Determination of Ferulic and *p*-Coumaric Acids in Wheat Straw and the Amounts Released by Mild Acid and Alkaline Peroxide Treatment

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Ferulic acid (FA) and *p*-coumaric acid (PCA) are two major phenolic acids in a wide variety of graminaceous plants. Particularly, FA has been implicated in cross-linking cell wall polymers. In the present paper, the variations in FA and PCA contents of wheat straw upon various treatments relevant to the process of producing paper-making fibers were investigated. Wheat straw contained 0.48% FA and 0.42% PCA on the basis of dry material. Among them, of the total FA, 56% was esterified only and 44% was etherified. In contrast, >80% of the PCA was esterified only. In general, the phenolic acids had a high tendency to dissolve in hot water or alkaline peroxide solution, as compared to lignin. Nevertheless, the two-step pulping process, a mild acid wash followed by alkaline peroxide bleaching, removed <40% of FA and <20% of PCA. The phenolic acids remaining attached to the lignin might have an effect on the properties of wheat straw fibers made according to this method.

Keywords: *Ferulic acid; p-coumaric acid; lignin; wheat straw; alkaline peroxide bleaching*

INTRODUCTION

Hydroxycinnamic acids, particularly ferulic (FA) and *p*-coumaric acids (PCA), occur widely in cell walls of graminaceous plants such as wheat straw (Yamamoto et al., 1989). As bifunctional molecules with carboxylic and phenolic bonding sites, these phenolic acids provide a means of cross-linking cell wall polymers. Such a cross-linking has been reported to have a profound influence on the growth of the plant cell wall and its mechanical properties and biodegradability (Fry, 1986; Hartley and Ford, 1989; Hartley, 1990; Iiyama et al., 1994; Jung and Deetz, 1993; Yamamoto et al., 1989).

It was proposed that FA ester−ether bridges played a predominant role in cross-linking between lignin and polysaccharides in wheat straw (Scalbert et al., 1985, 1986). This hypothesis was experimentally supported by a systematic investigation (Iiyama et al., 1990, 1994; Lam et al., 1990, 1992a,b, 1994), which showed that at all stages of wheat maturation PCA was mostly esterified to lignin or polysaccharides, whereas FA occurred almost equally in esterified and etherified forms. Furthermore, experimental evidence has proven the ester bonding of hydroxycinnamic acids or their dimers to polysaccharides, mainly arabinoxylans, by isolating and identifying hydroxycinnamoylated oligosaccharides released from the enzymatic hydrolysis of cell walls of graminaceous plants (Kato et al., 1983; Smith and Hartley, 1983; Shibuya, 1984; Mueller-Harvey and Hartley, 1986; Hartley et al., 1990; Ishii, 1991). On the other hand, there is less information on the attachment of FA to lignin. Earlier findings by NMR study suggested that FA associated with lignin through ether linkage (Nimz et al., 1981; Scalbert et al., 1985). From experiments with model compounds, Scalbert et al. (1986) proposed a mechanism of formation of benzyl aryl ether linkage through addition of PCA or FA to quinone methides. More recently, Ralph claimed that the bonding patterns between hydroxycinnamic acids and lignin do not just involve alkyl aryl ethers but, like their derived alcohols, PCA and FA may undergo oxidative coupling reactions to incorporate into lignins (Ralph, 1997). This new mechanism has been supported by Jacquet et al. (1995), who isolated and identified dimers associating FA to the β position of coniferyl alcohol in the saponification products of wheat and oat straws.

It is well documented that the cell wall cross-linking in which hydroxycinnamic acids function as bridging molecules has a detrimental effect on the microbial or enzymatic degradability of graminaceous plant polysaccharides (Hartley and Ford, 1989; Hartley, 1990). Release of these acids by alkaline or enzymatic treatment has been reported to result in increased biodegradability of the cell walls.

However, there is little knowledge about the behavior of FA and PCA in the process of producing paper-making fibers from straws. Traditional pulping processes involve chemical reactions using strong alkali or acid to solubilize lignin and separate fibers; under such conditions FA and PCA would be dissolved along with the lignin. However, the major disadvantages of chemical pulping include low fiber yield and a large amount of hazardous effluent. These problems are likely to be resolved by high-yield pulping. In high-yield pulping, straws are softened by mild chemical treatment and disintegrated by mechanical action, thereby retaining most of the fiber components. Therefore, it is of interest to understand the behavior of FA and PCA in the mechanical pulping process and their effect on the properties of the resulting fibers. This paper reports the variations of FA and PCA content of wheat straw in our

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newly developed process in which straws are sequentially treated with mild acid and alkaline peroxide solutions.

EXPERIMENTAL PROCEDURES

Materials. All chemicals were commercial products and were employed as received. Wheat straw (*Triticum aestivum*) supplied by the University of Alberta farm was cut by a hammer mill into lengths of 15−25 mm and screened to remove fines and dusts, which provided material that was suitable for the mechanical pulping process (Pan and Leary, 1998). The screened wheat straw was mainly composed of internodes.

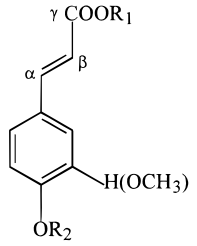
Preparation of Wheat Straw Samples. The cut wheat straw was soaked in distilled water at a water-to-straw ratio of 25 mL/g, at 60 °C for 1 h. For mild acid wash, the liquor was adjusted to pH 3 with sulfuric acid. The wheat straw, pretreated at pH 3, was washed to bring it to a neutral pH and subsequently treated with an alkaline peroxide solution at 60 °C for 2 h. To 100 g of wheat straw (dry weight before the pretreatment) were added 4 g of H_2O_2, 4 g of NaOH (or 5 g of Na_2CO_3), and 600 mL of water. The wheat straw samples, recovered from the above-mentioned treatments, were thoroughly washed with distilled water and air-dried. The dried materials were ground in a Wiley mill to pass a 0.4 mm sieve, extracted with toluene/ethanol (2:1, v/v) in a Soxhlet apparatus for 24 h, and then dried at 50 °C overnight.

Determination of Wheat Straw Composition. Klason lignin was measured as the material insoluble in 72% H_2SO_4, and acid-soluble lignin was determined by UV absorption at 205 nm of diluted acid hydrolysis solutions according to TAPPI standard methods (T222 os-74 and UM 250). Ash and extractive contents were estimated following TAPPI standard methods 211 om-93 and 204 om-88, respectively.

Isolation of Ester-Linked Phenolic Acids by Mild Alkaline Hydrolysis. The extractive-free and dried wheat straw samples (200 mg) were saponified with 20 mL of 2 N NaOH at 20 °C for 24 h under N_2 with magnetic stirring. Upon completion, the reaction mixtures were acidified to pH 2 using 10% HCl, quantitatively transferred to centrifuge tubes, and centrifuged at 10 000 rpm for 15 min. After the supernatants were collected, the straw residues were washed twice with pH 2 aqueous HCl solution, and these supernatants were also collected. An internal standard (0.5 mg of *o*-coumaric acid) in methanol solution was added, and the combined supernatants were extracted with 3 × 30 mL of ethyl ether. The ether extracts were dried over Na_2SO_4, evaporated under reduced pressure, redissolved in 1 mL of dimethylformamide/water (2:1, v/v), and analyzed by HPLC.

Isolation of Ether-Linked Phenolic Acids by Acid Hydrolysis. The aqueous layers from ether extraction and the straw residues from centrifugation were combined and freeze-dried. The recovered materials were refluxed for 1 h in 35 mL of dioxane/2 N HCl (9:1, v/v). Upon cooling, the residues were collected on filters and the filtrates collected. The same procedure was then followed as in the alkaline hydrolysis.

HPLC Analysis. The phenolic acids obtained as above were separated in a Hewlett-Packard HPLC 1090 instrument using a reverse phase Hypersil ODS (4.6 mm × 200 mm, 5 μm particle size) column with a diode array detector at room temperature. The samples were eluted by a gradient of acetonitrile (A) and 1% acetic acid in water (B) (0 min, B = 90%; 35 min, B = 70%; 37 min, B = 55%; 42 min, B = 55%; 44 min, B = 90%; 65 min, B = 90%) at a flow rate of 0.8 mL/min and monitored at 280, 320, 330, and 354 nm. Quantitative data were obtained from the chromatograms recorded at 354 nm. Injection volume was 50 μL. Calibration curves of the standard compounds were run at weight ratios of FA and PCA to *o*-coumaric acid (I.S.) of 2.0, 1.0, and 0.4. The methanol solutions of three makeups were diluted with water, adjusted to pH 2 using 10% HCl, and then subjected to the same procedure as in the preparation of alkaline and acid hydrolysis products.



A: Esterified only; R_1 = LG or PS; R_2 = H
B: Etherified only; R_1 = H; R_2 = LG
C: Ester-ether bridge; R_1 = PS; R_2 = LG

Figure 1. Schematic representation of linkages of FA and PCA to lignin (LG) and/or polysaccharides (PS).

RESULTS AND DISCUSSION

General Procedure. As illustrated in Figure 1, FA and PCA are linked to lignin and/or polysaccharides through ester or ether bonds in wheat straw cell walls. Clearly, only the molecules, except for dimerized ones, that are associated both with lignin by ether bond and with polysaccharides by ester bond contribute to the cell wall cross-linking. It is known that the alkyl aryl ether resisted mild alkaline hydrolysis but was acid-labile (Scalbert et al., 1985). The difference in stability of the ester and ether bonds allows a separation of ester- and ether-bonded FA and PCA. Accordingly, in a sequential treatment of wheat straw, mild alkaline hydrolysis serves to release ester-bonded FA and PCA (A in Figure 1), and then acid hydrolysis cleaves the alkyl aryl ether bond to release the remainders (B plus C). It should be noted that the method based on this concept cannot differentiate between etherified only (B) and esterified−etherified (C) molecules. To do this, a sophisticated multistep procedure was introduced (Lam et al., 1992a, 1994). Nevertheless, the ether-bonded FA was reported to be mainly linked to polysaccharides through ester bonds. (Scalbert et al., 1985; Lam et al., 1994). In the present paper, we quantitatively estimated FA and PCA only in two categories, that is, esterified (A) and etherified (B + C). Obviously, the FA and PCA incorporated into lignin through carbon−carbon bonds and ether bonds other than alkyl aryl ones, as proposed by Ralph (1997), are unlikely to be released by the method used in this investigation.

The phenolic acids separated by HPLC were identified by comparing their retention times and UV absorption characteristics with those of authentic compounds. Although dehydrodimers of FA and PCA having various coupling patterns were reported to be also released by saponification and separated by HPLC and GC (Jacquet et al., 1995; Ralph et al., 1994), this paper focused on the estimation of monomeric products. In addition to FA and PCA, the acid hydrolysis products contained two other components, possibly cinnamaldehyde-type compounds, as evidenced by matching their retention times and UV absorption characteristics with those of authentic compounds. The acid hydrolysis was likely to release cinnamaldehyde-type end groups bound to lignin through 4-O-alkyl linkages (Gellerstedt and Zhang, 1992).

Initially, we followed a procedure reported in the literature (Scalbert et al., 1985; Billa et al., 1993; Billa and Monties, 1995). By this procedure, wheat straw residues recovered by centrifugation were subsequently acid hydrolyzed, but the supernatants after extraction were discarded. We obtained a very low recovery of etherified FA and PCA and then speculated that sub-

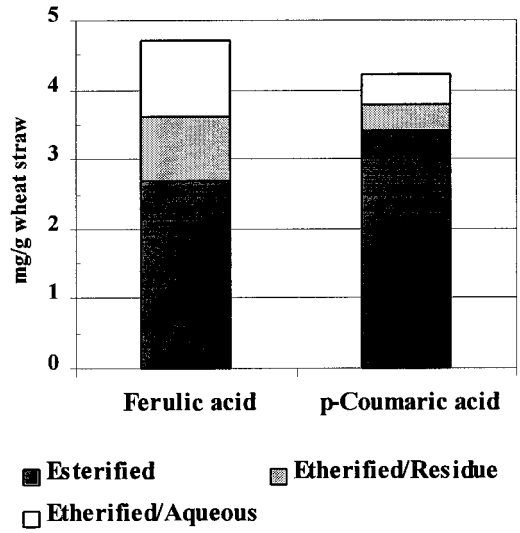


Figure 2. Distribution of FA and PCA in alkaline hydrolysate of wheat straw and saponified residues.

stantial amounts of those substances might have been lost by discarding the aqueous layers. This speculation was proven true by freeze-drying the aqueous fractions and then subjecting them to acid hydrolysis. The results of this set of experiments are shown in Figure 2.

As can be seen in Figure 2, the ether-bonded FA and PCA distributed almost equally in the extracted alkaline hydrolysate and saponified straw residue. It should be pointed out that, as described under Experimental Procedures, the alkaline hydrolysate was acidified so that dissolved lignins must have been precipitated and recovered together with the straw residue (Scalbert et al., 1985). Moreover, we did observe that the supernatant recovered by centrifugation was fairly clear. Therefore, it is thought that the etherified FA and PCA recovered from the acid hydrolysis of aqueous fractions were likely to attach to lignin fragments which could be bound to oligo- or polysaccharides. The lignin−carbohydrate complex (LCC) was dissolved as part of delignification of wheat straw in alkaline medium because mild alkaline treatment can dissolve substantial amounts of wheat straw lignin (Billa and Monties, 1995). The LCC fragments containing sufficient carbohydrate were rendered highly water-soluble.

Similar observations were made on the estimation of amounts of FA and PCA in fractions of a wheat straw milled wood lignin (MWL) (Iiyama et al., 1990). Throughout this multistep MWL preparation, the crude MWL (i.e., dioxane−water extract) contained a large amount of LCC that was rich in etherified FA, whereas the purified MWL contained a little etherified FA. The authors found that the purification procedure, including water precipitation, removed most of the LCC, thus removing etherified FA. In a previous report, Billa and Monties (1995) compared the results of determination of FA and PCA by two procedures. The first one was similar to that used here (Scalbert et al., 1985), that is, mild alkaline hydrolysis followed by acid hydrolysis. The second procedure (Iiyama et al., 1990) was based on the finding that mild alkaline hydrolysis (dilute alkali at room temperature) released only the ester-linked phenolic acids but that high-temperature (170 °C) alkaline treatment cleaved both the ester and ether linkages. Therefore, the etherified part was the difference of amounts of phenolic acids recovered from these two alkaline treatments. They observed that the amount of

Table 1. Various Treatments of Wheat Straw and Composition of the Residues (All Values Are Based on the Dry Weight of the Original Material)

treatment	original	pH 7 wash	pH 3 wash	H_2O_2/ NaOH	H_2O_2/ Na_2CO_3
straw recovery, %	100	93.5	95.2	86.8	90.5
ash content, %	6.48	4.23	4.66		
extractives content,[a] %	3.43	1.53	2.00	0.76	1.13
lignin content, %					
Klason	17.44	16.47	16.43	13.12	15.00
acid-soluble	1.87	1.51	1.58	1.48	1.53
total	19.31	17.98	18.01	14.60	16.53

[a] Toluene/ethanol (2:1, v/v).

etherified FA by the first method was only about one-fourth that by the second one. However, they did not fully explain where the remainder was. Our results suggest that FA and PCA attached to water-soluble lignin−polysaccharide fractions are substantial and cannot be ignored. Therefore, we adopted a modified procedure as described under Experimental Procedures.

It is also demonstrated that 56% of the recovered FA was esterified only and 44% was etherified, whereas >80% of the PCA was esterified only. These proportions are consistent with the data available in the literature (Scalbert et al., 1985; Lam et al., 1990, 1992a; Billa and Monties, 1995).

Treatments of Wheat Straw. In the present investigation, FA and PCA were quantitatively determined on various wheat straw samples after treatments relevant to the alkaline peroxide mechanical pulping (APMP). In the APMP process, wheat straw is softened and bleached by alkaline peroxide solution and subsequently defibrated mechanically to lignocellulosic pulp. As a high-yield pulping method, it is important to produce a pulp of acceptable brightness without significant dissolution of straw components. It is generally accepted that hydroperoxide ion (HOO^-), formed in alkaline media, is the principal active species in hydrogen peroxide bleaching systems. This anion is a strong nucleophile that, during bleaching, preferentially attacks ethylenic and carbonyl groups present in lignin. As a consequence, such chromophores as quinones, cinnamaldehyde, and ring-conjugated ketones are converted to nonchromophoric species (Dence, 1996). Obviously, the pH of the bleach liquor should be high enough to ensure an adequate concentration of hydroperoxide anion to promote bleaching reactions.

On the other hand, hydrogen peroxide is unstable in alkaline conditions and readily decomposes, particularly in the presence of certain transition metals such as manganese, iron, and copper. This metal-catalyzed decomposition of hydrogen peroxide is undesirable in the bleaching operation because it leads to a loss of bleaching capacity and generates more active radicals, such as hydroxyl radicals, participating in degradation reactions of lignin and carbohydrates and in chromophore-creating reactions. Our earlier study has shown that wheat straw could be more efficiently bleached when it was washed with water at a slightly acidic pH prior to alkaline peroxide treatment (Pan and Leary, 1998). Dissolution of metals by the washing contributes, at least in part, to enhanced bleaching.

Table 1 summarizes various treatments involved in the two-step process, that is, acid wash followed by alkaline peroxide treatment, and the corresponding solubility of wheat straw components. The washing at neutral and acidic pH resulted in a slight dissolution of

Table 2. Amounts of FA and PCA in Various Wheat Straw Samples

	FA, mg/g of wheat straw sample[a]			PCA, mg/g of wheat straw sample[a]		
sample source	esterified	etherified	total	esterified	etherified	total
original wheat straw	2.7	2.1	4.8	3.4	0.82	4.2
pH 7 wash	2.5	1.4	3.9	3.1	0.50	3.6
pH 3 wash	2.5	1.8	4.3	3.1	0.57	3.7
H_2O_2/NaOH	1.6	1.3	2.9	2.7	0.67	3.4
H_2O_2/Na_2CO_3	2.1	1.4	3.5	3.0	0.50	3.5

[a] All values are based on the dry weight of the original material and are an average of triplicate analyses (standard deviation < ±5%).

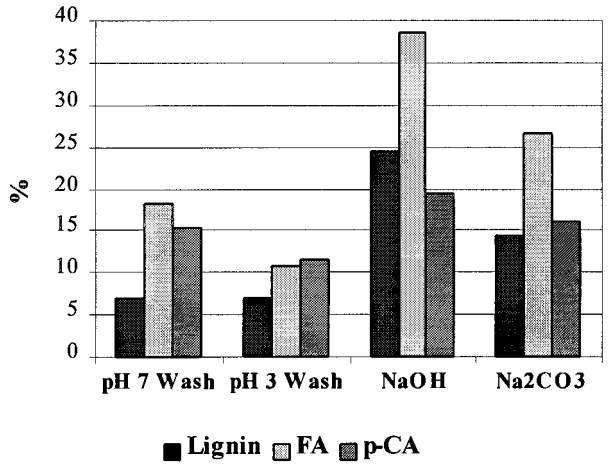


Figure 3. Percent of total lignin, FA, and PCA removed from wheat straw by different treatments.

wheat straw components, including ash, extractives, and lignin. Surprisingly, the water solubility of wheat straw was slightly reduced when the pH was lowered. In comparison to the neutral wash, the acid wash resulted in higher ash and extractive contents but a similar lignin content. In the subsequent alkaline peroxide treatment, the sodium hydroxide-based solution dissolved more substances than the sodium carbonate one.

Variations in Content of FA and PCA. Table 2 reports the amounts of esterified and etherified FA and PCA remaining in wheat straw after the treatments described in Table 1. Generally, the washing removed some FA and PCA, and the alkaline peroxide treatment resulted in a moderate decrease of these phenolic acids. The removal of FA and PCA by the washing could be due to the solubilization of lignin and hemicellulose fragments bearing FA or PCA. As Table 1 indicates, the washing resulted in the dissolution of 5−6.5% of wheat straw material, possibly including low molecular weight lignin and hemicelluloses. It is also shown that the washing caused a drop in lignin content, particularly the acid-soluble lignin. This portion of lignin technically includes FA and PCA dissolved in sulfuric acid because it is estimated by UV absorption. In this sense, removal of FA and PCA from wheat straw could indirectly be reflected by a reduced acid-soluble lignin content in the residue.

Overall, as can be seen in Figure 3, the extent of FA and PCA removal was greater than the degree of lignin solubilization by the treatments of wheat straw. These phenolic acids might be bound to "core lignins" as end-units and as well linked to easily solubilized lignin and polysaccharide fractions in wheat straw so that they are more easily removed than lignin. In the alkaline peroxide treatments, FA had a higher solubility than PCA. As mentioned earlier, FA is mostly attached to polysaccharides through ester bonds. Therefore, its tendency to be dissolved is higher because hemicelluloses are easily solubilized in alkaline solutions.

Comparison of the data in Table 2 shows differences in the esterified and etherified FA and PCA released by the two alkaline peroxide treatments. The treatment using NaOH as an alkali source removed more phenolic acids than the treatment using Na_2CO_3, except for etherified PCA, because the former is a stronger alkali than the latter. When NaOH was used, the dissolution of esterified FA and PCA was more substantial than that of etherified ones. This is not surprising because the ester bond is alkali-labile. However, only part of the esterified phenolic acids was dissolved by the alkaline peroxide treatments. This result seemed unexpected. It should be noted that wheat straw is a complex reaction substrate and many reactions could take place. As observed earlier (Pan and Leary, 1998), the pH of the solutions dropped rapidly as the bleach liquors were added to wheat straw. At the beginning, part of the applied alkali was likely to be consumed to neutralize acidic components in wheat straw. As well, oxidative reactions in bleaching would produce alkali-consuming acidic functional groups. Consequently, the amount of alkali, or the concentration of hydroxyl anion, in the alkaline peroxide solutions was reduced quickly and thus insufficient to perform complete saponification of ester linkages of wheat straw.

There could be another mechanism of eliminating FA and PCA through cleavage of their α,β double bonds by hydrogen peroxide. In hydrogen peroxide bleaching chemistry, the cleavage of the α,β double bonds in cinnamaldehyde structures is an important reaction (Dence, 1996). If similar reactions had occurred on cinnamic acids, there would have been formation of corresponding benzaldehyde or benzoic acid products. In fact, no appreciable amounts of such side-chain cleavage products were observed by HPLC. This result would suggest that FA and PCA may not undergo the side-chain cleavage reaction by alkaline peroxide. Unlike the aldehyde group at the γ position of cinnamaldehyde-type structures, the carboxylic acid or its ester group, as an electron donator, deactivates the α,β double bond in cinnamic acid structures, which is not likely to be attacked by HOO^- (Reeves and Pearl, 1965). Obviously, the removal of FA and PCA from wheat straw during alkaline peroxide treatment can only result from the cleavage of their linkages (e.g., esters) to lignin and hemicelluloses and the solubilization of lignin and hemicellulose fragments bearing FA or PCA.

Figure 4 shows the FT-IR spectra of lignins dissolved by the alkaline peroxide treatments. The lignins were recovered as dioxane-soluble fractions of the precipitates resulting from acidifying the bleaching liquors. As can be seen, the two lignin samples had strong absorbance at around 1730 and 1666 cm^{-1}, relative to the aromatic skeleton vibrations in lignin assigned at around 1600 cm^{-1} and around 1510 cm^{-1}. This result indicated that

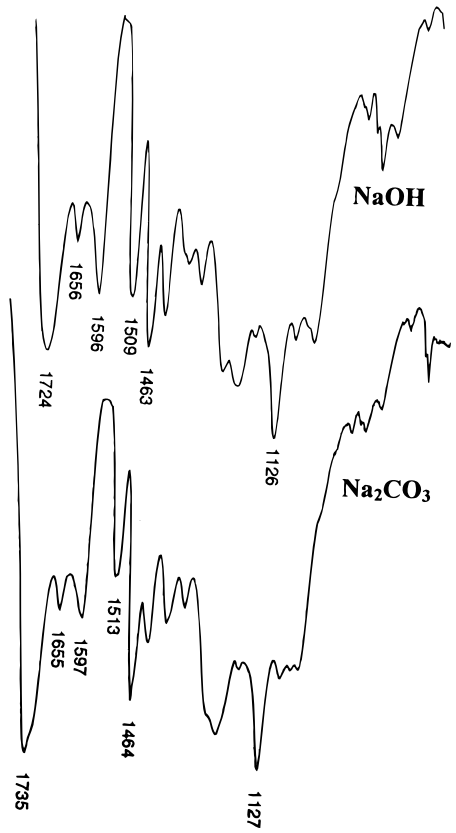


Figure 4. FT-IR spectra of dissolved lignins in alkaline peroxide treatments.

these lignins contained large quantities of carboxyl and carbonyl groups. In particular, these lignins are typified by the presence of a large peak at around 1730 cm^{-1}. In addition to those produced by hydrogen peroxide oxidation, carboxylic acids and their ester groups in FA and PCA attached to the dissolved lignins may contribute to this absorption band. It has been shown that the alkaline peroxide treatments removed part of FA and PCA from wheat straw. For this carboxylic band, absorption at 1710−1720 cm^{-1} is attributed to free carboxyl groups and absorption at 1730−1740 cm^{-1} is attributed to carboxylic esters. As shown in Figure 4, the lignin from the Na_2CO_3 treatment contained more ester groups so that the band shifted to the higher wavenumber. Na_2CO_3 is a weaker alkali, thereby hydrolyzing ester linkages to a smaller extent.

In summary, the solubility of FA and PCA was dependent on the alkalinity of peroxide bleach liquors. Under the conditions used in this paper, the bleaching removed <40% of FA and <20% of PCA from wheat straw. Billa et al. (1993) have also observed that the hydrogen peroxide bleaching of wheat straw mechanical pulps resulted in a moderate reduction in esterified FA and PCA but had little effect on etherified ones. Further study is needed to understand the impact of remaining phenolic acids on the fiber properties.

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