J Wood Sci (2003) 49:47–52

ORIGINAL ARTICLE

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Comparative study of organic solvent and water-soluble lipophilic extractives from wheat straw I: yield and chemical composition

Received: January 29, 2001 / Accepted: January 30, 2001

Abstract The chemical composition of six lipophilic extractives from wheat straw by extraction with toluene-ethanol (2:1, v/v), chloroform-methanol (2:1, v/v), methyl *tert*-butyl ether, hexane, petroleum ether, and dichloromethane, respectively, in a Soxhlet extractor, and one water-soluble lipophilic extract has been examined. Five main lipid classes (free fatty/resin acids, sterols, waxes, steryl esters, triglycerides) were identified and their individual components quantified by gas chromatography as their trimethylsilyl (TMS) esters (free fatty/resin acids) and TMS ethers (sterols). The abundant saturated fatty acids were palmitic acid (C16:0), myristic acid (C14:0), and pentadecanoic acid (C15:0). Palmitoleic acid (C16:1), linoleic acid (C18:2), and oleic acid (C18:1) were the major unsaturated free fatty acids. Abietic acid was detected as the only single component in the resin acids. Of the sterols identified, β-sitosterol was found to be the major compound together with minor amounts of cholesterol, ergosterol, stigmasterol, and stigmastanol. Palmityl palmitate and oleyl palmitate were identified as the major components in waxes. The steryl esters analyzed were composed of steryl laurate, steryl myristate, steryl palmitate, steryl heptadecanoate, and steryl oleate. Tripalmitin, dipalmitoyl-oleoylglycerol, and triolein were the major components of the triglycerides.

Key words Wheat straw · Extractives · Fatty acids · Sterols · Waxes · Steryl esters · Triglycerides

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Introduction

Lipophilic extractives are materials extracted from wood and pulp with organic solvents such as ethanol, acetone, or dichloromethane. The nonpolar extractives, commonly called pitch or resin, are composed mainly of fatty acids, resin acids, fatty acid esters (e.g., steryl esters, waxes, triglycerides), and neutral compounds exemplified by fatty alcohols and sterols.[1] These extractives are hydrophobic low-molecular-weight compounds; and the various classes of extractives have different chemical behavior during pulping. Some of the esters are hydrolyzed during storage.[2] Most of these esters survive the process of mechanical pulping.[2] It is difficult to remove the lipophilic extractives during neutral and acidic processing of wood. However, during alkaline processes, such as kraft pulping, the glycerol esters are completely saponified, and free fatty and resin acids are easily dissolved as soaps, although small amounts remain in the pulp. Sterols, some sterol esters, and waxes are not removed under the alkaline conditions of kraft pulping and therefore cause problems in pulp and paper mills.[3]

Conventional approaches to reduce the deposition of wood extractives, such as debarking or seasoning of logs, are often not sufficient to eliminate pitch troubles. Recent advances in biotechnology have demonstrated the ability of various wood-inhabiting fungi to degrade wood lipophilic compounds, but these methods appear effective only on certain wood types and under specific pulping conditions.[4] Furthermore, the pitch problems are likely to become more severe with the introduction of more environmentally friendly bleaching processes that have replaced chlorine gas with other reagents such as hydrogen peroxide or ozone.[3]

It is of considerable interest to identify extractives that may help resolve difficulties with pitch. A number of studies that analyzed of extractives present in softwoods and hardwoods commonly used in the pulp and paper industry have been reported,[3–6] but little is known about the potential application of the results to the qualitative and quantitative characterization of straw extractives. Published studies on

the chemical composition of extractives from wheat straw refer mainly to more polar fractions containing phenolics.[7] Studies referring to the composition of lipophilic extractives from wheat straw have been scarce, and only the occurrence of several free fatty acids and sterols was qualitatively reported.[8] In this study, we describe the chemical composition of lipophilic extractives from wheat straw. The methods include organic-solvent and hot-water extractions and subsequent chromatographic separation with gas chromatography (GC).

Experimental

Material and reagents

Wheat straw (*Tritican aestivum* L.) was obtained from Silsoe Research Institute (Silsoe, Bedfordshire, UK) and was ground using a Christie Laboratory mill (Compak, UK) to pass through a 5 mm screen. The ground straw was then dried in a cabinet oven with air circulation at 60°C for 16 h before extraction. Fatty acids are abbreviated with a notation indicating the number of carbon atoms:number of double bonds. All standard compounds were obtained from Aldrich or Sigma. All organic solvents were of analytical or reagent grade.

Extraction and silylation

Straw chip (50 g) was placed in a cellulose thimble and was extracted using a Soxhlet extractor with 1500 ml of toluene ethanol (2:1, v/v) to give fraction 1 (F1), chloroform-methanol (2:1, v/v) (F2), methyl *tert*-butyl ether (MTBE) (F3), hexane (F4), petroleum ether (boiling point <45°C) (F5), or dichloromethane (F6) for 6 h, respectively. The solvent was evaporated at 35°C, and flasks containing the extractives were dried in a nitrogen stream to determine the yield of extractives.

For a comparative study, chopped straw chip (1–3 cm, 50 g) was suspended in distilled water (800 ml), and the suspension was agitated for 4 h at 98°C. After filtering the residue, the supernatant was concentrated and then extracted twice with MTBE (1000 ml) for 12 h at room temperature. The two extracts were combined, and the solvent was removed on a rotary vacuum evaporator at 35°C. After being dried in a stream of nitrogen, the yield of the hot water-soluble extractives was determined gravimetrically, and the fraction was labeled F7.

The procedure for silylation was as follows. Bis-(trimethylsilyl)-trifluoro-acetamide (240 μl) and trimethylchlorosilane (120 μl) were added to the extractives. The solution was kept in an oven for 20 min at 75°C. When cooled, 360 μl toluene was added. The solution was then shaken and thereafter was ready for analysis by GC.

Gas chromatography

The extractives were analyzed with an ATI UNICAM 610 series gas chromatograph (UK) equipped with a split–splitless injector and a flame ionization detector (FID). The capillary column was a bonded dimethyl polysiloxane Rtx-1 column (15 m × 0.53 mm i.d.) (Thames Chromatography Fairacres Industrial Centre, UK) with a film thickness of 0.10 μm. Helium was the carrier gas, and the initial flow rate was 1.6 ml/min. The injector and detector temperatures were both set at 340°C. The oven was temperature-programmed from 70°C to 336°C (2 min) at 8°C/min. Triplicate samples (1 μl) were injected in the splitless mode. All samples were analyzed under the same conditions. Compounds were identified by comparing their retention times with those of authentic compounds.[9]

Individual compounds of free fatty and resin acids and sterols were identified by comparing their gas chromatographic retention times and total ion detection mass spectra with those of authentic compounds; whereas each component of the waxes, steryl esters, and triglycerides was verified only by GC retention times because GC-mass spectrometry (GC-MS) gave only the fragments arising from their moiety by electron-impact MS and rarely gave detectable molecular ions. In this study a mixture of standard compounds (palmitic acid, azelaic acid, abietic acid, β-sitosterol, palmitic acid palmitic ester, cholesteryl palmitate, 1-monopalmitoyl-rac-glycerol, 1,2-dipalmitoyl-sn-glycerol, 1,2-dipalmitoyl-3-oleoyl-rac-glycerol) was used to prepare a calibration for the quantitation of free fatty acids, azelaic acid/maleic acid, resin acids, sterols, waxes, steryl esters, monoglycerides, diglycerides, and triglycerides, respectively. All of the fatty acids were assumed to have the same response factor. Similar estimations were made for all of the resin acids, sterols, waxes, steryl esters, monoglycerides, diglycerides, and triglycerides.

Mass spectra were recorded with a 5970 Series instrument (Hewlett Packard, UK) equipped with the same capillary column. The spectrometer was operated with an electron energy setting of 70 eV. Compounds were identified by comparing the mass fragmentography with standard compounds.

Results and discussion

Yield and purity

Results show that extractions with toluene-ethanol or chloroform-methanol gave larger amounts of extractives (2.38% dry straw for F1, 2.32% for F2) (Table 1). The yields of MTBE (F3) and dichloromethane (F6) extracts from the straw accounted for 1.00% and 1.17%, respectively. Extraction with hexane (F4) or petroleum ether (F5) yielded lower amounts of extractives (0.74% for F4, 0.55% for F5). The yields of the extractives were consistent with the order of polarity of the solvent, such as dichloromethane (9.08), MTBE (3.06), hexane (1.89), and petroleum ether (1.84).[10] Similar results have been reported by Wallis and Wearne[10] for pine woods. The authors indicated that the amount of extracts decreased with reduced polarity of the solvent, in the order methanol (3.33%) > acetone > dichloromethane

Table 1. Yield and composition of identified compounds

Yield/composition	Peak no.[a]	Extraction[b]						
		F1	F2	F3	F4	F5	F6	F7
Yield (% dry straw)		**2.38**	**2.32**	**1.00**	**0.74**	**0.55**	**1.17**	**0.91**
Chemical composition (% extractives)								
Free fatty acids	P1–27	**32.3**	**28.2**	**26.6**	**19.1**	**15.4**	**11.6**	**33.8**
All compounds identified		31.7	26.2	23.3	15.5	11.6	10.9	23.6
Capric acid (C10:0)	P1	0.1	0.1	0.2	0.1	0.0	0.1	0.9
Lauric acid (C12:0)	P3	0.4	0.2	0.2	0.1	0.2	0.2	1.2
Myristic acid (C14:0)	P5	13.7	10.8	5.2	2.0	1.4	1.3	1.4
Pentadecanoic acid (C15:0)	P6	10.4	8.0	5.3	1.1	1.1	1.1	8.9
Palmitoleic acid (C16:1)	P8	0.3	0.9	1.7	1.0	0.3	0.6	0.9
Palmitic acid (C16:0)	P9	4.6	4.5	6.2	5.4	3.2	3.9	5.5
Heptadecanoic acid (C17:0)	P11	0.3	0.1	0.5	0.7	0.4	0.2	0.2
Linoleic acid (C18:2) + oleic acid (C18:1)	P12	0.5	0.8	2.1	2.9	3.3	2.0	1.3
Stearic acid (C18:0)	P13	0.0	0.1	0.5	0.5	0.4	0.3	0.3
Nonadecanoic acid (C19:0)	P15	0.1	0.1	N	N	N	N	0.1
Gondoic acid (C20:1)	P16	0.0	0.0	0.2	0.1	0.1	0.1	0.1
Arachidic acid (C20:0)	P17	0.2	0.1	0.4	0.5	0.3	0.3	0.4
Heneicosanoic acid (C21:0)	P19	0.0	0.1	0.1	0.1	0.1	0.1	1.2
Behenic acid (C22:0)	P21	0.2	0.1	0.4	0.6	0.4	0.4	0.7
Lignoceric acid (C24:0)	P25	0.8	0.4	0.4	0.4	0.4	0.3	0.5
Resin/other acids	P4–14	**23.4**	**20.1**	**5.1**	**4.3**	**4.2**	**2.7**	**5.5**
All compounds identified		23.4	20.1	5.1	4.3	4.2	2.7	5.5
Azelaic acid	P4	16.2	15.2	2.8	1.8	1.9	1.2	3.9
Maleic acid	P7	7.1	4.7	2.1	2.3	1.6	1.4	1.5
Abietic acid	P14	0.1	0.2	0.2	0.2	0.7	0.1	0.1
Sterols	P28–31	**4.1**	**4.3**	**15.5**	**24.0**	**24.5**	**17.8**	**6.1**
All compounds identified		4.1	4.3	15.5	24.0	24.5	17.8	6.1
Cholesterol	P28	0.2	0.2	1.5	2.3	2.1	1.1	0.5
Ergosterol + stigmasterol + β-sitosterol	P29,30	2.9	3.4	13.9	20.7	21.5	16.2	4.3
Stigmastanol	P31	1.0	0.7	0.1	1.0	0.9	0.5	1.3
Waxes	P32–38	**1.4**	**2.6**	**17.0**	**21.1**	**20.8**	**15.8**	**8.2**
All compounds identified		1.3	2.1	10.7	14.1	14.1	10.9	5.9
Palmityl palmitate	P32	0.6	1.0	4.7	8.1	7.6	4.6	2.5
Oleyl palmitate	P35	0.6	1.0	5.9	5.8	6.3	6.2	3.3
Oleyl oleate	P37	0.1	0.1	0.1	0.2	0.2	0.1	0.1
Diglycerides	P39,40	**0.8**	**0.5**	**0.2**	**0.2**	**0.4**	**0.4**	**0.2**
All compound identified		0.4	0.3	0.1	0.1	0.2	0.2	0.1
Dipalmitin	P39	0.4	0.3	0.1	0.1	0.2	0.2	0.1
Steryl esters (mainly sitosteryl esters)	P41–50	**13.9**	**10.4**	**11.3**	**12.5**	**12.6**	**19.1**	**6.8**
All compounds identified		8.7	7.6	8.0	10.2	10.2	15.2	4.3
Steryl laurate	P43	3.2	1.0	0.6	0.9	1.1	0.6	1.9
Steryl myristate	P45	2.6	2.5	2.5	2.7	2.9	4.9	1.4
Steryl palmitate	P47	1.4	1.4	1.3	2.2	2.2	4.5	0.5
Steryl heptadecanoate	P48	1.0	1.4	2.2	2.0	1.4	3.4	0.3
Steryl oleate	P49	0.5	1.3	1.4	2.4	2.6	1.8	0.2
Triglycerides	P51–59	**1.7**	**8.9**	**9.7**	**13.4**	**14.2**	**12.5**	**3.5**
All compounds identified		1.3	4.0	2.7	5.3	8.1	6.9	2.6
Tripalmitin	P52	0.5	0.8	0.1	0.7	0.8	0.5	0.5
Dipalmitoyl-oleoylglycerol	P53	0.4	1.5	1.0	1.8	3.0	2.8	1.0
Triolein	P56	0.4	1.7	1.6	2.8	4.3	3.6	1.1
Total lipophilic substances		54.2	54.9	80.3	90.3	87.9	77.2	58.6
Total substances		77.6	75.0	85.4	94.6	92.1	79.9	64.1

[a] Peak number in Fig. 1

[b] Fractions of the extractives obtained by extraction with toluene-ethanol (2:1, v/v; F1), chloroform-methanol (2:1, v/v; F2), methyl *tert*-butyl ether (F3), hexane (F4), petroleum ether (F5), and dichloromethane (F6) for 6 h in a Soxhlet, respectively. F7 represents the extractive fraction solubilized during the treatment of wheat straw with water at 98°C for 4 h

> hexane (1.55%). There is no single organic solvent to extract all the lipophilic substances individually, and different solvents removed different combinations. Extractions with MTBE, dichloromethane, hexane, and petroleum ether are used to determine such substances as sterols, waxes, fats, resins, and nonvolatile hydrocarbons. The extractable components of woods or straws with toluene-ethanol or chloroform-methanol are composed of certain other nonlipophilic components, such as azelaic acid (15.2%–16.2%) or maleic acid (4.7%–7.1%). As shown in Table 1, the purity of the lipophilic substances in the extracts obtained by MTBE, hexane, petroleum ether, and

dichloromethane accounted for 80.3%, 90.3%, 87.9%, and 77.2%, respectively, which were higher amounts than those in the extracts obtained by toluene-ethanol (54.2%) or chloroform-methanol (54.9%). This indicated that the latter two mixtures of solvents are extracting some nonlipophilic materials that are not analyzed by the GC system except for the azelaic and maleic acids. The hexane and petroleum ether gave high percentages of lipophilic extractives but did not extract significant amounts of azelaic or maleic acid. If only lipophilic extractives are of interest, hexane and petroleum ether are good solvents, giving extracts containing 88%–90% lipophilic extractives. The percentage of lipophilic components in the extractives identified by GC were lowest for the toluene-ethanol and chloroform-methanol extracts and highest for the hexane and petroleum ether extracts; MTBE and dichloromethane extracts gave similar values (77%–80%) (Table 1). This is particularly reflected in the amounts of sterols and waxes (4.3% and 2.6%, respectively) in the chloroform-methanol extract and 24.0% and 21.1%, respectively, in the hexane extract. However, if the purity of the lipophilic extractives is considered, MTBE and dichloromethane could be useful solvents for the extraction of wheat straw, although they did not have much more yield of lipophilic extractives.

A comparison was made between Soxhlet extraction with conventional organic solvent and extraction with hot water. The results showed that the amount of extract obtained with hot water treatment of wheat straw depended on the treatment time and the temperature (data not shown). In this study, 4 h of treatment at 98°C was found to be satisfactory, as shown by the yield of hot water-soluble extracts (0.91%), which corresponds to the values of the extracts obtained by the Soxhlet extractions. On the other hand, as shown in Table 1, the water extract gave less than 60% of lipophilic extractives. Similar results have been reported by Orsa et al.[11] after water treatment of spruce wood. We also found that vigorous mixing was essential for high extraction yields of lipophilic extractives. Evidently, strong mechanical action is required to obtain sufficient contact between lipophilic droplets and water.

Chemical composition

A capillary column with thin films was used for the GC analyses. It was found that the column was able to separate all classes of extractives and individual compounds. Identification of the components was based on a comparison of their retention times or mass spectra with authentic compounds by mass fragmentography (5970 Series, Hewlett Packard) (data not shown) or with library spectra. Equal amounts of the standards gave peak areas in ratios of 1.00:0.88:0.86:0.93:0.85:0.74:1.00:0.78:0.60 for fatty acid, azelaic acid/maleic acid, resin acid, sterol, wax, steryl ester, monoglyceride, diglyceride, and triglyceride, respectively. The ratios were reproducible and were not notably affected by small variations in injection, detector temperature, injection volume, or carrier gas flow. The use of these nine appropriate standards compensates for some of the discrimination possibly occurring with GC analysis and provides a control measure within each chromatogram. The FID response was assumed to be the same for the component group and the corresponding standard. In other words, the individual peak area of each fatty acid, azelaic acid or maleic acid, resin acid, sterol, wax, steryl ester, monoglyceride, diglyceride, and triglyceride in samples was divided by 1.00, 0.88, 0.86, 0.93, 0.85, 0.74, 1.00, 0.78, and 0.60, respectively, before calculating their concentrations in the samples.

The chromatogram of the total lipophilic extract, obtained by extraction with MTBE from wheat straw, is shown in Fig. 1, and the compositions of all the compounds

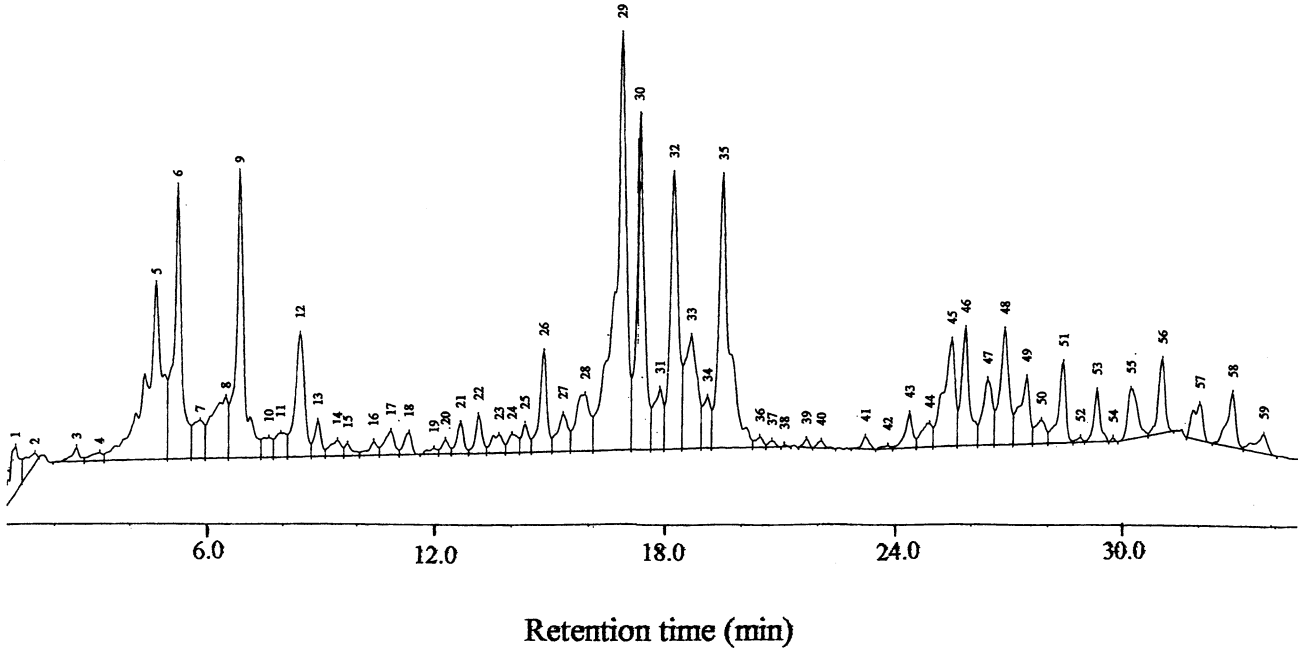

Fig. 1. Chromatogram of lipophilic extractives obtained by a Soxhlet extraction with methyl *tert*-butyl ether from wheat straw

identified quantitatively are listed in Table 1. Obviously, the extractives in wheat straw comprise a large number of components. Free fatty acids (11.6%–26.6%), sterols (15.5%–24.5%), waxes (15.8%–21.1%), sterol esters (11.3%–19.1%), and triglycerides (9.7%–14.2%) were the major lipid groups present in the extracts obtained by MTBE, hexane, petroleum ether, and dichloromethane, respectively. Although extractions with toluene-ethanol (2:1, v/v) or chloroform-methanol (2:1, v/v) gave extracts composed largely of free fatty acids (28.2%–32.3%) and azelaic acid (15.2%–16.2%), the major class of lipid in the boiling water extract was free fatty acids, comprising more than 50% of the total extractives identified.

The GC results showed a relatively high concentration of free fatty acids in all the lipophilic extractives from wheat straw. Their distributions were identified in the range from C10 to C24. Myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), linoleic acid (C18:2), and oleic acid (C18:1) were the major components; stearic acid (C18:0), capric acid (C10:0), lauric acid (C12:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), gondoic acid (C20:1), arachidic acid (C20:0), heneicosanoic acid (C21:0), behenic acid (C22:0), and lignoceric acid (C24:0) were detected in small amounts. The results were generally consistent with the data obtained from the lipophilic extractives in *Eucalyptus globulus* wood by Gutierrez et al.[3] They reported that free fatty acids accounted for 27.69 mg/100 g wood and occurred in the range from C14 to C26, the dominant component being palmitic acid (C16:0) followed by linoleic (C18:2), oleic (C18:1), and stearic (C18:0) acids.

Seven resin acids (pimaric, sandaracopimaric, isopimaric, palustric, dehydroabietic, abietic, and neoabietic acids) have been reported from slash pine wood (*Pinus elliottii* engelm) based on comparison of their GC retention times with those of model compounds and by GC-MS spectra.[12] The present findings showed that abietic acid was the only resin acid detected in the extractives and occurred in extremely low concentration in wheat straw. The relatively larger amount of fatty acids than resin acids is probably because the resin acids are transformed by oxidation into compounds containing additional oxygen functions and higher molar mass compounds that are not analyzed as resin acids by the techniques used.[10] Furthermore, thermal isomerization reactions of resin acids such as neoabietic, levopimaric, and palustric acids during the process of Soxhlet extraction may lead to the formation of abietic acid as the preferred end-product.[13]

It is interesting to note that the extracts contained 1.2%–16.2% azelaic acid and 1.4%–7.1% maleic acid. They were particularly high in the extracts obtained with toluene-ethanol (F1) or chloroform-methanol (F2), which yielded 15.2%–16.2% azelaic acid and 4.7%–7.1% maleic acid. Based on the study of active compounds in wheat straw extracts with allelopathic activity by high-resolution GC-MS (HRGC-MS) and HRGC-Fourier transform infrared spectrometry (HRGC-FTIR), Chaves das Neves and Gaspar[7] reported that azelaic acid was the dominant component in the wheat straw extractives, and maleic acid appeared in a noticeable amount.

Sterols were the second most important class of compounds in wheat straw extractives, accounting for 15.5%, 24.0%, 24.5%, and 17.8% in F3, F4, F5, and F6 extracts, respectively. β-Sitosterol was the main sterol in the extracts. Cholesterol, ergosterol, stigmasterol, and stigmastanol were also identified from the extracts but in small amounts. Gutierrez et al.[3] reported a similar observation for lipophilic extractives from *Eucalyptus globulus* labill. wood.

Waxes were another major class of lipids in wheat straw extractives, comprising 15.8%–21.1% of the total F3–F6 extracts. However, the extracts obtained with toluene-ethanol (F1) or chloroform-methanol (F2) gave only 1.4% and 2.6% of waxes, respectively. Palmityl palmitate and oleyl palmitate were found to be the major compounds and accounted for 62.4%–68.4% of the total waxes in the above four extracts (F3–F6) as revealed by GC. Oleyl oleate was identified in trace amounts (0.1%–0.2%) in all the extracts.

Steryl esters were also important constituents of the lipophilic extractives in wheat straw, accounting for 6.8%–19.1% of the extracts. During characterization of the composition of steryl esters, it was impossible to identify individual sterol esters by GC-MS, as they only show fragments arising from the sterol moiety by electron-impact MS and rarely give detectable molecular ions.[3,14] A similar phenomenon was observed in the GC-MS analyses for triglycerides. In this study, these two classes of lipids were therefore identified only by comparing retention times with authentic compounds of GC. The utilization of a high-temperature capillary column made it possible to increase the final temperature to 340°C, which is necessary for separation of steryl esters and triglycerides over a 15 m capillary column over a short period of time (34 min). Although equal amounts of steryl esters and triglycerides were eluted with small peak areas compared to the equal amounts of free fatty acids, sterols, and waxes, their separation was satisfactory in the 15 m GC column. To correct the analyses, the individual peak area of steryl esters and triglycerides was divided by 0.74 and 0.60, respectively, to calculate their concentrations. Furthermore, the major wood and straw steryl esters, β-sitosterol esters, were not available commercially and were replaced by closely related compounds (cholesteryl esters) as standards in this study.[8,15] It was possible to identify a series of steryl esters by comparing the GC retention times of the steryl esters in extracts with cholesteryl ester standards. The steryl esters consisted of combinations of the major fatty acids (lauric, myristic, palmitic, heptadecanoic, and oleic acids) with sterols (mainly β-sitosterol).[8] The distribution of esterified fatty acids was the same as that of the free fatty acids, as described above. Steryl laurate, steryl myristate, steryl palmitate, steryl heptadecanoate, and steryl oleate together comprised 62.5%–81.6% of the total steryl esters in seven lipophilic extractives.

Finally, the triglycerides identified among the straw extractives accounted for 8.9%–14.2% in F2–F6 fractions and only 1.7% and 3.5% in toluene-ethanol and water-soluble extracts, respectively. These triacylglycerols are commonly produced as energy reserves and carbon skeletons for growth and development, as they are twice as efficient for

metabolic energy than either proteins or carbohydrates.[16] However, in this study only approximately half of the triglycerides were identified, which were mainly comprised of tripalmitin, 1,2-dipalmitoyl-3-oleoylglycerol, and triolein (*cis*-9). Further work is in progress to study the composition of the triglycerides.

Diacylglycerols are present in trace amounts in fresh animal and plant tissues and may be intermediates in the synthesis of triacylglycerols.[16] In the present study, diglycerides were also identified among the lipophilic extractives from wheat straw, although in small amounts (0.2%–0.8%). Dipalmitoyl glycerol was the only single compound identified. Monoglyceride was not detected in any of the extracts in this study.

Acknowledgments We are grateful for the financial support of this research from the LINK program (fractionation of wheat straw) of the U.K. Ministry of Agriculture, Fisheries, and Food, and the prominent young scientist program of National Natural Science Foundation of China (no. 30025036). We also thank Mrs. Jianmin Fang for her help with extraction of the samples.

References

1. Sithole BB (1992) Modern methods for the analysis of extractives from wood and pulp: a review. Appita 45:260–264
2. Hardell HL, Nilvebrant NO (1999) A rapid method to discrimination between free and esterified fatty acids by pyrolytic methylation using tetramethylammonium acetate or hydroxide. J Anal Appl Pyrolysis 52:1–14
3. Gutierrez A, del Ril JC, Gonzalez-Vila FJ, Martin F (1999) Chemical composition of lipophilic extractives from *Eucalyptus globulus* labill. wood. Holzforschung 53:481–486
4. Gutierrez A, del Ril JC, Gonzalez-Vila FJ, Martin F (1998) Analysis of lipophilic extractives from wood and pith deposits by solid-phase extraction and gas chromatograph. J Chromatogr A 823:449–455
5. Hillis WE (1962) Wood extractives and their significance to the pulp and paper industry. Academic Press, San Diego
6. Rowe JW (1989) Natural products of woody plants II. Springer-Verlag, Berlin
7. Chaves das Neves HJ, Gaspar EMM (1990) Identification of active compounds in wheat straw extractives with allelopathic activity by HRGC-MS and HRGC-FTIR. J High Resol Chromatogr 13:550–554
8. Chaves das Neves HJ, Gaspar EMM (1995) HRGC-MS and HPLC-MS identification of new ketosterols in an extract of wheat straw. J High Resol Chromatogr 18:299–303
9. Orsa F, Holmbom B (1994) A convenient method for the determination of wood extractives in papermaking process waters and effluents. J Pulp Pap Sci 20(12):J361–J366.
10. Wallis AFA, Wearne RH (1997) Characterization of resin in radiata pine woods, bisulfite pulps and mill pith samples. Appita 50:409–414
11. Orsa F, Holmbom B, Thornton J, Ekman R (1993) Dissolution and dispersion of spruce wood components into water. In: Seventh international symposium on wood and pulping chemistry proceedings, Beijing, vol 3, pp 383–388
12. Quinde A, Paszner L (1991) Isomerization of slash pine (*Pinus elliottii* engelm) resin and fatty acids during seasoning. Appita 44:379–384
13. Quinde A, Paszner L (1992) Behavior of the major resin and fatty acids of slash pine (*Pinus elliottii* engelm) during organosolv pulping. Holzforschung 46:513–522
14. Evershed RP, Prescott MC, Spooner N, Goad LJ (1989) Negative ion ammonia chemical ionization and electron impact ionization mass spectrometric analysis of steryl fatty acyl esters. Steroids 53:285–309
15. Chen T, Breuil C, Carriere S, Hatton JV (1994) Solid-phase extraction can rapidly separate lipid classes from acetone extracts of wood and pulp. TAPPI J 77:235–240
16. Cooper LLD, Oliver JE, Vilbiss EDD, Doss RP (2000) Lipid composition of the extracellular matrix of *Botrytis cinerea* germlings. Phytochemistry 53:293–298