

## NOTE

Seiichi Yasuda · Kazuhiko Fukushima · Akihiro Kakehi

**Formation and chemical structures of acid-soluble lignin I: sulfuric acid treatment time and acid-soluble lignin content of hardwood**

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**Abstract** To elucidate the formation mechanism of acid-soluble lignin (ASL) formed in the Klason lignin determination, beech wood meals were treated with sulfuric acid (SA) under various conditions, and the ASL solution was extracted with  $\text{CHCl}_3$ . The results indicated the following: (1) wood components yielding ASL are dissolved in 72% SA during the initial stage; (2) the quantity of ASL is highest during the initial stage, then decreases with prolonged time of 72% SA treatment and finally reaches a constant value; (3) soluble lignin prepared by 72% SA treatment and subsequent standing in 3% SA again yield insoluble Klason lignin and ASL after boiling in 3% SA; and (4) about half the amount of ASL is dissolved in  $\text{CHCl}_3$ . The foregoing suggest that wood components yielding ASL are dissolved in 72% SA at the beginning and finally change to ASL after being subjected to depolymerization, hydrolysis, and other reactions. ASL may thus be composed of low-molecular-weight degradation products and hydrophilic derivatives of lignin.

**Key words** Acid-soluble lignin · Klason lignin · Sulfuric acid lignin · Hardwood

**Introduction**

The Klason method is the most typical lignin determination procedure. The procedure separates lignin as an insoluble material by depolymerization of cellulose and hemicellulose in 72% sulfuric acid (SA) followed by hydrolysis of the dissolved polysaccharides in boiling 3% SA. However, part of the lignin is dissolved in the filtrate as

so-called acid-soluble lignin (ASL). Because lignin possesses oxygen-containing functional groups at benzylic positions, it is sensitive to acidic media and therefore undergoes considerable changes during the lignin determination procedure. Chemical changes in 72% SA are known to be brought about by reactions such as condensation<sup>1</sup> between aromatic and benzylic carbons, cleavage<sup>2</sup> of  $\beta$ -syringyl ether linkage, and rearrangement<sup>3</sup> of  $\beta$ -aryl ether units. The main reaction in boiling 3% SA is probably hydrolysis of the depolymerized polysaccharides to soluble monosaccharides.

The higher ASL content<sup>4</sup> of syringyl lignin-rich woods and the higher reactivity<sup>2</sup> of the syringyl nucleus to SA than the guaiacyl nucleus suggest an important relation between ASL and syringyl lignin. Syringyl nuclei, furthermore, have higher reactivity during the condensation reaction with carbohydrates in 72% SA, yielding glycosides with carbon-to-carbon linkages (C-glycosides). To date, several investigators have tried to elucidate the structure and formation mechanism of ASL, but much remains unknown regarding its structure and other aspects. The present report examines the relation between SA treatment time and ASL content to further clarify the formation mechanism of ASL.

**Materials and methods****Lignin determination**

Klason lignin (KL) and ASL determinations were carried out by the modified JIS method (JIS P8008), with adjusted 72% SA treatment times. Absorptivities of 94.5<sup>5</sup> and 113<sup>6</sup> l·g<sup>-1</sup>·cm<sup>-1</sup> were used for the ultraviolet (UV) (205 nm) spectrophotometric determination of the ASLs of pine and beech, respectively.

**Extraction of ASL solution with  $\text{CHCl}_3$** 

A 25-ml portion of the ASL solutions (3% SA concentration) of pine and beech was extracted with  $\text{CHCl}_3$ .

S. Yasuda (✉) · K. Fukushima · A. Kakehi  
Division of Biological Material Sciences, Department of Biosphere Resources Science, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan  
Tel. +81-52-789-4159; Fax +81-52-789-4163  
e-mail: syasuda@agr.nagoya-u.ac.jp

The aqueous layer was again extracted with diethyl ether to remove the  $\text{CHCl}_3$ , evaporated under reduced pressure to about half the volume, and then adjusted to the original volume with distilled water.

## Results and discussion

The results of lignin determination under a variety of 72% SA treatment times are summarized in Table 1. Red pine (a softwood) showed a constant lignin determination value under 72% SA treatment times of 30 and 120 min; the hardwood beech had higher determination values, particularly higher ASL contents, than those of softwood under the same conditions. To obtain useful information on the relation between 72% SA treatment times and the determination values of KL and ASL, shorter treatment times were adopted. As shown in Table 1, reduction of the treatment time increased both KL and ASL values. The increase in KL can be explained by insufficient hydrolysis of insoluble polysaccharides in the acidic suspension while being boiled in 3% SA, as a consequence of insufficient swelling and insufficient destruction of the microfibril structure of cellulose in 72% SA. It is known that the chloroform extracts, except fulfural, of the ASL solution have a typical lignin UV absorptivity, and that the remaining ASL has a coefficient only one-third<sup>6</sup> as high, suggesting that the former are lignin degradation products on the basis of their high solubility in 3% SA and that the latter has hydrophilic groups formed during 72% SA treatment. ASL is probably composed of two components: lignin degradation products and secondarily formed hydrophilic materials such as lignin-carbohydrate compounds,<sup>7</sup> as described later. About half of the ASL from beech was extracted with  $\text{CHCl}_3$ .

It was also found that a 72% SA-soluble fraction<sup>8</sup> separated by a glass filter contained 58% syringyl lignin; the remaining 72% SA-insoluble fraction contained only 19% syringyl lignin. Probably the insoluble 19% syringyl lignin can be ascribed to covalent bonds between guaiacyl lignin. Furthermore, pine lignin,<sup>8</sup> composed of only guaiacyl lignin, was not dissolved in 72% SA. These findings indicate the high solubility of syringyl lignin in 72% SA. Experimental support<sup>2</sup> for this solubility was obtained based on the behavior of guaiacyl and syringyl lignin model compounds in 72% SA. The compound guaiacylglycerol- $\beta$ -guaiacyl ether changed to polymeric solids in 72% SA. In contrast, the compound syringylglycerol- $\beta$ -syringyl ether formed a homogeneous solution in 72% SA, suggesting complete dissolution of the formed polymeric material in 72% SA. Considering the high solubility of syringyl lignin in 72% SA, it is thought that the lignin initially dissolves in 72% SA during the Klason treatment and simultaneously undergoes intermolecular condensation, condensation with carbohydrates, degradation, and other reactions. Therefore, the yield of ASL is higher during the initial stages of 72% SA treatment and reaches a constant after sufficient reaction time. After acid hydrolysis of polysaccharides by boiling in 3% SA, the originally dissolved syringyl lignin formed insoluble KL and soluble ASL.

To better understand the form of lignin after treatment with 72% SA, the suspensions were allowed to stand at room temperature instead of boiling. Considering the results in Table 2, the ASL of beech in standing 3% SA after the treatment with 72% SA possesses the polysaccharides chain of the degraded lignin-carbohydrate compounds (LCCs) or secondarily formed LCC to some extent (or both). This result is not surprising if one considers that the purpose of boiling is to hydrolyze partially depolymerized polysaccharides. Prolonged treatment with 72% SA caused

**Table 1.** Treatment of red pine and beech wood meals with 72% sulfuric acid for different periods, followed by boiling in 3% sulfuric acid

Time (min) <sup>a</sup>	Yield (%) <sup>b</sup>				
	KL	ASL	Total lignin	Water-soluble ASL	$\text{CHCl}_3$ -soluble ASL
Red pine					
30	27.8	0.5	28.3	0.4	0.1
120	27.3	0.4	27.7	0.3	0.1
Beech					
5	48.0	5.1	53.1	2.7	2.4
10	29.1	4.6	33.7	2.5	2.1
20	21.6	4.0	25.6	2.6	1.6
30	21.6	3.7	25.3	2.4	1.1
60	21.4	3.4	24.8	2.3	1.1
120	21.5	2.9	24.4	1.8	1.1
240	21.8	2.5	24.3	1.7	0.7
		2.3 <sup>c</sup>		1.0	1.3

Treatment notes: Klason lignin (KL) and acid-soluble lignin (ASL) determinations were carried out by the modified JIS method (JIS P8008), with adjusted 72% sulfuric acid (SA) treatment times

<sup>a</sup>The 72% SA treatment time

<sup>b</sup>Percent on extractive-free wood meal

<sup>c</sup>ASL solution was neutralized (pH 3.0) with saturated  $\text{Ba}(\text{OH})_2$ , centrifuged to remove  $\text{BaSO}_4$ , and then concentrated under reduced pressure to the original volume

**Table 2.** Treatment of red pine and beech wood meals with 72% for different periods, SA followed by standing in 3% SA and retreatment of the formed KL and ASL by boiling in 3% SA

Wood or sample	Time (min) <sup>a</sup>	Yield (%) <sup>b</sup>				
		KL	ASL	Total lignin	Water-soluble ASL	CHCl <sub>3</sub> -soluble ASL
Red pine	30	29.2	1.7	30.9	1.3	0.4
	120	29.5	1.1	30.6	1.0	0.2
Beech	10	47.0 <sup>c</sup>	6.2 <sup>d</sup>	53.2	3.6	2.6
KL-10 <sup>e</sup>		27.7	0.7	28.4	–	–
ASL-10 <sup>e</sup>		1.2	4.2	5.4	1.9	2.3
Total		28.9	4.9	33.8 <sup>e</sup>		
Beech	30	14.1	9.7	23.8	5.8	3.9
KL-30		13.5	0.8	15.0	–	–
ASL-30		6.3	3.5	9.6	1.7	1.6
Total		19.8	4.3	24.6		
Beech	120	12.6	10.0	22.6	5.3	4.7
KL-120		12.5	0.1	12.6	–	–
ASL-120		8.2	3.1	11.3	1.5	1.6
Total		20.7	3.4	23.9		

Treatment notes: Wood meals were treated with 72% SA, changing the treatment times, then allowed to stand at room temperature for 3 h in 3% SA. After separating the suspension with a glass filter, the insoluble KLS (KL-10, -30, and -120; these KLS were prepared by 72% SA treatment for 10, 30, and 120 min, respectively) were suspended in the freshly prepared 3% SA, boiled for 3 h, and then again filtered with a glass filter. The ASL solutions (ASL-10, -30, and -120), after adjusting SA concentration to 3%, were boiled for 3 h and also filtered

–, not determined

<sup>a</sup>The 72% SA treatment time

<sup>b</sup>Percent on extractive-free wood meal

<sup>c</sup>KL and ASL were obtained by treating beech wood meal with 72% SA for 10 min followed by standing at room temperature for 3 h in 3% SA

<sup>d</sup>KL-10 and ASL-10 were boiled for 3 h in 3% SA

<sup>e</sup>Total of KL-10 and ASL-10

**Table 3.** Treatment of the 72% SA insoluble and soluble fractions, obtained after separation with a glass filter, by standing or boiling in 3% SA

Hydrolysis condition	Yield (%)					
	SA-insoluble fraction		Soluble fraction			
	KL	ASL	KL	ASL	H <sub>2</sub> O-ASL	CHCl <sub>3</sub> -ASL
Standing	–	–	0.1	10.5	5.8	4.0
Boiling	13.0	0.6	7.3	3.2	1.9	1.3

Treatment notes: Beech wood meals were treated with 72% SA for 30 min and then filtered with a glass filter as fast as possible to give insoluble (IS) and soluble (S) fractions. Half of the S solution was adjusted to a concentration of 3% SA, allowed to stand at room temperature for 3 h, and then filtered with a glass filter. The IS in the freshly prepared 3% SA and the other half of the S solution also adjusted to a concentration of 3% SA were boiled for 3 h and also filtered

–, not determined

an increase in ASL, in contrast with a significant decrease in KL. Experimental evidence<sup>7</sup> in support of this possibility was provided by the faster 72% SA-catalyzed condensation reactions of syringyl lignin model compounds with carbohydrates. In any case, hemicellulose may play an important role in the formation of ASL. Boiling the first ASLs (ASL-10, -30, and -120) in 3% SA solution again produced KL and ASL. Liberation of the second KL may be interpreted as being due to the hydrolysis of polysaccharides in the LCCs or the lower solubility of 3% SA solution by the hydrolysis of the partially depolymerized

polysaccharides dissolved in monosaccharides. The formation of ASL from KLS (KL-10 and KL-30) after standing in 3% SA at room temperature may be interpreted as insufficient separation of the 72% SA suspension with a glass filter or liberation of KL accompanying by hydrolysis of the remained LCC in KL (or both). It is difficult to find an explanation for the higher yields of CHCl<sub>3</sub>-soluble ASL after standing.

To confirm the hypothesis that ASL is dissolved in 72% SA during the initial stage, a 72% SA suspension of beech wood meals was separated with a glass filter to

give insoluble (IS) and soluble (S) fractions. Half of the filtrate (S) was then allowed to stand at room temperature, and the remaining half was boiled. Because the 72% SA suspension had a high viscosity after treatment for 10min and the suspension could not be separated in a short time, a longer treatment time (30min) was adopted. Because the yield (10.5%) of ASL from the S fraction in Table 3 is almost equal to that (9.7%) of ASL in Table 2, ASL was almost dissolved in 72% SA within 30min. Hydrolysis of the S fraction (which was prepared by standing at room temperature in 3% SA) in boiling 3% SA gave a large amount of IS, suggesting hydrolysis of glycosidic bonds between ligin and carbohydrates, as shown in Table 3. ASL was confirmed to be dissolved in 72% SA.

In summary, syringyl lignin is rapidly dissolved in 72% SA; and at the same time the dissolved components undergo intermolecular condensation, condensation with carbohydrates, degradation, and other reactions to yield ASL and insoluble KL with syringyl nuclei. Sufficient treatment times are required to reach constant ASL values.

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