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Chemical Characterization of Lignin and Lipid Fractions in Kenaf Bast Fibers Used for Manufacturing High-Quality Papers

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The chemical composition of lignin and lipids of bast fibers from kenaf (*Hibiscus cannabinus*) used for high-quality paper pulp production was studied. Pyrolysis−gas chromatography/mass spectrometry (Py-GC/MS) of fibers showed a lignin with a high syringyl/guaiacyl ratio (5.4) and minor amounts of *p*-hydroxyphenyl units. Simultaneously, sinapyl and coniferyl acetates were also identified, indicating that this lignin is partially acetylated. *p*-Hydroxycinnamic acids were found in only trace amounts. The main lipids identified by GC/MS of extracts from kenaf fibers were series of long-chain *n*-fatty acids, waxes, *n*-alkanes, and *n*-fatty alcohols. Free and esterified sterols and triterpenols, steroid hydrocarbons, and steroid and triterpenoid ketones, as well as steryl glycosides, were also found. Finally, the fate of the main constituents of kenaf fibers in alkaline pulping was also investigated.

KEYWORDS: Kenaf; *Hibiscus cannabinus*; bast fibers; lipids; lignin; acetylated lignin, steroids

INTRODUCTION

Nonwood plants are the common fiber source for paper pulp production in developing countries where wood fibers are not available. In the developed world, although wood is still by far the main raw material for paper pulp manufacture, a market exists for high-value-added papers from these fibers (*1*). Kenaf (*Hibiscus cannabinus*) is an annual dicotyledonous plant of the Malvaceae family, which grows in temperate and tropical areas and has received increasing interest in the pulp and paper industry (*2*, *3*). The kenaf plant contains moderately long fibers in its outer stem (bast) and short fibers in its core, both of which are suitable for paper and other products (*4*, *5*). However, the bast fibers are especially suitable for manufacturing specialty papers such as coffee filters and teabags.

Studies on the chemical composition of kenaf bast fibers are important for optimizing the pulping and bleaching processes of this raw material. Among the several parameters that may affect pulp production are the composition of lignin and lipids in fibers. In general, the efficiency of pulping is directly proportional to the amount of syringyl (S) units in lignin (*6*). The guaiacyl (G) units have a free C-5 position available for carbon−carbon interunit bonds, which make them fairly resistant to lignin depolymerization in pulping. On the other hand, it is well-known that lipophilic compounds present in raw materials cause significant environmental and technical problems in the manufacturing of paper pulp. During pulping, lipids are released from the fibers, forming colloidal pitch, which can deposit in either pulp or machinery causing production troubles (*7*, *8*). In the manufacture of alkaline pulps, a large part of the lipids originally present in the raw material is removed during the cooking. However, some chemical species survive these processes and are found as pulp extractives, suspended in process waters or forming the so-called pitch deposits in circuits, equipment, and final product (*9*). Moreover, such extractives might contribute to the toxicity of paper pulp effluents and products (*10*).

Several studies have been published regarding the composition of in situ or isolated lignin of kenaf fibers using different methodologies (*11*−*16*). On the other hand, studies regarding the composition of lipids from kenaf fibers have been scarce (*17*). In the present study we have characterized the lignin in kenaf bast fibers using analytical pyrolysis coupled to gas chromatography/mass spectrometry (Py-GC/MS). Py-GC/MS is a powerful analytical tool for the rapid analysis of complex polymer mixtures including lignocellulosic materials (*18*, *19*). It combines rapid thermal degradation and GC/MS of marker compounds from pyrolytic breakdown. We also performed a detailed analysis of the chemical composition of lipids from kenaf bast fibers. These analyses were carried out by GC and GC/MS using short- and medium-length high-temperature capillary columns, respectively, with thin films (*20*). This method enables the elution and analysis of intact high molecular weight lipids such as waxes, sterol esters, and triglycerides. Finally, the fate of kenaf lignin and lipids in alkaline pulping of these fibers was also studied.

MATERIALS AND METHODS

Samples. Kenaf (*H. cannabinus*) bast fibers (from China) and the alkaline pulp (kappa 6) made therefrom were supplied by CELESA pulp mill (Tortosa, Spain). Kenaf fibers contained 15% of core fibers, and the pulp was obtained by soda−anthraquinone (AQ) cooking (*21*). Kenaf bast fibers were air-dried, whereas the pulp sample was dried in an aerated oven at 50 °C. The dried samples were milled using a knife mill (Janke and Kunkel, Analysenmühle). For the study of lignin composition of fibers and pulp, milled samples were analyzed by Py-

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GC/MS. For the analysis of lipid composition, milled samples were extracted with acetone in a Soxhlet apparatus for 8 h. The extracts were evaporated to dryness and resuspended in chloroform for chromatographic analysis of the lipophilic fraction. Two replicates were used for each sample, and all samples were subjected to GC and GC/MS analyses. For hemicellulose analysis and lignin content estimation, milled samples were successively extracted with acetone (8 h in Soxhlet) and hot water (3 h at 100 °C). Klason lignin was estimated as the residue after sulfuric acid hydrolysis of the pre-extracted material, and sugars from polysaccharide hydrolysis were analyzed as alditol acetates by GC according to Tappi rules T222 om-88 and T249 om85 (*22*), respectively. Ash content was estimated as the residue after 6 h at 575 °C.

GC and GC/MS Analyses. An HP 5890 gas chromatograph (Hewlett-Packard, Hoofddorp, The Netherlands) equipped with a split−splitless injector and a flame ionization detector (FID) was used for GC analyses of the lipophilic compounds. The injector and the detector temperatures were set at 300 and 350 °C, respectively. Samples were injected in the splitless mode. Helium was used as the carrier gas. The capillary column used was a 5 m × 0.25 mm i.d., 0.1 μm, high-temperature, polyimide-coated fused silica tubing DB-5HT from J&W Scientific (Folsom, CA), especially processed for use at 400 °C. The oven was temperature programmed from 100 °C (1 min) to 350 °C (3 min) at 15 °C/min. Peaks were quantified by area, and a mixture of standards (octadecane, palmitic acid, sitosterol, cholesteryl oleate, and campesteryl, stigmasteryl, and sitosteryl 3-β-D-glucopyranosides) was used to elaborate calibration curves. The data from the two replicates were averaged. In all cases the standard deviations from replicates were below 10% of the mean values.

The GC/MS analyses were performed on a model GC 8000 Top gas chromatograph (Thermo Finnigan, San Jose, CA) coupled to a Voyager quadrupole mass spectrometer detector (ThermoQuest Finnigan) equipped with a 15 m × 0.25 mm i.d., 0.1 μm, DB-5HT fused silica capillary column (J&W). The oven was heated from 120 °C (1 min) to 380 °C (5 min) at 10 °C/min. The injector and transfer line temperatures were set at 300 and 350 °C, respectively. Helium was used as the carrier gas, and the injection was performed in splitless mode. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylation was used when required. Compounds were identified by comparing their mass spectra with mass spectra in the Wiley and NIST libraries, by mass fragmentography, and, when possible, by comparison with authentic standards.

Py-GC/MS. The pyrolysis of kenaf fibers and pulp was performed in duplicate with a Curie-point pyrolyzer (Horizon Instruments Ltd.) coupled to a Varian Saturn 2000 GC/MS, using a 30 m × 0.25 mm i.d., 0.25 μm, DB-5 column. Approximately 100 μg of finely divided sample was deposited on a ferromagnetic wire, then inserted into the glass liner, and immediately placed in the pyrolyzer. The pyrolysis was carried out at 610 °C for 4s. The chromatograph was programmed from 40 °C (1 min) to 300 °C at a rate of 6 °C/min. The final temperature was held for 20 min. The injector, equipped with a liquid carbon dioxide cryogenic unit, was programmed from −30 °C (1 min) to 300 °C at 200 °C/min, whereas the GC/MS interface was kept at 300 °C. For the pyrolysis in the presence of tetramethylammonium hydroxide (TMAH), to analyze *p*-hydroxycinnamic acids, ∼100 μg of sample was mixed with 0.5 μL of 25% TMAH. The wire was then inserted into the glass liner, which was subsequently placed in the pyrolyzer. The pyrolysis was carried out as described above. The compounds were identified by comparing the mass spectra obtained with those of the Wiley and NIST computer libraries and that reported in the literature (*18*, *19*). Relative peak molar areas were calculated for carbohydrate and lignin pyrolysis products. The summed molar areas of the relevant peaks were normalized to 100%, and the data for two repetitive pyrolysis experiments were averaged. The relative standard deviation for the pyrolysis data was <10%. No attempt was made to calculate the response factor for every single compound released. However, for most of the lignin-derived phenols, the response factors are nearly identical (*23*), with the exception of vanillin, but this is a minor peak here.

Table 1. Chemical Composition of Kenaf Fibers and Their Alkaline Pulp (Percent)

	fibers	pulp		fibers	pulp
ash	1.8	2.1	xylose	11.0	12.7
extractives	1.0	0.3	mannose	4.2	1.0
water-soluble		0.7	galactose	2.5	0
lignin	11.4	0.8	glucose	63.8	80.3
arabinose	2.4	1.1			

RESULTS AND DISCUSSION

The composition on the main constituents of kenaf bast fibers is shown in **Table 1**. The lignin content accounted for 11% of fibers. This value is higher than for other nonwood bast fibers such as hemp but lower than for wood (*24*). The content of lipids was ∼1%, similar to that of hemp but lower than that of other nonwood materials used for papermaking, such as flax (*25*, *26*). The hemicellulose fraction was mainly constituted by xylose and mannose. The composition of the kenaf alkaline pulp (kappa 6) selected for this study is also shown in **Table 1**. A decrease of the hemicellulose content could be observed after pulping, but most hemicellulose remained in the pulp due to xylan resistance toward alkaline cooking. The glucan content, mainly corresponding to cellulose, increased during pulping. The major part of the lignin in kenaf fibers was removed during pulping, although some residual lignin remained in the unbleached pulp. About one-third of the lipophilic extractives also remained in the pulp.

Lignin Composition. To analyze in situ the chemical composition of lignin, the kenaf bast fibers were subjected to Py-GC/MS. The Py-GC/MS chromatogram is shown in **Figure 1**, and the identities and relative abundances of the released compounds are listed in **Table 2**. Carbohydrate pyrolysis products represented 35% on average and phenols from lignin represented 65% of the total identified compounds from kenaf fibers. The pyrogram of kenaf bast fibers showed compounds derived from guaiacyl (G) and syringyl (S) lignin units, with a very strong predominance of the S units (S/G molar ratio of 5.4). Compounds derived from *p*-hydroxyphenyl (H) lignin units were found in minor amounts (1.4% of typical lignin-derived compounds). The main lignin-derived compounds identified were syringol (**34**), 4-methylsyringol (**39**), 4-vinylsyringol (**47**), 4-allylsyringol (**49**), and *cis*- and *trans*-4-propenylsyringol (**51** and **55**). Syringaldehyde (**52**), syringylacetone (**59**), *trans*-sinapaldehyde (**63**), and *cis*- and *trans*-sinapyl alcohol (**62** and **64**) were also identified. The guaiacyl counterparts were also detected, although in lower amounts. The high S/G ratio observed in the lignin of bast fibers upon Py-GC/MS is in agreement with the values reported by other authors (*11*, *14*−*16*). In contrast, some authors (*12*, *13*) have reported higher proportions of G over S lignin units of kenaf bast fibers. On the other hand, the low content of H units found after Py-GC/MS of kenaf fibers agrees with most previous works (*15*, *16*). However, Neto et al. (*12*) reported a high content of H lignin units in kenaf, as compared to other dicotyledoneous lignins. The authors claimed that the high H lignin content could be due to the presence of *p*-coumaric acid type structures. In the present work, we analyzed the presence of cinnamic acids using Py-GC/MS in the presence of TMAH, and methyl derivatives of *p*-coumaric and ferulic acids (4-methoxycinnamic acid and 3,4-dimethoxycinnamic acid methyl esters, respectively) were found in only trace amounts (cinnamic acids/lignin molar ratio of 0.03), with a predominance of ferulic acid over *p*-coumaric

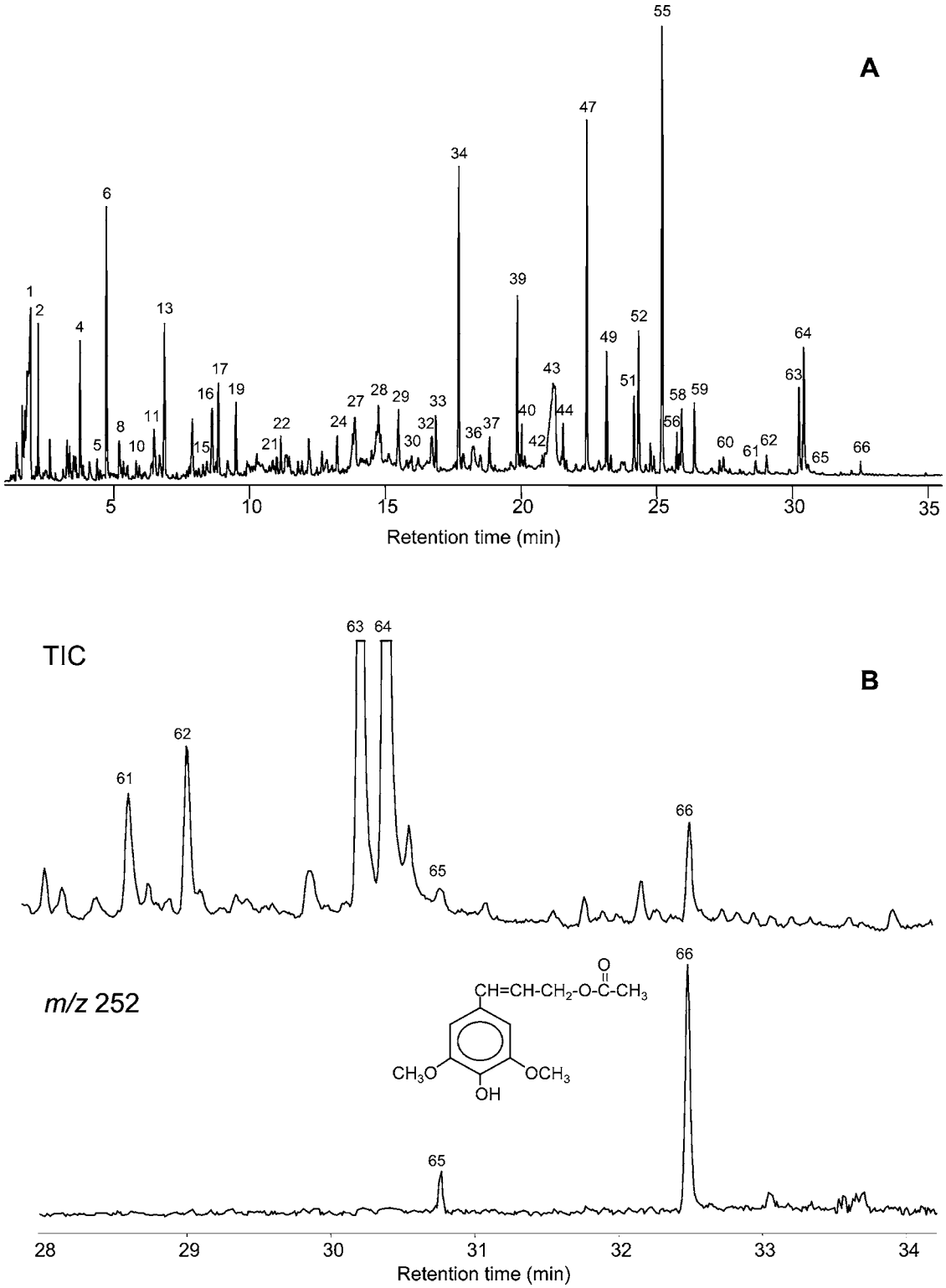

Figure 1. (**A**) Py-GC/MS chromatogram of kenaf bast fibers and (**B**) detail of the total ion chromatogram (TIC) and chromatogram of the ion at *m/z* 252 showing the presence of sinapyl acetates. The identities of the compounds are listed in **Table 2**.

acid (1.73 of molar ratio). This result agrees with the low amount of cinnamic acids in kenaf bast fibers reported in other works (*13*, *16*).

A detailed analysis of the compounds released after Py-GC/MS of kenaf bast fibers revealed the presence of sinapyl acetate [3-(4-hydroxy-3,5-dimethoxyphenyl)-2-propen-1-ol acetate] in cis and trans forms. Minor amounts of coniferyl acetates (cis and trans forms) were also detected. Acetylated lignin units have already been reported to occur in kenaf (*11*); however, this is the first time that the presence of acetylated lignin units has been shown using Py-GC/MS. Sinapyl and coniferyl acetates have also been detected for the first time in other nonwood fibers with high S/G ratios such as sisal, abaca, and jute (*27*). The presence of sinapyl acetates in kenaf bast fibers is shown in **Figure 1**. A predominance of the trans over the cis form was observed, similar to that of the respective nonacetylated sinapyl alcohols. Previous NMR and degradative studies (*11*, *28*) have shown that lignin in kenaf is acetylated at the γ-position of the

Table 2. Compounds Released after Py-GC/MS of Kenaf Fibers[a]

no.	compound	mass fragments	MW	formula	origin	%
1	hydroxyacetaldehyde	*42*/*60*	60	$C_2H_4O_2$	C	12.1
2	3-hydroxypropanal	73/*74*	74	$C_3H_6O_2$	C	1.1
3	(3*H*)-furan-2-one	*55*/84	84	$C_4H_4O_2$	C	0.2
4	unknown	*56*/57/87				2.9
5	(2*H*)-furan-3-one	55/*84*	84	$C_4H_4O_2$	C	0.3
6	2-furaldehyde	67/*95*/96	96	$C_5H_4O_2$	C	3.3
7	2-methylfuran	53/81/*82*	82	C_5H_6O	C	0.3
8	2-(hydroxymethyl)furan	*43*/70/81/98	98	$C_5H_6O_2$	C	1.0
9	cyclopent-1-ene-3,4-dione	54/68/*96*	96	$C_5H_4O_2$	C	0.3
10	4-methyltetrahydrofuran-3-one	*43*/72	100	$C_5H_8O_2$	C	0.6
11	(5*H*)-furan-2-one	*55*/84	84	$C_4H_4O_2$	C	1.2
12	acetylfuran	43/*95*/110	110	$C_6H_6O_2$	C	0.1
13	2,3-dihydro-5-methylfuran-2-one	55/69/*98*	98	$C_5H_6O_2$	C	3.6
14	5-methyl-2-furfuraldehyde	53/109/*110*	110	$C_6H_6O_2$	C	0.2
15	phenol	65/66/*94*	94	C_5H_6O	LH	0.2
16	5,6-dihydropyran-2,5-dione	*68*/98	98	$C_5H_6O_2$	C	1.6
17	4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	58/85/*114*	114	$C_5H_6O_3$	C	1.4
18	3-hydroxy-2-methyl-2-cyclopenten-1-one	55/84/*112*	112	$C_6H_8O_2$	C	0.5
19	2-hydroxy-3-methyl-2-cyclopenten-1-one	55/84/*112*	112	$C_6H_8O_2$	C	1.9
20	4-methylphenol	77/*107*/108	108	C_7H_8O	LH	0.3
21	2-furoic acid, methyl ester	67/*95*/126	126	$C_6H_6O_3$	C	0.4
22	guaiacol	81/*109*/124	124	$C_7H_8O_2$	LG	0.9
23	4-ethylphenol	77/*107*/122	122	$C_8H_{10}O_2$	LH	0.1
24	3,4-dihydroxybenzaldehyde	81/109/*137*/138	138	$C_7H_6O_3$	LM	0.6
25	5-hydroxymethyl-2-tetrahydrofuraldehyde-3-one	*43*/57/69/70/85	144	$C_6H_8O_4$	C	0.7
26	4-methylguaiacol	95/*123*/138	138	$C_8H_{10}O_2$	LG	0.2
27	catechol	64/81/92/*110*	110	$C_6H_6O_2$	LM/C	2.3
28	5-hydroxymethyl-2-furaldehyde	69/*97*/109/126	126	$C_6H_6O_3$	C	1.4
29	3-methoxycatechol	79/97/125/*140*	140	$C_7H_8O_3$	LM	1.5
30	4-ethylguaiacol	122/*137*/152	152	$C_9H_{12}O_2$	LG	0.3
31	4-methylcatechol	78/107/123/*124*	124	$C_7H_8O_2$	LM	0.2
32	1,4-dideoxy-D-glycerohex-1-enepyrenone-3-ulone	43/73/*87*/113/144	144	$C_6H_8O_4$	C	0.8
33	4-vinylguaiacol	107/*135*/150	150	$C_9H_{10}O_2$	LG	1.1
34	syringol	111/139/*154*	154	$C_8H_{10}O_3$	LS	6.2
35	eugenol	131/149/*164*	164	$C_{10}H_{12}O_2$	LG	0.5
36	pyrogallol	52/80/97/108/*126*	126	$C_6H_6O_3$	LM	2.1
37	vanillin	109/151/*152*	152	$C_8H_8O_3$	LG	0.6
38	*cis*-isoeugenol	131/149/*164*	164	$C_{10}H_{12}O_2$	LG	0.1
39	4-methylsyringol	125/153/*168*	168	$C_9H_{12}O_2$	LS	2.4
40	*trans*-isoeugenol	131/149/*164*	164	$C_{10}H_{12}O_2$	LG	0.8
41	homovanillin	122/*137*/166	166	$C_{10}H_{14}O_2$	LG	0.3
42	acetoguaiacone	123/*151*/166	166	$C_9H_{10}O_3$	LG	0.3
43	levoglucosane	57/*60*/73/98	162	$C_6H_{10}O_5$	C	10.0
44	4-ethylsyringol	*167*/182	182	$C_{10}H_{14}O_3$	LS	0.9
45	guaiacylacetone	122/*137*/180	180	$C_{10}H_{12}O_3$	LG	0.2
46	1,6-anhydro-β-D-glucofuranose	*73*/85/115	162	$C_6H_{10}O_5$	C	0.3
47	4-vinylsyringol	137/165/*180*	180	$C_{10}H_{12}O_3$	LS	6.3
48	guaiacyl vinyl ketone	123/*151*/178	178	$C_{10}H_{10}O_3$	LG	0.1
49	4-allylsyringol	167/179/*194*	194	$C_{11}H_{14}O_3$	LS	1.7
50	4-propylsyringol	123/*167*/196	196	$C_{11}H_{16}O_3$	LS	0.3
51	*cis*-4-propenylsyringol	167/179/*194*	194	$C_{11}H_{14}O_3$	LS	1.3
52	syringaldehyde	167/181/*182*	182	$C_9H_{10}O_4$	LS	2.4
53	*cis*-coniferyl alcohol	124/*137*/151/180	180	$C_{10}H_{12}O_3$	LG	0.3
54	4-propinylsyringol	106/131/177/*192*	192	$C_{11}H_{12}O_3$	LS	0.4
55	*trans*-4-propenylsyringol	167/179/*194*	194	$C_{11}H_{14}O_3$	LS	7.4
56	acetosyringone	153/*181*/196	196	$C_{10}H_{12}O_4$	LS	0.7
57	*trans*-coniferaldehyde	107/135/147/*178*	178	$C_{10}H_{10}O_3$	LG	0.3
58	*trans*-coniferyl alcohol	124/*137*/151/180	180	$C_{10}H_{12}O_3$	LG	1.7
59	syringylacetone	123/*167*/210	210	$C_{11}H_{14}O_4$	LS	1.1
60	propiosyringone	151/*181*/210	210	$C_{11}H_{14}O_4$	LS	0.3
61	dihydrosinapyl alcohol	167/*168*/212	212	$C_{11}H_{16}O_4$	LS	0.4
62	*cis*-sinapyl alcohol	154/*167*/210	210	$C_{11}H_{14}O_4$	LS	0.5
63	*trans*-sinapaldehyde	137/165/180/*208*	208	$C_{11}H_{12}O_4$	LS	1.8
64	*trans*-sinapyl alcohol	154/*167*/210	210	$C_{11}H_{14}O_4$	LS	3.7
65	*cis*-sinapyl acetate	*149*/161/192/209/252	252	$C_{13}H_{16}O_5$	LS	0.4
66	*trans*-sinapyl acetate	*149*/161/192/209/252	252	$C_{13}H_{16}O_5$	LS	0.9
	%H					1.3
	%G					15.4
	%S					83.3
	S/G					5.4

[a] Main mass fragments, molecular weight (MW), formula, origin, and relative molar abundances (%) are included. C, carbohydrates; LM, modified lignin; LH, *p*-hydroxyphenyl lignin units, H; LG, guaiacyl lignin units, G; LS, syringyl lignin units, S. Italic mass fragments indicate base peaks.

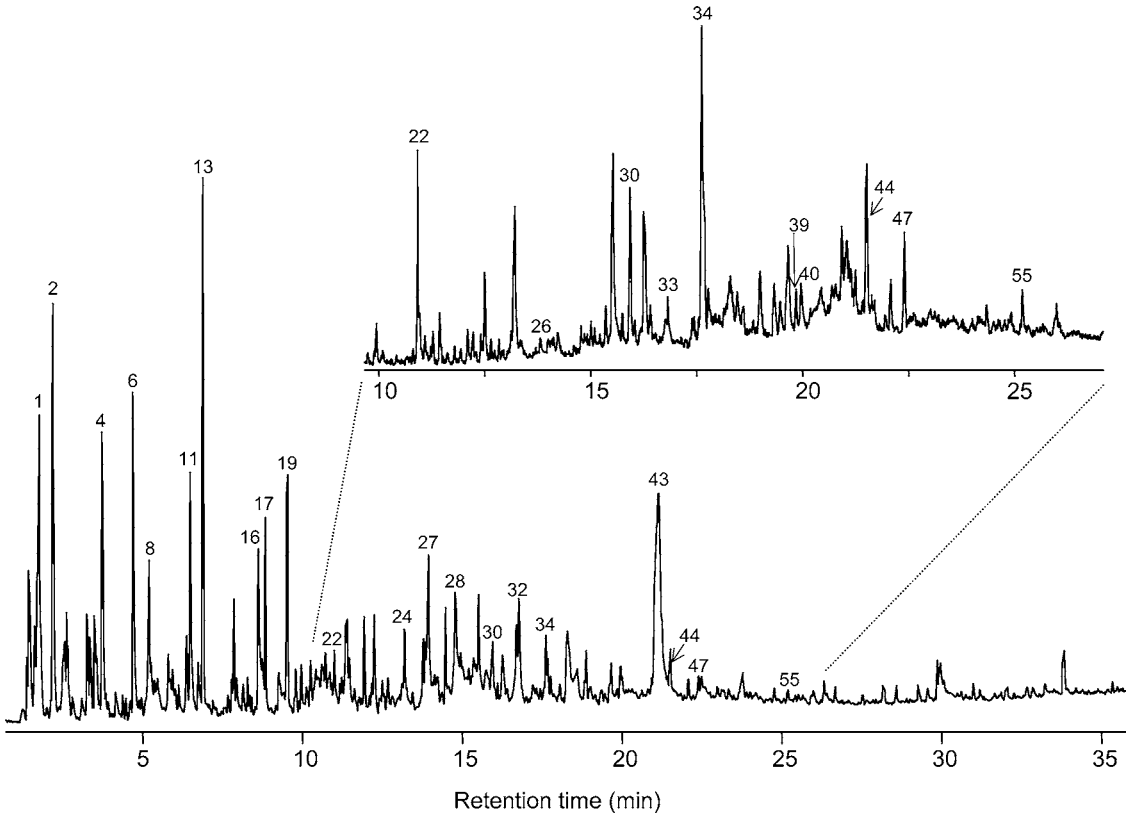

Figure 2. Py-GC/MS chromatogram of kenaf pulp. The inset illustrates a reconstructed ion chromatogram showing the main lignin markers. The reconstructed chromatogram was the sum of the molecular ions of guaiacol (**22**), 4-methylguaiacol (**26**), 4-vinylguaiacol (**33**), 4-ethylguaiacol (**30**), 4-propenylguaiacol (**35**), syringol (**34**), 4-methylsyringol (**39**), 4-vinylsyringol (**47**), 4-ethylsyringol (**44**), and 4-propenylsyringol (**51**). The identities of the compounds are listed in **Table 2**.

side chain and that this acetylation occurred predominantly on S units, in agreement with the present Py-GC/MS results. It has recently been suggested that sinapyl acetate is a lignin precursor in kenaf (*29*).

The chemical composition of the residual lignin in pulp after soda−AQ pulping of kenaf fibers was also studied using Py-GC/MS. The main peaks in the pyrogram after Py-GC/MS of kenaf pulp corresponded to carbohydrate-derived compounds, whereas the lignin-derived peaks were very minor (**Figure 2**), corresponding to the low lignin content of the kenaf pulp (<1%) (**Table 1**). However, by selecting characteristic ions (molecular ions) of the different lignin markers, more lignin-derived compounds could be detected in the pyrograms of the pulp sample (inset in **Figure 2**), which allowed the estimation of the S/G ratio (1.1). The strong decrease in the S/G ratio after cooking shows the preferential degradation of S lignin units (versus G units) upon alkaline pulping conditions. In this sense, the high S-lignin content observed in the kenaf bast fiber is advantageous for delignification during pulping because the S lignin is relatively unbranched and has a lower condensation degree than the G lignin (*30*, *31*).

Lipid Composition. The total lipid extract accounted for 1.0% of the kenaf bast fibers. The underivatized and silylated extracts were analyzed by GC and GC/MS. The GC/MS chromatogram of the underivatized kenaf lipids is shown in **Figure 3**, and the identities and abundances of the main compounds are summarized in **Table 3**. The most predominant lipid classes in kenaf fibers were series of *n*-fatty acids (28%), waxes (25%), *n*-alkanes (22%), and *n*-fatty alcohols (14%). Minor amounts of steroids and triterpenoids were also present in these fibers, and triglycerides were not detected.

Free fatty acids were identified in the kenaf fibers ranging from tetradecanoic (C_{14}) to dotriacontanoic (C_{32}) acids, with strong even-over-odd carbon atom predominance. Palmitic ($C_{16:0}$) and linoleic ($C_{18:2}$) acids were the most abundant, followed by oleic ($C_{18:1}$) and stearic ($C_{18:0}$) acids, in agreement with previous works (*17*).

Waxes were found in the range from C_{42} to C_{52} with the presence of only the even carbon atom number homologues, the C_{46} and C_{48} analogues being the most abundant. Each chromatographic peak consisted of a complex mixture of different long-chain fatty acids esterified to different long-chain fatty alcohols. The identification and quantitation of the individual long-chain esters in each chromatographic peak were resolved on the basis of the mass spectra of the peaks. The mass spectra of long-chain esters are characterized by a base peak produced by a rearrangement process involving the transfer of two hydrogen atoms from the alcohol chain to the acid chain, giving a protonated acid ion (*32*). Therefore, the base peak gives the number of carbon atoms in the acid moiety and the molecular ion the total number of carbon atoms in the ester. It is possible then to determine the individual contribution of esters to every chromatographic peak by mass spectrometric determination of the molecular ion and the base peak. Quantitation of individual esters was accomplished by integrating areas in the chromatographic profiles of ions characteristic for the acidic moiety. The detailed structural composition of the high molecular weight waxes identified in the kenaf fiber is shown in **Table 4**. The esterified fatty acids ranged from C_{16} to C_{30} and the esterified fatty alcohols from C_{16} to C_{34}. The acyl moiety of the waxes was exclusively constituted by saturated fatty acids with even carbon number, eicosanoic acid (C_{20}) being the most predomi-

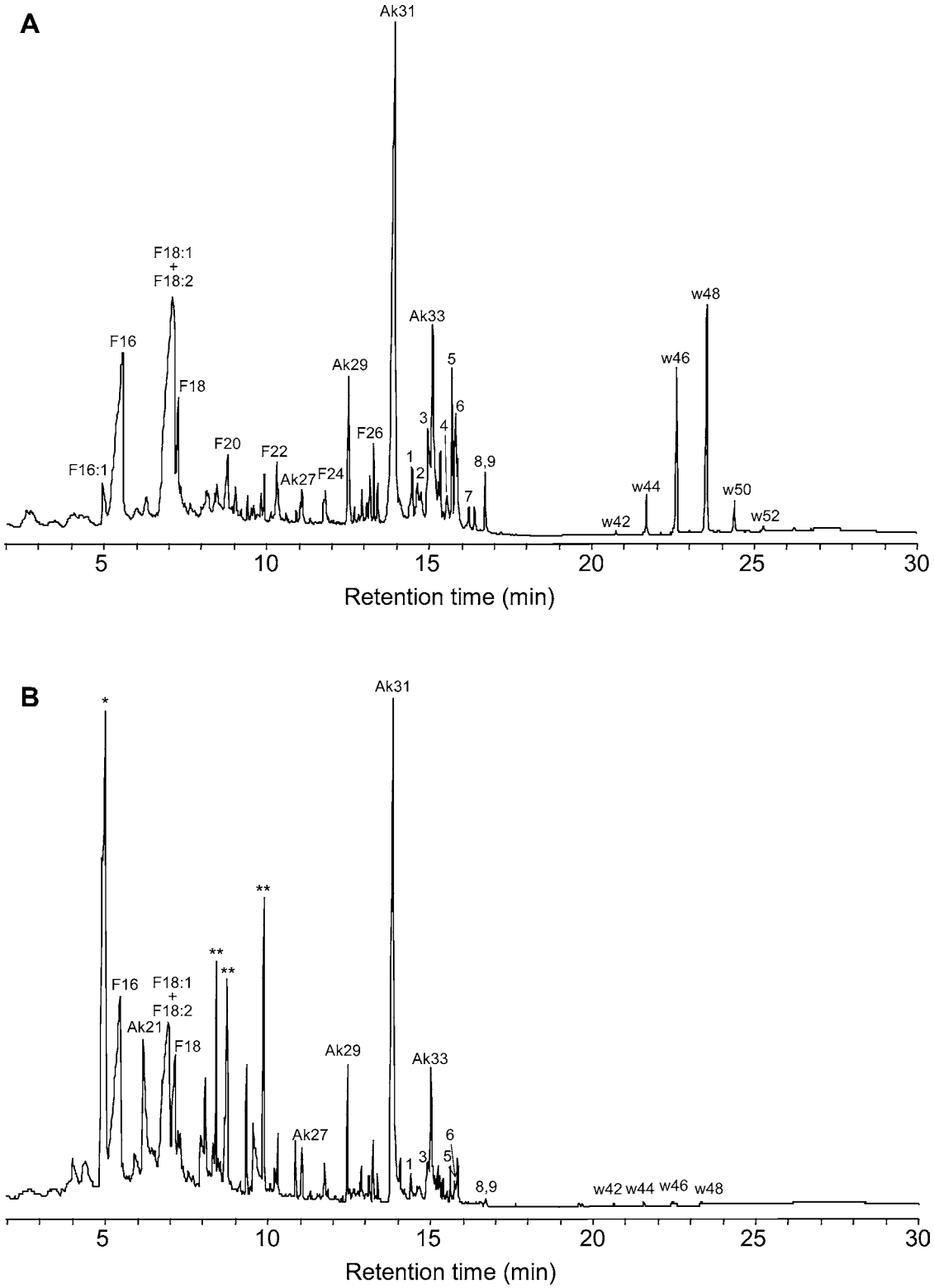

Figure 3. GC/MS chromatograms of the underivatized lipid extracts from (**A**) kenaf bast fibers and (**B**) kenaf pulp. F(*n*), *n*-fatty acid series; Ak(*n*), *n*-alkane series; w(*n*), wax series; *n* denotes the total carbon atom number. Other compounds reflected are (1) campesterol, (2) stigmasterol, (3) sitosterol, (4) motiol, (5) stigmast-4-en-3-one, (6) glutinol, (7) ergostane-3,6-dione, (8) stigmast-4-en-3,6-dione, and (9) stigmastane-3,6-dione. * is anthraquinone, and ** are contaminants.

nant. Waxes with unsaturated fatty acids could not be detected, despite the high amounts of oleic and linoleic acids present in free form. Among the esterified alcohols, the most predominant was octacosanol (C_{28}), followed by hexacosanol (C_{26}) and triacontanol (C_{30}). The predominant wax was C_{48}, mostly constituted by eicosanoic acid (C_{20}) esterified to octacosanol (C_{28}), followed by wax C_{46}, mostly constituted by eicosanoic acid (C_{20}) esterified to hexacosanol (C_{26}) and octadecanoic acid (C_{18}) esterified to octacosanol (C_{28}). Before the present study, octacosanyl eicosanoate was the only wax ester reported to occur in kenaf bark fibers (*17*).

Table 3. Composition of Lipids (Milligrams per 100 g) from Kenaf Fibers and Their Alkaline Pulp[a]

compound	mass fragments	MW	formula	fibers	pulp
n-alkanes				26.88	20.67
n-heneicosane	*57*/71/85/296	296	$C_{21}H_{44}$	0.29	3.36
n-tricosane	*57*/71/85/324	324	$C_{23}H_{48}$	0.07	0.96
n-pentacosane	*57*/71/85/352	352	$C_{25}H_{52}$	0.14	1.54
n-hexacosane	*57*/71/85/366	366	$C_{26}H_{54}$	0.20	0.29
n-heptacosane	*57*/71/85/380	380	$C_{27}H_{56}$	0.43	0.56
n-octacosane	*57*/71/85/394	394	$C_{28}H_{58}$	0.23	0.31
n-nonacosane	*57*/71/85/408	408	$C_{29}H_{60}$	1.93	1.03
n-triacontane	*57*/71/85/422	422	$C_{30}H_{62}$	0.46	0.24
n-hentriacontane	*57*/71/85/436	436	$C_{31}H_{64}$	20.48	11.20
n-tritriacontane	*57*/71/85/464	464	$C_{33}H_{68}$	2.65	1.18
steroid hydrocarbons				2.32	0.93
ergostatriene	*135*/143/380	380	$C_{28}H_{44}$	0.20	0.08
ergostadiene	*81*/147/367/382	382	$C_{28}H_{46}$	0.08	0.03
stigmastadiene	*81*/147/381/396	396	$C_{29}H_{48}$	0.46	0.22
stigmastene	*81*/215/383/398	398	$C_{29}H_{50}$	0.20	0.04
stigmasta-3,5,22-triene	*135*/143/394	394	$C_{29}H_{46}$	0.96	0.43
stigmasta-3,5-diene	*81*/147/381/396	396	$C_{29}H_{48}$	0.43	0.13
fatty acids				33.35	13.60
n-tetradecanoic acid	60/*73*/129/228	228	$C_{14}H_{28}O$	0.25	0.34
n-pentadecanoic acid	60/*73*/129/242	242	$C_{15}H_{30}O$	0.30	0.22
9-hexadecenoic acid	*55*/69/236/254	254	$C_{16}H_{30}O$	0.09	0.00
n-hexadecanoic acid	60/*73*/129/256	256	$C_{16}H_{32}O_2$	10.87	5.59
n-heptadecanoic acid	60/*73*/129/270	270	$C_{17}H_{34}O_2$	0.23	0.18
9,12-octadecadienoic acid	*67*/81/280	280	$C_{18}H_{32}O_2$	11.79	1.17
9-octadecenoic acid	*55*/69/264	282	$C_{18}H_{34}O_2$	5.29	2.53
n-octadecanoic acid	60/*73*/129/284	284	$C_{18}H_{36}O_2$	1.74	1.60
n-eicosanoic acid	60/73/129/312	312	$C_{20}H_{40}O_2$	0.86	1.13
n-docosanoic acid	60/73/129/340	340	$C_{22}H_{44}O_2$	0.91	0.46
n-tetracosanoic acid	60/73/129/368	368	$C_{24}H_{48}O_2$	0.37	0.17
n-hexacosanoic acid	60/73/129/396	396	$C_{26}H_{52}O_2$	0.17	0.08
n-octacosanoic acid	60/73/129/424	424	$C_{28}H_{56}O_2$	0.34	0.10
n-triacontanoic acid	60/73/129/452	452	$C_{30}H_{60}O_2$	0.12	0.03
n-dotriacontanoic acid	60/73/129/480	480	$C_{32}H_{64}O_2$	0.02	0.00
fatty alcohols				13.00	12.28
n-octadecanol	75/103/*355**	370*	$C_{23}H_{50}OSi$*	0.06	1.80
n-eicosanol	75/103/*383**	398*	$C_{25}H_{54}OSi$*	0.03	1.44
n-docosanol	75/103/*411**	426*	$C_{27}H_{58}OSi$*	0.02	1.09
n-hexacosanol	75/103/*439**	454*	$C_{29}H_{62}OSi$*	1.76	0.90
n-octacosanol	75/103/*467**	482*	$C_{31}H_{66}OSi$*	8.93	5.36
n-triacontanol	75/103/*495**	510*	$C_{33}H_{70}OSi$*	2.19	1.58
n-dotriacontanol	75/103/*523**	538*	$C_{35}H_{74}OSi$*	0.01	0.11
aldehydes				0.65	0.04
n-heneicosanal	*82*/96/292	310	$C_{21}H_{42}O$	0.11	0.00
n-docosanal	*82*/96/306	324	$C_{22}H_{44}O$	0.20	0.00
n-tricosanal	*82*/96/320	338	$C_{23}H_{46}O$	0.20	0.00
n-tetracosanal	*82*/96/334	352	$C_{24}H_{48}O$	0.05	0.00
n-hexacosanal	*82*/96/362	380	$C_{26}H_{52}O$	0.02	0.00
n-octacosanal	*82*/96/390	408	$C_{28}H_{56}O$	0.07	0.04
sterols/triterpenols				4.71	0.55
campesterol	*55*/145/213/382/400	400	$C_{28}H_{48}O$	0.07	0.00
stigmasterol	*55*/83/255/394/412	412	$C_{29}H_{48}O$	0.19	0.00
sitosterol	145/*213*/396/414	414	$C_{29}H_{50}O$	2.33	0.05
stigmastanol	*215*/416	416	$C_{29}H_{52}O$	0.27	0.01
motiol	*69*/83/241/259/411	426	$C_{30}H_{48}O$	0.31	0.00
glutinol	95/231/259/274	426	$C_{30}H_{48}O$	1.44	0.19
β-amyrin	203/*218*/426	426	$C_{30}H_{48}O$	0.07	0.15
α-amyrin	203/*218*/426	426	$C_{30}H_{48}O$	0.03	0.15
α-Tocopherol	*165*/205/430	430	$C_{29}H_{50}O_2$	0.86	0.01
triterpenoid and steroid ketones				3.97	0.63
β-amyrenone	189/203/*218*/409/424	424	$C_{30}H_{48}O$	0.23	0.04
α-amyrenone	189/203/*218*/409/424	424	$C_{30}H_{48}O$	0.07	0.02
stigmastan-3-one	*231*/232/414	414	$C_{29}H_{50}O$	0.07	0.04
stigmasta-7,22-dien-3-one	*55*/269/298/367/410	410	$C_{29}H_{46}O$	0.60	0.12
stigmasta-3,5-dien-7-one	*174*/269/410	410	$C_{29}H_{50}O$	0.06	0.09
stigmast-4-en-3-one	*124*/229/412	412	$C_{29}H_{48}O$	1.57	0.14
stigmastadienone isomer	*57*/136/174/269/410	410	$C_{29}H_{46}O$	0.29	0.18
ergostane-3,6-dione	137/245/*414*	414	$C_{28}H_{46}O_2$	0.23	0.00
stigmast-4-en-3,6-dione	137/398/408/411/*426*	426	$C_{29}H_{46}O_2$	0.30	0.02
stigmastane-3,6-dione	245/287/*428*	428	$C_{29}H_{48}O_2$	0.55	0.03
waxes				29.56	3.21
C_{42}	*57*/71/257/620	620	$C_{42}H_{84}O_2$	0.20	0.28
C_{44}	*57*/71/257/285/648	648	$C_{44}H_{88}O_2$	1.71	0.85
C_{46}	*57*/71/285/313/676	676	$C_{46}H_{92}O_2$	8.31	1.16
C_{48}	*57*/71/313/704	704	$C_{48}H_{96}O_2$	17.04	0.78
C_{50}	*57*/71/313/341/752	732	$C_{50}H_{100}O_2$	1.90	0.14
C_{52}	*57*/71/341/369/760	760	$C_{52}H_{104}O_2$	0.40	0.00

Table 3 (Continued)

compound	mass fragments	MW	formula	fibers	pulp
sterol/triterpenol esters				0.59	0.00
hexadecyl sitosterol	147/381/*396*	652	$C_{45}H_{80}O_2$	0.05	0.00
octadecyl sitosterol	147/381/*396*	680	$C_{47}H_{84}O_2$	0.04	0.00
hexadecyl β-amyrin	189/203/*218*	664	$C_{46}H_{80}O_2$	0.09	0.00
octadecyl β-amyrin	189/203/*218*	692	$C_{48}H_{84}O_2$	0.05	0.00
tetradecyl glutinol	*259*/274	636	$C_{44}H_{76}O_2$	0.13	0.00
hexadecyl glutinol	*259*/274	664	$C_{46}H_{80}O_2$	0.15	0.00
octadecyl glutinol	*259*/274	692	$C_{48}H_{84}O_2$	0.08	0.00
steryl glycosides				0.38	0.21
campesteryl 3-β-D-glucopyranoside	*204*/217/361/383*	850*	$C_{46}H_{90}O_6Si_4$*	0.04	0.02
stigmasteryl 3-β-D-glucopyranoside	*204*/217/361/395*	862*	$C_{47}H_{90}O_6Si_4$*	0.07	0.04
sitosteryl 3-β-D-glucopyranoside	*204*/217/361/397*	864*	$C_{47}H_{92}O_6Si_4$*	0.27	0.15

[a] Main mass fragments, molecular weight (MW), and formula are included. * as TMS ether derivatives. Italic mass fragments indicate base peaks.

Table 4. Composition of the Different Waxes (Milligrams per 100 g) Identified in Kenaf Fibers and Their Pulp

wax fatty acid:fatty alcohol	fibers	pulp
wax C_{42}	**0.21**	**0.28**
C_{16}:C_{26}	0.18	0.28
C_{20}:C_{22}	0.01	0.00
C_{22}:C_{20}	0.01	0.00
C_{24}:C_{18}	0.01	0.00
wax C_{44}	**1.71**	**0.86**
C_{16}:C_{28}	1.31	0.77
C_{18}:C_{26}	0.31	0.09
C_{20}:C_{24}	0.05	0.00
C_{22}:C_{22}	0.04	0.00
wax C_{46}	**8.12**	**1.15**
C_{16}:C_{30}	0.81	0.21
C_{18}:C_{28}	3.14	0.71
C_{20}:C_{26}	3.96	0.21
C_{22}:C_{24}	0.12	0.02
C_{24}:C_{22}	0.04	0.00
C_{26}:C_{20}	0.01	0.00
C_{28}:C_{18}	0.03	0.00
C_{30}:C_{16}	0.01	0.00
wax C_{48}	**16.97**	**0.78**
C_{16}:C_{32}	0.32	0.00
C_{18}:C_{30}	1.18	0.07
C_{20}:C_{28}	14.57	0.57
C_{22}:C_{26}	0.75	0.14
C_{24}:C_{24}	0.08	0.00
C_{26}:C_{22}	0.01	0.00
C_{30}:C_{18}	0.06	0.00
wax C_{50}	**1.90**	**0.14**
C_{16}:C_{34}	0.01	0.00
C_{18}:C_{32}	0.02	0.00
C_{20}:C_{30}	1.73	0.00
C_{22}:C_{28}	0.12	0.14
C_{24}:C_{26}	0.02	0.00
wax C_{52}	**0.40**	**0.00**
C_{20}:C_{32}	0.31	0.00
C_{22}:C_{30}	0.03	0.00
C_{24}:C_{28}	0.04	0.00
C_{26}:C_{26}	0.02	0.00

A series of *n*-alkanes ranging from C_{21} to C_{33} was also identified in the kenaf bast fibers with a strong odd-over-even carbon atom number predominance, hentriacontane (C_{31}) being the most predominant. *n*-Alkanes with even carbon atom numbers (C_{26}, C_{28}, and C_{30}) were also identified, albeit in lower amounts. Nonacosane (C_{29}) and hentriacontane (C_{31}) were the only alkanes previously reported in kenaf bast fiber (*15*), the latter being the most prominent, as found in the present work.

n-Fatty alcohols ranging from C_{18} to C_{32} were present in the kenaf extracts with strong even-over-odd carbon atom predominance, octacosanol (C_{28}) being the most abundant, as also reported by Seca et al. (*17*). Interestingly, the series of free fatty alcohols parallels the distribution of the esterified fatty alcohols in waxes. Minor amounts of a series of *n*-aldehydes ranging from C_{21} to C_{28} were identified in the kenaf fibers with docosanal (C_{22}) and tricosanal (C_{23}) predominating. This series of compounds has not been reported before in kenaf fibers.

Sterols and triterpenols were also present among the lipids of kenaf fibers. Sitosterol was the most abundant among the free sterols, with the presence of minor amounts of campesterol, stigmasterol, and stigmastanol. Lower amounts of sitosterol could also be found in ester form. Steryl glycosides, such as campesteryl, stigmasteryl, and sitosteryl β-D-glucopyranosides, were identified in minor amounts, the latter being the most predominant. The identification of steryl glycosides was accomplished (after BSTFA derivatization of the lipid extract) by comparison with the mass spectra and relative retention times of authentic standards (*33*). To our knowledge, the existence of the latter compounds in kenaf fibers is reported here for the first time. Among triterpenols, β- and α-amyrins occurred in free form, and a low amount of β-amyrin was also present in esterified form. Two other triterpenols, both characterized by a base peak at *m*/*z* 259 and molecular ions at *m*/*z* 426, were identified as motiol and glutinol. These compounds were also reported in kenaf bast fibers, and the structure of motiol was subsequently confirmed by NMR by Seca et al. (*17*). A series of high molecular weight compounds with mass spectra characterized by fragments at *m*/*z* 259 and 274, similar to that of free glutinol, were present in the extracts and were tentatively identified as glutinol esters. Lupeol, which was reported to occur in relatively high amount in kenaf (*17*), could not be detected here. Finally, several steroid hydrocarbons (such as stigmasta-3,5-diene and stigmasta-3,5,22-triene) and steroid and triterpenoid ketones (such as stigmast-4-en-3-one, stigmastan-3-one, stigmasta-7,22-dien-3-one, and β- and α-amyrenones) were also identified.

To investigate the fate of the different kenaf lipids during cooking, the lipid composition of an alkaline pulp with kappa 6 was studied. The GC/MS chromatogram of the underivatized pulp lipids, which accounted for 0.3% (**Table 1**), is shown in **Figure 3**, and the composition is listed in **Table 3**. It can be observed that the different lipid classes had different behaviors during cooking. The main difference in the lipid composition of kenaf pulp with respect to kenaf fibers concerned the amount of waxes, which showed a strong decrease, accounting for only 6% of the total lipids identified in pulp. The low wax content in the pulp of kappa 6 is due to the extensive hydrolysis of wax esters during alkaline cooking. The fatty acid content also decreased after cooking. At sufficiently high pH (as in alkaline pulping), the acids dissociate and form fatty acid soaps and can

thus dissolve in water to quite a high extent, forming fatty acid soaps. On the other hand, alkanes, fatty alcohols, sterols and triterpenols, steroid hydrocarbons and ketones, and steryl glycosides survived cooking. These compounds have a very low solubility in water and are difficult to remove and, therefore, can cause pitch problems. Steryl glycosides were present in minor amounts in the pulp. These compounds contain both hydrophilic and hydrophobic sites, have high melting points, and have very low solubility in water, alkali, and usual organic solvents. Due to these properties, they constitute protecting layers that prevent the cooking and bleaching chemicals from reaching the lipids (*34*). Problems caused by these compounds are increased in closed water systems.

In conclusion, the present work has shown different behaviors of lignin and lipids from the kenaf fibers during soda−AQ cooking. Knowledge of the chemical composition of the main components of nonwood plants and their behavior in pulping will be useful for a better utilization of nonwood plants. Nevertheless, further studies and efforts will be necessary to improve the industrial use of nonwood plants. The fiber demands of the industry should also be translated to agricultural production of high-quality fiber raw material.

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