JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 3697−3703 **3697**

Feruloyl Esterase Utilization for Simultaneous Processing of Nonwood Plants into Phenolic Compounds and Pulp Fibers

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Wheat and oilseed flax straws were studied as raw material for papermaking. Two different aspects were investigated to valorize these agricultural byproducts: the capacity to recover some phenolic compounds and the use of the resulting cellulose fibers in papermaking. Straw phenolic compound composition was analyzed to determine the different accessible molecules and their available quantity. Ferulic acid, coumaric acid, vanillic acid, and vanillin were found in both wheat and oilseed flax straws. To enhance the release of these phenolic compounds, enzymatic treatments with feruloyl esterase and xylanase, two enzymes known for their role in lignin destructuration, were tested. These treatments increased the amount of phenolic compounds released, destructured hemicellulose and lignin, and improved the soda cooking conditions of pulps with the reduction of chemical charge need in the papermaking process. Phenolic compound production from this process could enhance the cost-effectiveness of papermaking from annual plants.

KEYWORDS: Wheat straw; oilseed flax straw; lignin; pulp and paper; phenolic compounds

INTRODUCTION

Nonwood fibers look promising for economic and environmental reasons. As compared to wood, they are low-cost agricultural byproducts and represent a short-term renewable fiber source. Wheat straw is an agricultural raw material often used for the production of pulp and paper, mainly in the Southeast Asian countries (*1*−*3*). Nonwood fibers are usually short fibers. However, flax (*Linum usitatissimum*), an herbaceous annual plant grown mainly for the production of fibers (linen) or oilseeds, contains mainly long fibers and can be used to produce fibers of superior quality (*4*). Textile flax and oilseed flax are two different varieties of plants. Textile flax was more studied and has been largely used for the production of thin, strong sheet papers such as used in cigarette, Bible, and fine paper production (*5*). Oilseed flax is grown only for its seeds. The plant is smaller with shorter fibers but a more lignified stem than textile flax (*6*). Currently, seed flax straw is burned after harvest. A small part of production is used to produce a lower fiber grade for, for example, certain reinforced composites, geotextiles, and insulation materials (*7*−*9*). Therefore, the use of oilseed flax fibers seems to be a promising alternative in annual plant use. Several Canadian researchers published studies on the use of oilseed flax chemical pulps in blends with hardwood and/or softwood pulps (*10*, *11*). They reported improvement in pulp strength properties due to the incorporation of flax. However, the use of these byproducts for papermaking presents several disadvantages due to the cost of collection and storage of the straws after harvest, the significant size of installation required, and the accelerated wear of paper machinery due to the high silica content. Therefore, the cost-effectiveness of such a process depends on the development of additional value from the straw. The production of phenolic compounds such as hydroxycinnamic acids should enhance the overall profitability of the process. Hydroxycinnamic acids, particularly ferulic acid (4-hydroxy-3-methoxycinnamic acid) and *p*-coumaric acid (4-hydroxycinnamic acid), occur widely in lignin and hemicellulose, constituting with cellulose the cell walls of graminaceous plants such as cereal straw (*12*). Ferulic acid is of economic interest as it is a vanillin precursor and a strong antioxidative molecule. It is a highly valuable additive that is used in the food industry and has pharmacological and cosmetic applications (*13*, *14*).

As the production of paper pulps aims to remove lignin and hemicellulose to obtain mainly cellulose fibers, valorization of residual phenolic compounds should be considered.

The objectives of this work were (i) to determine the chemical composition of the wheat and oilseed flax straws in order to obtain a better knowledge of the phenolic content of raw material, (ii) to determine the value of the phenolic compounds that are potentially extractible as "natural" products by a

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10.1021/jf052725y CCC: $33.50
Published on Web 04/20/2006

nonchemical process such as enzymatic treatment, and (iii) to produce pulp and paper with enhanced properties allowing uses such as in specific papers.

MATERIALS AND METHODS

Raw Material. Oilseed flax straw used for these experiments was cultured in the Gers region (France) for oilseed production and harvested in 2000. It was not retted but was partially decorticated, and the dust was removed. Wheat straw was also produced in France and harvested in 2000. It was sliced into ~30 mm length fragments.

The whole stem of the plant after seed removal was used as raw material for phenolic compound recovery and papermaking. Straws were ground and allowed to work with homogeneous samples. Grinding was applied with industrial methods.

Lignin Determination. Lignin content of straws was determined by Klason lignin analysis according to Tappi standards (1983). The insoluble lignin residue was obtained after milled straw treatment with a mixture of 72% H_2SO_4 for 2 h at room temperature. Thereafter, the plant material was boiled in diluted (4%) acid, washed with water, and dried.

Lignin content of pulps was determined with kappa number according to the standard NFT 12007. This index characterizes the delignification degree of cellulosic pulps. It corresponds to the quantity of potassium permanganate N/10 in milliliters consummated in standard conditions per gram of pulp. Results are reported at a value corresponding to 50% of permanganate consummated.

The content of β-O-4 linked monomers was determined by the thioacidolysis method, developed by Lapierre et al. (*15*), on the milled straws. The released guaiacyl (G) and syringyl (S) monomers were separated by capillary gas chromatography as trimethylsilyl derivatives. The column used is a J&W Scientific DBS (30 m × 0.25 mm; film = 0.25 μm). Four milliliters of sample was injected. Injector and detector temperatures were, respectively, 260 and 270 °C. The program of the temperature gradient was as follows: 40 °C in 1 min; 40 °C/min to 160 °C; 4 °C /min to 260 °C; 20 min at 260 °C; 2 °C/min to 270 °C; 10 min at 270 °C.

Phenolic Compound Content. To determine the phenolic composition of the wheat and flax straws, milled straws were treated with 4 M NaOH for 2 h at 110 °C. After acidification and extraction with ethyl ether, the ether layers were dried and the pellet was resuspended in 50% methanol. The phenolic compounds extracted were analyzed by HPLC with a Hewlett-Packard HPLC 1050 instrument using a reverse phase Chromolith column with a photodiode array detector at room temperature. Compounds were separated by an acetonitrile gradient. Identification of the compounds was confirmed by a LC-MS analysis.

Supernatants obtained after enzymatic treatment were concentrated under vacuum to be at the proper concentration for HPLC analysis.

Enzymes. Xylanase from a genetically modified *Bacillus* strain was supplied by Novo Nordisk (Bagsvaerd, Denmark). One unit was defined by the supplier as the amount of enzyme liberating 1 μmol of reducing sugar (measured as xylose equivalent) from xylan per minute at 50 °C and pH 5.

Feruloyl esterase (FAE) was produced by an *Aspergillus niger* recombinant strain as described by Record et al. (*16*). For esterase production, cultures were inoculated with 1×10^6 spores/mL in 300 mL flasks. Supernatants were harvested after 7 days of cultivation at 30 °C and 120 rpm stirring. Esterase purification included ultrafiltration (10 kDa cutoff), dialysis [50 mM phosphate buffer, pH 7, with 1 mM EDTA and 0.6 $(NH_4)_2SO_4$], and phenyl-Sepharose 6 chromatography (same buffer) using a linear $(NH_4)_2SO_4$ gradient (0.6−0 mM in 50 mM phosphate buffer, pH 7, with 1 mM EDTA). FAE was assayed using methyl ferulate as substrate. One activity unit was defined as the amount of enzyme that catalyzes the release of 1 μmol of ferulic acid per minute.

Enzymatic Treatment of Straws. The FAE treatment was conducted using 20 units/g on wheat or oilseed flax straws, in pH 5 acetate buffer, at 40 °C, overnight at 3% consistency. The xylanase treatment was conducted at 1 unit/g, in pH 5 acetate buffer at 50 °C for 2 h at 3% consistency in agitated flasks. When the two enzymes were used together, FAE conditions were chosen as 20 units/g of FAE and 1 unit/g of xylanase.

After enzymatic treatment, a light alkaline extraction was applied. Straws were treated with 2% NaOH (w/w) at 70 °C for 90 min at 5% consistency.

Effluent Treatment. Effluents from enzymatic and light alkaline treatment were filtered. Filtrates were submitted to 2 N NaOH at 35 °C for 30 min. Phenolic acids were measured before and after alkaline treatment by HPLC analysis.

Cooking Conditions. Cooking experiments were carried out at laboratory scale with flax and wheat straws, in a digester containing six autoclaves of 4 L immersed in a temperature-regulated oil bath. Different alkali charges (20−22%) and cooking times (60−120 min) were tested for constant liquor-to-straw ratio (4:1), temperature (170 °C), and time to reach cooking temperature (60 min). The best conditions to cook the flax straw were determined to obtain a homogeneous chemical pulp with a kappa number of ~10, in which the long fibers were cooked enough. Cooking conditions retained for further experiments were 20% NaOH and 120 min for decorticated flax and 22% NaOH and 60 min for wheat straw.

After cooking and screening, pulps were refined with a PFI mill according to the international standard NF EN 25264-2. This PFI mill processes pulp under laboratory conditions that simulate the commercial refining process. The PFI mill develops the fibrillation and bonding potential of papermaking fibers. It consists of an internal roll with beating bars revolving inside a heavy bowl.

Pulp Quality and Fiber Characteristic Evaluation. Optical and physical measurements were performed on Rapid Kothen handsheets of 70−75 g/m^2, according to international standards by the following properties: freeness, ISO 5267-1; tensile, NF EN ISO 1924-2; tear index, NF EN 21974; bulk, ISO 5270; and brightness, ISO 2470.

RESULTS AND DISCUSSION

Straws were submitted to an enzymatic treatment (feruloyl esterase or xylanase) with or without steam pretreatment (**Figure 1**). Effluents were analyzed for phenolic compounds. Straws, enzymatically treated and untreated, were cooked with soda, and phenolic compounds were extracted from black liquors and analyzed. The physical properties of the paper fabricated from unbleached pulp were determined to check the influence of pretreatment on the pulp quality.

Cinnamoyl Acid Distribution in Wheat and Flax straws. *Lignin.* The wheat and oilseed decorticated flax straws contained 19% lignin and 11% lignin, respectively, as measured by the Klason method.

A thioacidolysis analysis was performed, and the guaiacyl−syringyl (G−S) composition of the lignin monomers of wheat and decorticated flax straws was determined. Hydroxyl monomers of lignin (H forms) represent only 10% of total lignin monomers. These results (**Figure 2**) are consistent with the results obtained by pyrolysis analysis by Martinez in 2001 (*17*) on wheat straw and by Morrison in 2003 (*18*) on flax straw.

Flax contained less lignin than wheat straw, and thioacidolysis gave mainly G subunits (75% G for 25% S). For wheat straw G and S subunits were 48 and 52%, respectively. The S/G ratio was lower for flax straw, indicating a more condensed lignin than for wheat straw.

Phenolic Compounds. Both wheat and flax straws were submitted to an alkaline hydrolysis to release phenolic monomers linked by a β-O-4 bond.

The phenolic compounds separated by HPLC were identified by their relative retention times and UV absorption spectra. Spectra observed by HPLC are presented in **Figure 3**. Identification of the compounds was confirmed by LC-MS analysis.

Four main monomeric phenolic compounds (**Figure 4**) were found in straw hydrolysates: ferulic acid (FA), *p*-coumaric acid (pCA), vanillic acid (VaA), and vanillin (Va), as shown in **Figure 5**. Wheat straw lignin contained more alkali extractable phenolic compounds than flax straw. This is probably related

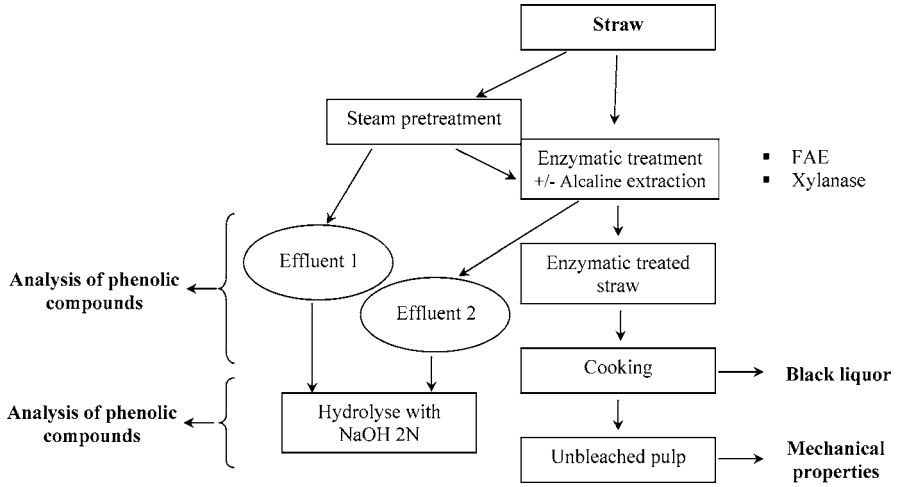

Figure 1. Diagram of straw treatment.

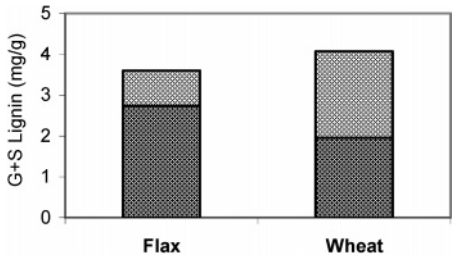

Figure 2. Distribution of G (heavy shading) and S (light shading) monomers of lignin in flax and wheat straws.

to the higher lignin content of wheat straw. However, flax straw contained more vanillin than wheat straw. This is probably related to differences in biosynthetic pathways of aromatic compounds. As seen in lignin composition, the S/G ratio is about 1 in wheat straw and about 0.33 in flax straw, indicating a preferential pathway to increasing proportionally ferulic acid related compounds in flax, including vanillin (*19*). The low content in ferulic acid could be explained by the low content in hemicelluloses (*20*), a source of easily extractable ferulic and coumaric acids.

Phenolic Compound Recovery from Enzymatic Treatment. *Effect of pH on Compound Release.* Enzymatic treatments have to be applied in media buffered at optimal pH to obtain an optimal enzyme activity. Three different pH conditions were studied to determine the influence of buffer in phenolic compound recovery: citrate buffer at pH 3, acetate buffer at pH 5, or phosphate buffer at pH 7. Trials were run at 5% consistency during 2 h at 50 °C under 200 rpm stirring. The quantities of the different phenolic compounds released as measured by HPLC are shown in **Figure 6**. The best condition for releasing these phenolic compounds in water was pH 5 for flax and wheat straws. This condition represents the best compromise between optimal pH of feruloyl esterase (*21*) and xylanase. Treatment in more acidic conditions seemed to be less efficient.

Effect of Enzyme Treatment. Two different enzyme treatments were applied on the straws, feruloyl esterase (FAE) and xylanase (X). FAE , an enzyme produced by the *Aspergillus niger* fungus, is able to cleave the ester bonds between ferulic acid and arabinose in hemicelluloses (*22*). Cleavage of these ester bonds allows the separation of hemicellulose layers and the release of fragments of lignin located between the layers (*23*). X degrades hemicelluloses due to cleavage of β-O-4 bonds (*24*).

The release of phenolic compounds was compared for both enzyme treatments, followed or not by alkaline extraction (AE), and for treatment using both enzymes simultaneously (**Figure 7**).

For wheat straw, a xylanase treatment seemed to have no effect on the release of phenolic compounds as compared to the reference. Moreover, X treatment followed by alkaline extraction (2% NaOH, 70 °C, 90 min) increased the release of FA and pCA by ∼14%. The same behavior could also be observed for FAE treatment. FAE without alkaline extraction allowed higher release of phenolic compounds than X in the same conditions. FAE followed by alkaline extraction increased the released of FA and pCA by ∼20%.

When the two enzymes were used together, results were similar to that obtained with xylanase alone, showing no synergistic effect.

Flax straw behaved similarly as wheat straw, but a lower extraction yield was observed, due to the lower initial content (**Figure 2**).

Effect of Steam Pretreatment. Straws were submitted to a steam pretreatment to increase the recovery of aromatic compounds. Erlenmeyer flasks containing straws at 6% consistency in water were autoclaved for 20 min at 120 °C. Acetate buffer (0.1 M, pH 5) and 1 unit of X or 20 units of FAE per gram of straw were added to this steam-treated straw.

Figure 8 shows the effect of presteaming on the concentration of coumaric acid released by enzymatic treatments or alkaline extraction from wheat straw. Only coumaric acid data are given, but the other phenolic compounds had the same behavior in these conditions. Similar results were obtained with the flax straw (data not shown) with an increase of the phenolic compounds released after the steam pretreatment.

The steam pretreatment facilitated the release of the four phenolic compounds. Heating by steam should have an effect on the hemicelluloses and lignin layers, opening the structures and enabling the phenolic compounds to be more accessible for enzymatic or chemical treatments.

Hydrolysis of the Enzymatic Effluent Treatment. After filtration, phenolic compound contents in effluents released by enzymatic and alkaline extraction were measured. HPLC allows only the determination of free monomers. Hydrolysis with 2 N NaOH at 35 °C for 30 min of the effluent increased the amount of phenolic compounds titrated by HPLC, showing the presence of esterified forms (released by the alkaline treatment). Ether linkages are stronger and cannot be cleaved by alkaline hydrolysis.

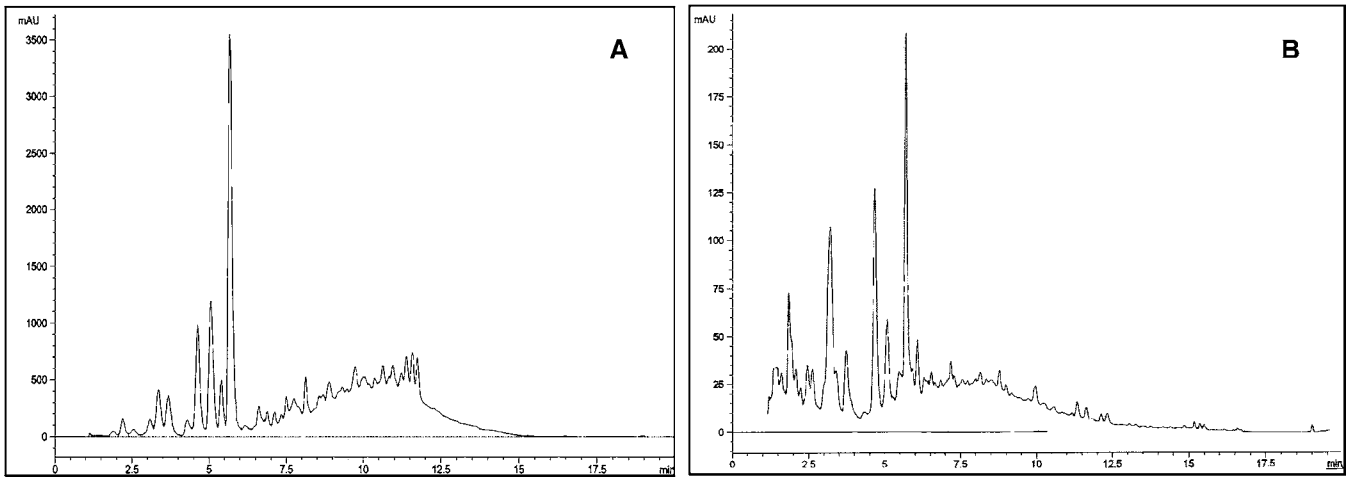

Figure 3. Graph obtained by a HPLC analysis of wheat straw (**A**) or flax straw (**B**) hydrolysate.

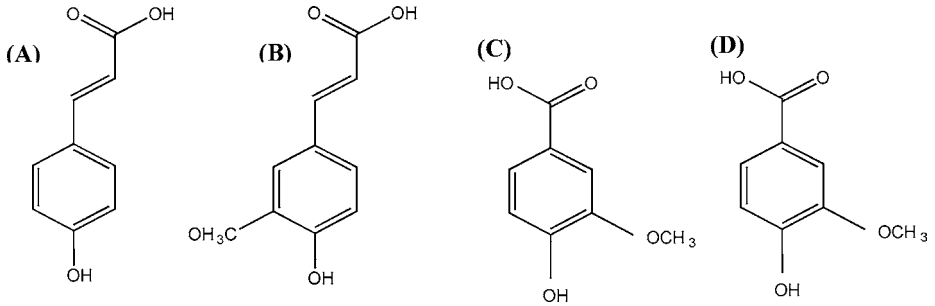

Figure 4. Phenolic compounds: coumaric acid (**A**), ferulic acid (**B**), vanillic acid (**C**), and vanillin (**D**).

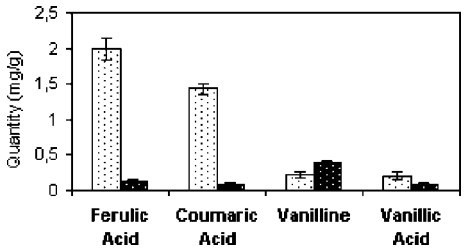

Figure 5. Phenolic compound composition of wheat (white bars) and decorticated flax (black bars) straws.

Figure 9 shows the results obtained for coumaric acid in wheat straw, but the concentration variations were very similar for the other phenolic compounds and also for the flax straw (data not shown). A light alkaline treatment (0.025 N NaOH) after the enzymatic treatment increased the coumaric acid recovery of ~50%. A strong alkaline hydrolysis by 2 N NaOH of effluent increased the amount of free phenolic compounds detected by HPLC from 13 to 76%. This effect is greater on sample first treated with the light alkaline treatment and reached a 76% increase for xylanase/alkaline extraction samples. A moderate alkaline treatment applied with enzymatic treatment seems to release hemicellulose fragments containing esterified coumaric acid liberated by alkaline hydrolysis and detected as free compounds by HPLC. The role of alkaline extraction in this case is to remove fragments cleaved by enzymatic treatment but not soluble in water. The strong alkaline hydrolysis of effluent allows the release of free coumaric acid from solubilized hemicellulose fragments.

Effect of FAE Pretreatment of Flax Straw on Chemical Pulping. After FAE treatment, flax straw was cooked at 20% consistency with 20% alkali charge.

After cooking, residual alkali concentration and kappa number were measured. Results presented in **Table 1** show that straw

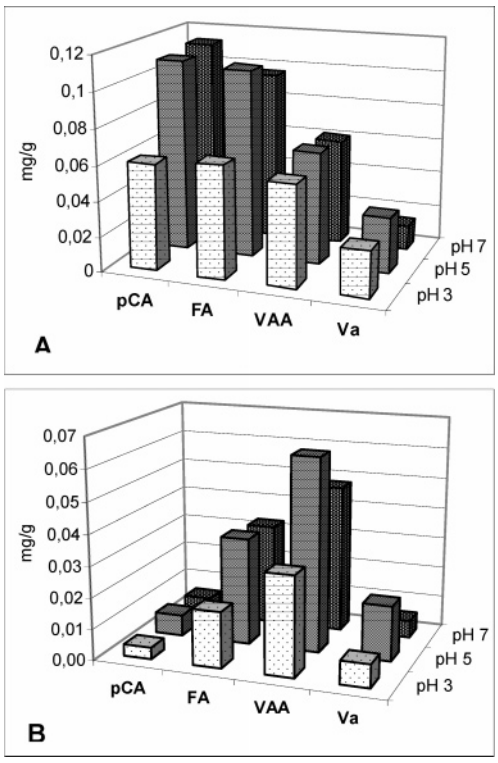

Figure 6. pH effect on the release of phenolic compounds in wheat (**A**) and flax (**B**) straws.

enzymatic pretreatment resulted in a reduced NaOH consumption during the cooking. Moreover, with the same active alkali charge, a lower kappa number was reached. Enzymatic treatment led to a 2 point drop in kappa number on flax, revealing a better delignification. Removal of phenolic compounds opened the

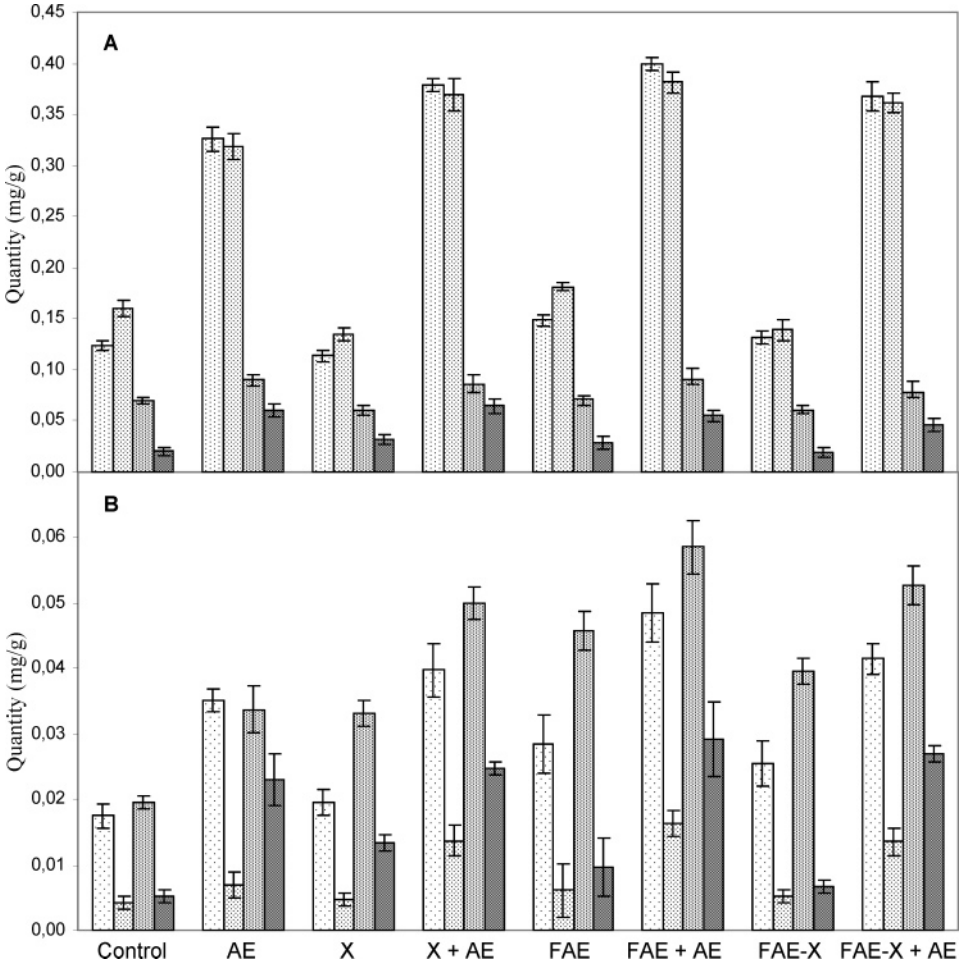

Figure 7. Phenolic compounds released by enzymatic treatments on wheat (**A**) and flax (**B**) straws. AE, alkaline extraction; X, xylanase treatment; X + AE, xylanase treatment followed by a light alkaline extraction; FAE, feruloyl esterase treatment; FAE + AE, feruloyl esterase treatment followed by a light alkaline extraction; FAE-X, treatment with the combination of xylanase and feruloyl esterase; FAE-X + AE, treatment with the combination of xylanase and feruloyl esterase followed by a light alkaline extraction. Bars represent, from left to right in each grouping, FA, ferulic acid; pCA, *p*-coumaric acid; VaA, vanillic acid; and Va, vanillin.

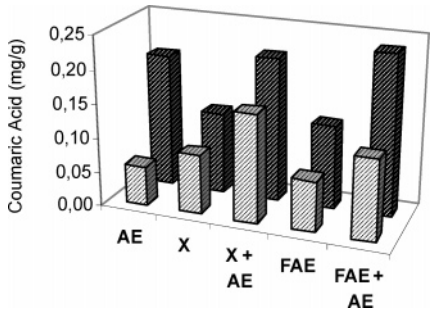

Figure 8. Concentration of coumaric acid released after enzymatic treatment on wheat straw (lighter bars) and presteamed wheat straw (darbker bars).

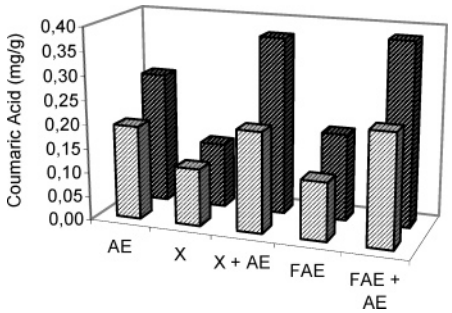

Figure 9. Coumaric acid concentration released by wheat straw enzymatic treatment after hydrolysis of the liquor (ligher bars, free coumaric acids; darker bars, solubilized by alkaline treatment).

fiber structure and weakened lignin−hemicelluloses linkages, facilitating lignin removal during cooking.

The mechanical properties of the unbleached pulp obtained from flax straw pretreated with FAE were evaluated (**Table 2**). The abiotic condition correspond to a pretreatment at pH 5 (acetate buffer), at 3% consistency and 40 °C overnight (same as FAE pretreatment but without enzyme).

The flax pulp pretreated with enzyme presented a higher bulk although the pulp was more refined, which could be interesting for some papers. This higher bulk can reveal an increase in fiber rigidity. Tensile strength is a physical characteristic that is dependent upon the interfiber bonding degree. The tensile index decreases with FAE pretreatment, which shows an enzyme effect on interfiber bonding. Flax pulp had very good tear index values. The tear index depends on individual fiber strength and interfiber

Table 1. Effect of FAE Impregnation on Cooking

	decorticated flax	decorticated flax
FAE impregnation	no	yes
residual alkali	3.4%	4.8%
kappa number	10.2	8.1

Table 2. Mechanical Properties of Flax

	flax	flax abiotic	flax FAE
freeness (°SR)	62 (3)	61 (3)	59 (3)
bulk (cm^3/g)	1.82 (0,06)	1.82 (0.09)	2.24 (0.1)
tensile index (N·m/g)	40.8 (1.1)	39.9 (1.1)	32.5 (0.9)
tear index ($mN·m^2/g$)	10.9 (1.2)	10.7 (1.1)	20.03 (2.6)

Table 3. Phenolic Compound Concentration in Black Liquor

	PCA (mg/g)	FA (mg/g)	Va (mg/g)
decorticated flax	0	0	0.35
raw flax	0	0	0.40
wheat	0.3	0.5	1.5

bonding. It is significantly higher than softwood kraft pulp, the fibers of which are used to increase the tear strength in paper. Although this property decreased with acid treatment (abiotic condition), it increases significantly with enzymatic treatments. FAE has a real activity on fibers and enhances flax mechanical characteristics.

Recovery of Phenolic Compounds from Black Liquors. Cooking experiments were carried out at laboratory scale with flax and wheat straws as described before. The best conditions to cook the flax straw were determined with the aim to obtain a homogeneous chemical pulp in which the long fibers were sufficiently cooked.

After cooking, black liquors from the chemical pulp production were analyzed to determine the potential recovery of the phenolic compounds (**Table 3**).

In these black liquors, coumaric and ferulic acid concentrations were lower than the concentrations obtained by alkaline treatment of the straws. The high concentration of vanillin obtained with wheat straw (~1.5 mg/g) should be explained by the transformation of ferulic acid into vanillin due to the more drastic alkaline treatment during cooking. This is consistent with vanillin concentration produced from flax straw, which is very similar in black liquor and in alkaline extraction (~0.4 mg/g).

Conclusion. Recovery of phenolic compounds (ferulic acid, *p*-coumaric acid, vanillic acid, and vanillin) in straws seems to be an interesting alternative to valorize nonwood plants. Indeed, if we consider the tonnage of raw material used in the papermaking industry, kilograms of high-value compounds could be produced. These phenolic compounds recovered with enzymatic treatment could benefit from the designation "natural" label and could be marketed at a higher price than their chemically derived analogues. Development of a new environmentally friendly process resulting in an enhanced value of the raw material would be very interesting. It would be possible to obtain high added-value phenolic compounds to be used in food, pharmaceutical, and cosmetological products and pulps for papermaking with the use of agricultural byproducts. Moreover, recovery of phenolic compounds will lead to cleaner waste and will reduce the ecological impact of the process.

ACKNOWLEDGMENT

We are grateful to F. Labalette (ONIDOL) and C. Jacquin (Arvalis Institut du Végétal) for supplying wheat and flax straws for this study.

LITERATURE CITED

(1) Escudero, E. Semichemical cereals straw pulp, with sulphur free lignin recovery. A clean and profitable process. *Paper Technol.* **2000**.

(2) Fiala, W.; Nardi, F. The NACO Process. *Paper Technol. Ind.* **1985**, *26*, 75−79.

(3) Madelung, H. Straw as a furnish component—Danish experience. *Paper Technol. Ind.* **1985**, *26*, 80−85.

(4) Morrison, W. H.; Archibald, D. D.; Sharma, H. S. S.; Akin, D. E. Chemical and physical characterization of water- and dew-retted flax fibers. *Ind. Crops Prod.* **2000**, *12*, 39−46.

(5) Sain, M.; Fortier, D. Flax shives refining, chemical modification and hydrophobisation for paper production. *Ind. Crops Prod.* **2002**, *15*, 1−13.

(6) Rennebaum, H.; Grimm, E.; Warnstorff, K.; Diepenbrock, W. Fibre quality of linseed (*Linum usitatissimum* L.) and the assessment of genotypes for use of fibres as a by-product. *Ind. Crops Prod.* **2002**, *16*, 201−215.

(7) van Voorn, B.; Smit, H. H. G.; Sinke, R. J.; de Klerk, B. Natural fibre reinforced sheet moulding compound. *Composites, Part A* **2001**, *32*, 1271−1279.

(8) Smeder, B.; Liljedahl, S. Market oriented identification of important properties in developing flax fibres for technical uses. *Ind. Crops Prod.* **1996**, *5*, 149−162.

(9) Bledzki, A. K.; Gassan, J. Composites reinforced with cellulose based fibres. *Prog. Polym. Sci.* **1999**, *24*, 221−274.

(10) Tschirner, U.; Satyavolu, N. Canadian oil seed flax fiber in traditional pulping. *TAPPI Pulping Conf.* **1999**, *2*, 683−688.

(11) Chute, W. Pulps from oilseed flax straw in mainstream pulp and paper applications. *Non Wood Fibres and Crop Residues*, Amsterdam, The Netherlands, Oct 17−18, 2001; Pira International: Leatherhead, U.K., 2001; 14 pp.

(12) Sun, R. C.; Sun, X. F.; Wang, S. Q.; Zhu, W.; Wang, X. Y. Ester and ether linkages between hydroxycinnamic acids and lignins from wheat, rice, rye, and barley straws, maize stems, and fast-growing poplar wood. *Ind. Crops Prod.* **2002**, *15*, 179−188.

(13) Yuan, X.; Wang, J.; Yao, H. Antioxidant activity of feruloylated oligosaccharides from wheat bran. *Food Chem.* **2005**, *90*, 759−764.

(14) Ou, S.; Kwok, K. C. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *J. Sci. Food Agric.* **2004**, *84*, 1261−1269.

(15) Lapierre, C.; Monties, B.; Rolando, C. Thioacidolysis of poplar lignins: identification of monomeric syringyl products and characterization of guaiacyl-syringyl lignin fractions. *Holzforschung* **1986**, *40*, 113−118.

(16) Record, E.; Asther, M.; Sigoillot, C.; Pagès, S.; Punt, P. J.; Delattre, M.; Haon, M.; Van den Hondel, C. A. M. J. J.; Sigoillot, J. C.; Lesage-Meessen, L.; Asther, M. Overproduction of the *Aspergillus niger* feruloyl esterase for pulp bleaching application. *Appl. Microbiol. Biotechnol.* **2003**, *4*, 349−355.

(17) Martinez, A. T.; Camarero, S.; Gutierrez, A.; Bocchini, P.; Galletti, G. C. Studies on wheat lignin degradation by *Pleurotus* species using analytical pyrolysis. *J. Anal. Appl. Pyrolysis* **2001**, *58−59*, 401−411.

(18) Morrison, W. H.; Himmelsbach, D. S.; Akin, D. E.; Evans, J. D. Chemical and spectroscopic analysis of lignin in isolated flax fibers. *J. Agric. Food Chem.* **2003**, *51*, 2565−2568.

(19) Meyermans, H.; Morreel, K.; Lapierre, C.; Pollet, B.; De Bruyn, A.; Busson, R.; Herdewijn, P.; Devreese, B.; Van Beeumen, J.; Marita, J. M.; Ralph, J.; Chen, C.; Burggraeve, B.; Van Montagu, M.; Messens, E.; Boerjan, W. Modifications in lignin and accumulation of phenolic glucosides in poplar xylem upon down-regulation of caffeoyl-coenzyme A O-methyltransferase, an enzyme involves in lignin biosynthesis. *J. Biol. Chem.* **2000**, *275*, 36899−36909.

(20) Jimenez, L.; Angulo, V.; Ramos, E.; De la Torre, M. J.; Ferrer, J. L. Comparison of various pulping processes for producing pulp from vine shoots. *Ind. Crops Prod.* **2005**, *23*, 122−130.

(21) Williamson, G.; Faulds, C. B.; Kroon, P. A. Specificity of ferulic acid (feruloyl) esterases. *Biochem. Soc. Trans.* **1998**, *26*, 205−209.

(22) Faulds, C. B.; Bartolome, B.; Williamson, G. Novel biotransformations of agro-industrial cereal waste by ferulic acid esterases. *Ind. Crops Prod.* **1997**, *6*, 367−374.

(23) Kroon, P. A.; Garcia-Conesa, M. T.; Fillingham, I. J.; Hazlewood, G. P.; Williamson, G. Release of ferulic acid dehydrodimers from plant cell walls by feruloyl esterases. *J. Sci. Food Agric.* **1999**, *79*, 428−434.

(24) Beaugrand, J.; Crônier, D.; Debeire, P.; Chabbert, B. Arabinoxylan and hydroxycinnamate content of wheat bran in relation to endoxylanase susceptibility. *J. Cereal Sci.* **2004**, *40*, 223−230.

Received for review November 3, 2005. Revised manuscript received February 17, 2006. Accepted March 16, 2006. This research was supported by the GIS-EBL (Region Provence Alpes Côte d′Azur and Conseil Général des Bouches du Rhônes, France). S.T. is grateful to the Centre Technique du Papier for a Ph.D. scholarship.

JF052725Y