Extraction of Wheat Germ Oil by Supercritical CO_2: Oil and Defatted Cake Characterization

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ABSTRACT: In this paper the working conditions for the extraction of wheat germ oil in a supercritical CO_2 pilot plant of 1-L extraction capacity were studied. The best conditions were: pressure, 38 MPa; temperature, 55°C; wheat germ particle size, about 0.35 mm; CO_2 flow rate, 1.5 L min^{-1}. These conditions gave yields of about 92% of total oil after 3 h of processing. The obtained oils and the partially defatted cake were investigated with regard to their FA, tocol (tocopherol and tocotrienol), carotenoid, and sterol compositions and to their quality characteristics (FFA, PV, *para*-anisidine value, and color of the by-product). Moreover, the oil quality was evaluated in relation to the progress of the supercritical extraction.

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KEY WORDS: Carotenoids, color, defatted cake, supercritical CO_2 extraction, tocols, wheat germ.

Wheat germ constitutes about 2–3% of the whole wheat kernel; it has an average moisture content of 14% and contains about 10% oil (1). Even though it is a minor milling by-product, it is valuable owing to its nutritional and pharmaceutical properties (2). The well-known beneficial effects of wheat germ oils are basically due to their high content of vitamin E (3) and unsaturated FA, mainly linoleic acid (18:2). Wheat germ oil has been shown to reduce plasma and liver cholesterol in animals and to delay aging (4,5). Nevertheless, because of its unfavorable baking properties and susceptibility to oxidation, the germ is removed from the endosperm during milling and is mainly used for fodder and for oil production (6).

Wheat germ oil is obtained either by solvent extraction, which recovers about 90% of the oil, or by mechanical pressing, which recovers about 50% (7). The extracted wheat germ oils usually need to be refined, owing to their high FFA content, varying from 5 up to 25%, depending on the conditions of milling, germ storage, and oil extraction (8). Thus, the conventional production methods either have a low recovery or can lead to degradation of heat-sensitive compounds as well as leave traces of toxic solvents in the oil. Supercritical CO_2 (SC-CO_2) extraction can overcome these negative factors; in fact, the oils are solvent-free and do not need the traditional refining processes, and extraction yields are similar to those obtained using solvent. Additionally, despite high production costs, the fractionating high-pressure extraction is suitable for separating different chemical components, for instance FFA, or for concentrating others, such as vitamin E (9), thus improving wheat germ oil quality. Finally, CO_2 is nontoxic, nonflammable, noncorrosive, cheap, and recyclable. Moreover, the quality and the storage characteristics of wheat germ oil cake are normally improved by SC fluid extraction, since the combined effect of CO_2 and high pressure can lead to decreases of peroxidase and lipoxygenase activities (10), to the inactivation of microrganisms (11), and to inhibition of the development of rancidity by limiting the contact with atmospheric oxygen. Thus, although it is possible to obtain a safer product and, in some cases, an improved by-product, the SC technology is still not widespread because of its production costs and because of the difficulty using it in a continuous cycle on an industrial scale.

The aim of this present research was to assess working conditions for wheat germ oil extraction in an SC-CO_2 pilot plant and for the production of a food-grade-quality defatted germ flour. The conditions of extraction were selected by considering their feasibility for industrial application.

EXPERIMENTAL PROCEDURES

Apparatus and extraction methods. The SC-CO_2 apparatus was provided by Tecnoprocess snc (Rome, Italy). Carbon dioxide was pumped under high pressure (up to 65 MPa) by a single-end diaphragm-type compressor. The extractor was equipped with a 1-L capacity extraction vessel and with three 0.2-L separation vessels. All vessels were made of 316 stainless steel and were capable of pressure ≤60 MPa, with an interspace for the circulation of water conditioned by heat exchangers. A thermocouple was inserted into the vessels to monitor their internal temperatures. The second and third vessels were provided with micrometric valves for the modulation of head loss. The plant allows the CO_2 recycling through a condenser and a feeding reservoir. The instantaneous flow rate of CO_2, as well as the total flow, were measured with a rotameter.

Materials. Flaky food-grade wheat germ was supplied by a commercial stock in Italy and stored at 5°C. The wheat germ was milled with a refrigerated mill (Cyclotec FOSS, Padova, Italy) to produce a fine powder varying from about 0.35 to 0.50 mm average particle diameter.

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Extraction conditions and sampling. In assessing the effectiveness of SC-CO_2 in dissolving oil fractions from wheat germ, three different experimental conditions of wheat germ pressure and particle size were tested. During the extraction a higher solubility was required, which was obtained with high temperature values. In our research, temperatures over 55°C were avoided in order to preserve oil quality. The different particle sizes and pressure in the monitored samples were chosen (i) with the aim of getting good CO_2 solvency under SC conditions using the largest possible wheat germ particle size (ø) in order to avoid feed caking, which would limit industrial scale extraction, and (ii) to make the SC-CO_2 extraction economically feasible for industrial use.

The experimental conditions adopted during extraction were as follows: (i) P = 25 MPa, T = 55°C, ø = 0.50 mm; (ii) P = 38 MPa, T = 55°C, ø = 0.50 mm; (iii) P = 38 MPa, T = 55°C, ø = 0.35 mm. The pressure and the temperature of separation (P = 6.5 MPa, T = 20°C) were maintained almost constant in the different tests. The oils from the separator vessels were sampled during processing in order to study the yield over time and to evaluate the selectivity of the extraction process.

Analytical procedures. The amount of oil extracted with SC-CO_2 was gravimetrically determined. Oil was also extracted from wheat germ by recycling petroleum ether in a Soxhlet apparatus for 9 h; this oil will be referred to as wheat germ oil. FFA and PV of oils were determined according to Association of Official Analytical Chemists' methods 28.032 and 28.025 (12), and *para*-anisidine value (AV) was determined by IUPAC method 2.504 (13). Moisture content and total nitrogen in wheat germ and in the residual cake were determined according to the International Association for Cereal Science and Technology's methods ICC 110/1 and ICC 105/2, respectively (14). The FA composition of oils was determined, after methylation, by using a gas chromatograph (Model HRGC 5300; Carlo Erba, Milano, Italy) fitted with a 30 m × 0.25 mm, 0.20 µm i.d. Rtx-2330 (Restek, Bellefonte, PA) capillary column and an FID. Color (b, yellowness) of the defatted cake was assessed with a Minolta CR-200 chromameter having an opening size of 10 mm (Minolta, Japan). Total dietary fiber was quantified by the enzymatic gravimetric procedures of Prosky *et al.* (15). Tocols and carotenoids in wheat germ, defatted cake, and extracted wheat germ oils were determined after hot saponification followed by extraction with solvent (16,17).

HPLC analysis. Chromatography of tocols was performed using a Waters (Milford, MA) HPLC analytical system comprising a Model 510 solvent delivery system equipped with a programmable Model 470 spectrofluorimeter. The chromatographic separation of the compounds was achieved with the normal-phase method of Shin and Godber (18), with a slight modification. A 250 × 4.6 mm, 5 µm i.d. Kromasil Phenomenex Si column (Torrance, CA) was used. The mobile phase was *n*-hexane/ethyl acetate/acetic acid (97.3:1.8:0.9 by vol) at a flow rate of 1.6 mL/min. Fluorimetric detection of all peaks was performed at an excitation wavelength of 290 nm and emission wavelength of 330 nm, attenuation 8, gain × 100, filter 1.5 s. A typical run lasted approximately 25 min.

Detection and quantification of carotenoids was carried out with a Waters HPLC on a Model 510 solvent delivery system equipped with a Model 991 photodiode array detector using a 250 × 4.6 mm i.d., 5 µm Kromasil Phenomenex Si column. The compounds were detected at 450 nm. The mobile phase was 5% isopropyl alcohol in *n*-hexane (vol/vol), with a flow rate of 1.5 mL/min and a total run of 15 min. Results were elaborated by a Waters Millennium Chromatography system.

Reagents and standards. HPLC and analytical-grade reagents and solvents were supplied by Carlo Erba. α-Tocopherol (α-T) and β-tocopherol (β-T) standards were from Merck (Darmstadt, Germany); α-tocotrienol (α-T3) and β-tocotrienol (β-T3) standards were purified by HPLC separation and collection from a saponified barley sample. Carotenoid standards (β-carotene, lutein, zeaxanthin) were purchased from Extrasynthèse (Z.I. Lyon-Nord, Genay, France).

Statistical design and analysis. SC-CO_2 extraction of wheat germ oil was carried out in triplicate for each different extraction process. The characterization of oils and of defatted wheat germ was made on samples obtained from the best experimental conditions. The oils obtained from the three repetitions of the best experimental conditions were pooled for the analysis and were compared with wheat germ oil and cake oil. The analyses were carried out in triplicate and data reported are the mean ± SD of three repetitions. A one-way ANOVA was conducted on the data using the Bonferroni test (McGraw-Hill Inc. Software, 1992). Student's *t*-test was used to differentiate mean values, and significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

The oil yields from wheat germ after solvent and SC-CO_2 extraction under different conditions are shown in Table 1. The higher oil recovery, about 92% of the liquid (petroleum ether) extraction, was obtained by adopting a smaller particle size (0.35 mm), higher conditions of pressure (P = 38 MPa, T = 55°C), and about 3 h of processing (19). Considering the above parameters, particle size appears to have a greater influence on yield than the pressure adopted during extraction. In our experiment, about 600–700 g of the milled wheat germ was placed in the extraction vessel; the bulk density of the wheat germ used was roughly 600–700 kg/m^3. The CO_2 diffusion process is characterized by a faster rate when the SC fluid flows in the void of the material particles and a slower rate in the intraparticle diffusion process, which limits the rate

TABLE 1
Effect of Pressure (P), Temperature (T), and Wheat Germ Particle Size on Oil Extraction Yields

Extraction medium	T-P conditions (°C)	T-P conditions (MPa)	Wheat germ size (mm)	Extracted oil (g/100 g wheat germ)	Yield (%)
SC-CO_2	55	25	0.50	6.4	50
SC-CO_2	55	38	0.50	7.2	57
SC-CO_2	55	38	0.35	11.7	92
Petroleum ether			0.50	12.7	100

TABLE 2
Combined FA Composition (%) of Wheat Germ Oil, of SC-CO_2 Oil, and of Cake Oil

Sample	Palmitic 16:0	Palmitoleic 16:1	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3
Wheat germ oil	18.0	1.3	1.3	21.6	51.3	6.4
SC-CO_2 oil	18.1	0.9	1.0	18.4	54.6	6.7
Cake oil	19.1	0.8	1.1	17.5	54.1	7.1

of the whole process (20). Thus, reduction of the particle size increases diffusivity to a critical point, in which the caking of feed makes oil extraction difficult in an industrial plant. The CO_2 flow rate of 1.5 L/min was able to guarantee maximal yield in the specified time interval (21). The pressure and temperature of separator vessels (P = 6.5 MPa; T = 20°C) were maintained almost constant in the different tests. In fact, this temperature corresponds to a low vegetable oil solubility in SC-CO_2 at any pressure condition.

The following results are related to the better yield conditions (P = 38 MPa, T = 55°C, ø = 0.35 mm) as reported above.

The percentage content of the major FA in wheat germ oil, in SC-CO_2 oils, and in the cake oil are shown in Table 2. The FA composition did not change significantly among the different test samples. Unsaturated FA represented over 80% of the FA composition, mainly due to linoleic acid, which is about 55% of the total FA.

Qualitative characteristics of oils from wheat germ are affected by the lipid hydrolysis, which produces FFA, as well as by the enzymatic oxidation of the polyunsaturated linoleic acid. These reactions occur at a rate governed by the temperature and the moisture content of the product during storage (22). In Figure 1, changes in yield, FFA, and PV during SC-CO_2 extraction of wheat germ oil are reported. Oil quality varied in relation to the progress of the SC-CO_2 extraction; in particular, the first fractions, sampled after 15 and 45 min, had the greatest PV and FFA contents, respectively. However, in the SC-CO_2 oil, low levels (FFA = 4.0%; PV = 3.8 meq O_2/kg oil) were found, indicating that the degree of lipid oxidation in the wheat germ was minimal; this low level of oxidation was confirmed by the low AV value (= 2.1) (23). These data also indicate that SC-CO_2 extraction does not increase oxidation. Moreover, PV and FFA levels in the SC-CO_2 oil decreased depending on extraction time. These findings confirm the report by Seidel *et al.* (24), who used black caraway seeds.

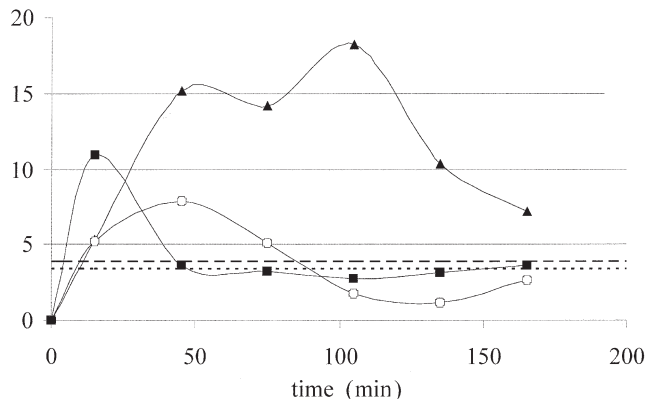

FIG. 1. Yield (g; ▲), FFA (%; ○), and PV (meq O_2/kg oil; ■) evolution during supercritical CO_2 extraction of wheat germ oil. (---) wheat germ FFA; (– – –) wheat germ PV. The CV was always less than 5%.

TABLE 3
Tocol Content (mg/kg oil) in Wheat Germ Oil, in SC-CO_2 Oils at Different Extraction Times, and in Cake Oil

Sample	Mean (SD) α-T	α-T3	β-T	β-T3	∑T	∑T3	Total
Wheat germ oil	1630 (65)	29 (0.8)	543 (12)	85 (1.0)	2173	114	2287
Oil (min)[a]							
15	1620 (21)	22 (0.9)	582 (15)	52 (2.5)	2202	74	2276
45	1789 (61)	22 (0.6)	562 (18)	48 (1.2)	2351	70	2421
75	2123 (32)	35 (0.9)	709 (23)	59 (2.2)	2832	94	2926
105	1471 (23)	29 (1.1)	580 (17)	77 (4.0)	2051	106	2157
135	665 (35)	21 (0.9)	330 (12)	63 (2.3)	995	84	1079
165	952 (48)	19 (0.6)	363 (15)	50 (1.2)	1315	69	1384
Cake oil	223 (12)	29 (1.5)	118 (3.0)	36 (1.2)	341	65	406

[a]Extraction time. Abbreviations: α-T, α-tocopherol; α-T3, α-tocotrienol; β-T, β-tocopherol; β-T3, β-tocotrienol; ∑T, total tocopherol; ∑T3, total tocotrienols.

The composition and content (mg/kg oil) of tocols and carotenoids of wheat germ oil, of oils extracted with SC-CO_2, and of the cake oil are given in Table 3 and Table 4, respectively. The greatest concentration of α-T, which is the most abundant tocol in wheat germ, was found in the fraction sampled at 75 min (Table 3). A different behavior between tocols was shown, depending on their different polarity, with maximal β-T3 content occurring after about 2 h of processing. In general, the highest tocol recovery was obtained after 75 min of extraction, achieving about 27% enrichment. The total tocol recovery at the end of the extraction was good, with a yield of about 87%. Relative to carotenoids (Table 4), the most representative carotenoid in wheat germ was lutein, followed by zeaxanthin and β-carotene. The concentration of total carotenoids in extracted oils reached its maximum after 165 min of extraction, with an enrichment of about 70%. The different carotenoids showed the same behavior during the whole process of extraction, with a total extraction yield as to

TABLE 4
Carotenoid Content (mg/kg oil) in Wheat Germ Oil, in SC-CO_2 Oils at Different Extraction Times, and in Cake Oil

Sample	Mean (SD) β-Carotene	Lutein	Zeaxanthin	Total
Wheat germ oil	8.3 (0.2)	24.6 (0.6)	22.8 (0.5)	55.7
Oil (min)[a]				
15	6.2 (0.4)	21.1 (0.8)	18.4 (0.5)	45.7
45	1.5 (0.0)	4.4 (0.2)	3.6 (0.1)	9.5
75	1.6 (0.0)	4.3 (0.2)	3.4 (0.1)	9.3
105	3.7 (0.1)	7.7 (0.3)	5.5 (0.2)	16.9
135	8.2 (0.3)	20.5 (0.5)	13.8 (0.5)	42.5
165	11.0 (0.3)	47.7 (2.1)	37.3 (1.5)	96.0
Cake oil	10.0 (0.4)	74.1 (3.2)	71.4 (2.5)	155.5

[a]Extraction time.

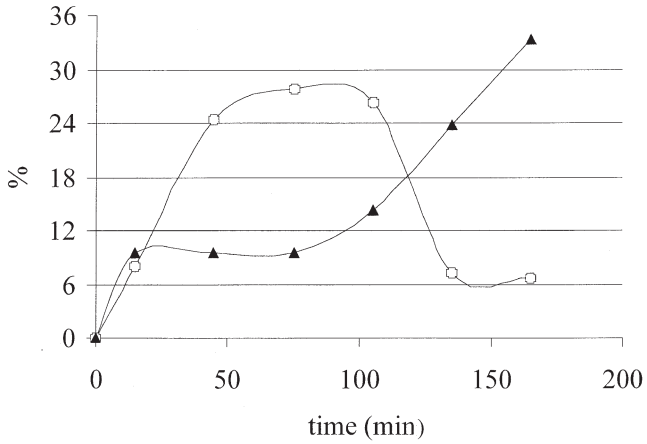

FIG. 2. Evolution of tocols (○) and carotenoids (▲) during supercritical CO_2 extraction of wheat germ oil. The CV was always less than 5%.

wheat germ of about 45%. The time course of tocol and carotenoid extraction with SC-CO_2 is shown in Figure 2. Recovery of tocols in each sampled fraction, expressed as a percentage of the total recovery, showed a parabolic trend, reaching the lowest value (%) after 165 min of extraction. In contrast, as extraction time increased, a larger amount of carotenoids was extracted with a maximal yield at the end of the processing.

These results demonstrate that fractionation using SC-CO_2 extraction is suitable to separate different chemical components and to improve wheat germ oil quality. In order to consider the possibility of using wheat germ as a protein-mineral food supplement, the quality of the defatted wheat germ after SC-CO_2 extraction was also evaluated. Proximate analysis of partially defatted wheat germ and of wheat germ before SC-CO_2 extraction is given in Figure 3, where the expected decrease in fat and in tocol content in defatted wheat germ is shown. The low lipid content (about 2%) could prevent autooxidative processes, thus improving the shelf life of this byproduct. Ash, fiber, and protein contents above all increased in defatted wheat germ, giving an additional nutritional value to the defatted cake. The decrease in carotenoids, which are of particular technological interest (color of cereal product), appeared remarkable, even if the index of the yellow parameter (b) varied to a lesser extent. In light of these results, partially defatted wheat germ could be used as an interesting by-product having desirable nutritional and technological properties.

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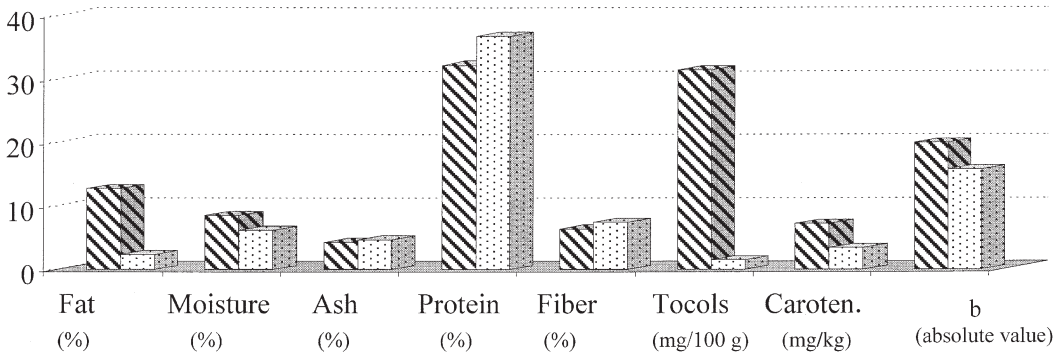

FIG. 3. Wheat germ (diagonally lined bars) and defatted wheat germ (stippled bars) composition. The CV was always less than 5%. Caroten.: carotenoids.

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