Quality of Wheat Germ Oil Extracted by Liquid and Supercritical Carbon Dioxide

A. Molero G mez and E. Mart nez de la Ossa*

Department of Chemical Engineering, Food Technology and Environmental Technology, University of Cádiz, E-11510 Puerto Real (Cádiz), Spain

ABSTRACT: The extraction of wheat germ oil by liquid and supercritical CO_2 is described from the point of view of both operative method and pretreatment of raw material. The best conditions for wheat germ oil extraction are: pressure, 150 bar; temperature, 40ºC; and solvent flow rate, 1.5 L/min at standard temperature and pressure. The yields and fatty acid compositions obtained are very similar to those resulting from the conventional extraction process using hexane as solvent (8.0 wt%), although a higher-quality oil is obtained by using CO_2 as solvent (free fatty acids, 12.4%; tocopherol content, 416.7 mg tocopherol/g wheat germ oil). These factors lead to the conclusion that the extraction process using CO_2 could be economically competitive with the conventional process, since it considerably simplifies the oil refinement stages and completely eliminates the solvent distillation stage, which are the most costly processing steps in terms of energy consumption.

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KEY WORDS: Linoleic acid, liquid carbon dioxide, supercritical carbon dioxide, supercritical fluid, supercritical fluid extraction, wheat germ oil.

In recent years, the application of supercritical extraction (SCE) and, in particular, the use of liquid and supercritical CO_2 have been of considerable interest in the food industry. The decaffeination of coffee (1,2), the preparation of hop extract for the beer industry (1,2), and the production of spice essences and extracts (3) are just three examples of commercially available processes. This separation technique offers extraction yields very similar to those obtained by conventional extraction processes using liquid solvents, but does require a certain combination of operating temperature and pressure. Its advantages, compared to organic solvents, are that CO_2 is nontoxic, nonflammable, and noncorrosive and that it is cheap and readily available in bulk quantity, with a high degree of purity. In processing terms, CO_2 has a low critical temperature and pressure (31.1°C and 73.8 atm, respectively) which make it the ideal solvent for natural products, since they do not suffer thermal degradation reactions during the process.

*To whom correspondence should be addressed at Department of Chemical Engineering, Food Technology and Environmental Technology, University of Cádiz, Apdo. 40, E-11510 Puerto Real (Cádiz), Spain.
E-mail: enrique.martinezdelaossa@uca.es

For several years now, the beneficial effects of vegetable oils in the human diet have been well known, basically due to their high content in unsaturated fats and their high energy value. But of all the fatty acids present in vegetable oils, linoleic acid (18:2) must be considered the most significant and valuable benefit to human health (4–6). It is essential for human physiology and must be included in our diet, because it cannot be synthesized by the cell or by cellular tissue from any other fatty acid, nor indeed from any other source (7). Also of major importance, wheat germ is one of the vegetable materials with the highest vitamin E content (8). The value of vitamin E lies in its antioxidant action, which, by acting against the formation of free radicals, prevents the premature aging of human tissue (9). At the present time, most of the production of vitamin E is obtained as a by-product of oils deodorization step of the refining process (10,11).

The extraction of wheat germ oil using liquid and supercritical CO_2 is studied here. The working conditions of the process (i.e., the operating pressure, temperature, and solvent flow rate) and the pretreatment of the raw material (i.e., grain size and humidity) have been optimized. Then a comparison is made between the relative qualities of the oils produced by SCE and by organic solvent extraction, with the aim of checking whether, in principle, the SCE process can be economically competitive with the conventional organic solvent process.

MATERIALS AND METHODS

The raw material used for the processes was wheat germ of 0.75 mm average grain size supplied by CODEC S.A. (Valladolid, Spain). If necessary, the wheat germ was crushed in a coffee mill "Futurmat" (Barcelona, Spain) model FP of 2-kg capacity and capable of milling to 18 grades of particle size. The milled material used in the study was a fine powder of 0.30 mm average particle diameter. Dehydration of the raw material was carried out by heating to 65°C for a period of at least 4 h, until constant weight was achieved.

In order to compare to the conventional extraction process with liquid solvents, a Soxhlet-type apparatus with hexane as solvent (250 mL of hexane and 40 g of wheat germ) was used. Extraction time was 16 h which guaranteed the full depletion

of the grain and allowed the maximum possible extractive yield to be determined. These values are considered very important to establish an indisputable basis for comparison with the high-pressure SCE process. The equipment used for the SCE process with CO_2 was the SCE Screening System model manufactured by Autoclave Engineers (Erie, PA), and it is shown (as a schematic diagram) in Figure 1.

Liquid CO_2 was cooled to prevent its gasification and introduced into a high-performance liquid chromatography (HPLC) pump of 46–460 mL/h capacity with cooled head. The extraction vessel was a 75-mL volume, 316 SS high-pressure cylinder, capable of operating up to 400 bar and 340°C, with an electrical heating jacket. A sample of about 25 g was placed in the extractor vessel. The pressure of the CO_2 cylinder was maintained throughout the system by opening valves V-1, V-2, V-3, and V-4 and closing the other valves (V-5, V-6, and V-7). Before the pump was switched on, valves V-1, V-2, V-3, and V-4 were closed. Then the pump was switched on and its output pressure increased by the pressure regulating valve to reach the required processing pressure. Then the compressed CO_2 was introduced into the extractor vessel by opening valves V-3 and V-2. When the desired pressure was reached by adjusting the pressure-regulating valve, the heating jacket was used to reach the operating temperature. When both the desired pressure and temperature were reached, the extraction was started by opening valve V-7. The flow rate of CO_2 through the extractor vessel was regulated by the flow micrometer valve. The oil dissolved in the supercritical CO_2 was separated from the CO_2 and collected in the separator of 100 mL capacity at ambient temperature and pressure. The CO_2 was passed through two filters to remove the entrained oils and then through an in-line volumetric flow meter, which controlled the quantity and flow rate of CO_2 used. The flow meter used was a model FC-70 supplied by EG&G Flow Technology (Phoenix, AZ).

The optimization of the extraction process involved testing the following ranges of operating conditions: pressure, from 50 to 300 bar; temperature, from 10 to 60°C; and solvent flow rate, from 0.5 to 2.0 L/min at standard temperature and pressure. For the extractions performed at 10 and 20°C, the electric heating jacket was replaced with another brass tube jacket for the circulation of water at these two temperatures.

The physicochemical characterization of the oils extracted was performed by using the standards set by AOAC for the analysis of all oils for human consumption (12). The parameters analyzed were: density, viscosity, and absorbance at 290 nm. The various indices determined were: refraction, acidity (expressed as the percentage of oleic acid), iodine (by the Hanus method), saponification, and peroxides. The percentage of unsaponifiables was also determined.

The fatty acid composition of the oils was determined, after methylation (13) by gas chromatography using a model 5890 Hewlett-Packard (Pittsburgh, PA) gas chromatograph fitted with a Carbowax 20M (Supelco, Bellefonte, PA) capillary column and a flame-ionization detector.

Analysis of tocopherol content and individual tocopherol species in the extract was accomplished by HPLC. A model 1100 Hewlett-Packard was used in conjunction with a 5 m RP-18, 250 × 4 mm column (Merck, Darmstadt, Germany) using methanol as mobile phase in an isocratic mode at a flow rate of 1.0 mL/min. Detection was accomplished by using an ultraviolet-visible detector at 290 nm.

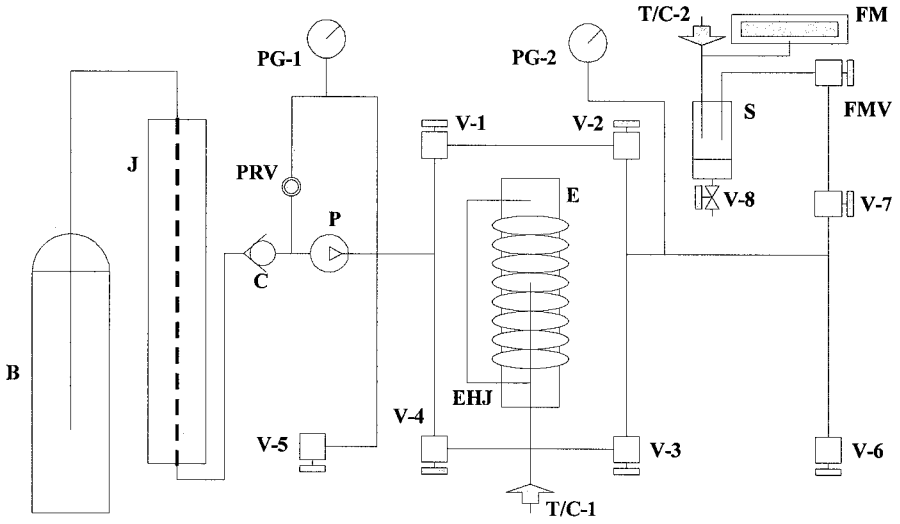

FIG. 1. Schematic diagram of supercritical CO_2 extraction apparatus. B: liquid CO_2 cylinder; J: cooler; C: check valve; P: high-pressure pump; PRV: pressure-regulating valve; E: extraction vessel; S: separator; EHJ: electrical heating jacket; FMV: flow micrometer valve; T/C-1,2: thermocouples; V-1,8: shut-off valves; PG-1,2: pressure gauges; FM: flow meter.

All the products and chemical reagents used were of analytical quality. Methanol used for the tocopherol analysis was HPLC-grade. The CO_2 used was of 99.95% purity (Carburos Metálicos, S.A., Seville, Spain).

RESULTS AND DISCUSSION

Extraction of wheat germ oil by liquid and supercritical CO_2. The yield of wheat germ oil obtained with supercritical CO_2 increases along the extraction time. However, the rate of extraction decreases over time. An extraction time of 3 h gives wheat germ oil recoveries above 95% of the total quantity. Although longer periods give slightly greater yields, they represent a significantly greater consumption of solvent. These data closely agree with those obtained by Taniguchi and colleagues (14).

Figure 2 shows the influence of the operating pressure for each of the six operating temperatures tested. In the vicinity of the critical region of CO_2 (around 50–150 bar for all temperatures studied), a dramatic change in the amount of extracted oil takes place. This change is due to variations in the physical properties of CO_2, particularly density which is closely related to its solvent capacity. As can be seen from this figure, for all temperatures, the extraction yield reaches a maximum at a certain pressure (around 100 bar, for the lower operating temperatures of 10 and 20°C; around 150 bar for 30 and 40°C; and around 200 bar for the higher temperatures of 50 and 60°C); beyond these practical limits, a considerable increase in pressure is required to achieve even a slight increase in the separation yield. At pressures below 150 bar, the amount of wheat germ oil dissolved in liquid CO_2 is greater than in its supercritical state. These results are confirmed by those presented in other studies (14–16).

Figure 3 shows the effect of CO_2 flow rate on the extraction yield, at operating conditions of 150 bar and 40°C. The oil extraction maximum is approached at about 1.5 L/min. A higher flow rate will give a somewhat higher yield but with a much higher solvent consumption; a lower flow rate will reduce solvent consumption but produce notably lower yields (and even for moderate yields requires processing times longer than 5 h, thus using considerably more solvent).

The flow rates of 1.5 and 2.0 L/min produce their maximal yields for processing times of about 3 h. The lower rate better maintains solubility equilibrium in the later stages of the extraction process when the quantity of oil still available is very small. Therefore, the flow rate of 1.5 L/min gives an increased yield in comparison to the 2.0 L/min rate (17).

No appreciable differences were detected in the yields obtained when the extraction process was carried out. Similar results were obtained when the influence of grain size on yield was studied. No significant difference in extraction yield was obtained between original wheat germ of 0.75 mm grain size and that milled to an average particle size of 0.30 mm. Given these results obtained for humidity and grain size of the raw material, wheat germ does not need pretreatment prior to extraction (14), in contrast to other natural products such as grape seed, for which the preparatory conditioning prior to

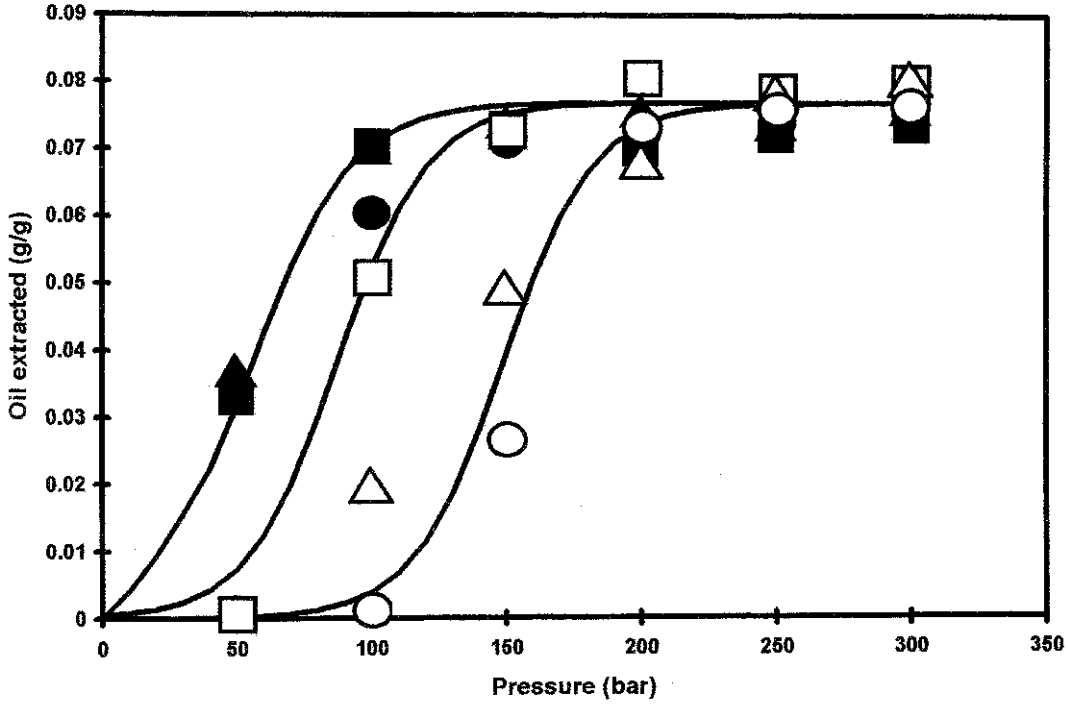

FIG. 2. Effect of pressure on the extraction of wheat germ oil in supercritical CO_2 at six operating temperatures. Operating conditions: extraction time, 3 h; solvent flow rate, 1.5 L/min (standard temperature and pressure); wheat germ humidity, 9.36%; size of wheat germ, flake (0.75 mm); temperature, (■) 10ºC, (▲) 20ºC, (●) 30ºC, (□) 40ºC, (△) 50ºC, (○) 60ºC.

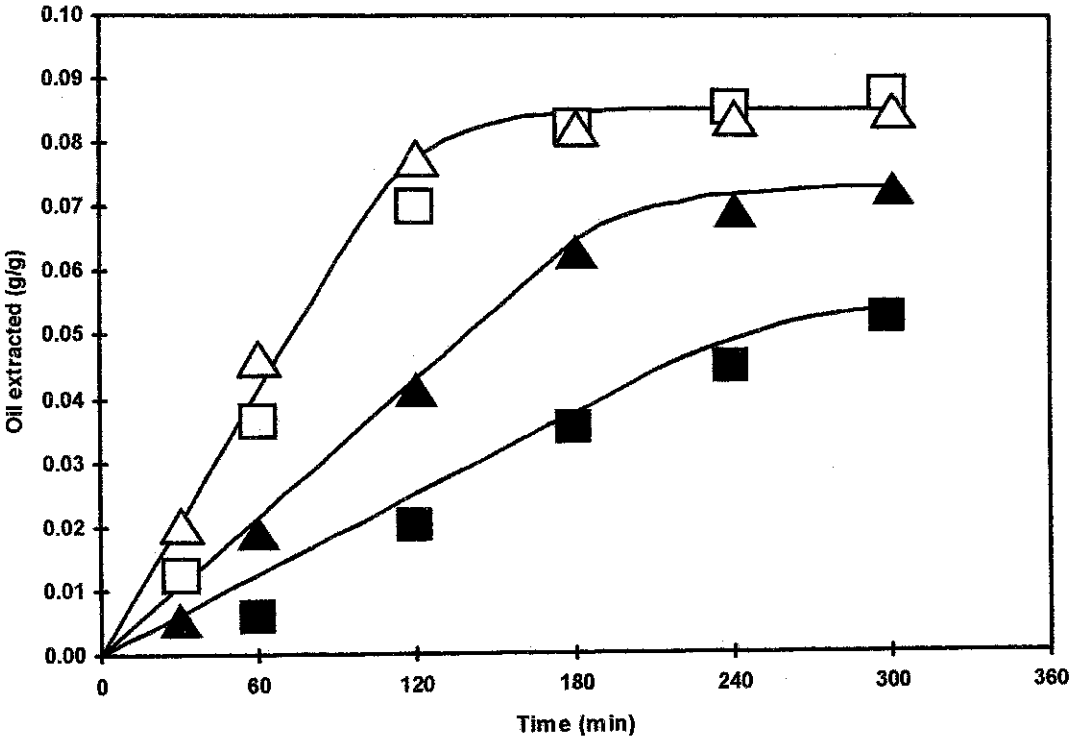

FIG. 3. Effect of flow rate of supercritical CO_2 on the extraction of wheat germ oil. Operating conditions: pressure, 150 bar; temperature, 40ºC; wheat germ humidity, 9.36%; size of wheat germ, flake (0.75 mm); solvent flow rate: (■) 0.5 L/min; (▲) 1.0 L/min; (□) 1.5 L/min; (△) 2.0 L/min.

supercritical extraction was studied and described in a previous paper (16).

Characterization of extracted oils. Table 1 gives the experimental yield values for the extraction of wheat germ oil, using both liquid and supercritical CO_2, under the optimized operating conditions, together with the corresponding data for conventional extraction using hexane. The apparent yield from the high-pressure extraction of wheat germ oil is slightly lower than with extraction by hexane. This difference has already been widely discussed (15–18), and it is interpreted that hexane is much less selective than CO_2 in the extracted oil and produces oil containing some undesirable compounds.

Taking the above into consideration, the results given in Table 1 indicate that the extraction process using liquid and supercritical CO_2 gives an extraction yield not significantly different from the Soxhlet equipment using hexane as solvent. If it is therefore accepted that SCE could be a replacement in the conventional extraction techniques, then the determination of the optimal operating conditions using liquid and supercritical CO_2 must be based on economic considerations and on the relative qualities of the oils obtained.

TABLE 1
Comparison of Wheat Germ Oil Extraction Yields Obtained Using Hexane and CO_2 as Solvents

Operating conditions	Yield (wt%)
CO_2, 100 bar, 10ºC, 3 h	7.3
CO_2, 100 bar, 20ºC, 3 h	7.6
CO_2, 150 bar, 30ºC, 3 h	7.5
CO_2, 150 bar, 40ºC, 3 h	8.0
CO_2, 200 bar, 50ºC, 3 h	8.0
CO_2, 200 bar, 60ºC, 3 h	7.8
Hexane, Soxhlet, 16 h	8.6

Table 2 presents a comparison of the physicochemical characterization of wheat germ oils produced by the SCE process under various operating conditions tested, and by the Soxhlet equipment using hexane as solvent. The analysis of these parameters for the different oils enables the optimal operating conditions for the SCE process to be selected.

Since there are appreciable variations between the oils obtained in terms of most of the analyzed indices, apart from refractive index, density and viscosity, the combination of pressure and temperature used in the SCE process plays an important role in the quality of extracted oil.

Acidity shows a parabolic trend, reaching the lowest values when the SCE process is carried out at temperatures of 30 and 40°C. If the free fatty acid content of oils produced by the two processes is discounted, the values of the iodine and saponification indices indicate that the quantity of triglycerides present in the oils extracted by both processes is similar, revealing that both extraction methods succeed in completely exhausting the wheat germ.

The values obtained for the unsaponifiable fraction do show a clear difference between oils extracted using both sol-

TABLE 2
Comparison of Extracted Wheat Germ Oils Obtained Using Hexane and CO_2 as Solvents

	Solvent						
	CO_2						Hexane
Physicochemical parameter	100 bar, 10°C, 3 h	100 bar, 20°C, 3 h	150 bar, 30°C, 3 h	150 bar, 40°C, 3 h	200 bar, 50°C, 3 h	200 bar, 60°C, 3 h	Soxhlet, 16 h
Refractive index	1.475	1.474	1.476	1.477	1.476	1.475	1.472
Density (g mL^{-1})	0.919	0.918	0.920	0.918	0.916	0.918	0.905
Viscosity (cP)	86	83	72	70	74	92	32
Absorbance at 290 nm (log ε)	0.676	0.665	0.757	1.157	0.841	0.878	0.969
Free fatty acid (%)	31.6	34.9	14.0	12.4	25.3	34.4	27.3
Iodine index (g/100 g)	111	112	102	100	107	115	107
Saponification index	217	181	185	185	197	204	207
Unsaponifiable fraction (%)	1.59	2.20	5.88	7.28	6.66	7.05	2.76
Peroxide index (meq/kg)	73	69	75	95	98	93	147

TABLE 3
Fatty Acid Composition (%) of Oil Extracted Using Hexane and Liquid and Supercritical CO_2

Oil	Palmitic 16:0	Palmitoleic 16:1	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
CO_2, 100 bar, 10ºC, 3 h	18.91	0.24	0.69	16.41	57.26	6.48
CO_2, 100 bar, 20ºC, 3 h	18.95	0.23	0.73	16.52	57.10	6.46
CO_2, 150 bar, 30ºC, 3 h	18.21	0.22	0.52	14.17	58.40	8.49
CO_2, 150 bar, 40ºC, 3 h	18.15	0.20	0.52	13.94	58.56	8.25
CO_2, 200 bar, 50ºC, 3 h	18.25	0.24	0.59	14.82	58.64	7.47
CO_2, 200 bar, 60ºC, 3 h	18.48	0.22	0.49	13.81	58.74	8.25
Hexane, Soxhlet, 16 h	18.09	0.22	0.50	13.69	58.99	8.51

vents. With the high-pressure extraction process, as the operating temperature is increased, more compounds comprising the unsaponifiable fraction are extracted (19).

Regarding the parameters of absorbance in the ultraviolet region and the peroxide index, the degree of oxidation of the lipids present in the wheat germ oil is minimal, and the only degradation in the oil is due to the oxidation of tocopherols, registered at around 290 nm and supported by the peroxide index values.

Composition of fatty acids. Table 3 gives the fatty acid composition of wheat germ oil obtained by extraction with liquid and supercritical CO_2, under the different operating conditions tested, and in comparison to that of oil extracted in the Soxhlet equipment using hexane as solvent. The results show that all the oils contain a high proportion of unsaturated fatty acids—81% unsaturated against 19% saturated—as occurs with other vegetable oils. Also, in all cases, the main fatty acid is linoleic, representing around 58% of the total.

The other conclusion from these data is that the different operating conditions tested did not produce any appreciable difference in the fatty acid composition of wheat germ oil extracted by SCE.

Content of tocopherol. Table 4 shows the tocopherol composition of extracted wheat germ oils with liquid and supercritical CO_2 compared to that of oil extracted in the Soxhlet equipment using hexane as solvent. As can be seen, the amount of tocopherol found in the wheat germ oil extracted with CO_2 is very similar under the different operating conditions tested. These quantities are higher in comparison to the amount obtained when oil was extracted using hexane as solvent, due to the higher temperature used during the extraction (a difference of the supercritical process).

Owing to the amount of tocopherols found in wheat germ oil extracted with liquid and supercritical CO_2, using SCE coupled with supercritical fluid chromatography can achieve the enrichment of tocopherols from wheat germ (20). Enrich-

TABLE 4
Composition of Tocopherol in Wheat Germ Oil (mg tocopherol/g wheat germ oil)

Extraction method	α-Tocopherol	β-Tocopherol
CO_2, 100 bar, 10ºC, 3 h	213.7	75.5
CO_2, 100 bar, 20ºC, 3 h	173.4	70.9
CO_2, 150 bar, 30ºC, 3 h	191.6	76.8
CO_2, 150 bar, 40ºC, 3 h	319.2	97.5
CO_2, 200 bar, 50ºC, 3 h	178.8	74.2
CO_2, 200 bar, 60ºC, 3 h	287.0	121.0
Hexane, Soxhlet, 16 h	166.0	66.6

ment and concentration of tocopherols are of vital importance from the point of view of the alimentary and pharmacology industries.

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