Characterization of Supercritical Fluid Extracts from Raw Wool by TLC-FID and GC-MS

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ABSTRACT: Extraction selectivity of whole wool wax from raw wool by using ethanol-modified carbon dioxide at constant pressure (100 bar) and temperature (60°C) was studied. A kinetic study was carried out to optimize the percentage of ethanol and the extraction time. The modified carbon dioxide was decompressed in ethanol as a collection solvent, leading to the formation of two different fractions as a function of their solubility. Qualitative and quantitative analyses of the lipid classes present in the collected fractions were performed by TLC coupled to an automated FID system (TLC-FID). Moreover, a detailed structural comparison was carried out between the aliphatic high-molecular-mass esters and the steryl ester patterns by means of subambient pressure GC-MS in both the EI and the ammonia positive CI modes. Considerable differences in the lipid composition of the two wool wax fractions collected were observed by TLC-FID and GC-MS.

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KEY WORDS: Lanolin, supercritical fluid extraction, supercritical fluid fractionation, wool wax characterization and fractionation.

Wool wax, which forms a natural protective coating on wool fibers, is a unique substance secreted by the sebaceous glands of sheep. It constitutes 10–25% of the weight of the sheared greasy wool. Lanolin, the refined form of wool wax, is an important ingredient used in pharmaceutical or cosmetic formulations. Chemically, it consists of a complex mixture of monoesters, diesters, and hydroxy-esters of high-M.W. alcohols (aliphatic and steroidal) and FA (aliphatic, α-hydroxy, and ω-hydroxy) as well as free fatty alcohols, FFA, and sterols (1–5).

Nowadays, the crude wool wax is removed from raw wool by an industrial process using aqueous nonionic surfactant solutions at a neutral or alkaline pH and is subsequently recovered by centrifuging (6). For commercial wool testing and wool wax extraction, the most commonly used method is a solvent extraction method with dichloromethane (DCM) (7). Recently, the use of supercritical fluid extraction (SFE) with carbon dioxide (CO_2) as a solvent has been studied in an attempt to avoid or minimize the use of chlorinated organic solvents to determine the wool wax in wool fibers (8–10). Although the extraction selectivity of the different wool lipid classes has already been studied under different experimental conditions such as pressure, temperature, extraction time, and modifier (11–13), fractionation in the postextraction steps has not yet been attempted.

A study of wool wax extracted from raw wool fibers with pressurized CO_2 at constant pressure and temperature (100 bar, 60°C) was carried out. Based on an earlier paper, ethanol was chosen as a modifier given that it provides the highest extraction yields (14). In this paper, in order to optimize the percentage of ethanol used and the extraction time, a kinetic study was performed using two different percentages of ethanol. Under optimal conditions, two different fractions were obtained as a function of their solubility in ethanol: a white solid and an amber liquid. The lipid composition of the fractions collected was analyzed by a thin-layer chromatograph coupled to an automated FID (TLC-FID) and a gas chromatograph coupled to a mass spectrometer (GC-MS). TLC-FID enables a rapid separation and precise quantification of different lipid classes without sample pretreatment (15); in fact, this method has been used to study the lipid composition of different wool extracts (10,13,16–18). GC-MS permitted characterization at a molecular level, whereas TLC-FID enabled characterization in accordance with chemical classes, thereby providing complementary information. Subambient pressure GC coupled with MS (19,20) was chosen as an identification technique because it was the most suitable method to analyze the complex high-molecular-mass mixture of wool wax at lower elution temperatures compared with conventional GC (19,20). Prior to the MS analysis, SFE extracts were prefractionated by gel permeation chromatography (GPC) to obtain two fractions in accordance with their M.W. distribution. In this way, a cut-off time was defined to enrich the first fraction with target compounds such as steryl and aliphatic esters, and to remove the second fraction, which contains lower-M.W. compounds such as FFA, fatty alcohols, hydroxy acids, and diols. Owing to the high-molecular-mass compounds contained in wool wax, subambient pressure GC (20,21) was needed to carry out extract characterization. GC-MS methodologies have already been developed for characterization of steryl esters (21) and aliphatic esters (22), and they were applied in this study.

EXPERIMENTAL PROCEDURES

Materials and reagents. Australian Merino sheared raw wool (diameter of wool fibers ≅ 21 μm) was obtained from local sources. Prior to its extraction, the raw wool was hand

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homogenized and equilibrated in a conditioning room (24°C, 60% relative humidity). DCM (reagent grade) was obtained from Scharlau (Barcelona, Spain); formic acid (85%) was obtained from Probus (Badalona, Spain); and chloroform, methanol, *n*-hexane, diethyl ether, and benzene (all for analysis) were purchased from Merck (Darmstadt, Germany). SFE grade CO_2 (99.998%) was kindly supplied by Praxair España (Barcelona, Spain). Analytical grade ethanol from Merck was used as a modifier. Palmitic acid behenyl ester (≈99%) (mono-ES), behenyl alcohol (98%) (AL), behenic acid (99%) (FFA), cholesterol (>99%) (S) (all supplied by Sigma, St. Louis, MO), and synthesized dipalmitic acid hexadecyl ester (di-ES) were used as standard compounds for the TLC-FID lipid analysis. Ethyl acetate and cyclohexane (Licrosolv® grade), isooctane (Suprasolv® grade), and bis-silyl-trifluoroactamide (BSTFA) were supplied by Merck.

Extraction procedures. (i) Soxhlet extraction. A raw wool sample (≈3.5 g) was Soxhlet-extracted with DCM (220 mL) for 4 h according to a Woolmark Company standard (7). A minimum siphoning rate of 4–5 cycle/h was maintained. The recovered extracts were immediately filtered [glass fiber filter (GF/F) 0.7 µm] and concentrated to dryness by rotary evaporation. To eliminate traces of moisture, the extracts were maintained under vacuum overnight in a desiccator over P_2O_5 and then weighed. This assay yielded the total amount of matter extractable using DCM.

(ii) SFE extraction. Wool wax samples were extracted from raw wool by SFE with CO_2 using ethanol as an organic modifier at 60°C and 100 bar. The SFE apparatus was an SFC 3000 (Carlo Erba, Milan, Italy) equipped with two 200-mL syringe pumps.

Prior to SFE extraction, the solvent used as an organic modifier was filtered through a 0.2-µm nylon membrane purchased from Lida (Kenosha, WI). About 3.5 g of raw wool was inserted into a 7-mL stainless steel extraction cell (Keystone Scientific, Bellefonte, PA). A 1.5 mL/min flow rate was held and controlled by a microvalve regulator (Hoke, Cresskill, NJ) and delivered by two high-precision syringe pumps (Carlo Erba). The decompression system was heated at 90°C to limit the Joule–Thompson effect. CO_2 was vented through a cryogenic trap to minimize solvent trapping losses. Extract was recovered in a small amount of ethanol.

Extract analysis by TLC-FID. Qualitative and quantitative analyses of the lipid classes present in the different collected extracts were performed by TLC coupled with an automated FID system (Iatroscan MK-5; Iatron Laboratories, Tokyo, Japan). Standard compounds or an aliquot of dry extracts (25–60 mg) was redissolved in chloroform/methanol (2:1, vol/vol) (5–12 mg/mL). Samples (0.8 µL) were spotted onto silica gel-coated Chromarods (type S-III) from Iatron Laboratories with an SES 3202/IS-02 semiautomatic sample spotter (Nieder-Olm, Germany) equipped with a 2-µL precision syringe (Iatron Laboratory, Inc.). The rods (in sets of 10 mounted semipermanently on stainless steel racks) were developed four consecutive times using the following mobile phases: (i) 70 mL of chloroform/methanol/water (57:12:0.6, vol/vol/vol) up to 1 cm (twice); (ii) 70 mL of hexane/diethyl ether/formic acid (58:12:0.3, vol/vol/vol) up to 9 cm; (iii) 70 mL of hexane/benzene (35:35, vol/vol) up to 10 cm. After each development, the rods were then heated for 5–10 min at 60°C to dry the remaining solvent and run through an FID in the Iatroscan by using a flow rate of 2 L/min of atmospheric air, a hydrogen flow of 160 mL/min (high-purity hydrogen, C50), and a scanning speed of 3.0 s/cm. A total scan was performed to identify all the lipid components. Data were processed with Boreal software, version 2.5 (Boreal Software Development, Grenoble, France).

GC-MS characterization. (i) Sample preparation. Wool wax extracts, obtained under optimal SFE conditions, were dissolved in the GPC mobile phase, ethyl acetate/cyclohexane (1:1), and filtered through a 0.45-µm nylon membrane (Lida, Kenosha, WI). The LC system used (Shimadzu, Kyoto, Japan) was equipped with a Rheodyne high-pressure valve with a 100-µL loop, LC-10AT pumps, a UV detector (SPD-10AV, SCL-10A), and class-VP software. The GPC column used (450 × 10 mm i.d.) was packed with Bio Beads SX-3 (200–400 mesh) from Bio-Rad (Hercules, CA). Ethyl acetate/cyclohexane (1:1) at a 2 mL/min flow rate was used as the mobile phase. A total of 20 mg of wool wax was injected into the column. The first 8-min elution fraction, corresponding to the high-molecular-mass compounds, was used for the GC-MS characterization. After rotary evaporation to *ca.* 1 mL, extract silylation was performed. Ten microliters of the solution was placed in a 2-mL conic vial, to which 10 µL of BSTFA was added. The closed vial was maintained at 70°C for 1 h and then evaporated to dryness under a gentle nitrogen stream. Isooctane (50 µL) was added to the vial to reconstitute the sample and analyzed before 48 h to avoid hydrolysis of the trimethylsilyl group. The main polar constituents of wool wax such as FFA, hydroxy acids, and diols were derivatized prior to the GC-MS determination.

(ii) Instrumental analysis. A subambient-pressure CP Sil 8 model CB/MS capillary GC column (5% diphenyl-dimethylpolysiloxane) of 10 m × 0.53 mm i.d. and 0.25 µm film thickness, fitted to a deactivated restrictor of 50 cm length and 0.1 mm i.d. at the injection port, was obtained from Chrompack (Middelburg, The Netherlands). One microliter of sample was injected into the splitless mode at 320°C by activating the injector purge at 90 s from injection. Initial column temperature was held at 90°C for 1 min, and programmed to increase at 10°C/min to 320°C, keeping the final column temperature for 20 min (44 total min each run). Chromatographic analysis was performed in the constant flow mode at 1.2 mL/min.

In the CI mode (CI-MS), a 6890A gas chromatograph (Agilent Technologies, Palo Alto, CA), coupled to a 5973N mass spectrometer also from Agilent, was used. The quadrupole was maintained at 150°C and the transfer line at 280°C. The MS detector was used in the positive polarity mode with ammonia (electronic grade) as a reagent gas, and the ion source temperature was maintained at 200°C.

Also, an MD 800 mass spectrometer (Fisons, Loughborough, United Kingdom) was used in the EI mode. The transfer line and ion source temperatures were maintained at 320

and 230°C, respectively. The EI system was used for aliphatic ester determination, and the CI system was used for cholesteryl ester determination.

RESULTS AND DISCUSSION

Optimization of the CO_2 modifier content and extraction time. To obtain the best wool wax recovery using an optimal extraction time, a kinetic study was carried out using two different percentages of ethanol as the CO_2 modifier (Fig. 1). The results highlight the importance of this parameter. When the pressurized CO_2 contained 15% ethanol (vol/vol), a low proportion of wool wax was extracted during the first 15 min. However, when the percentage of ethanol was increased (CO_2/30% ethanol, vol/vol), the extraction rate of lanolin increased. The content of the modifier did not have any influence on the extraction rate after 25 min, but the resultant extraction profiles suggest that the extraction rate is limited by the solubility of some wool wax components. A total wool wax yield of CO_2/15% ethanol (vol/vol) and CO_2/30% ethanol (vol/vol) gave 15.1 and 20.7 wt%, respectively, calculated based on the weight of scoured and dried wool.

This extraction behavior shows that the limiting parameter in this experiment was the solubility of wool wax in the extractant fluid. In fact, during the first minutes of extraction, the fluid was saturated with wool wax. By raising the proportion of modifier, and hence the fluid polarity, the solubility of wool wax was significantly enhanced. When solute (wool wax) saturation was not achieved (after the first few minutes), the proportion of modifier was less important, producing a similar extraction rate.

TLC-FID analysis of the different fractions obtained as a function of the number of compressed fluid extraction cell volumes was carried out to determine the lipid composition and to observe the preferential extraction of lipids in accordance with the polarity of the extractant agent (CO_2/15% ethanol or CO_2/30% ethanol).

The TLC-FID technique was used to study the lipid composition of wool extracts (15–18), but in our experiment some nonpolar lipids in wool wax extracts were coeluted. The

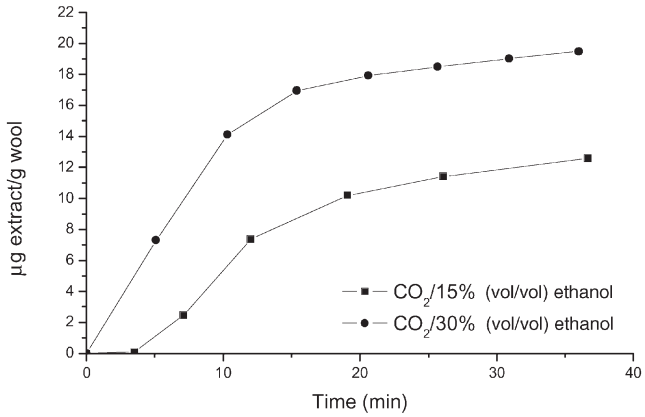

FIG. 1. Wool wax yield as a function of extraction time by using pressurized CO_2 containing different amounts of modifier at 60°C and 100 bar.

mobile phase composition was optimized to achieve the best resolution of the standard lipid compounds representing wool wax composition. In addition to single-standard compounds, a standard mixture containing mono-ES, di-ES, AL, FFA, and S was used. The best TLC-FID resolution (Fig. 2A) was obtained by developing the Chromarods according to the method indicated in the Experimental Procedures section. This methodology enabled us to identify (Fig. 2B) and quantify (Fig. 3) the different lipid classes present in wool wax extracts.

Table 1 shows the retention times of the different lipid classes present in the TLC-FID chromatograms of wool lipid extracts. In accordance with the retention time of the standard compounds, the 2, 4, 6, 7, and 8 peaks of the wool extract were identified as mono-ES, di-ES, FFA, AL, and S, respectively. Peak 1 was identified as the solvent front. Peak 5 was identified as TG (17; Jover, E., C. Domínguez, P. Erra, and J.M. Bayona, unpublished results). Peak 9, termed polar lipids (PL), included a strong peak and two unresolved peaks. In peak 9, we identified the presence of polar lipids such as ceramides, 7-hydroxycholesterol, cholesterol derivatives, and glucoceramides (Domínguez, C., J.M. Bayona, and P. Erra, unpublished results). Peak 10 corresponded to salts, and included cholesterol sulfate (Csulf) (Domínguez, C., J.M. Bayona, and P. Erra,

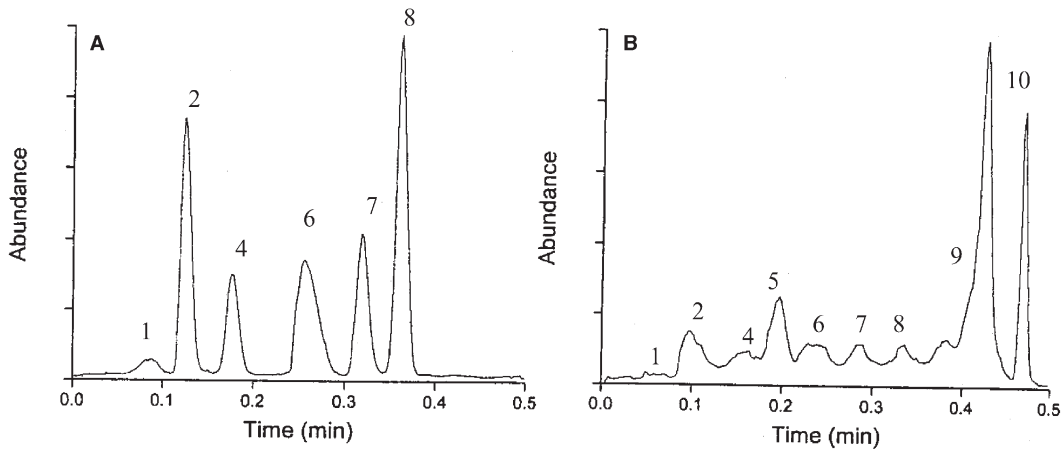

FIG. 2. TLC-FID chromatogram of the mixture of standard lipid compounds (A) and total CO_2/15% ethanol (vol/vol) wool wax extract (B).

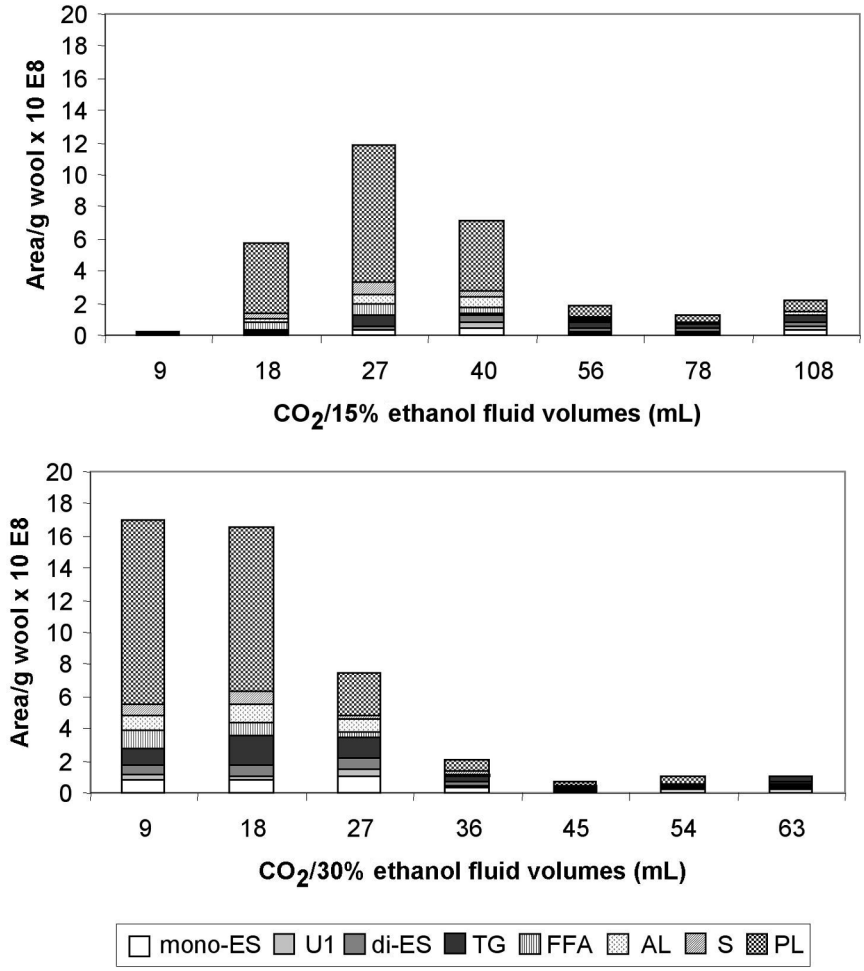

FIG. 3. Average area value of each lipid class extracted from raw wool with different percentages of ethanol in pressurized CO_2 as a function of the extractant volume. mono-ES, palmitic acid behenyl ester; U1, unknown compound; di-ES, dipalmitic acid hexadecyl ester; AL, behenyl alcohol; S, cholesterol; PL, polar lipids.

unpublished results). These polar lipid classes occur in the internal wool lipids (17,18,23). Therefore, it may be inferred that the internal lipids of the wool fibers were partially extracted under our experimental extraction conditions.

The relative amounts of the lipid classes present in the different wool wax extracts obtained by using CO_2 containing two different percentages of ethanol are plotted in Figure 3 (mean value from three TLC-FID replicates). The percentage of ethanol exerted an important influence on the extraction rate and on the composition of extracts. When the CO_2 contained 15% ethanol, a minimal amount of lipids was extracted within the first volume of extractant (9 mL), followed by a significant increase in the extraction yield from 18 to 40 mL of the mobile phase, and then showed a stepwise decrease in lipid content down to 78 mL, followed by a slight increase up to 108 mL. Although each fraction collected contained all of the lipid classes, the proportion of these varied as the extractant volume increased. For CO_2/15% ethanol, PL, S, AL, and FFA were preferentially extracted in 18 to 40 mL of extractant, whereas mono-ES and di-ES were formed predominantly from 27 to 108 mL of the extractant volume. When the percentage of ethanol in CO_2 reached 30% (vol/vol), the fractionation of lipids was poorer. However, in this case it was observed that PL, S, and FFA were extracted with 9 and 18 mL of extractant and that AL, TG, mono-ES, and di-ES were found throughout the fractions eluted with 9 to 27 mL of extractant.

The total quantity of each lipid class extracted with CO_2/30% ethanol was higher than its counterpart extracted with CO_2/15% ethanol (Table 2). This result is in accordance with the yields obtained for both extractant agents, revealing

TABLE 1
Retention Time (t_r) of Lipid Classes in the TLC-FID Chromatogram of the Different Wool Wax CO_2/Ethanol Fractions[a]

Peak	Lipid class	t_r (min)	Peak	Lipid class	t_r (min)
1	SF	0.08 ± 0.01	6	FFA	0.27 ± 0.01
2	Mono-ES	0.12 ± 0.01	7	AL	0.32 ± 0.01
3	U1	0.15 ± 0.01	8	S	0.36 ± 0.01
4	Di-ES	0.18 ± 0.01	9	PL	0.38–0.42 ± 0.01
5	TG	0.24 ± 0.01	10	Salts	0.47 ± 0.01

[a]SF, solvent front; Mono-ES, monoesters; U1, unknown compound; Di-ES, diesters; AL, free fatty alcohols; S, sterols; PL, polar lipids.

TABLE 2
Relative Abundance (area/g wool × 10^8) of Each Lipid Class in the TLC-FID of the Wool Wax Extracted with CO_2/15% Ethanol (vol/vol) and CO_2/30% Ethanol (vol/vol)[a]

Lipid class	15% Ethanol	30% Ethanol	Ratio 30%/15%
Mono-ES	195.8 ± 17.6	421.1 ± 37.9	2.2
U1	112.6 ± 10.1	167.4 ± 15.1	1.5
Di-ES	195.2 ± 17.6	280.9 ± 25.3	1.4
TG	245.3 ± 22.1	538.2 ± 48.4	2.2
FFA	199.7 ± 17.8	237.0 ± 21.3	1.2
AL	215.4 ± 19.4	356.9 ± 32.1	1.7
S	181.7 ± 16.4	195.9 ± 17.6	1.1
PL	2073.2 ± 186.5	2705.9 ± 243.5	1.3

[a]For abbreviations see Table 1.

that, for each lipid class, additional polar compounds were extracted by increasing the polarity (addition of ethanol) of the extractant agent.

It was observed that, during the extraction, a white insoluble suspension appeared in the collection solvent and that the ethanol layer became progressively yellow. To study the chemical composition of both wool wax fractions accurately, raw wool was extracted with an intermediate proportion of ethanol modifier (20%) and with an extraction time of 30 min, corresponding to 56 mL of extractant.

Fractionation of wool wax in the collection system. SFE extract collection was carried out in ethanol, given that it was the modifier used during SFE wool wax extraction. However, during extract collection, the CO_2 was decompressed and evaporated so that the wool wax lipids remained in pure ethanol. Owing to the change in polarity of the extractant agent (CO_2/ethanol) and the collection solvent (100% ethanol), a fractionation of lipids occurred spontaneously during the decompression of the extractant agent. Once the extraction was completed, an amber liquid was separated from the white solid and the solvent was removed with a rotary evaporator. A white insoluble wool wax (72.9%) and an amber wool wax (27.1%) were obtained. A TLC-FID analysis of aliquots of the two different fractions collected showed they had different compositions (Fig. 4). It should be pointed out that, even though the two fractions contained similar lipid classes, the proportion of these classes differed considerably. The white solid wool wax fraction was enriched in nonpolar lipids [mono-ES, an unknown compound (U1), di-ES, and TG], whereas the amber wool wax fraction contained mostly polar lipids.

(i) TLC-FID analysis. The total area value of lipid classes present in both fractions from three replicate TLC-FID analyses is plotted in Figure 5A. The two wool wax fractions contain most of the lipid classes. However, it should be pointed out that mono-ES, U1, and di-ES were preferentially present in the white solid wool wax fraction, and S, PL, and salts were found in the amber liquid wool wax fraction. Moreover, the other lipid classes were present in both wool wax fractions in similar proportions. Accordingly, it is likely that the two fractions collected can be used in different applications.

The total area of each lipid class present in the two different wool wax fractions collected, in comparison with the DCM extract, is plotted in Figure 5B. It is known that DCM largely removes wool wax and small amounts of internal wool lipids (8).

The biggest difference observed between the two fractions with respect to the DCM extract was in the proportion of mono-ES and PL. On the other hand, the composition of the amber wool wax fraction showed the smallest difference with respect to the DCM extract, particularly in the least polar fraction (mono-ES, U1, and di-ES). It was richer in polar lipids (FFA, AL, S, PL, and salts) than the DCM extracts, whereas the white wool wax fraction was enriched in nonpolar lipids compared with DCM (mono-ES, U1, di-ES, and TG). Moreover, both wool wax fractions had a higher proportion of AL than the DCM extract. It should be pointed out that the lowest portion of FFA in the amber fraction in comparison with the DCM extract could be attributed to their hydrophobicity rather than to their retention times.

(ii) GC-MS characterization. The two fractions obtained in the solvent collection system contained the same families of compounds as the DCM Soxhlet wool extract. Molecular characterization has been focused on a detailed identification of esters, which are the main components of lanolin (1–5). The

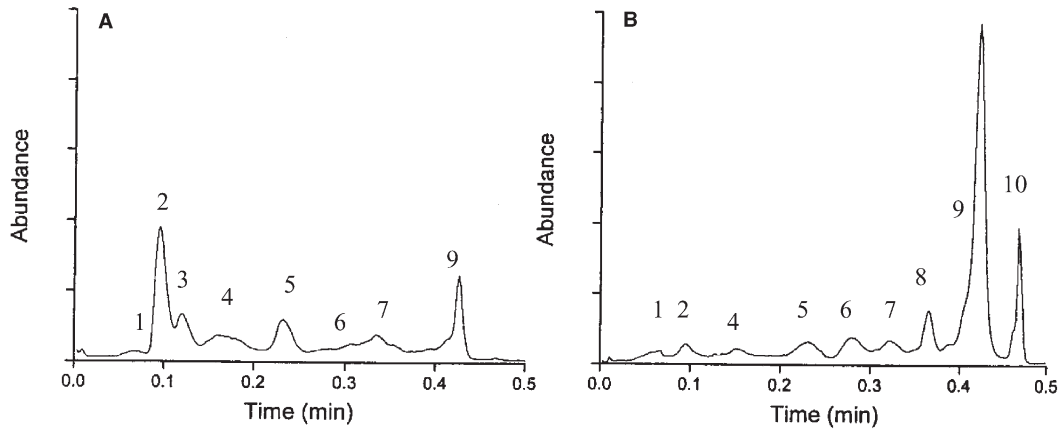

FIG. 4. TLC-FID chromatogram of the white wool wax fraction (A) and amber wool wax fraction (B) obtained in the collection system using CO_2/20% ethanol (vol/vol) at 60°C and 100 bar.

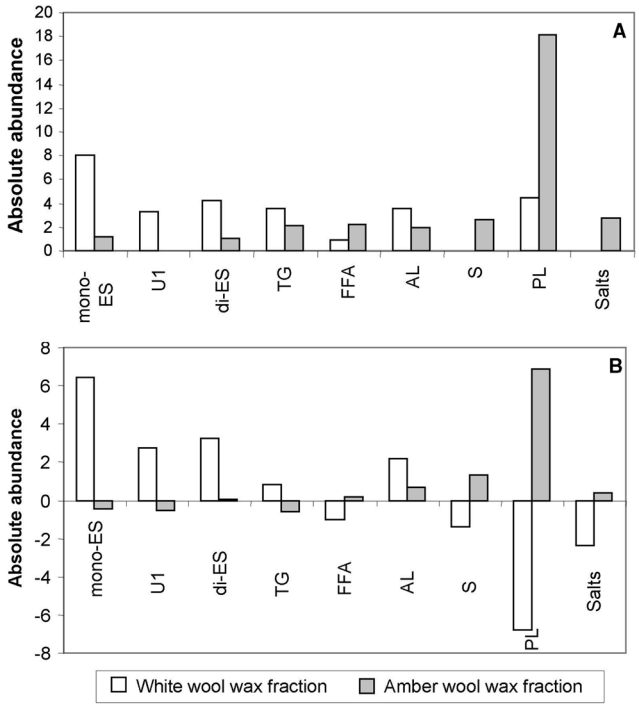

FIG. 5. Average area value of each lipid class present in the white and amber wool wax fractions (A) and lipid class differences between the dichloromethane (DCM) reference extract and the white and amber wool wax fractions (B). For abbreviations see Figure 3.

TLC-FID ester peak was constituted by the coelution of steryl and aliphatic esters. First, the molecular distribution of steryl esters, for the two fractions obtained, was determined by using subambient pressure GC-CI-MS since steryl esters, and particularly cholesteryl esters are thermally labile (24). Steryl esters present in wool wax can be determined by their sterol composition (i.e., lanosterol, dihydrolanosterol, or cholesterol), acid moiety, and isomeric form, namely, normal, *iso* and *anteiso,* with *iso* corresponding to (ω-1)-monomethyl-substituted FFA and *anteiso* to (ω-2)-monomethyl-substituted (21). In Figure 6A, the normalized homologous series of the cholesteryl ester are compared as a function of the acid moiety of the two wool wax extracts and the reference DCM extract without considering the isomeric distribution. The cholesteryl ester distribution in the amber fraction was similar to that of commercial lanolin, which has been reported (21) to have a monomodal distribution and a maximum centered at the C_{19} acid chain. However, the distribution in the white fraction component was totally different, exhibiting a shift toward a higher M.W. with a maximum equivalent to a C_{24} acid chain length.

The molecular distribution of aliphatic esters was characterized and, as can be seen in Figure 6B, the white fraction was more similar to the DCM extract than was the amber fraction. Only slight differences could be observed between the white fraction and the DCM extract. Instead of a Gaussian monomodal distribution, as in the Soxhlet DCM extract, the pattern of the white fraction showed an enrichment in high-molecular-mass compounds, maintaining the highest abundance for the C_{42} ester. The distribution of aliphatic esters in the amber fraction was found to be richer in shorter-chain esters, with an increased proportion of C_{38}–C_{39} homologs.

However, the largest difference observed between lipid classes was that between the aliphatic ester and cholesteryl ester content. Indeed, the amber fraction was richer in steryl esters than the white fraction. In addition to the differences between the two fractions, a comparison of these extracts was carried out with a reference wool wax obtained by DCM-Soxhlet extraction. We compared the steryl ester/aliphatic ester ratio of the two fractions corrected by the DCM reference extract ratio to highlight the existing differences (Fig. 7) according to Equation 1:

$$\left(\frac{C_nOO-\text{chol.}}{C_{16}OO-V_{22\text{aliph}}}\right)_{\text{extract}}-\left(\frac{C_nOO-\text{chol.}}{C_{16}OO-C_{22\text{aliph}}}\right)_{\text{DCM}} \quad [1]$$

$$= \text{relative abundance index}$$

On one hand, the amber fraction consistently had a higher ratio than the DCM, which could exceed 700% of the relative steryl ester enrichment for the cholesteryl nonadecanoate. On the other hand, in the white fraction a lower ratio than that of the DCM reference was observed for the shorter-acid chain cholesteryl esters and a higher ratio than that of the reference was observed for the longer ones, attaining 300% for the cholesteryl esters with the longest acid chains (e.g., cholesteryl tetracosanoate).

As one can observe in Figure 6A, the molecular distribution of cholesteryl esters differed more in the white fraction than in the amber fraction compared to the DCM reference extract. In contrast, the steryl ester/aliphatic ester ratio in the white fraction was more similar to the reference extract than was the amber one.

Therefore, differences between the two fractions in their patterns of cholesteryl esters and aliphatic esters can readily be explained by means of their relative solubility in ethanol. In the two families of compounds studied, when the alkyl chain became longer, the solubility of the compound decreased, with the result that the equilibrium of partitioning of this compound between the white and amber fractions favored displacement toward the insoluble part corresponding to the white fraction.

Owing to the complex composition of raw wool wax, it was not possible to obtain a pure lipidic fraction with SFE CO_2/ethanol, but some enrichment in certain lipidic classes was observed. Despite the fact that the two isolated fractions contained all the lipid classes, their proportions varied as a function of their M.W., given its influence on relative solubility. Therefore, fractionation of the lipids probably occurs because of their molecular mass and not only as a function of their chemical class.

In this study we developed a methodology that enabled us to carry out, in a single step, the extraction and fractionation of wool wax. This fractionation process allowed us to obtain two enriched fractions, an amber fraction containing smaller and more polar compounds and a white fraction enriched with

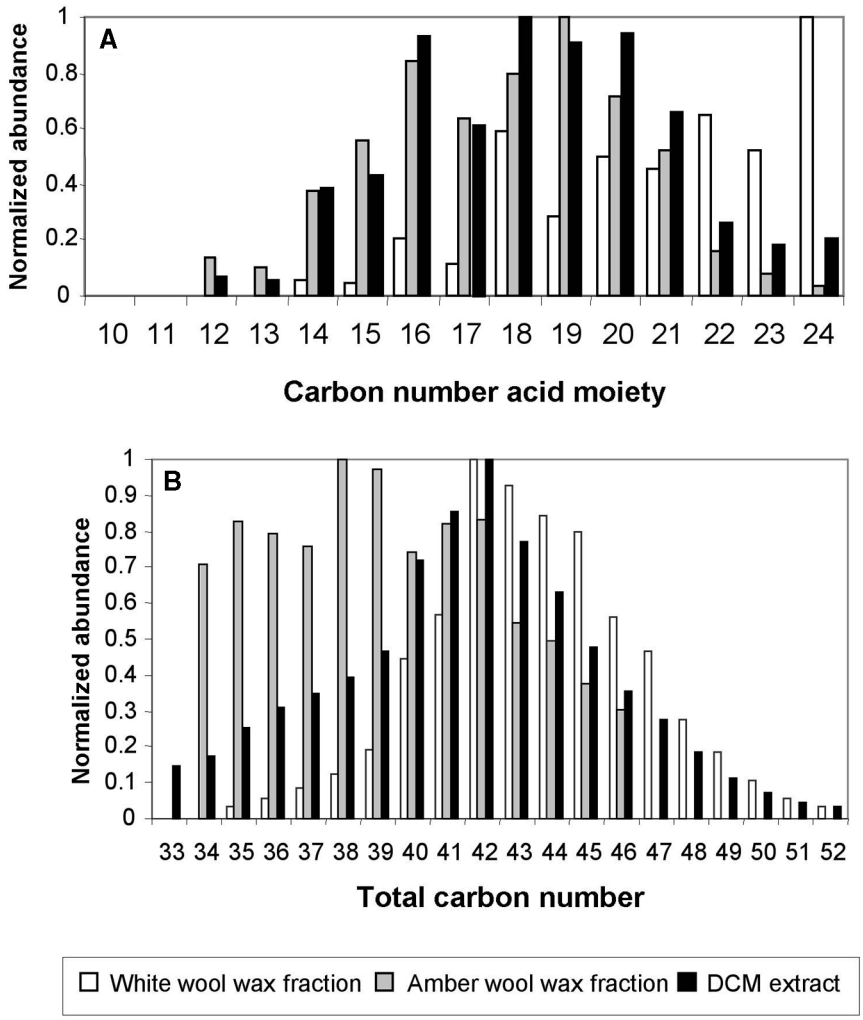

FIG. 6. Cholesteryl ester acid chain moiety distribution of the white and amber wool wax fractions and the DCM reference extract (A); aliphatic ester distribution depending on the total carbon number of the two fractions and the DCM reference extract (B). For abbreviation see Figure 5.

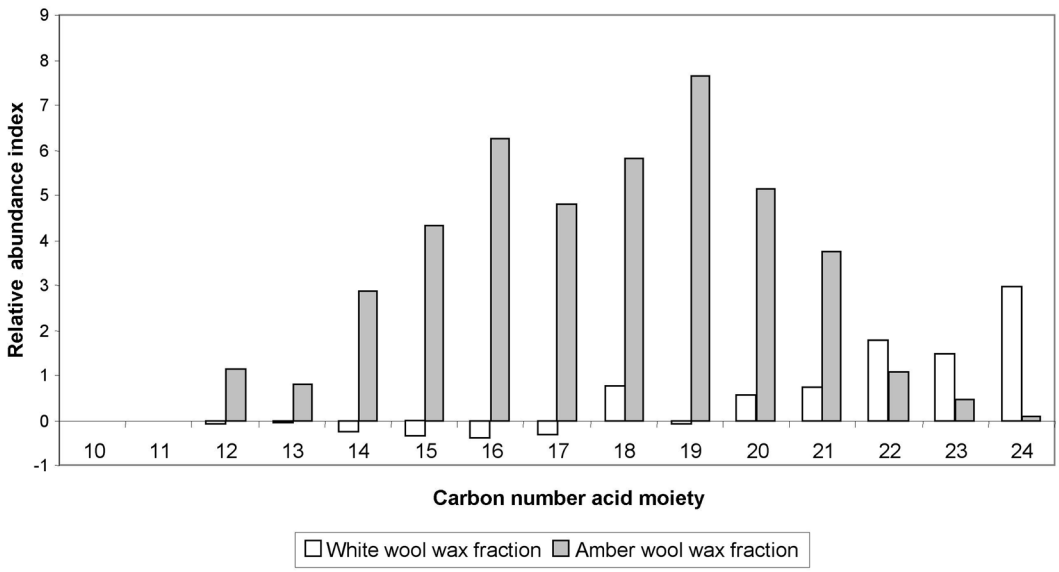

FIG. 7. Cholesteryl ester/aliphatic ester ratio in the DCM reference extract as compared to the white and amber wool wax fractions.

larger and less-polar compounds. This methodology could be of major interest to the textile industry to minimize wastewater production and to the cosmetic and pharmaceutical industries to generate a new enriched base product.

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