Pilot-Scale Supercritical Carbon Dioxide Extraction and Fractionation of Wheat Germ Oil

Michael Eisenmenger[a,b], Nurhan T. Dunford[a,b,*], Fred Eller[c], Scott Taylor[c], and Jose Martinez[d]

[a]Department of Plant and Soil Sciences and [b]Agricultural Products Research and Technology Center, Oklahoma State University, Stillwater, Oklahoma 74078, [c]USDA, ARS, National Center for Agricultural Utilization Research, Peoria, Illinois, and [4]Thar Technologies, Pittsburgh, Pennsylvania

ABSTRACT: There is a need for the development of new processing techniques to facilitate vegetable oil extraction and refining while sustaining the nutritional components naturally present in edible oils and reducing the adverse impact of oil processing on the environment. In this study supercritical carbon dioxide ($SC\text{-}CO_2$) extraction and fractionation techniques were examined as alternative methods to obtain wheat germ oil (WGO) of high quality and purity. It was shown that the $SC\text{-}CO_2$ extraction technique is effective in extraction of WGO. There was no significant difference in the FA composition of $SC\text{-}CO_2$- and hexane-extracted WGO. Both hexane- and $SC\text{-}CO_2$-extracted WGO were rich in α-tocopherol. Moisture content of the $SC\text{-}CO_2$-extracted oil was higher than that of the hexane-extracted oil. Solvent/feed ratio had a significant effect on the $SC\text{-}CO_2$ extraction yields. This study demonstrated that supercritical fluid fractionation was a viable process to remove FFA efficiently from both hexane- and $SC\text{-}CO_2$-extracted WGO while retaining bioactive oil components in the final product.

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KEY WORDS: Fractionation, free fatty acid, oil refining, supercritical carbon dioxide, wheat germ oil.

Wheat germ oil (WGO) is rich in PUFA and bioactive compounds. These compounds are prone to oxidation and degradation under the conditions used for conventional edible oil extraction and refining methods. Hence there is a need for development of new processing techniques that will maintain WGO quality and biological activity of the oil components during processing. Supercritical fluid technology is an alternative method to conventional hexane extraction and refining. Several research studies reporting supercritical carbon dioxide ($SC\text{-}CO_2$) extraction of WGO have been published (1–4). Taniguchi *et al*. (4) reported that WGO solubility in $SC\text{-}CO_2$ was 0.35% (w/w) at 40°C and 20 MPa. $SC\text{-}CO_2$-extracted oil had a lighter color and contained less phosphorus than hexane-extracted oil. According to Panfili *et al*. (3), FFA content and PV of the oils collected during the first 45 min of extraction were higher than those of the oil fractions collected at the later stages of the process. Extraction of wheat germ with liquid and $SC\text{-}CO_2$ (5–40 MPa) at relatively low temperatures (10–60°C) indicated that pressure had a significant effect on the oil yields whereas the effect of temperature was insignificant (4). Dunford and Martinez (1) studied the effect of pressure and temperature on the $SC\text{-}CO_2$ WGO extraction yields in the range of 10–55 MPa and 40–80°C. Yields of $SC\text{-}CO_2$ extracts varied significantly with temperature and pressure in the 2 to 20% (w/w) range. Soxhlet extraction using hexane as a solvent yielded 11% (w/w) WGO. These results indicate that $SC\text{-}CO_2$ at high pressure extracts some of the wheat germ components that are not soluble in hexane. At higher temperature and pressures, moisture can be co-extracted with oil, resulting in higher extraction yields (5,6). The highest $SC\text{-}CO_2$ extraction yield was obtained at the highest pressure used (55 MPa). The temperature dependence of the extract yield was more pronounced at higher temperatures (60 and 80°C) and the lowest pressure examined in that study (10 MPa).

The literature on utilization of a columnar supercritical fluid fractionation (SFF) technique for vegetable oil processing is relatively limited as compared with many other topics that focus simply on the use of supercritical fluid extractions. A U.S. patent describes enrichment of phytosterols in rice bran oil using a columnar $SC\text{-}CO_2$ fractionation process (7). When a continuous countercurrent SFF was used for deacidification of rice bran oil at 14 MPa and 80°C, FFA were effectively removed without any oryzanol loss in the extract fraction (8–10). When $SC\text{-}CO_2$-extracted corn fiber oil, which is also rich in phytosterol esters (especially oryzanol), was fractionated using SFF, it was possible to obtain phytosterol-enriched TAG fractions (>15% phytosterol content) (11).

To our knowledge, SFF of WGO has not been reported in the literature. The objectives of this research were to examine pilot-scale $SC\text{-}CO_2$ extraction of WGO and to investigate the potential of using SFF for the removal of FFA from both hexane- and $SC\text{-}CO_2$-extracted WGO.

EXPERIMENTAL PROCEDURES

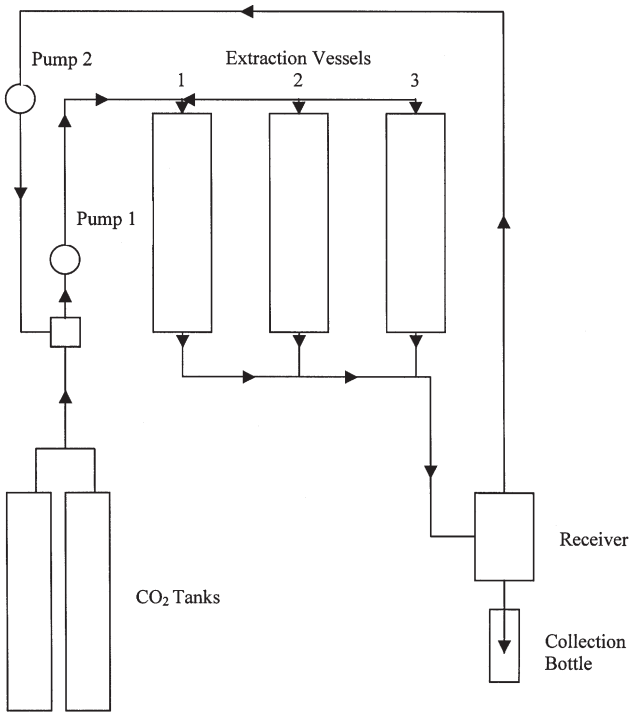
Wheat germ samples were supplied by ADM Milling Co. (Enid, OK). Germ was obtained from milling of winter wheat (20% Kansas-80% Oklahoma-grown winter wheat). Germ samples were used for extraction runs with no pretreatment. Hexane extraction of WGO was carried out according to the

*To whom correspondence should be addressed at Oklahoma State University, Department of Plant and Soil Sciences, Agricultural Products Research and Technology Center, Room 103, Stillwater, OK 74078.

E-mail: Nurhan.Dunford@okstate.edu

AOCS Official methods Ba 3-38 using *n*-hexane (12) to determine the oil content of the material used for SC-CO_2 extraction runs. Reagent-grade *n*-hexane (Pharmco, Brookfield, CT) was used as a solvent for the extraction.

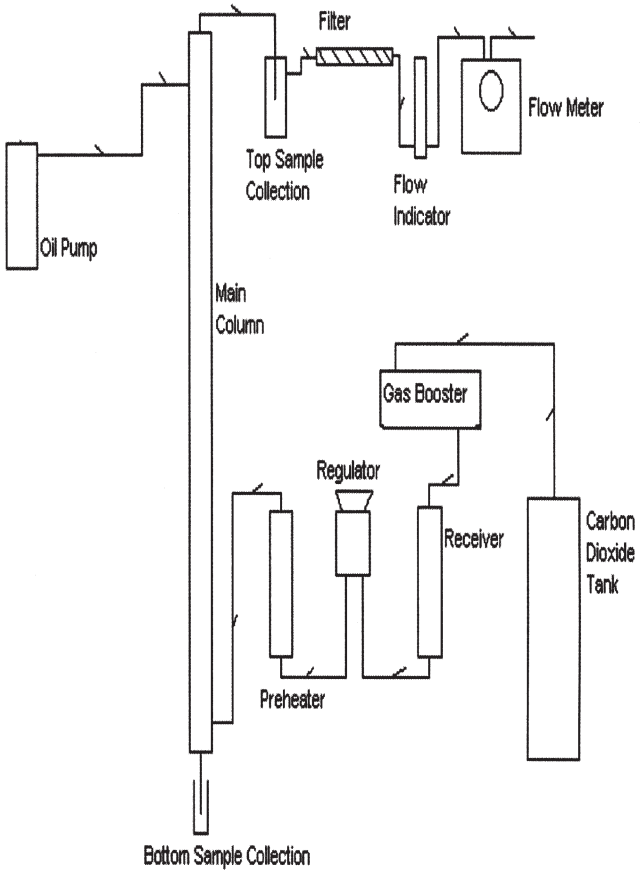
The small-scale SC-CO_2 extraction experiments were carried out in a system manufactured by Thar Technologies (Pittsburgh, PA). A schematic diagram of the system was given by Dunford and Martinez (1). The carbon dioxide and the pump heads were chilled (5°C) to avoid cavitations and compressibility problems. The liquid CO_2 was compressed by a high-pressure pump to the operating pressure (10–55 MPa) at constant flow rate. The high-pressure pump (flow rate: 20–200 g/min) featured dual stainless steel heads with cam-driven sapphire pistons, cartridge check valves, and pressure gauge and rupture disc assemblies. The high-pressure CO_2 flowed through a pre-heater to ensure that the extraction temperature (40–80°C) had been attained before it reached the extraction vessel (100 mL). A sample of about 35 g was loaded into the vessel, and the pressure was controlled by a back-pressure regulator. The solvent and the extracted compounds leaving the vessel from the top passed through a pressure reduction valve, and the extract was collected in a separation vessel. The total amount extracted was calculated based on the difference between the weight of the extraction vessel before and after each extraction.



SCHEME 1

The pilot-scale SC-CO_2 extraction of wheat germ was carried out at the National Center for Agricultural Utilization Research facilities of the USDA, Agricultural Research Service (Peoria, IL). The extraction was conducted with three vessels (4 L each) in parallel (Scheme 1). Each vessel contained 1.5 kg of wheat germ. The total of 4.5 kg of wheat germ was extracted at 80°C and 69 MPa using 16 kg CO_2 (measured at atmospheric conditions). The CO_2 flow rate was 0.18 kg/min. The extract was collected in a receiver maintained at 60°C and 11 MPa. The recovery of the extract was accomplished by pressure drop across a back-pressure relief valve followed by condensation and recovery of the precipitate. Extraction was conducted for a total of 45 min. The extraction was assumed complete when no more extract was collected in the receiver. The amount of extract collected was determined gravimetrically by draining the oil accumulated in the separator into a bottle and then weighing the bottle with oil.

SFF experiments were carried out using both commercially hexane-extracted WGO and SC-CO_2-extracted oil. Hexane-extracted crude WGO was a donation from Vitamins, Inc. (Chicago, IL). Oil samples were stored in sealed containers at 4°C away from the light. Both SC-CO_2- and commercially hexane-extracted WGO samples were used for SFF experiments without further purification except that they were centrifuged at 29,000 × *g*, at 4°C for 30 min and vacuum-filtered through #2 Whatman filter paper. The SFF experiments were conducted at the Food and Agricultural Products Research and Technology Center's pilot plant facility on the Oklahoma State University campus (Stillwater, OK). The SFF column, which was designed in-house, is 3 m long and has a 2.5 cm inner diameter (Temco, Inc., Tulsa, OK). A schematic flow diagram of the SFF system is shown in Scheme 2. The fractionation column had a pre-heater and four independently controlled temperature zones. The temperature of the main column was maintained by an HS-4ZC Heating System (Temco, Inc.). The heating system consisted of a Watlow temperature controller, which uses cascade-style heating (Watlow Electric Manufacturing Company, Winona, MN). The temperature of the column was maintained automatically at ±2°C of the set point by WatView Run-Time software (Version: 2.3.7, 1999–2002; Watlow Anafaze, Inc., Watsonville, CA) run on a Dell Inspiron 8100 laptop computer. The pre-heater set temperature was maintained by a PID type controller (Model TC-11-K; Watlow Electric Manufacturing Company). The temperature sensing was from type-K thermocouples inserted inside and on the surface of the vessels. The main column was packed with protruded 316 SS packing material (0.4 cm Pro-Pak; Cannon Instrument Company, State College, PA). There were two separate ports for CO_2 inlet and raffinate removal at the bottom of the column. The lower section of the column (about 30 cm) below the inlet was used for raffinate collection. The fractionation experiments were carried out in a continuous countercurrent mode of operation. Initially the column was pressurized with CO_2 (Research Grade, min. purity 99.998%; Matheson Tri-Gas, Houston, TX) and allowed to equilibrate at the desired temperature and pressure. An air-driven gas booster pump (Model ACT-62/152; Haskel Inc., Burbank, CA) was used to deliver CO_2 into the column. The pressure fluctuations in the column were minimized by placing a high-pressure gas receiver (volume 570 cm^3, Model #157-12; Haskel Inc.) and a back-pressure valve (Model 26-2091B44S172; Tescom Corporation, Elk River, MN) after the



SCHEME 2

booster pump and before the column. Carbon dioxide entered at the bottom of the column just above the raffinate reservoir. Oil was introduced from the top of the column by a syringe pump (ISCO model 100DX pump; Teledyne Isco, Inc., Lincoln, NE) controlled by an ISCO SFX 200 controller (Teledyne Isco). Solute-laden SC-CO_2 rose upward and was recovered as the extract fraction from the top of the column. Compounds with lower solubility in SC-CO_2 than that of the extract and/or components with larger M.W. moved downward and collected as raffinate at the bottom of the column. The extract and raffinate fractions were expanded through micrometering valves (Part no.30VRMM4812; Autoclave Engineers, Inc., Erie, PA) and the precipitate was collected into vials cooled by two Microban ICE-PAKs (Fisher Scientific, Pittsburgh, PA). Then CO_2 passed through a custom gas filtration device (a tube filled with glass wool), a flow indicator, and finally through a dry gas test meter (Model DMT-200A-3; American Meter Company, Philadelphia, PA) for recording the total amount of CO_2 used for the fractionation process.

Fractionations of crude WGO were carried out at 14 MPa, 80°C, and 4 and 8 L CO_2/min flow rates. Oil was introduced to the column at a constant flow rate of 0.3 mL/min, giving S/F ratios of approximately 25:1 and 50:1 (w/w). Fractionations of SC-CO_2-extracted WGO were performed under the same conditions but only at one flow rate, 8 L CO_2 /min (S/F ratio = 50:1). The total run time for the fractionation experiments was 6 h. The system was allowed to reach steady-state conditions for the first 4 h. The steady-state conditions in the column were confirmed by attaining constant weight and composition of the extract fraction collected in 30-min intervals through several testing fractionations over various time intervals. The data on the fractionation experiments reported in this study were collected in 1-h intervals after steady-state operation was established in the column (fifth and sixth hour of the fractionation runs). These samples were characterized for their chemical composition. Insoluble oil components that had collected in the reservoir were drained every half hour. After the completion of each experimental run the column was depressurized and residual oil was drained off. The column was cleaned between runs at a pressure of 34 MPa and temperature of 80°C by flowing CO_2 at 8–10 L/min for more than 6 h.

Analytical tests. Moisture content of oil samples was determined by using a Karl Fischer Titrator (758 KFD Titrino, Metrohm; Brinkman Instruments, Inc., Westbury, NY). The 34811 Hydranal Titrant-2 was used as a titrant and the 34812 Hydranol Solvent was the component solvent. Both solvents were purchased from Sigma-Aldrich Corporation (St. Louis, MO).

The FFA content of the oil samples was determined by using a colorimetric method (13). Cupric acetate-pyridine solution was prepared by adjusting the pH of the filtered 5% (wt/vol) aqueous cupric acetate (99.9% purity, JT Baker, Phillipsburg, NJ) solution to 6.0–6.2 with pyridine (99% purity; Fisher Chemicals, Fairlawn, NJ). About 0.03–0.05 g of oil samples were weighed into a 5-mL volumetric flask and brought to 5 mL volume with benzene (ACS grade; EMD Manufacturing, Savannah, GA). Color development was initiated by addition of 1 mL cupric acetate-pyridine reagent into the oil-benzene mixture. After mixing and centrifugation, absorbance of the top layer was read at 715 nm using a UV/vis spectrophotometer (Beckman DU 520; Beckman Coulter, Fullerton, CA). FFA contents of the samples were determined from the calibration curve. Oleic acid (90% purity; Aldrich, Milwaukee, WI) was used for the preparation of the standard curve.

Total FA compositions of the oil samples were determined by GC (14). The HPLC method used for analysis of tocopherol content of the oil samples was described in detail by Jonnala *et al.* (15). The analytical methods used for determination of lipid composition of WGO samples were explained in detail elsewhere (16). In summary, analytical separations of TAG, FFA, and free and FA esters of phytosterols in oil samples were achieved by using an HPLC method developed by Moreau *et al.* (17). A LiChrosorb Diol, 5 μm, 100 × 3.0 mm (Chrompack Inc., Raritan, NJ) column was used for the analysis. The mobile phase consisted of the following: A: hexane/acetic acid (1000:1); B: 2-propanol. The solvent gradient system was as follows: 100% A for 8 min, 100% A to 99% A (1% B) in 2 min, hold for 20 min, from 99% A to 100% A in 1 min, and hold for 29 min, resulting in 60 min total analysis time. The mobile phase flow rate was 0.5 mL/min. Oil samples were dissolved in HPLC-grade hexane.

Statistical analysis. All fractionation runs and analyses were carried out at least in duplicate and in randomized order with

the mean values being reported. ANOVA of the results was performed using the General Linear Model procedure of SAS (Software Version 8.1; SAS Institute Inc., Cary, NC). Multiple comparisons of the various means were carried out by LSD test at $P = 0.05$.

RESULTS AND DISCUSSION

The effect of pressure and temperature on the wheat germ extraction yield was examined in a previous study by using a small-scale SC-CO_2 extraction system (100 mL vessel and 35 g wheat germ) (1). The highest extract yield was obtained at the highest temperature and pressure examined in the study, 80°C and 55 MPa, respectively. The effect of solvent (CO_2)/feed (WGO) ratio (S/F) as a function of system pressure and temperature on extraction yield was investigated in the present study on the same system used for the first study (1). An increase in the S/F (w/w) beyond 40 did not have a significant effect on the extraction yield at 40°C and 55 MPa (Fig. 1). However, at 80°C an increase in S/F and pressure resulted in a significant increase in extract yield, indicating that for large-scale SC-CO_2 extraction of wheat germ at high pressure, temperature and S/F ratio would improve process efficiency. The use of a larger amount of CO_2 would not increase the processing cost significantly since CO_2 would be recycled in an industrial-scale supercritical fluid processing system.

Based on the results obtained by using the small-scale SC-CO_2 extraction system, wheat germ extraction was scaled up at 69 MPa and 80°C to determine the chemical composition of SC-CO_2-extracted WGO and to collect enough feed material to study SFF of SC-CO_2-extracted WGO. The amount of extract collected was about 11% (w/w), which was similar to conventional hexane extraction yield. However, SC-CO_2-extracted WGO was found to contain significantly higher moisture than the hexane-extracted oil, 4.4 ± 0.05 and 0.49 ± 0.01% (w/w), respectively. The apparent lower oil yield (amount of extract collected) from the large-scale SC-CO_2 extraction process is due to extract loss in the system during the collection process. In a previous study we reported that wheat germ extract yield was 20% when SC-CO_2 extraction was carried out at 80°C and 55 MPa, and that the extract yield was determined by the difference in the weight of the extraction vessel before and after extraction (1). It was not practical to determine the extract yield by the weight loss from wheat germ during the pilot-scale extraction process owing to the large size of extractors. Hence, the "amount of extract collected" rather than "extract yield" was reported for the pilot-scale WGO extraction. The co-extraction of moisture with oil from natural materials had been examined previously (5,6). Commercially refined soybean oil contained 0.05% moisture (determined in this laboratory). High moisture content in the SC-CO_2-extracted oil would not be a problem because, during industrial-scale SC-CO_2 extraction of vegetable oils, water would be easily separated in a high-pressure separator prior to precipitation of lipids from CO_2.

Total unsaturated FA and PUFA contents of the WGO were about 82 and 67%, respectively (Table 1). There was no significant difference in FA composition of SC-CO_2- and hexane-extracted WGO. Similar results were reported for other vegetable oils (18–20).

The FFA content of WGO is usually very high and quite variable (5–25% is typical), depending on conditions of germ separation, germ storage, and oil extraction. FFA often contribute to bitter and soapy flavors in food as well as to instability; hence, they need to be removed during the edible oil-refin-

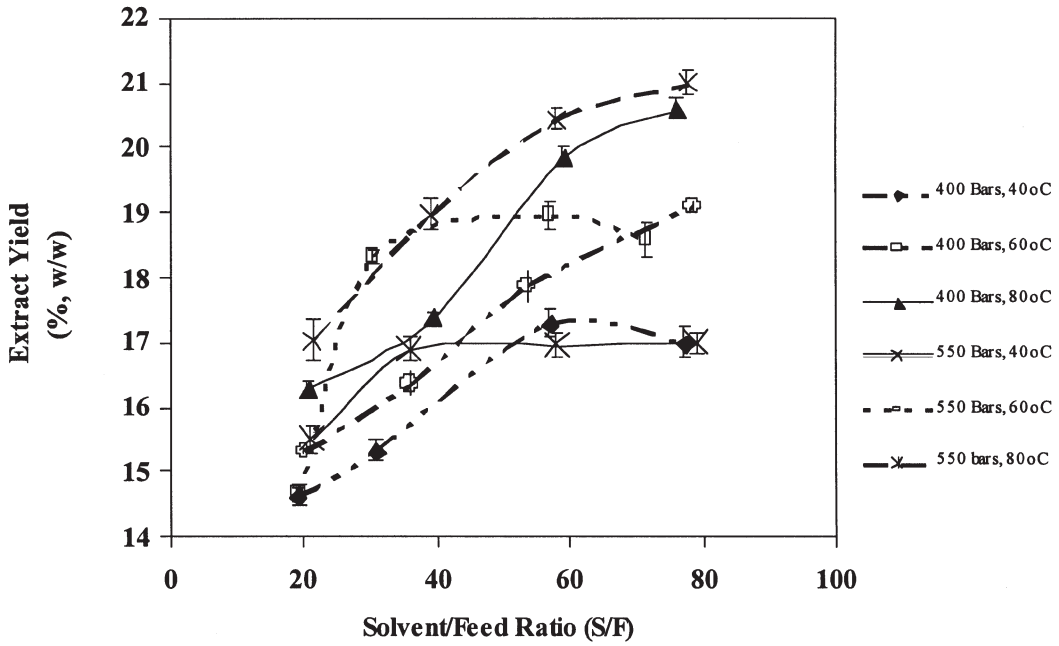


FIG. 1. Effect of solvent/feed ratio on extract yield as a function of temperature and pressure.

TABLE 1
FA Composition (%, w/w) of WGO Samples Extracted with Hexane and SC-CO_2[a]

FA	Crude WGO	SFE WGO
14:0	0.09 ± 0.001	0.09 ± 0.001
16:0	16.7 ± 0.04	16.8 ± 0.3
16:1	0.18 ± 0.001	0.15 ± 0.001
18:0	0.77 ± 0.01	0.5 ± 0.01
18:1	16.9 ± 0.01	13.6 ± 0.02
18:2	57.6 ± 0.06	59.7 ± 0.27
20:0	0.19 ± 0.001	0.11 ± 0.001
20:1	1.7 ± 0.01	1.45 ± 0.001
18:3	6.4 ± 0.01	7.3 ± 0.08
22:0	0.11 ± 0.001	0.78 ± 0.001
22:1	0.28 ± 0.02	0.23 ± 0.01
24:0	0.10 ± 0.001	0.06 ± 0.001

[a]Crude WGO, commercially hexane-extracted crude wheat germ oil (WGO); SFE WGO, pilot scale supercritical carbon dioxide (SC-CO_2)-extracted WGO (69 MPa and 80°C). Values are presented as mean ± SD.

TABLE 2
FFA Composition of WGO Processed with Various Methods[a]

Designation	Description	FFA[a] (%, w/w)
Crude WGO	[b]	7.9 ± 0.09
SFE WGO	[c]	6.2 ± 0.4
SFF 1	Crude WGO fractionated using SFF[d]	77.9 ± 0.6
SFF 2	Crude WGO fractionated using SFF[e]	78.0 ± 1.0
SFF 3	SFE WGO fractionated using SFF[f]	78.7 ± 0.2

[a]Values are presented as mean ± SD.
[b]Crude WGO, commercially hexane-extracted crude wheat germ oil (WGO).
[c]SFE WGO, pilot-scale supercritical carbon dioxide (SC-CO_2)-extracted WGO (69 MPa and 80°C).
[d]SFF 1, crude WGO fractionated using supercritical fractionation (SFF) technique [14 MPa, 80°C,and 25:1 solvent to feed (S/F) ratio (extract)].
[e]SFF 2, crude WGO fractionated using SFF technique [14 MPa, 80°C, and 50:1 S/F ratio (extract)].
[f]SFF 3, SFE WGO fractionated using SFF technique [14 MPa, 80°C, and 50:1 S/F ratio (extract)].

ing process. Although the FFA content of the hexane-extracted oil sample used in this study was higher than that of the SC-CO_2-extracted oil, the difference was not statistically significant (Table 2). In this study, both commercially hexane-extracted and SC-CO_2-extracted WGO were used for SFF experiments. The fractionation experiments were carried out at 80°C and 14 MPa because high temperature and low pressure improves FFA removal and minimizes TAG loss during the SFF process (8). The amounts of SFF extract (fraction collected from the top of the column) collected during the fractionation runs were as follows: 40 mg extract/1 h from crude WGO at 4 L/min CO_2 flow rate and 80 mg extract/1 h from crude WGO and 70 mg extract/1 h from SFE WGO at 8 L/min CO_2 flow rate. Increasing the CO_2 flow rate from 4 to 8 L/min doubled the amount of extract collected under the same fractionation conditions—14 MPa, 80°C, and 0.3 mL/min oil flow rate—indicating that SC-CO_2 was saturated under the experimental conditions. Although further increase in the S/F ratio above 50:1 would improve the FFA removal from crude oils, higher CO_2 flow rates were beyond our SFF system limits.

Chemical characterization of the SFF products was carried out on products that were collected at the fifth and sixth hours of the fractionation experiments after steady state had been achieved in the system. The extract fractions from the SFF process contained over 77% (w/w) FFA (Table 2). Increasing the solvent (CO_2)/feed (oil) ratio from 25:1 to 50:1 did not significantly improve the FFA removal efficiency. This might be due to higher selectivity of SC-CO_2 for other oil components under the chosen processing conditions. The FFA removal efficiency can be improved by lowering the system pressure, i.e., 10 MPa. However, lower system pressure results in reduced extraction yield. In this study our preference was to collect enough extract for chemical characterization of the product rather than to maximize FFA removal. Process optimization for SFF of WGO requires further research.

WGO is rich in α-tocopherol (Table 3). SC-CO_2-extracted oil contained a significantly higher total tocopherol content than hexane-extracted oil. Tocopherol compositions of extracts collected during SFF were very low (0.05 mg/g). These results indicate that tocopherols were retained with the TAG in the raffinate fraction. It appears that the S/F ratio did not have a significant effect on the extract tocopherol concentrations. Compositions of SFF extracts obtained from hexane- and SC-CO_2-extracted WGO were similar, containing a very low amount of α-tocopherol and no γ- and β-tocopherols.

Phytosterol FA ester content in the SFF extracts was lower than that of the feed oil, indicating that phytosterols were enriched in the raffinate fraction (Table 4). These results support data in the literature (7). Moisture analysis of the SFF raffinate fraction indicated that the SFF process removed a significant amount of moisture from SC-CO_2-extracted WGO. The moisture content of SC-CO_2- and hexane-extracted WGO decreased from 4.4 to 0.46% and from 0.49 to 0.11%, respectively, during the SFF process. Although we did not have enough oil samples

TABLE 3
Tocopherol Compositions (mg/g oil) of WGO Extracted and Refined Through Various Methods[a]

Sample	α-Tocopherol	β-Tocopherol	γ-Tocopherol
Crude WGO	13.9 ± 0.8	1.1 ± 0.02	0.08 ± 0.001
SFE WGO	25.6 ± 0.4	1.2 ± 0.1	0.06 ± 0.001
SFF 1	0.05 ± 0.001	ND	ND
SFF 2	0.05 ± 0.01	ND	ND
SFF 3	0.04 ± 0.01	ND	ND

[a]Values presented as mean ± SD. ND, not detected; see Table 2 for other abbreviations.

TABLE 4
Lipid and Phytosterol Compositions of Wheat Germ Oil Samples (HPLC area %) Before and After SFF Process[a]

Lipid/phytosterol	Crude WGO	SFE WGO	SFF 1	SFF 2	SFF 3
Phytosterol esters	8.4 ± 0.02	10.1 ± 0.4	3.7 ± 0.4	2.2 ± 0.04	3.2 ± 0.3
TAG	79.5 ± 0.2	81.5 ± 0.9	2.4 ± 0.3	2.7 ± 0.04	2.1 ± 0.9
FFA	11.4 ± 0.1	7.7 ± 0.5	92.7 ± 0.2	94.6 ± 0.04	93.9 ± 0.8
Free sterols	0.8 ± 0.03	0.7 ± 0.2	1.2 ± 0.1	0.5 ± 0.04	0.8 ± 0.2

[a]Values presented as mean ± SD. For abbreviations see Tables 1 and 2.

to analyze the moisture content in the SFF extracts, a mass balance on moisture clearly shows that a significant amount of water ends up in the SFF fractions or is carried out with the CO_2 stream.

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