Extraction of Woolgrease with Supercritical Carbon Dioxide

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Supercritical carbon dioxide was used to extract unrefined woolgrease. The resulting product had no color and little odor, resembling high-grade commercial lanolin. The amounts of woolgrease extracted for fixed volumes of supercritical fluid solvent were measured at 60, 70 and 80°C and at pressures from 200–520 bar. In this study, the highest recoveries were obtained at 80°C and pressures in excess of 380 bar. Analysis of the extracts by capillary supercritical-fluid chromatography and thin-layer chromatography indicated that the composition of the extract changed with extraction time. The earliest fractions were enriched in cholesterol and contained relatively small amounts of species with higher molecular weight. The results suggest that supercritical fluid extraction may be used to obtain an enriched cholesterol fraction from woolgrease.

KEY WORDS: Cholesterol fractionation, lanolin, supercritical-fluid chromatography.

Supercritical fluid extraction has been considered for a wide variety of separations, including coffee decaffeination, the extraction of hops, spices and flavor oils (1), and the removal of edible oils from seeds (2). Some of the earliest work on the use of compressed gas solvents was performed by Zhuse *et al.* (3) who demonstrated the feasibility of using compressed propane, propylene, butane and butylene to extract lanolin from raw woolgrease. The extractions were done at 100°C and pressures from 60–103 bar. Lanolin is derived from woolgrease commercially by a process of deodorizing, bleaching and neutralizing, to yield a light-colored material with little odor and low free fatty acid and moisture contents (4). Due to its inertness and ease of emulsification, lanolin is widely used in the cosmetic and pharmaceutical industries. Extraction of lanolin with supercritical carbon dioxide (CO_2) may offer advantages over the current methods because a light-colored, low-odor product may be obtained by properly adjusting the extraction temperature and pressure. In this study, we consider the use of CO_2, a nontoxic, nonflammable solvent, to obtain lanolin from woolgrease. We performed extractions from 250–550 bar and 60–80°C.

EXPERIMENTAL PROCEDURES

Extraction experiments. The experiments in this study were performed with a supercritical-fluid extraction unit designed and manufactured by Newport Scientific (Jessup, MD) (Fig. 1). The unit consisted of a 1000-mL extraction vessel connected by high-pressure tubing to a series of two metering valves and a 250-mL glass separation flask. The unit included a 700-bar double-ended diaphragm compressor, a flow meter and a flow totalizer. The pressure was controlled with a back-pressure regulator. The pressure and temperature of the extraction vessel were kept constant, and the metering valves were heated to prevent the extracted woolgrease from precipitating and blocking the tubing.

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To conduct the experiments, 40–60 g of commercially obtained woolgrease (Amerchol Corporation, Edison, NJ) was held between pieces of clean glass wool and placed in the extractor. CO_2 gas from the supply tank was compressed to the desired pressure and slowly pumped through the extractor. The pressure of the CO_2 was controlled by a back-pressure regulator. Upon leaving the extractor, the CO_2 stream, enriched in the woolgrease, flowed through the metering valves, where the resulting change in pressure caused the extracted woolgrease to precipitate in the collection flask.

The amount of woolgrease extracted as a function of temperature and pressure was determined by measuring the solute that was collected from a known volume of CO_2 (100 L measured at room temperature and atmospheric pressure). The measurements were made at 60, 70 and 80°C and pressures from 200–550 bar. The flow rate was controlled between 0.8 and 0.11 L/s. At least three measurements were made at each condition.

An exhaustive extraction was also performed to study the changes in extract yield and composition with extraction time. In this experiment, 60.4 g of woolgrease was held between pieces of clean glass wool, and CO_2 was passed continuously through the extractor until the appearance of solute in the collection flask stopped. The experiment was run at 80°C and 520 bar. Four samples were collected. Fractions 1, 2 and 3 were collected during the initial 210, 215 and 217 min of the experiment, respectively, and Fraction 4 was collected during the final 290 min of the experiment. The unextracted residue that remained in the extractor was removed from the glass wool by Soxhlet extraction with hexane. Melting point curves were obtained for each fraction by differential scanning calorimetry.

Sample analysis. The fractions and the unextracted residue were analyzed by capillary supercritical-fluid chromatography in a Lee Scientific Model 501 chromatograph (Dionex Corp., Salt Lake City, UT). The mobile phase was CO_2 (Scott Specialty Gases, Plumsteadville,

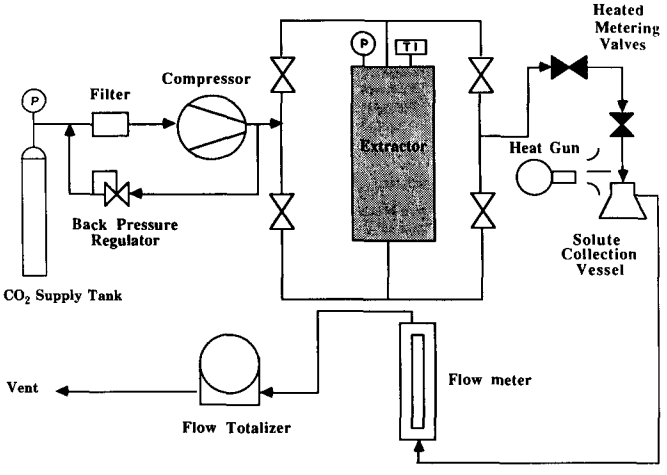

FIG. 1. Experimental apparatus to extract woolgrease with supercritical carbon dioxide (CO_2). P, pressure gauge; TI = temperature indicator.

PA), which was delivered by a computer-controlled syringe pump. Several capillary columns, consisting of cross-linked polysiloxane bonded polymer phases, were evaluated for separation of the woolgrease fractions. These included the SB-Methyl, SB-Octyl, SB-Biphenyl-30 and SB-Cyano-50 columns (Lee Scientific). An SB-Octyl capillary column, 15 m long, 50 micron internal diameter with a stationary phase film thickness of 0.25 micron, produced the best resolution of the complex mixtures.

Several density and pressure programs were evaluated to produce optimum chromatographic fractionation of the lanolin mixtures. The best separation was obtained with a density program that consisted of holding the density constant at 0.28 g/mL for 15 min, followed by a linear increase in density at 0.006 g/mL/min to a density of 0.66 g/mL with a subsequent 5-min hold. The oven temperature was maintained at 120°C, with the flame-ionization detector set at 350°C. Samples were dissolved in *n*-hexane with gentle warming at the 3.0-wt% level and injected with a modified, cooled Valco (Valco Instruments, Houston, TX) injection valve. All injections were made from the contents of a 200-nL fixed-volume internal loop within the Valco valve.

The fractions were also analyzed by thin-layer chromatography (TLC). The samples were initially dissolved in methylene chloride in concentrations of approximately 40 mg/mL and chromatographed with a solvent system composed of 80:20:0.5 petroleum ether, diethyl ether and glacial acetic acid on a plate of Silica Gel G. Each sample solution was applied in a 5-μL aliquot.

RESULTS AND DISCUSSION

The amount of woolgrease extracted as a function of temperature and pressure is shown in Figure 2. Note that the amount of woolgrease in the supercritical CO_2 increases with increasing pressure up to 450 bar. Note also that more woolgrease is extracted at higher temperature, probably due to the increased vapor pressure of the woolgrease components. Zhuse *et al.* (3), who used compressed ethane, propane, propylene and butane, achieved higher recoveries. Their results ranged from 0.062 g/L at 105°C and 76 bar with a mixture of propane, ethane and butane to 1.25 g/L at 100°C and 71 bar for a mixture of propane, propylene, butane and butylene. Zhuse *et al.* (3), however, noted serious discoloration of the product at higher pressures. Moreover, the flammable nature of these gases raises safety concerns. Although the highest recovery obtained in this study was 0.013 g/L at 80°C and 450 bar, the product had light color and little odor. Therefore, despite greater solubility with other compressed solvents, the use of CO_2 may be advantageous because it yields a high-quality product and does not present a flammability danger.

The results of the differential scanning calorimetry analysis of the samples, collected during the exhaustive extraction experiment, are shown in Table 1. Because the complex melting curves did not show a sharp minimum, the values listed should be viewed as the temperature for complete melting. Note that the melting points of the fractions increased with increasing extraction time.

A typical supercritical-fluid chromatogram obtained from the analysis of unextracted woolgrease is shown in Figure 3. The solvent peak (*n*-hexane) is followed by the

TABLE 1

Melting Points of Extracted[a] Woolgrease Fractions as Determined by Differential Scanning Calorimetry

Fraction	Volume of CO_2 used (L)	Weight of extract recovered (g)	Melting point (°C)
Unrefined woolgrease	—	—	39
1	1192	18.7	40
2	1284	14.7	40
3	1681	9.5	44
4	1549	3.8	46
Residue	—	7.9	56

[a]Extraction at 80°C, 520 bar. Flow rate of carbon dioxide (CO_2) was 0.08–0.11 L/s. Total extraction time, 920 min. Fractions 1–3 collected at approximately 210-min intervals, fraction 4 collected during final 290 min.

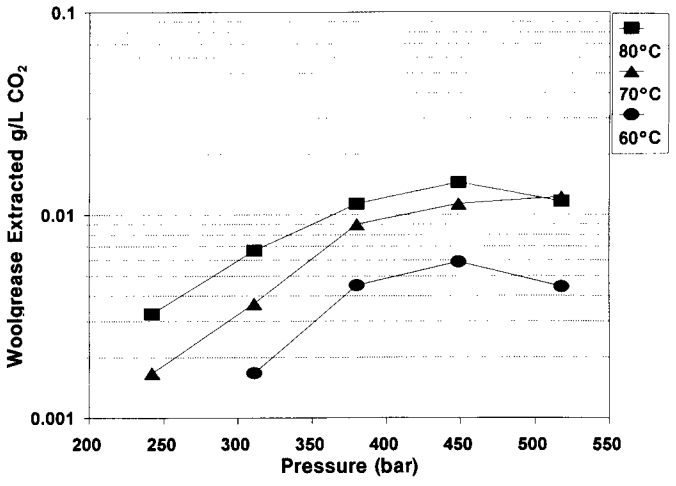

FIG. 2. Amount of woolgrease recovered per L CO_2 as a function of temperature and pressure. CO_2 flow rate: 0.08–0.11 L/s. See Figure 1 for abbreviation.

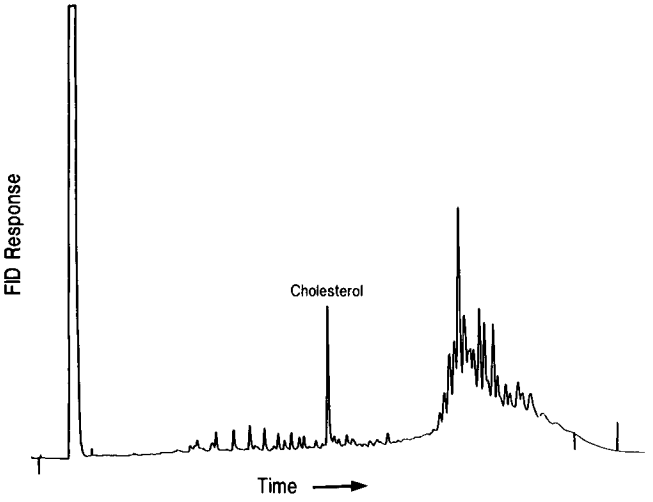

FIG. 3. Supercritical-fluid chromatogram of unrefined woolgrease. Chromatographic conditions are given in the text. FID, flame-ionization detector.

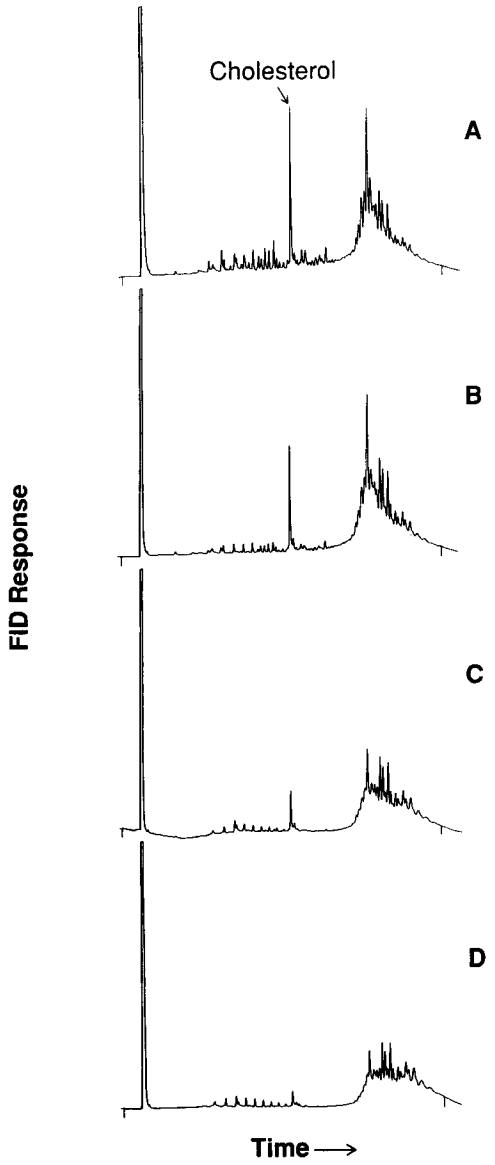

FIG. 4. Supercritical-fluid chromatograms of extracted woolgrease fractions. A–D, Fractions 1–4. Extraction conditions are the same as in Table 1. See Figure 3 for abbreviation.

woolgrease components, each succeeding component being eluted at higher mobile-phase gas density as the control program progresses. Standard solutes were injected and chromatographed under the same elution conditions, permitting the identification of cholesterol and several cholesteryl esters on the basis of retention times. The partially resolved profile at the end of the chromatogram contains the wool wax esters. Attempts to further resolve these moieties by different columns or chromatographic programs were not successful.

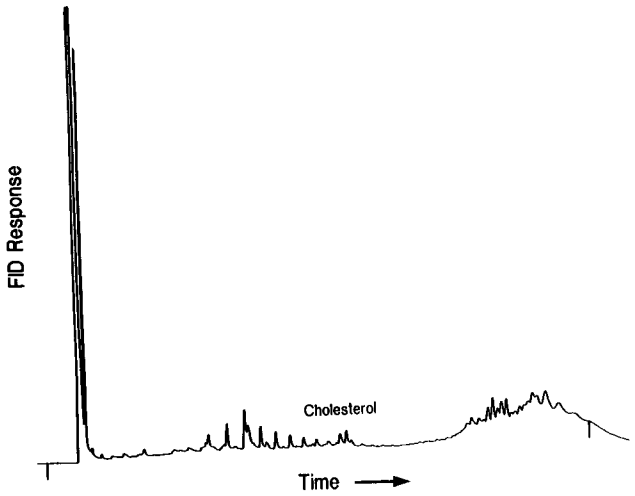

FIG. 5. Supercritical-fluid chromatogram of unextracted residue from exhaustive extraction experiment. Extraction conditions are the same as in Table 1. See Figure 3 for abbreviation.

The chromatographic profiles of the fractions collected during the exhaustive extraction experiment are shown in Figure 4. Inspection of the chromatograms indicates a gradual decrease in the cholesterol and lower-molecular weight compounds as a function of the extraction time. The wool wax ester profiles show a subtle shift in composition, primarily due to an enrichment of the higher-molecular weight components. This result explains the higher melting points of the last fraction and the unextracted residue. Note that there is only a trace of cholesterol remaining in the chromatograph of the unextracted residue (Fig. 5).

These trends were confirmed by TLC. The TLC plates (data not shown) indicated that unrefined woolgrease is composed mainly of long-chain esters with small quantities of free sterols (including cholesterol) and long-chain fatty acids. In contrast, the residue from the supercritical fluid extraction contained little of the long-chain esters and was comprised primarily of high-molecular weight, polar materials, which remained at the origin of the TLC plate.

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