ISOLATION AND CHARACTERIZATION OF HOT WATER-SOLUBLE LIPOPHILIC EXTRACTIVES FROM WHEAT STRAW. PART II. SPECTROSCOPIC AND THERMAL CHARACTERIZATION

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ABSTRACT

The chemical composition of the six extractives, isolated with hot water at 80–95°C for 0.5 h at pH 6.0–8.0 from wheat straw, consists mainly of free fatty acids, waxes, sterols, triglycerides, and sterol esters, together with minor amounts of diglycerides, resin acid, and phenolic compounds. In this continuing study, the six lipophilic extractives were further investigated by Fourier transform infrared, and ^{1}H and ^{13}C nuclear magnetic resonance as well as thermal analysis, and the results are reported.

Keywords: Wheat straw, lipophilic extractives, FT-IR, ^{1}H and ^{13}C-NMR, thermal stability.

INTRODUCTION

Wheat straw is one of the main agricultural residues and is produced in large quantities worldwide every year. In developing countries such as China and India, these large quantities of fibrous crop residues are currently underutilized as raw materials for papermaking (Sun et al. 1995). During the mechanical pulping of straw by grinding or refining, noticeable amounts of hemicelluloses, together with small quantities of low molecular weight lignin fragments and considerable amounts of lipophilic extractives, are dissolved or dispersed in colloidal form, into the water. The liberated components are then carried over to the paper machine white-water system since mechanical pulping normally does not include washing operations (Ekman and Holmbom 1989). It was also found that more of these components can be dissolved and/or dispersed in bleaching operations, particularly, in alkaline peroxide bleaching. This dissolution and dispersion of various components should be kept at a minimum in order to maintain a high pulp yield and to decrease the effluent load (Orsa et al. 1993).

In recent years, considerable interest has been directed to the agricultural waste in developed countries because of its environmental problems. As we attempt to resolve the pitch problems and to reduce the impact on the environment from the effluents in the papermaking industry, the development of effective technologies for investigation of lipophilic extractives from wheat straw is considered to be both important and significant. A number of methods for isolation and separation of extractives in wood pulp and paper mill waters have been developed (Orsa et al. 1993; Orsa and Holmbom 1994). However, most methods are too laborious and, hence, too expensive for process studies where a large number of samples need to be analyzed in a reasonable time

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(Orsa and Holmbom 1994). Besides gas chromatography, high performance liquid chromatography (HPLC) has been adapted to the separation and identification of total extractives in softwoods (Suckling and Ede 1990) and to resin acids in aqueous samples (Richardson et al. 1992). The related technique, high performance size exclusion chromatography, has allowed separation of extracts from effluents of softwood processing (Sjöstrom and Holmbom 1987), while high performance thin-layer chromatography has provided a convenient method for analysis of resin in wood, pulp and paper (Sandstrom et al. 1996). Other approaches to the characterization have involved Fourier transform infrared (FTIR) (Ajuong and Breese 1998) and ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopies from wood resin and pitch samples (Suckling and Ede 1990; Gunstone 1993; Medina et al. 1994).

FT-IR, ^{1}H, and ^{13}C-NMR spectroscopies are extremely powerful analytical techniques for both qualitative and quantitative studies of structural properties of the lipophilic extractives. They may give important complementary information regarding the samples, particularly giving an overall view of their structures. In recent years, FTIR spectroscopy has been shown to have potential applications in the pulp and paper industry for quantitative analysis of wood resin and pitch deposit extractives (Sithole 1992). In addition, ^{1}H or ^{13}C NMR spectroscopy is also a useful tool in the study of various problems related to extractives technology. Complementary information about the extractive class composition and the total acyl profile can be inferred simultaneously from the same ^{1}H or ^{13}C NMR spectrum (Medina et al. 1994). The method was found to be as accurate as conventional methods for the analysis of extractives. Furthermore, thermal analysis has been proved to be a very useful method in the routine analysis of lipophilic substrates, particularly for measuring their melting points (Blanco and Almendros 1994).

The first paper of this series reported the chemical composition of the six water-soluble lipophilic extractives separated by gas chromatography GC on a medium-length high-temperature capillary column without derivatization. It was found that treatment of wheat straw with hot water at 80–95°C for 0.5 h at pH 6.0–8.0 released 41.0–53.0% of the original lipophilic extractives. The extracts contained 68.7–75.8% lipophilic substances, comprising mainly free fatty acids (25.8–48.4%), waxes (9.4–27.0%), sterols (4.1–8.0%), triglycerides (3.3–11.0%), and sterol esters (2.6–5.1%). Minor amounts of diglycerides (0.3–0.5%), resin acid (0.5–3.1%), and phenolic compounds (0.9–3.6%) were also quantitatively determined in the extractives (Sun et al. 2003). In this continuing study, the six preparations of the lipophilic extractives solubilized in hot water at 80–95°C for 0.5 h at pH 6.0–8.0, were further characterized by FTIR and ^{1}H and ^{13}C NMR spectroscopies as well as thermal analysis.

MATERIALS AND METHODS

Materials

Wheat straw was kindly supplied by Silsoe Research Institute (Silsoe, Bedfordshire). The straw was first ground using a Christie Laboratory mill to pass through a screen with 5-mm-diameter apertures. The ground straw was dried in a cabinet oven with air circulation at 60°C for 16 h and then kept in a refrigerator at 5°C before treatment.

Isolation of lipophilic extractives released during the hot water treatment

Ground straw meal (50 g) was suspended in 2 liters distilled water (pH 7.1) for 0.5 h at 80°C, 85°C, 90°C, 95°C, and at 90°C for 0.5 h at pH 8.0 adjusted by 2% NaOH, and at pH 6.0 adjusted by 2% HCl, respectively. After filtration, each of the supernatants was extracted with 600 mL dichloromethane three times at room temperature for 6 h, and the combined extract was evaporated to remove the solvent under vacuum in a rotary evaporator at 30°C. The released extractives were further dried in a nitrogen steam and then weighed to deter-

TABLE 1. *The yield (% dry straw) and chemical composition (% extractives) of water-soluble extractives in wheat straw.*

Yield/composition	F_1[a]	F_2[a]	F_3[a]	F_4[a]	F_5[a]	F_6[a]
Yield	0.48	0.50	0.54	0.56	0.62	0.60
Chemical composition						
Free fatty/resin/other acids	50.46	45.46	37.29	28.12	33.56	27.32
Sterols	4.05	4.37	6.13	7.95	7.33	7.81
Waxes	9.36	12.53	15.59	27.03	18.93	17.13
Diglycerides	0.41	0.43	0.44	0.45	0.26	0.44
Steryl esters	3.12	3.66	5.26	5.61	4.89	6.87
Triglycerides	3.34	5.52	7.29	7.79	6.70	10.96
Total substance	70.74	72.97	72.00	76.95	71.67	70.53
Total lipophilic substance	68.68	70.89	70.87	75.75	69.31	68.99

[a] Represent fractions of the extractives solubilized during the treatment of the straw with water at pH 7.1 for 0.5 h at 80°C (F_1), 85°C (F_2), 90°C (F_3), 95°C (F_4), and at 90°C for 0.5 h at pH 8.0 (F_5), and pH 6.0 (F_6).

mine the yield. Note that the extract released during the treatment with distilled water for 0.5 h at 80, 85, 90, and 95°C was labeled for fraction 1 (F_1), 2 (F_2), 3 (F_3), and 4 (F_4), respectively, and those dissolved during the treatment with pH 8.0 and 6.0 aqueous solution for 0.5 h at 90°C were considered to be fractions 5 (F_5) and 6 (F_6), respectively. The dried extract (8 mg) was re-dissolved in a 1 mL dichloromethane and was thereafter ready for separation and identification by GC without derivatization. All the experiments were performed in duplicate. The detailed method for analysis of hot water-soluble lipophilic extractives by gas chromatography was reported in a previous paper of this series (Sun et al. 2003). The yield, purity, and chemical composition of lipophilic extractives solubilized during the hot water treatment at different pH and various temperatures are listed in Table 1. The yield is calculated based on the mass of untreated starting material, whereas the purity and chemical composition are calculated based on the mass of dried extract.

FTIR and ^{1}H and ^{13}C NMR spectroscopies

FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750) using a KBr disc 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 was obtained on a Bruker 250 AC spectrometer at 62.4 MHz in deuteriochloroform. ^{1}H NMR spectrum was recorded at 25°C from 20 mg of sample dissolved in 1.0 mL deuteriochloroform for a total of 150 scans using an 8 μs (~90°) pulse and a 4-s delay time between scans. Solution ^{13}C-NMR spectrum was recorded at 25°C from 100 mg of sample dissolved in 1.0 mL chloroform-d after 5000 scans. A 70° pulse flipping angle, a 10-μs pulse width and a 15-s delay time between scans were used.

Thermal analysis

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of the extractives were performed with a simultaneous thermal analyzer (STA 625). This apparatus provides for a continuous measurement of sample weight at a range of temperatures between ambient and 600°C. Samples of approximately 10 mg weight were heated in a platinum crucible to 600°C at a heating rate of 10°C min^{-1}.

RESULTS AND DISCUSSION

FTIR spectra

Figure 1 shows FTIR spectra of hot water-soluble lipophilic extractives F_1 (spectrum a),

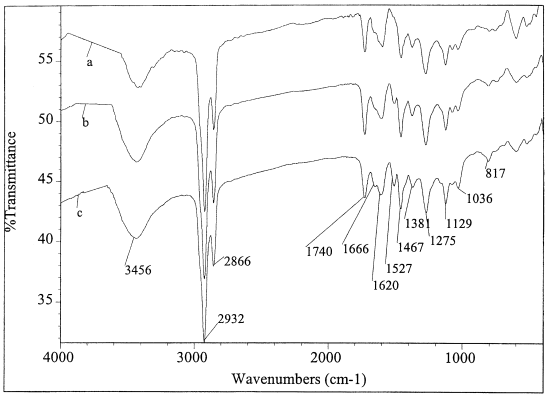

FIG. 1. FT-IR Spectra of extractives F_1 (spectrum a), F_2 (spectrum b), and F_3 (spectrum c).

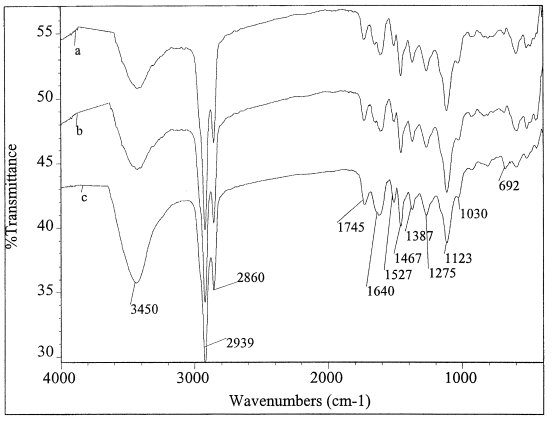

FIG. 2. FT-IR Spectra of extractives F_4 (spectrum a), F_5 (spectrum b), and F_6 (spectrum c).

F_2 (spectrum b), and F_3 (spectrum c). Obviously, all three spectra exhibit similar absorption bands characteristic of the main functional groups, indicating similar mixtures of the extractives. A broad band at 3456 cm^{-1} is assigned to the hydroxyl group stretching vibration in sterols, mono-, and diglycerides, co-extracted polysaccharides, or water in samples (Man and Setiowaty 1999). The very strong methylene and methyl stretching frequencies give peaks at 2932 and 2866 cm^{-1}, respectively. The peak at 1740 cm^{-1} is attributed to absorption by carbonyl bonds in esters (waxes, sterol esters, triglycerides). The carbonyl bonds in free fatty and resin acids exhibit a band at 1712 cm^{-1}, and are strongly overlapped with the previous one. Two bands at 1666 and 1620 cm^{-1} are characterized by the carbon double bond stretching (C=C in unsaturated fatty acids and their esters or in sterols and steryl esters). Two sharp peaks at 1467 and 1381 cm^{-1} represent the methylene bending vibration and methyl symmetrical bending, respectively. A much stronger intensity at 1467 cm^{-1} than at 1381 cm^{-1} revealed typical long chain fatty acids or their esters. The carbon single bonded oxygen (C-O) or bonded hydroxyl (C-OH), bending or stretching vibration gives an absorption band at 1275 cm^{-1}. A very weak band at 1180 cm^{-1} in F_3 (data not shown) is due to the C-O stretching in the aliphatic esters (O=C-O-CHCH$_2$-) (Ajuong and Breese 1998), indicating a small amount of esters in the extract. This confirmed the results obtained by GC analysis (Sun et al. 2003). The small band at 1129 cm^{-1} is indicative of the C-C stretching in extractives such as in sterols and steryl esters. The C-OH stretching in sterols occurs at 1036 cm^{-1}. A band at 817 cm^{-1} corresponds to the carbon single-bonded hydrogen (C-H) bending vibrations in the extractives. The occurrence of a very weak absorption band at 1527 cm^{-1} in extracts F_2 and F_3 is undoubtedly due to the co-extracted phenolic ring skeletal vibrations in the lipophilic extractives, which corresponded to the data obtained by HPLC analysis (Sun et al. 2003).

The FTIR spectra of hot water-soluble extracts F_4 (spectrum a), F_5 (spectrum b), and F_6 (spectrum c) are illustrated in Fig. 2. Prominent peaks for lipids include strong absorptions at 3450 cm^{-1} (OH stretching), 2934 and 2860 cm^{-1} (CH_2 and CH_3 stretching), 1745 cm^{-1} (C=O stretching of esters), 1712 cm^{-1} (C=O stretching of acids, overlapped strongly with the previous one), 1640 cm^{-1} (C=C stretching of unsaturated lipids), 1467 cm^{-1} (CH_2 bending vibration), 1387 cm^{-1} (CH_3 bending vibration), 1275 cm^{-1} (C-O or C-OH bending or stretching vibration), 1123 cm^{-1} (C-C stretching), and 1030 cm^{-1} (C-OH stretching). In the light of these absorptions, it

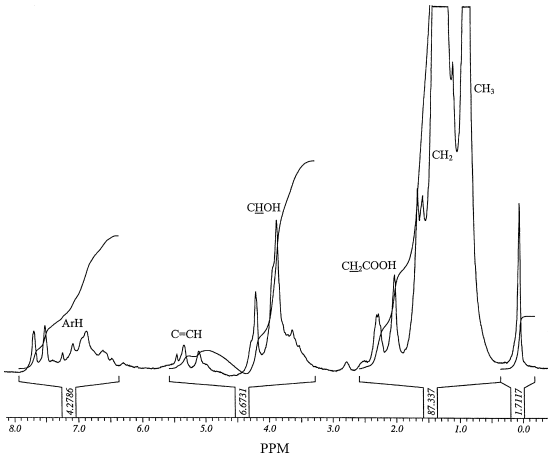

FIG. 3. ^{1}H NMR Spectrum of extractives F_3.

is possible that the strong band at 1123 cm^{-1} is also indicative of C-O-C stretching of water-soluble polysaccharides in the extracts, a typical of xylans (Sun et al. 2000). Similarly, a small band at 1527 cm^{-1} in three spectra implied minor amounts of co-extracted phenolic compounds in the lipophilic extractives.

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

To obtain further information on the whole extractives and characterize their functional groups, ^{1}H and ^{13}C NMR spectra were performed. Figure 3 gives the ^{1}H NMR spectrum of extractives F_3. The most intense signal at 1.24 ppm originates from the methylene aliphatic protons, and a very strong signal at 0.85 ppm is assigned to the methyl protons in lipids. In addition, the spectrum gives peaks at 2.0 ppm for protons on carbons adjacent to carbonyl in esters (CH_2-C=O), 2.3 ppm for protons on carbons adjacent to an carboxylic acid group (C$\underline{H}_2$-COOH), 3.9–4.2 ppm for protons on carbons adjacent to alcohols (C$\underline{H}$OH) or ethers (C$\underline{H}$-O-C), and 5.1–5.5 ppm for protons on carbons adjacent to alkene (C=C$\underline{H}$) (Wallis and Wearne 1997). Note that the peaks between 6.5 and 7.8 ppm are tentatively attributed to the aromatic protons (ArH) from co-extracted phenolic compounds or arise from the residual chloroform present in $CDCl_3$.

A solution state ^{13}C NMR spectrum of lipophilic extractives F_2 is illustrated in Fig. 4. The carbon atoms from unsaturated compounds (110–160 ppm), aliphatic (0–34 ppm) and C-O substituted (40–90 ppm) structures, and functional groups (170–200 ppm) were clearly observed (Gonzalez-Vila et al. 1997). The carbonyl group gives a signal at 192.8 ppm. Two signals at 183.5 and 178.4 ppm are indicative of carbonyl groups in free resin and fatty acids, respectively (Suckling and Ede 1990). The signal at 156.3 ppm is attributed to unsaturated carbon (>C=) in resin acids, sterols or steryl esters, and two signals at 130.8 and 128.7 ppm are assigned to unsaturated carbon in double bond (-CH=CH-) from fatty acids or their esters. The carbon-bonded oxygen group (>C-O) in sterol or steryl esters exhibits a signal at 68.1 ppm. The peak at 14.0 ppm relates to methyl end of the chain, whereas the methylene units successively further from the methyl group give the signals between 31.9 and 22.6 ppm, in which the most strong signal occurs at 29.6 ppm (Musser and Kilpatrick 1998). The peaks at 38.6 ppm are presumed due to the carbon in methine group of resin acids, sterols, or steryl esters.

Thermal analysis

The thermal stability of the lipophilic extractives and their melting temperatures were determined by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), and their curves for extracts F_4 and F_6 are shown in Fig. 5, a and b, respectively. As illustrated in two curves of TGA, both of their decomposition temperatures started at 177°C. However, the decomposition temperature at 10% weight loss occurred at 262°C in F_4 and 254°C in F_6. Furthermore, when the temperature rose to 400°C, the weight loss increased significantly to 63% in F_4 and 60% in F_6, indicating a slightly more stable of the extractives F_6 released at 90°C (pH 6.0) than the extractives F_4 solubilized at 95°C (pH 7.1). Non-volatile residues at 600°C were small, 11% of the total extractives in F_4 and

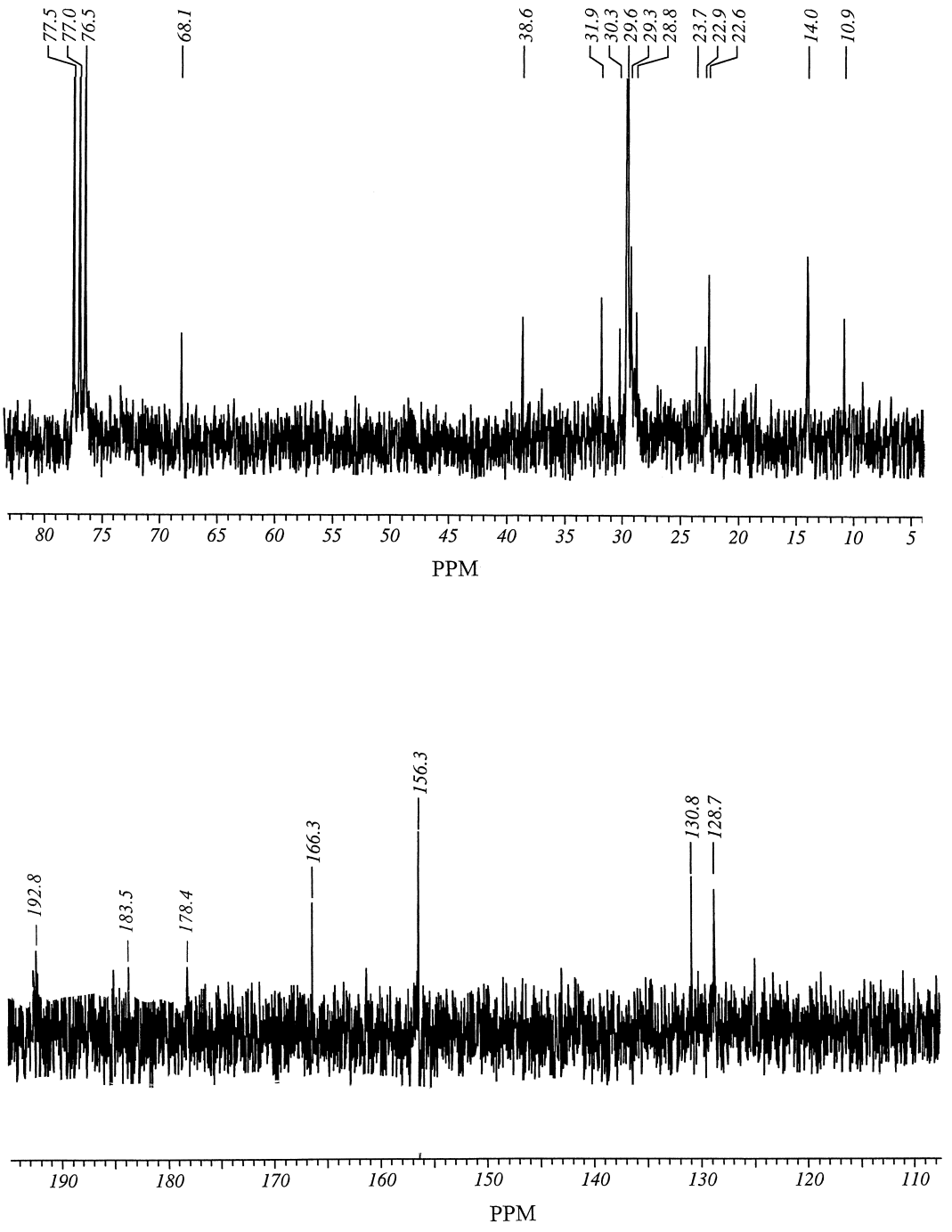

FIG. 4. The ^{13}C NMR Spectrum of extractives F_2.

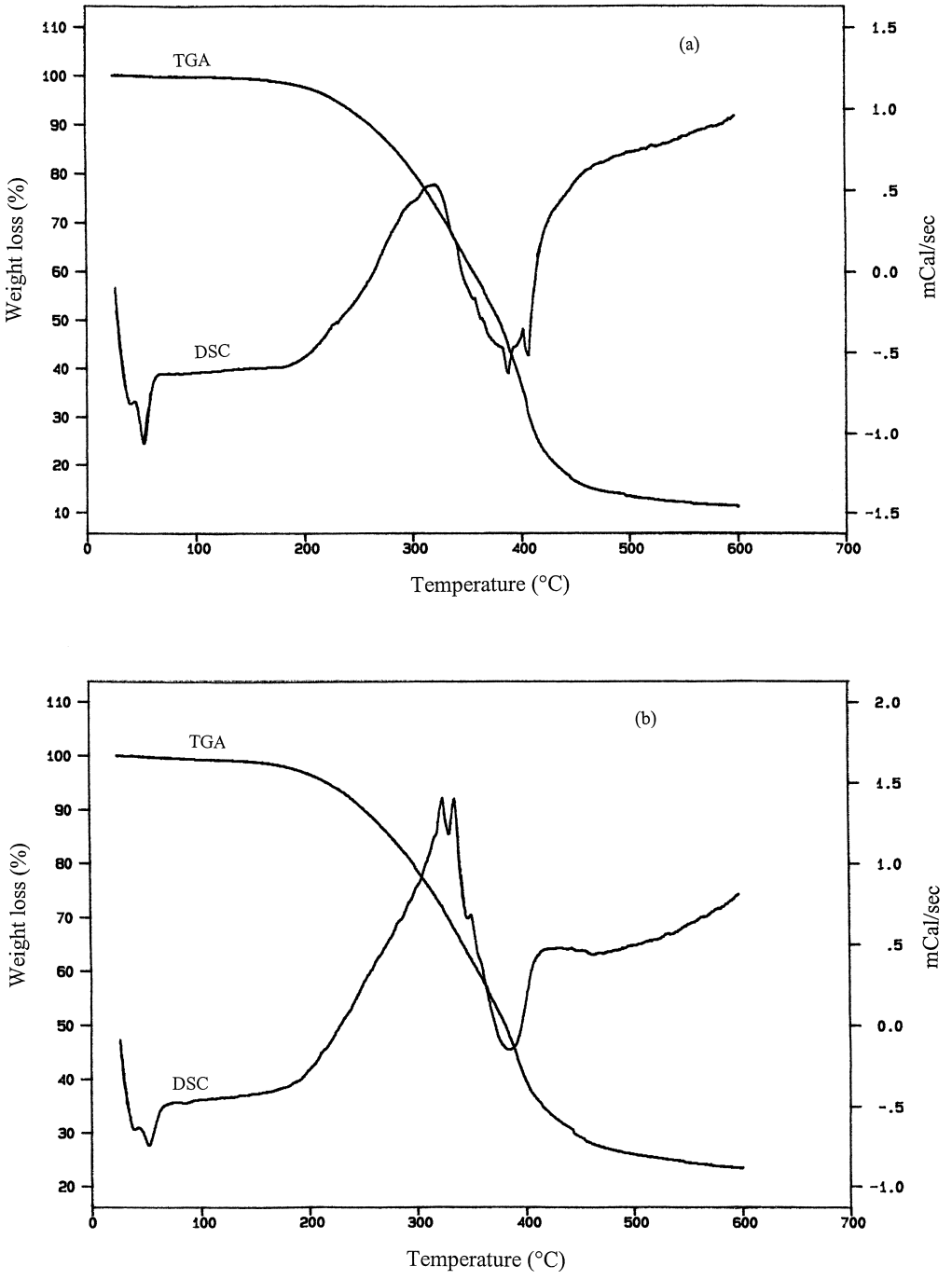

FIG. 5. Thermograms of extractives F_4 (a) and F_6 (b).

22% in F_6, which are probably due to the contaminates such as ash and salts from the straw. These results supported the data analyzed by GC and corresponded to the purity of lipophilic extractives F_4 (77.0%) and F_6 (70.5) (Sun et al. 2003). The melting temperatures, as shown in DSC curves, occurred at 38.1–52.4°C for both of the preparations, which implied that both extractives were a mixture of the lipophilic substances. Such typical melting points for lipophilic extractives have been also observed from wood extractives, 30–70°C (Severtson et al. 1999).

In short, in the FTIR spectra of six lipophilic extractives, an intense band at 1740 or 1745 cm^{-1} is attributed to ester carbonyl of triglycerides or steryl esters or waxes, whereas occurrence of carboxylic groups in free fatty acids or resin acids at 1710 cm^{-1} is strongly overlapped with the previous one. Two bands at 1666 and 1640 or 1620 cm^{-1} are indicative of the carbon double bond stretching in unsaturated fatty acids and their esters or in sterols and steryl esters. The C-C stretching such as in sterols and steryl esters occurs at 1129 or 1123 cm^{-1}. Signals at 183.5 and 178.4 ppm in the ^{13}C NMR spectrum of F_2 correspond to carbonyl groups in free resin and fatty acids, respectively. All the extractives were to varying degrees thermally unstable at temperature ~180°C, and the melting temperature occurred between 38 and 54°C.

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