Influence of Operation Variables on Quality Parameters of Olive Husk Oil Extracted with CO_2: Three-Step Sequential Extraction

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ABSTRACT: Supercritical CO_2 extraction is a viable alternative process for the extraction of high-quality oil from olive husk (also known as olive pomace), a residue obtained in the production of olive oil. We analyzed the effect of pressure (100–300 bar), temperature (40–60°C), solvent flow (1–1.5 L/min), and particle size (0.30–0.55 mm) on four important quality parameters of the oil extracted with CO_2: tocopherol concentration, extinction coefficients at 232 and 270 nm, and saponification value. Response surface methodology was used to obtain mathematical expressions related to the operating variables and parameters studied. Results from these experiments were also used to design a three-step sequential CO_2 extraction procedure to obtain a higher-quality extract. The optimal operational sequence consisted of a first extraction step at 75 bar for 1 h using 1% (vol/vol) ethanol modifier, followed by a second extraction stage at 350 bar for 2.5 h without ethanol and a third step, also at 350 bar, for 2.5 h but using ethanol. These extraction conditions obtained an intermediate fraction of oil with 64% yield and all normal parameters according to European Commission food legislation. This fraction is suitable without any further refining. On the contrary, the oils obtained by hexane extraction and by conventional supercritical CO_2 extraction at optimal conditions are suitable for human consumption after further refining. This last finding may result in improved economics of the sequential CO_2 extraction process compared to the conventional extraction method with hexane.

Paper no. J10354 in *JAOCS 80*, 181–188 (February 2003).

KEY WORDS: Olive husk, olive oil, quality parameters, response surface methodology, sequential extraction, supercritical extraction.

Olive husk (also known as olive pomace) is a by-product obtained from the industrial process for olive oil production. It comprises water, oil, olive peel, and kernels. Its oil content is extracted with hexane to obtain crude olive husk oil in an industrial process that consists of successive steps including drying, grinding, extraction, and distillation of the solvent. This process causes several problems related to the use of organic solvents and the necessity of recovering them by distillation, an energy-intensive operation. Furthermore, the crude husk oil thus obtained must be refined to be suitable for human consumption. This refining step is expensive, and thermal degradation and loss of highly valuable compounds usually result (1,2). The refined oil must comply with strict limits related to oil quality and purity (defined as parameters) that have been established as authenticity criteria and that are used for classifying the oil (3).

Owing to its favorable selectivity, supercritical fluid extraction has been applied for the extraction and refining of vegetable oils, producing high-quality extracts (2,4). In the case of olive oil, little about this process has been reported in the literature, although it has been applied to the continuous countercurrent extraction (5) or refining (6) of olive husk flakes and to olive husk oil previously extracted with hexane (6–8). However, in most of these reports the effects of operating variables on oil quality parameters have not been analyzed. We previously (9) reported on the effects of the most common operational variables on the extraction yield (Y) as well as oil acidity (OA), PV, and phosphorus content (PC).

In the present work the effects of pressure (P), temperature (T), solvent flow, and particle size on additional quality and purity parameters not considered previously were analyzed: tocopherol (TO) concentration, extinction coefficients at 232 and 270 nm (K_{232} and K_{270}, respectively), and saponification value (SV).

EXPERIMENTAL PROCEDURES

Materials. Olive husk, provided by Aceites Pina Bajo Aragón, S.A. (Villarta de S. Juan, C. Real, Spain), was milled and dried to final moisture of 6% (w/w). The dried husk was sieved to obtain several size fractions with the following average diameters: 0.108, 0.175, 0.240, 0.300, 0.425, 0.550, 0.610, 0.845, 1.500, and over 2.000 mm. These fractions were frozen and stored in an N_2 atmosphere. Liquid CO_2 (purity, 99.5%) was supplied by Carburos Metalicos S.A. (Madrid, Spain). Ethanol (purity 99.95%) and analytical-grade hexane were supplied by Panreac (Montplet & Esteban S.A., Barcelona, Spain).

Apparatus and extraction procedure. The experiments were completed in a batch-type apparatus, as shown in Scheme 1 (described in detail in Ref. 10). Liquid CO_2 from a stainless-steel cylinder (SC) was cooled (C), filtered (F), and

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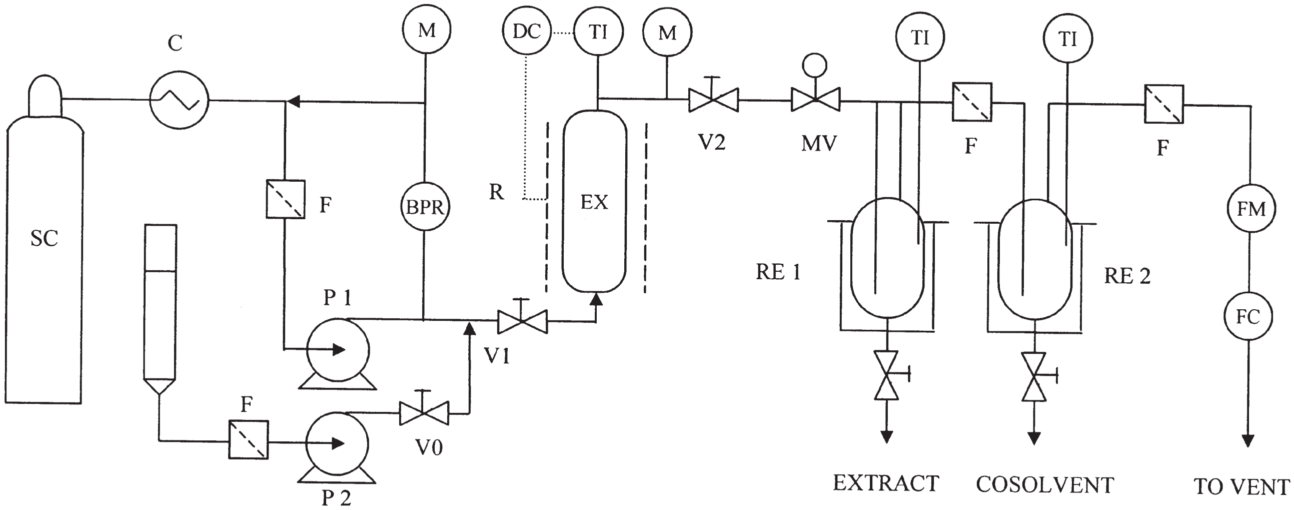

SCHEME 1

compressed by a positive-displacement HPLC pump (P 1). The pressure was regulated by a back-pressure regulator (BPR) and checked by a manometer (M). The compressed fluid was passed through a 75-mL stainless-steel cylinder (EX) from the bottom. To keep the extractor temperature at the desired value, a digital controller (DC) regulated the electric current through a resistor (R) that surrounded the extractor cylinder. The temperature in the extractor was displayed by a temperature indicator (TI). The oil-laden gas from the extractor was passed through a heated metering valve (MV) where the supercritical CO_2 was depressurized, and the extracted oil was collected in a cooled receiver (RE 1) at 0°C. The gas flow through the extractor was measured by a turbine flow meter (FM) and totalled by a digital flow computer (FC).

In all experiments the extraction was run on 25-g olive husk samples placed in the extractor between two layers of glass wool to prevent loss of small particles. The response surface experiments were accomplished in 2.5 h because the yield obtained after this time period was negligible. For sequential extractions the ethanol was fed using a second pump (P 2). Ethanol was recovered separately from the extract in the receiver (RE 2).

Soxhlet extractions. To compare the supercritical extracts to those obtained with hexane, 25-g samples of olive husk flakes were extracted in a 250-mL Soxhlet apparatus with analytical-grade hexane for 2.5 h, using the industrial particle-size distribution of 0.55 mm average diameter. After extraction, the solvent was evaporated and the oil content was determined gravimetrically. The residual solvent in the extract was lower than 1 mg/kg, determined according to the European Standard (3) corresponding to AOCS Official Method Ca 3b-87 (12).

Analyses. The quality parameters of the oil were determined according to standard methods specified in European Commission Regulation 2568/91 (3), in accordance with the following AOCS official methods (11): Ch 5-91 for extinction coefficients, Cd 3-25 for SV, Cd 3d-63 for OA, Cc 7-25 for refractive index, Cd 1-25 for iodine number, Ca 12b-92 for PC, Ce 1e-91 for oleic acid content, and Cd 8-53 for PV.

HPLC analyses of TO. The analyses were performed by HPLC using a Hewlett-Packard 1100 liquid chromatograph as outlined in Reference 12. TO was measured using 1 mL/min methanol as the mobile phase at 25°C (isocratic), a 5-μm RP-18 column (4 × 25 cm, Lichrosphere; Merck, Darmstadt, Germany) as the stationary phase, and a UV-vis detector set at 293 nm.

RESULTS AND DISCUSSION

Response surface experiments. Response surface methodology (RSM) is commonly used to study empirical relationships between measured responses and independent variables (also called factors), minimizing experimentation and leading to correlations that can be used for process optimization (13).

The responses studied in the first part of the work were TO concentration, K_{232} and K_{270}, and SV. The TO are extremely valuable compounds because of their activity as vitamin E and antioxidants. TO concentration is related to oil oxidation. The extinction coefficients give the UV absorbance at 232 and 270 nm. The maximum absorbance for hydroperoxides and conjugated dienes is 232 nm, whereas 270 nm is the maximum for conjugated trienes, aldehydes, and ketones. All these compounds are formed as a result of oxidation reactions. SV is related to the M.W. of oil TG and is expressed as mg KOH required to saponify 1 g of oil (1).

The independent variables selected for the first part of the study were P, T, solvent flow, and particle size. The levels of each factor (Table 1) were determined in a previous work (9): 100–300 bar P, 40–60°C T, 0.30–0.55 mm particle diameter, and CO_2 flow at standard conditions from 1 to 1.5 L/min. For a direct comparison of each variable and to avoid the effect produced in calculations by their different magnitudes, the independent variables were normalized in the range of – 1 to + 1 according to Equation 1:

$$x_i = 2(X_i - X_{\min})/(X_{\max} - X_{\min}) - 1 \qquad [1]$$

TABLE 1
Levels of Factors

Factor	Lower level (–1)	Higher level (+1)
Temperature (T, ºC)	40	60
Pressure (P, bar)	100	300
CO_2 flow (Q, L/min)	1.0	1.5
Particle diameter (D, mm)	0.30	0.55

where x_i is the normalized value of variable X at condition i, X_i is the dimensional value, and X_{min} and X_{max} represent the dimensional inferior and superior limits, respectively.

The RSM analysis consisted of a full factorial 2^4 experimental design to which two central points were added to evaluate the curvature effect. Experiments were run at random. The standard experimentation matrix is shown in Table 2. Column 2 indicates the number of replications performed in each experiment to obtain significant oil quantity for the analyses according to the European Standard (3). Columns 3 to 6 give the variable levels coded –1 to +1 in the dimensionless coordinate, and columns 7 to 10 give the dimensional variable levels. Table 2 also shows the experimental values obtained for the selected responses (columns 11 to 14).

The Commercial Statgraphics 5.0 Program (Manugistics, Inc., Rockville, MD) was used to perform the statistical analysis of the experimental results. The analysis of the main and interaction effects for the chosen responses, together with the curvature check results, are shown in Table 3. The test of statistical significance (*P* value) was determined according to the total error criteria considering a confidence level of 95%. It can be seen in Table 3 that the curvature effect was not significant for any parameter studied. This result indicated the validity of the pure factorial design in the experimental range analyzed, so second-order models were not necessary.

For TO concentration, Table 3 shows that the pressure–temperature interaction (PT) was the only significant factor. Fitting the data to a first-order model gave the following equation, where the independent variables were codified in the –1 to +1 range according to Equation 1 (see Table 1). Equation 2 reproduced experimental data with a SD of 13.90 ppm:

$$\text{TO (ppm)} = 105.14 - 22.14\ \text{PT} \qquad [2]$$

Figure 1A shows the influence of these variables on TO concentration. The independent variables were represented in dimensional coordinates. The optimal conditions for the selective extraction of TO were 100 bar and 60°C. TO in extracts obtained at lower P were attributed to the decrease in relative recovery of other diluting materials (mainly TG). This enhancement in the selective extraction of TO from a multicomponent mixture at low P was also observed by Brunner *et al.* (14), who extracted palm oil at 300 to 500 bar; King *et al.* (15), who extracted rice bran at 250 to 700 bar; Ikushima *et al.* (16) in the extraction of α-tocopherol and linoleic acid methyl ester mixtures; and Lucas *et al.* (17), who extracted olive tree leaves at 250 to 450 bar. In all these reports the effect of P on the selective extraction of TO was attributed to a decrease in the recovery of other oil components. We observed an increased TO concentration at 100 bar when T was increased from 40 to 60ºC. In this P range, the increase in TO solubility with increasing temperature was higher than that corresponding to TG (18). The same effect was also observed by King *et al.* (15) when extracting rice bran at 250 bar and 40 to 80°C.

TABLE 2
Experimental Matrix and Results for the Full 2^4 Factorial Design[a]

Experiment	Number of extractions	P	T	Q	D	P (bar)	T (°C)	Q (L/min)	D (mm)	TO (ppm)	K_{232}	K_{270}	SV (mg/g)
1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14
1	5	–1	–1	–1	–1	100	40	1.0	0.300	138	8.3	2.3	180
2	3	+1	–1	–1	–1	300	40	1.0	0.300	120	4.7	1.9	181
3	5	–1	+1	–1	–1	100	60	1.0	0.300	73	14.7	4.8	176
4	3	+1	+1	–1	–1	300	60	1.0	0.300	73	5.8	1.4	187
5	5	–1	–1	+1	–1	100	40	1.5	0.300	173	9.0	1.7	198
6	3	+1	–1	+1	–1	300	40	1.5	0.300	56	6.7	1.7	194
7	5	–1	+1	+1	–1	100	60	1.5	0.300	162	11.3	3.5	201
8	3	+1	+1	+1	–1	300	60	1.5	0.300	148	4.1	1.5	182
9	5	–1	–1	–1	+1	100	40	1.0	0.550	30	8.7	2.9	196
10	3	+1	–1	–1	+1	300	40	1.0	0.550	127	8.6	1.8	170
11	5	–1	+1	–1	+1	100	60	1.0	0.550	231	14.6	3.5	189
12	3	+1	+1	–1	+1	300	60	1.0	0.550	23	3.5	0.9	193
13	5	–1	–1	+1	+1	100	40	1.5	0.550	23	9.6	3.3	201
14	3	+1	–1	+1	+1	300	40	1.5	0.550	193	9.4	1.3	180
15	5	–1	+1	+1	+1	100	60	1.5	0.550	57	12.0	3.8	183
16	3	+1	+1	+1	+1	300	60	1.5	0.550	56	5.9	0.7	195
17	4	0	0	0	0	200	50	1.25	0.425	111	7.7	1.2	190
18	4	0	0	0	0	200	50	1.25	0.425	124	7.0	1.4	196

[a] Conditions: time of extraction, 2.5 h; weight of olive husk, 25 g; TO, tocopherol content; K_{232} and K_{270}, extinction coefficients at 232 and 270 nm, respectively; SV, saponification value. For other abbreviations see Table 1.

[b] Numbers 1–14 indicate column numbers used in text.

TABLE 3
Main Effects and Interaction from the 2^4 Factorial Design[a]

Factor or interaction	Effect TO	*P* value	Effect K_{232}	*P* value	Effect K_{270}	*P* value	Effect SV	*P* value
1	2	3	4	5	6	7	8	9
P	−11.3	0.823	−4.34	0.020	−1.80	0.012	1.8	0.286
T	−4.7	0.925	0.29	0.750	0.39	0.291	1.6	0.914
Q	6.4	0.899	−0.21	0.890	−0.28	0.445	15.6	0.164
D	−25.5	0.617	1.08	0.490	−0.07	0.841	−0.2	0.863
PT	−44.3	0.049	−3.97	0.029	−0.95	0.027	13.2	0.182
PQ	20.9	0.681	0.85	0.380	0.06	0.862	−4.0	0.539
PD	25. 8	0.614	0.68	0.478	−0.39	0.290	−6.0	0.600
TQ	−1.0	0.984	−1.07	0.269	−0.02	0.950	−3.5	0.467
TD	3.2	0.948	−1.03	0.283	−0.50	0.185	5.0	0.611
QD	−27.5	0.592	−0.09	0.917	0.24	0.513	−9.6	0.339
Curvature	16.9	0.817	−0.10	0.323	1.01	0.188	29.8	0.512

[a] See Tables 1 and 2 for abbreviations.

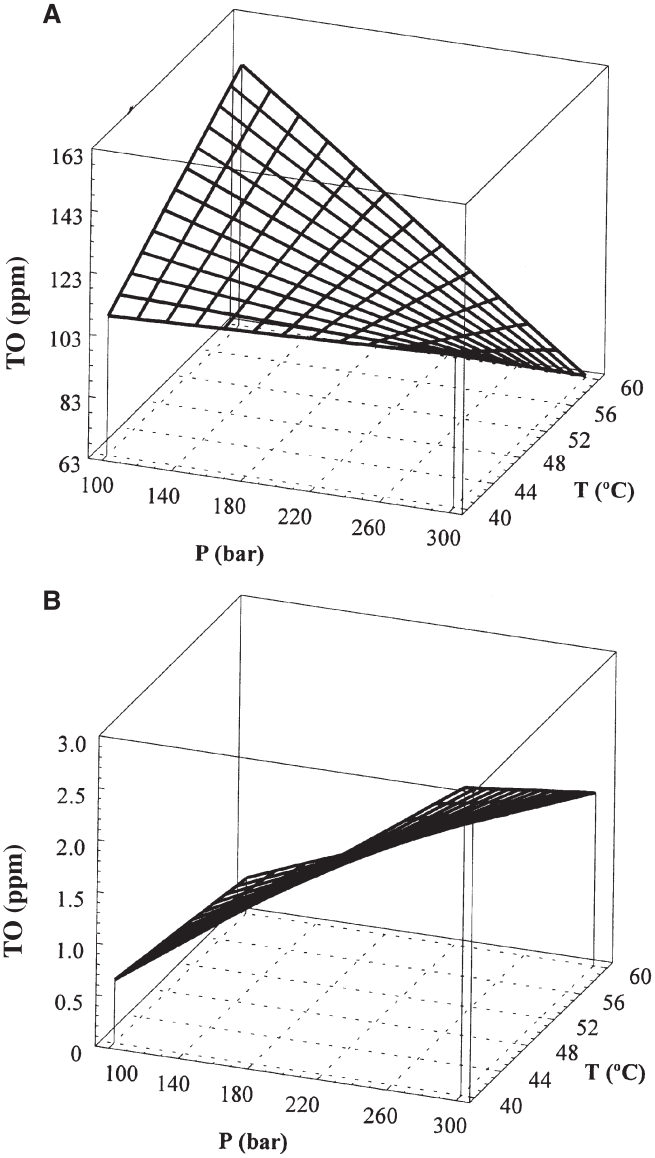

FIG. 1. Full 2^4 factorial design. Pressure (P)–temperature (T) interaction for (A) tocopherol (TO) concentration and (B) mass of TO extracted. Conditions: Q (CO_2 flow) = 1.25 L/min; D (particle diameter) = 0.425 mm.

Figure 2B presents the total amount of TO extracted, instead of their concentration, as a function of P and T. It can be seen that TO extraction increased with increasing P owing to the increase in CO_2 density and hence its extraction capacity. Figure 2B also shows a slight decrease in TO recovery with an increase in T. Considering that the solubility of pure TO in CO_2 increases with an increase in T (18), this T effect may show that coextraction of other oil components modifies the TO solubility in supercritical CO_2 (14).

For K_{232}, data in Table 3 were fitted to the following relationship, where the independent variables were codified in the range of –1 to +1 according to Equation 1 (see Table 1) with a SD of 0.36.

$$K_{232} = 8.67 - 2.17\ P - 1.99\ PT \qquad [3]$$

Figure 2A shows the influence of P and T over K_{232}. Since K_{232} is related to the concentration of hydroperoxides and conjugated dienes, their selective extraction was favored at 100 bar and 60°C, indicating that these compounds were preferably extracted with respect to TG at these conditions. As observed for TO, increasing the extraction capacity of CO_2 resulted in a greater increase in the recovery of TG and then in a dilution of hydroperoxides and conjugated dienes in the extract.

In Figure 2B, the effect of P and T on K'_{232} (the total amount of hydroperoxides and dienes extracted instead of their concentrations in the extracts) is shown. K'_{232} was obtained by multiplying K_{232} by the corresponding oil yield (9). It can be observed in Figure 2B that the recovery of these compounds was favored at higher P and lower T. The effect of P was related to the increase in CO_2 extraction capacity with P, whereas the slight influence of T may be attributed to the fact that the two factors affecting the solubilities of these solutes (namely, CO_2 density and solute vapor P) are balanced in the experimental range analyzed.

For K_{270}, the corresponding relationship obtained by fitting the experimental data to the results shown in Table 3 was:

$$K_{270} = 2.26 - 0.90\ P - 0.48\ P \qquad [4]$$

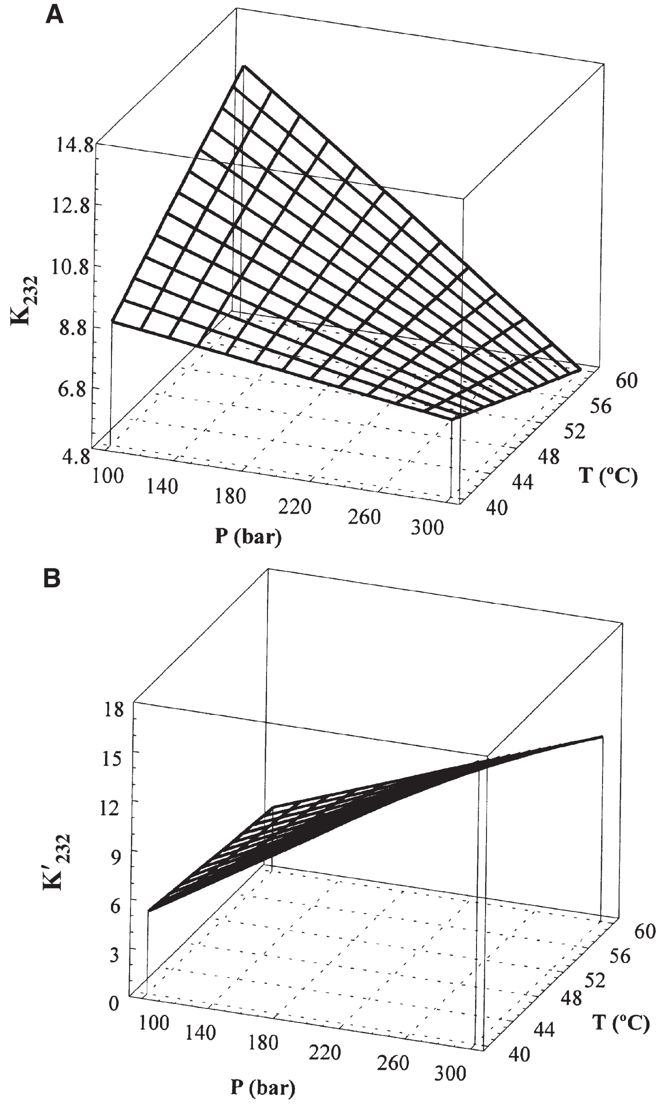

FIG. 2. Full 2^4 factorial design. P–T interaction for (A) K_{232} and (B) K'_{232}. Conditions: Q = 1.25 L/min, D = 0.425 mm. See text for description of K'_{232}. For abbreviations see Figure 1.

where the independent variables are codified in the range of –1 to +1 according to Equation 1 (see Table 1). Equation 4 reproduced experimental data with a SD of 0.14.

The influence of the experimental variables on the extinction coefficient K_{270} was analogous to that obtained for K_{232}. This result was not unexpected since K_{270} is related to the composition in conjugated trienes, aldehydes, and ketones. All these oxidation products have very similar polarity and M.W. properties compared to hydroperoxides and conjugated dienes, related to K_{232}, and will be extracted from the olive husk showing a similar trend. This justifies the fact that Equation 4 is analogous to Equation 3. As observed for K_{232}, the selective extraction of oxidation compounds is favored at 100 bar and 60°C owing to the selective extraction of other husk compounds with respect to TG.

There is no significant effect of any operating variable on the SV (Table 3). This means that the scatter in our results was due to experimental error and not to the influence of any operating variable. The mean value of this response in the experimental range analyzed was 189 mg/g, within the range permitted (182–193) by the European Commission Standard (3). These results were not unexpected since several investigators have found no significant variations in SV with either supercritical or hexane extracts (19,20).

Comparison with hexane extracts and best single-step extraction conditions. Column 2 in Table 4 shows the quality parameters of the olive husk oil extracted with hexane. Table 4 also summarizes the results obtained for these parameters in the supercritical extraction of olive husk oil. Columns 3 to 6 show both maximum and minimum experimental and calculated values of the parameters in the experimental range studied. The equations used to obtain the calculated values are presented in column 7. Finally, to compare the quality of the oil with the specifications given in the European Commission regulation (3), these are included in column 8 of the table.

From data in column 2 it can be observed that the hexane extract matches the European regulation. However, it shows a very low TO concentration and a value for K_{232} very close to the permitted limit (3). These results agree with the poor values that we obtained (9) for other quality parameters, namely, OA, PV, and PC of the husk oil extracted with hexane. For these parameters to be in accordance with the European Commission regulation, it was necessary to refine the crude oil (3).

Regarding the supercritical extracts, notable differences shown in columns 3 to 6 for extracts obtained at different conditions confirm the possibility of modifying the selectivity of the solvent for different oil compounds. The best supercritical oil, matching the European Commission regulation and with the maximal TO concentration, was obtained at 300 bar and 55°C, these conditions being calculated from Equations 2 to 4. Their quality characteristics were TO = 94 ppm, K_{232} = 5.5, K_{270} = 1.12, and SV = 189 mg/g. Regarding these parameters and those studied in our previous work (9), the quality of the oil thus extracted is superior to that obtained in industrial hexane extraction.

Three-step sequential extraction. In the single-step extraction we have obtained an extract with increased quality that is suitable for consumption according to the parameters studied (TO, K_{232}, K_{270}, and SV). For other parameters such as OA, PV, and PC, it was previously determined (9) that the supercritical extract required only a soft refining step. A survey of the literature shows that the selective extraction of FFA occurs at low P levels in the first stages of extraction (6,7). It also has been reported that the addition of modifiers decreases the selectivity of the solvent but increases the extraction rate (21,22), and that oil recovery is favored when using either high P or modifiers (22,23). Considering all these facts together with our previous results (9), it appears that by applying a sequential extraction to olive husks we can obtain oil fractions with different qualities.

In the sequential extraction proposed here, we attempted to obtain olive husk oil in three extraction stages. The objective in the first step was to recover and concentrate most of

TABLE 4
Comparison of Hexane and Supercritical Extracts

Response	Soxhlet extraction	Supercritical extraction					
	Experimental value	Minimum	Maximum	Mimimum[b]	Maximum[b]	Equation	Standard[c]
1[a]	2	3	4	5	6	7	8
TO (ppm)	60	23	231	83	127	2	—[d]
K_{232}	5.3	3.5	14.6	4.5	12.8	3	≤5.5
K_{270}	1.3	0.7	4.8	0.88	3.6	4	≤2.5
SV (mg/g)	184	147	201	189	189	[e]	182–193

[a] Numbers 1–8 indicate column numbers used in text. See Tables 1 and 2 for abbreviations.
[b] For equation see text.
[c] CEE/2568/91(Ref. 3).
[d] —, not applicable
[e] Equation: SV = 189.

the FFA, whereas the aim of the second was to obtain the maximum amount of oil ready to be commercialized. Finally, the last step was applied to recover the residual oil still remaining in the olive husk.

The independent variables studied in the first step were P (in the range 75–100 bar), extraction time (1–1.5 h), and ethanol concentration in CO_2 (0–1%, vol/vol) used as co-solvent. In the second step, the P was set at 350 bar and the variable investigated was extraction time in the range 2–2.5 h. The final stage consisted of a 2.5-h extraction period at 350 bar using 1% (vol/vol) ethanol in CO_2. The rest of the operating conditions were those leading to the best values of the quality parameters considered in both this work and our previous investigation (9): T 55°C, solvent flow 1.25 L/min, and husk particle diameter 0.30 mm. The operating conditions of the sequential extraction experiments are summarized in Table 5.

TABLE 5
Operational Conditions of Sequential Extractions[a]

Step	Conditions	Experiment			
		E-I	E-II	E-III	E-IV
1	P (bar)	75	100	75	75
	Time of operation (h)	1	1	1.5	1
	Ethanol (1%, vol/vol)	NO	NO	NO	YES
2	P (bar)	350	350	350	350
	Time of operation (h)	2.5	2.5	2	2.5
	Ethanol (1%, vol/vol)	NO	NO	NO	NO
3	P (bar)	350	350	350	350
	Time of operation (h)	2.5	2.5	2.5	2.5
	Ethanol (1%, vol/vol)	YES	YES	YES	YES

[a] Conditions: Q = 1.25 L/min; D = 0.30 mm; weight of olive husk = 25 g (see Experimental Procedures section for details of each experiment). E, experiment; for other abbreviations see Table 1.

The dependent variables selected for determining the best conditions to perform each extraction step were Y, determined as the weight percentage of the oil extracted with respect to that obtained by hexane Soxhlet extraction, and OA. They were chosen because they are the most sensitive to variations in the operating variables. Table 6 shows the corresponding results obtained in each fraction of the oil.

Results from experiment E-I presented in Table 6 (a reference test for comparison purposes) show that in the first extraction period, most of the total amount of FFA in the husk was concentrated in a relatively low amount of oil (7%, w/w), so this step can be successfully used to eliminate the acidity of the samples. In the second stage, most of the oil (72%) was recovered with low OA (3.7%, w/w), but despite this important observation, this value was still over the norm (0.5%). During the last extraction period a co-solvent (ethanol) was used to increase the recovery of the remaining oil, and an important amount of FFA still fixed to the natural matrix (6,9,23) was extracted. In E-II, increasing the P to 100 bar in the first step increased extraction and concentration of FFA. Because of that, the increase in FFA solubility in this P range was higher, corresponding to that of TG compounds (6). The high removal of FFA in the first stage reduced the FFA content of the remaining oil and, as a result, the OA of the second-stage extract was only 1.1%. From the results of experiment E-III, we observed that increasing the length of the first stage, with respect to the reference test, increased oil recovery and decreased OA. The slight reduction in OA observed was probably due to diluting FFA (which are mostly extracted at the beginning of the step) in the rest of the oil compounds. This exhaustive elimination of FFA at low P produced oil in the second step with only an OA = 0.34%, matching the

TABLE 6
Sequential Extraction Oil Analyses[a]

Test	E-I			E-II			E-III			E-IV		
Step	1	2	3	1	2	3	1	2	3	1	2	3
Y (%)	7.0	72.0	21.0	11.8	70.2	18.0	13.8	61.5	27.0	21.0	64.0	15.0
OA (%)	39.0	3.7	24.5	44.7	1.1	24.5	37.4	0.34	19.6	38.5	0.22	15.5

[a] See Table 5 for experimental conditions. Y, oil yield; OA, oil acidity; for other abbreviations see Table 5.

TABLE 7
Analytical Parameters of Extracts Obtained by Different Techniques

Parameter	Hexane extraction	One-step extraction optimal conditions[a]	Three-step sequential extraction[b]	Standard[c]
Y (%)	100	80	64	—[d]
OA (%)	16	14	0.2	≤0.5
TO (ppm)	60	94	61	—
Refraction index	1.4677	1.4676	1.4678	1.4650–1.4707
Iodine number (%)	58	83	84	75–90
Phosphorus content (%)	$3 \cdot 10^{-2}$	$2.3 \cdot 10^{-3}$	$2.1 \cdot 10^{-3}$	—
Oleic acid content (%)	68	64	65	55–83
K_{232}	5.3	5.5	4.4	≤5.5
K_{270}	1.3	1.1	0.86	≤2.5
SV (mg/g)	184	189	190	182–193
PV (meq/kg)	14	10	4	≤5

[a] P = 300 bar; T= 55°C; Q = 1.25 L/min; D = 0.30 mm.
[b] Conditions corresponding to E-IV (see Table 5).
[c] CEE/2568/91(Ref. 3).
[d] —, not applicable. See Tables 1, 2, and 6 for abbreviations.

European Commission specifications. Finally, compared to the reference test, the addition of a modifier in the first stage of experiment E-IV produced the same effect as increasing the extraction time in experiment E-III. OA slightly decreased due to the dilution of the FFA because of the increased oil yield (14% with respect to experiment E-I). In the second stage, the OA was lower than normal because of the efficient elimination of acidity in the first step.

According to the results expressed above, the optimal operation sequence leading to higher fractions of oil complying with specifications was that corresponding to experiment E-IV. These conditions permitted us to obtain an intermediate fraction of oil with 64% yield and 0.22% OA. A complete analysis of this fraction for all quality parameters is shown in Table 7. Results obtained for hexane extracts and for the best single-step supercritical extraction are also shown in Table 7. The best single-step extraction was calculated by using Equations 2 to 4 to obtain the optimal oil characteristics according to normal and maximal TO concentrations. The sequential extraction process produced oil suitable for human consumption without the need for any further refining procedure. However, the extracts obtained with hexane and in the single-step supercritical CO_2 extraction at optimal conditions should be refined. This process must be more rigorous for hexane extracts because their quality parameters differ more from the standard than those corresponding to the optimal CO_2 extract. This finding is advantageous for the economy of the process.

REFERENCES

1. Boskou, D., *Olive Oil: Chemistry and Technology*, AOCS Press, Champaign, 1996, pp. 25–66 and 267–282.
2. Maxwell, R.J., Solubility Measurements of Lipids Constituents in Supercritical Fluids, in *Supercritical Fluid Technology in Oil and Lipid Chemistry*, edited by J.W. King and G.R. List, AOCS Press, Champaign, 1996, pp. 20–34.
3. European Commission (ECC) Regulation 2568/91, On the Characteristics of Olive Oil and Olive-Residue Oil on the Relevant Methods of Analysis, *Offic. J. Eur. Commun. L248*:1–82 (1991).
4. Stahl, E., K.W. Quirin, and D. Gerard, *Dense Gases for Extraction and Refining*, Springer-Verlag, Berlin, 1988, pp. 72–210.
5. Brunner, G., and P.C. Simoes, Multicomponent Phase Equilibria of an Extra-Virgin Olive Oil in Supercritical Carbon Dioxide, *J. Supercrit. Fluids 9*:75–81 (1996).
6. Gonçalvez, M., A.M.P. Vasconcellos, E.J.S. Gomes de Azevedo, H.J. Chaves das Neves, and M. Nunes da Ponte, On the Applications of SFE to the Deacification of Olive Oil, *J. Am. Oil Chem. Soc. 68*:474–480 (1991).
7. Esquivel, M.M., and G. Bernardo-Gil, Extraction of Olive Husk Oil with Compressed Carbon Dioxide, *J. Supercrit. Fluids 6*: 91–94 (1993).
8. Demirbas, A., Liquefaction of Olive Husk by Supercritical Fluid Extraction, *Energy Convers. Manage. 41*:1875–1883 (2000).
9. Lucas, A., J. Rincón, and I. Gracia, Influence of Operation Variables on Yield and Quality Parameters of Olive Husk Oil Extracted with Supercritical CO_2, *J. Am. Oil Chem. Soc. 79*:237– 243 (2002).
10. Lucas, A., A. García, J. Rincón, A. Alvarez, I. Gracia, and M.A. García, Supercritical Carbon Dioxide Extraction of Fatty and Waxy Material from Rice Bran, *J. Am. Oil Chem. Soc. 73*:1127–1131 (1997).
11. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, AOCS Press, Champaign, 1993.
12. Koskas, J.P., J. Cillard, and P. Cillard, Separation of α-Tocopherol, α-Tocopherolquinone, and α-Tocopherol Dimer by Reversed-Phase High-Performance Liquid Chromatography, *J. Chromatogr. 287*:442–446 (1984).
13. Box, J.E.P., W.G. Hunter, and J.S. Hunter, *Statistics for Experimenters: An Introduction to Design, Data Analysis and Model Building*, Spanish edn., Reverte Publishing, Barcelona, Spain, 1993, pp. 317–361.
14. Brunner, G., T. Malchow, K. Sturken, and T. Gottschau, Separation of Tocopherol from Deodorizer Condensates by Countercurrent Extraction with Carbon Dioxide, *J. Supercrit. Fluids 4*: 72–80 (1991).
15. King, J.W., F. Favati, and S.L. Taylor, Production of Tocopherol Concentrates by Supercritical Fluid Extraction and Chromatography, *Sep. Sci. Technol. 31*:1843–1857 (1996).
16. Ikushima, Y., M. Arai, and K. Arai, *In situ* Monitoring of Extraction and Separation Behavior of Lipids with SC-CO_2, *Ind. Eng. Chem. Res. 31*:568–574 (1992).
17. Lucas, A., E. Martinez de la Ossa, J. Rincón, M.A. Blanco, and I. Gracia, Supercritical Fluid Extraction of Tocopherol Concentrates from Olive Tree Leaves, *J. Supercrit. Fluids 22*:221–228 (2002).
18. Oghaki, K., I. Tsukahara, K. Semba, and T. Katayama, A Fundamental Study of the Extraction with a Supercritical Fluid. Solubilities of α-Tocopherol, Palmitic Acid and Tripalmitin in

Compressed Carbon Dioxide at 25 and 40°C, *Int. Chem. Eng. Jpn. 29*:303–308 (1989).
19. Martínez de la Ossa, E., A. Molero, and M.D. Gordillo, Caracterización del Aceite de Germen de Trigo Extraído con CO_2 Líquido y Supercrítico, *Grasas Aceites 48*:87–92 (1997).
20. Bondioli, P., C. Mariani, A. Lanzani, E. Fedeli, A. Mossa, and A. Muller, Lampante Olive Oil Refining with Supercritical Carbon Dioxide, *J. Am. Oil Chem. Soc. 69*:477–480 (1992).
21. King, J.W., G.R. List, and A.J. Heaking, Selective Extraction of Phospholipid Mixtures by Supercritical Carbon Dioxide and Cosolvents, *J. Food Sci. 61*:1230–1233 (1996).
22. Cocero, M.J., and L. Calvo, Supercritical Fluid Extraction of Sunflower Seed Oil with CO_2–Ethanol Mixtures, *J. Am. Oil Chem. Soc. 73*:1573–1578 (1996).
23. Turkay, S., M.D. Burford, M.K. Sangun, E. Ekinci, K.D. Bartle, and A.A. Clifford, Deacidification of Black Cumin Seed Oil by Selective Supercritical CO_2, *Ibid. 73*:1265–1270 (1996).

[Received June 11, 2002; accepted October 20, 2002]