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Supercritical fluid extraction of tocopherol concentrates from olive tree leaves

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Abstract

Olive tree leaves, a residue obtained during the harvest of olives for oil production were treated with supercritical carbon dioxide to analyze the possibility of obtaining tocopherol concentrates. Oil and tocopherol extraction rates were determined as a function of pressure (25–45 MPa), particle size (0.25–1.5 mm), solvent flow (0.5–1.5 SL/min) and temperature (313–333 K). Two optimal extraction conditions were determined, considering the maximum recovery or concentration criterion. Those conditions led to a highly valuable extract of 74.5 and 97.1% (w/w) tocopherol concentration, respectively. Results obtained were compared to hexane soxhlet extraction. © 2002 Elsevier Published by Science B.V

Keywords: Tocopherol; Supercritical carbon dioxide; Supercritical extraction; Concentration

1. Introduction

Tocopherols are extremely valuable compounds because of their activity as vitamin E and capacity as anti-oxidizing agent. The isomer with the highest vitamin E activity is α-tocopherol and it has become an important additive to all kind of food products [1]. Today, most of the tocopherols are obtained by vacuum distillation of deodorizing-step residues generated in the refining of vegetable oils. Throughout this process, that includes several steps such as solvent recovery and purification, copious amounts of organic solvents and energy are required, and thermal degradation of tocopherol is commonly encountered [2].

Recently an increasing interest in both, detection and search for new alternative tocopherol extraction and isolation techniques has been observed. Among them, supercritical fluid technology has been applied to extract tocopherols from natural materials such as palm oil [3], rice bran or soybean [4], obtaining enrichment factors up to 4

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with respect to the solvent-obtained extracts. Residues and by-products have also been used for extraction purposes. Thus, Brunner et al. [5] extracted deodorizer condensates in a countercurrent column and Shishikura et al. [6] and Lee et al. [7] obtained tocopherol concentrates from soybean sludge. In all cases, the tocopherol content of the extracts depended on the composition and properties of the natural matrix.

Trying to enhance concentration different techniques have been employed. Direct carbon dioxide extraction reported 10% (w/w) contents [6], while coupled to techniques of esterification or adsorption yielded up to 64% (w/w) [6,7]. When high purity is required, preparative supercritical fluid chromatography (SFC) has been used, leading to concentrations up to 95% [8]. The industrial application will depend on the economics of the coupled-technique operation and further purification process, being of special interest the search of a direct-extraction application that result in high tocopherol concentration.

In this work, we have used supercritical CO_2 for the extraction of olive tree leaves, a residue obtained during the harvest of olives for oil production. The aim of the work is to analyze the effect of pressure, particle size, solvent flow and temperature on the extraction rates of tocopherol from olive tree leaves. These results permitted us to determine those conditions leading to both maximum tocopherol recovery and concentration.

2. Experimental procedures

2.1. Materials

Liquid carbon dioxide (purity 99.5%) was supplied by Carburos Metalicos, S.A. (Madrid, Spain).

Olive tree leaves were collected in the Ciudad Real area (Spain). The samples were maintained at 328 K for 2 h to reduce their humidity to 6% by weight in a dry basis. Next, they were allowed to reach ambient temperature and, finally, grilled and sieved to obtain three size fractions whose average diameters were 1.5, 0.5, and 0.25 mm, respectively.

α-, β-, γ- and δ-Tocopherol standards were provided by SIGMA S.A. The rest of the reagents used for tocopherol determination were in HPLC grade (Panreac, Montplet & Esteban, S.A., Barcelona, Spain).

2.2. Apparatus and extraction procedure

The flow diagram extraction equipment is shown in Fig. 1 and it has also been described previously in Ref. [9]. Liquid carbon dioxide was provided from a steel cylinder (SC). After cooling (LC) and filtering (F), the CO_2 was compressed (P) by a positive displacement HPLC type pump. The pressure was regulated by a back-pressure-regulator (BPR) and checked by a manometer (M). The compressed fluid was passed through a

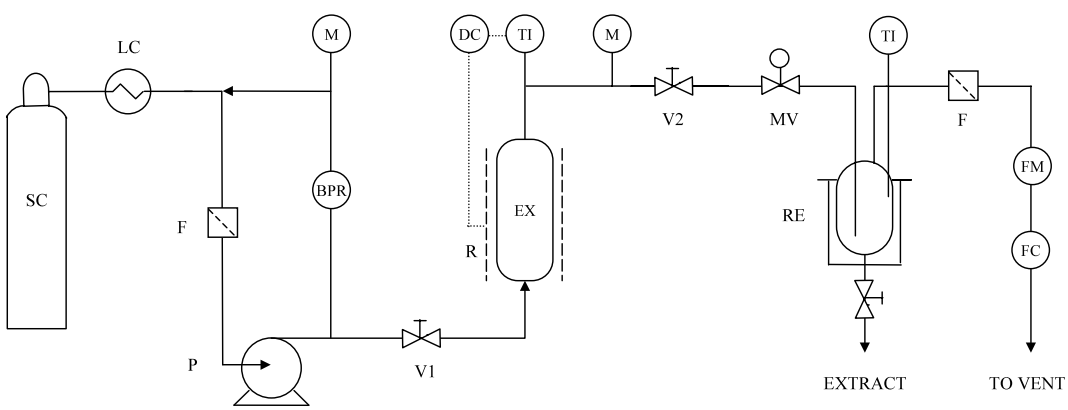

Fig. 1. Extraction apparatus.

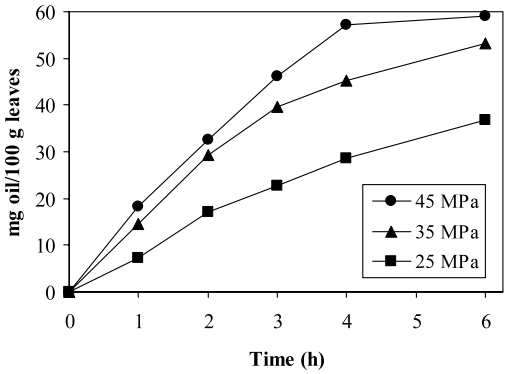

Fig. 2. Effect of pressure on oil yield. Conditions: $T = 313$ K; $Q = 1$ SL/min; Pd = 0.5 mm.

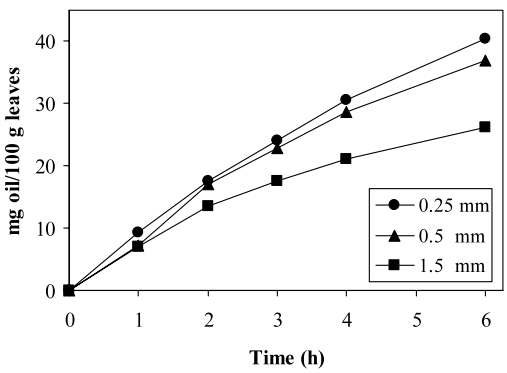

Fig. 3. Effect of particle size on oil yield. Conditions: $P = 25$ MPa; $T = 313$ K; $Q = 1$ SL/min.

vertically mounted extractor (EX) from the bottom. The extractor was a 75 ml stainless steel cylinder (17.48 mm i.d. × 304.8 mm). To keep the extractor temperature at the desired value a digital controller regulated the electric current through a resistor (R) that surrounded the extractor cylinder. The oil-laden gas from the extractor was passed through a heated metering valve (MV) where the supercritical CO_2 was depressurized, and the separated oil was collected in a cooled receiver (RE). The gas flow through the extractor was measured by a turbine flow meter (FM) and totalized by a digital flow computer (FC). In all experiments, the extraction was run on 15 g olive tree leaf samples, placed in the extractor between the two layers of glass wool to avoid losses of small particles.

2.3. Soxhlet extractions

Samples of 25 g of olive tree leaves were also extracted in a 250 ml Soxhlet apparatus with analytical grade hexane and methanol (Panreac, Montplet and Esteban, S.A., Barcelona, Spain) for 6 h to compare the quality of this oil with that extracted using SC-CO_2. After the evaporation of the solvent, the oil content was determined gravimetrically.

2.4. HPLC analysis of tocopherol

The tocopherol analyses were performed by HPLC using a Hewlett–Packard 1100 liquid chromatograph as outlined in Ref. [10]. The tocopherol was measured using MeOH as the mobile phase and a 5 μm RP-18 column 4 × 250 mm (Lichrosphere, Merck) as the stationary phase. Solute detection was accomplished by using a UV–Vis detector set at 293 nm. Conditions were: temperature 298 K (isocratic), mobile phase at 1 ml/min.

3. Results and discussion

3.1. Oil yield

The effect of pressure on the extraction of oil from olive tree leaves was investigated with supercritical CO_2 at pressures of 25, 35 and 45 MPa. The results are presented in Fig. 2, where the oil yield (expressed as mg of extracted oil/ 100 g of leaves) is plotted against operation time. As expected, it was observed that extraction yield strongly increased with pressure, according to the basic principles of supercritical fluid extraction [11,12] and similar to the other studies on oil extraction from seeds and leaves [13–15]. These studies showed that, in general, at a constant temperature, the solubility of a substance in a SCF increases with pressure.

The three extractions showed in Fig. 3 were performed to check out the effect of particle size on the oil recoveries. As expected [16], oil yield increased with decreasing particle size.

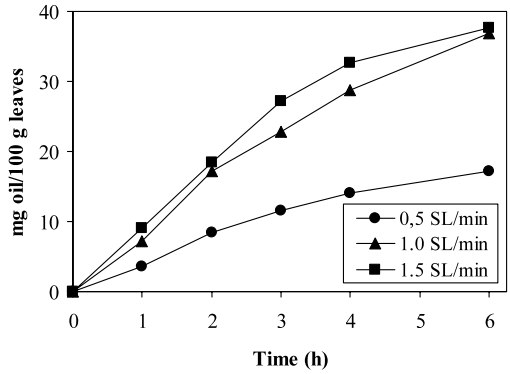

Fig. 4. Effect of solvent flow on oil yield. Conditions: $P = 25$ MPa; $T = 313$ K; Pd = 0.5 mm.

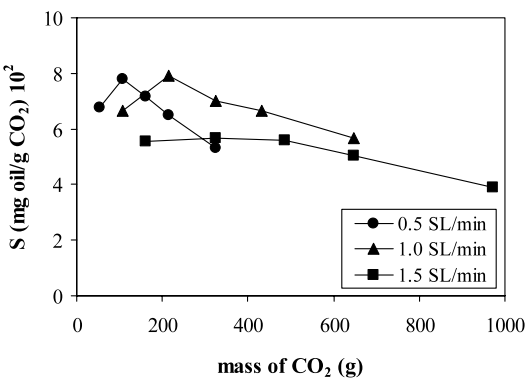

Fig. 5. Oil loading of supercritical CO_2 at different flow rates. Conditions: $P = 25$ MPa; $T = 313$ K; Pd = 0.5 mm.

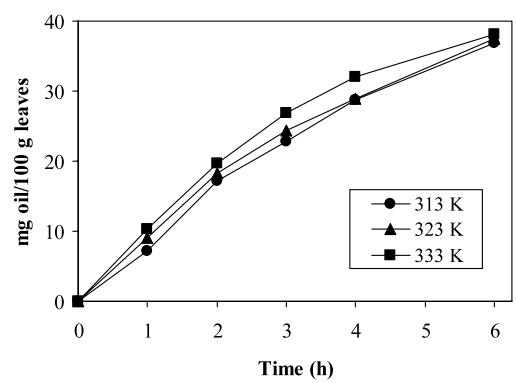

Fig. 6. Effect of temperature on oil yield. Conditions: $P = 25$ MPa; $Q = 1$ SL/min; Pd = 0.5 mm.

The effect of solvent flow is shown in Fig. 4. It can be observed that increasing CO_2 flow from 0.5 to 1 SL/min (l/min at standard conditions) increased oil yield. In the range of 1–1.5 SL/min only a slight increase is observed, indicating that for higher flow the carbon dioxide leaving the extractor is not saturated. This evidence is better observed in Fig. 5, when representing the apparent solubility of the oil, *s*, against the mass of solvent used. For both 0.5 and 1 SL/min, the oil load in the outgoing CO_2 during the first stages of extraction (as far as 200 g) remains nearly constant at a mean value of 0.072 mg oil/g CO_2. Since this result is in good agreement with the oil solubility data predicted from del Valle–Aguilera correlation [17], 0.06 mg oil/g CO_2, we may suggest that the outgoing CO_2 remains saturated when operating at these lower flows. However, at a fluid flow of 1.5 SL/min conditions, the initial oil load decreases, indicating that the residence time of CO_2 in the extractor is not enough to reach equilibrium conditions. Another possible explanation to the effect of this variable could be by-passing the cells by some of the solvent due to the existence of microscopic fluid flow effects in the plant matrix, as reported by Hortaçsu et al. [18].

The influence of temperature in the cumulative content of oil extracted is showed in Fig. 6. A slight increase in oil yield was observed by increasing the temperature. This result suggests that the effect of the increased volatility of oil compounds with temperature on oil solubility is more important than that of the drop of CO_2 density in the experimental range analyzed (313–333 K). This fact has been previously reported mainly at conditions over the critical point of the solvent [14,19].

3.2. Tocopherol yield

The effect of operational variables on the extraction rate of tocopherol from olive tree leaves is showed in Figs. 7–10. In all analyses, α-tocopherol was the major isomer (> 90%) against the γ-isomer. β- and δ-Tocopherols was not detected in the extracts.

The effect of pressure was investigated at pressures of 25, 35 and 45 MPa. The experimental

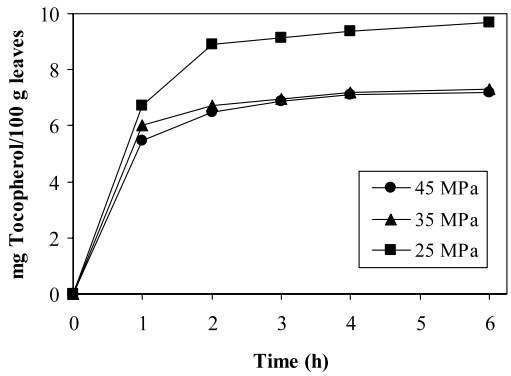

Fig. 7. Effect of pressure on tocopherol yield. Conditions: $T = 313$ K; $Q = 1$ SL/min; Pd = 0.5 mm.

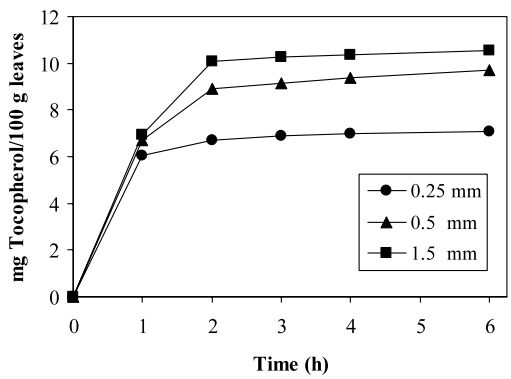

Fig. 8. Effect of particle size on tocopherol yield. Conditions: $P = 25$ MPa; $T = 313$ K; Q = 1 SL/min.

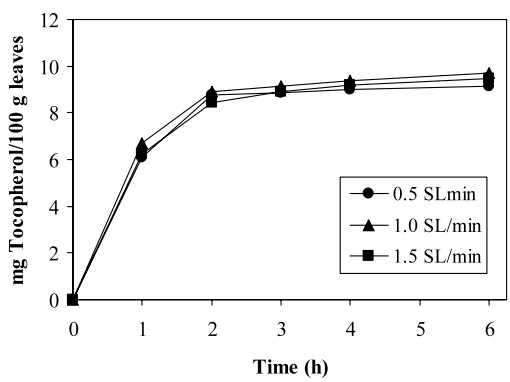

Fig. 9. Effect of solvent flow on tocopherol yield. Conditions: $P = 25$ MPa; $T = 313$ K; Pd = 0.5 mm.

results are presented in Fig. 7, where the tocopherol yield (expressed as mg of α- plus γ-tocopherol/100 g of leaves) is plotted against extraction time. As it can be observed, the maximum amount of tocopherol was obtained at 25 MPa and a further increase of pressure up to 35 and 45 MPa decreased tocopherol recovery. This result may be explained by considering that the extraction of tocopherol is related to the extraction capabilities of other matrix compounds. Then, a competitive extraction of other diluting materials should occur with increasing pressure. Brunner [3] and King [4] observed a similar behavior in the supercritical extraction of tocopherols from palm leaves at 363 K and pressures from 30 to 50 MPa and soybean flakes at temperatures from 313 to 353 K and pressures of 25 and 70 MPa, respectively.

The influence of particle size is presented in Fig. 8, showing that tocopherol recovery increases with increasing particle diameter, probably due to tocopherol decomposition during sample preparation and the waiting period before the experiments. In order to confirm this assertion, additional extraction experiments with different particle sizes were made to extinction (SFE conditions). These experiments showed that tocopherol exhaustion yields also increased with increasing particle sizes and, therefore, that tocopherol decomposition could well be the reason why tocopherol recovery increased with increasing particle size.

The effect of solvent flow on the extraction rate of tocopherol was also investigated. As observed

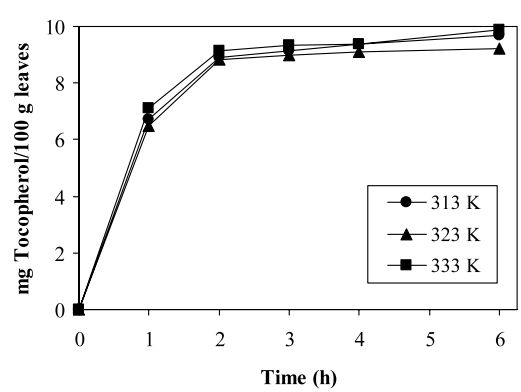

Fig. 10. Effect of temperature on tocopherol yield. Conditions: $P = 25$ MPa; $Q = 1$ SL/min; Pd = 0.5 mm.

in Fig. 9, no significant influence of this variable was found in the experimental range analyzed, probably because the effect of this variable is so small that it can only be detected when a much wider range of fluid flow is analyzed.

In the same way, the influence of temperature in the tocopherol content of extracts in the range analyzed (Fig. 10) was not observed. Considering the previous results for the temperature effect on oil yield showed in Fig. 6, it can be affirmed that the slight increase in oil yield is a result of a large increase in the temperature of vapor pressure of compounds other than tocopherol. This fact clearly indicates that tocopherol is an easily extractable compound, even at low temperatures [4,19].

3.3. Determination of optimal extraction conditions

In order to obtain optimal extraction conditions two criteria were used: the first criterion was established regarding maximum tocopherol recovery, while the second criterion was concerned with the maximum concentration of tocopherol in extracts.

Attending the first criterion, the optimal time of extraction was determined in 2 h. As observed in Figs. 2–10, longer experiments did not increase the oil or tocopherol recoveries significantly and produced diluted tocopherol extracts. The optimal pressure selected was 25 MPa since at higher pressures the amount of tocopherol extracted diminished due to the larger increase in the pressure of the solubility of oil components other than tocopherol. The particle size selected was 1.5 mm to avoid tocopherol decomposition. Solvent flow was determined in 1 SL/min in order to obtain the maximum amount of extract in equilibrium conditions. Finally, based on energy requirements, the temperature selected was 313 K. At these optimal conditions, the tocopherol concentration in the extract was 74.5%.

In order to establish the optimal conditions for the second criterion, it must be considered that tocopherol is preferably recovered from the natural matrix in the initial stages of extraction [4]. Then, it is reasonable to assume that optimal conditions would be close to those corresponding to the first criterion, but using a lower operation time. In fact, these conditions correspond to 1 h operation time and the rest of the conditions overlapping with those obtained for the first criterion. Thus, at conditions selected above, in the first hour of extraction a 97% tocopherol concentrate is obtained.

Conditions selected in this work are very similar to those obtained for the different natural materials summarized in Table 1, despite that the selective partitioning of tocopherol widely varied with the type of seed been extracted. In general, it has been found that the higher fatty acid and glyceride concentration in the material produces a higher dilution in the product as a result of the coelution [4,6,15]. Our results confirm that statement and show that olive oil leaves-type matrixes are ideal for supercritical fluid extraction of tocopherol, due to that its low *oil content*, 0.4% w/w, determined as outlined in Ref. [20], producing highly concentrated tocopherol extracts without the need of coupling SFE to another enrichment technique.

3.4. Comparison of the supercritical fluid extraction of olive tree leaves with soxhlet extraction

In order to compare our results with those obtained by solvent extraction, a soxhlet extraction was performed using hexane as described before. The results are presented in Table 2 together with those obtained for CO_2 at the two optimal conditions selected.

As it can be observed from Table 2, the hexane extract showed no tocopherol content, probably due to the polar character of tocopherol and the facility of the thermal and oxidative degradation described before [2]. Compared with the SFE, the higher yields obtained with hexane indicates the presence of high molecular weight non-polar compounds in leaves, more difficult to extract with carbon dioxide than with hexane, specially at low pressures [11,12]. These compounds are typically waxy materials located in the outer part of the leaves [13]. Thus, the portion of the oil content of leaves feasible to extraction by CO_2 is still re-

Table 1
Results obtained in the supercritical fluid extraction of tocopherol from different natural materials

Author	Material	Technique	Optimal conditions	Tocopherol in enriched fraction (% w/w)
Brunner et al. [3]	Palm leaves Soybean flakes	SC-CO_2	30 MPa, 343 K	11.3
King et al. [4]	Rice Bran	SC-CO_2	25 MPa, 313 K	0.6
		SC-N_2O	15.2 MPa, 323 K	10.4
Shishikura et al. [6]	Soybean sludge	Esterification-SC-CO_2-pressure ramp	10.1–20.3 MPa, 323 K	19.5
		Esterification-silicic acid adsorption	10.1–20.3 MPa, 323 K	64.3
Lee et al. [7]	Soybean sludge	Esterification-SC-CO_2	35.5 MPa, 318 K	40.0
Saito and Yamamuchi [8]	Wheat germ	SC-CO_2-Preparative SFC	25 MPa	95.0
	Olive tree leaves	SC-CO_2 [a]	25 MPa, 313 K	74.5
This Work		SC-CO_2 [b]	25 MPa, 313 K	97.1

SC: supercritical; SFC: supercritical fluid chromatography.
[a] Optimal extraction conditions, recovery criterion.
[b] Optimal extraction conditions, concentration criterion.

Table 2
Comparison the SFE of olive tree leaves with soxhlet extraction

Extraction technique	Oil Yield (mg oil/100 g leaves)	Total amount of tocopherol (mg/100 g leaves)	Tocopherol concentration (% w/w)
Hexane	0.41×10^3	–	0.00
SC-CO_2 [a]	7.15	6.94	97.10
SC-CO_2 [b]	13.56	10.10	74.48

[a] $P = 250$ MPa, $T = 313$ K, Pd = 1.5 mm, $Q = 1$ SL/min for 1 h of extraction.
[b] $P = 250$ MPa, $T = 313$ K, Pd = 1.5 mm, $Q = 1$ SL/min for 2 h of extraction.

duced, being responsible to obtain high tocopherol concentrates (97%). According to these results, SFE arises as an alternative technique for enrichment extractions, specially indicated for natural matrixes with low content of non-polar compounds.

4. Conclusion

In this work, we have used supercritical CO_2 for the direct extraction of olive tree leaves, a residue obtained during the olive harvest for olive oil production. We have analyzed the effect of pressure, particle size, solvent flow and temperature on the extraction rates of oil and tocopherol. Two optimal extraction conditions were determined, considering the maximum recovery or concentration criterion. These conditions led to high valuable extracts of 74.5 and 97.1% (w/w) tocopherol concentration, respectively.

Direct SFE of matrixes with low content of non-polar compounds allows obtaining high tocopherol concentrates without coupling SFE to another enrichment technique. This finding results in an interesting advantage in the economics of an alternative process to the classical extraction methods.

Acknowledgements

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