Characterization and Supercritical Carbon Dioxide Extraction of Walnut Oil

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ABSTRACT: Walnut (*Juglans regia* L.) oil was extracted with compressed carbon dioxide (CO_2) in the temperature range of 308 to 321 K and in the pressure range of 18 to 23.4 MPa. The influence of particle size was also studied at a superficial velocity of 0.068 cm/s, within a tubular extractor of 0.2 L capacity (cross-sectional area of 16.4 cm^2). FFA, sterol, TAG, and tocopherol compositions were not different from those of oil obtained with *n*-hexane. The main FA was linoleic acid (56.5%), followed by oleic acid (21.2%) and linolenic acid (13.2%). The main TAG was LLL (linoleic, linoleic, linoleic) (24.4%), followed by OLL (oleic, linoleic, linoleic) (19.6%) and LLLn (linoleic, linoleic, linolenic) (18.4%). The main component of sterols was β-sitosterol (85.16%), followed by campesterol (5.06%). The amount of cholesterol was low (0.31 and 0.16% for oils extracted by *n*-hexane and supercritical fluid extraction, respectively. The CO_2-extracted oil presented a larger amount of tocopherols (405.7 µg/g oil) when compared with 303.2 µg/g oil obtained with *n*-hexane. Oxidative stability determined by PV and the Rancimat method revealed that walnut oil was readily oxidized. Oil extracted by supercritical CO_2 was clearer than that extracted by *n*-hexane, showing some refining. A central composite, nonfactorial design was used to optimize the extraction conditions using the software *Statistica*, Version 5. The best results were found at 22 MPa, 308 K, and particle diameter (Dp) = 0.1 mm.

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KEY WORDS: *Juglans regia* L., lipids, oil characterization, supercritical CO_2, walnut.

Walnut oil has long been used in France as an alternative to olive oil for salad dressing (1). Lipid content of walnut kernels (*Juglans regia* L.) can vary from 52 to 70% depending on the cultivar, location grown, and irrigation rate (2–5). The major FA found in walnut oil are linoleic, oleic, and linolenic acids. Linoleic acid is considered the most significant and valuable benefit to human health (6).

The preventive role of monounsaturated FA (MUFA) and PUFA in cardiovascular disease has been recognized. It has been reported that walnut consumption (kernel and oil) reduces blood cholesterol levels (7–11). These advantages come from the high levels of MUFA and PUFA and possibly the tocopherol content. The experiments were conducted using specific foods with walnuts or almonds to lower total plasma and LDL cholesterol.

Owing to the high commercial value of both whole walnuts and walnut oil, extreme care needs to be taken to prevent oxidation of the unstable PUFA in the oil. Studies showed that a considerable range of stability in the Rancimat tests occurs among different cultivars of walnut (6).

Nearly all of the referred oils were obtained by a conventional oil extraction process with *n*-hexane at boiling temperature or by cold-pressing.

Several oils were obtained by using supercritical extraction with carbon dioxide (12–19). This separation technique offers extraction yields very similar to those obtained by conventional extraction processes using liquid solvents but does require a certain combination of operating conditions, depending on pressure, temperature, particle size, or superficial velocity. Its advantages, compared to organic solvents, are that CO_2 is nontoxic, nonflammable, and noncorrosive and that it is cheap and readily available in bulk quantity with a high degree of purity. In processing terms, CO_2 has a low critical temperature and pressure (304.1 K and 7.38 MPa, respectively), making it the ideal solvent for natural products, which tend to be susceptible to thermal degradation during processing.

The aim of this work was to study the characterization of walnut oil extracted by using mainly supercritical CO_2 as the solvent. Characteristics of oils obtained under supercritical fluid extraction (SFE) conditions were compared to those of Soxhlet *n*-hexane-extracted oils.

MATERIALS AND METHODS

Raw material. The walnuts were acquired from a Portuguese producer in 1999 and were preserved under nitrogen atmosphere until they were ground using an Armfield FT2 hammer mill (Armfield Technical Education Company Limited, Ringwood Hampshire, England). The particle fractions were separated in a set of sieves (ASTM type) by using a Retsch KS 1000 horizontal sieving machine (Retsch, Haan, Germany), and fractions with medium linear dimensions (Dp) of 0.01 to 0.5 mm were studied. The total oil content was determined to be 71.0 ± 0.1%.

Solvents. The carbon dioxide used was CO_2 N45 (purity ≥99.995%) and was supplied by Ar Liquido-Portugal (Lisbon, Portugal). All other solvents and reagents used in analytical determinations were Merck, pro analysis type.

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SFE. (i) Equipment. Extraction measurements were carried out in a semi-batch-flow extraction apparatus built at Instituto Superior Técnico (Lisbon, Portugal). The apparatus was identical to that described by Esquível and Bernardo-Gil (14). The extraction experiments were performed with a tubular extractor of 0.2 L of capacity (cross-sectional area = 16.4 cm^2), assembled as described (14).

Liquid CO_2 from the supply cylinder was passed through a cold bath (about 275 K) and then pumped with an air-driven liquid pump, model MCP-71 (Haskel International Inc., Burbank, CA) and heated by a tubular heat exchanger to the extraction temperatures. Pressure was controlled with a back-pressure regulator (model number 26-1722-024-043; Tescom Co., Elk River, MN).

The extractor containing the raw material was placed in a controlled temperature water bath, the temperature inside the extractor being controlled by a digital controller (Ero Electronic, Novara, Italy) within an accuracy of ±0.05 K. The pressure at the exit of the extractor was measured using a differential manometer (Skalenwert 2; FBM, Freiburg, Germany) with an accuracy of ±0.2 MPa. After leaving the extractor, the stream of CO_2 loaded with extract flowed through an on/off valve and a sequence of pressure expansion valves (Hoke needle valve; Hoke Inc., Glendale, WI). The stream pressure was reduced in this way in three successive stages down to atmospheric pressure, and the oily extract was recovered in a glass collector. Water and volatile components were deposited in a second collector that was immersed in a dry ice–acetone bath at approximately 203 K.

A Dry Test Meter (DTM-200A; American Meter Company, Philadelphia, PA) with an accuracy of ±0.005 L was used to measure the delivered volume of CO_2. Pressure and temperature conditions were measured at the end of assembly. The estimated accuracy of the pressure measurement was ±0.01 MPa, and temperature was measured with a mercury thermometer to within ±0.1 K.

Using the above equipment, the mass of oily extract (and hence the yield of extract) was determined as a function of extraction time and the mass of CO_2 passed at each of the conditions of temperature, pressure, and particle size.

(ii) Extraction procedure. The extractor was manually filled with a weighed quantity of ground walnuts, and CO_2 was pumped into the extractor up to the desired extraction pressure value. After ensuring that there were no leaks in the equipment, the expansion valves were opened and a steady stream of the solvent was allowed to pass upward through the bed of ground particles at predetermined pressure and temperature values. After a given extraction period, these valves were closed for another period (the "stagnant period"), and the weight of the oil deposited in the first collector and the combined weight of the water and other volatile material obtained in the second collector were determined.

The expansion valves and pipes leading from the extractor to the first collector were washed with *n*-hexane to remove any oil trapped in this region. To wash the pipes, the *n*-hexane was fed through a cap fitting placed just after the first expansion valve (which was closed during the washing operation). The *n*-hexane was left in contact with the inside surfaces of the pipe

for about 10 min and was then sucked out with a vacuum pump. The washings were collected, and the oil was subsequently separated from the solvent by using a rotary evaporator VV2000 (Heidolph, Schwabach, Germany) and weighed. This weight was added to that of the oil obtained in the first collector.

After the first stagnant period, the valves leading from the extractor were reopened, and extraction was resumed as above. This procedure was repeated until sufficient oil had been extracted to define the form of the extraction curve at the temperature and pressure studied.

To check the overall efficiency of the collectors, at the end of each run the ratio of the total weight of extract collected to the weight loss of the solid bed was calculated. The above set of tests was repeated as sets of temperature, pressure, and particle size at a superficial velocity of 0.068 cm s^{-1}.

The oily extracts obtained in the first collector were analyzed to determine their FA, TAG, sterol, and tocopherol compositions.

n-*Hexane extraction*. Oils were *n*-hexane-extracted by using a Soxhlet apparatus (0.5 L).

Analytical methods. (i) FAME analysis. The FAME were prepared by transesterification of walnut oil with 2 N KOH in methanol and *n*-heptane. GC analysis of FAME was performed in a PerkinElmer chromatograph equipped with a fused-silica capillary column with a stationary phase of 70% cyanopropyl polyphenylene-siloxane (BPX70, S.G.E.; PerkinElmer, Norwalk, CT), 50 m × 0.22 mm, 0.25 µm film thickness, a split injector at 240ºC, and an FID at 250ºC. Helium was used as carrier gas (pressure of 20 psig, retention time of *n*-heptane = 5.75 min). The programmed temperature was 20 min at 175ºC, 5ºC/min to 220ºC, and a final isotherm at 220ºC for 30 min. The identification of FAME was based on external standards using commercial reference compounds (Sigma, St. Louis, MO). Each FAME sample was analyzed three times.

(ii) TAG analysis. The TAG were determined by HPLC. The walnut oil was dissolved in acetone, and 10 µL was injected in an HPLC system (LC 30; PerkinElmer) equipped with a refractive index detector and an RP18 column. The mobile phase was a mixture of acetone and acetonitrile (50:50, vol/vol).

(iii) Sterol analysis. The sterol fraction of walnut oil was determined by GC after pretreatment (20). The sterols were then analyzed in a Hewlett-Packard 5890 Series II chromatograph (Palo Alto, CA) with a split/splitless injector and an FID. A Hewlett-Packard 3396 Series II integrator was used for data collection. The column was an HP5 (5% biphenyl, 95% dimethylpolysiloxane), 50 m × 0.32 mm, and the thickness of the film was 0.17 µm. The injector temperature was 200ºC, the detector temperature 250ºC, and the column temperature was held at 275ºC. The carrier gas was N_2 at 2 mL/min. A split ratio of 1:20 was used.

(iv) Tocopherol analysis. The tocopherols were determined by HPLC. The walnut oil was dissolved in *n*-hexane at 36 mg/mL of concentration, and 20 µL was injected in an HPLC (JASCO PU980; Tokyo, Japan) equipped with an automatic injector (JASCO AS-950-10) in a silica column (250 × 3.0

mm i.d. Lichrosorb). The mobile phase was a mixture of *n*-hexane and isopropanol (99.8:0.2, vol/vol) with a flow rate of 0.5 mL/min. A fluorescence detector (JASCO FP-920) was used at an excitation wavelength of 292 nm and an emission wavelength of 324 nm. The identification of tocopherols was made by comparison with the retention times of standards (Sigma), and quantification was done using a calibration curve for α-tocopherol and relative areas of other tocopherols to α-tocopherol. JASCO/Borwin chromatographic software, Version 1.2, was used for the integration of peaks.

(v) Rancimat method. Extracted oil samples (5 g) were weighed into test tubes and connected to a Rancimat 679 (Metrohm, Herisau, Switzerland). Air was passed through the samples at 20 L/min while being heated at 110ºC. The gases released during oxidation were carried into a cell containing water, and the change in conductivity of the water was plotted on a graph during the necessary time. The oxidative stability was measured as the time corresponding to the inflection point of the curve, named the induction time (IT).

(vi) PV. The PV, expressed as meq oxygen/kg fat, was determined by the iodine titration method. Extracted oil samples (2 g) were weighed into test tubes. The oxidation of the potassium iodide, in acetic acid medium, by the active oxygen of the fat was followed by titration of the free iodine with sodium thiosulfate, using starch as indicator.

RESULTS AND DISCUSSION

Figures 1, 2, and 3 show the SFE extraction curves for the yields of walnut oil as a function of the ratio g CO_2/g of ground walnut at several conditions of pressure, temperature, and median particle diameter of ground walnut. As expected, yield increased with the increase in pressure and the decrease in temperature. The particle size was also a very important parameter. At the conditions studied, the yields increased with the increase in the median particle diameter to an optimal value.

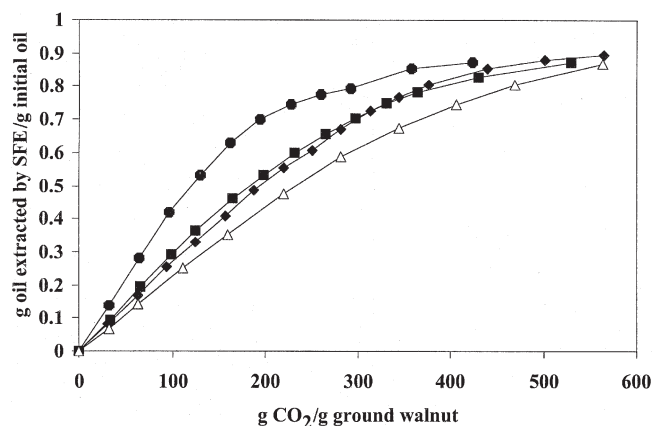Studies using *Statistica,* Version 5, software (Statsoft,

FIG. 2. Walnut oil recovered by supercritical CO_2 as a function of the ratio g CO_2/g ground walnut at 22 MPa. (●), 308 K and Dp = 0.1 mm; (■), 308 and Dp = 0.01 mm; (◆), 318 K and Dp = 0.1 mm; (△), 318 K and Dp = 0.01 mm. For abbreviations see Figure 1.

Tulsa, OK) were developed to obtain functional relations between the yield of walnut oil (YD) at 150 and 390 min of extraction time and process parameters such as pressure (*P*), temperature (*T*), and median particle diameter (Dp). The slopes of the extraction curves (initial extraction velocities) were also studied. Some of these relations are shown in Figures 4, 5, and 6.

The response surfaces shown in Figure 4 are described as

$$YD_{150\ min} = 327.07 - 0.2581\ P^2 - 1.520\ T + 2193.4\ Dp$$
$$- 205.69\ Dp^2 + 0.0462\ PT - 6.634\ TDp \quad [1]$$
$$R^2 = 0.985;\ Adj = 0.971\ (P < 0.05)$$

where Adj is the adjusted R^2 value.

At 150 min of extraction time, an optimal value of the yield occurred for the particle diameter range studied, recommending values of about 0.25 to 0.3 mm. At 150 min, the yield exhibited high dependence on the pressure and temperature, with high values for the yield at this extraction time at higher values of pressure and lower values of temperature.

FIG. 1. Walnut oil recovered by supercritical CO_2 as a function of the ratio g CO_2/g ground walnut at 18 MPa. (●), 308 K and particle diameter (Dp) = 0.1 mm; (■), 308 K and Dp = 0.01 mm; (◆), 318 K and Dp = 0.1 mm; (△), 318 K and Dp = 0.01 mm. SFE, supercritical fluid extraction.
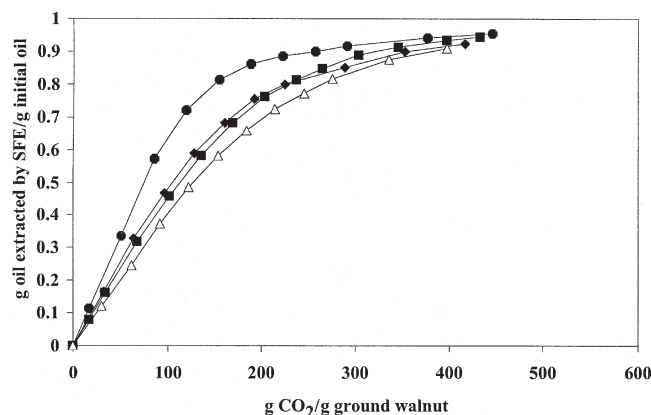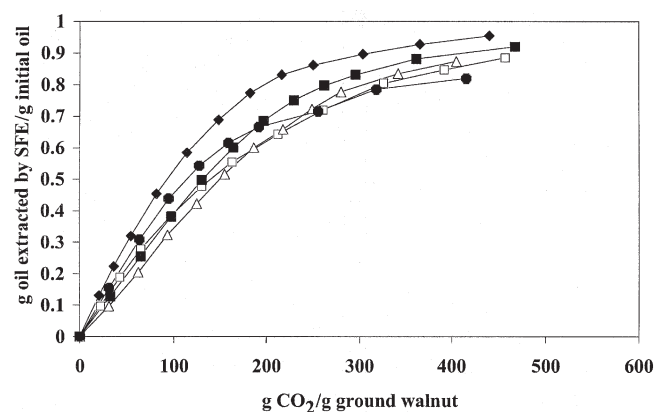
FIG. 3. Walnut oil recovered by supercritical CO_2 as a function of the ratio g CO_2/g ground walnut at several conditions. (■), 20 MPa, 313 K, and Dp = 0.05 mm; (□), 20 MPa, 313 K, and Dp = 0.05 mm; (●), 20 MPa, 313 K, and Dp = 0.5 mm; (◆), 23.4 MPa, 313 K, and Dp = 0.05 mm; (△), 20 MPa, 321 K, and Dp = 0.5 mm. For abbreviations see Figure 1.
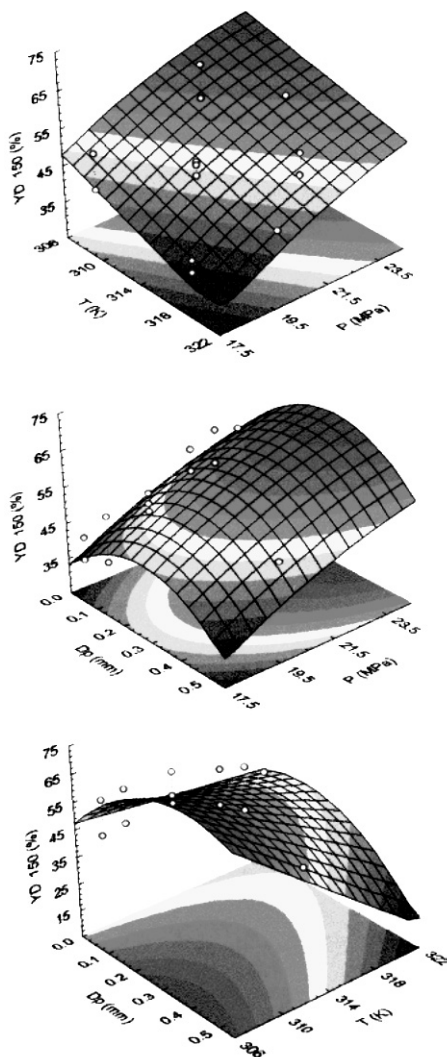
FIG. 4. Response surfaces showing the relation between the yield (YD) of walnut oil at 150 min of extraction as a function of pressure (*P*), temperature (*T*) and Dp. For other abbreviation see Figure 1.
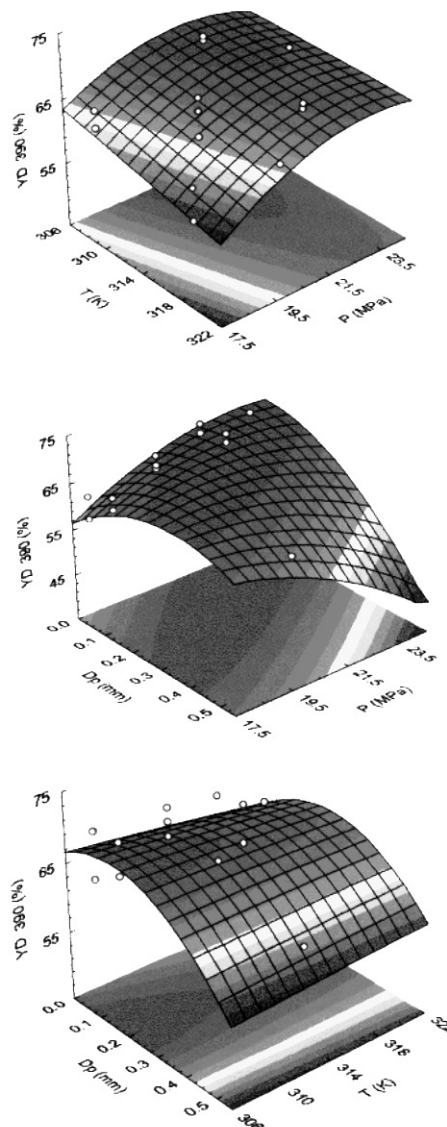

FIG. 5. Response surfaces showing the relation between the YD of walnut oil at 390 min of extraction as a function of *P, T* and Dp. For abbreviations see Figures 1 and 4.

The response surfaces shown in Figure 5 are described as

$$YD_{390\ min} = 90.158 - 0.3566\ P^2 - 0.00232\ T^2 - 99.363\ Dp^2$$

$$+ 0.0542\ PT - 10.8832\ PDp + 0.8289\ TDp \qquad [2]$$

$$R^2 = 0.974;\ Adj = 0.974\ (P < 0.01)$$

At 390 min of extraction time, an optimal value of the yield occurred within the pressure and particle diameter ranges studied. The values 22 MPa for pressure and 0.1 mm for particle diameter can be recommended. At this extraction time the temperature is not a very significant parameter.

The initial extraction velocities can be represented by the equation (Fig. 6)

$$Y\ (g\ oil/g\ walnut/kg\ CO_2) = 0.14567 + 0.007009\ P$$

$$- 0.000763\ T + 5.0994\ Dp - 0.40146\ Dp^2 - 0.01555\ TDp \quad [3]$$

$$R^2 = 0.983;\ Adj = 0.971\ (P < 0.01)$$

The surface showed that an optimal value for the initial

extraction velocity—the slope of the extraction curves—occurred within the experimental particle diameter range. Again, the initial extraction velocities exhibited high dependence on the pressure and temperature, being high values at higher values of pressure and lower values of temperature.

From Figures 1–6 we see that the best results were obtained for 22 MPa, 308 K, and Dp = 0.1 mm. At these conditions, and at 150 min of extraction time, about 85% of oil was extracted, whereas at 390 min, it was 95%. Although an economic optimization must be recommended, it seems that probably 150 min of extraction is a good time for preparation of walnut oil, since less CO_2 was spent and, as the residence time is smaller, a greater potential extractor throughput can be achieved.

No significant differences were found when the oils extracted by *n*-hexane in Soxhlet or by SFE were analyzed by FAME analysis. The results are presented in Table 1. The
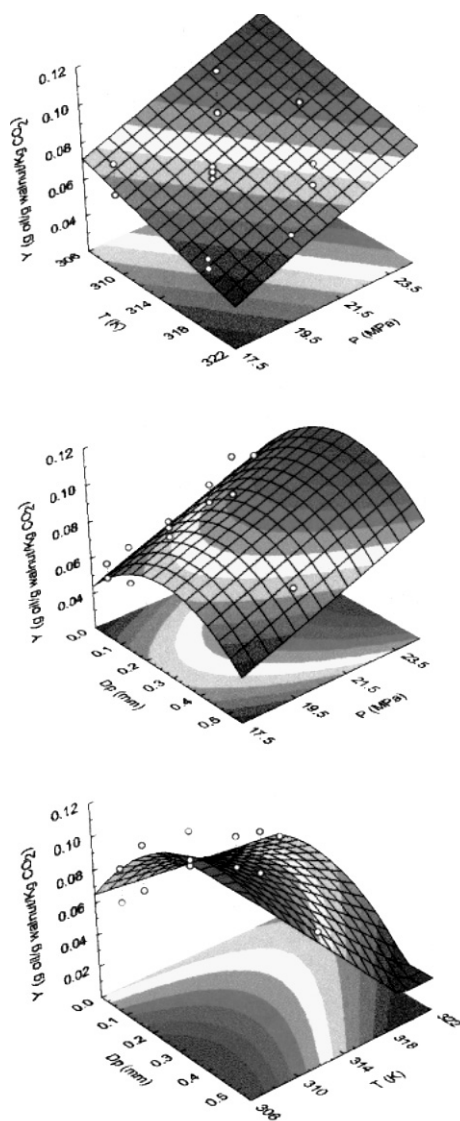
FIG. 6. Response surfaces showing the relation between the initial extraction velocities as a function of *P, T* and Dp. For abbreviations see Figures 1 and 4.

main component was linoleic acid (56.46%), followed by oleic acid (21.22%) and linolenic acid (13.16%). This agrees with results presented by Savage *et al.* (4) for walnut oils of several cultivars of walnut trees grown in Europe, the United States, and New Zealand.

Table 1 also presents the results of TAG analysis for walnut oils extracted by both methods. The main TAG found in walnut oils was the LLL TAG, followed by the OLL and LLLn. Results were similar for walnut oils extracted by *n*-hexane and by SFE.

Table 2 presents the results obtained for the analysis of sterols in walnut oil. The main component was β-sitosterol (85.16%), followed by campesterol (5.06%). The amount of cholesterol was low (0.31 and 0.16% for the oil extracted by *n*-hexane and by SFE, respectively).

Tocopherol amounts presented in Table 2 were similar to those found by Savage *et al.* (4). It was not possible to sepa-

TABLE 1
FA and TAG Compositions of Walnut Oils Extracted by Supercritical Fluid Extraction (SFE) and *n*-Hexane

		SFE	*n*-Hexane
FA (mol%)			
Myristic	14:0	0.01	0.01
Palmitic	16:0	6.49	6.08
Palmitoleic	16:1	0.07	0.07
Stearic	18:0	2.13	2.10
Oleic	18:1	21.22	20.98
Linoleic	18:2	56.46	56.88
Linolenic	18:3	13.16	13.41
Gadoleic	20:1	0.02	0.01
Behenic	22:0	0.06	0.07
TAG (mol%)[a]			
LLnLn		4.2	4.4
LLLn		18.4	18.1
LLL		24.4	23.7
OLLn		7.3	7.2
PLLn		3.5	3.3
OLL		19.6	19.3
PLL		7.8	7.7
OOL		7.2	7.3
POL		4.5	4.4
SLL		1.3	1.4
OOO		1.2	1.3
SOL		0.3	0.2

[a]P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid.

rate the β- and the γ-tocopherols, but a perceptible small peak revealed that the amount of β-tocopherol was very small. This is in agreement with the results of Savage *et al.* (4) and Lavedrine *et al.* (21). The SFE oil presented a larger total amount of tocopherols (405.7 µg/g oil) compared with the 303.2 µg/g of oil obtained with *n*-hexane.

TABLE 2
Sterol Composition, Total (µg/g oil) and Individual (%) Tocopherol Contents, Induction Time (IT), PV, and Acidity Index (AI) of Walnut Oils Extracted by SFE and *n*-Hexane[a]

	SFE	*n*-Hexane
Sterols (mol%)		
Cholesterol	0.16	0.31
Campesterol	5.06	4.43
Campestanol	0.00	0.00
Stigmasterol	0.40	0.39
Chlerosterol	0.74	1.05
β-Sitosterol	83.86	85.16
Δ5-Avenasterol	8.45	7.35
Δ5,24-Stigmastadienol	0.63	0.60
Δ7-Stigmasterol	0.06	0.15
Δ7-Avenasterol	0.10	0.13
Tocopherols		
α- (%)	6.3	2.7
β- and γ- (%)	82.5	82.0
δ- (%)	11.2	15.3
Total (µg/g oil)	405.7	303.2
IT (h)	1.33	3.62
PV (meq O$_2$/kg oil)	5.7	6.4
AI	0.3	0.3

[a]For other abbreviation see Table 1.

Oxidative stability tests using both Rancimat (IT) and PV revealed that walnut oil is not significantly protected against oxidation (Table 2). The SFE oil presented a lower IT, however, indicating greater susceptibility to oxidation than that extracted by *n*-hexane. The PV and the acidity indexes are similar for oils extracted by the different methods (Table 2). Oil extracted by SFE was clearer than that extracted by *n*-hexane, showing some refining.

ACKNOWLEDGMENTS

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