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Release of ferulic acid from corn cobs by alkaline hydrolysis

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ABSTRACT

The process of corn cobs alkaline hydrolysis to produce solutions with high hydroxy-cinnamic acids content was investigated. In particular the attention was focused on the solubilisation of ferulic acid (FA) and related compounds, mainly *p*-coumaric acid (*p*-CA). Although these compounds have applications as antioxidants, the purpose of this work was to obtain FA solutions that can be used as feedstock for the biotechnological production of vanillin in future studies. The effects of different concentrations of NaOH ($0.2 \leq C_a \leq 2.0$N) and solid/liquid ratios ($0.028 \leq S/L \leq 0.168$ g/g) on the solubilisation of FA versus time have been investigated at room temperature. Optimal hydrolysis conditions ($C_a = 0.5$N, S/L = 0.084 g/g after 6 h) ensured the production of hydrolysates with relatively high contents of both FA (1171 ± 34 mg/L) and *p*-coumaric acid (2156 ± 64 mg/L), which can be used in future studies for the microbial transformation into vanillin.

1. Introduction

Corn cobs is an important by-product of corn industry: for every 100 kg of corn grain approximately 18 kg of corn cobs are produced. A large quantity of corn cobs remains unused as lignocellulosic waste or used as animal feed. Such a feedstock contains 31.7% cellulose, 34.7% hemicellulose, 20.3% lignin and 3.4% acetyl groups (oven-dry basis) [1], which can be fractioned in separate streams.

Lignin is a well-known component of secondary (non-growing) cell walls, where it replaces water and completely prevents further growth. It is mainly made of phenolic acids linked through ester, ether, or acetal bonds to other components of the plant cell wall [2]. The major phenolic compounds identified in both primary and secondary cell walls of graminaceous plants, and in particular of cereals, are cinnamic acids such as ferulic acid (4-hydroxy-3-methoxycinnamic acid; FA) and *p*-coumaric acid (4-hydroxycinnamic acid; *p*-CA) [3]. These cinnamic acids act in the cross-linking of plant cell walls and are precursors of a variety of compounds that play an important role in plant defence responses [4]. FA and *p*-CA are very abundant, representing together up to 1.5% by weight of cereal cell walls [5].

Since *p*-CA and FA are bifunctional, they are able to form ester or ether linkages by reaction of their carboxyl or phenolic groups, respectively. Whereas the former is principally associated with lignin, the latter is mainly esterified with hemicellulose [6]. The linkage between FA and lignin is not yet fully understood, because it largely depends on the raw material, and the results are influenced by the fractionation methods employed. Mature wheat endosperm contains a feruloylated arabinoxylan in the secondary cell wall, where ferulic acid seems to be bound via ester linkages to the structural polysaccharides [6,7], while dimers of ferulic acid can serve to cross-link hemicellulose [8]. In cereals FA is ester-linked to O-5 of arabinose of cell wall-bound arabinoxylans [6,9], whereas in sugar beet it is associated mainly with the pectine side chains and linked to the O-2 of arabinose (50–60%) and the O-6 of galactose (40–50%) [8,10]. Cell walls of cereal straw such as wheat straw and grass are characterized by high levels of ester-linked *p*-CA and FA [11]. Higher proportions of ferulates are located mainly in cereal bran, maize bran being an especially rich source of FA [5], while *p*-CA is predominant in cereal stems [12].

Alkaline treatments dissolve lignin by cleavage of ester linkages in lignin–polysaccharide complexes, thus releasing phenolic acids [13]. The high solubility of cereal lignin in alkaline solution might originate either from alkali cleavage of ferulic acid cross-link between lignin and hemicelluloses or from modification of lignin polyelectrolyte properties induced by free carboxyl groups of phenolic acids ethers [14].

Chemical and enzymatic treatments of agro-industrial wastes or surpluses can allow FA and *p*-CA to be released and then to use in further transformations. There is great interest in the potential of FA and related compounds either as antioxidants, i.e. in food preser-

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vation because of their ability to inhibit fatty acid peroxidation [15], or as feedstocks for the high-added value vanillin bio-production, one of the main flavourings and aroma compounds [16], through an integrated, eco-sustainable process.

In this work we investigated the solubilisation of FA and other compounds from corn cobs by alkaline hydrolysis employing NaOH as a catalyst. The effects of NaOH concentration and solid/liquid ratio on the solubilisation process were assessed. Hydrolysates of alkaline treatments were characterized in their content of free and total phenolic compounds, sugars, oligosaccharides (OS) and organic acids, with the aim of optimizing FA release for future vanillin production.

2. Materials and methods

2.1. Raw material and chemical characterization

Corn cobs were collected locally (Ourense, Galicia, Spain), milled to a particle size smaller than 1 mm, homogenized in a single lot and stored until use. Aliquots from the above homogenized lot were subjected to moisture determination (ISO 638 method) and to quantitative acid hydrolysis (TAPPI T13m method) with 72% sulphuric acid. The solid residue after hydrolysis was expressed as Klason lignin. Hydrolysates were analyzed for monosaccharides (glucose coming from cellulose; xylose and arabinose from hemicelluloses) and acetic acid (from acetyl groups) by high performance liquid chromatography (HPLC) as reported elsewhere [17]. The results allowed the determination of the sample contents of cellulose and starch (based on glucose present in liquors), hemicellulosic polysaccharide constituents (based on xylose and arabinose), and acetyl groups (based on acetic acid).

Corn cobs were subjected to non-isothermal treatments with water (auto-hydrolysis) using 8 g water/g dry solid in a Parr reactor model 4563 M (Parr Instrument Company, Moline, IL). The reaction medium was heated according to the standard temperature profile to reach a final temperature of 202 °C. The solid and liquid phases were separated by filtration. The solid residue of corn cobs from auto-hydrolysis treatment (CAR) was subjected to moisture determination and to quantitative acid hydrolysis with 72% sulphuric acid following standard methods and was utilized as substrate for the alkaline hydrolysis experiments performed in this study.

2.2. Alkaline hydrolysis

Alkaline hydrolysis experiments were carried out by employing either raw corn cobs or CAR. Reactions were performed at room temperature using NaOH as catalysts in a solid/liquid ratio between 0.028 and 0.084 g of raw material/g of NaOH solution. NaOH concentration and reaction times were varied in the ranges 0.2–2.0N and 1–24 h, respectively. All the treatments were conducted in triplicate, and the standard deviation of each determination did not exceed 3%.

The liquors obtained from alkaline treatments were separated from the solid fraction by vacuum filtration through common laboratory paper filters. Aliquots from liquors were characterized through the determination of free and total phenolic compounds, sugars, oligosaccharides, organic acids and solubilised non-volatile compounds (SNVC) content. Solid residues from treatments were washed with water to remove the residual alkalinity, air-dried, and analyzed for the moisture content to measure the raw material dissolved fraction, and subjected to the same analytical determinations as for the raw material.

2.3. Analytical methods

Free phenolic acids concentrations were determined by HPLC, model 1100 (Agilent, Palo Alto, CA), using a UV detector (at 280 nm) and a Vydac 201TP54 C18 reverse-phase column (Grace, Deerfield, IL) with a C18 guard column. Samples were neutralized and filtered through 0.45 μm-pore membranes (Millipore, Vimodrome, Italy). Separation was achieved using a linear gradient of two solvents: solvent A (1.0% acetic acid in water, v/v), solvent B (1% acetic acid, 9% water, 90% methanol, v/v). The linear gradient was run at 35 °C in 60 min from 0% to 52% of B at a flow rate of 1 mL/min.

Glucose, xylose, arabinose and acetic acid were determined directly by the same HPLC as above, using a Refractive Index detector and a Supelcogel H59304-U column (Sigma–Aldrich, Bellefonte, PA). Samples were neutralized and filtered through 0.45 μm-pore membranes (Millipore). Separation was achieved using as mobile phase 0.005 M H_2SO_4 at a flow rate of 0.5 mL/min at 50 °C.

Oligosaccharides were measured by an indirect method based on quantitative acid hydrolysis of the liquors. For analytical purposes, samples of liquors were subjected to quantitative posthydrolysis (treatment with 4% sulphuric acid at 121 °C for 20 min), and the reaction products were assayed by the same HPLC method as for direct monomers determination. The increase in the concentrations of monosaccharides and acetic acid caused by posthydrolysis provided a measure of the oligomer concentration, expressed as xyloligoosaccharides, arabinooligosaccharides, glucooligosaccharides, and acetyl groups concentration [17].

Total phenolic compounds (TPC) concentration was measured using the Folin–Ciocalteu assay [18]. Briefly, 4.8 mL of distilled water, 0.2 mL of sample, and 0.5 mL of Folin–Ciocalteu reagent were added to 25 mL graduated flasks. After mixing, 1 mL of 20% sodium carbonate solution was added, and distilled water was added to reach the final volume of 10 mL. Solutions were mixed and allowed to stand in dark at room temperature for 1 h. Sample aliquots were used to determine total phenols concentration using a spectrophotometer, model Lambda 25 (PerkinElmer, Waltham, MA), at a wavelength of 725 nm. TPC concentration was standardized against caffeic acid and expressed in mg/L of caffeic acid equivalents (CAE). To this purpose, we used a calibration straight line described with the equation $ABS_{725} = 2.06 \times 10^{-3}$ CAE within the range 100–1000 mg/L of CAE with $R^2 = 0.9962$.

The content of unidentified phenolic compounds (UPC) was calculated from the TPC concentration and the free phenolic compounds concentrations using the equation:

$$\mathrm{UPC} = \mathrm{TPC} - C_{\mathrm{FA}}\beta_{\mathrm{FA}} - C_{p\text{-CA}}\beta_{p\text{-CA}} \quad (1)$$

where C_{FA} and $C_{p\text{-CA}}$ are the ferulic and *p*-coumaric acid concentrations (mg/L), while β_{FA} (1.2477 mg of CAE mg^{-1} of FA) and $\beta_{p\text{-CA}}$ (1.4057 mg of CAE mg^{-1} of *p*-CA) are the conversion factors to express the concentrations of ferulic and *p*-coumaric acids as mg/L of CAE. These conversion factors resulted from the ratios between the extinction coefficients of caffeic acid ($\varepsilon_{\mathrm{CAF}} = 2.1\ \mu g\ L^{-1}\ cm^{-1}$), ferulic acid ($\varepsilon_{\mathrm{FA}} = 1.7\ \mu g\ L^{-1}\ cm^{-1}$) and *p*-coumaric acid ($\varepsilon_{p\text{-CA}} = 1.5\ \mu g\ L^{-1}\ cm^{-1}$), respectively, obtained by the Folin–Ciocalteu assay.

The content of solubilised non-volatile compounds (SNVC) of the liquor was measured by oven drying until constant weight, taking into account the contribution of NaOH to the dry weight.

The solid phase from alkaline hydrolysis was recovered by filtration, washed with distilled water to remove the residual alkalinity, dried at room temperature and subjected to the same chemical analysis as for the feedstock material.