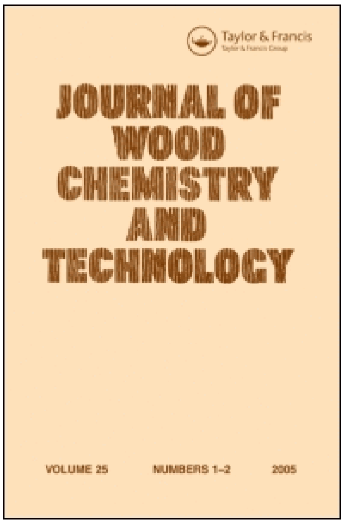

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Eiichi Maekawa[a]; Taizo Ichizawa[a]; Testuo Koshijima[a]
[a] Wood Chemistry Section, Wood Research Institute Kyoto University, Kyoto, Japan

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AN EVALUATION OF THE ACID-SOLUBLE LIGNIN DETERMINATION IN ANALYSES OF LIGNIN BY THE SULFURIC ACID METHOD

Eiichi Maekawa, Taizo Ichizawa, and Testuo Koshijima

Wood Chemistry Section, Wood Research Institute
Kyoto University, Gokasho, Uji, Kyoto 611 Japan

ABSTRACT

The acid-soluble lignin determination used in analyses of lignin by the sulfuric acid method was evaluated. The conventional UV spectrophotometric method using the absorption at 205 nm is appropriate for hardwoods, whereas for softwoods and bamboo the absorption at 205 nm should be corrected, since the absorption maximum is shifted to a shorter wavelength in sulfuric acid solution. Therefore, in the latter cases, the actual absorption maximum should be used.

INTRODUCTION

The sulfuric acid method[1] is the most widely accepted analytical method for determining lignin in woody plants, although the method is tedious and time-consuming. The method, which uses 72% (wt) sulfuric acid for the hydrolysis of the associated polysaccharides, is based on the fact that the lignin remains as an acid-insoluble residues referred to as Klason lignin or sulfuric acid lignin. The commonly accepted procedure for the determination of Klason lignin has been adopted as a Tappi standard method[2] in the United States and as a JIS testing method[3] in Japan. It is generally believed that the sulfuric acid method gives the most reliable value for the native lignin of woody plants, especially conifers, because the recovery of lignin as sulfuric acid lignin is essentially quantitative. However, many investigators have pointed out that treatment of wood with 72% sulfuric acid results in part of the total lignin being dissolved in the filtrate, i.e. acid-soluble lignin.[4] The acid-soluble lignin content of hardwoods is usually 3-4%, while in the softwoods the amount is typically less than 1% on the original wood. The amount of acid-soluble lignin is typically determined by a UV spectrophotometric method because the method is simple and convenient. The amount of acid-soluble lignin can be determined in principle by measuring the absorbance at either 200-205 nm or 280 nm. However, the use of the absorbance at 280 nm involves a few potential sources of error. Klason lignin filtrates contain furfural and hydroxymethyl furfural (polysaccharide degradation products) which absorb near 280 nm.[5] Therefore, this region of the spectrum should not be used for the determination of acid-soluble lignin, and, alternatively, use of the absorbance at 205 nm is recommended.[6-8] As a result, absorption in this region has been exclusively applied for the determination of acid-soluble lignin in various wood species.[9,10] The total lignin in wood is expressed as the sum of the acid-insoluble Klason lignin and the spectrophotometrically-determined acid-soluble lignin.[9] In the UV spectrophotometric method, 110 L g^{-1} cm^{-1} is used as the absorptivity (extinction coefficient) at 205 nm, representing an average for several wood species,[10,11] e.g. 113 and 106 L g^{-1} cm^{-1} for the acid-soluble lignin of birch and eucalyptus, respectively.[12]

This study was undertaken to reevaluate the UV spectrophotometric method for determining the acid-soluble lignin in the Klason lignin filtrate. Of particular interest was the question of whether the absorbance at 205 nm should be used in the analysis of all wood species.

EXPERIMENTAL

Materials and Methods

Wood meals (60-80 mesh) from the heartwood of Japanese beech (Buna, *Fagus crenata* BLUME), maple (Itayakaede, *Acer pictum Subv. eupictum Pax.*), Japanese red pine (Akamatsu, *Pinus densiflora S. et Z.*) and bamboo (Mosochiku, *Phyllostachys edulis Riv.*) were extracted with benzene-ethanol (2:1, vol) and the oven-dry weight of each sample was determined by standard methods. Milled wood lignins (MWL) of Buna, Akamatsu and Mosochiku were used as the standard native lignin samples. The MWL were prepared according to the procedure of Björkman,[13] and contained 27.2%, 19.4%, and 13.2% carbohydrate for Buna, Akamatsu, and Mosochiku, respectively. The extracted wood meals were partially delignified by the acid-chlorite method[14] and the peracetic acid method[15] described previously. Ultraviolet spectra of p-hydroxy benzoic acid, vanillic acid and syringic acid were obtained from commercially available materials.

Sulfuric Acid Method for Lignin Determination

Klason lignin filtrates from wood meals and partially-delignified wood meals were prepared according to the JIS method for lignin determination in wood. Klason lignin contents of the wood meals were: Buna, 21.09%; Itaya-kaede, 24.95%; Akamatsu, 26.87%; and Mosochiku, 25.66%. Ultraviolet spectra of the acid-soluble lignin in the filtrates were taken over the range of 190-350 nm after suitable dilution with distilled water. Acid-soluble lignin contents were determined from the absorbances at 205, 240, and 280 nm, according to the following equation:

$$\text{Acid-soluble Lignin (\%)} = \frac{DV(A_s - A_b)}{aW} \times 100$$

where D is the dilution ratio, V is the filtrate volume (L), A_s is the absorbance of the sample, A_b is the absorbance of the solvent, **a** is absorptivity of the lignin (L g^{-1} cm^{-1}), and W is the oven-dry weight of the wood meal sample (g). The absorptivities used were: 110 at 205 nm, 30 at 240 nm, 23.6 at 280 nm (hardwoods), and 23.3 at 280 nm (softwoods).

Whatman CF-1 cellulose powder (hexosan standard) and glucuronoxylan (9.3% uronic anhydride by decarboxylation) prepared from Japanese beech wood (pentosan standard) were hydrolyzed under the same conditions used for the Klason lignin analyses. The UV spectra of the acid hydrolysates, suitably diluted, were recorded over the range of 190-350 nm (Figure 1) to determine the wavelengths of absorption maxima and their absorbances.

RESULTS AND DISCUSSION

UV Spectra of Klason Lignin Filtrates

The UV spectra of Buna, Akamatsu, and Mosochiku MWL have patterns characteristic of native lignin (Figure 2), although the Mosochiku MWL spectrum has two weak broad absorptions near 285 and 315 nm. These may be due to the presence of ester linkages of p-coumaric acid observed in lignins of Gramineae.

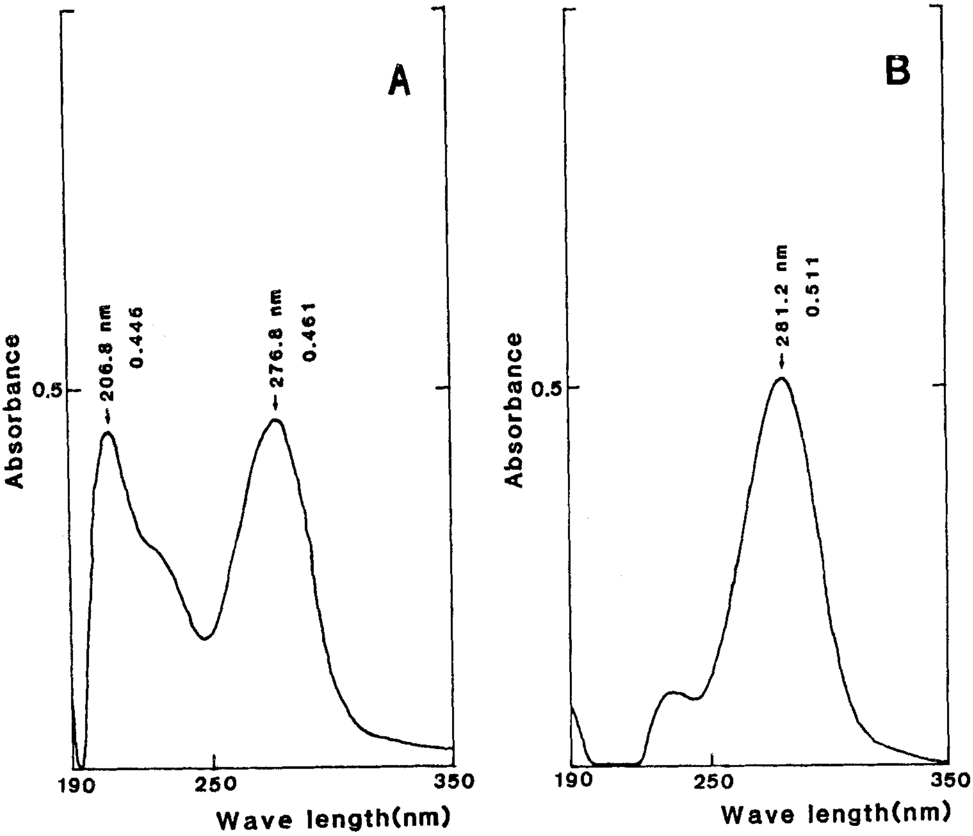

Figure 1. Ultraviolet spectra of the products arising from cellulose and Japanese beech xylan in the Klason lignin determination. A - Beech xylan. B - Cellulose.

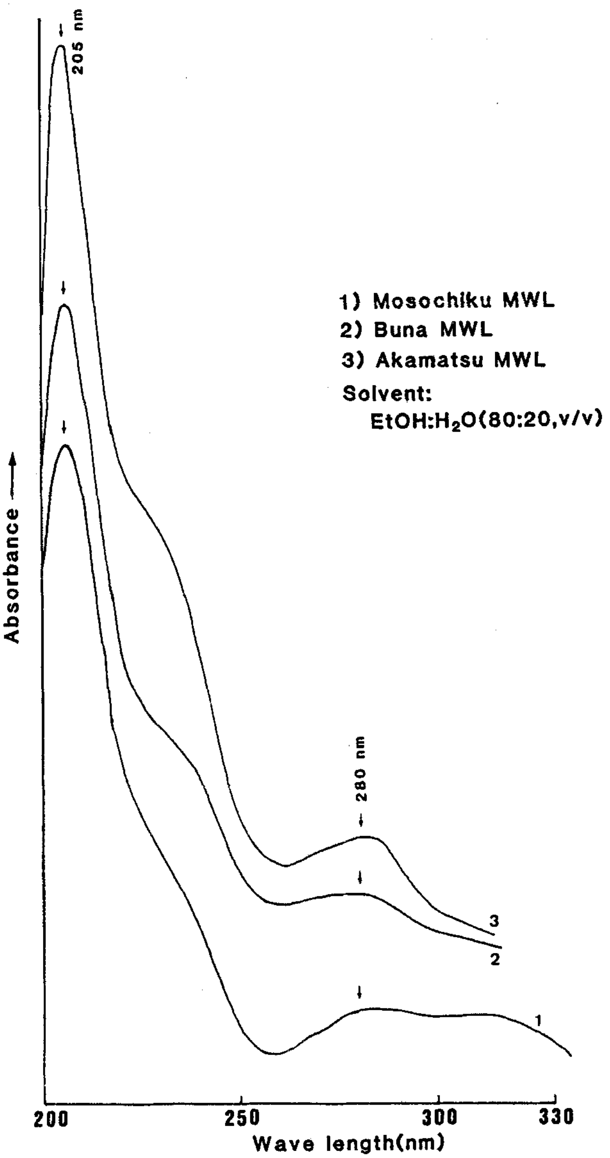

Figure 2. Ultraviolet spectra of Japanese buna, akamatsu, and mosochiku milled-wood lignins (MWL).

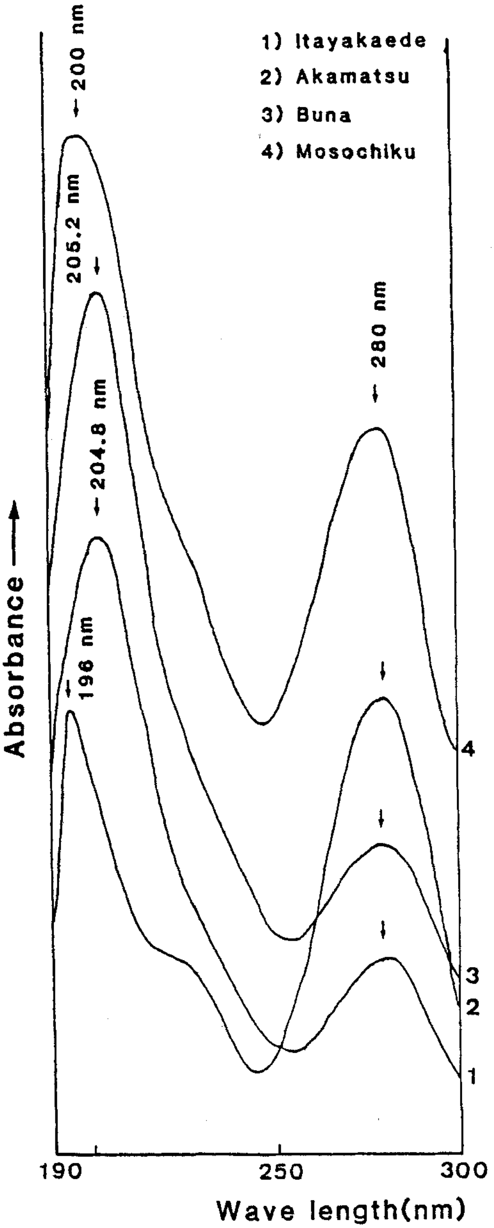

Figure 3. Ultraviolet spectra of Klason lignin filtrates from wood meals of Japanese buna, itayakaede, akamatsu, and mosochiku.

The UV spectra of the MWL are characterized by strong absorptions near 205 nm, pronounced shoulders near 230 nm, and weak absorptions near 280 nm.[16] The UV spectra of the Klason lignin filtrates prepared from the wood meals also show patterns similar to those of the MWL, i.e. absorption maxima near 205 nm and 280 nm, but for Mosochiku and Akamatsu the lower wavelength maximum occurred at 200 and 196 nm, respectively. Similarly, the Klason lignin filtrates obtained from other softwoods such as Japanese cedar (sugi), Japanese cypress (hinoki) and Japanese larch (karamatsu) had absorption maxima at 196-197 nm rather than 205 nm (Figure 4). This implies that use of the absorption at 205 nm results in an artificially low value for the acid-soluble lignin in the softwoods and bamboo which must be corrected to obtain an accurate value.

All of the samples had characteristic absorption maxima near 280 nm attributable primarily to the benzenoid moieties of lignin, but also partially due to furfural and hydroxymethyl furfural obtained from polysaccharide degradation. The acid-soluble lignin spectra had a primary absorption at 196-205 nm and a secondary absorption near 280 nm. Similar UV spectra are also observed for acid-soluble lignins from holocelluloses partially delignified by the acid-chlorite[14] and peracetic acid methods[15] as shown in Figures 5 and 6. However, in this case, a shift of the absorption maximum at 205 nm was not observed.

The ratios of the absorbance at 196-205 nm to that at 280 was calculated for the Klason lignin filtrates from several wood species. As is evident in Table 1, the ratio Max $A_{196-205}$/Max A_{280} for the hardwoods was generally 2-3 with the exception of Japanese cherry and red lauan, whereas for the softwoods the ratio tended to be centered around 1.0. This means that the Klason lignin filtrate from the softwoods contained carbohydrate degradation products tending to give a strong absorption at 280 nm, while those from the hardwoods contained degradation products having a stronger absorption at 205 nm.

UV Spectra of Chloroform and Ether Extracts

Spectra of the diethyl ether extracts of Klason lignin filtrates of various wood meals had strong absorption maxima at 205.2 nm and weaker absorption near 280 nm in ethanol (Figure 7). This suggests that the extracts consist of the similar degradation products, independent of the wood species. In the spectra of the chloroform extracts (Figure 8), the first absorption maxima was shifted to

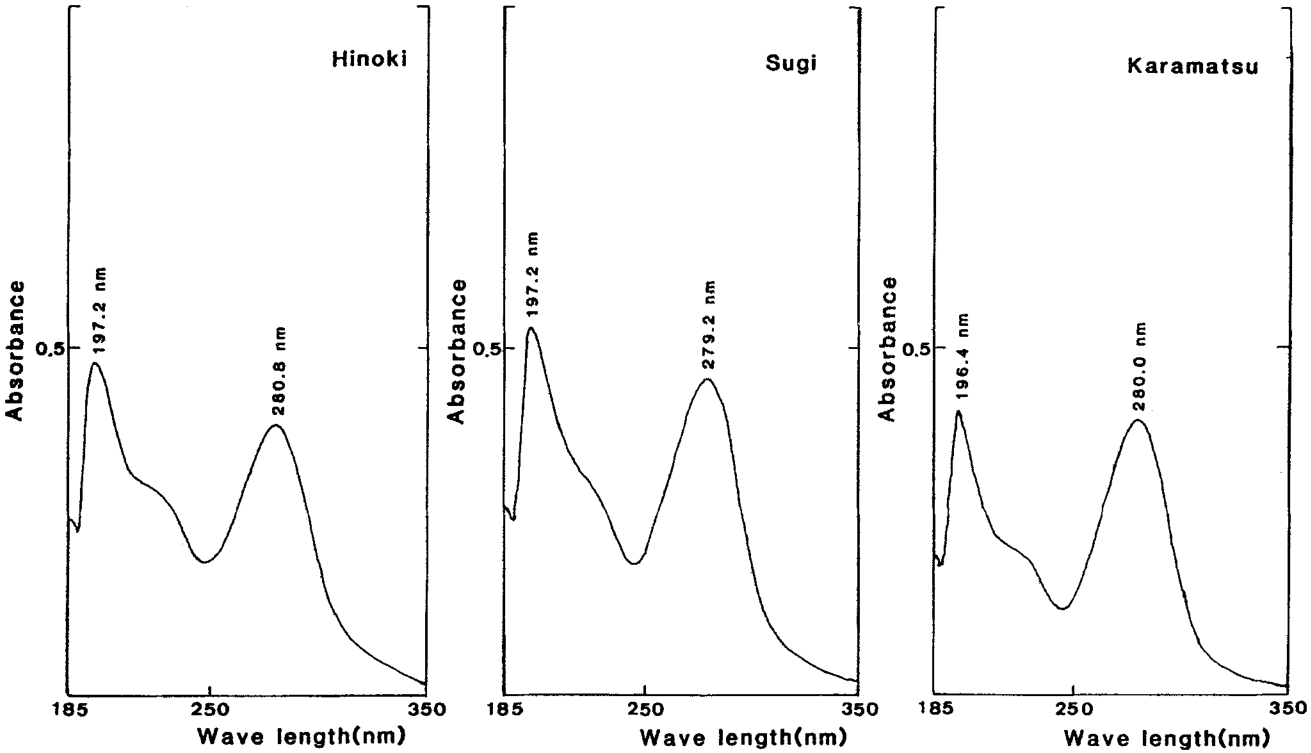

Figure 4. Ultraviolet spectra of Klason lignin filtrates prepared from wood meals of Japanese hinoki, sugi, and karamatsu.

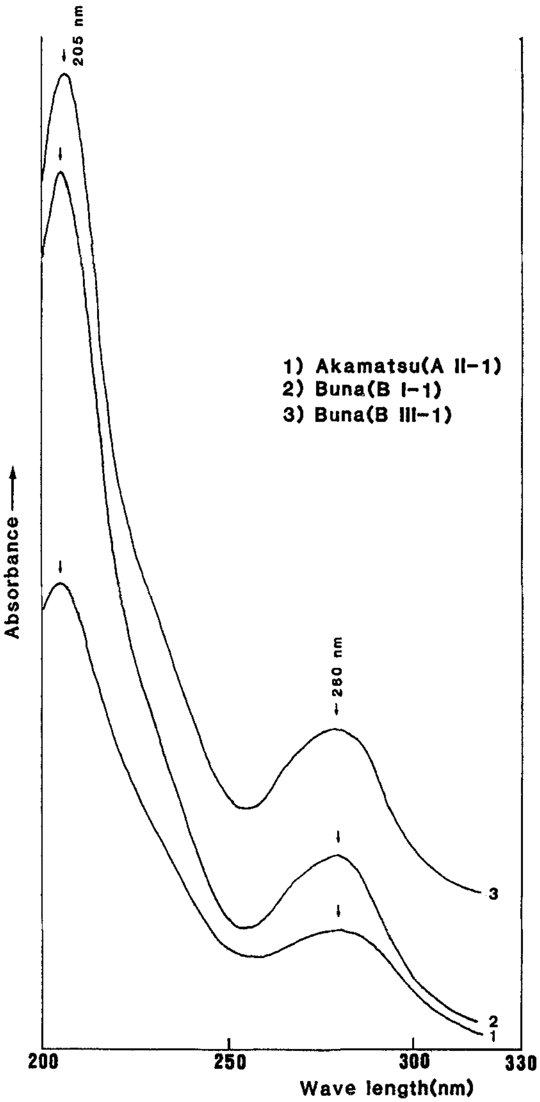

Figure 5. Ultraviolet spectra of Klason lignin filtrates from partially-delignified (acid-chlorite) wood meals of akamatsu and buna with varying Klason lignin contents: BI-1 (8.95%), BIII-1 (9.81%), and AII-1 (15.98%).

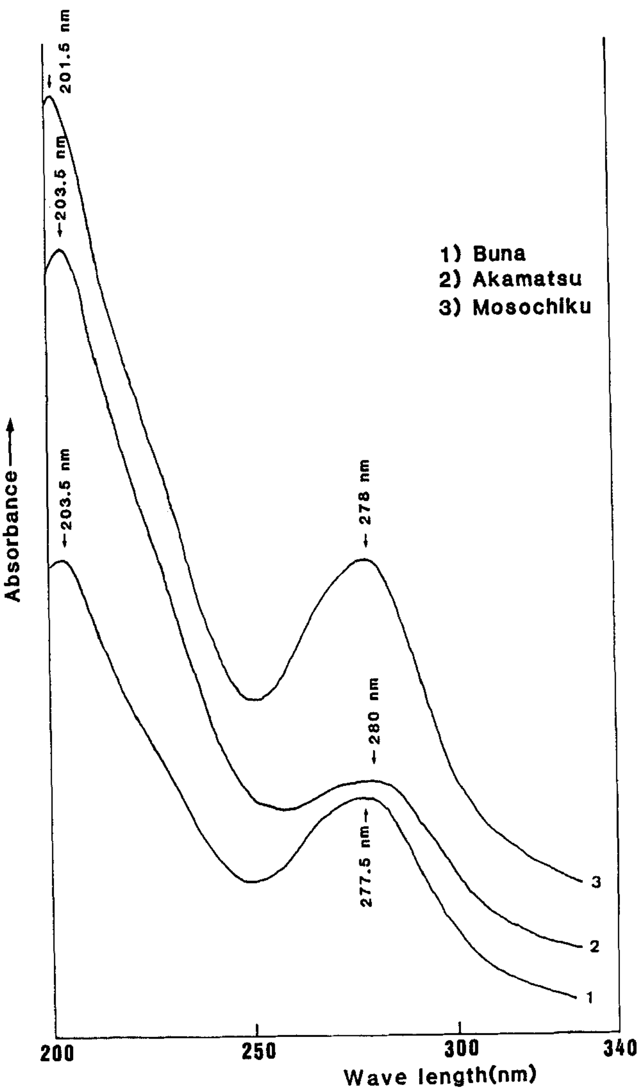

Figure 6. Ultraviolet spectra of Klason lignin filtrates from partially-delignified (peracetic acid) wood meals with varying lignin contents: buna (1.89%), akamatsu (8.20%), and mosochiku (1.42%).

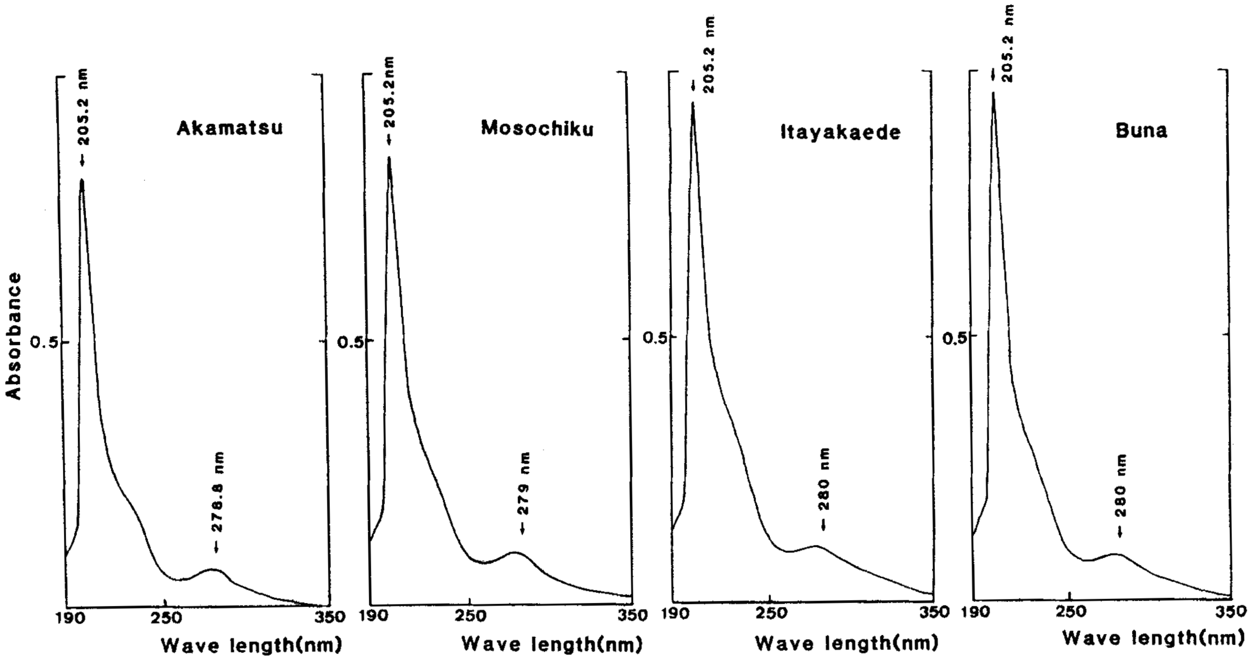

Figure 7. Ultraviolet spectra of diethyl ether extracts of Klason lignin filtrates from wood meals of buna, itayakaede, akamatsu, and mosochiku.

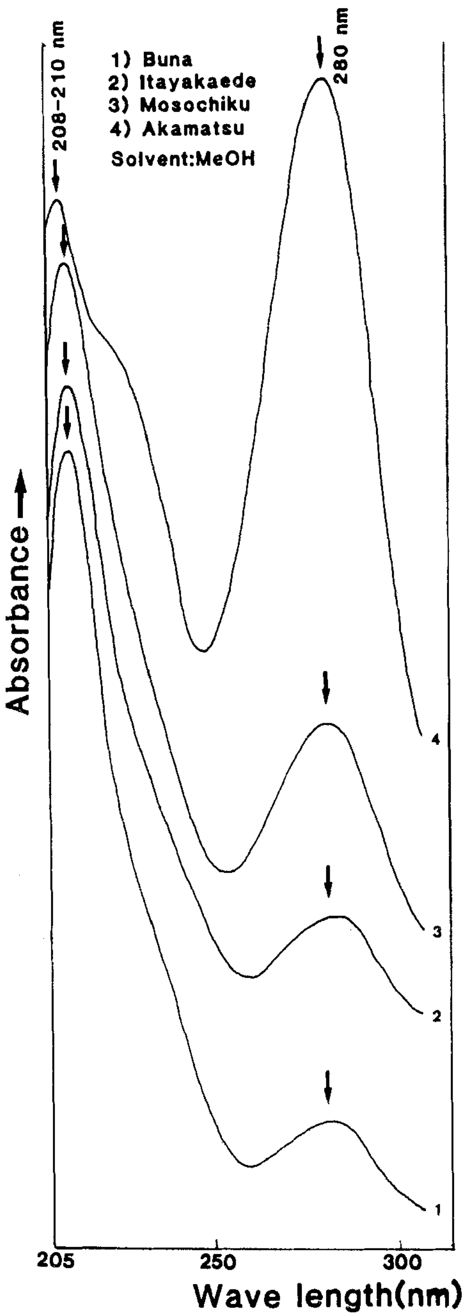

Figure 8. Ultraviolet spectra of chloroform extracts of Klason lignin filtrates from wood meals of buna, itayakaede, akamatsu, and mosochiku.

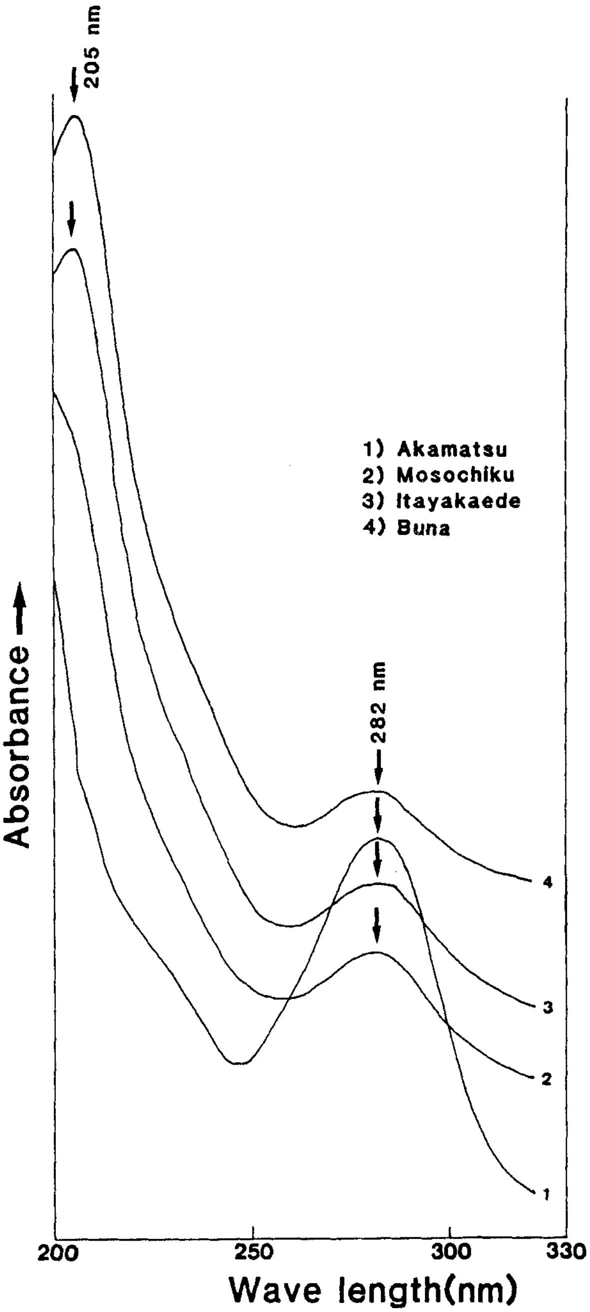

Figure 9. Ultraviolet spectra of Klason lignin filtrates after extraction with diethyl ether.

TABLE I

$A_{196-205}$ and A_{280} in Ultraviolet Spectra of Klason Lignin Filtrates

	$A_{196-205}$	A_{280}	$A_{196-205}/A_{280}$
Japanese beech (Buna)	0.658	0.200	3.29
Maple (Itayakaede)	0.538	0.186	2.89
White Birch	0.465	0.242	1.92
Japanese cherry (Sakura)	0.791	0.477	1.66
Bamboo (Mosochiku)	0.674	0.442	1.52
Red Lauan	0.948	0.668	1.42
Japanese red pine (Akamatsu)	0.446	0.452	0.99
Japanese cypress (Hinoki)	0.475	0.390	1.22
Japanese cedar (Sugi)	0.531	0.457	1.16
Japanese larch (Karamatsu)	0.410	0.396	1.04

TABLE 2

Acid-soluble Lignin Contents of Partially-delignified (Acid Chlorite) Wood Meals of Buna and Akamatsu

	Klason Lignin (%)	A_{205}	Acid-soluble lignin[a] Dilution	(%)
Buna[b]				
B-O	21.09	0.616	5	3.05
B-I	9.81	0.559	50/3	7.76
B-II	6.87	0.578	50/3	8.01
B-III	5.03	0.554	50/3	7.65
B-IV	3.03	0.534	50/3	7.35
Akamatsu				
A-O	26.87	0.325	1	0.32
A-I	16.45	0.538	10	4.50
A-II	15.98	0.573	10	4.74
A-III	4.94	0.626	10	5.20
A-IV	3.54	0.626	10	5.31

[a] Calculated using 110 L $g^{-1}cm^{-1}$ as the absorption coefficient.
[b] B-O and A-O are the extractive-free, original wood meals.

TABLE 3

Acid-soluble Lignin Analyses Based on Absorbances Measured at Various Wavelengths

	Itayakaede	Buna	Mosochiku	Akamatsu
A_{196}				0.571
SL_1 (%)				0.55
A_{200}			0.666	
SL_2 (%)			1.53	
A_{205}	0.472	0.601	0.567	0.330
SL_3 (%)	2.37	2.93	1.39	0.33
A_{240}	0.164	0.198	0.225	0.184
SL_4 (%)	2.60	3.18	1.76	0.60
A_{280}	0.178	0.192	0.420	0.434
SL_5 (%)	4.09	4.36	4.84	2.03

SL_1, SL_2, SL_3: Calculated using 110 L g^{-1} cm^{-1} as the absorptivity.[11]

SL_4: Calculated using 30 L g^{-1} cm^{-1} as the absorptivity.[21]

SL_5: Calculated using absorptivities of 23.6 L g^{-1} cm^{-1} for hardwoods and 23.3 L g^{-1} cm^{-1} for softwoods.[22]

Acid-soluble lignin analyses are based on the oven-dry wood.

208-210 nm in methanol, and the maximum at 280 nm was unusual high for Akamatsu. However, after extraction with diethyl ether the spectra of the Klason lignin filtrates were similar to the original spectra (Figure 9).

The Origin of the Absorption at 205.2 nm in Klason Lignin Filtrates

The UV spectra of Klason lignin filtrates (Figure 7) are characterized by a strong absorption at 205.2 nm and a weak absorption at 280 nm. Spectra from 190-350 nm were obtained for three constitutive elements of lignin , i.e. a guaiacyl moiety (vanillic acid), a syringyl moiety (syringic acid), and a p-hydroxybenzoyl moiety (p-hydroxybenzoic acid). Vanillic acid had absorption maxima at 206.8 (log ϵ 4.52), 217.6 (log ϵ 4.54), 260 (log ϵ 4.28), and 290 nm (log ϵ 2.97), syringic acid at 217.2 (log ϵ 4.30) and 272.8 nm (log ϵ 3.89), and p-hydroxybenzoic acid at 205.2 (log ϵ 4.40) and 253.2 nm (log ϵ 4.37). p-Hydroxybenzoic acid gave the same absorption maximum at 205.2 nm as the Klason lignin filtrate. Pearl et al.[17] pointed out previously that the acid-soluble lignin in the Klason lignin filtrate of aspen wood (*Populus tremuloides*) is of the p-hydroxybenzoyl type. A similar conclusion is probably warranted for the acid-soluble lignins in this study. This is further supported by the findings of Yasuda et al. who found that lignin model compounds were demethylated in sulfuric acid.[18]

Acid-soluble Lignin in Partially-delignified Woodmeals

The acid-soluble lignin content of Klason lignin filtrates is reported to increase as delignification proceeds.[14,19,20] The data in Table 2 shows that the acid-soluble lignin content of partially-delignified Buna (hardwood) woodmeal increased to 2-3 times the initial value as delignification progressed; in Akamatsu (softwood) the increase was more than 10 times.

Comparison of acid-soluble lignin analyses using various wavelengths

As already shown in Figure 1, the absorption of acid-soluble lignin near

280 nm overlaps with absorption of the acid-catalysed degradation products of the wood polysaccharides, e.g. furfural (λ_{max} 278 nm) and hydroxymethylfurfural (λ_{max} 284 nm). Use of the absorbance at 205 nm to determine acid-soluble lignin may be appropriate for the hardwoods, but for the softwoods and bamboo the values obtained may be too low because of the species-dependent variation in the absorption maximum in this region. The shift of λ_{max} to lower wavelengths for the softwoods and bamboo seems to be due to the low lignin content of the sulfuric acid solution, because the absorption maximum in the 205 nm region is variable, depending on the concentration of lignin products.[9] The absorbance at 240 nm has been used by Ahlgren and Goring[19] to determine the acid-soluble lignin in Klason lignin filtrates. In Table 3, the results obtained from acid-soluble lignin analyses using the absorbances measured at various wavelengths are reported. The use of the absorbance at 280 nm gave much higher values for the acid-soluble lignin in every wood sample, probably because of the contribution of the carbohydrate degradation products at this wavelength. Values obtained for the acid soluble lignin of Buna and Itayakaede using the absorbance at 205 nm were within ~ 0.2% (wood basis) of the values obtained using 240 nm. For Mosochiku and Akamatsu, use of 200 and 196 nm for λ_{max} gave values very close to those obtained using 240 nm. For Akamatsu, use of the absorbance at 205 nm would yield a value for the acid-soluble lignin which is approximately one-half of the value obtained using the true absorption maximum. These results indicate that the conventional UV spectrophotometric method for determining acid-soluble lignin which uses the absorbance at 205 nm is probably appropriate for the hardwoods, but for the softwoods and bamboo the actual λ_{max} in the 200 nm region should be used.

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