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The Kismet of Residual Lignins During LMS Delignification
of High-Kappa Kraft Pulps

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F.S. Chakar and A.J. Ragauskas: Laccase-mediator studies on high-kappa kraft pulps

The Kismet of Residual Lignins During LMS Delignification of High-Kappa Kraft Pulps

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Summary

A series of laccase-mediator treatments (LMS) with 1-hydroxybenzotriazole (HBT) and *N*-acetyl-*N*-phenylhydroxylamine (NHAA) as the mediators were performed on a laboratory prepared southern softwood conventional kraft pulp (kappa # 75.4). Subsequent to the LMS treatments, the treated pulps were subjected to various oxidatively reinforced alkaline extraction stages (E*). The kappa results suggested that both LMS_{HBT} and LMS_{NHAA} treatments delignified this high-kappa pulp. The E* stages were beneficial in countering the darkening effect observed after the LMS treatments. Structural changes in residual lignins isolated before and after laccase-mediator (LMS_{NHAA} (E*) and LMS_{HBT} (E*)) treatments were explored. The spectral analysis of phosphitylated residual lignins revealed an increase in carboxylic acid content and a depletion of phenolic hydroxyl groups in non-condensed at C-5 lignin moieties. Aliphatic hydroxyl groups were substantially decreased when NHAA was used. Overall, it appears that LMS_{HBT} and LMS_{NHAA} treatments on high-kappa kraft pulps primarily attack phenolic hydroxyl groups in non-condensed at C-5 lignin structures.

Keywords

Laccase

Mediator

Delignification

Oxidatively reinforced alkaline extraction stages

³¹P NMR spectroscopy

Introduction

As pulp producers continue to address environmental concerns, other areas of interest are re-emerging. In particular, wood utilization practices are becoming consequential as the availability of and accessibility to inexpensive fibers will diminish in the long run. These issues will be vital if pulp producers are to remain competitive in this global market.

Research efforts have begun to focus on developing novel manufacturing technologies that address these issues. One of the most promising approaches to improving the economics of kraft pulp production consists of increasing overall pulp yields. This can be achieved by halting the kraft cook at a relatively high kappa (> 45) prior to reaching the terminal phase. The pulp is then subjected to a single or double oxygen stage before it is bleached. Jameel *et al.* (1997) and others have shown that this approach can improve the overall yield of bleached kraft pulps by 2-4 % (Parthasarathy 1997; Bokstrom and Norden 1998).

As an alternative to oxygen delignification, we have recently begun exploring the feasibility of delignifying high-kappa pulps via lignin degrading enzymes, more specifically with laccase (Chakar *et al.* 1998; Chakar and Ragauskas 1999). Historically, the use of laccase for delignifying kraft pulps was limited due to the size of the enzyme and its inability to diffuse into pulp fibers to oxidize the residual lignin (Jurasek 1995).

Fortunately, this barrier was overcome when Bourbonnais and Paice (1992) realized that laccase in the presence of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), a mediator, could delignify both hardwood and softwood kraft pulps with high selectivity. The introduction of 1-hydroxybenzotriazole (HBT) by Call (1994) further demonstrated the high delignification capability and high selectivity of a laccase-mediator system (LMS) on conventional pre- and post O_2 kraft pulps. Research into the delignification chemistry of kraft pulps *via* an LMS_{ABTS} and an LMS_{HBT} system has been and continues to be extensively examined (Balakshin *et al.*; Bourbonnais *et al.* 1997a,b; 1997; Muheim *et al.* 1992; Poppius-Levlin *et al.* 1997a,b; Potthast *et al.* 1999; Potthast *et al.* 1997; Sealey *et al.* 1999; Sealey and Ragauskas 1997).

More recently, several other compounds, such as *N*-acetyl-*N*-phenylhydroxylamine (NHAA), violuric acid (VA), and others have been reported to act as mediators (see Fig. 1) (Amann 1997; Schneider 1995). Despite these advancements, much remains to be learned about the chemistry of laccase mediated delignification systems.

The purpose of this study was to examine the delignification chemistry of LMS systems on a high-lignin content kraft pulp using NHAA and HBT as mediators. The LMS_{HBT} system served as a reference to the understudied LMS_{NHAA} system. The conditions of the alkaline extraction stage were varied so that the effects of peroxide (E+P), oxygen (E+O), and peroxide and oxygen (E+P+O) on the LMS treated pulp could be established. The outcome of the LMS(E*) treatments was determined by measuring the changes in lignin content and brightness of the treated pulps. In addition, the structural changes in residual lignins isolated after LMS_{NHAA}(E), LMS_{HBT}(E) and LMS_{NHAA}(E+P+O), LMS_{HBT}(E+P+O) treatments were ascertained by ³¹P NMR.

Experimental

Materials and Methods

All materials were purchased from Aldrich Chemical Co., Milwaukee, WI, and used as received, except for *p*-dioxane, NHAA and laccase. *p*-Dioxane was freshly distilled over NaBH₄ prior to using it for the lignin isolation experiments. NHAA was synthesized in accordance with Oxley's method (Oxley *et al.* 1989). A conventional southern USA softwood kraft pulp was prepared from *Pinus taeda* chips at Potlatch Corp facilities in Cloquet, MN. The chips were cooked to an H-factor of 573 using 18.5 % active alkali. The pulp was thoroughly washed, screened, centrifuged, fluffed, and stored at 4°C prior to LMS bleaching treatments. Laccase, from *Trametes villosa*, was donated by Novo Nordisk Biochem, Franklinton, NC.

Enzyme assay

Laccase activity was measured by monitoring the rate of oxidation of syringaldazine. One unit of activity (U) was defined as the change in absorbance at 530 nm of 0.001 per minute per ml of enzyme solution, in a 100 mM potassium phosphate buffer (2.2 ml) and 0.216 mM syringaldazine in methanol (0.3 ml, pH 6.7). The procedure was carried out at 23°C. The activity of the laccase was 1.87E+06 U/ml of enzyme solution.

Laccase-mediator delignification procedure

A 1000-ml capacity Parr reactor equipped with a stirrer, a pressure gauge, a heating mantle, and connected to a Parr 4842 temperature controller was charged with 15 g of o.d. fibers. The pulp consistency was adjusted to 9 % by adding distilled water. The slurry was then heated to a temperature of 45°C and was maintained at this temperature throughout the incubation period. HBT (2.2×10^{-3} moles) was then added (or 2.2×10^{-3} moles of NHAA) to the heated slurry. Subsequent to mixing the slurry (ca. 5 minutes), the pH was adjusted to 4.5 with glacial acetic acid. Laccase was then added (372,000 U per gram of o.d. pulp) and the reactor was sealed and pressurized with oxygen to 145 psi. After the four- hour treatment, the pulp was thoroughly washed and subjected to various oxidatively reinforced alkali extraction stages (E*). All E* stages were performed for one hour at 80°C in 4mm thick heat-sealable Kapak pouches. The E* conditions are summarized in Table 1. Kappa and brightness measurements were performed on the extracted pulps in accordance with TAPPI methods T236 and T452, respectively (TAPPI Test Methods 1999).

Hexenuronic acid content in brownstock

The content of hexenuronic acids in the brownstock was indirectly measured in accordance with a modified procedure reported by Vuorinen *et al.* (1996). In brief, a 1000-ml round bottom flask

was charged with 25 g of pulp (o.d. basis). The pulp consistency was adjusted to 3 % by adding distilled water. The pH was then lowered to 3 using a 4.0 N solution of sulfuric acid. The slurry was refluxed for three hours at 100°C. The change in kappa number before and after the treatment was then determined and served as an indirect measurement of hexenuronic acids (see Table 2).

Control experiments

Control experiments (see Table 3) were performed on the brownstock in accordance with the LMS experimental protocol, except no laccase was employed. The treated pulps were then subjected to the E* stages under the conditions outlined in Table 1.

Laccase-mediator procedure for lignin isolation purposes

In order to isolate the residual lignin from the LMS treated pulps, larger batches were needed. A 2000-ml capacity Parr reactor was employed and was charged with 60 g of never-dried fibers (solid basis). The experimental protocol for the larger batches was identical to the one described above, except 8.9×10^{-3} moles of HBT and NHAA were added instead of 2.2×10^{-3} moles.

Isolation of residual lignins

The isolation of residual lignins was carried out following standard literature methods (Gellerstedt and Lindfors 1991; Gellerstedt *et al.* 1994; Froass *et al.* 1996). In brief, a 5000-ml three-necked round bottom flask was charged with 50 g of o.d. pulp and the consistency was adjusted to 4 % by adding a 0.10 N HCl 9:1 *p*-dioxane:water solution. The slurry was then refluxed for two hours under an argon atmosphere. The pulp was filtered and the filtrate was filtered through celite, neutralized, and concentrated under reduced pressure to approximately 10 % of the original volume. Water (ca. 400 ml) was added and the mixture was concentrated again under reduced pressure to remove the last traces of *p*-dioxane. The solution's pH was then adjusted to 2.5 with 1.00 N HCl. The precipitated lignin was collected, washed several times, and freeze-dried. Lignin yields ranged from 45.4 to 48.3 %. Lignin yields were calculated as follows:

$\% \text{ lignin yield} = \{ \text{mass of lignin isolated} / (\text{initial kappa of brownstock}) \times 0.15 \} \times 100.$

Characterization of residual lignins

The residual lignins isolated from the brownstock (kappa # 75.4) and from LMS_{HBT} (E), LMS_{HBT} (E+P+O), LMS_{NHAA} (E) and LMS_{NHAA} (E+P+O) treated pulps were phosphitylated and characterized by ³¹P NMR in accordance with established literature methods (Granata and Argyropoulos 1995; Jiang and Argyropoulos 1998). NMR data were acquired with a DMX400 MHz Bruker spectrometer.

NMR error analysis

The NMR error analysis was conducted by isolating the residual lignin from the brownstock three separate times under identical conditions and comparing the results. The isolated lignin samples were then phosphitylated and analyzed by ³¹P NMR. A least significant difference (LSD) value at a 95 % confidence interval was obtained by using the standard deviation along with the Student-t value. The calculated LSD values for the functional groups acquired by ³¹P NMR are illustrated in Table 4.

Results and Discussion

To date, research efforts into laccase-mediator systems have focused on the bio-delignification of low-lignin content kraft pulps with kappa numbers ranging from 10-33. These studies have been primarily directed at developing novel environmentally compatible bleaching technologies. Our research interests lie in applying LMS towards kraft process improvements. We have previously shown that an LMS treatment could efficiently delignify high-lignin content kraft pulps (Chakar *et al.* 1998; Chakar and Ragauskas 1999). The utilization of such technology may have positive ramifications on wood utilization practices, since LMS treatments have been repeatedly shown to exhibit a high selectivity towards lignin and not towards carbohydrates. The purpose of this study

was to further understand the fundamental LMS delignification chemistry of high-kappa kraft pulps with HBT and NHAA. All LMS_{NHAA} (E*) and LMS_{HBT} (E*) treatments were performed under identical conditions. This enabled us to relate the changes in biobleaching to the mediator. These changes were assessed by determining structural differences in the residual lignins as well as by measuring the kappa and brightness of the treated pulps.

Hexenuronic acids in the brownstock

It is well known that the presence of hexenuronic acids (HexA) in kraft pulps, especially in hardwood kraft fibers, has an impact on the kappa number. Indeed, Vuorinen *et al.* (1996) reported that HexA contribute as much as 50% to the kappa number of Scandinavian hardwood kraft pulps. Clearly, in such cases, a kappa number would not be a good reflection of the lignin content. In this study, we proceeded to indirectly measure the HexA content in the brownstock in accordance with the literature (Vuorinen *et al.* 1996). The change in kappa number before and after the acid hydrolysis treatments averaged 2.15% (see Table 2). Consequently, this indicates that the kappa number is a good reflection of the lignin content in the pulp used in this study.

Extent of delignification and brightness results

Delignification results from control experiments

It has been shown that in order to achieve substantial delignification with an LMS treatment, both the mediator and the laccase must be present in the system, and that a treatment in the presence of only laccase has a minimal effect on delignification (Paice *et al.* 1995a). In this study, we performed a series of control experiments in the absence of laccase. The pulps were first treated in the presence of HBT and/or NHAA (MS_{HBT} and MS_{NHAA}) and then subjected to the E* stages. In addition, an alkaline extraction (E) was carried out on the untreated brownstock (BS(E)). The kappa numbers measured subsequent to the $MS_{HBT}(E)$, $MS_{NHAA}(E)$, and BS(E) treatments were the same (see Fig. 2). Clearly, this indicates that the presence of the mediator alone does not delignify the pulp. Furthermore, we can conclude that the decrease in kappa number of pulps treated with

either mediator and followed by an E+O, E+P, and E+P+O stage is attributed to the oxidative reinforcement and not to the mediator.

Delignification results from LMS treated pulps

The LMS (E*) delignification kappa data are depicted in Figure 2. Clearly, these results suggest that both an LMS_{HBT} and an LMS_{NHAA} delignified the high-kappa pulp. However, based on the experimental conditions employed in this study, the use of HBT yielded a higher degree of delignification than NHAA. The use of oxidatively reinforced alkali extractions after both the LMS_{HBT} and LMS_{NHAA} treatments further enhanced this effect. The use of an (E+P+O) alkaline extraction stage seems to narrow the difference of the kappa number after an LMS_{NHAA} and an LMS_{HBT} treatment. The addition of peroxide in the alkaline extraction stage lead to both brightening and delignification. This differs from the typical response of D₀ pulps to E+P treatments, where the peroxide essentially brightens the pulp and does not significantly delignify it (Runge and Ragauskas 1999). In our case, the alkaline peroxide response could be due to several factors, including the presence of transitional metals in the pulp.

A general comparison of the LMS delignification results and the control studies summarized in Figure 2 indicate that both the mediator and laccase must be present in the system in order to achieve substantial delignification.

Brightness results from LMS treated pulps

Figure 3 illustrates the brightness values for the LMS (E) and LMS (E*) treated pulps. Pulp darkening was observed subsequent to the LMS_{HBT} (E) and LMS_{NHAA} (E) treatments. However, the loss in brightness was more pronounced when NHAA was used. Pulp darkening after LMS_{HBT} and LMS_{ABTS} stages on low-lignin content pulps has been reported by several researchers (Poppius-Levlin *et al.* 1997a; Paice *et al.* 1995b). We speculate that this may be attributed to a greater content of quinone type structures generated during an LMS_{NHAA} than during an LMS_{HBT}

treatment. The oxidatively reinforced alkali extraction stages were effective at countering this loss in brightness, especially when peroxide was used (i.e., E+P and E+P+O). Evidently, the oxidative reinforcement of an alkaline extraction stage leads to the destruction of chromophores.

Analysis of phosphitylated residual lignins

Having characterized the LMS (E*) treated pulps for lignin content and brightness, we proceeded further with our study by examining the structural changes in the residual lignins. The residual lignins from the brownstock and from LMS_{HBT} (E), LMS_{HBT} (E+P+O), LMS_{NHAA} (E), and LMS_{NHAA} (E+P+O) treated pulps were isolated, phosphitylated, and characterized *via* ³¹P NMR. This facile and effective technique enabled us to canvass several important lignin functional groups, including carboxylic acid groups, aliphatic hydroxyl groups, and phenolic hydroxyl groups in non-condensed and condensed at C-5 lignin moieties. Figure 4 illustrates phenolic lignin moieties that were quantified using this procedure.

Carboxylic acid groups

The results shown in Figure 5 clearly indicate that relative to the brownstock residual lignin, the carboxylic acid groups content increased after an LMS_{HBT} (E) and LMS_{NHAA} (E) treatment on the high-kappa pulp. An analogous increase in carboxylic acid groups has been reported in previous LMS_{HBT} work using low-lignin content kraft pulps (Sealey and Ragauskas 1998).

Our results also showed that the reinforcement of the alkaline extraction stage with peroxide and oxygen further increased the content of carboxylic acid groups. The content of carboxyl groups after an LMS_{NHAA} (E+P+O) was greater than after an LMS_{HBT} (E+P+O). This difference must be due to the different delignification chemistry of the two mediators.

Phenolic hydroxyl groups in lignin structures non-condensed at C-5

The data shown in Figure 6 indicate that the residual lignins isolated after an LMS_{NHAA} (E) and LMS_{HBT} (E) treatment were depleted of non-condensed at C-5 lignin structures with respect to the brownstock lignin. Nonetheless, the decrease in this moiety was more pronounced with NHAA than with HBT. The oxidative reinforcement of the alkaline stage after an LMS_{HBT} further decreased the non-condensed groups by an additional 31.5%. The depletion of these lignin structures has also been noted on low-lignin content pulps (Poppius-Levlin *et al.* 1997b; Sealey and Ragauskas 1998). An (E+P+O) stage after the LMS_{NHAA} treatment did not yield any further decrease of phenolic hydroxyl groups in non-condensed at C-5 lignin structures, as one would anticipate when additional oxidants are introduced in an alkaline extraction stage. These results seem to suggest that there are differences in the fundamental chemistry between an LMS_{NHAA} and an LMS_{HBT} treatment. Further studies will be required to elucidate these differences.

Phenolic hydroxyl groups in C-5 condensed lignin structures

Inspection of Figure 7 reveals that relative to the brownstock residual lignin, the concentration of phenolic hydroxyl groups in C-5 condensed lignin structures after an LMS_{HBT} (E) and an LMS_{NHAA} (E) treatment was comparable. The reinforcement of the alkaline extraction stage after an LMS_{HBT} treatment substantially decreased the content of phenolic hydroxyl groups in these condensed lignin structures. A decrease of this magnitude was not observed after an LMS_{NHAA} treatment.

Overall, the phenolic hydroxyl data in non-condensed and condensed lignin structures seem to suggest that the LMS delignification chemistry on high-kappa pulps exhibits a higher selectivity towards non-condensed at C-5 lignin structures than towards condensed C-5 lignin moieties.

These results are in general agreement with recent studies on LMS_{HBT} (E) treatments on low-lignin content pulps, which highlight the oxidative selectivity of an LMS towards phenolic hydroxyl groups (Sealey and Ragauskas 1998; Poppius-Levlin *et al.* 1997b).

Aliphatic hydroxyl groups

The results shown in Figure 8 illustrate a decrease in the content of aliphatic hydroxyl groups after an LMS_{NHAA} (E) and an LMS_{HBT} (E) treatment relative to the brownstock residual lignin.

However, the decrease was greater with NHAA than with HBT. This observation is indicative of side chain oxidation, and is consistent with Freudenreich *et al.* (1998) and Li *et al.* (1997) recent observation of side chain oxidation and fragmentation of model compounds during LMS treatments. The benefits associated with oxidatively reinforcing the alkali extraction stages were not evident.

Conclusions

In summary, the delignification response of the pulps clearly indicates that an LMS treatment can be effectively employed on high-lignin content kraft pulps. Oxidative reinforcement of the alkali extraction stages is beneficial in delignifying and countering the darkening phenomenon observed after LMS treatments. Based on the spectral analysis of residual lignins, an LMS_{NHAA} (E) treatment led to a greater decrease in the content of phenolic hydroxyl groups in non-condensed at C-5 lignin structures than an LMS_{HBT} (E) stage. Nonetheless, the results seem to indicate that both an LMS_{NHAA} and an LMS_{HBT} principally favors the oxidation of free phenolic moieties. Oxidation of side chain aliphatic hydroxyl groups was more pronounced after an LMS_{NHAA} than after an LMS_{HBT} treatment.

In conclusion, our LMS studies on high-kappa kraft pulps suggest that differences exist in the delignification chemistry of NHAA and HBT, despite the fact that both mediators operate *via* nitroxyl radicals (Potthast *et al.* 1997). As previously discussed, the formation of quinones could be occurring during the LMS treatment. This hypothesis is supported by the brightness response of the pulps to E vs. (E+P+O). In addition, the structural changes in the phenolic hydroxyl content of the LMS (E*) residual lignin are consistent with our hypothesis. Further studies are ongoing to confirm this important issue.

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Table 1. Summary of extraction stage conditions

Extraction Stage (E*)	%NaOH (o.d. basis)	%H ₂ O ₂ (o.d. basis)	O ₂ (psi)
E	2.5	-	-
E+O	2.5	-	60
E+P	2.5	0.5	-
E+P+O	2.5	0.5	60

Table 2. Changes in kappa # after acid treatment

Replicate #	Initial kappa number of brownstock	Kappa number after treatment	% Change
1	75.4	73.6	2.4
2	75.4	74.0	1.9

Table 3. Summary of control experiments

Experiment ^a
Brownstock followed by E stage
Brownstock treated with HBT and followed by E stage
Brownstock treated with NHAA only and followed by E stage
Brownstock treated with HBT only and followed by E+O stage
Brownstock treated with NHAA only and followed by E+O stage
Brownstock treated with HBT only and followed by E+P stage
Brownstock treated with NHAA only and followed by E+P stage
Brownstock treated with HBT only and followed by E+P+O stage
Brownstock treated with NHAA only and followed by E+P+O stage

^a: Mediator treatments were performed without the laccase (see LMS and extraction procedures for experimental details).

Table 4. ³¹P NMR Least significant difference values

Functional group	Average (mmol/g lignin)	SD.	LSD
Carboxyl OH	0.19	0.006	0.037
Non-condensed at C-5 phenolic OH	0.91	0.013	0.078
Condensed at C-5 phenolic OH	0.69	0.022	0.133
Aliphatic OH	1.73	0.007	0.042

Figure 1. Structures of mediators employed in this study

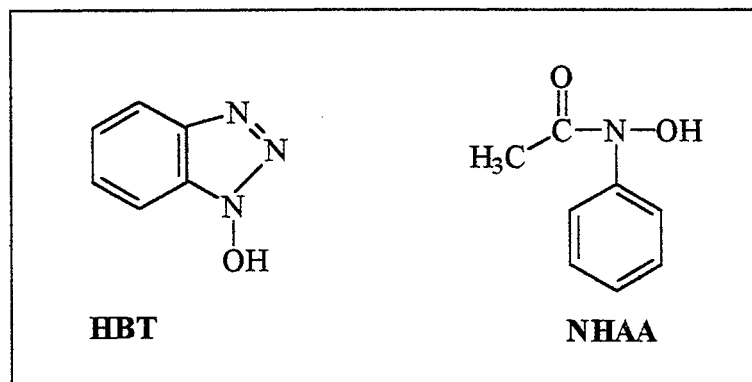
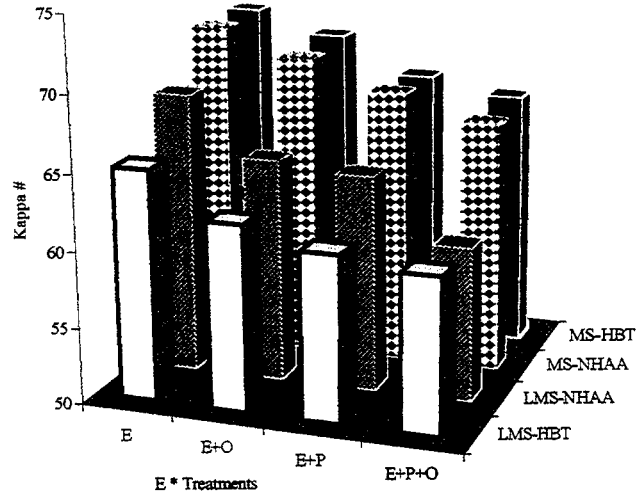
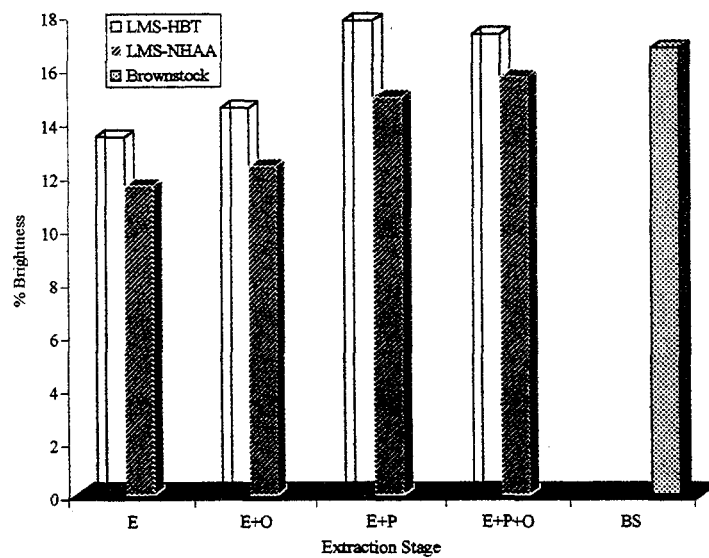


Figure 2. Kappa results of control treatments in the absence of laccase (MS-NHAA and MS-HBT) and LMS treated pulps using NHAA and HBT(LMS-NHAA and LMS-HBT) followed by the alkaline extraction stages E, E+O, E+P, and E+P+O



N.B.: Initial kappa # of brownstock was 75.4. The kappa of the brownstock subsequent to an alkaline extraction stage (E) was 72.3.

Figure 3. TAPPI Brightness results of brownstock as well as of LMS treated pulps using NHAA and HBT (LMS-NHAA and LMS-HBT) followed by the alkaline extraction stages E, E+O, E+P, and E+P+O



N.B: Each data point represents the average of 5 brightness readings.

Figure 4. Phenolic hydroxyl groups in C-5 condensed and non-condensed at C-5 lignin structures

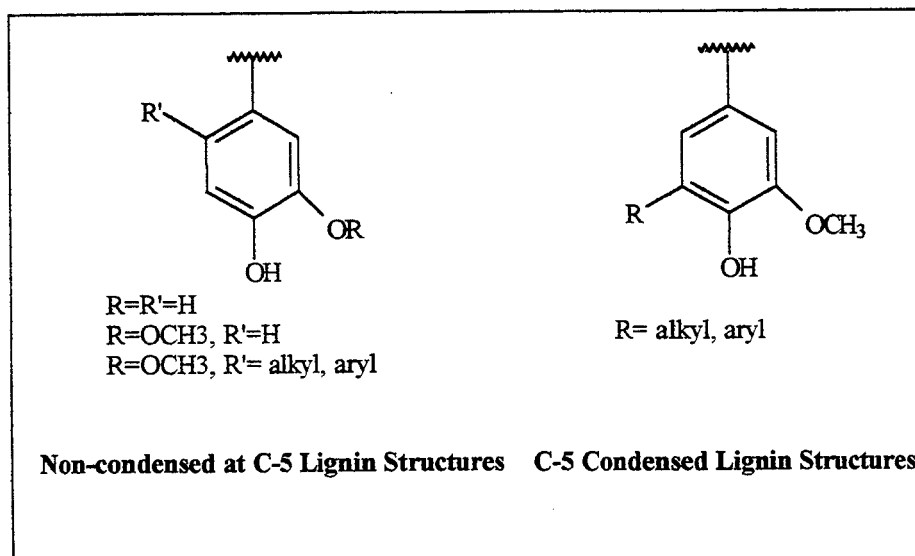


Figure 5. Carboxylic acid hydroxyl groups in residual lignins isolated from the brownstock as well as from LMS treated pulps using NHAA and HBT (LMS-NHAA and LMS-HBT) followed by the alkaline extraction stages E and E+P+O

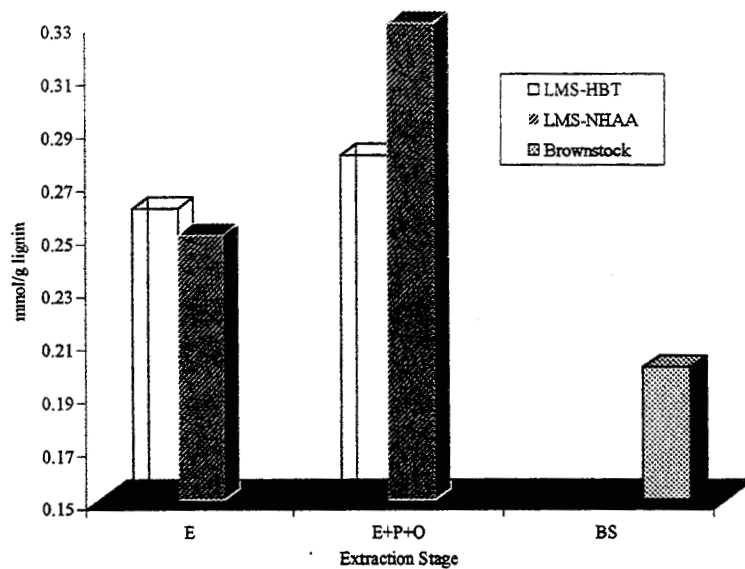


Figure 6. Phenolic hydroxyl groups in non-condensed lignin structures at C-5 in residual lignins isolated from the brownstock as well as from LMS treated pulps using NHAA and HBT (LMS-NHAA and LMS-HBT) followed by the alkaline extraction stages E and E+P+O

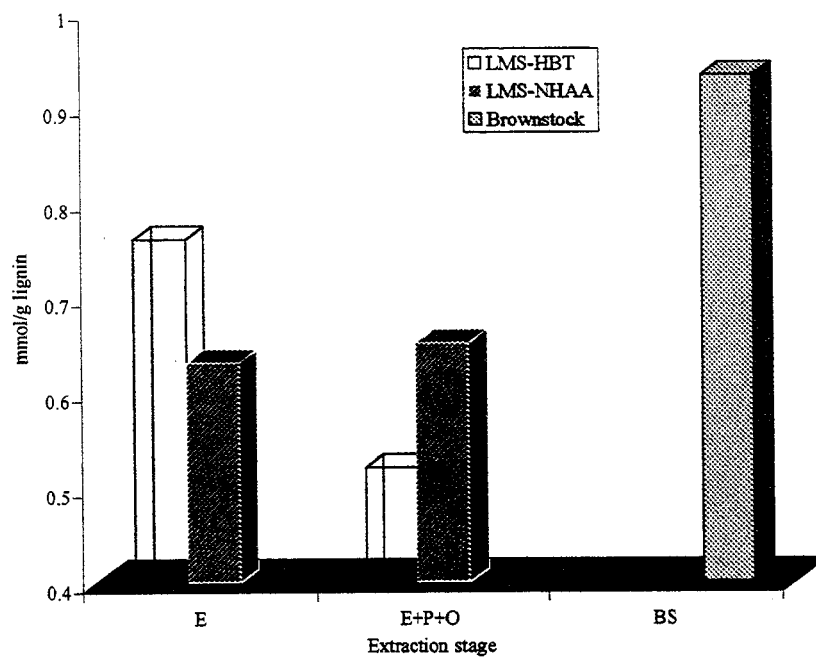


Figure 7. Phenolic hydroxyl groups in C-5 condensed lignin structures in residual lignins isolated from the brownstock as well as from LMS treated pulps using NHAA and HBT (LMS-NHAA and LMS-HBT) followed by the alkaline extraction stages E and E+P+O

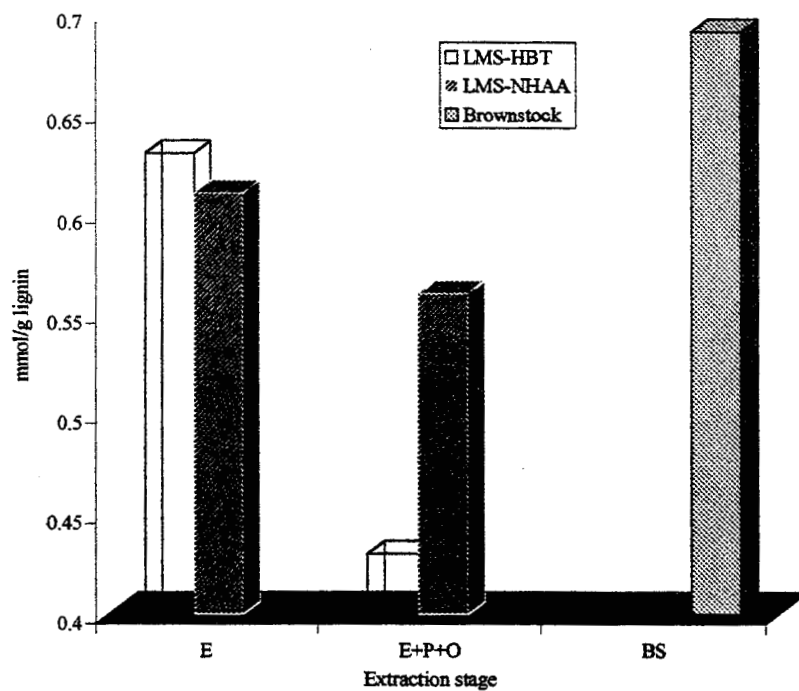


Figure 8. Aliphatic hydroxyl groups in residual lignins isolated from the brownstock as well as from LMS treated pulps using NHAA and HBT (LMS-NHAA and LMS-HBT) followed by the alkaline extraction stages E and E+P+O

