solvent instead of the sample. The tubes were incubated at 40 °C for 30 min in a water bath. The absorbance was read at 750 nm against the blank using a spectrophotometer (Perkin-Elmer, Lambda Bio 40). A calibration curve with

standard gallic acid in water (Fluka, 100–800 mgGA/L, R^2 = 0.999) was used to express the results as gallic acid equivalents (GAE), as both concentration in the liquor and as yield referred to initial wheat straw dry weight.

Total phenolic content was also evaluated based on the direct absorbance of the liquor at 280 nm (280 Index) (eventually after proper dilution) [47] and expressed as GAE using a calibration curve with standard gallic acid in water (Fluka, 100–800 mgGA/L, R^2 = 0.995).

2.5. Total Cinnamic Acids Content

Total cinnamic acids content was evaluated based on the direct absorbance of the liquor at 320 nm (eventually after proper dilution) [47] and expressed as equivalents of ferulic acid (FAE using a calibration curve with standard ferulic acid in water (Fluka, 100–800 mgFA/L, R^2 = 0.995).

2.6. Ferric Reducing/Antioxidant Power (FRAP Assay)

The ferric reducing/antioxidant power (FRAP) was evaluated according to the procedure described by [48]. The FRAP reagent was freshly prepared and incubate at 37 °C with 2.5 mL of 20 mM TPTZ solution (2, 4, 6-Tris (2-pyridyl)-s-triazine, Sigma-Aldrich) in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 M acetate buffer (pH 3.6). An aliquot of this reagent (3.7 mL) was mixed with 360 µL of distilled water and 120 µL of the liquor (or water for the blank). The test samples and reagent blank were incubated at 37 °C for 30 min in a water bath and then the absorbance of the sample is read at 593 nm against the blank. A calibration curve was prepared with $FeSO_4 \cdot 7H_2O$ (Carlo Erba), covering the range of 50–400 mgFe(II)/L (R^2 = 0.999). Then the results were calculated and expressed both as molFe(II)/molGAE (based on Folin's assay) and µmolFe(II)/$g_{dm\ wheat\ straw}$.

2.7. Antioxidant Power (ABTS Assay)

The ability of the extract to reduce ABTS radical was analyzed as reported by Vadivel et al. [7]. A radical solution (7 mM ABTS by Fluka and 2.45 mM potassium persulfate) was prepared and kept in dark at room temperature for 12–16 h before use. The solution was then diluted with aqueous ethanol (50%) to an absorbance of 0.70 (±0.02) at 734 nm against ethanol 50% and equilibrated at 30 °C just before the analysis. An aliquot of the diluted radical solution (2 mL) was mixed with 20 µL of different dilutions of the liquor and kept in the dark for 6 min at 30 °C. The absorbance was finally read at 734 nm, and through a calibration curve with Trolox® standard (Tr) in aqueous ethanol (50%) (Sigma-Aldrich; 100–500 mgTr/L, R^2 = 0.999), the percent of absorbance reduction was expressed as molTr/molGAE and µmolTr/$g_{dm\ wheat\ straw}$.

2.8. Radical Scavenging Activity (Superoxide Assay)

This assay measured the capacity of compounds to scavenge the superoxide anion radical and was performed as reported by Vadivel et al. [7]. The reaction mixture was prepared as 3×10^{-6} M riboflavin (Sigma-Aldrich), 1×10^{-2} M methionine (Sigma-Aldrich), 1×10^{-4} M nitroblue tetrozolium chloride (Sigma-Aldrich) and 0.1 mM EDTA (Sigma-Aldrich) in phosphate buffered saline (pH 7.4). An aliquot of this mixture (3.0 mL) was mixed with 100 µL of liquor in closed tubes and illuminated for 40 min under a fluorescent lamp (18 W). The absorbance was then read at 560 nm against the un-illuminated reaction mixture. Based on the volume of liquor used for the analysis and on the total phenols content (based on Folin's assay), the superoxide radical scavenging activity (SRSA) results are expressed both as %SRSA/µMGAE and %SRSA/$mg_{dm\ wheat\ straw}$.

2.9. Phenolic Profile and Sugar Degradation Products

The acid liquors were analyzed for the content in specific phenolic compounds and in sugar degradation products by chromatographic analysis performed on an HPLC system (Perkin Elmer, Norwalk, CT, USA) equipped with a 200 Series pump, a diode array detector (DAD), a Jasco LC-Net

II/ADC (Oklahoma City, OK, USA) communication module and operated by ChromNAV Control Center software. For the evaluation of phenolic compounds, samples of liquors were extracted with a SupercleanTM ENVITM-Chrom P SPE Tube, 6 mL–0.50 g column (Supelco, Bellefonte, PA, USA) before injection into the HPLC equipped with a SupelcosilTM LC-18, 250 × 4.6 mm, 5 µm particles fitted column (Supelco). Details for the samples pretreatment and analysis conditions are reported in Vadivel et al. [7]. To assess the concentration of sugar degradation products, 5-HMF, furfural and 5-MF, the auto-hydrolyzed liquors were 4-time diluted, filtered (0.45 µm) and injected. Then, the same analytical conditions used for phenolic compounds were applied. Both principal phenolic compounds and sugar degradation products were identified and quantified through external calibration curves with chemical standards [7].

2.10. Free Sugars and Acetic Acid

The concentration of free glucose, fructose, xylose and acetic acid was evaluated directly on the liquid recovered after the first step of auto-hydrolysis by specific enzymatic kits (Megazyme kit, GOPOD-format K-Glucose, K-Xylose and K-Acetrm) after sample neutralization according to kit instructions.

3. Results and Discussion

Wheat straw showed a 5.85 ± 0.73% moisture content and a content of cellulose, hemicellulose and lignin (as % on dry matter) of 33.78 ± 0.20%, 19.47 ± 0.22% and 21.53 ± 0.73%, respectively, that is in line with the average values reported in the literature [49]. The extractives were 5.62 ± 0.32% and the ash 7.60 ± 0.05% (on dm).

3.1. Cellulose Isolation

Table 1 reports the results of the cellulose isolation from wheat process following the two different protocols of Figure 1, based on the characterization of the solid residue obtained at the end of the whole process, then after acid or auto-hydrolysis, basic hydrolysis and bleaching.

Table 1. Characterization of the final fiber residue obtained after acid/alkaline fractionation process of wheat straw (with initial auto-hydrolysis or conventional acid hydrolysis) in terms of cellulose, hemicellulose, lignin and acetic acid content. Values are reported as mean values (n = 3) ± sd.

	Auto-Hydrolysis	Acid Hydrolysis
Fiber residue yield (g/100 $g_{\text{wheat straw dm}}$)	20.41 ± 0.22	17.56 ± 0.37
Cellulose (g/100 $g_{\text{fiber residue dm}}$)	77.79 ± 0.85	85.98 ± 1.84
Cellulose yield (g/100 $g_{\text{wheat straw dm}}$)	15.88 ± 0.17	15.10 ± 0.32
Cellulose recovery (g/100 $g_{\text{cellulose wheat straw dm}}$)	47.01 ± 0.51	44.70 ± 0.92
Hemicellulose (g/100 $g_{\text{fiber residue dm}}$)	1.01 ± 0.02	2.05 ± 0.11
Acetic acid (g/100 $g_{\text{fiber residue dm}}$)	0.19 ± 0.02	0.59 ± 0.01
Acid soluble lignin (g/100 $g_{\text{fiber residue dm}}$)	0.90 ± 0.04	1.09 ± 0.03
Acid insoluble lignin (g/100 $g_{\text{fiber residue dm}}$)	5.47 ± 0.50	12.43 ± 0.24

Even though the fiber residue from auto-hydrolysis appears to be better in color as well as texture (Figure 2), its purity (cellulose content) is slightly lower in the auto-hydrolysis process when compared to the acid hydrolysis method (Table 1). Interestingly, the hemicellulose, acetic acid, acid soluble and acid insoluble lignin content of auto-hydrolyzed fiber is lower than those of the acid hydrolyzed sample. This means that the auto-hydrolysis process leads to a better delignification of the final cellulose material, which results both in a better quality of the cellulose and in a possible higher lignin recovery form the obtained liquor [36]. On the other hand, the final lower purity of cellulose coming from auto-hydrolysis could be explained by the formation of pseudo-lignin compounds during biomass pretreatment processes [50]. These compounds, derived from sugar degradation products, cannot be

evaluated using structural carbohydrates analytical method. For these reasons, furthers studies need to be conducted in order to properly assess these compounds. However, the cellulose recovery is comparable and so the greener auto-hydrolysis process could be very interesting from an industrial point of view.

Figure 2. Cellulose residues obtained from acid hydrolysis (**A**) and auto-hydrolysis (**B**) of wheat straw.

These kinds of pretreatment processes were often used to recover cellulose with lower purity to be used for successive fermentation, for instance for bioethanol production [51] or for further enzymatic hydrolysis [52]. For this reason, there are few scientific works concerning the recovery of high pure cellulose from wheat straw. Sun and Tomkinson [53] studied a process to produce highly purified cellulose from wheat straw using sequential treatments of dewaxed straw (i.e., wheat straw after extraction with ethanol and toluene) with potassium hydroxide under ultrasonic irradiation. The final fiber residue yield is equal to 37%, then higher than in the present study (Table 1). The main issue is that the proposed process was performed using ultrasound technology, which presents very high costs at industrial scale. Moreover, the final fiber residue yield of auto-hydrolysis process proposed in this work can be further increased by improving both solids recovery yield and optimizing the operating conditions of the process. Montané et al. (1998) [54] investigated a fractionation of wheat straw by steam-explosion technique, instead of auto-hydrolysis, followed by an alkali delignification and a final bleaching step to produce a high pure cellulose. In this case, the final fiber residues yield is slightly higher (23.9%) than the one obtained in this work, but it must be taken into account that, as already mentioned, auto-hydrolysis process has not been optimized yet. Moreover, steam explosion pretreatment presents the main disadvantage of using critical operating conditions as high pressure and temperature involved [55] with the related high costs and safety problems. In a more recent work [56], Lopes et al., studied a pre-treatment methodology of wheat straw with 1-ethyl-3-methylimidazolium acetate (i.e., an ionic liquid) and its subsequent fractionation to cellulose, hemicellulose and lignin. The fractionation of completely dissolved biomass led to cellulose-rich and hemicellulose-rich fractions. The main differences from the auto-hydrolysis process are related to the use of methylimidazolium acetate and to the pre-treatment operating conditions (i.e., 120 °C and 6 h of stirring), while using alkaline step to recover high pure cellulose was similar. This process had a high final fiber cellulose yield equal to 41.8%. On the other hand, this process has some major disadvantages, such as the long pretreatment operational time (6 h) and the use of methylimidazolium acetate as reagent, which increases the environmental impact and the cost of the process. Concerning this, a life cycle assessment (LCA) comparison between these two processes should be conducted as a future study. For what has been explained above, auto-hydrolysis has many technological advantages compared to other pretreatment processes. These include low by-product generation, limited problems derived from equipment corrosion, and the reduction of capital and operational costs, making it one of the main interesting choices for industrial applications.

3.2. Total Phenols and Cinnamic Acids Content

The different first hydrolysis step led to different concentrations and yields of total phenols and cinnamic acids based on spectrophotometric analyses (Table 2). Total phenols content of the samples was evaluated with two different methods. The Folin Index is widely adopted to study natural antioxidants and estimate the total phenols content even though it actually measures the capacity of a compound to reduce the Folin's reagent, therefore it already gives an estimation of the antioxidant capacity. On the other hand, the 280 index is based on the characteristic absorption of the benzene rings of the majority of phenols at 280 nm, and is less influenced by the oxidative status of the analyzed molecules, but it is also related to the release of acid soluble lignin and sugar degradation products.

Table 2. Total phenols and cinnamic acids content of wheat straw (WS) auto-hydrolysate and acid hydrolysate liquors. GAE: gallic acid equivalents. FAE: ferulic acid equivalents. Values are reported as mean values (n = 3) ± sd.

Parameter	Auto-Hydrolysis		Acid Hydrolysis	
	mg/L	mg/g_{dmWS}	mg/L	mg/g_{dmWS}
Total phenols (Folin_GAE)	1532.28 ± 61.56	20.06 ± 0.77	1211.86 ± 71.66	4.70 ± 0.29
Total phenols (280_GAE)	4990.78 ± 401.01	65.32 ± 5.12	6932.85 ± 240.63	27.24 ± 0.95
Cinnamic acids (FAE)	137.91 ± 52.42	1.81 ± 0.69	41.64 ± 11.49	0.16 ± 0.05

It is important to underline that the pH of the acid liquor obtained from the auto-hydrolysis reactor was mildly acidic (4.28 ± 0.05), when compared to the liquor coming from the autoclave acid hydrolysis (0.31 ± 0.01). Even though only distilled water was used, the acetic acid released during the auto-hydrolysis process from delignification is the catalyzer of the reaction and the reason for this acidity [42]. The total phenols yield, calculated considering the exact volume of the recovered liquor, was higher for auto-hydrolysis, from 2.4 to 4.3 times based on the 280 Index and Folin index, respectively, and 11 times for total cinnamic acids. This might be due to the higher efficiency of this kind of hydrolysis together with a lower severity that could have avoided the degradation of the released phenolic compounds. On the other hand, there might also have been an effect of the higher liquid-to-solid ratio applied.

3.3. Antioxidant Power (FRAP, ABTS and SRSA Assays)

The antioxidant capacity of the liquors was assessed based on three assays (Table 3). In general, for all the applied assays, it was confirmed that for the total phenols, there was a higher release of antioxidant compounds with the auto-hydrolysis process, by almost 5 times for the FRAP and Superoxide test, and more than 20 times for the ABTS test. In the literature (Vadivel et al., 2017), it is generally observed a high correlation between this kind of tests and the Folin assay, since, as already explained, the Folin also measures the antioxidant capacity (as reducing power) of a sample. Evaluation of the specific antioxidant power showed a high correlation in the case of the FRAP and Superoxide assays, since the calculated values were comparable for the two liquors, while the ABTS test showed a higher specific antioxidant capacity for the phenolic compounds contained in the auto-hydrolysis liquor. Concerning acid hydrolysis, the antioxidant power obtained reflects the trend assessed by Akpinar et al. [14], who studied the antioxidant activity of dilute acid hydrolysate of wheat straw. Moreover, FRAP and ABTS and also Folin assays confirm that wheat straw, a raw material with high content of cellulose and lignin, releases less antioxidant compounds compared to other biomasses, such as fruit pomace, and so is more suitable for cellulose recovery [55,56]. Indeed, FRAP and Folin assay test values are about 160 times and 120 times lower, respectively, than those reported by Bassani et al. [35], who analyzed the antioxidant recovery from grape pomace using auto-hydrolysis process in similar operating conditions. On the other hand, Gullon et al. [57] analyzed the liquors obtained from the auto-hydrolysis at different operating temperatures of vine shoots, that are a more

lignocellulos biomass. Gullon et al. obtained 12.2 mgGAE/$g_{vine\ shoot}$ of total phenol content which is similar to the value obtained for wheat straw extracts. Another example is the spent coffee grounds, that are expected to release a low amount of antioxidant compounds due to a previous extraction with hot water during coffee preparation. Ballesteros et al. [58] tested different auto-hydrolysis conditions to optimize the process for antioxidant compounds recovery from spent coffee grounds, finding values of about 25 mg GAE/g, similar to the one obtained in this work for wheat straw.

Table 3. Antioxidant power of wheat straw (WS) auto-hydrolysis and acid hydrolysate liquors measured with different assays (FRAP, ABTS and Superoxide). Values are reported as mean values (n = 3) ± sd.

Parameter	Auto-Hydrolysis	Acid Hydrolysis
FRAP μmolFe/g_{dmWS}	309.26 ± 32.42	67.27 ± 2.00
FRAP molFe/molGAE	2.62 ± 0.27	2.44 ± 0.07
ABTS μmolTrolox/g_{dmWS}	93.89 ± 6.47	4.06 ± 1.32
ABTS molTrolox/molGAE	0.80 ± 0.05	0.15 ± 0.05
Superoxide (%SRSA/mg_{dmWS})	387.15 ± 79.94	80.49 ± 11.91
Superoxide (%SRSA/μMGAE)	0.34 ± 0.072	0.29 ± 0.048

3.4. Phenolic Profile, Sugars and Sugar Degradation Compounds

The analysis of glucose and xylose in the liquors (Table 4) coming from cellulose and hemicellulose hydrolysis, respectively, seems to confirm the higher severity of the conventional acid hydrolysis, particularly regarding the effect on hemicellulose, since xylose concentration was nearly 100 times that in the auto-hydrolysis liquor, while the glucose concentration was 15 times higher. Even considering the double liquid to solid ratio adopted in the autohydrolysis process, the sugars yield was higher with the acid hydrolysis. Moreover, acetic acid content was higher both in concentration and in overall yield. Acetic acid originates from the removal of acetyl groups of hemicellulose and oxidation of released monosaccharides; therefore, its values agree with those of glucose and xylose. However, HPLC analysis revealed a lower yield of sugar degradation products for conventional acid hydrolysis. This means a higher severity of the auto-hydrolysis from the point of view of sugar degradation. This might indicate both that the auto-hydrolysis is less aggressive than the acid hydrolysis step in causing the degradation of fibers into monomers, and that the high temperatures involved in auto-hydrolysis leads to a higher conversion of released sugars into furans. This might be negative if the liquors are meant to be used as substrate fermentation due to the typical anti-fermentative potential of these compounds, but it could be positive due to the important role of furans as starting molecules for many other compounds [59].

Acid liquors were also analyzed for the phenolic profile (Table 4), looking for the phenolic acids and aldehydes that typically originate from lignin depolymerization and solubilization [60]. A few phenolic acids were detected, at very low concentration, only in the acid liquor, maybe due to their degradation under the high temperature reached in the auto-hydrolysis. Apart from these compounds, HPLC analysis confirmed the higher antioxidant content and power previously reported for the auto-hydrolysis based on the higher vanillin and syringaldehyde yield. Moreover, the data reported in Table 4 are in agreement with the values obtained from the analysis of Carvalheiro et al. [15] and of Akpinar et al. [61], despite the first being started with a lower liquid to solid inlet ratio (i.e., 1:10 compared to 1:20), while the second operated with milder conditions (i.e., 180 °C compared to 190 °C).

Table 4. Phenolic profile and sugar and sugar degradation compounds content of auto-hydrolysis and acid hydrolysis liquors of wheat straw. HMF: hydroxyl-methyl-furfural; MF: methyl furfural. Values are reported as mean values (n = 3) ± sd.

	Auto-hydrolysis		Acid Hydrolysis	
	mg/L	**mg/g$_{dw}$**	**mg/L**	**mg/g$_{dw}$**
Glucose	61.96 ± 1.70	0.81 ± 0.02	958.33 ± 16.95	3.71 ± 0.07
Xylose	169.56 ± 19.56	2.22 ± 0.26	15993.18 ± 538.20	61.97 ± 2.08
Acetic acid	132.27 ± 8.54	1.73 ± 0.11	2491.69 ± 4.86	9.66 ± 0.67
5-HMF	112.15 ± 0.23	1.47 ± 0.01	22.30 ± 0.46	0.17 ± 0.01
Furfural	1389.59 ± 1.13	18.23 ± 0.02	1139.61 ± 31.72	8.84 ± 0.25
5-MF	18.21 ± 0.16	0.24 ± 0.002	2.38 ± 0.07	0.02 ± 0.01
p-hydroxibenzoic acid	ND	ND	ND	ND
Gentisic acid	ND	ND	ND	ND
Vanillic acid	ND	ND	1.30 ± 0.09	0.01 ± 0.01
Caffeic acid	ND	ND	1.62 ± 0.16	0.01 ± 0.01
Siringic acid	ND	ND	1.65 ± 0.07	0.01 ± 0.01
Vanillin	29.34 ± 0.09	0.385 ± 0.01	5.33 ± 0.15	0.04 ± 0.01
Syringaldehyde	3.53 ± 0.26	0.046 ± 0.01	3.06 ± 0.04	0.02 ± 0.01
Ferulic acid	ND	ND	ND	ND
Sinapic acid	ND	ND	ND	ND

3.5. Kinetics of the Acid Hydrolysis and Auto-Hydrolysis Step

Application of a non-conventional process with auto-hydrolysis instead of the acid hydrolysis step, allowed to obtain apparently a better purer cellulose residue (Figure 2). For this reason, it could be interesting to further investigate and optimize the auto-hydrolysis step from the point of view of improving the cellulose yield. As explained in the materials and methods, for the kinetics study, the conditions of samples cooling were standardized as much as possible, and this might have led to slightly different results than in the previous trials. It was then decided to monitor the sugars (Table 5) release, since this first step is meant to hydrolyze the hemicelluloses with release of pentoses (mainly xylose), but also a fraction of cellulose can be hydrolyzed (giving glucose) depending on the severity of the working conditions. Furthermore, a too-severe process may lead to the thermal degradation of the released sugars with the formation of furans, and their concentration was monitored as well (Table 6). Indeed, as reported in a review work by Jönsson and Martin [62], the pentoses and uronic acids, which come from the hydrolysis of the hemicelluloses, decompose, leading to the formation of furfural, while the hexoses degradation leads to HMF (5-hydroxymethyl-2-furaldehyde) formation. This was confirmed by xylose concentration profile (Table 5) which shows a maximum. This trend can be explained considering that, initially, the hemicellulose hydrolysis into xylose is the dominant reaction, while, when hemicellulose is almost completely hydrolyzed, the degradation of xylose becomes the dominant reaction. This results in higher concentrations of sugar degradation products in case of auto-hydrolysis (Table 6). On the other hand, xylose concentration of acid hydrolysis is higher and increases its value until a plateau. This means that hemicellulose was hydrolyzed, and that xylose was not completely converted into the related degradation products due to the lower temperature compared to auto-hydrolysis. Regarding glucose, the concentration is lower in case of auto-hydrolysis (Table 6) and this means that auto-hydrolysis is less aggressive and so it allows a higher cellulose recovery. It is interesting to highlight that the glucose concentration shows a maximum trend profile in case of acid hydrolysis. This could be due to the fact that glucose reaches a concentration such that glucose degradation reactions become relevant.

Table 5. Xylose and glucose concentration variation during the conventional acid hydrolysis and the auto-hydrolysis. Values are reported as mean values (n = 3) ± sd.

Time [min]	Acid Hydrolysis		Auto-Hydrolysis	
	D-Glu [g/L]	D-Xyl [g/L]	D-Glu [g/L]	D-Xyl [g/L]
0	1.64 ± 0.24	12.92 ± 0.17	0.06 ± 0.2	0.02 ± 0.01
10	2.02 ± 0.10	13.51 ± 0.25	0.11 ± 0.2	0.43 ± 0.05
20	0.86 ± 0.01	15.11 ± 0.49	-	-
30	0.93 ± 0.06	15.76 ± 0.57	-	-
45	0.96 ± 0.02	15.99 ± 0.54	-	-
180	-	-	0.14 ± 0.3	0.32 ± 0.02

Regarding the sugar degradation products, Jönsson and Martin [62] reported that under severe pretreatment conditions, such as long reaction time, high temperature or acid concentration, HMF is further degraded to levulinic and formic acids. Moreover, as HMF, furfural can further degrade to formic acid. These aspects are in good agreement with the data reported in Table 6. Indeed, furfural increases until 30 min and then remains constant, while 5-HMF increases and then decreases in cases of acid hydrolysis, because of its conversion into levulinic acid and formic acid (which, in fact, appears after half of the reaction time) or because of its reduction to 5-MF which constantly increases until 30 min. The phenolic components showed different behaviors depending on the hydrolysis type and the compound. For all the detected compounds (vanillin, syringaldehyde, ferulic acid and p-coumaric acid), the auto-hydrolysis allowed for higher concentration, in agreement with the results from total phenols and antioxidant power. Since the liquid to solid ratio was double in the auto-hydrolysis, the yields were even higher suggesting a higher straw delignification. As for the sugar degradation products, the concentrations of all the detected phenolic compounds of the auto-hydrolyzed liquor increased over time, in contrast to acid hydrolysis, showing the progress of all the involved reactions: lignin solubilization, hemicellulose hydrolysis, sugars degradation. In the auto-hydrolysis, the concentrations increased over time indicating a progressive release of the compounds, while in the acid hydrolysis, it looks like a certain amount is released immediately and then the compounds degrade over time (or the degradation rate is higher than the release rate). Vanillin is the only exception, showing a slight increase also in the acid hydrolysis, probably linked to progressive solubilization of the acid soluble lignin fraction, since it is one of the most relevant lignin degradation products. Finally, acetic acid, which comes from the hydrolysis of the acetyl groups of hemicelluloses, is another acid found in the liquor [62].

Table 6. Phenolic profile and sugar degradation compounds content of auto-hydrolysis and acid hydrolysis liquors of wheat straw at different hydrolysis times. HMF: hydroxyl-methyl-furfural; MF: methyl furfural. Values are reported as mean values (n = 3) ± sd.

Compound (mg/L)	Time of Hydrolysis (min)					
	0	**10**	**20**	**30**	**45**	**180**
5-HMF Acid hydrolysis	67.73 ± 11.68	90.14 ± 2.49	97.51 ± 2.98	83.28 ± 3.43	58.91 ± 4.22	-
Auto-hydrolysis	nd	18.77 ± 3.37	-	-	-	80.07 ± 5.86
Furfural Acid hydrolysis	155.65 ± 5.34	388.55 ± 21.97	458.42 ± 32.38	785.49 ± 7.73	785.44 ± 53.49	-
Auto-hydrolysis	6.37 ± 0.02	275.40 ± 15.09	-	-	-	828.48 ± 140.30
5-MF Acid hydrolysis	0.79 ± 0.14	1.22 ± 0.06	2.15 ± 0.06	3.68 ± 0.16	3.23 ± 0.01	-
Auto-hydrolysis	n.d.	9.67 ± 0.14	-	-	-	17.36 ± 2.04
Vanillin Acid hydrolysis	2.98 ± 0.38	4.26 ± 0.26	4.72 ± 0.27	5.66 ± 0.26	5.51 ± 0.04	-
Auto-hydrolysis	5.31 ± 0.18	17.74 ± 0.15	-	-	-	26.43 ± 1.73
Syringaldehyde Acid hydrolysis	18.78 ± 1.45	9.64 ± 0.54	9.61 ± 0.27	7.32 ± 0.34	6.39 ± 0.17	-
Auto-hydrolysis	3.42 ± 0.42	15.33 ± 1.27	-	-	-	20.44 ± 2.59
p-cumaric acid Acid hydrolysis	8.83 ± 0.92	2.97 ± 0.30	3.22 ± 0.48	1.67 ± 0.20	1.19 ± 0.15	-
Auto-hydrolysis	n.d.	n.d.	-	-	-	5.94 ± 0.80
Ferulic acid Acid hydrolysis	58.44 ± 4.26	26.27 ± 2.52	23.97 ± 3.12	9.55 ± 0.50	6.38 ± 0.74	-
Auto-hydrolysis	604.40 ± 5.04	1064.71 ± 148.47	-	-	-	1264.32 ± 244.39
Acetic acid Acid hydrolysis	1827.51 ± 225.99	2498.57 ± 195.67	2561.61 ± 322.03	2440.16 ± 98.33	2333.79 ± 267.20	-
Auto-hydrolysis	1495.80 ± 12.95	3114.70 ± 23.70	-	-	-	3081.95 ± 670.20
Formic acid Acid hydrolysis	n.d.	n.d.	n.d.	2029.26 ± 221.39	2969.06 ± 156.66	-
Auto-hydrolysis	n.d.	n.d.	-	-	-	80.07 ± 5.86

4. Conclusions

In this work, an auto-hydrolysis process for cellulose and antioxidant recovery from wheat straw, an agriculture residue, was carried out and compared with the traditional acid hydrolysis in terms of some key parameters, such as cellulose yield and recovery. The main goal was to evaluate auto-hydrolysis as a greener and cleaner process to replace hydrolysis. Auto-hydrolysis resulted as being more effective in separating cell-wall bound phenolic compounds, antioxidants and cellulose from WS sample than conventional acid hydrolysis. Hence, it could be recommended for replacing the aggressive acid hydrolysis step in the isolation of natural antioxidants and fibers from WS samples. Further auto-hydrolysis trials at different temperatures will be required to optimize the cellulose recovery process, together with their application on other different biomasses to evaluate the flexibility of the technology. Moreover, a first kinetics study was conducted in order to investigate the sugars, their degradation products and the phenolic compounds release in the auto-hydrolysis and acid hydrolysis step. Indeed, some sugar degradation products, such as furfural or formic acid, can inhibit downstream biochemical processes or fermentation substrates. On the other hand, phenolic compounds and sugar degradation product have high interest in the food industry—for example, because of their antioxidant capacity. For these reasons, auto-hydrolysis seems to be better for the recovery of these compounds, maintaining the same quality of the final cellulose recovered. This study will be the base for further developments of this novel process and for its optimization in terms of cellulose recovery yield. Finally, future investigations concerning the Life Cycle Assessment will also be necessary to effectively evaluate the environmental impact of such a process and to optimize it from this point of view.

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