

# COMPARATIVE STUDY OF METHYL-TERT-BUTYL ETHER EXTRACTIVES FROM RYE AND RICE STRAW

*Xiao Feng Sun*

Postgraduate Student  
Centre for Straw Utilization  
The North-Western University of Agricultural and Forest Sciences and Technology  
Yangling, People's Republic of China

and

*RunCang Sun*

Professor  
State Key Laboratory of Pulp and Paper Engineering  
College of Pulp and Paper Sciences and Environmental Engineering, South China University of Technology  
Guangzhou, People's Republic of China

(Received June 2000)

## ABSTRACT

The chemical composition of lipophilic extractives from rye and rice straws has been comparatively examined. Free fatty acids (19.04–22.95%), sterols (12.54–14.60%), waxes (9.53–27.14%), steryl esters (16.02–18.19%), and triglycerides (5.72–11.38%) were identified as the five major classes of lipids in the two straw extractives. Minor components of diglycerides (0.23–0.42%) and resin acids (0.05–0.12%) were also verified from the two straw lipophilic extracts. Of the individual compounds in each group, fifteen free fatty acids, four sterols, three waxes, five steryl esters, and three triglycerides were quantitatively determined. The most abundant saturated free fatty acids were palmitic acid (C16:0, 3.96–4.24%) and tetradecanoic acid (C14:0, 2.95–3.62%), whereas linoleic (C18:2) and/or oleic (C18:1) acids (1.87–2.09%) were the most dominant unsaturated free fatty acids.  $\beta$ -Sitosterol was identified as a predominant component, accounting for 83.89% of the total sterols in rye straw extract and 94.45% in rice straw extractives. Palmitic acid palmityl ester was verified as a dominant component in a group of waxes, accounting for approximately 70% of the waxes analyzed in the two extracts. The steryl esters analyzed were composed mainly of steryl laurate (0.29–0.95%), steryl myristate (3.20–3.56%), steryl palmitate (1.86–2.28%), steryl margarate (2.20–2.93%), and steryl oleate (2.13%). Of the triglycerides verified, glyceryl tripalmitate (0.23–1.64%), 1,2-dipalmitoyl-3-oleoyl-glycerol (1.06–2.08%), and triolein (*cis*-9) (0.77–1.61%) were identified in this group.

**Keywords:** Rye straw, rice straw, methyl tert-butyl ether, lipophilic extractives, phenolics.

## INTRODUCTION

Lipophilic extractives from wood are composed of a large number of different compounds. These compounds may conveniently be grouped into six main classes: free fatty acids, resin acids, sterols, waxes, steryl esters, and triglycerides (Suckling et al. 1990). Some non-polar extractives such as alcohols and terpenes also appear in noticeable amounts (Sithole et al. 1992). Smaller quantities of a variety of phenolic compounds may be also found in the extracts, particularly those from heartwood

(Suckling et al. 1990). The composition and amount of the extractives are dependent on the wood species, tree age, and environmental conditions (Chen et al. 1994). Considerable amounts of the wood extractives can be dispersed or dissolved during mechanical fibrillation and mechanical pulp bleaching operations (Ekman and Holmbom 1989). However, these dispersed extractives are difficult to remove, and they cause pitch problems. During kraft pulping, the triglycerides are completely saponified, and the fatty and resin acids dissolve as soluble soaps. However, sterols and

some sterol esters and waxes do not form soluble soap under the alkaline conditions, and therefore have a tendency to deposit and cause pitch problems (Gutierrez et al. 1999). The accumulation of these extractives can cause significant technical and economic problems for pulp and paper manufacturers (Gutierrez et al. 1998).

Efforts to find a solution to this problem begin with developing a rapid, simple, and quantitative method for separating and identifying the complex lipid extractives in wood samples. Such a method would be useful in understanding pitch formation and optimizing reactions to remove the extractives prior to pulping. Classical methods for quantitatively analyzing extractives require many steps including solvent extraction, saponification, separation of the acid and neutral fractions, and analysis of the derivatized acids by gas chromatography (GC). This technique can separate the lipid complex into resin acids, free and combined fatty acids, and non-saponifiables (Sithole 1992; Orsa and Holmbom 1994; Wallis and Wearne 1997). A number of other techniques such as thin layer chromatography (Suckling et al. 1990), high performance liquid chromatography (HPLC) (Richardson et al. 1992), high performance size exclusion chromatography (Sjostrom and Holmbom 1987), and  $^{13}\text{C}$  nuclear magnetic resonance ( $^{13}\text{C}$  NMR) spectroscopic analysis (Suckling and Ede 1990; Gunstone 1993; Medina et al. 1994), for analysis of extractives in wood samples have been developed. However, most methods are too laborious and some are too expensive for analysis of a large number of samples. On the other hand, during the past ten years, with the use of modern gas chromatographs with short capillary columns, it has become possible to analyze free fatty and resin acids, sterols, waxes, steryl esters, and triglycerides in a single chromatographic run (Quinde and Paszner 1992; Chen et al. 1994; Orsa and Holmbom 1994; Wallis and Wearne 1997; Gutierrez et al. 1998, 1999).

Rye and rice straws are major agricultural residues and are currently used as raw mate-

rials for papermaking in developing countries such as China and India. More than 9 million tons of straw pulp are produced annually in China, which accounts for about 90% of the world's total straw pulp (Atchison 1996). However, these two plants and all Gramineae are covered on both culm and leaf with semi-crystalline wax particles 1–2  $\mu\text{m}$  across and 2–3  $\mu\text{m}$  high. These waxes are mainly long-chain alkane, ester and alcohol waxes together with noticeable amounts of free fatty acids (Juniper 1979). As we attempt to resolve the pitch problems in soda-anthraquinone pulping and to reduce the impact on the environment from the effluents in the paper industry, the development of effective technologies for investigation of lipophilic extractives from straw is considered to be both important and significant. Furthermore, even though the content of waxes in the straw crop comprises only 1.0–2.0% of the dry weight, this still constitutes 15–30 million tons of valuable by-products of waxes each year in the world. It might be also worth looking at this lipophilic material as a potential supply source for many industrial processes.

In the present study, the lipophilic extractives in rye and rice straws were analyzed by a rapid method that enables convenient quantitative determination of individual components in all six classes of free fatty acids, resin acids, sterols, waxes, steryl esters, and triglycerides. The method includes solvent extraction, silylation, and gas chromatography using a medium-length, high temperature capillary column with a thin film. Minor amounts of phenolic compounds in the extractives were quantitatively determined by HPLC at 280 nm.

## MATERIALS AND METHODS

### *Materials and reagents*

Rye and rice straws were kindly supplied by the experimental farm of The North-Western University of Agricultural and Forest Sciences and Technology (Yangling, P. R. China). They were dried in sunlight and then cut into

small pieces. The cut straw was ground to pass a 1.2-mm-size screen. The ground straws were further dried in a cabinet oven with air circulation for 16 h at 60°C and then stored at 5°C until used. The yield of extractives was measured on an oven-dried basis. All standard compounds used were purchased from Sigma (Xian). Analytical grade methyl-tert-butyl ether (MTBE) was used. The fatty acids mentioned below are abbreviated with a code indicating the number of carbon atoms:number of double bonds. For example, C18:1 indicates *cis*-9-octadecenoic acid (oleic acid).

#### *Extraction and silylation*

Dried rye and rice straw samples (~50 g) were extracted with 1,800 ml of methyl tert-butyl ether (MTBE) for 12 h in a Soxhlet. After solvent removal with a rotary vacuum evaporator at 35°C, the residue was dried in nitrogen steam and then weighed to determine the yield of extractives. Note that the extract obtained from rye straw was labeled for fraction 1 (F1) only, and that obtained from rice straw was considered to be extract fraction 2 (F2). The dried lipophilic extracts (~3 mg) were then silylated with 120  $\mu$ l bis-trimethylsilyl-trifluoroacetamide and 60  $\mu$ l trimethylchlorosilane. The reactions were completed by keeping the glass-stoppered test tubes in an oven at 75°C for 20 min. When cooled, 180  $\mu$ l toluene was added. The solution was shaken and was then ready for analysis by GC.

#### *GC analysis*

The silylated sample was analyzed by GC on an Rtx-1 capillary column coated with crosslinked 100% dimethyl polysiloxane (15 m, 0.53 mm i.d., 0.10  $\mu$ m film thickness, purchased from Hewlett-Packard Company, Beijing) with a flame ionization detector (FID). Helium was used as the carrier gas. The injector and detector temperatures were held at 340°C. The oven was temperature-programmed from 70°C to 340°C (2 min) at 8°C/min. A sample of solution (1  $\mu$ l) was injected splitless mode and changed to split mode after

0.5 min. The total analysis time including cooling of the column oven and injector, followed by temperature stabilization, was about 45 min.

Each compound of free fatty and resin acids and sterols was identified both by comparison of their gas chromatographic retention times and by total ion detection mass spectra with those in authentic compounds. Individual components of the waxes, steryl esters, and triglycerides were verified only by GC retention times since GC-MS gave only fragments by electron-impact MS and rarely gave detectable molecular ions (Gutierrez et al. 1999). On the other hand, previous studies found that the major components of steryl esters in the wood and wheat straw were  $\beta$ -sitosterol esters (Chen et al. 1994; Chaves das Neves and Gaspar 1995). 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 standard compounds (palmitic acid, abietic acid,  $\beta$ -sitosterol, palmitic acid palmitic ester, cholesteryl palmitate, 1-monopalmitoyl-rac-glycerol, 1,2-dipalmitoyl-sn-glycerol, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol) was used to calibrate for the quantification of all the free fatty acids, resin acids, sterols, waxes, steryl esters, monoglycerides, diglycerides, and triglycerides, respectively. The FID response was assumed to be same for the component group corresponding to each class of standards used.

#### *HPLC analysis*

Determination of minor amounts of phenolic compounds in the extractives was performed on a Hichrom H50DS HPLC silica-based column with dimensions of 250  $\times$  4.6 mm (purchased from Phenomenex Co., Beijing). Samples (~10 mg) were dissolved in 1 ml methanol and separations 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. Note that 2,2'-dihydroxybiphenyl was used as a standard compound to calibrate the content of lignans (phenyl propane dimers) in the extractives.

## RESULTS AND DISCUSSION

### *Yield and purity*

Traditional methods for the extraction of extractives from wood and pulp samples use organic solvents such as acetone, ethanol, ether, or dichloromethane in a Soxhlet extractor (Sithole et al. 1991). The results showed that the mass of substances extracted from fresh wood samples decreased as the polarity of solvents decreased, in the order methanol > acetone > dichloromethane > hexane. However, the ratios of individual components in the lipophilic extractives did not vary substantially using different extraction solvents (Wallis and Wearne 1997). Interestingly, based on an extensive study of wood extractives in papermaking process waters and effluents, Orsa and Holmbom (1994) found that MTBE is an effective solvent for extraction of fatty and resin acids in pulp mill effluents. After preliminary experiments with several solvents, we also chose MTBE as a solvent for extraction of lipophilic extractives from rye and rice straw samples because it not only gave a high purity of lipophilic extractives but also extracted the total lipophilic substances to a high degree. Extraction with 2/3 toluene-1/3 ethanol released high amounts of total extractives but gave low lipophilic purity (~50%) of the extract, which contained substantial amounts of non-lipid components such as low-molecular-weight polysaccharides, ash or salts (data not shown). Results in Table 1 showed that extraction of rye and rice straws with MTBE yielded 1.01% and 0.97% extractives, which contained 84.65% and 73.28% lipophilic substances determined by GC analysis, respectively. This suggested again that MTBE is a

good solvent, giving extracts predominately of lipophilic extractives.

### *Chemical composition*

The analysis by GC using a medium-length capillary column resolved almost all of the individual compounds in the two lipophilic extractives, although a few of them co-eluted, such as linoleic acid (C18:2) and oleic acid (C18:1). Each compound in five major classes of lipids (free fatty acids, sterols, waxes, sterol esters, and triglycerides) was clearly differentiated as shown by a typical chromatogram (Fig. 1) for five authentic compounds (palmitic acid,  $\beta$ -sitosterol, palmitic acid palmitic ester, cholesteryl heptadecanolate, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol). Fifty-six compounds in the MTBE extract of rye straw were resolved by GC under the condition given, as illustrated by the chromatogram in Fig. 2. The designation of the peak numbers is given in Table 1. The peaks representing individual components were identified with the authentic compounds and quantitated against a standard mixture of equal amounts of palmitic acid, abietic acid,  $\beta$ -sitosterol, palmitic 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 1.0:0.86:0.92:0.85:0.74:0.96:0.79:0.61. This eight standard mixture was used to calibrate the peak areas of free fatty acids, resin acids, sterols, waxes, sterol esters, monoglycerides, diglycerides, and triglycerides, respectively. Interestingly, no significant differences in the peak areas were found between the equal amounts of authentic compounds in the same group of the lipids. However, there appeared noticeable variations in peak areas between the different classes of lipids. Thus, the peak areas of free fatty acids in the extractives were quantitated against palmitic acid by division of 1.0 before calculation of their concentrations, and resin acids, sterols, waxes, sterol esters, monoglycerides, diglycerides, and triglycerides were quantitated against abietic acid,  $\beta$ -sitosterol,

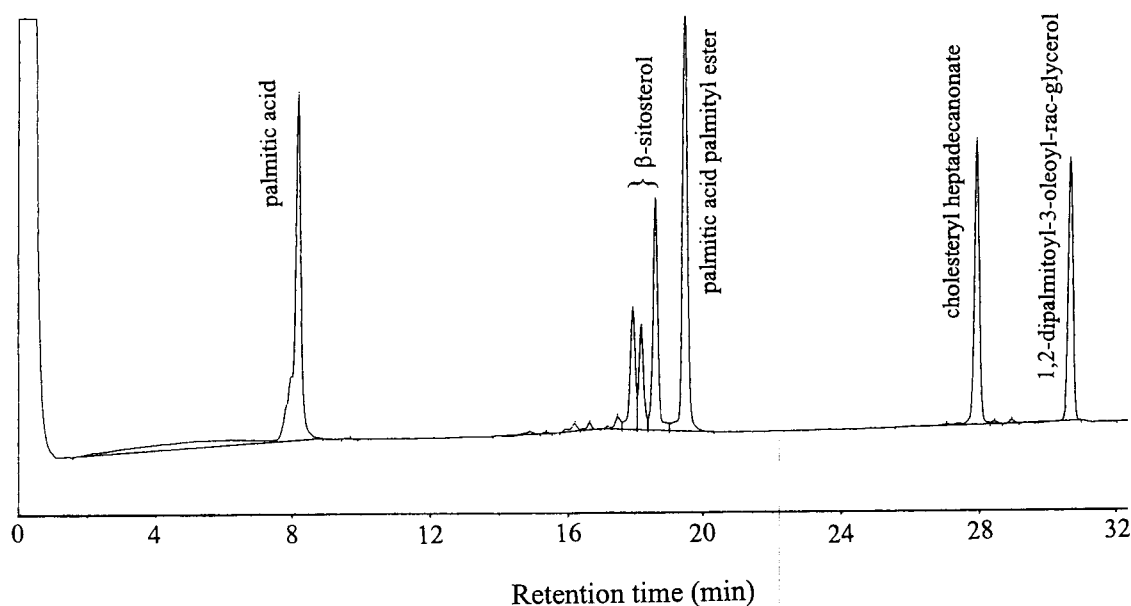


FIG. 1. Typical chromatogram for a standard mixture.

palmitic acid, palmitate, cholesteryl palmitate, 1-monopalmitoyl-rac-glycerol, 1,2-dipalmitoyl-sn-glycerol, and 1,2-dipalmitoyl-3-oleoyl-rac-glycerol by division of 0.86, 0.92, 0.85, 0.74, 0.96, 0.79, and 0.61, respectively, prior to calculation of their concentrations.

Obviously, as Table 1 shows, waxes (27.14%), free fatty acids (22.95%), sterol esters (16.02%), and sterols (12.54%) were dominant in rye straw extractives, whereas triglycerides (5.72%) were present in small quantities in the fraction. On the other hand, the rice

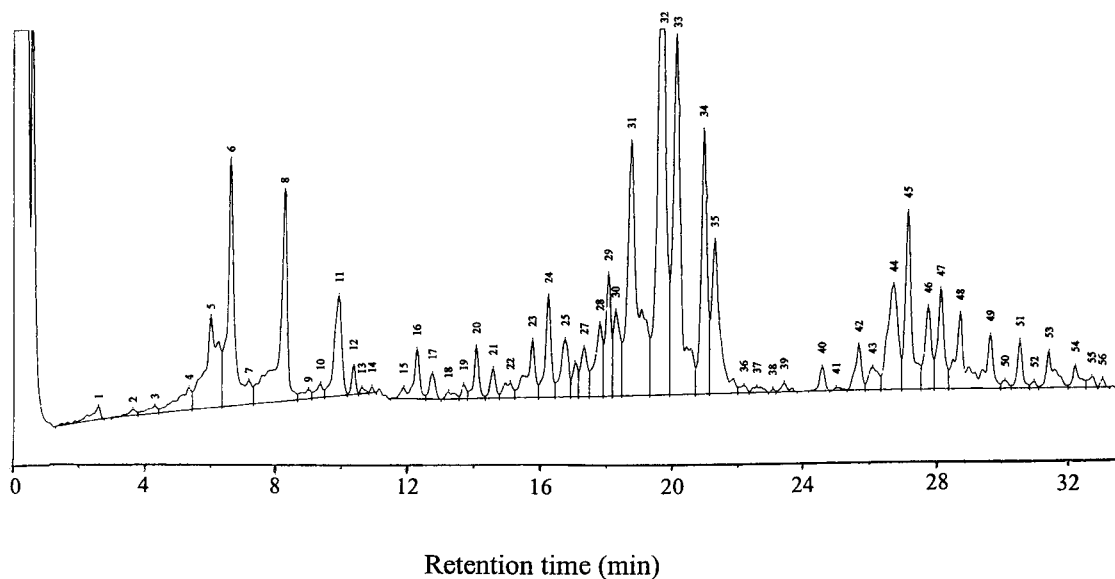


FIG. 2. The chromatogram of methyl tert-butyl ether extractives (F1) from rye straw.

TABLE 1. The yield (% dry straw) and chemical composition (% dry extractives) of extractives in rye and rice straws.

Yield/composition	F1 <sup>a</sup>	F2 <sup>a</sup>	F1	F2
Yield	1.01	0.97		
Chemical composition			Continued for chemical composition	
<b>Free fatty/resin acids (P 1–26)<sup>b</sup></b>	23.00	19.16	<b>Waxes (P 32–37)</b>	27.14
Compounds analysed	13.57	14.84	Compounds analysed	15.06
Decanoic acid, C10:0 (P 1)	0.37	0.67	Palmitic acid palmitate (P 32)	10.51
Dodecanoic acid, C12:0 (P 3)	0.21	0.16	Palmitic acid oleyl ester (P 34)	4.39
Tetradecanoic acid, C14:0 (P 5)	2.95	3.62	Oleic acid oleyl ester (P 36)	0.16
Pentadecanoic acid, C15:0 (P 7)	0.39	0.57		
Hexadecanoic acid, C16:1	N	0.42	<b>Diglycerides (P38–39)</b>	0.23
Hexadecanoic acid, C16:0 (P 8)	4.24	3.96	Compounds analyzed	0.05
Heptadecanoic acid, C17:0 (P 10)	0.25	0.37	Dipalmitin (P 38)	0.05
Linoleic acid (C18:2) + oleic acid (C18:1)(P 11)	1.87	2.09		
Octadecanoic acid, C18:0 (P 12)	0.30	0.42	<b>Sterol esters (P 40–48)</b>	16.02
Abietic acid (P 14)	0.05	0.12	Compounds analyzed	10.70
Gondoic acid, C20:1 (P 15)	0.18	0.21	Steryl laurate (P 42)	0.95
Eicosanoic acid, C20:0 (P 16)	0.70	0.60	Steryl myristate (P 44)	3.56
Heneicosanoic acid, C21:0 (P 18)	0.20	0.18	Steryl palmitate (P 46)	1.86
Docosanoic acid, C22:0 (P 20)	0.66	0.47	Steryl heptadecanoate (P 47)	2.20
Tetracosanoic acid, C24:0 (P 23)	1.20	0.98	Steryl oleate (P 48)	2.13
<b>Sterols (P 27–31)</b>	12.54	14.60	<b>Triglycerides (P 49–56)</b>	5.72
Compounds analyzed	12.54	14.60	Compounds analyzed	2.06
Cholesterol (P 27)	0.84	0.32	Tripalmitin (P 50)	0.23
Ergosterol (P 28)	1.18	0.49	Dipalmitoyl-oleoylglycerol (P 51)	1.06
Stigmasterol (P 30)	N <sup>c</sup>	T <sup>d</sup>		
β-Sitosterol (P 29–31)	10.52	13.79	Triolein (P 54)	0.77
			<b>Total</b>	<b>84.65</b>
				<b>73.28</b>

<sup>a</sup> Represent fractions of the extractives obtained by extraction with methyl-tert-butyl ether from rye straw (F1) and rice straw (F2) for 12 h in a Soxhlet, respectively.

<sup>b</sup> Represent peak numbers in Fig. 1.

<sup>c</sup> N, not detectable.

<sup>d</sup> T, trace.

straw extract was enriched in all of the five classes of lipids as shown by free fatty acids (19.04%), steryl esters (18.19%), sterols (14.60%), triglycerides (11.38%), and waxes (9.53%). Diglycerides were detected in minor amounts (0.23% in F1, 0.42% in F2). No monoglycerides were verified in the extracts. Similar trace amounts of diglycerides were also previously identified from wood extractives (Gutierrez et al. 1999; Cooper et al. 2000).

Free fatty acids were the most dominant group of lipids in rice straw extract and the second most abundant class of lipophilic substances in rye straw extractives. As shown in Table 1, fifteen free fatty acids were verified in the extracts based on comparison of their

retention times and mass spectra with authentic compounds. Palmitic acid (C16:0, 3.96–4.24%) and tetradecanoic acid (C14:0, 2.95–3.62%) were the major saturated free fatty acids, which together comprised 34.40% and 46.45% of the total saturated free fatty acids in rye straw extract and rice straw extractives, respectively, whereas linoleic (C18:2) and/or oleic (C18:1) acids (1.87–2.09%) were the dominant unsaturated free fatty acids, which together accounted for over 90% of the total unsaturated free fatty acids in rye straw extract and more than 75% in rice straw extractives, respectively. Tetracosanoic acid (C24:0, 0.98–1.20%), eicosanoic acid (C20:0, 0.60–0.70%), docosanoic acid (C22:0, 0.47–0.66%), decanoic acid (C10:0, 0.37–0.67%), pentadecanoic

acid (C15:0, 0.39–0.57%), octadecanoic acid (C18:0, 0.30–0.42%), heptadecanoic acid (C17:0, 0.25–0.37%), gondoic acid (C20:1, 0.18–0.21%), heneicosanoic acid (C21:0, 0.18–0.20%), and dodecanoic acid (C12:0, 0.16–0.21%) were detected with small amounts in the two extractives. Hexadecenoic acid (C16:1) was absent in rye straw extract, while it occurred in a minor quantity in rice straw extract (0.42%). The results obtained coincided with the studies of free fatty acid composition from *Eucalyptus globulus* Labill. wood extractives, in which palmitic acid (C16:0), linoleic (C18:2), and oleic (C18:1) were dominant (Gutierrez et al. 1999). In addition, trace amounts of abietic acid (0.05–0.12%) were identified as the only resin acid in the two extracts. It is probable that a portion of resin acids such as neoabietic and palustric acids isomerized into a more 'thermostable' form like abietic acid during the Soxhlet extractions (Quinde and Paszner 1992) or only a few types of resin acids such as abietic acid are present in two straws.

Peaks 27–31 in Fig. 2 were identified as sterols, another important group of lipids in the extractives. Clearly,  $\beta$ -sitosterol was verified as a predominant component, accounting for 83.89% and 94.45% of the total sterols in rye straw extract and rice straw extractives, respectively. Ergosterol (1.18% in F1, 0.49% in F2) and cholesterol (0.84% in F1, 0.32% in F2) were also identified in this group, but in minor amounts. Stigmasterol was not detected in rye straw extract, while it occurred in trace quantities in rice straw extractives. Similar results have been reported from *Eucalyptus globulus* Labill. wood by Gutierrez et al. (1999) and wheat straw by Chaves das Neves and Gaspar (1995), who stated that sterols were the major compounds in the wood and wheat straw extractives, in which  $\beta$ -sitosterol was the main sterol present in this group.

Waxes were the most abundant group of lipids in rye straw extract, comprising 27.14% of the total extract. They were also the important class of the lipophilic extractives in rice straw extractives, accounting for 9.53% of the total

extractives. Based on comparison of their GC retention times with the authentic compounds, palmitic acid palmityl ester was identified as a dominant component in wax class, accounting for approximately 70% of the total waxes analyzed in the two extracts. Noticeable amounts of palmitic acid oleyl ester (4.39% in F1 and 0.96% in F2) and minor quantities of oleic acid oleyl ester (0.16–0.34%) were also identified from the two extractives.

Steryl esters (16.02–18.19%) were the third most dominant group of lipids in rye and rice straw lipophilic extractives as shown in Table 1. Obviously, the use of a medium-length, high-temperature capillary column made it possible to separate the individual components in groups of waxes, steryl esters, and triglycerides from the straw extractives, although equal amounts of waxes, steryl esters, and triglycerides gave only 85, 74, and 61% peak areas as compared to the equal quantity of the free fatty acids. In this study, the individual compounds were, therefore, 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. It was found that the steryl esters consisted of combinations of the major fatty acids (Lauric, myristic, palmitic, margaric, and oleic acids) with sterols mainly  $\beta$ -sitosterol (Chaves das Neves and Gaspar 1995). Five components of steryl laurate, steryl myristate, steryl palmitate, steryl margarate, and steryl oleate were detected from the two lipophilic extractives, which together amounted to 66.79 and 59.54% of the total steryl esters in the rye straw extract and rice straw extractives, respectively. Obviously, it can be concluded that the distribution of esterified fatty acids is the same as that of the free fatty acids as described above.

Finally, other lipophilic components verified from the two straw extractives were triglycerides, together with minor amounts of diglycerides. As shown in Fig. 2 and Table 1,

triglycerides consisted of eight individual components and accounted for 5.72 and 11.38% of the total rye and rice straw extractives, respectively. Glycerol tripalmitate, 1,2-dipalmitoyl-3-oleoyl-rac-glycerol, and triolein (*cis*-9) were identified in this group, which together comprised 36.01% of the total triglycerides in F1 and 46.84% in F2. Diglycerides were identified in minor amounts, ranging from 0.23% in rye straw extract and 0.42% in rice straw extractives. 1,2-Dipalmitoyl-rac-glycerol was the only compound identified and accounted for trace amounts in F1 (0.05%) and minor quantities in F2 (0.22%). No monoglycerides were detected in the two extractives of rye and rice straws. Similar trace amounts of diglycerides have been also reported from fresh animal and other plant tissues (Cooper et al. 2000).

It is well known that phenolic substances are widely distributed in plants. For example, cinnamic acids have been found in various combined forms, such as with glycerides and polysaccharides in the cell walls (Salomonsson et al. 1978; Chaves das Neves and Gaspar 1990). In the present study, minor amounts of phenolic compounds released in the two extracts were determined by HPLC at 280 nm and the results are given in Table 2. As can be seen from Table 2, rye straw extract had three to four times more phenolic compounds than that of rice straw extractive, although they accounted for very small amounts, 0.37% in F1 and 0.10% in F2. This implied that the phenolics in the cell walls of rye straw were more easily soluble than those from the rice straw cell walls during the organic solvent extraction in a Soxhlet extractor. 1-Naphthoic acid, *p*-coumaric acid, lignan, vanillic acid, protocatechuic acid, vanillin, ferulic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, syringaldehyde, benzoic acid, and syringic acid were determined as the major phenolic compounds identified, whereas acetovanillone, acetosyringone, cinnamic acid, *m*-toluic acid, and fumaric acid were detected in trace amounts in the rye straw extract.

TABLE 2. The content (% dry extractives, w/w) of phenolic acids and aldehydes, lignan, and other compounds identified in the rye and rice straw extractives by HPLC.

Phenolic acids and aldehydes, lignan, and other compounds	F1 <sup>a</sup>	F2 <sup>a</sup>
Protocatechuic acid	0.022	0.011
<i>p</i> -Hydroxybenzoic acid	0.018	0.008
<i>p</i> -Hydroxybenzaldehyde	0.011	0.006
Vanillic acid	0.023	0.007
Syringic acid	0.010	0.003
Vanillin	0.020	0.006
Syringaldehyde	0.017	0.004
Acetovanillone	0.008	0.002
Acetosyringone	0.005	0.001
<i>p</i> -Coumaric acid	0.043	0.011
Ferulic acid	0.018	0.006
Sinapic acid	0.016	0.002
Cinnamic acid	0.003	0.001
Fumaric acid	0.001	N
Benzoic acid	0.021	0.003
<i>m</i> -Toluic acid	0.003	0.001
1-Naphthoic acid	0.33	0.008
Lignan	0.040	0.015
Total (%)	0.57	0.10

<sup>a</sup> Corresponding to the extractive fractions in Table 1; N, not detectable.

#### CONCLUSIONS

Overall, the results obtained indicated that waxes (27.14%) and free fatty acids (22.95%) were the two most dominant classes of lipids in the rye straw extract. Steryl esters (16.02%) and sterols (12.54%) were also two important groups of lipids, and triglycerides (5.72%) occurred in small amounts in the lipophilic extractives from rye straw. The MTBE extractives of rice straw were composed mainly of free fatty acids (19.04%), steryl esters (18.19%), sterols (14.60%), triglycerides (11.38%), and waxes (9.53%). Diglycerides (0.23% in F1, 0.42% in F2) accounted for minor amounts in rye and rice straw extractives. Abietic acid was the only component identified in a group of resin acids, and appeared in minor amounts (0.12%) in rice straw extract and trace quantities (0.05%) in rye straw extractives. The non-lipid substances in the two extractives consisted mainly of noticeable amounts of ash or salts (16.8% in F1 and ~25% in F2), together with minor amounts of phenolic substances and co-extracted polysac-



charides. It was found that MTBE was an effective solvent for extraction of lipophilic extractives with high purity from the two straws. This solvent may be used as a standard for the determination of lipophilic extractives from both straw and wood samples.

#### ACKNOWLEDGMENTS

The funding of this project by the Cheung Kong Scholars Programme and National Natural Science Foundation of China is gratefully acknowledged.

#### REFERENCES

- ATCHISON, J. E. 1996. Twenty-five years of global progress in non-wood plant fibre repulping. *Tappi J.* 79(10):87-95.
- CHAVES DAS NEVES, H. J., AND E. M. M. GASPAR. 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.
- , AND ———. 1995. HRGC-MS and HPLC-MS identification of new ketosterols in an extract of wheat straw. *J. High Resol. Chromatogr.* 18:299-303.
- CHEN, T., C. BREUIL, S. CARRIERE, AND J. V. HATTON. 1994. Solid-phase extraction can rapidly separate lipid classes from acetone extracts of wood and pulp. *Tappi J.* 77:235-240.
- COOPER, L. L. D., J. E. OLIVER, E. D. D. VILBISS, AND R. P. DOSS. 2000. Lipid composition of the extracellular matrix of *Botrytis cinerea* germings. *Phytochemistry* 53:293-298.
- EKMAN, R., AND B. HOLMBOM. 1989. Analysis by gas chromatography of the wood extractives in pulp and water samples from mechanical pulping of spruce. *Nordic Pulp Pap. Res. J.* 1:16-24.
- GUNSTONE, F. D. 1993. High-resolution <sup>13</sup>C NMR spectra of long-chain acids, methyl esters, glycerol esters, wax esters, nitriles, amides, alcohols and acetates. *Chem. Phys. Lipids* 66:189-193.
- GUTIERREZ, A., J. C. DEL RIL, F. J. GONZALEZ-VILA, AND F. MARTIN. 1998. Analysis of lipophilic extractives from wood and pith deposits by solid-phase extraction and gas chromatography. *J. Chromatogr. A* 823:449-455.
- , ———, ———, AND ———. 1999. Chemical composition of lipophilic extractives from *Eucalyptus globulus* Labill. wood. *Holzforschung* 53:481-486.
- JUNIPER, B. E. 1979. The structure and chemistry of straw. *Agric. Progress* 54:18-27.
- MEDINA, I., R. SACCHI, AND S. AUBOURG. 1994. <sup>13</sup>C Nuclear magnetic resonance monitoring of free fatty acid release after thermal processing. *JAOCs* 71:479-482.
- ORSA, F., AND B. HOLMBOM. 1994. A convenient method for the determination of wood extractives in papermaking process waters and effluents. *J. Pulp Pap. Sci.* 20:J361-J366.
- QUINDE, A., AND L. PASZNER. 1992. Behavior of the major resin- and fatty acids of slash pine (*Pinus elliottii* engelmann) during organosolv pulping. *Holzforschung* 46:513-522.
- RICHARDSON, D. E., J. B. BREMNER, AND O. GRADY. 1992. Quantitative analysis of total resin acids by high-performance liquid chromatography of their coumarin ester derivatives. *J. Chromatogr.* 595:155-159.
- SALOMONSSON, A. C., O. THEANDER, AND P. AMAN. 1978. Quantitative determination by GLC of phenolic acids as ethyl derivatives in cereal straws. *J. Agric. Food Chem.* 26(4):830-835.
- SITHOLE, B. B. 1992. Modern methods for the analysis of extractives from wood and pulp: a review. *Appita* 45:260-264.
- , P. VOLLSTAEDT, AND L. H. ALLEN. 1991. Composition of Soxtec and Soxhlet systems for determining extractives content. *Tappi J.* 74(11):187-191.
- , J. L. SULLIVAN, AND L. H. ALLEN. 1992. Identification and quantitation and acetone extractives of wood and bark by ion exchange and capillary GC with a spreadsheet program. *Holzforschung* 46:409-416.
- SJOSTROM, J., AND B. HOLMBOM. 1987. Size-exclusion chromatography of deposits in pulp and paper mills. *J. Chromatogr.* 411:363-367.
- SUCKLING, I. D., AND R. M. EDE. 1990. A quantitative <sup>13</sup>C nuclear magnetic resonance method for the analysis of wood extractives and pitch samples. *Appita* 43:77-80.
- , S. S. GALLAGHER, AND R. M. EDE. 1990. A new method for softwood extractives analysis using high performance liquid chromatography. *Holzforschung* 44:339-345.
- WALLIS, A. F. A., AND R. H. WEARNE. 1997. Characterization of resin in radiata pine woods, bisulfite pulps and mill pith samples. *Appita* 50:409-414.