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Analysis of lipophilic extractives from wood and pitch deposits by solid-phase extraction and gas chromatography

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Abstract

An analytical procedure for the analysis of lipophilic extractives from wood and pitch deposits is described in this paper. It comprises a rapid gas chromatographic method that enables the analysis of a high number of samples in a short period of time. Short-length, high-temperature capillary columns with thin films and high temperature- programming rates were preferred for the rapid analysis of wood extractives since they enable elution and separation of compounds within a wide molecular mass range (from fatty acids to sterol esters and triglycerides) in the same chromatographic analysis in a short period of time. Several examples of analysis of extractives from pine and eucalypt woods and pitch deposits in an eucalypt kraft pulp, are shown. On the other hand, a simple fractionation method using solid-phase extraction (SPE) in aminopropyl cartridges is described for the preparative scale separation and fractionation of wood lipophilic extractives into major lipid classes. The SPE advantages include smaller sample and solvent requirements and ease of use compared to conventional solvent extraction techniques.

Keywords: Wood; Lipids; Fatty acids; Sitosterol; Waxes; Sterol esters; Triglycerides

1. Introduction

Lipophilic extractives from wood consist of complex mixtures of many different compounds, from the low-molecular-mass resin and fatty acids to the high-molecular-mass waxes, sterol esters and triglycerides. The accumulation of wood lipophilic extractives during pulping and papermaking (the so-called pitch deposits) cause significant technical and economic troubles in pulp and paper manufacture [1]. Conventional approaches used to reduce wood extractive deposition, such as debarking or seasoning of logs, are often not sufficient to eliminate pitch problems. Recent advances in biotechnology have shown the ability of different wood-inhabiting fungi to degrade wood lipophilic compounds [2,3]. Fungi and fungal enzymes are already being used in commercial products for wood and pulp depitching [4,5]. However, these methods appear effective only on certain wood types and under specific pulping conditions. Efforts for larger and more selective fungal screening and optimization of the biotechnological approaches are being performed. However, due to the complex lipid mixture present in the wood extractives, their analysis frequently involves initial separation of the sample into the various lipid components, that is too laborious and hence time-consuming for process studies where a large number of samples need to be analyzed in a reasonable time. Thus, it is of considerable interest to develop rapid and simple analytical procedures to evaluate biological pretreatments and, on the other hand, to monitor wood extractives previous the pulping process in order to predict and control pitch problems.

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In this work, a rapid method for the analysis of complex mixtures of wood lipophilic extractives with no prior fractionation, is reported. Gas chromatography (GC) and GC–mass spectrometry (MS) using short- and medium-length high-temperature capillary columns (with thin films), respectively, that enable elution and separation of high-molecular-mass lipids [6–11], are employed.

On the other hand, for a more detailed characterization, a simple solid-phase extraction (SPE) method using aminopropyl phase cartridges is described for the fractionation of the complex mixture of lipids isolated from woods and pitch deposits into major lipid classes, which can be subsequently characterized and quantified by GC and GC–MS. The advantages of SPE over traditional sample preparation methods include increased speed and simplicity, reduced solvent usage and improved selectivity [12]. The method outlined in this paper is based on that previously developed by Kaluzny et al. [13] for the separation of lipids from bovine adipose tissue. However, a different fractionation scheme with reduced number of steps and cartridges, as well as a different solvent system, have been used for the fractionation of wood lipids.

2. Experimental

2.1. Samples

The samples selected for this study were eucalypt and pine wood and a pitch deposit taken from an eucalypt kraft pulp. The woods (2 g), previously ground to sawdust, and the pitch deposit were Soxhlet-extracted with 150 ml of acetone (Panreac, Barcelona, Spain) for 6 h. The dried acetone extracts were subsequently redissolved in chloroform (Merck, Darmstadt, Germany) and evaporated under nitrogen to dryness.

2.2. Gas chromatography

A Hewlett-Packard HP 5890 gas chromatograph equipped with a split–splitless injector and a flame ionization detection (FID) system was used (Hewlett-Packard, Hoofddorp, Netherlands). The injector and the detector temperatures were set at 300°C and 350°C, respectively. Sample volumes of 1 μl were injected in the splitless mode. Helium was used as the carrier gas. The capillary column used was a high-temperature, polyimide-coated fused-silica tubing DB5-HT (0.25 mm I.D., 0.1 μm film thickness) from J&W Scientific (Folsom, CA, USA), specially processed for an extended temperature of 400°C. Different column lengths and temperature programs were tried as will be discussed later. For the 5 m capillary column selected for the analysis of wood extractives, the oven was temperature-programmed from 100°C (1 min) to 350°C (3 min) at 15°C/min. A mixture of standard compounds (palmitic acid, dehydroabietic acid, sitosterol, cholesteryl oleate and triheptadecanoin) supplied by Sigma (St. Louis, MO, USA) was used to elaborate a calibration curve for the quantitation of wood extractives. All peaks were quantified by peak area.

2.3. Gas chromatography–mass spectrometry

The GC–MS analyses were performed on a Varian Star 3400 gas chromatograph (Varian, Walnut Creek, CA, USA) with an ion trap detector (Varian Saturn 2000) using a high-temperature capillary column (DB-5HT, 15 m×0.25 mm I.D., 0.1 μm film thickness; J&W Scientific). Helium was used as the carrier gas. The samples were injected with an autoinjector (Varian 8200) directly onto the column using a SPI (septum-equipped programmable injector) system. The temperature of the injector during the injection was 120°C, and 0.1 min after the injection was programmed to 380°C at a rate of 200°C/min and hold 10 min. The oven was temperature-programmed from 120°C (1 min) to 380°C (5 min) at 10°C/min. The temperatures of the ion trap and the transfer line were set at 200°C and 300°C, respectively. Compounds were identified by computer comparison of the mass spectra with those in the Wiley and NIST libraries, by mass fragmentography and when possible by comparison with standard compounds.

2.4. SPE fractionation

Aminopropyl phase cartridges (500 mg) from Waters (Division of Millipore, Milford, MA, USA) were used in this study. All solvents (hexane,

chloroform, diethyl ether and acetic acid) were supplied by Merck. The column separations described below were performed with duplicates and with different quantities of lipid extract (5–20 mg). The procedure for the fractionation of eucalypt wood lipids was as follows: the dried chloroform extract was taken up in a minimal volume (<0.5 ml) of hexane–chloroform (4:1) and loaded into the cartridge column previously conditioned with hexane (4 ml). The cartridge was loaded and eluted by gravity. Gravity flow is essential when the full resolving power of the sorbent is required [14] and it demands little additional equipment. However, when a major flow-rate is needed, a positive displacement or vacuum can be applied. The column was first eluted with 8 ml of hexane and subsequently with 6 ml of hexane–chloroform (5:1), then with 10 ml of chloroform and finally with 10 ml of diethyl ether–acetic acid (98:2). Each isolated fraction was dried under nitrogen and weighed. GC and GC–MS were used to monitor the purity and to determine the amount of solvent needed to elute each fraction.

3. Results and discussion

3.1. Rapid analysis of wood lipophilic extractives by GC and GC–MS

Several authors have used short GC conventional capillary columns, which allow the elution and separation of high-molecular-mass lipids, for the analysis of wood lipophilic extractives, although they do not allow the best resolution [10,11]. In the present study, the range of temperature of the analysis have been extended by using high-temperature capillary columns. On the other hand, capillary columns with thin films, which are necessary for an optimal analysis of high-molecular-mass lipids such as waxes, sterol esters and triglycerides [15] were preferred for this study. Therefore, the column finally selected for the chromatographic analyses was a DB5-HT capillary column (J&W Scientific) of 0.25 mm I.D. and with a film thickness of 0.1 μm. Previous procedures for the analysis of wood extractives used conventional temperature columns and different film thickness or internal diameters [10,11].

Different column lengths (from 25 to 2 m) and different temperature programs were investigated for the analysis of the lipid extract from eucalypt wood by GC–FID. As the main objective was to obtain short elution times, high temperature-programming rates, which enhance the speed of the analysis, were preferred. Fig. 1 shows the chromatograms of eucalypt wood lipophilic extractives obtained with different column lengths and temperature program rates. It can be observed that the high-molecular-mass lipids (sterol esters and triglycerides) start to elute as the column length decreases. Shorter lengths (2 m) were also attempted but the resolution was not good enough for quantitative purposes. The length of the column used should be a compromise between

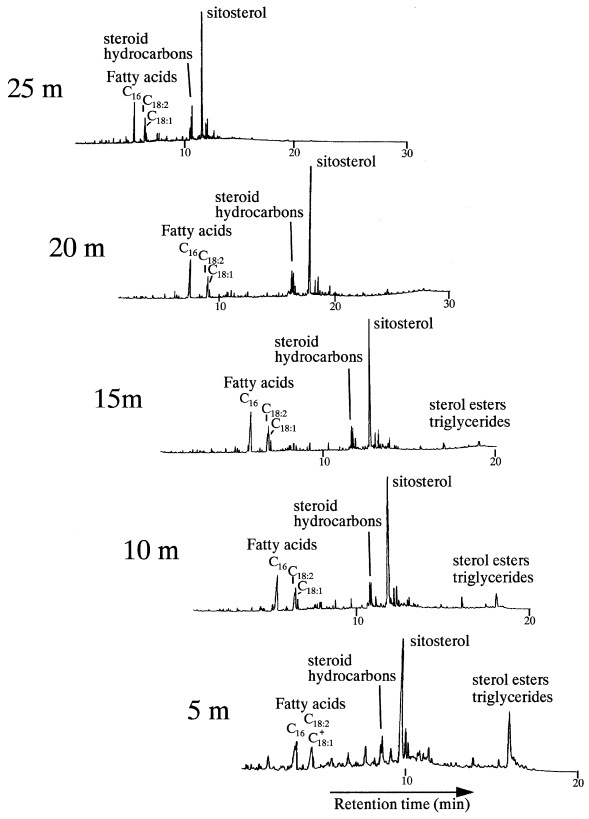

Fig. 1. GC–FID chromatograms of the lipid extract of eucalypt wood obtained with high-temperature capillary columns of different lengths. The oven was temperature-programmed as follows: for the 5 m, 10 m and 15 m columns, from 100°C (1 min) to 350°C (3 min) at 15°C/min; for the 20 m column, from 100°C (1 min) to 350°C (5 min) at 10°C/min; and for the 25 m column, from 100°C (1 min) to 380°C (15 min) at 20°C/min. The identity of major compounds is shown in the chromatograms.

the optimum, in terms of resolution, with a need to limit the exposure time of the sample to high temperatures to the minimum [15].

After this comparative study, a 5 m capillary column was selected for the rapid analysis of wood extractives, since it enables elution and separation of compounds in a wide range of molecular masses (from fatty and resin acids to sterol esters and triglycerides) in the same chromatographic analysis in a short period of time (20 min) and with resolution enough for the quantitation of the different component groups. As an example of the analysis of lipophilic wood extractives by GC using the 5 m high-temperature capillary column, the chromatograms corresponding to the extractives isolated from eucalypt wood and pine sapwood and a selected pitch deposit, are shown in Fig. 2.

A mixture of standard compounds (palmitic acid, dehydroabietic acid, sitosterol, cholesteryl oleate and triheptadecanoin) with a concentration range between 0.1 and 1 mg/ml was used to elaborate a calibration curve for the quantitation of wood extractives (shown in Fig. 3). The correlation coefficient was higher than 0.99 in all the cases. As an example of the quantitation of the GC analyses, the composition of the main lipid classes from eucalypt wood and pine sapwood is shown in Table 1.

The chromatograms obtained by GC–FID using the 5 m capillary column had to be reproducible in GC–MS in order to identify the different compounds. However, GC–MS systems cannot support short-length columns. The minimum column length that could be used in the Varian Saturn 2000 GC–MS system for a capillary column of 0.25 mm I.D. was

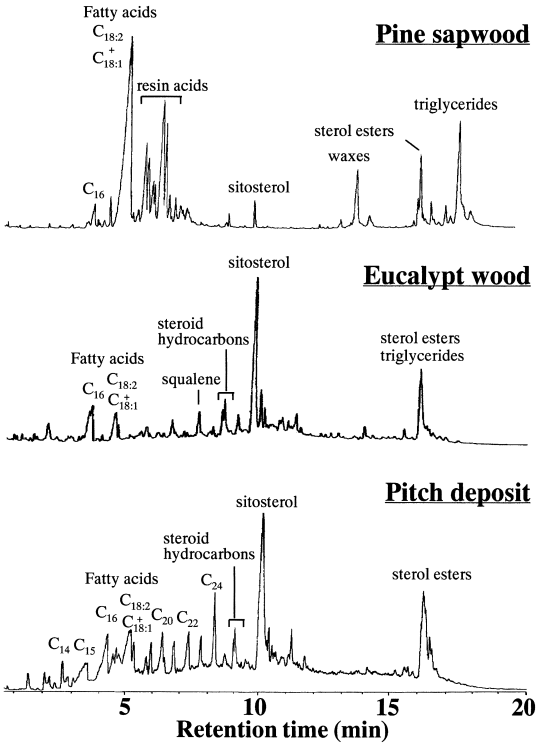

Fig. 2. GC–FID chromatograms of lipophilic extractives from pine sapwood, eucalypt wood and a pitch deposit in an eucalypt kraft pulp, obtained with the short capillary column (5 m). The oven was temperature-programmed from 100°C (1 min) to 350°C (3 min) at 15°C/min. The identity of major compounds is shown in the chromatograms.

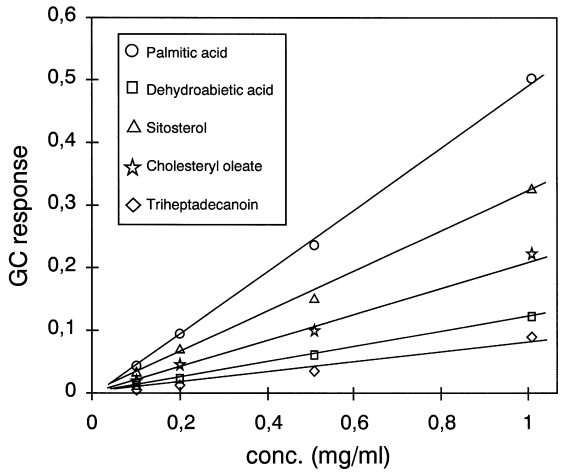

Fig. 3. Calibration graph obtained with the different lipid standards.

Table 1
Composition of the main lipid classes present in the extractives of eucalypt wood and pine sapwood

	Eucalypt wood (mg/100 g wood)	Pine sapwood (mg/g wood)
Fatty acids	27.7±1.0	4.0±1.0
Resin acids	0	8.9±0.9
Sitosterol	49.4±1.0	0.2±0.02
Waxes	5.8±0.5	1.6±0.3
Sterol esters	51.7±1.7	1.2±0.2
Triglycerides	13.2±0.4	7.3±2.0

Standard deviations were calculated with three replicates.

limited to 15 m. Although the elution of sterol esters and triglycerides was not optimal with this column length, it was sufficient for allowing their identification by mass spectra. The utilization of a high-temperature capillary column made it possible to increase the final temperature up to 380°C necessary for the detection of sterol esters and triglycerides in a 15 m capillary column in a short period of time (30 min). The good reproducibility of chromatograms of the eucalypt wood extractives obtained by GC–FID with a 5 m column and by GC–MS with a 15 m column, is shown in Fig. 4. Although some triglycerides and sterol esters from eucalypt wood may elute closely, their differentiation is possible in the 15 m column by their mass spectra. On the other hand, this has not been a problem for the quantitation in GC–FID when the purpose was categorizing the extractives into chemical classes as in the case of the quantitation of wood extractives degradation attained by fungal treatment. However, when a more accurate characterization of some compounds was required, the extract was fractionated by a simple SPE procedure that is described below.

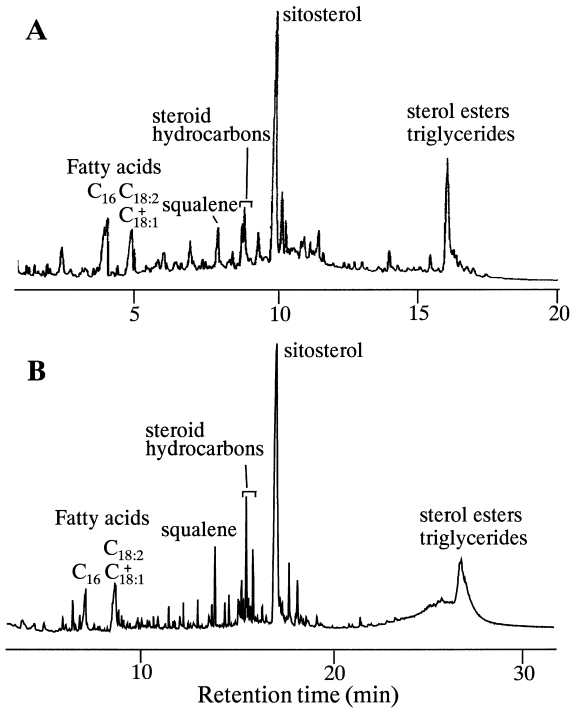

Fig. 4. Chromatograms of the lipid extract of eucalypt wood obtained in (A) GC–FID (DB5-HT, 5 m), temperature-programmed from 100°C (1 min) to 350°C (3 min) at 15°C/min, and (B) GC–MS (DB5-HT, 15 m), temperature-programmed from 120°C (1 min) to 380°C (5 min) at 10°C/min. The identity of major compounds is shown in the chromatograms.

3.2. SPE fractionation of complex wood lipid mixtures

A basic method for the separation of individual neutral and polar lipid classes using aminopropyl-bonded SPE cartridges was developed by Kaluzny et al. [13] for the separation of lipids from bovine adipose tissue. Chen et al. [16] adapted this SPE method to the fractionation of wood extractives with little success. Indeed, it was too time-consuming and involved the use of three different SPE cartridges and a multi-step fractionation that resulted in seven final fractions.

In the present work, wood lipophilic extractives are also fractionated in aminopropyl-bonded SPE cartridges, however only one cartridge is used per sample and some elution steps have been eliminated resulting only in four final fractions. The SPE method outlined here have been optimized for the case of eucalypt wood extractives. The recovery rate was approx. 95% and the purity of each isolated fraction was confirmed by GC and GC–MS using the chromatographic methods described above. The proportions of the different fractions isolated from eucalypt wood lipophilic extractives (means of two samples) were the following: fraction A: 34±1%, fraction B: 8±0.8%, fraction C: 32±1% and fraction D: 26±1.5%.

Fig. 5 shows the GC–MS chromatograms of the different SPE fractions isolated from eucalypt wood lipophilic extractives. Sterol esters, waxes and hydrocarbons (predominantly squalene) were recovered in fraction A, whereas triglycerides eluted in fraction B, sterols in fraction C, and free fatty acids in fraction D. In the SPE procedure, lipids elute from the aminopropyl column in order of increasing polarity. Polar lipids such as fatty acids or those having a polar group such as sterols are likely to interact more strongly with the aminopropyl group on the columns through hydrogen bonding to the primary amine group [12].

On the other hand, when the sample is taken up in

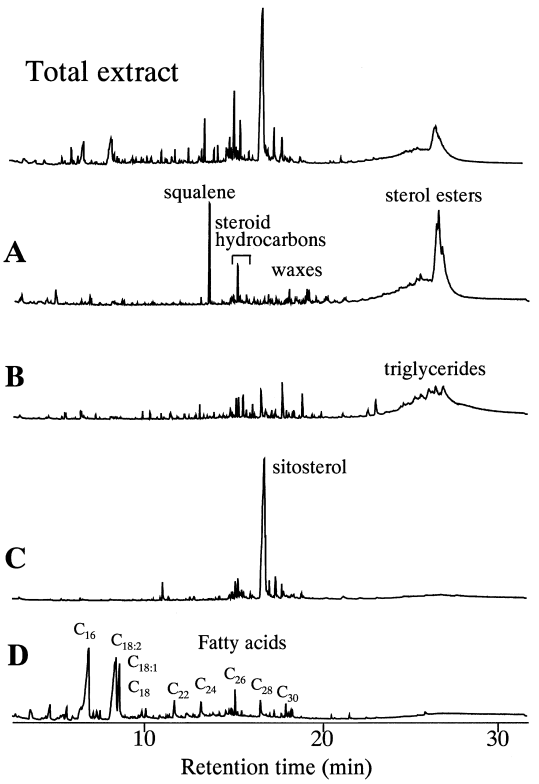

Fig. 5. GC–MS chromatograms of the fractions obtained from eucalypt wood extractives after SPE fractionation in aminopropyl columns. The oven was temperature-programmed from 120°C (1 min) to 380°C (5 min) at 10°C/min. The identity of major compounds is shown in the chromatograms.

pure chloroform and the column subsequently eluted with chloroform–hexane (1:5), as described by Chen et al. [16], triglycerides and sterols may coelute in fraction A. This does not occur if the sample is taken up in pure hexane and subsequently eluted with the same solvent. However, since the eucalypt wood extractives were not totally soluble in pure hexane, it was necessary to use the mixture hexane–chloroform (4:1) in which these samples were completely soluble. The proportion of chloroform should not be increased, since higher amounts of the more polar solvent may result in some triglyceride elution in fraction A.

The SPE fractionation method described here has also been successfully applied to the fractionation of the extracts of pitch deposits occurring during the Kraft pulping of eucalypt wood (not shown here).

4. Conclusions

High-temperature, short-length capillary columns have been used for the rapid identification and quantitation of lipophilic wood extractives with no prior derivatization nor fractionation. This analytical method is being routinely used in our laboratory for the evaluation of biological removal of extractives from eucalypt and pine woods, which implies the analysis of a great number of samples. Analysis of lipophilic extractives from pine and eucalypt woods, and a pitch deposit have been carried out. For a more detailed characterization of some compounds that are in minor proportion in the extracts or elute jointly, a simple SPE fractionation procedure has been developed for the separation of complex lipid mixtures into major fractions. Although the SPE procedure has been developed specifically for the fractionation of lipids from eucalypt wood, it can also be applied to other wood species or pitch deposit extracts. The advantages of using SPE include smaller sample and solvent requirements and simpler use than conventional solvent extraction techniques.

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