J Wood Sci (2002) 48:222–226

ORIGINAL ARTICLE

Run Cang Sun · Jeremy Tomkinson

Comparative study of organic solvent-soluble and water-soluble lipophilic extractives from wheat straw 2: spectroscopic and thermal analysis

Received: January 29, 2001 / Accepted: August 1, 2001

Abstract As a continuing study of six organic solvent-soluble lipophilic extractives and one water-soluble lipophilic extract from wheat straw, seven extracts were further compared using Fourier transform-infrared (FT-IR) analysis, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, and thermal analysis. In the FT-IR spectra of two-thirds chloroform/one-third methanol, methyl *tert*-butyl ether (MTBE), hexane, petroleum ether, and dichloromethane extracts, a strong band at 1746 cm^{-1} is indicative of the ester carbonyl of triglycerides or steryl esters or waxes, whereas the occurrence of carboxylic carbonyl of free fatty acids or resin acids at 1712–1719 cm^{-1} is verified in the spectra of two-thirds toluene/one-third ethanol and hot water extracts. Signals at 187.9, 178.4, and 173.3 ppm in the ^{13}C NMR spectrum of MTBE extract correspond to carbonyl groups of resin acids, fatty acids, and fatty acid/steryl esters, respectively. All the extractives were to varying degrees thermally unstable at a temperature of about 200°C, and the melting temperature occurred at about 54°C.

Key words Wheat straw · Extractives · FT-IR; ^{1}H and ^{13}C NMR · Thermal analysis

Introduction

The extractives, commonly called pitch or wood resin, are comprised mainly of fatty acids, resin acids, waxes, alcohols, terpenes, sterols, steryl esters, and glycerides.[1–3] Because of the complexity of the chemical composition, it is difficult to find a single technique to analyze their structures. It is therefore thought that the most precise way to study the extractives from wood or straw is to use a combination of several techniques, each providing partial but complementary information. Among these methods, Fourier transform-infrared (FT-IR) spectroscopy has received much attention for quantitative analysis of wood resin and contaminants in pulp as well as pitch deposit extractives over the years. It has a major advantage over the conventional grating instruments, having more energy, excellent reproducibility, and accuracy from the laser source. With increasing use of the microcomputer, FT-IR spectroscopy is capable of manipulating spectral information (subtraction, ratioing, derivative spectra, deconvolution) and advanced chemometric software to handle calibration development.[4–6] In addition, the FT-IR method is nondestructive and often permits a sample to be analyzed in situ. The region between 780 and 2526 cm^{21} has been shown to have potential applications in the pulp and paper industry. It has been used to determine wood resin directly in ground wood pulp.[3]

Carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopy is another useful tool for studying various problems related to lipid technology. Complementary information about lipid class composition and total acyl profile can be inferred simultaneously from the same ^{13}C NMR spectrum.[7,8] This technique enables quantitation of the extractives in terms of total fatty acids, resin acids, triglycerides, and fatty acid esters. The method was found to be as accurate as conventional methods for analyzing extractives. The advantages of the method are that it is nondestructive and analysis is fast.[1] Furthermore, thermal analysis has proved useful for analyzing of lipids. Thermogravimetric analysis (TGA) can be employed to monitor the weight loss of the extractives as they are heated, cooled, or held isothermally; and differential scanning calorimetry (DSC) was performed to determine the melting temperature and enthalpies of the extractives.[9]

The first paper of this series reported the chemical composition of the seven lipophilic extractives from wheat straw.[10] It was found that the lipophilic extractives obtained by methyl-*tert*-butyl ether (MTBE), hexane, petroleum

---

R.C. Sun (✉) · J. Tomkinson
The BioComposites Centre, University of Wales, Bangor LL57 2UW, UK
Tel. +44-1248-370588; Fax +44-1248-370594
e-mail: bcs00a@bangor.ac.uk

R.C. Sun
State Key Laboratory of Pulp and Paper Engineering, College of Paper and Environment Engineering, South China University of Technology, Guangzhou 510640, China

ether, and dichloromethane from wheat straw consist mainly of free fatty acids (11.6%–26.6%), sterols (15.5%–24.5%), waxes (15.8%–21.1%), steryl esters (11.3%–19.1%), and triglycerides (9.7%–14.2%). Extraction with two-thirds toluene/one-third ethanol or with two-thirds chloroform/one-third methanol resulted in a higher yield of extractives but lower purity of the lipophilic substances (54.2%–54.9%); enriched free fatty acids were identified (28.2–32.3%), as were azelaic and maleic acids (19.9%–23.3%) and steryl esters (10.4%–13.9%).

First, the straw is treated with water for 4h at 98°C and then extracted with MTBE; the extract contained the major classes of lipids such as free fatty acids (33.8%), sterols (6.1%), waxes (8.2%), steryl esters (6.8%), and triglycerides (3.5%) together with noticeable amounts of phenolics (5.2%).[10] In this study, the major functional groups of the various extractive components recovered using various organic solvents and hot water treatment prior to MTBE extraction were further identified and assigned by means of FT-IR spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, and thermal analysis; the results are reported.

Experimental

Material and extractions

Wheat straw selected for this study was obtained from Silsoe Research Institute (Silsoe, Bedfordshire, UK) and was chopped using a Christie Laboratory mill (Compak Co., UK) to pass through a screen with 5mm diameter apertures. The chopped and dried straw chip (50g) was placed in a cellulose thimble and was Soxhlet-extracted with 1500ml of toluene-ethanol (2:1, v/v), chloroform-methanol (2:1, v/v), MTBE, hexane, petroleum ether, and dichloromethane for 6h, respectively. The solvent was removed by rotary evaporation at 35°C, and flasks containing the extractives were dried in a nitrogen stream and then weighed to determine the yield of extractives. Note that extraction was performed with toluene-ethanol for fraction 1 (F1); extractions were done with chloroform-methanol, MTBE, hexane, petroleum ether, and dichloromethane for fractions 2 (F2), 3 (F3), 4 (F4), 5 (F5), and 6 (F6), respectively. To isolate the soluble extractives in MTBE after hot water treatment, the ground straw meal (50g) was suspended in 800ml distilled water in a flask, and the suspensions were agitated for 4h at 98°C. After filtration of the residues, the supernatants were concentrated and then extracted twice with 1000ml MTBE at room temperature for 12h. The two extracts were combined, and the solvent was removed using 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.

Spectroscopic and thermal analyses

The FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet 750, UK) using a KBr disk containing 1% finely ground samples. Thirty-two scans were taken of each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode. The solution-state ^{1}H and ^{13}C NMR spectra were obtained on a Bruker 250 AC spectrometer at 62.4MHz in deuterochloroform. The ^{1}H NMR spectrum was recorded at 25°C from 20mg of sample dissolved in 1.0ml deuterochloroform for a total of 32 scans using an 8μs (~90°) pulse and a 4s delay time between scans. The solution-state ^{13}C NMR spectrum was recorded at 25°C from 100mg of sample dissolved in 1.0ml chloroform-d$_6$ after 5000 scans. A 70° pulse flipping angle, 10μs pulse width, and 15s delay time between scans were used.

Thermogravimetric analysis of the extractives was performed with a simultaneous thermal analyzer (STA 625, PL Thermal Sciences, UK). This apparatus provides a continuous measurement of sample weight at a range of temperatures between ambient and 600°C. Samples of approximately 10mg were heated in a platinum crucible to 600°C at a heating rate of 10°C min^{-1}. Provision was made for electronic differentiation of the weight signal to give the rate of weight loss.

Results and discussion

FT-IR spectra

Figure 1 shows the FT-IR spectra of the toluene-ethanol extract (spectrum 1), chloroform-methanol extract (spectrum 2), and MTBE extract (spectrum 3). The significant difference between the spectra lies in the weak absorptions at 2926–2866 cm^{-1} (CH stretching) and 1746 cm^{-1} (C=O stretching for esters) and the strong absorbances at 3436 cm^{-1} (OH stretching) and 1045 cm^{-1} (C—O—C stretching) in spectrum 1; the opposite absorption trend occurred in spectra 2 and 3. These results also confirm the

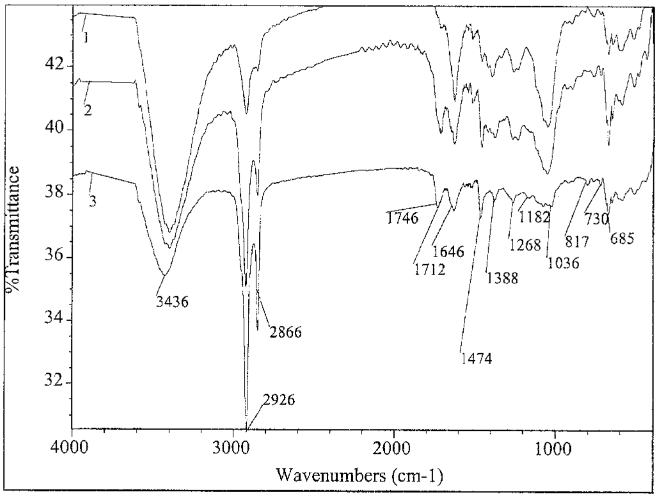

Fig. 1. Fourier transform-infrared (FT-IR) spectra of extractives obtained by extraction with toluene-ethanol (2:1 v/v, spectrum *1*), chloroform-methanol (2:1 v/v, spectrum *2*), and methyl *tert*-butyl ether (MTBE) (spectrum *3*) for 6h in a Soxhlet apparatus

data obtained by gas chromatography (GC) analysis, in which the toluene-ethanol extract (spectrum 1) contained only minor amounts of waxes (1.42%) and triglycerides (1.72%), whereas the extracts obtained by chloroform-methanol and MTBE contained noticeable amounts of waxes (2.57% in F2, 17.00% in F3) and triglycerides (8.92% in F2, 9.67% in F3).[10] The strong band at 1045 cm^{-1} in spectra 1 and 2 implied that the extractions with two-thirds toluene/one-third ethanol or with two-thirds chloroform/one-third methanol may dissolve some of the low-molecular-weight polysaccharides.

In spectra 2 and 3, the absorption band at 3436 cm^{-1} was given by the OH stretching vibration mode of sterols or monoglycerides/diglycerides or water in samples.[5] Extremely strong methylene and methyl stretching frequencies, particularly in spectra 2 and 3, appeared at 2926 and 2866 cm^{-1}, respectively. A carboxylic carbonyl stretching peak was produced at 1712 cm^{-1} as a "shoulder" to the ester carbonyl stretching peak seen at 1746 cm^{-1}. These bands arise from the carboxylic carbonyl of free fatty acids or resin acids that absorb at 1712 cm^{-1} and the ester carbonyl of triglycerides or steryl esters or waxes that frequently absorb at 1746 cm^{-1}. The C$=$C *cis* stretching, arising from double bonds in unsaturated fatty acids such as linoleic acid and oleic acid and their esters or in sterols, gave an absorption band of 1646 cm^{-1}. Two bands at 1474 and 1388 cm^{-1} were due to the CH absorption bending vibration of CH_2 and CH_3, respectively. A broad, strong, carbon single-bonded oxygen (C—O) stretching vibration appears at 1268 cm^{-1}. This band could be an interaction band between carbon single-bonded oxygen stretching and in-plane carbon single-bonded hydroxyl bending in carboxylic acids. The broad band appearing at 1182 cm^{-1} may be arising from saturated esters in conformity with ester carbonyl stretching. The weak shoulder appearing at 1122 cm^{-1} in spectrum 3 may arise from aliphatic ether stretching.[4] The prominent bands at 1045 cm^{-1} in spectra 1 and 2 or at 1036 cm^{-1} in spectrum 3 are attributed to the symmetrical stretching of an ether bond (C—O—C), which absorbs usually between 1070 and 1020 cm^{-1}.[11] The far peaks at low frequencies of 817 and 730 cm^{-1} are assigned to the ring out-of-plane carbon single-bonded hydrogen bending vibrations associated with two adjacent hydrogen atoms and in-plane methylene (CH_2) rocking absorption in straight-chain lipids, respectively.[5]

The spectra of hexane extract (spectrum 1), petroleum ether extract (spectrum 2), and dichloromethane extract (spectrum 3) of the wheat straw sample are illustrated in Fig. 2. Despite some differences among the spectra, all three samples exhibited similar absorption bands. Prominent peaks include strong absorption at 3443 cm^{-1} (OH stretching), 2932 cm^{-1} (CH_2 stretching), 2860 cm^{-1} (CH_3 stretching), 1746 cm^{-1} (C$=$O stretching of esters), 1712 cm^{-1} (C$=$O stretching of acids), strongly overlapped with the previous one, 1640 cm^{-1} (C$=$C stretching of unsaturated acids or sterols, or C$=$O stretching of conjugated acid), 1474 cm^{-1} (CH_3 and CH_2 asymmetrical stretching), 1381 cm^{-1} (CH_3 symmetrical bending), 1268 cm^{-1} (C—O or C—OH stretching), 1196–1168 cm^{-1} (C—O in O$=$C—O—CHCH$_2$— stretching), 1030 cm^{-1} (C—O—C symmetrical stretching), 817 cm^{-1} (CH_2 bending vibration), and 731 cm^{-1} [$—(CH_2)_{n\geq4}—$ rocking vibration].

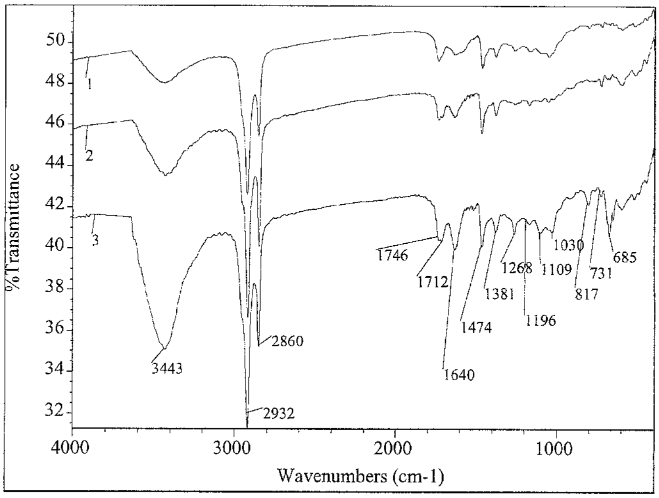

Fig. 2. FT-IR spectra of extractives obtained by extraction with hexane (spectrum *1*), petroleum ether (spectrum *2*), and dichloromethane (spectrum *3*) for 6 h in a Soxhlet apparatus

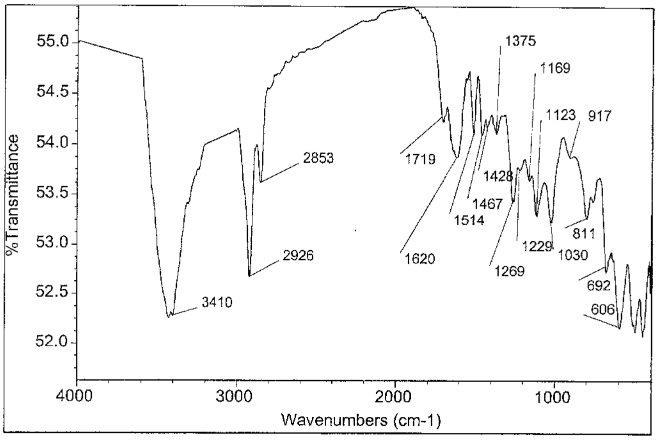

Fig. 3. FT-IR spectrum of extractives solubilized during the treatment of wheat straw with water at 98°C for 4 h and isolated by extraction with MTBE at room temperature

Treatment of the straw with water would be expected to release materials from within the wall structure, including, among others, condensed tannins and phenolics. The spectrum produced by this extract is presented in Fig. 3. In addition to the main vibration bands for lipophilic substances, the spectrum shows the typical ring skeletal bands at about 1620, 1514, and 1428 cm^{-1} for phenolics. Thus it may be recognized that the water-soluble components of this straw comprise noticeable amounts of aromatic substances, as shown by the predominance of ring vibrations in the spectrum. This observation was confirmed by the results obtained by high-performance liquid chromatography (HPLC) analyses, in which the extract contained 5.2% phenolics, resulting from depolymerization of the lignin.

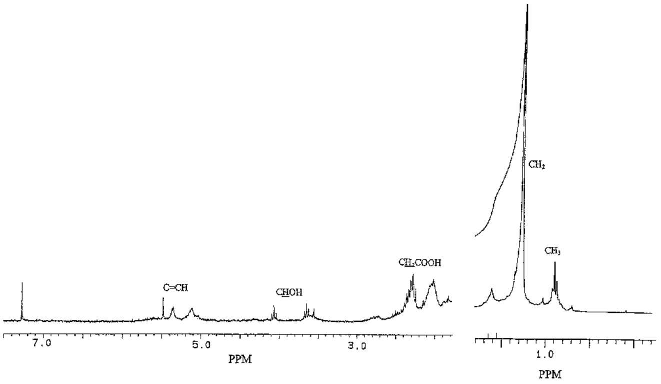

Fig. 4. ^{1}H nuclear magnetic resonance (NMR) spectrum of hexane extract

^{1}H and ^{13}C NMR spectra

The ^{1}H NMR spectrum of the hexane extract is given in Fig. 4. The most intense signal, occurring at 1.28 ppm, is attributed to the methylene aliphatic protons; the signal centered at 0.87 ppm is assigned to methyl protons. Minor signals from additional structural features [protons on carbons adjacent to carbonyl in esters (CH_2—C=O) or carboxyl in fatty acids ($C\underline{H}_2$—COOH) and hydroxyl groups ($C\underline{H}OH$); alkene (CH=C) protons] appear at 2.0–2.4, 3.6–4.1, and 5.1–5.5 ppm, respectively, as shown in Fig. 4.[12] The peak at 7.26 ppm is residual chloroform present in $CDCl_3$ and should be ignored.

Figure 5 shows the ^{13}C NMR spectrum of MTBE extract from wheat straw. The carbonyl group is obviously characterized by a strong signal at 194.6 ppm. Signals at 187.9, 178.4, and 173.3 ppm correspond to carbonyl groups of resin acids, fatty acids, and fatty acid/steryl esters, respectively. The signals at 156.3, 140.7, 134.9, 124.2, and 121.7 ppm originate from >C=in sterols and steryl esters. The signal attributed to an unsaturated carbon double bond (—CH=CH—) in fatty acids or fatty acid esters appears at 130.2 ppm. The groups of CH_2—O in glycerol carbons of triglycerides and >CH—O in sterol or steryl esters were identified with signals between 75 and 55 ppm. A small signal at 64.3 ppm (alkoxy C1 in ester) and a reduced signal at 63.0 ppm (alkoxy C1 in alcohol) were observed in the spectrum (data not shown).[8] The strong signals assigned to methyl and methylene groups appear in the spectrum between 14.1 and 39.7 ppm. The peak at 14.1 ppm is the methyl end of the chain. The peaks at 22.7, 31.9, and 29.3 ppm were methylene units successively farther from the methyl group. The large peak at 29.7 ppm is long internal methylene. The peak at 19.8 ppm is indicative of methyl branches on a straight methylene backbone, and the peak at 32.8 ppm is the methine associated with that methyl branch.[13]

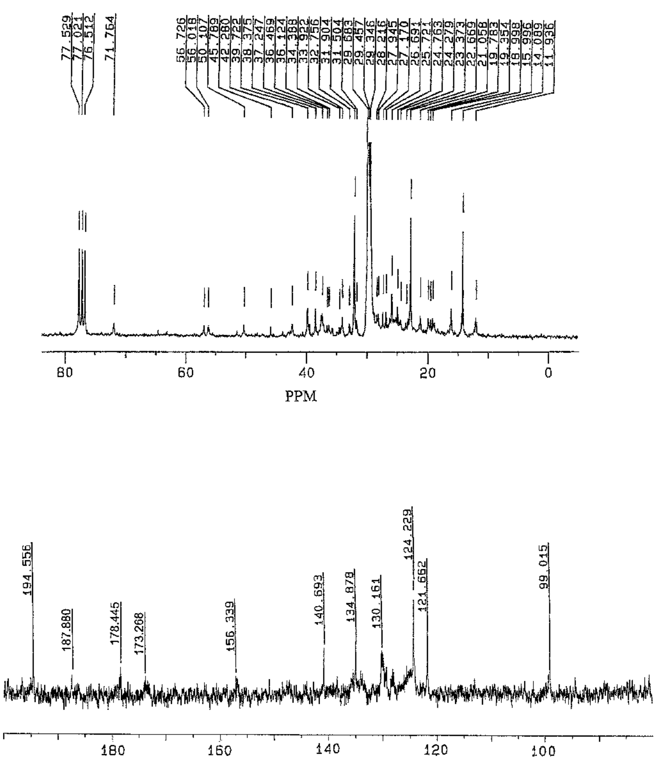

Fig. 5. ^{13}C NMR spectrum of MTBE extract

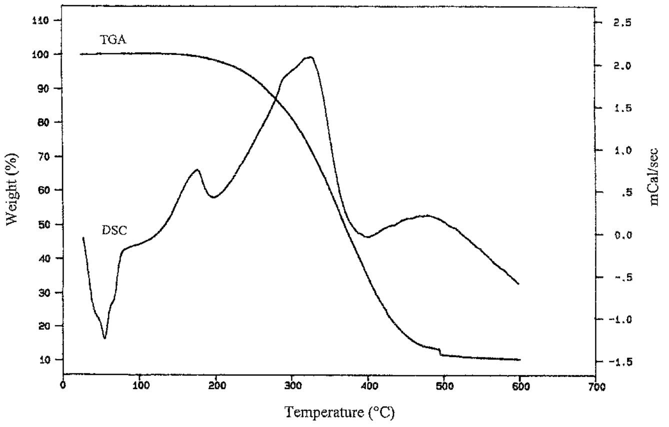

Fig. 6. Thermogram of hexane extract *TGA*, thermogravimetric analysis; *DSC*, differential scanning calorimetry

Thermal analysis

The thermal properties of the extractives were studied by thermogravimetric analysis and differential scanning calorimetry. The thermogram of hexane extract is shown in Fig. 6. As can be seen, the extract started to decompose at about 200°C, and its maximum rate of weight loss occurred between 300°C and 450°C. The decomposition temperature at 10% weight loss appeared at 271°C. When the temperature increased to 300°C, the weight loss accounted for 15.6%, and at 450°C it increased significantly to 83.0%. There was little non-volatile residue at 600°C (<10% of the total extract material). In Fig. 6 DSC was also performed to determine the melting temperatures and enthalpies of the extract. The melting temperature, due to a solid–liquid phase transition from the hexagonal crystal structure to a melt, occurred at about 54°C. This is consistent with previously reported values for wood resins from recycled fiber.[9] In addition, the DSC thermogram of the extractives gave one big broad exothermic peak with a maximum at 328°C.

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).

References

1. Suckling ID, Ede RM (1990) A quantitative ^{13}C nuclear magnetic resonance method for the analysis of wood extractives and pitch samples. Appita 43:77–80
2. Sithole BB, Vollstaedt P, Allen LH (1991) Composition of Soxtec and Soxhlet systems for determining extractives content. TAPPI J 74:187–191
3. Sithole BB (1992) Modern methods for the analysis of extractives from wood and pulp: a review. Appita 45:260–264
4. Ajuong EMA, Breese MC (1998) Fourier transform infrared characterization of pai wood (*Afzelia africana* smith) extractives. Holz Roh Werkstoff 56:139–142
5. Man YBC, Setiowaty G (1999) Application of Fourier transform infrared spectroscopy to determine free fatty acid contents in palm olein. Food Chem 66:109–114
6. Man YBC, Moh MH, van de Voort FR (1999) Determination of free fatty acids in crude palm oil and refined-bleached-deodorized palm olein using Fourier transform infrared spectroscopy. JAOCS 76:485–490
7. Medina I, Sacchi R, Aubourg S (1994) ^{13}C-Nuclear magnetic resonance monitoring of free fatty acid release after thermal processing. JAOCS 71:479–482
8. Gunstone FD (1993) High-resolution ^{13}C NMR spectra of long-chain acids, methyl esters, glycerol esters, wax esters, nitriles, amides, alcohols and acetates. Chem Phys Lipids 66:189–193
9. Guo XY, Douek M (1996) Analysis of deposits/stickies from newsprint mills using recycled fibre. J Pulp Pap Sci 22:431–438
10. Sun RC, Tomkinson J (2002) Comparative study of organic solvent and water soluble lipophilic extractives from wheat straw. 1. Yield and chemical composition. J Wood Sci (in Press)
11. Sun RC, Fang JM, Tomkinson J, Geng ZC, Liu JC (2001) Fractional isolation, physico-chemical characterization and homogeneous esterification of hemicelluloses from fast-growing poplar wood. Carbohydr Polym 44:29–39
12. Wallis AFA, Wearne RH (1997) Characterization of resin in radiata pine woods, bisulfite pulps and mill pitch samples. Appita 50:409–414
13. Musser BJ, Kilpatrick PK (1998) Molecular characterization of wax isolated from a variety of crude oils. Energy Fuels 12:715–725