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Analytical Methods

Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurised solvents

Anvar U. Buranov, G. Mazza *

Pacific Agri-Food Research Center, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, BC, Canada V0H 1Z0

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ABSTRACT

Extraction of ferulic acid and vanillin from flax shives, wheat bran and corn bran were carried out using two extraction methods, non-pressurised alkaline hydrolysis (0.5 M NaOH) and pressurised solvents (0.5 M NaOH, water, ethanol and ammonia). There were no differences in the content of products extracted with non-pressurised and pressurised 0.5 M NaOH solution yielding mostly ferulic acid, p-coumaric acid and small amounts of vanillin. Pressurised low-polarity water (PLPW), pressurised aqueous ethanol (PAE) and pressurised aqueous ammonia (PAA) were efficient in the one-step production of vanillin from ferulic acid in flax shives (guaiacyl-rich), wheat bran and corn bran (ferulic acid-rich). Vanillin was produced from the bound-ferulic acid *via* cleavage of the aliphatic double bond under the pressurised conditions. Higher content of ferulic acid in the corn bran yielded higher amounts of vanillin compared to wheat bran and flax shives. A simple and efficient purification procedure for ferulic acid from the alkaline extracts is presented. This procedure exploits the solubility of ferulic acid at different ethanol concentrations.

1. Introduction

Extraction of major phenolic compounds such as ferulic acid and vanillin from agricultural crop residues is important for the development of value-added products from renewable byproducts. Ferulic acid exhibits beneficial physiological effects such as antioxidant, anti-microbial, anti-thrombosis and anti-cancer activities (Ou & Kwok, 2004). Thus, it is widely used in food, pharmaceutical and cosmetic industries (Graf, 1992). Ferulic acid is present in many crop residues including wheat bran (0.5%), sugar-beet pulp (0.9%) and corn kernel (5%) (Barberousse et al., 2008; Mathew & Abraham, 2004). Ferulic acid is cross-linked with lignin and polysaccharides *via* ester and ether bonds, forming lignin/phenolics–carbohydrate complexes in crop residues and is released with dilute (0.1–4 M) NaOH solution at 50–70 °C (Barberousse et al., 2008; Fry, 1982; Saulnier, Chanliiaud, & Thibault, 1995; Sun, Sun, Wang, Zhu, & Wang, 2002). Ferulic acid can also be released enzymatically from plant materials by feruloyl esterases (Mathew & Abraham, 2004). However, the purification of ferulic acid from the brown alkaline extracts and fermentation broth is challenging. Complicated adsorption and desorption processes with activated charcoal and resin chromatography are employed for the purification of ferulic acid (Couteau & Mathaly, 1997), whilst natural ferulic acid is commercially produced from γ-oryzanol in rice oil (Ou et al., 2007).

Vanillin is an important compound widely used by the food industry for its aroma. Total consumption of vanillin is estimated to be 12,000 metric tonnes/year (Li & Rosazza, 2000). Also, a significant amount of vanillin is used as raw material by the pharmaceutical industry for the synthesis of drugs such as L-DOPA and methyl L-DOPA (Clark, 1990). Commercial production of vanillin from lignosulfonates, formed during pulping processes, is no longer allowed due to environmental concerns (Hocking, 1997). Currently, natural vanillin is produced enzymatically from ferulic acid in sugar-beet pulp, rice bran oil and maize bran (Lesage-Meessen et al., 1999; Priefert, Rabenhorst, & Steinbuchel, 2001; Zheng et al., 2007). This "nature identical" vanillin from plant sources is currently priced at about $1000/kg by Rhodia Organics Company (Cranbury, NJ). Thus, developing an alternative method for the production of natural vanillin at lower cost is desirable.

Pressurised low-polarity water extraction, also known as subcritical water extraction, modifies the properties of water by heating above 100 °C and keeping the pressure sufficiently high to

* Corresponding author. Tel.: +1 250 494 6376; fax: +1 250 494 0755.
E-mail address: mazzag@agr.gc.ca (G. Mazza).

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maintain water in a liquid state and thus improves its extraction capability. PLPW extraction has been compared and reported to be superior to conventional extraction techniques, due to its higher selectivity, cleanliness, speed, and cost savings in terms of material and energy consumption (Cacace & Mazza, 2006; Hawthorne, Grabanski, Martin, & Miller, 2000; Kim & Mazza, 2006). Flax shives are known to be rich in guaiacyl units (75%) (Buranov & Mazza, 2008; Day et al., 2005) that are the precursors of vanillin. Also, wheat and corn bran both contain a significant amount of ferulic acid (5 and 50 g/kg, respectively) which is also the precursor of vanillin (Ou & Kwok, 2004).

The purpose of this study was to investigate the extraction and purification of ferulic acid from agricultural crop residues, in particular flax shives, wheat and corn bran using pressurised low-polarity water (PLPW) extraction. Hydrothermal conversion of bound-ferulic acid in the crop residues into vanillin was also explored.

2. Materials and methods

2.1. Materials

Flax shives were provided by Biolin Research Inc. (Saskatoon, Canada) and stored at −35 °C until used. Flax shives were ground to a particle size of 0.5 mm with a rotor mill (Retsch Company, Germany). Wheat and corn bran were purchased from a local market (Penticton, BC) and stored at −35 °C until used. Wheat and corn bran were used as received for the extractions.

2.2. Extraction of phenolic compounds from biomass samples in a PLPW extractor

Extraction with pressurised solvents was carried out in a PLPW extractor as described previously (Buranov & Mazza, 2007). Milled flax shives (5 g), wheat bran (5 g), or corn bran (5 g) was packed into a stainless steel column (20 × 2 cm) with frits at both ends. Extraction commenced by pumping the solvent (0.5 M NaOH, 30% ethanol, 15% ammonia or water) directly into the PLPW extraction system and the pressure was increased to 5.2 MPa. The extracting solvent was pumped at a flow rate of 5 ml/min for 40 min. This was determined as optimal for 0.63 M NaOH and was previously described (Kim & Mazza, 2006). After extraction, the column was washed by pumping deionized water at room temperature, and the solid residue was recovered from the column. The liquid extracts were centrifuged at 16,282*g* for 5 min. The supernatant was filtered with a 0.45 μm syringe nylon filter. The filtrate was immediately analysed by HPLC as described in Section 2.5 to minimise/avoid the oxidation of vanillin to vanillic acid.

All alkaline extracts were neutralised with 6 M HCl to pH 7 prior to analysis. Due to the viscosity of alkaline extracts, 3X the volumes of 95% ethanol were added to precipitate hemicelluloses and glucomannans (Bobleter, 1994). The hemicellulose (from flax shives) and glucomannan (from wheat bran) precipitates were separated from the liquid phase by centrifugation at 11,657 *g* for 20 min at room temperature. The supernatant was vacuum evaporated until the ethanol concentration reached 30% as ferulic acid is soluble at this concentration. Further explanation is provided in Section 2.4. The extract was filtered with 0.45 μm nylon filter and the filtrate was analysed by HPLC for phenolic compounds as described in Section 2.5.

2.3. Ferulic acid extraction with non-pressurised alkaline solution and purification

Each biomass sample (5 g) was placed in an Erlenmeyer flask and attached to a condenser. A 150 ml aliquot of 0.5 M NaOH solution was added. The flask was heated and stirred continuously for 4 h at 50 °C (Ou et al., 2007). After hydrolysis, the extract was allowed to cool down to room temperature and the alkaline extract was neutralised with 6 M HCl. The hemicelluloses and glucomannans were precipitated by diluting 3-fold with 95% ethanol and the precipitate was separated with filtration. The filtrate was vacuum evaporated to remove excess ethanol. This resulted in the development of a brown extract containing ferulic acid. To determine the content of ferulic acid in the extract, 1 ml of extract was diluted with 1 ml anhydrous ethanol and filtered with a 0.45 μm nylon filter. The filtrate was analysed by HPLC for phenolic compounds according to Section 2.5.

Attempts to precipitate ferulic acid from the concentrated brown extract at room temperature and at temperatures below ambient were unsuccessful, and this may have been due to the presence of a lipophilic brown substance. The purification of ferulic acid was based on its solubility in different aqueous ethanol solutions and will be further discussed in Section 2.4. Ferulic acid is readily soluble in aqueous ethanol at concentrations ⩾30%. Therefore, 95% ethanol was added to the brown extract until the concentration reached 30%. This resulted in the development of a murky solution. The murky solution was centrifuged at 11,657 *g* for 20 min. The precipitate was vacuum dried at 45 °C and its FT-IR spectrum was determined. The supernatant was further vacuum evaporated leaving 20 ml of water extract and the precipitated ferulic acid became visible. To purify ferulic acid further, it was dissolved by adding 6 ml anhydrous ethanol, resulting in a less intense murky solution which was again further centrifuged at 11,657 *g* for 20 min. The supernatant (mostly ethanol) was vacuum evaporated to precipitate ferulic acid. The FT-IR spectra of ferulic acid and the precipitate from the murky solution were compared.

2.4. Determination of the solubility of pure ferulic acid in aqueous ethanol

The solubility of pure ferulic acid at different concentrations of ethanol was determined according to the equilibrium solubility method (Loftsson & Hreinsdóttir, 2006). For this, an excess amount of pure ferulic acid (∼100 mg) was added to 1 ml of water and aqueous ethanol of different concentrations. The suspension was heated in an autoclave at 121 °C for 20 min. After cooling to room temperature, a small amount of ferulic acid (∼1 mg) was added to promote precipitation and the vial resealed (Yalkowsky & Banerjee, 1991). After equilibration at room temperature for 3 days, the suspension was filtered through a 0.45 μm syringe nylon filter and the solution was analysed by HPLC according to Section 2.5.

2.5. Analysis of phenolic compounds in the extracts by HPLC

The neutral extracts were analysed for free phenolics (ferulic acid, p-coumaric acid and vanillin) by HPLC. The analytical HPLC system employed consisted of an Agilent 1100 high performance liquid chromatograph equipped with a diode array detector. The HPLC pump (model G1312A), autosampler (model G1329), column temperature, and diode array system were monitored and controlled using Agilent Chemstation Plus software (Agilent Technologies, Palo Alto, CA). Phenolic acid separation was performed on a reversed phase Zorbax SB-C18 (5 μm, 3 × 250 mm) column, preceded by a guard column with a cartridge (4.6 × 12.5 mm, Polypore CA 10 μm). The mobile phases were 50 μM phosphoric acid (solvent A) and methanol (solvent B). The methanol gradient was 5–55% from 0 to 51 min, 55–100% from 51 to 61 min, 100% from 61 to 68 min, 100–5% from 68 to 73 min, 5% from 73 to 83 min. Data were collected with a diode array detector between 210 and 400 nm and absorbance was monitored at 280 nm. Concentrations of phenolic compounds were calculated using standards

(0.2–5 mg/ml). Authentic phenolic compounds were obtained from Sigma–Aldrich Canada Ltd. (Ontario, Canada).

2.6. FT-IR spectra

FT-IR spectra of isolated ferulic acid and brown precipitate (wax) were acquired on a FT-IR spectrometer "Nicolet-380" (Thermo Fisher Scientific, Madison, WI, USA) using a KBr disc containing 1% finely ground samples. KBr discs were prepared by pressing the mixture of KBr and samples with a manual Qwik handi-press (Hamilton, 1995; Kunst & Samuels, 2003).

2.7. Separation of polymeric and oligomeric hemicelluloses via ultrafiltration

Separation of polymeric and oligomeric hemicelluloses from the neutralised alkaline extracts *via* ultrafiltration was carried out. Alkaline extracts were neutralised with HCl and 95% ethanol was added to make the ethanol concentration in the extract 35%, thereby allowing ferulic acid to be soluble in higher amounts (15 mg/ml) whilst wax and glucomannans remained insoluble. The precipitated wax and glucomannans were centrifuged at 16,282 *g* for 20 min at 20 °C. The supernatant was placed in the ultrafiltration unit under nitrogen pressure at 0.345 MPa (AMICON 8400, Millipore Corp., MA, USA). A polysulfone ultrafiltration (UF) membrane with a molecular weight cutoff of 30,000 (Sterlitech Corp., Kent, Washington) was used, in sequence, to separate polymeric hemicelluloses, oligomeric hemicelluloses and monomeric compounds (ferulic acid, p-coumaric acid, etc.). Polymeric hemicelluloses remained in the vessel and were dried at room temperature. Oligomeric hemicelluloses were recovered from the concentrated aqueous filtrate *via* ethanol precipitation. Pure ferulic acid was recovered through precipitation and by vacuum evaporation of water.

2.8. Thin-layer chromatography test

Thin-layer chromatography (TLC) was performed on 10 × 20 cm silica gel TLC plates (13179 SILICA GEL No. 6061, Rochester, NY). Plates were dried at 110 °C for 30 min before use. After sampling by a capillary tube, TLC plates were saturated in a developing chamber containing benzene–ethyl acetate–formic acid (12:4:1, v/v/v) and detection was performed with a UV lamp.

3. Results and discussion

3.1. Extraction of phenolic compounds from flax shives, wheat bran and corn bran

The yields of phenolic compounds from flax shives, wheat and corn bran by non-pressurised alkaline hydrolysis and pressurised solvent extraction procedures are shown in Table 1. Non-pressurised alkaline hydrolysis has been used for total release of ferulic acid by breaking the ester bonds in the lignin/phenolics–carbohydrate complexes in agricultural crop residues (Lam, Iiyama, & Stone, 1992; Smith & Hartley, 1983; Sun et al., 2002). The alkaline hydrolysis used in this study was for the quantification of ferulic acid in the biomass as proposed by Ou et al. (2007). The results indicated that the content of ferulic acid in flax shives (25 mg/100 g) was much lower than in wheat (391 mg/100 g) and corn bran (2510 mg/100 g).

Also, the overall content of hydroxycynnamic acids (ferulic and p-coumaric acids) in flax shives was lower (86 mg/100 g) than in wheat (402 mg/100 g) and corn bran (2860 mg/100 g). However, flax shives contained more p-coumaric acid (61 mg/100 g) than ferulic acid (25 mg/100 g). Our results are in agreement with the published results (Barberousse et al., 2008). These clear differences in the contents of ferulic acid served as the main criterion to initiate the current study on the effect of pressurised conditions. Optimal conditions for the pressurised extractions were chosen, based on previous optimisation studies on pressurised alkaline extraction of phenolic compounds from flax shives (Buranov & Mazza, 2007; Kim & Mazza, 2006). In these studies, a temperature of 180 °C and a flow rate of 5 ml/min for 40 min were optimal for the pressurised alkaline extraction (0.63 M NaOH) of phenolic compounds from flax shives. These conditions were kept constant throughout the current study and compared with other pressurised solvents namely PLPW, PAA and PAE.

Pressurised alkaline extractions gave results similar to those from the non-pressurised alkaline extraction for all flax shives,

Table 1
Yields of phenolic compounds extracted from flax shives, wheat bran and corn bran by non-pressurised alkaline solution and pressurised solvents.

Exp.#	Extraction methods	Biomass	Mass (g)	Extracting solvents and temperature, (°C)	Yields (mg/100 g)				
					Ferulic acid	p-Coumaric acid	Vanillin	Vanillic acid	Acetovanillone
1[a]	Non-pressurised	Flax shives	5	0.5 M NaOH; 50 °C	25 ± 10	61 ± 10	48 ± 3	14 ± 1	4 ± 0.5
2	Non-pressurised	Wheat bran	5	0.5 M NaOH; 50 °C	391 ± 50	20 ± 5	11 ± 2	–	–
3	Non-pressurised	Corn bran	5	0.5 M NaOH; 50 °C	2510 ± 50	350 ± 50	55 ± 5	–	–
4[b]	Pressurised	Flax shives	5	0.5 M NaOH;180 °C	18 ± 10	46 ± 10	46 ± 4	14 ± 2	11 ± 1
5	Pressurised	Flax shives	5	PAA; 180 °C	–	–	57 ± 3	16 ± 1	19 ± 2
6	Pressurised	Flax shives	5	PLPW;180 °C	8.4 ± 3	–	44 ± 3	41±1	18 ± 2
7[c]	Pressurised	Flax shives	5	PLPW;220 °C	7.8±3	–	102 ± 3	74±3	42 ± 2
8	Pressurised	Flax shives	5	PAE;180 °C	9.0 ± 3	–	24 ± 3	34 ± 2	15 ± 1
9	Pressurised	Flax shives	5	PAE 220 °C	8.5 ± 3		91 ± 3	76 ± 3	45 ± 2
10	Pressurised	Wheat bran	5	0.5 M NaOH;180 °C	391 ± 50	20 ± 5	11 ± 2	7 ± 1	–
11	Pressurised	Wheat bran	5	PLPW; 180 °C	97 ± 20	2 ± 0.5	20 ± 5	7 ± 1	–
12	Pressurised	Wheat bran	5	PLPW; 220 °C	63 ± 20	1 ± 0.5	34 ± 5	8±1	–
13	Pressurised	Wheat bran	5	PAE; 180 °C	65 ± 10	0.00	23 ± 5	6±1	–
14	Pressurised	Wheat bran	5	PAE; 220 °C	115 ± 5	2 ± 0.5	34 ± 5	8±1	–
15	Pressurised	Corn bran	5	0.5 M NaOH; 180 °C	2510 ± 50	350 ± 50	55 ± 5	–	–
16	Pressurised	Corn bran	5	PLPW; 180 °C	404 ± 30	68 ± 10	132 ± 20	–	–
17	Pressurised	Corn bran	5	PLPW; 220 °C	334 ± 20	70 ± 10	168 ± 30	–	–
18	Pressurised	Corn bran	5	PAE; 180 °C	197 ± 20	17 ± 5	41 ± 10	–	–
19	Pressurised	Corn bran	5	PAE; 220 °C	423 ± 30	68 ± 5	165 ± 20	–	–

[a] Conditions for non-pressurised alkaline-hydrolysis: T = 50 °C, P = Atm., biomass/alkali = 1:30, time = 4 h, n = 4.
[b] Conditions for pressurised extractions: T = 180 °C, P = 5.2 MPa, flow rate = 5 ml/min, time = 57 min, n = 4.
[c] Conditions for the extractions at 220 °C: T = 220 °C, P = 8 MPa, flow rate = 5 ml/min, time = 57 min, n = 4.

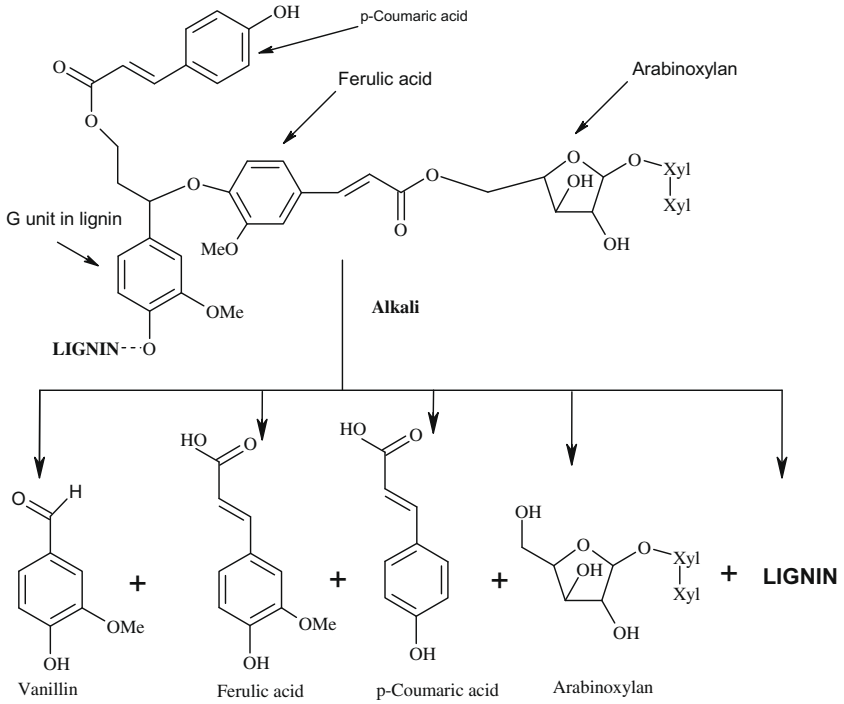

Fig. 1. Cleavage of lignin/phenolics–carbohydrate complexes in flax shives with alkali (Adapted from Buranov and Mazza, 2008).

wheat bran and corn bran (Table 1 and Fig. 2A, B, D, E, G and H). Both pressurised and non-pressurised alkaline extractions show vanillin in the biomass samples and also ferulic and p-coumaric acids (Figs. 1 and 2A, B). This confirms that flax shives and corn bran contain more vanillin (48 mg/100 g and 55 mg/100 g) than wheat bran (11 mg/100 g). Vanillin yields were similar with the non-pressurised and pressurised alkaline extractions, but it increased significantly during the PLPW, PAE and PAA extractions, confirming the formation of vanillin from bound-ferulic acid under pressurised conditions (Figs. 2C,F,I and 3). The vanillin yield from flax shives with PLPW, PAE and PAA conditions increased slightly (~100 mg/100 g at 220 °C), confirming that additional vanillin is probably formed from the bound-ferulic acid, the content of which is significantly lower in flax shives (25 mg/100 g) (Table 1 and Fig. 2C). Traces of hydroxycynnamic acids were detected. In flax shives, the major source of vanillin must be from bound-ferulic acid not from the degradation of the lignin polymer. The lignin polymer in flax shives belongs to hardwood lignins and it is mainly composed of guaiacyl (G) (75%) and syringyl (S) (25%) units (Day et al., 2005; Tapin, Sigoillot, Asther, & Petit-Conil, 2006). The G units in the lignin polymer might be the major source of vanillin (Fig. 1). However, pressurised conditions are not adequate for the formation of vanillin from the lignin polymers of flax shives, but ferulic acid was degraded into vanillin under these pressurised

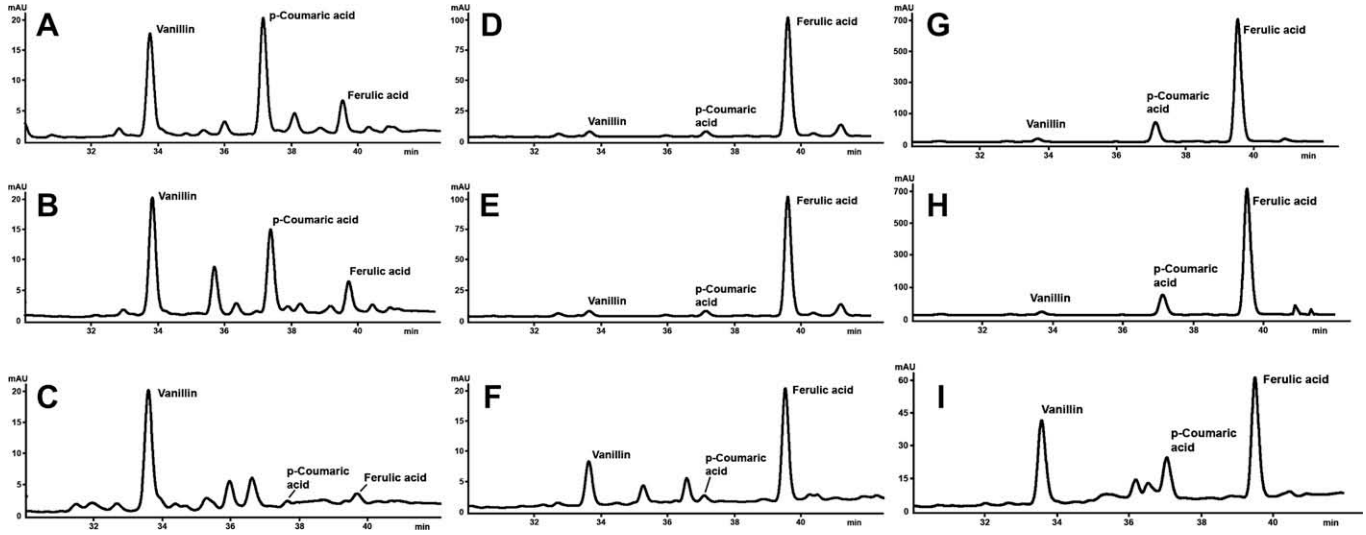

Fig. 2. Comparison of HPLC chromatograms of phenolic compounds from flax shives, wheat bran and corn bran. Extraction conditions: non-pressurised alkaline hydrolysis: 200 ml of 0.5 M NaOH, 50 °C for 4 h; pressurised alkaline extraction: 200 ml of 0.5 M NaOH, 180 °C, 5.2 MPa, 40 min, flow rate of 5 ml/min; PLPW: 200 ml of water, 180 °C, 5.2 MPa, 40 min, flow rate of 5 ml/min. A–C – Non-pressurised alkaline hydrolysis, pressurised alkaline extraction and PLPW extraction of flax shives; D–F – Non-pressurised alkaline hydrolysis, pressurised alkaline extraction and PLPW extraction of wheat bran; G–I – Non-pressurised alkaline hydrolysis, pressurised alkaline extraction and PLPW extraction of corn bran.

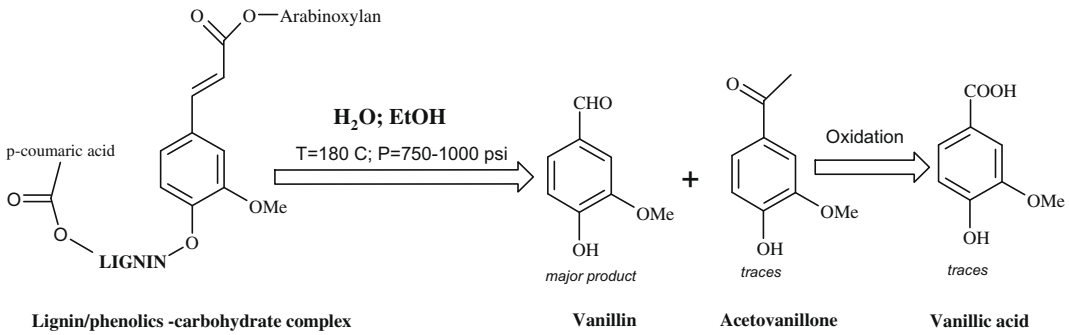

Fig. 3. Proposed reaction for degradation of the bound-ferulic acid in the biomass into vanillic acid, vanillin and acetovanillone under pressurised conditions.

conditions, which was also confirmed with the wheat and corn bran.

Alkali extraction (both non-pressurised and pressurised) of wheat bran indicated the presence of small amounts of vanillin (11 mg/100 g) which increased further under PLPW and PAE. Pressurised and non-pressurised NaOH solution was found inadequate for converting bound-ferulic acid into vanillin (Fig. 2D,E). PLPW and PAE lead to an increased formation of vanillin in the wheat bran (34 mg/100 g at 220 °C). This is a clear indication that vanillin is formed from the bound-ferulic acid in wheat bran (Fig. 3). However, there is still a significant amount of ferulic acid (115 mg/100 g) that was not converted into vanillin (34 mg/100 g) at 220 °C with both PLPW and PAE.

This same trend was observed with corn bran resulting in higher yields of vanillin (165 mg/100 g) reflecting its higher content of ferulic acid (2510 mg/100 g). The vanillin yield increased to 165 mg/100 g at 220 °C with PAE and PLPW. Similar to the wheat bran, significant amounts of ferulic acid in the corn bran (423 mg/100 g) were not converted into vanillin. Additional work is required to improve the conversion of the bound-ferulic acid in the biomass into vanillin, perhaps with the use of oxidants such as air, oxygen, hydrogen peroxide and ozone. Currently, the vanillin production is mainly done enzymatically in a two-step procedure, (1) enzymatic release of ferulic acid from agricultural crop residues and (2) enzymatic conversion of ferulic acid into vanillin, which requires considerable efforts to increase the yield and purity (Lesage-Meessen et al., 1999; Zheng et al., 2007).

Pressurised conditions can be used to extract vanillin from the bound-ferulic acid in crop residues through a one-step procedure which would help reduce the production cost greatly. PLPW, PAE and PAA were shown to be more efficient and superior to both pressurised and non-pressurised 0.5 M NaOH solution in the production of vanillin from the lignin/phenolics–carbohydrate complexes in flax shives, wheat bran and corn bran (Table 1). Under pressurised conditions, vanillin is likely formed from the cleavage of a double bond in the ferulic acid unit and ether bond between lignin and ferulic acid in the lignin/phenolics–carbohydrate complexes (Fig. 3). Thermal decomposition of free cinnamic, p-coumaric, ferulic acid, and sinapic acids was previously observed between 230 and 500 °C in a self-generated atmosphere. The products of thermal degradation were identified as vanillin and guaiacol (Shopova & Milbova, 1998).

The pressurised extraction conditions are ideal for the cleavage of the double bond in the bound-ferulic acid leading to higher amounts of vanillin (an aldehyde) and acetovanillone (a ketone) without an oxidant (Lee & Chen, 1991). Weak nucleophiles (water, ethanol and ammonia) are an efficient medium for selective cleavage of double bonds in the bound-ferulic acid at high temperature (180 °C) and pressure 5.17 MPa (750 psi). Good nucleophiles, such as sodium hydroxide, seem inefficient for the selective cleavage of double bonds; instead they hydrolyse ester groups leading to the formation of free ferulic acid. Perhaps, the use of "green" oxidants as additives can improve the vanillin yield, but the use of strong oxidants may be undesirable because it will oxidise vanillin into vanillic acid (Bailey, 1978). Therefore, pressurised extraction conditions may serve as an economical way of producing vanillin from agricultural crop residues with higher ferulic acid content, such as wheat bran, corn kernel, and sugar-beet pulp in one-step by choosing the right extraction/reaction conditions. The vanillin produced *via* pressurised solvent extraction is considered "natural", since it is extracted from crop residues with water or aqueous ethanol. The production cost of vanillin should be quite low, similar to synthetic vanillin ($20/kg), because enzymes are not required. However, in order to make this process commercially attractive the yield needs to be improved *via* "green" additives.

3.2. Purification of ferulic acid

The purification of ferulic acid from alkaline extracts was based on the solubility of ferulic acid in ethanol. Ferulic acid is insoluble in water at room temperature but is soluble in hot water, ethanol, ethyl acetate and ethyl ether (The Merck Index, 1996). Ethanol (60%) was reported to be suitable for the extraction of ferulic acid (Guo, Sun, & Li, 2003) and our strategy was focused on its solubility in aqueous ethanol. The solubility of pure ferulic acid was determined using different ethanol concentrations (Fig. 4). Significant amount of ferulic acid (~13 mg/ml) were dissolved in between 30 and 35% ethanol at room temperature.

In purifying ferulic acid, hemicellulose was precipitated from the neutralised extracts by diluting it 3-fold with 95% ethanol. Attempts to precipitate ferulic acid from the concentrated extract after vacuum evaporation were unsuccessful due to the presence of a lipophilic brown substance. This is the main concern in alkaline extracts of crop residues, because the purification procedure of ferulic acid from this brown substance uses activated charcoal and resin exchange chromatography (Couteau & Mathaly, 1997; Ou et al., 2007). This purification process is costly and precludes the production of ferulic acid from crop residues. In this study, an alternative and simple approach was used to overcome this problem. This oily substance was precipitated from the extracts by adding anhydrous ethanol to an ethanol concentration of 30%. Since ferulic acid is soluble in 30% aqueous ethanol at room temperature (Fig. 4) and the lipophilic brown substance remained insoluble, separation was readily achieved by centrifugation. The FT-IR spectra of this oily brown substance revealed that it was wax. Repeating this procedure twice enriched the ferulic acid content. Perhaps the precipitation of ferulic acid in the presence of wax (lipophilic brown substance) was not possible due to the adsorptive forces of the polymeric wax. The TLC tests also indicated that the purity of ferulic acid was high and had the same

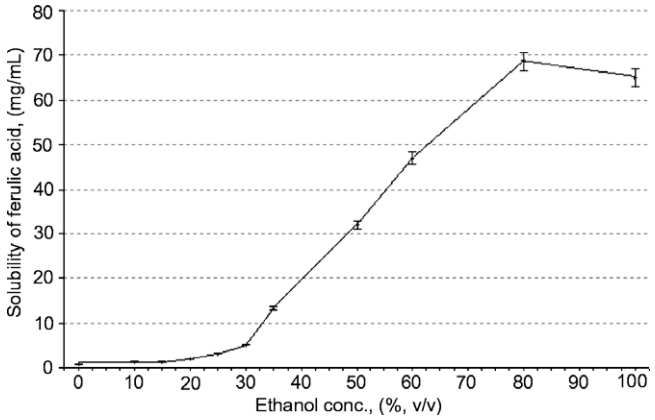

Fig. 4. Solubility of ferulic acid in various ethanol concentrations at room temperature.

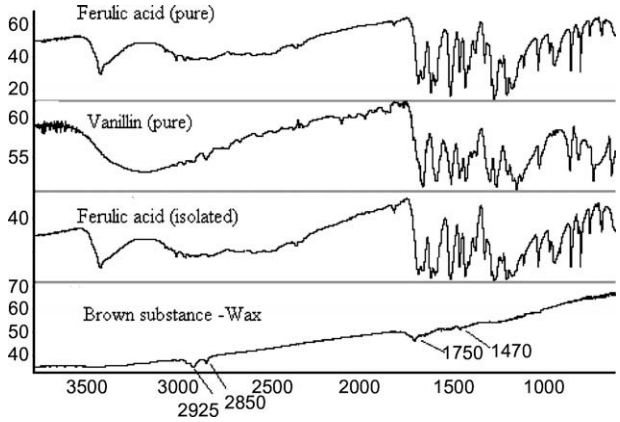

Fig. 5. FT-IR spectra of ferulic acid and wax fractions.

R*f* value as the standard. Traces of impurities (wax and oligomeric hemicelluloses) on the TLC plates completely disappeared. Defatting biomass prior to extractions would require additional cost and may modify the bound-ferulic acid.

Furthermore, the purification was carried out *via* ultrafiltration technique in order to minimise the use of ethanol and to separate polymeric and oligomeric hemicelluloses. Keeping ferulic acid in solution, wax and glucomannans were removed from the extract, containing 30% ethanol. Further polymeric hemicelluloses were isolated *via* ultrafiltration and oligomeric hemicelluloses were precipitated with ethanol. Finally, the enriched ferulic acid was precipitated from the vacuum evaporated extract.

The FT-IR spectra of isolated ferulic acid and wax are illustrated in Fig. 5. Isolated ferulic acid produced a similar spectrum with the pure ferulic acid, confirming its high purity. The precipitated wax gave signals similar to the functional groups of waxes, which contain mainly ester groups and carbonyl groups, giving clear signals at 2925 cm^{-1} and 1750 cm^{-1}, respectively. The C–H bending is observed at about 1470 cm^{-1} (Hamilton, 1995; Kunst & Samuels, 2003). FT-IR spectrum of the wax showed that there was no trace of ferulic acid which further confirms the efficiency of the purification procedure.

4. Conclusions

Pressurised water and ethanol are promising for the production of vanillin from crop residues in a one-step process. The source of vanillin in the biomass under pressurised conditions of water and ethanol is derived from ferulic acid in the lignin/phenolics–carbohydrate complexes. Production of vanillin from crop residues containing higher amounts of ferulic acid such as corn bran and sugar-beet pulp with pressurised solvents may be practical and cost-effective. However, more work needs to be done to increase the yield of vanillin.

Purification of ferulic acid from alkaline extracts is greatly simplified by exploiting its solubility. The brown alkaline extracts, which were primarily waxes, can be easily precipitated with 30% aqueous ethanol and therefore, the commercial production of ferulic acid from crop residues seems feasible.

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