Concentration of Sterols and Tocopherols from Olive Oil with Supercritical Carbon Dioxide

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ABSTRACT: A process based on the use of a semicontinuous countercurrent supercritical fluid extraction has been developed to isolate and concentrate minor compounds, such as sterols and tocopherols, from olive oil. In the present work, an evaluation of the efficiency of different random packing materials (Raschig rings, Dixon rings, Fenske rings, and glass beads) to selectively separate sterols and tocopherols from olive oil has been performed. Parameters such as recovery, enrichment, and selectivity vs. TG are discussed. Considering the importance of supercritical fluid extraction as a clean processing technology and the interest in minor compounds with nutraceutical properties from olive oil, the process studied represents an alternative to the reuse of low-quality olive oil to extract high added-value products.

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KEY WORDS: Countercurrent, nutraceutical, olive oil, sterols, supercritical fluid extraction, tocopherols.

Olive oil has an extraordinary economic and social importance in the Mediterranean Basin, especially in Spain and Italy, which are the main producers of this oil in the world. The costs associated with olive harvesting and oil extraction are very high. Therefore, it is essential to obtain good use of all the products derived from the olive grove to make cultivation profitable (1).

Minor compounds are of great importance in the final composition of olive oil because they influence the stability and overall acceptability as well as the nutritional and health-related properties of the olive oil. Compounds such as sterols, squalene, and tocopherols are of great interest as high value-added products because of their nutraceutical activities.

Tocopherols are well known as components of vitamin E; their presence in olive oil has been extensively described (2). At present, tocopherols are of increasing interest in the food industry due to their antioxidant activity and other nutraceutical effects (3).

Fractionation of fats and oils with supercritical carbon dioxide has been used to obtain products with improved functionality for specific applications or with better nutritional values. Several processes have been developed, for example, to obtain n-3 FA from fish oil (4–6) or to deacidify olive oil (7–9). Because of its efficiency and mild operating conditions, supercritical fluid extraction (SFE) also has been recommended, along with preparative supercritical fluid chromatography, for selectively isolating tocopherols from natural products (10–12) and squalene from deodorizer distillates (13). In a previous study (14) we demonstrated the feasibility of a process for extracting and fractionating tocopherol analogs from olive by-products with supercritical carbon dioxide.

The objective of this work is to improve the technology for extracting high value-added products from olive oil using a semicontinuous process employing a countercurrent column. The study evaluates the efficiency of different packing materials toward the selective separation of sterols and tocopherols from olive oil. The effects of recovery, enrichment, and selectivity of tocopherols and sterols vs. TG are presented and discussed.

EXPERIMENTAL PROCEDURES

Samples. Spanish olive oil (grade 1º, a mixture of virgin and refined olive oil) purchased in a local market was used for the present work. Vitamin E (97% α-tocopherol) and sterols (50% β-sitosterol, 30% campesterol) were obtained from Sigma-Aldrich (St. Louis, MO). The packings evaluated in this work were glass beads and Raschig rings supplied by Symta Ltd. (Madrid, Spain) as well as Fenske and Dixon rings supplied by Afora, S.A. (Madrid, Spain). All of the packings had the same dimensions, 3 mm diameter. CO_2 N-38 (99.98%) was kindly supplied by AL Air Liquide España S.A. (Madrid, Spain).

Countercurrent SFE (CC-SFE). A diagram of the CC-SFE system used in this study is shown in Figure 1. The CC-SFE liquid feed pilot plant had the following features: a countercurrent extraction column (316 stainless steel, 17.6 mm i.d. and 180 cm length) with three levels of sample introduction (top, medium, and bottom as shown in Fig. 1) that was packed with the different packing materials noted previously, two separator cells (270 mL capacity each), and a cryogenic trap at atmospheric pressure. The CO_2, modifier, and liquid sample pumps were from Dosapro Milton Roy (Madrid, Spain).

During extraction, a continuous flow of CO_2 was introduced into the column at the bottom level. When the operating pressure and temperature were reached (after approximately 30 min), liquid sample was pumped in at the selected flow rate for the entire extraction time through the middle

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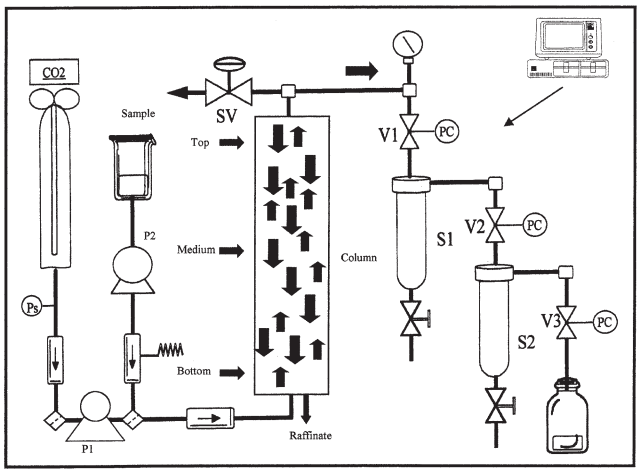

FIG. 1. Scheme of the countercurrent supercritical fluid extraction pilot plant scale employed. PC = pressure controller; V1, V2, V3 = micrometering valves; S1 = separator 1; S2 = separator 2; P1 = CO_2 pump; P2 = liquid sample pump; Ps = pressure sensor; SV = security valve; Top = top level of sample introduction; Medium = middle level of sample introduction; Bottom = bottom level of sample introduction.

level of the packed column (Fig. 1) located over the CO_2 inlet to create a countercurrent flow of sample (downward) to the CO_2 flow (upward). The ratio between the CO_2 flow rate and the sample flow rate was between 23.1 and 41.7 kg CO_2/kg oil; the flow rate of olive oil was constant at 0.1 L/h and the solvent (CO_2)/flow rate was 2.0 to 3.6 L/h. Extraction and fractionation conditions were kept constant throughout the experiments: Extraction pressure was 200 bar, and the cascade fractionation was achieved by setting pressures in separators 1 and 2 equal to 100 and 30 bar, respectively. Extraction temperature was maintained at 40°C, whereas temperatures in separators 1 and 2 were fixed at 40 and 0°C, respectively. The total extraction time was 60 min for each experiment.

LC analysis. A Varian (Walnut Creek, CA) Prostar series HPLC equipped with photodiode array detector was used to analyze sterols and tocopherols in the olive oil and in the extracts obtained using the CC-SFE system described above. The separation column was a Kromasil C4 (Hichrom, Ltd., Reading, United Kingdom) of 25 cm × 10 mm and 10-µm particle diameter. The samples were injected with no previous treatment into the 20-µL injection loop of the HPLC system. The mobile phase was 100% methanol at a flow rate of 4 mL/min over 30 min. Identification of compounds was achieved by comparing their retention times and spectra with those of standards. For quantitative analysis, calibration curves were prepared by analyzing different concentrations of tocopherol and sterol standards and by representing peak area (counts) vs. concentration (%w/w); correlation coefficients were 0.995 in both cases. Detection for vitamin E and sterols was performed at their absorption maxima in the UV, that is, 296 and 205 nm, respectively.

RESULTS AND DISCUSSION

The analysis of the original olive oil and the extracts obtained after CC-SFE was performed by direct injection into the HPLC system with no previous sample treatment, allowing fast analysis of the extracts and quantification of the sterols and tocopherols. Although more complex methods (in terms of sample preparation and analysis) have been developed to quantify the individual composition of sterols and tocopherols, the goal of the present research was to study the behavior of the different families of minor compounds found in the olive oil in terms of enrichment, concentration, and selectivity; therefore, the global composition of sterols and tocopherols was considered.

TABLE 1
Concentrations of Sterols in the Extracts and Raffinates (%w/w) for Different Packings

CO_2 flow rate (L/h)	kg CO_2	kg CO_2/kg oil	Sterols (%w/w)			
			Glass beads	Dixon rings	Raschig rings	Fenske rings
Extract of separator 1						
2.0	2.1	23.1	0.65	0.67	0.63	0.70
2.4	2.5	27.8	0.65	0.58	0.64	0.70
2.8	2.9	32.4	0.65	0.62	0.63	0.69
3.2	3.4	37.0	0.64	0.62	0.61	0.70
3.6	3.8	41.6	0.66	0.64	0.63	0.64
Extract of separator 2						
2.0	2.1	23.1	0.66	0.68	0.68	0.76
2.4	2.5	27.8	0.68	0.69	0.65	0.75
2.8	2.9	32.4	0.70	0.64	0.62	0.71
3.2	3.4	37.0	0.71	0.66	0.66	0.75
3.6	3.8	41.6	0.69	0.68	0.71	0.75
Raffinate						
2.0	2.1	23.1	0.32	0.31	0.32	0.31
2.4	2.5	27.8	0.31	0.31	0.32	0.31
2.8	2.9	32.4	0.31	0.31	0.32	0.31
3.2	3.4	37.0	0.31	0.31	0.32	0.30
3.6	3.8	41.6	0.30	0.30	0.31	0.30

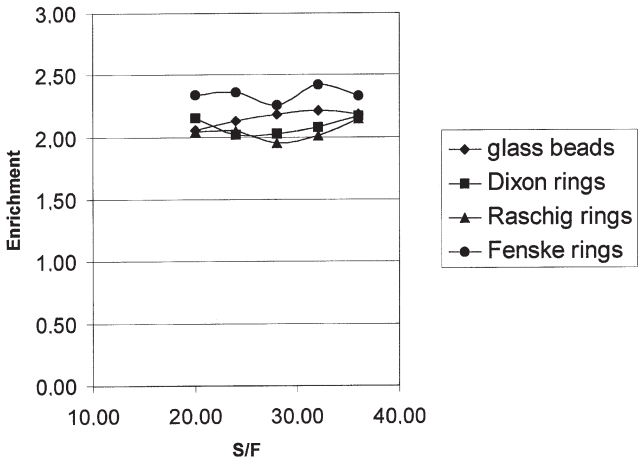

FIG. 2. Enrichment of sterols for different packing materials used as a function of the solvent-to-feed (S/F) ratio.

Sterols. The initial amount of sterols found in the original olive oil (before extraction) was 3340 ppm. This value is in agreement with the concentration range described the literature (1). Table 1 shows the concentrations of sterols in the extracts of separator 1, separator 2, and raffinate (%w/w). The concentration of sterols can be used to obtain enrichment factors, which can be calculated by dividing the average concentration of sterols in the extracts obtained in separators 1 and 2 (X_E) with respect to the concentration in the raffinate (X_R).

The enrichment of sterols using the different packing materials is shown in Figure 2 as a function of the solvent-to-feed (S/F) ratio. From these data it can be seen that when Fenske rings were used, the enrichment values were always higher relative to the other packings, independent of the S/F ratios used to perform the extractions. Small differences in enrichment values can lead to higher differences in production when processing large amounts of oil. This can be decisive in the economic evaluation of an industrial process because we are considering both a higher enrichment (and therefore a product with specific characteristics) and also the possibility of achieving an even more important enrichment by recycling the extracts in a continuous process. Fenske rings also offer an additional advantage: the possibility of providing a more or less constant enrichment value for sterols independent of the S/F ratio, which can also be very important to scaling-up.

The recoveries of sterols in the extract with respect to the content of sterols in the original olive oil were calculated at the different S/F ratios tested and for the different random packings used (Table 2). The maximal recovery that could be obtained with Dixon and Fenske rings at high flow rates (3.6 and

TABLE 2
Recoveries of Sterols in the Extracts (% w/w) for Different Packings

CO_2 flow rate (L/h)	kg CO_2	kg CO_2/kg oil	Recovery of sterols (%)			
			Glass beads	Dixon rings	Raschig rings	Fenske rings
2.0	2.1	23.1	8.65	12.52	8.61	12.28
2.4	2.5	27.8	12.42	11.05	10.66	14.116
2.8	2.9	32.4	12.95	12.96	8.62	13.92
3.2	3.4	37.0	15.44	15.13	10.54	17.74
3.6	3.8	41.6	17.06	17.88	12.29	16.61

TABLE 3
Concentration of Tocopherols in the Extracts and the Raffinate (%w/w) for the Different Random Packings Evaluated

CO_2 flow rate (L/h)	kg CO_2	kg CO_2/kg oil	Tocopherols (%w/w)			
			Glass beads	Dixon rings	Raschig rings	Fenske rings
Extracts of separator 1						
2.0	2.1	23.1	0.19	0.08	0.06	0.15
2.4	2.5	27.8	0.06	0.05	0.06	0.12
2.8	2.9	32.4	0.06	0.06	0.06	0.08
3.2	3.4	37.0	0.06	0.06	0.05	0.09
3.6	3.8	41.6	0.05	0.06	0.05	0.06
Extracts of separator 2						
2.0	2.1	23.1	0.27	0.10	0.09	0.20
2.4	2.5	27.8	0.06	0.06	0.08	0.19
2.8	2.9	32.4	0.05	0.07	0.09	0.12
3.2	3.4	37.0	0.07	0.08	0.09	0.15
3.6	3.8	41.6	0.05	0.09	0.09	0.10
Raffinate						
2.0	2.1	23.1	0.01	0.01	0.02	0.01
2.4	2.5	27.8	0.02	0.02	0.02	0.01
2.8	2.9	32.4	0.02	0.02	0.02	0.01
3.2	3.4	37.0	0.01	0.01	0.02	0.01
3.6	3.8	41.6	0.02	0.01	0.02	0.01

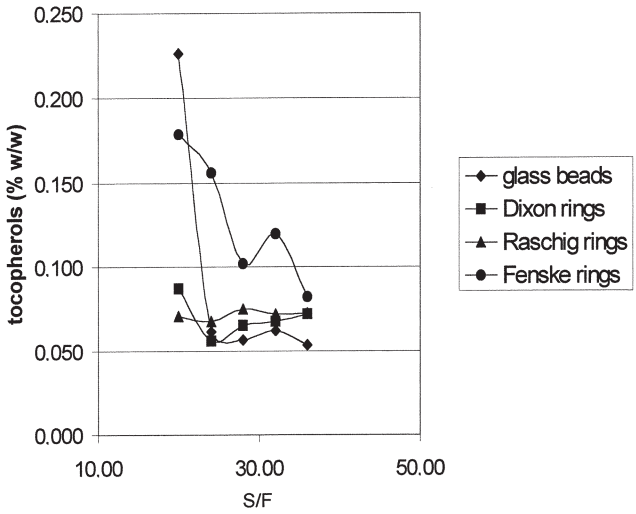

FIG. 3. Average concentrations of tocopherols in the extracts (%w/w) vs. S/F ratio. For abbreviation see Figure 2.

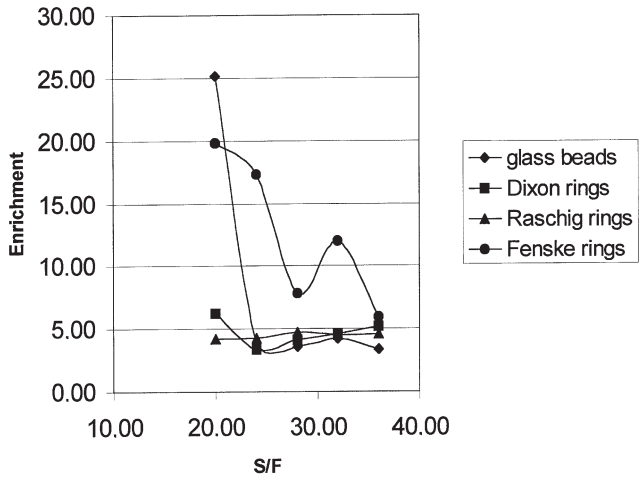

FIG. 4. Enrichment of tocopherols for different packing materials used as a function of the S/F ratio. For abbreviation see Figure 2.

3.2 L/h, respectively) was 18%. Recoveries were lower at lower S/F ratios (between 8 and 12%, depending on the packing material considered). These data must be analyzed along with the enrichment values (Fig. 2). When considering the possibility of implementing an industrial process, it is important to be able not only to enrich the extract in the compound (or group of compounds) of interest but also to extract the highest amount of compound from the original raw material.

Tocopherols. Analyzing the tocopherol content in the original oil produced a value of 190 ppm, which is also in agreement with previous results (1). Table 3 shows the concentration of tocopherols in the extracts and the raffinate for all packing materials tested and at different S/F ratios studied. A higher concentration of vitamin E was observed in the extracts of separator 2 for all the packings studied. The difference between the extracts of separator 1 and 2 was greater for the Fenske rings and glass beads than for the other two packings that were studied. A trend that is common to all materials tested is a decreased tocopherol concentration and an increased S/F ratio; this can be related to decreased extraction efficiency at higher CO_2 flow rates.

Figure 3 shows the average composition of the extracts (%w/w) vs. S/F ratio. The most promising results are obtained by using either Fenske rings or glass beads at the lowest CO_2 flow rate (2.0 L/h). As mentioned before, this decrease in CO_2 flow rate leads to a longer residence time of CO_2 inside the countercurrent column and therefore to greater contact time between the liquid phase (oil) and the gas or supercritical phase (CO_2). On the other hand, the chemical nature of both glass beads and Fenske rings (also glass) allow a uniform film of oil to be formed, thus taking advantage of the high specific surface area of these packing materials.

By measuring the amount of tocopherols in the amount of CO_2 used for SFE, it is possible to obtain the concentration of vitamin E in the gas phase, that is, the concentration that leaves the column and enters the separators. The highest value corresponds to 0.436 mg vitamin E/100 g CO_2 obtained with the Fenske rings at 2.0 L/h. This value is the same one obtained with the glass beads and is considered to correspond to a saturating or equilibrium concentration for the system olive oil–CO_2.

Figure 4 shows the values of enrichment obtained for tocopherols in the extracts calculated as described previously. In general, the global vitamin E enrichment tends to decrease with increasing S/F ratio. This behavior can easily be observed for both glass beads and Fenske rings. The maximal value (25.1) corresponds to the experiment performed with glass beads at 2.0 L/h followed by Fenske rings at 2.0 L/h where an enrichment value equal to 19.8 was observed.

Even though the enrichment obtained with Dixon and Raschig rings was in general four to five times lower (at CO_2 flow rate equal 2.0 L/h), it is important to consider the high degree of concentration in vitamin E obtained with all the packing materials tested.

TABLE 4
Recoveries of Tocopherols in the Extracts (%w/w) for Different Packings

CO_2 flow rate (L/h)	kg CO_2	kg CO_2/kg oil	Recovery of sterols (%)			
			Glass beads	Dixon rings	Raschig rings	Fenske rings
2.0	2.1	23.1	52.39	28.65	16.47	53.09
2.4	2.5	27.8	20.22	17.17	19.62	53.56
2.8	2.9	32.4	19.05	23.50	18.31	35.94
3.2	3.4	37.0	25.07	28.20	20.95	51.72
3.6	3.8	41.6	24.45	34.57	23.41	34.17

TABLE 5
Selectivities of Sterols and Tocopherols Toward Triglycerides for Different Packings

CO_2 flow rate (L/h)	kg CO_2	kg CO_2/kg oil	Glass beads	Dixon rings	Raschig rings	Fenske rings
Selectivity for sterols vs. TG						
2.0	2.1	23.1	2.66	2.60	2.52	3.21
2.4	2.5	27.8	2.62	2.47	2.50	3.11
2.8	2.9	32.4	2.76	2.52	2.34	2.85
3.2	3.4	37.0	2.74	2.53	2.46	3.02
3.6	3.8	41.6	2.61	2.63	2.64	2.84
Selectivity for tocopherols vs. TG						
2.0	2.1	23.1	32.49	7.55	5.15	27.27
2.4	2.5	27.8	4.73	4.04	5.17	22.84
2.8	2.9	32.4	4.49	5.07	5.65	9.93
3.2	3.4	37.0	5.17	5.51	5.50	14.96
3.6	3.8	41.6	4.03	6.24	5.58	7.18

Table 4 shows the percentage recovery of vitamin E obtained for different packings. The best results were achieved with Fenske rings, which allowed extraction of up to 53.6% of the vitamin E in the olive oil. Conversely, the worst values were obtained with Raschig rings.

Selectivity. The selectivity (S) is an 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. To obtain a high selectivity, the S value has to be high, and it must be higher than 1 to achieve a practical separation.

In general, the selectivity of the compound i with respect to the compound j can be described by using the following equation (15,16):

$$S = \frac{(X_E / X_R)_i}{(X_E / X_R)_j} \quad [1]$$

where X_E and X_R are the composition of the extract and the raffinate, respectively. In this study, the selectivity was calculated with respect to TG because the main goal of the investigation was to enrich the extracts in the minor components of the oil while leaving these major components mainly in the raffinate.

Table 5 shows the selectivity of the different compounds (sterols and tocopherols) toward TG. The Fenske rings provided the best results for the minor compounds in terms of selectivity and therefore in terms of separation efficiency.

The selectivity values obtained in all the experiments (mainly with the Fenske rings) show the possibility of separating and enriching the minor compounds from the TG, the major components of the olive oil.

The selectivity of CO_2 at the extracting conditions evaluated toward the compounds studied were the following: S tocopherols > S sterols > S TG (S = 1).

With the CC-SFE process studied, it is suitable to concentrate minor compounds from olive oil. This study shows the importance of the packing material used in the countercurrent column with respect to selectivity, enrichment, and recovery. Therefore, the oil, and even more specifically, low-quality (high-acidity) olive oil, can be considered a natural source of nutraceutical compounds, such as antioxidants, with a high added value. A more refined fractionation of the compounds, such as tocopherol isomers and sterols, can be achieved by applying new supercritical fluid technologies, such as the combined use of SFE and preparative-scale supercritical fluid chromatography. These studies are presently being conducted in our laboratory based also on the experience acquired for the extraction and fractionation of tocopherol isomers from olive pomace (14).

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