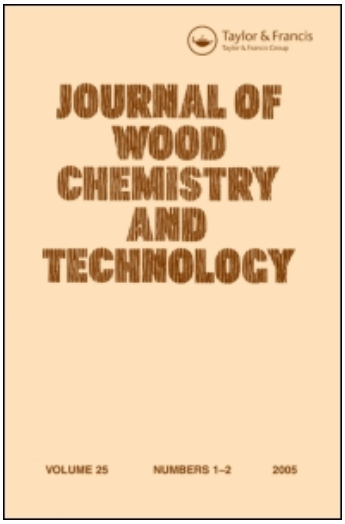

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EXTRACTION AND CHARACTERIZATION OF LIPOPHILIC EXTRACTIVES FROM RICE STRAW. I. CHEMICAL COMPOSITION

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ABSTRACT

A rapid analytical procedure, which enables convenient quantitative determination of individual components in rice straw lipophilic extractives, has been developed in this study. The method comprises a Soxhlet extraction with toluene–ethanol (2 : 1, v/v), chloroform, petroleum ether, dichloromethane, or hexane, silylation, and gas chromatography on a medium-length capillary column. The chemical composition of five

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lipophilic extractives has been examined. Free fatty/resin acids, sterols, waxes, steryl esters, and triglycerides were identified as the major five classes of lipids. Over all the free fatty acids, the most abundant free fatty acids were palmitic acid (C16 : 0, 3.82–8.11%), linoleic acid (C18 : 2) and/or oleic acid (C18 : 1, 1.22–3.35%), hexadecenoic acid (C16 : 1, 1.36–2.96%), and heptadecanoic acid (C17 : 0, 0.86–1.50%). β-Sitosterol and stigmasterol were the predominant components identified in a class of sterols, comprising over 90% of the total sterols. Palmitic acid palmity ester and palmitic acid oleyl ester were the major components analyzed in a group of waxes. The steryl esters identified were composed mainly of steryl palmitate, steryl oleate, steryl myristate, steryl heptadecanoate, and steryl laurate. Of the triglycerides identified, triolein was the dominant compound. Tripalmitin and dipalmitoyl-oleoylglycerol were also found in small amounts in this class of lipids. Diglycerides accounted for only minor amounts (0.19–0.33%) of the total extractives.

INTRODUCTION

Wood extractives, commonly called pitch or wood resins, are hydrophobic low molecular weight compounds present in low concentrations in wood and pulp.[1] The amount and composition of extractives are dependent on the wood species.[2] The lipophilic extractives in hardwood consist mainly of fatty acids, which are esterified with glycerol, fatty alcohols, sterols, and terpene alcohols, exception for small amounts of free fatty acids, free alcohols, and hydrocarbons. The resin acids and other diterpenoids typical of pine and spruce are completely absent in birch. In contrast, birch contains more neutral extractives than pine.[3] In addition, hardwood species like aspen and birch contain higher concentrations of steryl esters and waxes than softwood species.[4] The major extractives of softwood are resin acids and fatty acids, fatty acid esters such as steryl esters, waxes, and triglycerides, and neutral compounds as exemplified by fatty alcohols and sterols.[5] These extractives when liberated during the pulping process can cause significant problems for pulp and paper manufactures as they are deposited as pitch, either alone or in combination with fibers, fillers, defoaming agents, or coating binders.[6] In neutral to acidic processing of the wood, the lipophilic extractives are difficult to remove. However, during alkaline processing

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such as kraft pulping, the triglycerides are completely saponified and fatty and resin acids dissolved. In contrast to glycerol esters, sterols and some steryl esters and waxes do not form soluble soap under the alkaline conditions, and therefore, have a tendency to deposit on equipment and cause pitch problems.[7] In addition, these extractives have also a detrimental impact on the environment as contributors to effluent toxicity.[5]

Current methods for reduction of pitch problems include the use of wood species low in extractives, the seasoning of wood chips, and the addition of alum or talc to the pulp.[3] During chip storage, extractives undergo volatilization, enzymatic hydrolysis, and air oxidation. Unfortunately, these reactions are slow, particularly in the colder weather conditions.[6] Recently new biological methods using fungal pretreatment have been developed to reduce extractives levels with a minimal period of storage. However, the fungi are poorly adapted to low temperatures and to some of the wood species used, and some of them appear effective only on certain wood types and under specific pulping conditions.[6,8]

Conventional analyses of lipophilic extractives from woods or pulps involve multi-stage procedures, which include an extraction step and a determination process. The techniques, such as gas chromatography (GC), high performance liquid chromatography (HPLC), and supercritical fluid chromatography (SFC), were often used for separation of the extractives into free and esterified acids. When the composition of the fatty acids is necessary to know, a complete ester hydrolysis was performed prior to GC analysis.[1] From 1970 onwards, the ease and speed of GC techniques have simplified the analyses of extractives.[9] Particularly, with the use of modern gas chromatographs with short capillary columns, it is now possible to analyse free fatty and resin acids, sterols, and the total contents of non-volatile esters such as waxes, steryl esters, and triglycerides in a single chromatographic run. However, the individual components in waxes, steryl esters, and triglycerides are often eluted together, which limited their analysis by GC.[10,11]

Rice straw is currently used as potential raw material for papermaking in China. For studies involving the role of extractives in soda-anthraquinone or alkaline sulfite pulping we required a quick and effective method for analysis of the straw extractives in order to resolve the pitch problems and reduce the impact on the environment from the effluents. In the present study, the analysis of extractives in rice straw was carried out by GC using a medium-length high-temperature capillary column with thin films, that enable elution and separation of the individual compounds from high-molecular-mass lipids.

RESULTS AND DISCUSSION

Yield and Purity

Solvent extraction (e.g., with acetone, chloroform, dichloromethane, etc.) is routinely used to determine the wood resin or extractives content in samples of wood, pulp, paper, or pitch deposits. Traditionally, this has been carried out in a Soxhlet extractor.[12] No signal solvent is capable of removing all the lipophilic substances, and different solvents remove different combinations.[13] For a comparison study, we chose toluene–ethanol (2/1, v/v), chloroform, petroleum ether, dichloromethane, and hexane as the extraction solvents for testing their efficacy for extraction of rice straw sample. As shown in Table 1, a mixture of toluene–ethanol gave highest yield (3.42%), and signal solvent of petroleum ether and hexane yielded approximately equal lowest amounts of extractives (0.45, 0.65%), whereas both chloroform and dichloromethane gave similar medium values (1.19, 1.37%). However, the reverse trend was found in their purity (content of lipophilic extracts) of the extracts. Petroleum ether and hexane gave the higher total amounts of lipophilic extractives than those in the extractives obtained by toluene–ethanol (2/1, v/v), chloroform, and dichloromethane. This indicated that the extract from 2/3 toluene–1/3 ethanol consisted of substantially other non-lipid components such as low-molecular-weight carbohydrates, ash, and salts as shown by less than 40% of the lipophilic extractives identified by GC, although the extraction gave highest yield of the total extractives. Undoubtedly, only small amounts of non-lipid substances (~15%) were recovered in the petroleum ether extract as supported by 84.44% of the lipophilic extractives in the extract. Similarly, the relative high portions of 70.45% lipophilic substance in hexane extract, 59.04% in chloroform extract, and 59.25% in dichloromethane extract implied that noticeable amounts of non-lipid extracts were co-extracted in these three fractions, which are not analysed by the GC system used here exception for the azelaic and maleic acids. If only lipophilic extractives are of interest, petroleum ether or hexane is a good solvent, giving extracts containing over 70% of the lipophilic extractives. This is particularly reflected in the higher amounts of sterols (19.83–28.48%) waxes, (12.62–14.01%) and triglycerides (11.31–14.83%) analysed in the petroleum ether and hexane extractives, although care must be taken when interpreting results of total extracts. However, if both total extractives and lipophilic substances are considered, the preferred solvents are chloroform and dichloromethane since both gave relatively high yields of total extractives (1.19, 1.37%) with a acceptable lipid purity (~60%).

Table 1. The Yield (% Dry Straw) and Chemical Composition (% Dry Extractives) of Extractives in Rice Straw

Yield/Composition	F1[a]	F2[a]	F3[a]	F4[a]	F5[a]
Yield	3.42	1.19	0.45	1.37	0.65
Free fatty/resin/other acids (P 1–24)[b]	29.27	19.42	26.77	23.66	18.25
Compounds analysed	28.96	16.86	24.29	17.51	16.71
Decanoic acid, C10 : 0 (P 1)	0.28	0.15	0.16	0.12	0.11
Dodecanoic acid, C12 : 0 (P 3)	0.49	0.15	0.71	0.15	0.12
Azelaic acid (P 5)	3.98	1.45	1.01	0.69	0.66
Tetradecanoic acid, C14 : 0 (P 6)	4.88	0.47	0.87	0.28	0.24
Pentadecanoic acid, C15 : 0 (P 7)	3.02	0.36	0.73	1.28	0.38
Maleic acid (P 8)	6.47	0.89	1.68	0.58	1.19
Hexadecenoic acid, C16 : 1 (P 9)	1.94	1.38	2.96	1.36	2.08
Hexadecanoic acid, C16 : 0 (P 10)	3.82	5.01	8.11	4.18	4.65
Heptadecanoic acid, C17 : 0 (P 11)	0.86	1.05	1.38	1.50	1.15
Linoleic acid (C18 : 2) + oleic acid (C18 : 1) (P 12)	1.22	2.18	3.35	2.41	2.52
Octadecanoic acid, C18 : 0 (P 13)	0.23	0.49	0.68	0.78	0.61
Abietic acid (P 14)	0.25	0.52	0.59	1.21	0.16
Nonadecanoic acid, C19 : 0	0.16	0.18	0.26	0.53	N
Eicosanoic acid, C20 : 0 (P 15)	0.19	1.15	1.25	1.08	1.12
Heneicosanoic acid, C21 : 0 (P 17)	0.39	0.18	0.24	0.19	0.21
Docosanoic acid, C22 : 0 (P 18)	0.29	0.47	0.72	0.49	0.70
Tetracosanoic acid, C24 : 0 (P 22)	0.49	0.78	0.97	0.68	0.84
Sterols (P 25–28)	4.10	10.50	28.48	8.98	19.83
Compounds analysed	4.10	10.50	28.48	8.98	19.83
Cholesterol (P 25)	0.24	0.27	0.57	0.27	1.23
Stigmasterol + β-sitosterol (P 26–28)	3.86	10.23	27.91	8.61	18.60
Waxes (P 29–37)	4.68	8.63	14.01	6.58	12.62
Compounds analysed	2.06	3.20	6.13	2.38	3.47
Palmitic acid palmity ester (P 30)	1.55	2.48	4.82	1.65	2.28
Palmitic acid oleyl ester (P 33)	0.35	0.52	1.08	0.45	0.81
Oleic acid oleyl ester (P 35)	0.16	0.20	0.23	0.28	0.38
Diglycerides (38–39)	0.23	0.19	0.30	0.33	0.32
Compound analysed	0.14	0.11	0.19	0.18	0.17
Dipalmitin (P 38)	0.14	0.11	0.19	0.18	0.17
Sterol (mainly sitosterol) **esters** (P 40–48)	6.35	12.44	6.26	12.38	6.45
Compounds analysed	3.69	8.79	4.88	8.58	5.01
Steryl laurate (P 41)	0.46	0.28	0.42	0.29	0.32

(*continued*)

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Table 1. Continued

Yield/Composition	F1[a]	F2[a]	F3[a]	F4[a]	F5[a]
Steryl myristate (P 43)	1.26	2.82	0.72	2.26	0.58
Steryl palmitate (P 45)	0.64	1.84	1.76	1.95	1.58
Steryl heptadecanoate (P 46)	0.76	2.21	0.59	2.35	0.92
Steryl oleate (P 48)	0.57	1.64	1.39	1.73	1.61
Triglycerides (P 49–63)	5.59	10.20	11.31	8.59	14.83
Compounds analysed	2.45	4.91	3.94	3.51	3.62
Tripalmitin (P 50)	0.66	1.14	0.29	0.78	0.35
Dipalmitoyl-oleoylglycerol (P 52)	0.59	0.98	0.42	0.41	0.48
Triolein (P 55)	1.20	2.79	3.23	2.32	2.79
Total lipophilic substances	39.77	59.04	84.44	59.25	70.45
Total substances	50.22	61.38	87.13	60.52	72.30

[a]Represent fractions of the extractives obtained by extraction with toluene–ethanol (2 : 1, v/v, F1), chloroform (F2), petroleum ether (F3), dichloromethane (F4), and hexane (F5) for 12 h in a Soxhlet from rice straw.
[b]Represent peak numbers in Figure 1.

Chemical Composition

More than sixty individual compounds were resolved by capillary GC using medium-length column as illustrated by the chromatogram in Figure 1. The designation of the peak numbers is given in Table 1. The individual components were identified based on a comparison with GC retention times and mass spectra from authentic compounds and quantified against a standard mixture of palmitic acid, azelaic acid, abietic acid, β-sitosterol, palmitic acid palmitic ester, cholesteryl palmitate, 1-monopalmitoyl-rac-glycerol, 1,2-dipalmitoyl-sn-glycerol, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol. Practically no degradation of fatty and resin acid silyl esters or sterol silyl ethers occurred after 12 h storage at room temperature. After three days, degradation was noticeable. Similar degradations were also observed for the trimethylsilyl esters of fatty and resin acids from wood resins.[2] It is therefore all the silylated samples were analyzed by GC within 12 h in this study. Furthermore, previous studies demonstrated that the major components of steryl esters in wood and wheat straw were β-sitosterol esters.[6,14] However, these compounds were not available commercially and were, therefore, replaced by the closely related compounds, cholesteryl esters as standards in this study. A mixture of equal amounts of the standard compounds with palmitic acid, azelaic acid, abietic acid, β-sitosterol, palmitic

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acid palmitic ester, cholesteryl palmitate, 1-monopalmitoyl-rac-glycerol, 1,2-dipalmitoyl-sn-glycerol, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol, which gave peak areas in ratios of 1.0 : 0.88 : 0.86 : 0.92 : 0.85 : 0.74 : 1.0 : 0.79 : 0.61, was used to elaborate a calibration curve for the quantitation of free fatty acids, azelaic and maleic acids, resin acids, sterols, waxes, steryl esters, monoglycerides, diglycerides, and triglycerides, respectively. No significant differences in response factors were observed among the individual free fatty acids. Similar observations were found in all of the resin acids, sterols, waxes, steryl esters, monoglycerides, diglycerides, and triglycerides identified. The FID response was, therefore, assumed to be same for the component group and the corresponding standard.

Obviously, free fatty acids (16.40–24.08%), sterols (4.10–28.48%), waxes (4.68–14.01%), steryl esters (6.35–12.44%), and triglycerides (5.59–14.83%) were the major five lipid groups present in the rice straw lipophilic extractives. This is particularly reflected in the higher amounts of sterols (19.83–28.48%), waxes (12.62–14.01%), and triglycerides (11.31–14.83%) in the petroleum ether (F3) and hexane (F5) extracts, while the chloroform (F2) and dichloromethane (F4) extracts contained higher quantities of steryl esters (12.38–12.44%). Noticeable amounts of non-lipid substances such as azelaic and maleic acids (10.45%) were also determined in the toluene–ethanol extract (F1), whereas they appeared only minor amounts in the other four extracts (0.86–2.55%).

Fifteen free fatty acids were identified in detectable amounts in the five lipophilic extractives. The dominant free fatty acids in the four fractions between F2 and F4 were palmitic acid (C16 : 0, 4.18–8.11%), linoleic (C18 : 2) and/or oleic (C18 : 1) acids (2.18–3.35%), hexadecenoic acid (C16 : 1, 1.36–2.96%), heptadecanoic acid (C17 : 0, 1.05–1.50%), and eicosanoic acid (C20 : 0, 1.08–1.25%), which together comprised 63.06% of the total free fatty acids in F2, 70.80% in F3, 47.03% in F4, and 70.25% in F5, respectively. While in the toluene–ethanol extract (F1), tetradecanoic acid (C14 : 0, 4.88%) and pentadecanoic acid (C15 : 0, 3.02%) were also found as the major free fatty acid components exception for the palmitic acid (C16 : 0, 3.82%), hexadecenoic acid (C16 : 1, 1.94%), linoleic (C18 : 2) and/or oleic (C18 : 1) acids (1.22%), and heptadecanoic acid (C17 : 0, 0.86%). Decanoic acid (C10 : 0, 0.11–0.28%), dodecanoic acid (C12 : 0, 0.12–0.71%), octadecanoic acid (C18 : 0, 0.23–0.78%), heneicosanoic acid (C21 : 0, 0.18–0.39%), docosanoic acid (C22 : 0, 0.29–0.72%), and tetracosanoic acid (C24 : 0, 0.49–0.97%) were detected with small amounts in all of the five extractives. Nonadecanoic acid (C19 : 0) was not identified in the hexane extract (F5), whereas it appeared in a minor amount in other four extracts, ranging between 0.16% in F1 and 0.53% in F4. Similar free fatty acid composition was found in a study of *Eucalyptus globulus* labill.

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wood extractives by Gutierrez et al.,[7] who stated that palmitic acid (C16 : 0), linoleic (C18 : 2), and oleic (C18 : 1) were the dominant components in free fatty acids.

Based on studies of behavior of the major resin- and fatty acids of slash pine wood during organosolv pulping, seven resin acids present in the wood extractives were identified on the basis of comparison of their GC-retention times with those of standard compounds and by GC-MS spectra. Among them, pimarane (pimaric) and isopimarane (isopimaric and sandaracopimaric) types were almost completely removed from the wood chips and their chemical structure remains unchanged in organosolv pulping, whereas with the exception of abietic acid, resin acids of the abietane type (palustric, dehydroabietic, and neoabietic) were found in lower amounts in the cooking liquor than those originally present in wood.[15] Apparently, as shown in Table 1, only a minor amount of abietic acid (0.25–1.21%) was identified from the rice straw extractives in the present studies. The reason for this only abietic acid detected in rice straw extractives is presumed due to the isomerization of some portions of resin acids such as neoabietic and palustric acids into a more 'thermostable' form like abietic acid during the Soxhlet extraction conditions given in this study.

In a previous study of wheat straw extractives, analyses with high resolution gas chromatography coupled with mass spectrometry (HRGC-MS) and Fourier transform infrared spectrometry (HRGC-MS-FTIR) revealed that azelaic acid was the major component in the wheat straw acidic extractives, and maleic acid appeared in a noticeable amount.[14] In our case, the contents of azelaic acid and maleic acid were rather different between fraction F1 and fractions F2–F5. In toluene–ethanol extract considerable amounts of maleic acid (6.47%) and azelaic acid (3.98%) were quantitatively identified, comprising 20.81% of the total extractives from rice straw, whereas only minor amounts of azelaic acid (0.66–1.45%) and maleic acid (0.58–1.68%) were detected as non-lipid components by GC in other four extracts. This implied that the mixture of solvents such as toluene and ethanol favoured extraction of both lipophilic substances and non-lipid compounds, which resulted in low purity of lipophilic extractives in F1 extract, while the extraction using a signal solvent e.g., chloroform, petroleum ether, dichloromethane, or hexane separately isolated the lipophilic substances to a high level in rice straw extractives.

Among the sterols identified as peaks 25–28 in Figure 1, β-sitosterol and stigmasterol were the predominant compounds in this group, amounting for 94.15–98.00% of the total sterols. Cholesterol was determined in a minor amount, comprising 2.00–5.83% of the total sterols. The results obtained were consistent with the studies from *E. globulus* labill. wood by

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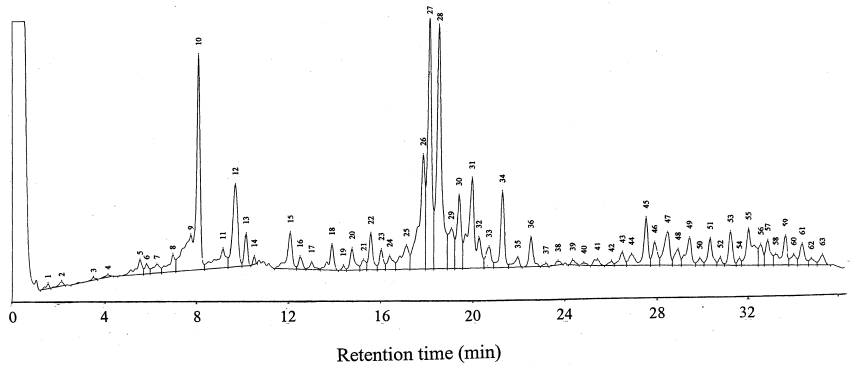

Figure 1. The chromatogram of hexane extractives (F5) from rice straw.

Guiterrez et al.[7] They revealed that sterols were the major compounds in this wood lipophilic extractives (64.50 mg sterols/100 g wood), in which β-sitosterol was the dominant sterol present in this group. Similar results have also been reported from aspen wood, in which α_1- and β-sitosterol, both in free form and as steryl esters, were the major sterols found in the wood extractives.[4]

Waxes were found to be another important group of the lipophilic extractives from rice straw, which were higher in petroleum ether (F3) and hexane (F4) extracts (12.62–14.01%) than in the extracts of toluene–ethanol (F1, 4.68%), chloroform (F2, 8.63%), and dichloromethane (F4, 6.58%). As illustrated in Figure 1, the waxes were composed of nine individual components as shown in peaks 29–37. Palmitic acid palmityl ester, palmitic acid oleyl ester, and oleic acid oleyl ester were identified in this class of lipids from the straw extractives, which together comprised 44.02% of the total waxes in F1, 37.08% in F2, 43.75% in F3, 36.17% in F4, and 27.50% in F5, respectively. Further analyzing work is needed on the other unknown 6 constituents to obtain whole composition of the waxes.

Steryl esters were also a important class of constituents in rice straw extractives. They accounted for 6.35% of the total extract in F1, 12.44% in F2, 6.26% in F3, 12.38% in F4, and 6.45% in F5. Interestingly, in this study, the utilization of a medium-length high-temperature capillary column made it possible to separate the individual components in groups of steryl esters, waxes, and triglycerides from rice straw extractives. From such a chromatogram the individual compounds were qualitatively identified by comparing their GC-retention times with those of authentic compounds and quantitatively determined by their peak areas relatively to palmitic

acid palmitic ester for waxes, cholesteryl palmitate for steryl esters, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol for triglycerides. The major components identified were steryl laurate, steryl myristate, steryl palmitate, steryl margarate, and steryl oleate, which together comprised 58.11–77.96% of the total steryl esters. It is very interesting to note that the distribution of esterified fatty acids in steryl esters is the same as that of the free fatty acids as described above. In addition, based on the studies of major wood and wheat straw steryl esters, Chen et al.[6] and Chaves das Neves and Gaspar[14] have independently reached similar conclusions by quite different way. They indicated that β-sitosterol esters were the dominant steryl esters in *E. globulus* labill. wood and wheat straw extractives. Similarly, it is therefore very likely that the steryl esters in the extractives of rice straw are composed mainly of combinations of β-sitosterol with major fatty acids such as lauric, myristic, palmitic, margaric, and oleic acids.

Finally, fifteen components were identified in a group of triglycerides. These triglycerides are commonly produced as storage reserves of energy and carbon skeletons for growth and development.[16] Triolein (*cis*-9) occurred as a major compound, accounting for 21.47% of the total triglycerides in F1, 27.74% in F2, 28.56% in F3, 27.01% in F4, and 18.81% in F5, respectively. Glyceryl tripalmitate and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol analyzed, appeared in minor amounts, ranging between 5.60% of the total triglycerides in F5 and 22.36% in F1. Besides the major five classes (free fatty and resin acids, sterols, waxes, steryl esters, and triglycerides) of lipophilic substances in the extractives, Diglycerides were also identified among the five lipophilic extractives from rice straw although in very minor amounts (0.19–0.33%). 1,2-Dipalmitoyl-rac-glycerol was the only one compound identified and accounted for 0.11–0.19% of the total extractives.

Table 2 exhibits the content and composition of the phenolic components in the extractives detected by HPLC at 280 nm based on comparison with the their authentic compounds. Obviously, extraction with 2/3 toluene and 1/3 ethanol resulted in dissolution of noticeable amounts of phenolic compounds, 8.22%, whereas extraction with petroleum ether, dichloromethane, or hexane released only trace quantities of phenolic acids and aldehydes (0.069–0.14%), and chloroform extract gave minor amounts of the phenolics (0.94%). This is particularly reflected in the amounts of lignan (2.24%) and *p*-coumaric acid (1.34%) analyzed in the toluene–ethanol extract. The results indicated again that the non-lipid substances in toluene–ethanol extract enriched not only in maleic and azelaic acids but also in phenolic compounds. *p*-Coumaric acid, ferulic acid, syringaldehyde, vanillin, vanillic acid, syringic acid, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, fumaric acid, *m*-toluic acid, and 1-naphthoic acid were

Table 2. The Content (% Dry Extractives, w/w) of Phenolic Acids and Aldehydes, Lignan, and Other Compounds Identified in the Rice Straw Extractives by HPLC

Phenolic Acids and Aldehydes, Lignan, and Other Compounds	F1[a]	F2[a]	F3[a]	F4[a]	F5[a]
Gallic acid	0.10	0.010	N	N	N
Protocatechuic acid	0.23	0.034	N	N	N
p-Hydroxybenzoic acid	0.31	0.042	N	0.013	0.011
p-Hydroxybenzaldehyde	0.41	0.062	0.002	0.006	0.003
Vanillic acid	0.31	0.081	0.002	0.044	0.005
Syringic acid	0.17	0.038	0.003	0.005	0.003
Vanillin	0.26	0.054	0.006	0.004	0.011
Syringaldehyde	0.19	0.068	0.008	0.011	0.013
Acetovanillone	0.13	0.005	N	N	N
Acetosyringone	0.005	0.002	N	N	N
p-Coumaric acid	1.34	0.12	0.006	0.006	0.005
Ferulic acid	0.38	0.041	0.004	0.007	0.005
Sinapic acid	0.12	0.038	N	N	N
Cinnamic acid	0.18	0.021	N	0.007	0.003
Fumaric acid	0.34	0.013	0.003	0.005	0.003
Benzoic acid	0.32	0.047	N	N	N
m-Toluic acid	0.56	0.11	0.011	0.010	0.013
1-Naphthoic acid	0.62	0.021	0.011	0.010	N
Lignan	2.24	0.13	0.013	0.010	0.011
Total (%)	8.22	0.94	0.069	0.14	0.086

[a]Corresponding to the extractive fractions in Table 1; N, not detectable.

determined as the major compounds identified, however, they all occurred in trace amounts in fractions F3, F4, and F5. Gallic acid, protocatechuic acid, acetovanillone, sinapic acid, cinnamic acid, and benzoic acid were found in minor amounts in toluene–ethanol extract, in trace quantities in chloroform extract, and absent in petroleum ether, dichloromethane, or hexane extract. These suggested that petroleum ether, dichloromethane, or hexane could be a useful alternative solvent for extraction of lipophilic extractives from rice straw, although care must be taken when interpreting yields of total extracts.

Release of lignan, characterized by two phenylpropane units bound together at their β-carbon, were found during the thermomechanical pulping process of heartwood[17] or Soxhlet extractions of Norway spruce wood.[18] Hydroxymatairesinol and its isomer allohydroxymatairesinol were the most abundant lignans in heartwood extractives.[18] In this study, minor amounts of lignan identified by HPLC using 2,2′-dihydroxybiphenyl as a standard, were found in toluene–ethanol extract (2.24%) and chloroform

extract (0.13%). Extraction with petroleum ether, dichloromethane, or hexane yielded only trace of lignan as shown in Table 2 (0.010–0.013%).

EXPERIMENTAL

Materials and Reagents

Rice straw was obtained from the experimental farm of The North-Western Science and Technology University of Agricultural and Forestry (Yangling, P. R. China). It was dried in sunlight and then cut into small pieces. The cut straw was ground to pass a 2.5 mm size screen. The yield of extractives was made on an oven-dried (60°C, 16 h) basis. The fatty acids mentioned below are abbreviated with a code indicating the number of carbon atoms : number of double bonds. All standard compounds used were obtained from Sigma (Xian). All organic solvents used were of analytical or reagent grade.

Extraction and Silylation

Fifth grams of straw meal was extracted with 2200 mL of toluene–ethanol (2/1, v/v), chloroform, petroleum ether, dichloromethane, and hexane for 12 h using a Soxhlet extraction apparatus, respectively. The extraction solution was evaporated to dryness at 35°C using a rotary evaporation, and the mixture was taken to further dryness in a nitrogen steam and then weighed to determine the yield of extractives. Note that the extract released in toluene–ethanol (2/1, v/v) was labeled for Fraction 1 (F1) only, and those dissolved in chloroform, petroleum ether, dichloromethane, and hexane were considered to be Fractions 2 (F2), 3 (F3), 4 (F4), and 5 (F5), respectively. The five lipophilic extracts (~3 mg) were silylated with 120 μL bis-trimethylsilyltrifluoroacetamide and 60 μL trimethylchlorosilane in an oven at 70°C for 25 min. When cooled, 180 μL toluene was added. The solution was shaken and thereafter ready for analysis by GC.

GC Analysis

The derivatized lipophilic extractives were analyzed on an Rtx-1 capillary column (15 m, 0.53 mm i.d., 0.10 μm film thickness, purchased from Hewlett-Packard Company, Beijing) with a flame ionization detector (FID). Helium was used as the carrier gas, and the initial flow was

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1.8 mL/min. The injector and detector temperatures were set at 340°C. The oven was temperature-programmed from 70 to 340°C (2 min) at 8°C/min. Injections were 1 μL splitless. The total analysis time, including cooling of the column oven and injector, followed by temperature stabilization, was about 45 min.

Analytes were identified by comparison of their gas chromatographic retention times or mass spectra with those in authentic compounds, in which free fatty and resin acids and sterols were both qualitatively identified by total ion detection from mass spectra and GC retention times with authentic compounds, whereas identification of the waxes, steryl esters, and triglycerides was carried out by only GC since GC-MS gave only the fragments arising from the their moiety by electron-impact MS and rarely gave detectable molecular ions. Similar methods for the detection of above high-mass lipids have been reported from wood extractives.[7] For quantitative analyses, palmitic acid was used as a representative standard for all of the free fatty acids, azelaic acid for all of the non-fatty and resin acids, abietic acid for all of the resin acids, β-sitosterol for all of the sterols, palmitic acid palmitic ester for all of the waxes, cholesteryl palmitate for all of the steryl esters, 1-monopalmitoyl-rac-glycerol for all of the monoglycerides, 1,2-dipalmitoyl-sn-glycerol for all of the diglycerides, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol for all of the triglycerides.

HPLC Analysis

Minor amounts of phenolic compounds in the extractives, were quantitatively analyzed on a Hichrom H5ODS column of dimensions 250 × 4.6 mm (purchased from Phenomenex Co., Beijing). Samples (~10 mg) were dissolved in 1 mL methanol and separation were obtained using a linear gradient of two solvent systems: solvent A (water–methanol–acetic acid, 84 : 15 : 1) and solvent B (methanol–water–acetic acid, 90 : 9 : 1). A linear gradient was run over 30 min from 0 to 40% B at a flow rate of 1 mL/min. The compounds were detected at 280 nm by computer comparison of the retention times and peak areas with the authentic phenolics. 2,2′-Dihydroxybiphenyl was used as a standard compound to calibrate the content of lignans (phenyl propane dimers) in the extractives.

CONCLUSIONS

It is clear from the above studies that the GC analyses of silylated extracts with a medium-length capillary column is an excellent technique for

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quantitatively determining individual free fatty and resin acids, sterols, waxes, steryl esters, and triglycerides. It was found that lipophilic extractives from rice straw consisted mainly of free fatty acids (16.40–24.08%), sterols (4.10–28.48%), waxes (4.68–14.01%), steryl esters (6.26–12.44%), and triglycerides (5.59–14.83%) together with minor amounts of diglycerides (0.19–0.33%). Extraction with petroleum ether gave lowest yield of total extractives (0.45%), but contained highest amounts of lipophilic extractives, enriching in sterols (28.48%), free fatty acids (24.08%), waxes (14.01%), triglycerides (11.31%), and steryl esters (6.26%). In contrast, extraction with 2/3 toluene and 1/3 ethanol produced highest quantities of total extractives (3.42%), but gave lowest purity of the lipid substances (39.77%). Maleic and azelaic acids (10.45%), phenolic substances (8.22%), and noticeable amounts of released polysaccharides, ash or slats were the major nonlipid constituents in this extract, whereas the extracts from chloroform, petroleum ether, dichloromethane, and hexane contained only minor amounts of maleic and azelaic acids (1.27–2.34%) and traces of phenolic compounds (0.07–0.94%). If both total lipophilic extractives and purity of the extract are considered, dichloromethane and chloroform could be the useful alternative solvents for extraction of rice straw, which yielded approximately 60% of lipophilic extractives.

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