Fatty and Resin Acid Analysis in Tall Oil Products via Supercritical Fluid Extraction–Supercritical Fluid Reaction Using Enzymatic Catalysis

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Abstract

Supercritical fluid extraction (SFE) is combined with supercritical fluid reaction (SFR) in an analytical mode to assess tall oil products for their fatty or resin acid content or both. The SFR consists of an inline enzymatically catalyzed reaction in which a lipase transesterifies specific lipids with methanol. The SFE–SFR sequence is conducted employing commercially available extractors using supported lipases in the extraction cell to form methyl esters. In this study, six different commercially available lipases are screened for activity. The SFE–SFR extracts are analyzed by capillary gas chromatography and supercritical fluid chromatography and then compared with tall oil products derivatized by conventional chemical derivatization techniques.

Introduction

Tall oil is a dark, odorous liquid that is a by-product from the sulfate (kraft) process of paper manufacturing. It is obtained by chemically treating the cooking liquor used in the operation of pulping wood for paper. Fatty acids, rosin acids, sterols, and other compounds principally make up this resinous material, which is used after refining to make coatings, sizing for paper, paint, varnish, linoleum, drying oils, emulsions, lubricants, and soaps (1). Recently, Jones et al. (2) even reported that humans who fed on a diet enriched in micronized tall oil phytosterols had reductions in total cholesterol and low-density lipoproteins.

The chemical composition of tall oil varies with the age, species, and geographical location of the source coniferous trees. The resin acids are diterpene carboxylic acids based on an alkyl-substituted perhydrophenanthrene ring structure, and the fatty acids are predominantly 18-carbon, straight-chain mono- or diunsaturated fatty acids. Essentially, all of the crude tall oil produced today is refined by fractional distillation for use in the previously specified applications. For many of theses applications, it is desirable to know the level of the individual fatty and resin acids present in these products (3).

Capillary gas chromatography (GC) has been routinely used to analyze crude tall oil (4–8), distilled tall oil (6,8), and tall oil rosin (9) after the fatty acids and resin acids have been converted to methyl esters via diazomethane (a somewhat tenuous reagent). However, McGuire and Powis (3) have successfully employed an alternative derivatizing agent (*N,N*-dimethylformamide dimethylacetal) for the preparation of the methyl esters of fatty acids and resin acids in tall oil fractionation products prior to GC. This reagent provides safe, rapid, and reproducible derivatization that eliminates the need to isolate the methyl esters from the derivatization reaction mixture.

Supercritical fluid extraction (SFE) and supercritical fluid reaction (SFR) (i.e., derivatization) methods are becoming increasingly popular as a result of the use of environmentally benign supercritical carbon dioxide (SC-CO_2). This interest in such methods is a result of the reduction or elimination (or both) of organic solvents, improvements in reaction equilibrium, and faster reaction times (10–12). Recently, fatty acid methyl ester (FAME) formation under supercritical conditions has been reported (13–19). Many of these studies used an immobilized lipase to enzymatically catalyze the transesterification of the lipids with methanol to FAMEs.

In this study, SFE coupled with an inline enzymatically catalyzed reaction was used to assess the fatty and resin acid content of tall oil products (crude tall oil, crude fatty acid, and rosin acid). The resultant extracts were analyzed by capillary GC and supercritical fluid chromatography (SFC). The SFE–SFR results were compared with results obtained by the chemical derivatization of the previously mentioned corresponding tall oil products.

Experimental

Materials

Crude tall oil, crude fatty acid, and crude rosin acid were provided by Dr. David McMahon (formerly of Union Camp Corporation, Princeton, NJ). Abietic acid was purchased from

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the products to the exclusion of others that may also be suitable.

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Aldrich Chemical Company (Milwaukee, WI), and dehydroabietic acid was acquired from Helix Biotech (Richmond, BC, Canada). A BF_3–MeOH reagent (14%, w/v) was obtained from Alltech Associates (Deerfield, IL), and Methyl-8 Concentrate (*N,N*-dimethylformamide dimethylacetal) was acquired from Pierce Chemical Company (Rockford, IL).

The lipases used in this study—Novozym SP 435 (derived from *Candida antarctica*) and Lipozyme IM (derived from *Mucor miehei*)—were gratis samples from Novo Nordisk (Franklington, NC). Chirazyme L-1 (a lipase derived from *Burkholderia sp.*) was provided by Boehringer Ingelheim Corp. (Indianapolis, IN). Lipase Type VII from *Candida cylindracea* (70% protein) and two Type II lipases (crude 25% protein and crude 70% protein) from porcine pancreas were purchased from Sigma Chemical (St. Louis, MO). Accurel EP100 (macroporous polypropylene, particle size of 200–1000 µm), which was used as an enzyme support, was acquired from Akzo Nobel (Orenberg, Germany), and mono- and dibasic phosphate were obtained from EM Science (Gibbstown, NJ).

SFE–SFR

SFE–SFR with an inline lipase catalyst was performed with an Isco (Lincoln, NE) Model SFX 3560 extractor whenever Novozym SP 435, Lipozyme IM, or Chirazyme L-1 lipases were used. SFE–SFC-grade CO_2 (Air Products and Chemicals, Inc., Allentown, PA) was used for all SFE–SFR experiments. Novozym SP 435, Lipozyme IM, and Chirozyme L-1 were used as received. Approximately 1.5–2.0 g of each enzyme (supported) were added to a 10-mL extraction cell. The excess cell volume was then filled with approximately 0.4 g Chem-Tube Hydromatrix (Varian, Harbor City, CA), and the various tall oil products (approximately 0.2 g) were applied to the top (inlet) of the bed. SFE–SFR as previously described by Snyder et al. (17) was then initiated, and the extract was collected after the fluid was depressurized through the heated restrictor.

SFE–SFR was also performed using an Isco Model SFX 2-10 extractor when using the Type VII and the porcine pancreas lipases. Lipase Type VII and the two porcine pancreas lipases were immobilized on Accurel EP100 according to the procedure of Bosley and Peilow (20) prior to SFE–SFR . SFE–SFR with porcine pancreas lipase Type II (crude 25% protein) was performed as described previously, except that a flow rate of 0.6 mL/min was used. SFE–SFR employing porcine pancreas lipase Type II (crude 70% protein) was performed using a 0.5-mL cell with approximately 20 mg of immobilized enzyme, approximately 30 mg of crude tall oil, and 60 mL of extraction/reaction fluid at a 0.6-mL/min flow rate. SFE–SFR with Lipase Type VII was performed in a 2.5-mL cell using approximately 50 mg of immobilized enzyme, approximately 30 mg of crude tall oil, and 60 mL of extraction/reaction fluid at 0.6-mL/min. Chem-Tube Hydromatrix was used in all cases to fill the cell void volume and act as the inert support for the crude tall oil samples.

Chemical analyses

SFC

SFC was performed on a Lee Scientific Series Model 600 chromatograph (Dionex Corporation, Sunnyvale, CA) equipped with a flame ionization detector held at 350°C. A Dionex SB-Phenyl-50 capillary column (10-m × 100-µm i.d., 0.5-µm film thickness) held at 100°C was used with the following pressure gradient: 100 atm held for 5 min, then raised 4 atm/min to 280 atm, and then held for 5 min. Injection of the samples was accomplished by a timed split automatic injector with a Valco valve (Valco, Houston, TX) for 0.5 s with a 200-nL loop. SFE–SFC-grade CO_2 (Air Products and Chemicals, Inc.) was used as the carrier fluid. Chromatograms were analyzed with a Data Jet integrator (Spectra-Physics Analytical, San Jose, CA).

GC

Extracts were analyzed by a 0.5-min split-delay splitless injection onto a Hewlett-Packard (Santa Clarita, CA) 6890 Series GC equipped with an HP-5 column (30-m × 0.32-mm i.d., 0.25-µm film thickness) using helium as the carrier gas at a linear flow velocity of 33 cm/s. The temperature program began at 150°C for 5 min and then raised to 280°C at 5°C/min, with a final hold time of 5 min. The injector temperature was 250°C and the flame ionization detector was held at 300°C. Injections were made using an HP Model 6890 series autoinjector with a sample volume of 1 µL. The chromatographic data were acquired using HP GC ChemStation software.

GC–mass spectrometry

Extracts were analyzed by a 0.5-min split-delay splitless injection onto a Hewlett-Packard 5890 Series II GC equipped with an SP-2380 column (60-m × 0.25-mm i.d., 0.20-µm film thickness) (Supelco, Bellefonte, PA) using helium as the carrier gas at a linear flow velocity of 18 cm/s. The temperature program was 150°C for 5 min and then increased by 5°C/min to 250°C. The injector temperature was set at 235°C. Injections were made using an HP Model 7683 auto-injector with a sample volume of 1 µL. Electron-impact mass spectra were obtained using an HP Model 5971 mass-selective detector at an ionization potential of 70 eV. The chromatographic data were acquired using an HP Vectra VL2 computer and ChemStation software.

Results and Discussion

The catalytic activity of lipases in SC-CO_2 has been investigated previously (13–19), but it was limited to the transesterification of lipids in oil seed and meat matrices. This study attempted to successfully extend SFE–SFR methodology to nonedible oil matrices containing resin acids along with fatty acids.

The initial SFE–SFR experiments were performed on the crude tall oil and crude fatty acid samples. The analyses were conducted using SFC to compare the starting material with the final SFE–SFR extract. As shown in Figure 1, the SFC of an SFE–SFR extract clearly showed the formation of FAMEs. However, the area of the chromatogram in which the resin acids and resin acid methyl esters (RAMEs) eluted was quite complex, and resin acid standards were not initially available for identification purposes. The extract was also examined by GC using the methodology of House et al. (21) developed for nutritional FAME-based analysis. Again, without standards it was impossible to tell if the resin acids

or RAMEs or both were even being eluted from the column.

Abietic and dehydroabietic acid were then obtained, and chemical derivatization used to form the methyl esters was attempted with BF_3–MeOH according to the procedure of House et al. (21). However, even using the BF_3–MeOH at full strength (14%, w/v), complete methyl-ester formation was not accomplished, as shown by the SFC in Figure 2. The resin acid standards were then subjected to chemical derivatization using Methyl-8 Concentrate according to McGuire and Powis (3), and total methyl ester formation was achieved, as shown by SFC. The tall oil products were then derivatized with Methyl-8 Concentrate to form the methylated standards for SFE–SFR extract comparison.

The crude tall oil, fatty acid, and rosin acid products were mixtures of multiple peaks. The two predominate resin acids were abietic and dehydroabietic acid. Oleic, linoleic, palmitic, and stearic acids were the major fatty acids, with oleic acid and linoleic acid being prevalent. This was confirmed via SFC, GC, and GC–mass spectrometry (MS) using the acid standards (neat and methylated). Minor rosin components (isopimaric, pimaric, and neoabietic acids) were also tentatively identified through the use of the Wiley mass-spectral library of the GC–MS.

SFE–SFR with the six lipases exhibited a range of enzymatic activity. The three lipases that were immobilized on Accurel EP100 provided very little conversion to the methylated products. This result is not surprising because Frykman et al. (14) have previously reported contrasting lipase activity under supercritical conditions with various lipases immobilized on the same support. In that study, some of the lipases exhibited decreased activity when immobilized on Accurel EP100 under supercritical conditions, and other lipases showed strong activity in facilitating methylation.

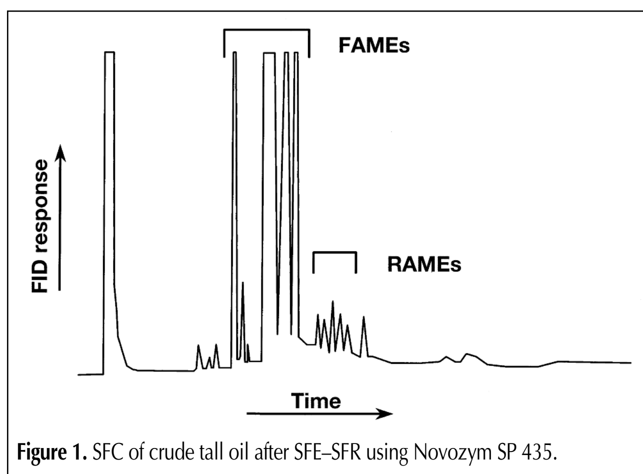

Figure 1. SFC of crude tall oil after SFE–SFR using Novozym SP 435.

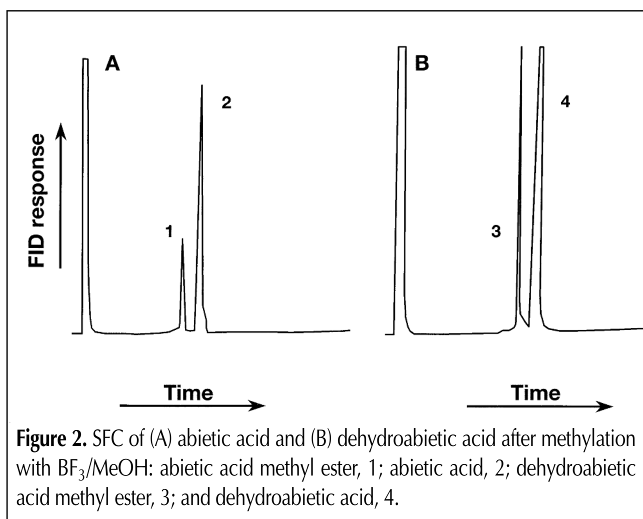

Figure 2. SFC of (A) abietic acid and (B) dehydroabietic acid after methylation with BF_3/MeOH: abietic acid methyl ester, 1; abietic acid, 2; dehydroabietic acid methyl ester, 3; and dehydroabietic acid, 4.

The Type VII lipase and the Type II porcine pancreas lipase (70% protein) did not form the methyl esters for either of the fatty or resin acids when crude tall oil was the starting matrix. In order to further confirm the low activity of these lipases, the SFE–SFR of soyflakes was conducted using both lipases. Soyflakes were selected as a test sample because of our experience with this matrix (15,19). In both cases, SFC analysis demonstrated a very minor amount of FAME formation (i.e., a large amount of triglyceride remained underivatized).

The Type II porcine pancreas lipase (25% protein) did form some FAMEs with crude tall oil as the matrix, but some (approximately 30%) of the original fatty acids remained. However, no RAMEs were formed. Again, the lipase was subjected to SFE–SFR using soyflakes to check its activity. FAMEs were formed, but a small amount of triglyceride remained, thus affirming with a second matrix that total FAME formation did not occur with this enzyme.

All three of the enzymes that were immobilized prior to receiving them yielded complete FAME formation when SFE–SFR was performed on the tall oil matrices. Only Novozym SP 435 formed RAMEs, as shown via SFC and GC–MS analysis. However, RAME formation was very minor, and the material balances for the reaction (derivatization) did not equal unity. It was noted that a small amount (10% wt) of the resin acids adsorbed onto the Chem-Tube Hydromatrix, and the other 90% wt was being retained by the Novozym SP 435. This was shown by recovering the enzyme and Hydromatrix materials from the extraction cell and washing them with chloroform and then performing subsequent analyses of these washes via SFC. Upon concentration of the enzyme and Hydromatrix washes, the resultant collected masses were recorded. It was found that the mass of unconverted tall oil material in addition to the original SFE–SFR extract yielded 100% mass balance for the reaction.

Lipozyme IM also behaved similarly in retaining the resin acid portion of the tall oil products. Again, the resin acids could be desorbed with a chloroform washing. As stated previously, Chirazyme L-1 did not form RAMEs, but it did not adsorb resin acids such as Novozym SP 435 and Lipozyme IM either. Such differential derivatizations, as well as an assessment of the converted and unconverted tall oil products, provides valuable quantitative information.

Conclusion

Transesterification of the fatty acids in the tall oil matrices with SC-CO_2 using enzymatic catalysis was successful to varying degrees. The three lipases that were immobilized on the Accurel EP100 had very little activity under the supercritical conditions, whereas the lipases that were received commercially immobilized exhibited strong transesterification activity. However, the resin

acids remained unesterified, with the exception that Novozym SP 435 produced RAMEs to a limited extent. This lack of RAME formation via enzymatic methanolysis may be because of a steric inhibition of the resin acid into the active site of the enzyme resulting from their tertiary structure. The presence of bulky groups near the carboxylic acid group of the resin acids inhibits the transesterification, because substrate reactivity follows the order of primary > secondary > tertiary acids (22).

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