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Countercurrent Supercritical Fluid Extraction and Fractionation of High-Added-Value Compounds from a Hexane Extract of Olive Leaves

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Countercurrent supercritical fluid extraction (CC-SFE) at a pilot scale plant was used for fractionation of high-added-value products from a raw extract of olive leaves in hexane. Compounds found in the raw extract were waxes, hydrocarbons, squalene, β-carotene, triglycerides, α-tocopherol, β-sitosterol, and alcohols. The CC-SFE extraction process was investigated according to a 2^3 full factorial experimental design using the following variables and ranges: extraction pressure, 75−200 bar; extraction temperature, 35−50 °C; and ethanol as modifier, 0−10%. Data were analyzed in terms of extraction yield, enrichment, recovery, and selectivity. Higher extraction yields were attained at 200 bar. For most of the compounds analyzed enrichment was attained at the same conditions, that is, 75 bar, 35 °C, and 10% ethanol. Hydrocarbons were usually recovered in the separators, whereas waxes and α-tocopherol remain in the raffinate. Selectivity data reveal that α-tocopherol is the most easily separable compound. The influence of the experimental factors on the recovery of all the compounds was studied by means of regression models. The best fitted model was attained for β-sitosterol, with $R^2 = 99.25\%$.

KEYWORDS: Olive leaves; solid−liquid (S−L) extraction; fractionation; countercurrent supercritical fluid extraction (CC-SFE); supercritical CO_2; full factorial experimental design; waxes; hydrocarbons; squalene; β-carotene; triglycerides; α-tocopherol; β-sitosterol; total alcohols

INTRODUCTION

Olive culture is one of the most important agricultural activities, mainly in the Mediterranean area where there are ~8 million ha of cultivated olive trees (98% of the world crop). This points out the great economic and social importance of this crop and the possible benefits that can be derived from utilization of its byproducts. Olive leaves are one of the byproducts of the farming of the olive grove and can be found in high amounts in the olive oil industries (10% of the total weight of the olives) and during pruning of olive trees. This raw material has low cost and high availability because it is concentrated in the olive oil production centers, but at present a very limited productivity is obtained from it because the main interest is concentrated in the olive oil production and not in the reuse of its byproducts. Several studies dealing with the chemical composition of the olive fruits and their oil have been carried out; however, only few works have been focused on the isolation and identification of some compounds found in olive leaves. In recent studies (*1*) some compounds such as α-tocopherol, β-carotene, and β-sitosterol, among others, have been identified in olive leaf hexane extract. All of them have biological activity capable of influencing human physiological systems. These compounds have multiple applications in the pharmaceutical, cosmetic, and food industries. There are many publications on the antioxidant activity of α-tocopherol, and also the protective effect of α-tocopherol and β-carotene in atherogenesis (*2*) and cancer (*3*, *4*), the potential clinical uses of squalene (*5*) and its chemopreventive effect on colon cancer (*6*, *7*), the potential health benefits of phytosterols (β-sitosterol reduces in vitro human breast cancer cells) (*8*, *9*), and the antiinflammatory activity of sterols from olive leaf (the same of the olive oil) (*10*) have been studied. In the past few years, the suspected toxicity of some synthetic compounds of food

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use (*11*−*13*) has raised the interest in natural products. Some industries, such as those related to food additives production, cosmetics, and pharmaceuticals, have increased their efforts in preparing bioactive compounds from natural products by extraction, fractionation, and purification.

Supercritical fluid extraction (SFE) technology uses an extracting agent in the supercritical region, which combines solvent properties similar to those of liquids with viscosities and diffusivities similar to those of gases. By changing operation conditions, the solvent power toward different compounds can be easily tuned. In addition, when CO_2 is the extracting agent (as in this work), the extraction can be accomplished in nonoxidant conditions and at temperatures low enough to preserve the integrity of both antioxidants and thermolabile compounds. SFE has been used from two different perspectives, the direct extraction of compounds of interest from natural sources to provide products with minimal deterioration in their functionalities (*14*−*20*) and the development of end-processes (fractionation, concentration, dearomatization, etc.) able to upgrade materials extracted from natural sources by conventional methods, such as solid−liquid (S−L) extraction (*21*−*23*). One of the most important advantages of SFE, in terms of being used as an end-process, is that it affects to a lesser extent the quality of the products compared to other techniques such as evaporation and distillation usually applied with those purposes. This second approach is used in this work because there are some technical and economic advantages in processing the solid olive leaves with the existent solvent extraction plants (for pomace olive oil production) in the region where olive trees are growing, instead of building new plants or transporting huge amounts of raw material to another location. Once the leaves have been processed, the solvent extract may be concentrated prior to SFE, to minimize the amount processed with this more expensive and specialized technology.

The present study describes a procedure to obtain high-added-value products from olive leaves. First, the compounds of interest are recovered from the olive leaves by S−L extraction with hexane. This raw extract is then fractionated by means of countercurrent SFE in a pilot scale plant. The raw extract is put in contact with supercritical carbon dioxide in a packed column, and the supercritical fluid extracts are recovered in two different fractionation cells after cascade depressurization. The effects of the main variables that influence SFE selectivity, such as extraction pressure, extraction temperature, and the presence of a modifier of CO_2 polarity, have been studied. Considering that each countercurrent (CC)-SFE fraction composition can be changed depending on the operation conditions of the experimental run, the experiments were planned to cover a wide range of the above-mentioned variables.

The objective of this work was to develop a procedure to obtain different fractions of high-added-value compounds present in a hexane extract from olive leaves by means of CC-SFE.

MATERIALS AND METHODS

Plant Material, Reagents, and Standards. Fresh green leaves were collected from 10-year-old trees. The plants (*Olea europaea* L.), Picual variety, were grown in the orchard of the Instituto de la Grasa (CSIC), Sevilla, Spain. Field collections were made in December 2000. Diethyl ether, *n*-hexane, and diisopropyl ether were purchased from Merck. *n*-Eicosane, cholesterol arachidate, lauryl arachidate, tripalmitin, heneicosanol, cholesterol, α-tocopherol, and *β*-sitosterol standards were all purchased from Sigma Aldrich. Silica gel G plates 20 × 20 cm, 0.25 mm thick, were obtained from Macherey-Nagel. All chemicals and solvents were of analytical grade. CO_2 N48 (99.998% purity) was kindly supplied by AL Air Liquide España, S.A. (Madrid, Spain). Ethanol (96%) was obtained from Panreac (Barcelona, Spain).

Table 1. Conditions of CC-SFE, Extracts Obtained, and Extraction Yield (EYI, Equation 1) for the Different Experiments

	SF extraction conditions				wt of extract obtained		
expt	P_{ext} (bar)	T_{ext} (°C)	modif (%)	CO_2 density (kg/L)	S1 (g)	S2 (g)	EYI (%)
1	75	35	0	0.27	13.2	0	9.9
2	75	35	10	0.27	93.1	0	28.2
3	75	50	0	0.19	22.0	0	16.5
4	75	50	10	0.19	59.5	0	18.0
5	200	35	0	0.87	9.3	103.0	84.4
6	200	35	10	0.87	48.3	5.0	29.3
7	200	50	0	0.78	8.5	89.7	73.8
8	200	50	10	0.78	45.2	115.1	48.5

Preparation of Hexane Raw Extract. Olive leaves (10 kg) were crushed in a laboratory blade cutter (Robot Coupe R6VV, Vincennes, Cedex. France) and extracted by maceration with 50 L of hexane (1:5 w/v), for 48 h at 25 °C. The material was filtered, and the solvent was partially removed (up to 5 L) under reduced pressure at 40 °C.

SFE Instrumentation and Extraction Method. The CC-SFE pilot plant used in this study (Iberfluid, Barcelona, Spain) has been described elsewhere (*24*). The extraction column (AISI 316 stainless steel, 3 cm i.d.) is divided into three sections (bottom, middle, and top) of 50.4 cm each, with independent temperature control. The column is packed with 3 mm diameter Fenske rings, which have previously demonstrated their usefulness in CC-SFE for sterol and tocopherol concentration (*24*). A sample introduction port is fixed at the top of each column section. Throughout all of the experimental work the liquid sample introduction was carried out through the middle point of the packed column, located over the inlet of the CO_2, creating a countercurrent between the flow of sample (downward) and the CO_2 flow (upward). The CC-SFE pilot plant has also two separator cells (270 mL each), where a cascade decompression takes place, and a cryogenic trap at atmospheric pressure. The CO_2, modifier, and liquid sample pumps were from Dosapro Milton Roy (Madrid, Spain). Both sample and extracting solvent were heated to enter in the extraction column at the extraction temperature. The plant has a computerized control system based on PLC instrumentation. A typical CC-SFE run started with the introduction of a continuous flow of CO_2 at the bottom of the extraction column. When the operating pressures and temperatures in the column and in the separators were reached, liquid sample was pumped at the selected flow rate during the entire extraction time.

Several CC-SF extractions of the hexane raw extract were performed using the pilot scale plant at the conditions of pressure (P_{ext}) and temperature (T_{ext}) shown in **Table 1**, according to a 2^3 full factorial experimental design (*25*). Extraction conditions were designed to explore a wide range of CO_2 densities (from 0.19 to 0.87 kg/L) at a gentle thermal handling of the sample to avoid degradation of thermally labile compounds such as tocopherols. A cascade fractionation of the supercritical fluid extract was achieved by setting pressures in separators 1 and 2 equal to 60% of the P_{ext} and 10 bar, respectively. Temperature in both separators was fixed at 35 °C. Thus, three fractions were obtained after CC-SFE and fractionation of the hexane raw extract, those in separators 1 and 2 (S1 and S2) and the raffinate (R), which is the byproduct of the extracted samples collected at the bottom of the column. Solvent (CO_2) flow rate was 2500 mL/h, and sample feed rate was 200 mL/h (133 g/h) throughout the complete experimentation. Therefore, the solvent-to-feed ratio (S/F) was maintained constant at 12.5 v/v. In experiments 2, 4, 6, and 8, ethanol was used as a modifier of CO_2 polarity and was fed at 10% of the solvent flow rate. The total extraction time in all cases was 60 min except in experiment 6, when 33 min was used due to the lack of a raw extract of the same batch. The obtained fractions were maintained at 2 °C until analysis.

Analysis of Hexane Raw Extract and SFE Fractions. Each sample (2 mL) was fractionated by column chromatography on silica gel (Kieselgel 60, 90−230 mesh, Merck) of mass 20 g (45 × 1.5 cm i.d. column). The elution was carried out sequentially, first by *n*-hexane

and then by *n*-hexane/diethyl ether mixtures of increasing polarity (95:5, 87:13, and 65:35 v/v). A total of four fractions (100 mL each) resulted from this elution pattern. The elutions were monitored by thin-layer chromatography (TLC).

The first fraction of 100 mL (hexane), containing saturated hydrocarbons, was concentrated to ~1 mL and then was analyzed by gas chromatography (GC) (*26*). The β-carotene and wax esters (eluted during 95:5 chromatography of the extract) were determined by visible spectrophotometry (*27*) and by GC (*1*), respectively. The triacylglycerols and tocopherols (eluted during 87:13 chromatography of the extract) were determined by GC (*28*) and by high-performance liquid chromatography (HPLC) (*29*), respectively. The fourth fraction of 100 mL (hexane/diethyl ether, 65:35), containing sterols and aliphatic alcohols, was concentrated to ~1 mL and first fractionated by preparative TLC. The sterols were determined by GC, according to an analytical procedure described in Regulations of the European Union Commission (*30*). The aliphatic alcohol fraction was silanized by adding a freshly prepared pyridine/hexamethyldisilazane/trimethylchlorosilane (9:3:1, v/v/v) mixture. An aliquot was taken from the clear solution and injected into the gas chromatograph. Aliphatic alcohol analyses were performed on a Hewlett-Packard 5890 series II gas chromatograph (Palo Alto, CA), equipped with a split/splitless injector and fitted with a glass insert filled with stationary phase and silanized glass wool, as well as a flame ionization detector. A capillary column of fused silica SPB-5 (Supelco, Inc., Bellefonte, PA), 30 m long, 0.25 mm i.d., and 0.25 μm film thickness, was also used. The chromatographic conditions employed were as follows: injection in split mode; split ratio, 1:50; pressure at column head, 120 kPa; carrier gas, hydrogen; injector temperature, 285 °C; detector temperature, 325 °C; initial oven temperature, 210 °C; initial time, 4 min; ramp, 2 °C /min; final temperature, 275 °C.

CC-SFE Performance Characterization. Some useful parameters to characterize CC-SFE performance have been used in the present work (*24*, *31*−*33*). These parameters are defined (see **Figure 1**) as follows:

Extraction yield (EYI, %) is the weight of supercritical fluid extract obtained from each 100 g fed:

$$\mathrm{EYI} = \frac{S_1 + S_2}{F + M} \times 100 \qquad (1)$$

Enrichment (ENR) is defined as the relationship between the overall concentration of the compound considered in the supercritical fluid extract (X_E) and its concentration in the raffinate (X_R):

$$\mathrm{ENR} = \frac{X_E}{X_R}; \qquad X_E = \frac{S_1X_1 + S_2X_2}{S_1 + S_2} \qquad (2)$$

Recovery (RCV, %) is the percent weight of a compound present in the feed that is recovered in the supercritical fluid extract:

$$\mathrm{RCV} = \frac{S_1X_1 + S_2X_2}{FX_F} \times 100 \qquad (3)$$

From an industrial point of view, recovery is a very important paramenter because it involves both a good yield and a high concentration of the compound of interest.

Selectivity (SEL). The selectivity of a compound *i* toward a compound *j* can be calculated as the enrichment of compound *i* divided by the enrichment of compound *j*:

$$\mathrm{SEL} = \frac{(X_E/X_R)_i}{(X_E/X_R)_j} \qquad (4)$$

A high (or low) selectivity value of compound *i* toward compound *j* denotes enough differential affinity of the solvent to attain an easy separation between them. Values of selectivity different from 1 are needed for a separation to be possible. For fractionation purposes, selectivity is a parameter of evident importance.

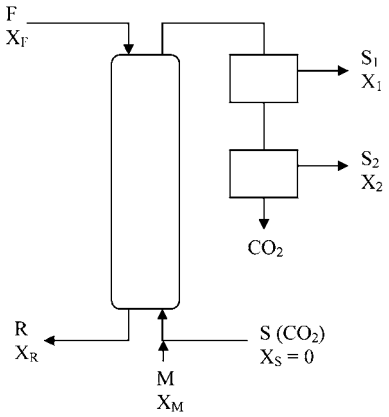

Figure 1. Flow diagram for mass balance and SFE performance parameters claculation: F, sample feed (g); S_i, SF extract in separator i (g); S, SFE solvent (CO_2, g); M, modifier feed (ethanol, g); X_i, w/w concentration of compound of interest (μg/g).

Statistical Calculations. Statistical experimental design and data analysis were performed using Statgraphics Plus 3.1 for Windows software (Statistical Graphics Corp., Manugistics Inc., Rockville, MD, 1997).

RESULTS AND DISCUSSION

Table 1 shows, along with the experimental conditions used, the weights of fractions S1 and S2 obtained in each CC-SFE run and the extraction yield (EYI, eq 1).

Fraction S2 was obtained only when CC-SFE was performed at high pressure. Higher extraction yields were attained at 200 bar, especially when no modifier was added. Therefore, in terms of fractionation (presence of extract in both separators) and yield of the supercritical fluid extracts, high extraction pressure would be preferred.

Concentration data of all compounds analyzed for each CC-SFE experiment and fraction (S1 or S2) and for the hexane raw extract are shown in **Table 2**. Data for S1 and S2 of experiments 2, 4, 6, and 8 have been corrected to overcome the dilution effect of the modifier. In experiment 6, fraction S2, all compounds except α-tocopherol increase their concentration over that of the raw extract, mainly hydrocarbons, β-sitosterol, and total alcohols. As a general trend, in experiments 5−8, fraction S1, six compounds show an increase of their concentration (waxes, triglycerides, and α-tocopherol do not). Finally, fraction S1 of experiment 2 exhibited clear concentration increments over raw extract in all compounds except α-tocopherol, which reached only a very small increment. Therefore, for concentration purposes in S1, the suitable CC-SFE conditions were that of experiment 2, whereas if concentration was preferred in S2, an extraction pressure equal to 200 bar had to be used. On the other hand, α-tocopherol seems to remain mainly in the raffinate independent of the experiment considered.

Throughout the experimentation, difficulties were undergone in some cases to quantitatively collect the raffinate from the bottom of the extraction column. To improve the accuracy of the SFE data, R and X_R were determined by mass balance.

Enrichment data (ENR, eq 2) are given in **Table 3**. The most favorable CC-SFE conditions are those of the experiment 2, with significant enrichment in most of the compounds analyzed. Hydrocarbons reached high values in some of the extraction conditions, followed by β-sitosterol. Another remarkable result was obtained for triglycerides, with an enrichment in experiment 4 that more than triples that of all other compounds at the same extraction conditions.

Table 2. Concentrations of the Analyzed Compounds in the Raw Extract and in the Countercurrent Supercritical Fluid Extracts (S1 and S2)[a]

	waxes		hydrocarbons		squalene		β-carotene		triglycerides		α-tocopherol		β-sitosterol		total alcohols	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
raw extract (μg/g)	5639		1910		75.2		63.9		154.1		962.4		123.5		256.8	
SF extracts (μg/g)																
expt 1	2714		1469		68.9		21.1		136.3		402.9		95.4		0.0	
expt 2	12460		5537		215.4		196.8		295.2		1044		270.0		371.8	
expt 3	285.7		107.1		17.7		0.0		19.3		62.0		0.0		0.0	
expt 4	5384		4142		106.0		61.0		603.0		368.8		237.9		530.1	
expt 5	5929	5357	2979	1793	96.4	50.3	102.9	48.3	139.3	114.3	410.7	210.7	279.1	112.3	35.7	0.0
expt 6	2732	7948	3626	22190	59.9	496.1	113.3	125.8	169.2	206.3	262.3	199.7	143.1	1179	480.2	2000
expt 7	6214	3429	2814	2029	86.1	39.3	107.9	32.0	146.4	121.6	296.4	182.1	129.6	146.1	535.7	188.6
expt 8	728.9	3386	3353	3694	156.4	77.2	56.0	97.1	93.4	177.9	150.8	65.3	176.3	49.7	269.4	491.0

[a] Data S1 and S2 of experiments 2, 4, 6, and 8 have been corrected to overcome the dilution effect of the modifier.

Table 3. Enrichment (ENR, Equation 2) in Different Compounds of the Countercurrent Supercritical Fluid Extracts

expt	waxes	hydrocarbons	squalene	β-carotene	triglycerides	α-tocopherol	β-sitosterol	total alcohols
1	0.46	0.75	0.91	0.31	0.87	0.39	0.75	0.00
2	4.20	11.36	10.65	16.69	2.99	1.12	4.09	1.76
3	0.04	0.05	0.20	0.00	0.11	0.05	0.00	0.00
4	0.95	2.92	1.55	0.94	10.85	0.34	2.42	2.69
5	0.78	0.94	0.29	0.43	0.32	0.05	1.16	0.00
6	0.49	11.33	1.56	2.67	1.18	0.20	3.21	5.94
7	0.33	1.52	0.26	0.28	0.52	0.06	2.28	0.60
8	0.31	11.26	1.90	1.96	1.00	0.05	0.54	4.50

Table 4. Recovery (RCV, Equation 3) of Different Compounds in the Countercurrent Supercritical Fluid Extractions

expt	waxes (%)	hydrocarbons (%)	squalene (%)	β-carotene (%)	triglycerides (%)	α-tocopherol (%)	β-sitosterol (%)	total alcohols (%)
1	7.63	9.09	3.28	8.78	4.15	7.67	0.00	7.20
2	81.66	80.69	86.75	53.95	30.56	61.61	40.77	39.29
3	0.93	3.90	0.00	2.07	1.07	0.00	0.00	0.00
4	39.04	25.39	17.17	70.43	6.90	34.69	37.16	40.40
5	83.61	60.76	69.76	63.74	19.94	86.25	0.97	86.31
6	82.45	39.35	52.53	32.87	7.82	57.09	71.13	42.38
7	81.06	42.56	44.56	59.27	14.73	86.54	62.85	79.36
8	91.38	64.20	64.88	48.49	4.50	33.55	80.92	39.01

Table 5. Selectivity Values (SEL, Equation 4) of Different Compounds toward α-Tocopherol

expt	waxes	hydrocarbons	squalene	β-carotene	triglycerydes	β-sitosterol	total alcohols
1	1.2	1.9	2.3	0.8	2.2	1.9	0.0
2	3.7	10.1	9.5	14.9	2.7	3.6	1.6
3	0.8	0.9	3.8	0.0	2.0	0.0	0.0
4	2.8	8.6	4.6	2.8	32.1	7.2	8.0
5	17.0	20.5	6.2	9.3	7.1	25.2	0.0
6	2.4	55.4	7.7	13.1	5.8	15.7	29.1
7	5.4	24.8	4.3	4.7	8.4	37.2	9.8
8	6.2	224.8	38.0	39.2	20.0	10.7	89.9

Table 4 shows recovery data (RCV, eq 3) for all of the compounds analyzed at each set of CC-SFE conditions. Recovery values >75% indicate that the compound was preferentially extracted, at certain CC-SFE conditions, and therefore collected into the separators. Conversely, recoveries lower than 25% denote that the compound was not extracted and thus mainly found in the raffinate. Experiments 1 and 3 are the most favorable to concentrate the compounds in the raffinate (thus, CC-SFE would be used for hexane elimination pourposes), whereas conditions of experiment 2 and 5 would concentrate the compounds in the separators. If the data are examined by compounds, hydrocarbons usually concentrate in the separators, whereas waxes and α-tocopherol usually remained in the raffinate. The other compounds did not show general trends in their distribution, exhibiting a great dependence on the CC-SFE conditions.

Selectivity calculations (SEL, eq 4) for every pair of compounds at all CC-SFE conditions reveal that α-tocopherol was the compound more easily separable from all others. This is in agreement with the fact that α-tocopherol is the compound more retained in the raffinate. **Table 5** shows selectivity values of the remaining seven compounds toward α-tocopherol. Experiments 5−8, carried out at higher pressures, lead in general to better selectivity values than experiments 1−4, especially experiment 8. As for the compounds, hydrocarbons were the compounds more easily separable from α-tocopherol, followed by total alcohols and β-sitosterol. The selectivity is a useful tool to compare the degree of separation achieved between different components at different conditions and represents the possibility of separating two components found in the same sample. Because selectivities are ratios of partition coefficients, they provide information about the maximum possible degree

Table 6. Experimental Values for Recovery of β-Sitosterol and Values Fitted by the Regression Model

expt	exptl value	fitted value
1	7.67	6.49
2	61.61	59.56
3	0.00	2.80
4	34.69	34.73
5	86.25	89.05
6	57.09	57.13
7	86.54	85.36
8	33.55	31.90

of separation of any pair of components under given conditions in a countercurrent process where the components can be preferentially extracted by the dense CO_2 phase (solubility driven) or can remain unextracted in the raffinate (or liquid residue obtained after extraction of the feed). The different selectivities observed by the different components can be explained by the different partition coefficients of the compounds between the extract and the raffinate.

As previously stated, recovery (RCV, eq 3) is a very important parameter characterizing a CC-SFE process because it embraces a good yield and a high concentration of the compound of interest. For this reason, recovery was the response selected to analyze the influence of the experimental factors (P_{ext}, T_{ext}, and modif) studied in this work. Also, empirical models were developed to describe the recovery of the different compounds found in the raw extract. The best fit model was attained for β-sitosterol and is reported below. An analysis of variance (ANOVA) was performed to detect the operating factors with significant effect on the recovery of β-sitosterol by CC-SFE. P_{ext} and T_{ext} have statistically significant ($p < 0.05$) influence on the selected response, while modif has not. However, although modif by itself is not an influential factor, it affected the recovery of β-sitosterol through the interactions $P_{ext} \times$ modif and $T_{ext} \times$ modif, which were also statistically significant at the 95% confidence level. By means of multiple regression techniques, the following empirical model, only with significant terms, was developed:

$$\text{RCV}\ (\beta\text{-sitosterol}) = 45.93 - 7.23T_{ext} + 19.93P_{ext} - 5.39\text{modif} \times T_{ext} - 21.35\text{modif} \times P_{ext} \quad (5)$$

Factors are expressed in coded values (i.e., for P_{ext}, -1 for 75 bar and $+1$ for 200 bar). This model provided a $R^2 = 99.25\%$ (adjusted for degrees of freedom). **Table 6** compares experimental data for β-sitosterol recovery with those obtained by using the empirical model shown above. A good agreement can be seen among the experimental and calculated values. **Figure 2** shows a response surface plot for the response selected (recovery of β-sitosterol) constructed from the empirical model. In the plot, the surface response is the connection of the values in the Z axis (values of response) corresponding to each pair of values in the X and Y axes (CC-SFE conditions). Response surface plots are used for visually predicting future responses and for determining factor values that optimize the response function. In **Figure 2a** the surface response is represented versus the two more influential factors (P_{ext} and T_{ext}), the third factor (modif) being fixed at the central value of its experimental range (5%). It can be seen that a combination of low T_{ext} (35 °C) with high P_{ext} (200 bar) yields the highest recovery for β-sitosterol. In **Figure 2b** the surface response is represented versus P_{ext} and modif, T_{ext} being fixed at the central value of its experimental range (42.5 °C). Notice that when modif goes

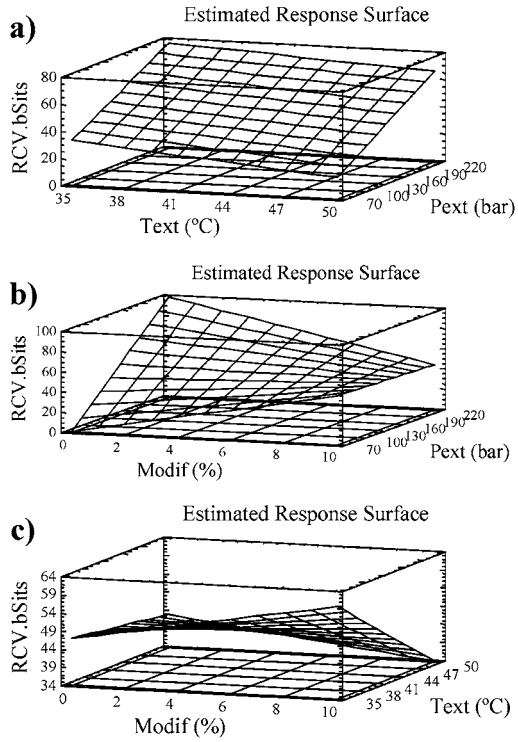

Figure 2. Response surface plots for recovery of β-sitosterol.

from 0 to 10%, an increase in the response is produced if P_{ext} is fixed at the lowest extraction pressure (75 bar), whereas a decrease is produced if P_{ext} is fixed at the highest extraction pressure (200 bar). Conversely, when P_{ext} goes from 75 to 200 bar, an increase in the response is produced if no modifier is added, whereas no change is produced if 10% of modifier is added. This different behavior of the influence of a variable on the response depending on the value set for another variable occurs when interaction exists between these variables. This is the case of modif and P_{ext}, the interaction (modif $\times$ P_{ext}) of which appears as a statistically significant factor in the regression model. Similar considerations can be made for the interaction modif $\times$ T_{ext} (**Figure 2c**).

In this paper the feasibility of using CC-SFE, at a pilot plant scale, for fractionation of high-added-value products from a raw extract of olive leaves in hexane has been demonstrated. Products of different composition can be obtained depending on the fraction collected (S1, S2, or R) and the SFE operative conditions used.

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