Squalene Recovery from Olive Oil Deodorizer Distillates

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Olive oil deodorization distillate contains squalene in a concentration range of 10 to 30 wt%. A process for its recovery by supercritical carbon dioxide extraction is described. The process consists mainly of converting the free fatty acids and the methyl and ethyl esters normally occurring in this by-product into their corresponding triglycerides. The latter are then extracted with supercritical carbon dioxide to provide a highly enriched squalene fraction. The process has been carried out on a pilot-plant scale with a column operating in the contercurrent mode. The relationship between the experimental conditions and squalene purity and yield has been studied. Analytical methods were used for the determination of squalene and other components in the fractions. By use of this process, squalene can be recovered in high purity and yields of about 90%.

KEY WORDS: By-product, carbon dioxide, countercurrent column, fractionation, liquid-liquid extraction, squalene, supercritical fluids.

Squalene (2,6,10,15,19,23-hexamethyl 2,6,10,14,18,20-tetracosahexaene) is a naturally occurring terpenoid hydrocarbon in fish liver oils (1). It is normally used in its natural or hydrogenated form (squalane) in cosmetic preparations as a moisturizing or emollient agent (2,3). Academic interest arises because it is a precursor in cholesterol biosynthesis (4–7).

One reason that limits the use of squalene in cosmetic applications is the uncertainty of its availability as a result of international concern for the protection of marine animals that are a primary source of natural squalene. This consideration turned our interest toward a vegetable origin source of squalene, namely a by-product of the olive oil industry, the distilled fraction obtained from the deodorization step.

It is known (8,9) that the unsaponifiable fraction of olive oil contains squalene (60–75%) but, for economical reasons, its recovery directly from the oil is not feasible. On the other hand, olive oil deodorization distillate is inexpensive and readily available and contains squalene in high concentration, so that its purification and isolation by distillation have been proposed (10,11). Olive oil deodorization distillate is a by-product of olive oil refining and represents 0.05–0.1% of the total processed oil. Its use is limited because it is typically mixed with olive oil neutralization by-products and hence has a low market value (0.3–0.5 United States $/kg).

In our previous paper on olive oil refining with supercritical carbon dioxide (12), we demonstrated the feasibility of separating squalene from the glyceride and nonglyceride substances normally found in olive oil. From this information we developed a process for squalene extraction in high yield and purity. In that study we extracted olive oil with supercritical CO_2 to eliminate the free fatty acid fraction at 40°C and 130 bar by means of a flux ratio of 100 kg CO_2/kg oil. Under these conditions we found that the enrichment ratio (ER) in CO_2 extraction, calculated for the pair squalene/fatty acid, was 168:85 = 2. The same ER for the pair squalene/methyl or ethyl ester was not calculated, but from molecular weight, polarity and vapor pressure data for the monoesters indicated that their ER values should be lower and, for this reason, those separations seemed more difficult. On the other hand, if we take the calculated ER values for mono- and diglycerides (168:22 and 168:4, respectively) along with the previously demonstrated ability to selectively and quantitatively extract squalene from olive oil, we considered the possibility of using the deodorizer distillate residue after converting it to a mixture of squalene and glycerides as described below.

EXPERIMENTAL PROCEDURES

Materials. Olive oil deodorizer distillate (OOD) was supplied by an Italian factory (Oleifici Fasanesi, Fasano, Italy) as collected directly from oil scrubbers. Glycerol, methanol, phosphoric acid, powdered zinc, *p*-toluenesulfonic acid and sodium hydroxide were obtained from Carlo Erba (Milano, Italy). Silica gel thin-layer chromatography (TLC) plates, 20 × 20 cm, thickness 0.25 mm and silica gel (70–230 mesh) used for flash chromatography were obtained from E. Merck (Darmstadt, Germany).

Analytical methods. Free fatty acids (FFA) were determined by NGD method C10-1976 and saponification number by NGD method C12-1976 (13). Squalene was determined as follows: an analytically weighed amount of sample (1 g) was placed into a 100-mL volumetric flask and diluted to volume with hexane. Exactly 1 mL of solution was transferred to a 10-mL test tube and 1 mL docotriacontane (100 mg/mL) was added. The solvent was partly evaporated under nitrogen flow, and the solution was deposited at the bottom of a preparaqtive TLC plate. TLC was carried out with hexane/diethyl ether (95:5) as eluant. After drying, the plate was sprayed with 2,7-dichlorofluorescein reagent in alcoholic solution, and the hyrocarbon fraction was located by ultraviolet detection, scraped off the plate and extracted twice with hot chloroform. The solvent was evaporated under reduced pressure, and the sample was dissolved in heptane and analyzed by gas–liquid chromatography (GLC) under the following conditions: GLC apparatus was a Carlo Erba model 4160 high-resolution gas chromatograph equipped with an SE 52 capillary column, the carrier gas was hydrogen at a flow rate of 1 mL/min. Injection technique was on-column with an oven temperature program of 130 to 250°C at 5°C/min. Detector temperature was 260°C. Flash column chromatography as described by Clark Still *et al.* (14) was used to estimate the main components of the OOD starting material. The elution solvent consisted of hexane/diethyl ether (95:5). FFAs were determined by titration.

Working methods. Saponification occurred: 500 g of OOD starting material was refluxed with 500 mL methanol, 500 mL distilled water and an amount of sodium hydroxide calculated on the basis of saponification number to be in 100% excess. The alkaline solution obtained was acidified with 85% H_3PO_4 and heated at 70°C to

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neutralize the FFA soaps. The organic layer was separated by means of a separatory funnel without solvent. The FFAs obtained from the previous step were transferred to a 1-L flask equipped with a magnetic stirrer and connected to a water vacuum pump. After moisture elimination at 100°C under vacuum, the temperature was raised to 180°C, and glycerol (35 g) and catalyst (2 g of *p*-toluenesulfonic acid or 2.5 g of powdered zinc) were added. The reaction progress was checked periodically by FFA titration. The reaction was stopped when FFA content reached 1%.

CO_2 extraction. A Muller Extract Co. GmbH (Coburg, Germany) continuous countercurrent apparatus was used. It consists mainly of an extraction column (3 m × 30 mm i.d.) in three sections, each of which can be held at a different temperature. Because the three sections have separate heating controls, it is possible to have different temperature conditions along the column, thus varying the solvent power of supercritical CO_2 within the column. Packing material was Sulzer stainless-steel rings (Sulzer, Winterthur, Switzerland). For further details refer to our paper on olive oil refining (12).

RESULTS AND DISCUSSION

The composition of the starting olive oil distillate residue is shown in Table 1. It consists primarily of hydrocarbons (mainly squalene), FFAs and methyl and ethyl esters in equal amounts. Olive oil naturally contains small amounts of methyl and ethyl esters, which are in part responsible for the distinctive aroma of olive oil (15). These compounds, because of their volatility, are lost during refining and concentrate in the deodorization distillate.

As we ascertained in our previous study (12), squalene could not be separated from the other two classes of compounds by supercritical CO_2 extraction, whereas squalene separation from a triglyceride matrix is feasible. It is apparent that the differences in molecular weight between FFAs, the corresponding methyl or ethyl esters and triglycerides play a key role in the separation efficiency under our supercritical extraction conditions. For this reason we set up a preliminary step that transforms free and esterified fatty acids to their corresponding triglyceride structures.

The starting olive oil distillate residue was completely saponified within three hours as ascertained by TLC by the absence of esterified fatty acids. The FFAs were isolated by acidifying the soaps, and the reaction mixture, containing all components, was esterified with glycerol and acid catalyst. In a first experiment we used *p*-toluenesulfonic acid, but the squalene structure was altered as determined by GLC. In Figure 1 is shown a gas chromatographic comparison of the hydrocarbons present in the esterified product with that obtained by direct supercritical CO_2 extraction of the starting material.

In a second test with powdered zinc as catalyst, good results were obtained. The reaction was rapid, and squalene in this case was not isomerized.

The glycerol esterified product was then subjected to supercritical CO_2 extraction. In these experiments we ascertained the best operating conditions by studying the effect of pressure and temperature on the extraction efficiencies. We decided to use a flux ratio of 30 kg CO_2/kg sample as the best compromise between cost and yield of product. In our previous work (12) we checked different flux ratios and found that there was no direct relationship between CO_2 used and extraction yield. Accordingly, we ascertained that for a given CO_2 flux ratio the increase of cost related to the higher amounts of CO_2 used was not accompanied by a corresponding increase in yield or purity of squalene. The results obtained are reported in Table 2. The best operating conditions were at a pressure of 150 bar and a temperature of 40°C, corresponding to the highest purity of extracted squalene (83.7%) and to the highest extraction yield (83.7%).

We also investigated the use of a temperature gradient inside the extraction column. Two different temperature gradient profiles were used: 50:40:30°C and 60:50:40°C (top/middle/bottom column sections), in comparison to the isothermal condition of 40:40:40°C. Results are reported in Table 3. For the gradient condition of 50:40:30°C, the

TABLE 1

Composition of Olive Oil Deodorizer Distillate[a]

Free fatty acids (as oleic acid)	34.2
Hydrocarbons[b]	31.5
Fatty acid esters (methyl and ethyl)	24.1
Sterols	4.6
Others (glycerides, tocopherols, etc.)	5.6

[a]The wt% as determined by flash column chromatography.
[b]The deodorizer distillate contains 28% squalene.

TABLE 2

Influence of Temperature and Pressure on Supercritical CO_2 Extraction of Squalene from Olive Oil Deodorizer Distillate[a]

Pressure (bar)	Temperature (°C)	Extract (%)[b]	Squalene in extract[b]	Squalene in residue[b]	Extraction yield[b]
110	40	7.1	67.6	24.9	17.2
130	40	20.3	50.7	22.2	36.8
150	40	28.0	83.7	6.3	83.7
130	60	9.0	67.2	24.1	21.6
150	60	15.5	61.8	21.8	34.3
170	60	26.0	57.3	17.7	53.3

[a]Column flux ratio = 30 kg CO_2/kg oil, 28 wt% squalene in olive oil distillate.
[b]As wt%.

TABLE 3

Temperature Gradient Supercritical CO_2 Extraction of Squalene from Olive Oil Deodorizer Distillate[a]

Column gradient °C[b]	50:40:30	60:50:40	40:40:40
Extract (%)	28.3	22.5	28.0
Squalene in extract (%)	90.0	82.1	83.7
Squalene in residue (%)	3.5	12.3	6.3
Free fatty acids (%) in extract	3.0	3.8	3.1
Esters (%)[c] in extract	7.0	14.1	13.2
Extraction yield (%)	91.1	66.0	83.7

[a]Column flux ratio = 30 kg CO_2/kg oil at 150 bar, 28 wt% squalene in olive oil distillate.
[b]Temperature of top/middle/bottom of extraction column.
[c]Mono- and diglycerides.

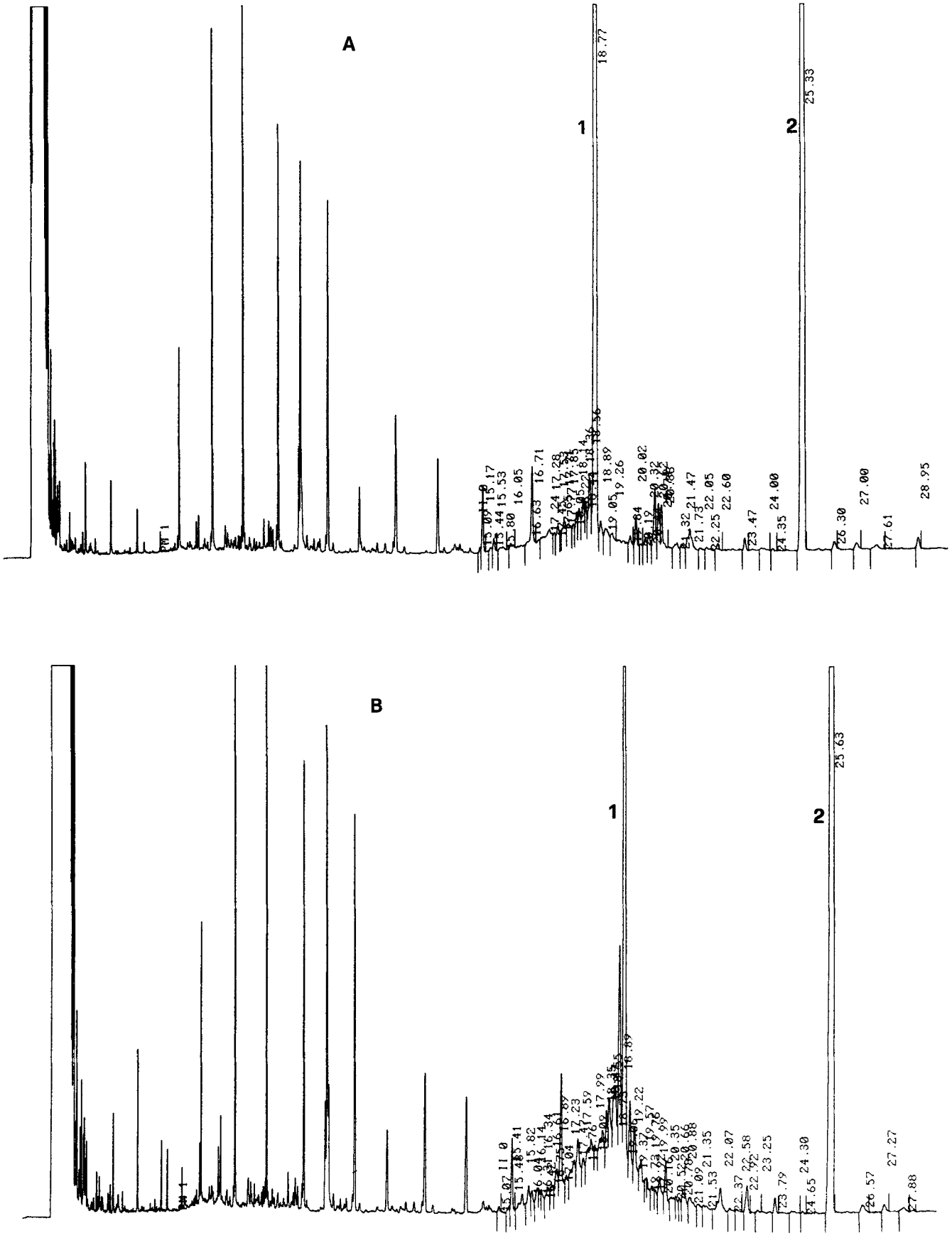

FIG. 1. High-resolution gas chromatography analysis of hydrocarbon fraction of olive oil deodorization distillate. A—untreated sample; B—reaction with *p*-toluenesulfonic acid. 1—squalene; 2—internal standard.

TABLE 4

Temperature Gradient Supercritical CO_2 Extraction of Low Squalene from Olive Oil Deodorizer Distillate[a]

Column gradient °C[b]	50:40:30	60:50:40	40:40:40
Extract (%)[c]	15.6	19.2	16.0
Squalene in extract (%)	46.3	27.0	40.2
Squalene in residue (%)	6.2	9.1	7.2
Free fatty acids (%)	5.8	6.3	6.0
Esters (%)[d]	47.9	66.6	53.8
Extraction yield (%)	57.8	41.5	51.4

[a]Column flux ratio = 30 kg CO_2/kg oil at 150 bar, 12.5 wt% squalene in olive oil distillate.

[b]Temperature of top/middle/bottom of extraction column.

[c]As wt%.

[d]Mono- and diglycerides.

yield and purity of squalene are improved, whereas for the 60:50:40°C gradient the yield was lower and squalene purity was unchanged.

The best extraction results obtained under the above conditions were with the column temperature gradient of 50:40:30°C. In this case the extraction column operates similarly to a distillation unit, in that the extract, produced in the middle part of the column at the optimal conditions of 40°C and 150 bar, is submitted to a subsequent refining step in the upper part of the column, where, at a temperature of 50°C, CO_2 extraction power is lowered but the selective extraction of squalene from the triglyceride matrix is enhanced. Furthermore, in the lower section of the columnn operating at near subcritical conditions (30°C and 150 bar), the extract is submitted to an exhaustive washing with liquid CO_2. This could be attributed to the fact, as pointed out by Stahl *et al.* (16) and by Zosel (17), that under these conditions both liquid-liquid extraction and distillation conditions are met. This hypothesis of extraction mechanism was confirmed in a subsequent series of experiments, carried out under the same experimental conditions but with an OOD of low squalene content (Table 4). In this case, owing to the lower squalene content, the obtained extract was richer in glycerides.

In conclusion, from the data obtained in this study, we propose a process for squalene recovery in high yields and in high purity by starting with a low-cost and readily available industrial by-product. This process is feasible from an economic point of view, based upon the two experimental conditions: (i) the zinc catalytic esterification reaction of FFAs present in the olive oil distillate residue to their corresponding glycerides, thus avoiding squalene isomerization and (ii) the optimized extraction conditions obtained with the supercritical CO_2 fluid process described. In regard to the latter, we can state that an extraction plant given to squalene recovery is expensive. However, based on the quality of the squalene obtained, the supercritical extraction process is, in our opinion, a viable process.

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