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Bioactive Components of Commercial and Supercritical Carbon Dioxide Processed Wheat Germ Oil

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Abstract Wheat germ oil (WGO) is a specialty product with a very high nutritional value. The chemical composition of both commercial and pilot scale supercritical carbon dioxide (SC-CO_2) processed WGO was examined. This study showed that methods used for oil extraction and refining did not have a significant effect on the fatty acid composition of the oil. SC-CO_2 extracted oil had a higher tocopherol content than that of commercially hexane extracted oil. The phospholipid content of the SC-CO_2 extracted oil was very low indicating that the SC-CO_2 extraction method could eliminate the degumming step from edible oil refining processes. Although the conventional chemical oil refining technique reduced the tocopherol content of the WGO, it was possible to concentrate tocopherols in WGO by using physical refining methods such as molecular distillation.

Keywords Wheat germ oil · Processing · Supercritical carbon dioxide extraction · Supercritical fluid fractionation · Bioactive compounds

Introduction

Wheat germ is a unique source of highly concentrated nutrients. It offers three times as much protein of high biological value (26%), 7 times as much fat (11%), 15 times as much sugar (17%), and 6 times as much mineral content (4%) when compared with flour from the endosperm [1]. In addition, wheat germ is the richest known source of α-tocopherols (vitamin E) of plant origin and also a rich source of phytosterols, polycosanols (POC), thiamine, riboflavin, and niacin [1]. Wheat germ oil (WGO) has a number of nutritional and health benefits such as reducing plasma and liver cholesterol levels, improving physical endurance/fitness, and possibly helping to delay effects of aging [2]. These effects are attributed to the high concentration of bioactive compounds present in the oil. Hexane extracted WGO consists of about 56% linoleic acid (18:2 n6), which is an essential fatty acid [3]. Total unsaturated and polyunsaturated fatty acid (PUFA) content of WGO is about 81 and 64%, respectively. Very high PUFA content makes WGO processing very challenging. Highly unsaturated fatty acids are prone to oxidative damage. Data on WGO phospholipids (PLs) is limited. It has been reported that phosphatidylcholine (PC) represents about 40–60% of total PLs in dissected wheat germ. Phosphatidylethanolamine (PE) (9–15%) and phosphatidylinositol (PI) (13–20%) are also present in significant amounts [4].

Wheat germ oil is rich in unsaponifiable compounds, in particular phytosterols and tocopherols. WGO has been reported to improve human physical fitness, an effect attributed to its high POC, specifically its high octacosanol (OC) content [5, 6]. The POC contents and compositions of wheat grain fractions were studied by Irmak and Dunford [7, 8]. The PC content of wheat bran was higher than that of the germ, shorts and flour. Tetracosanol (C24), hexacosanol (C26) and OC (C28) were the major POC components in all the varieties. WGO contains a higher amount of phytosterols than do the other common commercial oils [9]. Sitosterol (60–70%) and campesterol

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(20–30%) are the two major phytosterols present in WGO [9, 10].

Crude vegetable oils need to be refined to produce high quality and highly stable oils through elimination of undesirable compounds. A significant portion of the nutritional oil components is lost during conventional refining processes [11]. Wang and Johnson [12] examined the effect of conventional oil refining processes on the WGO quality. According to this study tocopherol content of WGO did not change significantly during degumming, neutralization and bleaching processes. However, deodorization conditions reduced the tocopherol content of WGO significantly. An U.S. patent describes molecular distillation of WGO [13]. Vitamin E concentrates can be prepared from refined WGO by a molecular distillation process [13]. Supercritical fluid extraction (SFE) and Supercritical fluid fractionation (SFF) techniques have been examined as alternatives to conventional methods to retain and enhance bioactive components in the refined product [14–16]. SFE of WGO has been reported by several research groups [15, 17–19]. According to Taniguchi et al. [17] the α- and β-tocopherols content of supercritical carbon dioxide (SC-CO_2) extracted oil were similar to those of hexane-extracted oil. However, Gomez and Ossa [18] reported higher tocopherol content in the SC-CO_2 extracted WGO as compared to that of the hexane extracted oil.

Chemical characterization of the products obtained from conventional and supercritical fluid processing is essential to establish the relative merits of these techniques. The objectives of this research were as follows: (1) To chemically characterize WGO samples that have been commercially extracted and refined through conventional methods, (2) To characterize SFE WGO, (3) To compare the composition of WGO processed with supercritical fluid technology to that obtained by conventional techniques.

Materials and Methods

Oil Selection and Sample Preparation

Commercial WGO samples were obtained directly from a processor. Four commercial WGO samples consisted of hexane extracted crude (HE WGO), two batches of refined (CR WGO, PR WGO) and tocopherol concentrated WGO (TC WGO). The hexane extracted crude HE WGO was centrifuged at 14,000 rpm, 4 °C for 30 min and vacuum filtered through a #2 Whatman filter paper before the analytical tests. CR WGO was commercially hexane extracted and further processed by using conventional chemical refining processes (degumming, chemical neutralization, bleaching and deodorization). The oil sample PR WGO was also hexane extracted and had undergone physical refining (degumming, molecular distillation and bleaching). The last sample, TC WGO was concentrated in tocopherols by molecular distillation of PR WGO [13]. Samples were used as is with no pretreatment unless stated otherwise. A commodity oil, soybean oil, was analyzed for comparison to WGO. Soybean oil was purchased at a local grocery store. Wheat germ was obtained from milling of winter wheat (20% Kansas, 80% Oklahoma-grown winter wheat) and supplied by ADM Milling Co. (Enid, OK, USA). The SC-CO_2 extraction of wheat germ was carried out at the USDA-Agricultural Research Services, National Center for Agricultural Utilization Research in Peoria, IL, USA [20]. In summary, a total of 4.5 kg of wheat germ was extracted at 80 °C and 68 MPa using 16 kg CO_2 (measured at atmospheric conditions). The extract was collected in a receiver maintained at 60 °C and 11 MPa.

Analytical Methods

Moisture Analysis

Moisture content of oil samples was determined by utilizing a Karl Fischer Titrator (758 KFD Titrino, Metrohm, Brinkman Instruments, Inc. Westbury, NY, USA). 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 (Sigma-Aldrich Corporation, St. Louis, MO, USA).

Free Fatty Acid Determination

The free fatty acid (FFA) content of the oil samples were determined by utilizing a colorimetric method [21]. About 0.03–0.05 g oil samples were weighed into a 5-mL volumetric flask and brought to 5 mL volume with benzene (ACS grade, EMD Manufacturing, Savannah, GA, USA). Cupric acetate-pyridine solution was prepared by adjusting the pH of the filtered 5% (w/v) aqueous cupric acetate (99.9% purity, RJ Baker, Phillipsburg, NJ, USA) solution to pH 6.0–6.2 using pyridine (99% purity, Fisher Chemicals, Fairlawn, NJ, USA). Color development was initiated by addition of 1 mL cupric acetate-pyridine reagent into an oil–benzene mixture. After mixing and centrifuging, absorbance of the top layer was read at 715 nm using a UV/vis spectrophotometer (Beckman DU 520, Fullerton, CA, USA). FFA contents of the samples were determined from the oleic acid (90% purity, Aldrich, Milwaukee, WI, USA) standard curve. This method was validated against Official American Oil Chemists' Society free fatty acid analysis method, AOCS Official Method Aa-6 [22].

Fatty Acid Composition

Fatty acid compositions of the oil samples were determined by gas chromatography (GC). Methylation of the fatty acids was carried out according to the AOCS Official Method Ce 2-66 [22]. Oil samples (0.26 g) were hydrolyzed using sodium hydroxide prior to methylation as explained in the same method. Undecanoic acid (11:0) was added to the oil sample as an internal standard for quantification prior to hydrolysis. The GC unit was a HP 6890 Plus system equipped with a flame ionization detector (FID) (HP Company, Wilmington, DE, USA). A Supelco SP-2560 fused silica capillary column with 100 m × 0.25 mm × 0 0.2 μm film thickness (Supelco, Bellefonte, PA, USA) was used for fatty acid analysis. The helium carrier gas flow rate was 19 cm/s. The injector temperature was maintained at 250 °C. A temperature program with total run time of 45 min was used. The initial column temperature 140 °C was maintained for 5 min. Then oven temperature was increased to 240 °C at a 4 °C/min ramp rate and kept constant at this temperature for 15 min. The detector conditions were as follows: temperature 260 °C, H_2 flow 40 mL/min, air flow 400 mL/min and make-up gas (He) 30 mL/min. Oil samples (1 μL) were injected by an autosampler (HP 7683, HP Company, Wilmington, DE, USA). Peak areas were calculated and data collection was managed using an HP Chemstation (Revision. A.09.01, Agilent Technologies, Palo Alto, CA, USA). The split ratio was 150:1. Fatty acid peaks were identified using a standard 36 FAME mixture (Supelco 37 component FAME mix, Supelco, Bellefonte, PA, USA).

Tocopherols

Tocopherol content of the oil samples were analyzed by using an HPLC method [23). The oil samples were dissolved in hexane (0.025 mg oil/mL) and filtered through a 0.2 μm filter (Iso-Disc filter, Supelco, Bellefonte, PA, USA). A normal phase HPLC column, Zorbax RX-SIL (5 μm particle size, 4.6 × 250 mm, Agilent Technologies, Santa Clara, CA, USA) was used for separation of tocopherol isomers. Analytical separation of oil components on the column was achieved by using a mobile phase consisting of hexane:isopropyl alcohol (99:1 v/v) on isocratic mode. Total run time and flow rate were 15 min and 1.3 mL/min, respectively. The HPLC system (Alliance 2690 Waters Corp., Milford, MA, USA) consisted of a separations module (Model 2695), a Photodiode Array Detector (PDA) (Model 2996, Waters, Milford, MA, USA) and a Multi Wavelength Fluorescence Detector (FD) (Model 2475, Waters, Milford, MA, USA). The fluorescence detector was set at 290 nm excitation wavelength and 400 nm emission wavelength. The fluorescence detector gain was set for 1. The column temperature was 35 °C. The injection volumes of the both, individual standards and the oil sample were 2 μL. An external calibration curve was prepared for each tocopherol standard (α, β, γ and δ tocopherol standards, CN Biosciences Inc., La Jalla, CA, USA) to calculate the amount of tocopherols present in the oil sample.

Polycosanols and Phytosterols

Oil samples (0.5 g) were hydrolyzed by refluxing with 10 mL of 1.0 M NaOH in methanol for 45 min. The hydrolyzed solution was extracted with HPLC grade diethyl ether (Burdick & Jackson, Muskegon, MI, USA) three times using equal volumes of diethyl ether. The ethyl ether phase collected from three extractions was combined and washed with deionized water until neutrality. Heptadecanol (1 mL, 100 μg/mL in ether) was added to the ether extract as an internal standard and solvent was evaporated to dryness under nitrogen using a Reacti-Vap evaporation unit (Model 18780, Pierce, Rockford, IL, USA) after drying over anhydrous sodium sulfate (ACS grade, EMD Chemicals Inc., Gibbstown, NJ, USA). The residue was transferred to a 1-mL volumetric flask and 0.5 mL chloroform and 250 μL silylation reagent (MSTFA) were added. The solution was heated at 60 °C for 15 min for derivatization. Chloroform was added to reach a total sample volume of 1 mL before analysis.

Trimethylsilyl derivatives of POCs and phytosterols were analyzed using a HP 6890 Series Gas Chromatography (GC) system coupled with a 5973 Network Mass Selective Detector (Agilent Technologies, Palo Alto, USA). A fused silica capillary Equity-5 (30 m × 0.25 mm × 0.5 μm film thickness) from Supelco (Bellefonte, USA) was used for the analysis. The oven temperature was programmed from 150 to 320 °C with a 4 °C/min heating rate and maintained at 320 °C for 15 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The inlet temperature was 300 °C. Mass Spectrophotometer (MS) parameters were as follows: MS transfer line 280 °C, ion source 230 °C and MS quadruple temperature 150 °C. The ionization energy was 70 eV. The scan range and rate were 100–600 AMU and 2 scans/s, respectively. One microliter of sample was injected into the GC–MS by an autosampler (HP 7683, HP Company, Wilmington, DE, USA). The split ratio was 1:10. The data collection and analysis were managed using HP Chemstation (Enhanced Chemstation G1701 DA Version D.00.00.38, Agilent Technologies, Palo Alto, CA, USA). The POC and phytosterol compositions of the samples were identified by direct comparison of their chromatographic retention times

and the mass spectra with those of the authentic compounds using external calibration curves. The peaks were also confirmed with NIST/EPA/NIH Mass Spectral Library (Version 2.0).

Phospholipids

The oil samples were dissolved in chloroform (15 mg oil/mL) and filtered through 0.2-μm Iso Disc filters (Supelco, Bellefonte, PA, USA) for further analysis. A normal phase silica column, μPorasil 10 μm (3.9 mm i.d × 300 mm) from Waters (Milford, MA, USA) was used for the analytical separation of the compounds. The mobile phase consisted of two mixtures: A: hexane:water:isopropyl alcohol (40:58:2) and B: hexane: water: isopropyl alcohol (40:50:10). The solvent gradient system was as follows: 100% A to 100% B in 7 min, then held for 6 min followed by returning to 100% A in 1 min and held for 11 min. Total run time was 25 min. The detector system consisted of a photodiode array detector (PDA) (Model 2996, Waters Milford, MA, USA) in series with an evaporative light scattering detector (ELSD) (Model 2000, All tech associates Inc., Deerfield, IL, USA). The ELSD set points were as follows: nitrogen flow rate 3.5 mL/min, impactor ON and drift tube temperature of 80 °C. Identification and quantification of chromatographic peaks were based on external standard curves prepared for individual standards. Phospholipid standards L-α-Phosphatidylcholine (PC) from soybean, 3-sn-Phosphatidic acid (PA) sodium salt from egg yolk lecithin, Phosphatidylserine (PS) from bovine, and L-α-Phosphatidylethanolamine (PE) were purchased from Sigma Inc. (St. Louis, MO, USA). Plant based Phosphatidylinositol (PI) was purchased from Matreya (State College, PA, USA). Standards were dissolved in chloroform. All solvents utilized for HPLC mobile phase were HPLC grade and filtered using a GH Polypro (47 mm, 0.45 μm) hydrophilic polypropylene membrane filter (Pall Life Sciences, Ann Arbor, MI, USA) before use.

Statistical Analysis

All fractionation runs and analyses were carried out in duplicate and in randomized order with the mean values being reported. Analysis of variance (ANOVA) of the results was performed using the General Linear Model procedure of SAS (Software Version 8.1. SAS Institute Inc., Cary, NC, USA). Multiple comparisons of the various means was carried out by least significant difference (LSD) test at $P = 0.05$.

Results and Discussion

The fatty acid composition of WGO reported in this study is in agreement with data published in the literature [24–26]. Linoleic acid (18:2) consisted of 57–58% of the total fatty acids in WGO (Table 1). Although SFE WGO had significantly higher linoleic acid content (59.7%) ($P < 0.05$) than the commercial oils, the variations were not large. Soybean oil had significantly lower linoleic acid content (54%) than WGO. Palmitic (16:0), oleic (18:1) and linolenic acids (18:3) were also present in significant amounts in all the oils. Saturated fatty acid content of SFE WGO was lower (about 16%, w/w) than the other oils (>17%). However, the difference was not large enough to affect the oil quality for practical applications. Similar results were reported in the literature for other vegetable oils [27, 28]. Mono and polyunsaturated oil contents of SFE WGO were about 23 and 61%, w/w, respectively. Utilization of different refining techniques did not have a significant effect on the fatty acid composition of WGO.

Free fatty acid levels of WGO samples are given in Table 2. HE WGO and SFE WGO contained substantial amount of FFAs, 7.9 and 6.2%, respectively. FFAs contribute to bitter and soapy flavor in foods; hence they are undesirable in edible oils. FFAs are removed during oil refining. Both physical and chemical refining reduced FFAs content of the oil to 0.4%. Refined soybean oil had significantly lower FFA (0.03%) than that of WGO. This result was expected for two reasons. First, crude soybean oil contains only 1–2% FFA as compared to >6% FFA in

Table 1 Fatty acid composition (%, w/w) of WGO samples extracted and refined through various methods as compared to soybean oil

Fatty acid[a]	HE WGO	CR WGO	PR WGO	TC WGO	SFE WGO	Soybean
14:0	0.09b	0.09b	0.09b	0.23a	0.09b	0.08b
16:0	16.7a	15.8b	16.8a	16.9a	16.8a	10.7c
16:1	0.18a	0.17a	0.16b	0.16b	0.15b	0.09c
18:0	0.77b	0.72b	0.72b	0.68b	0.5c	4.56a
18:1	16.9b	15.8c	15.9c	15.2c	13.6d	22.1a
18:2	57.6c	58.4b	57.7c	58.1b	59.7a	54.0d
18:3	6.4b	6.7b	6.5b	6.9c	7.3a	7.2a
20:0	0.19b	0.17b	0.16bc	0.15c	0.11d	0.36a
20:1	1.7a	1.6a	1.6a	1.52ab	1.45c	0.46c
22:0	0.11c	0.11c	0.11c	0.11b	0.78a	0.36b
22:1	0.28a	0.27a	0.26ab	0.26ab	0.23c	0d
24:0	0.10b	0.10b	0.10b	0.09b	0.06c	0.12a

Means in the same row with the same letter are not significantly different at $P > 0.05$

[a] The first number before the semicolon refers to the number of carbon atoms in the fatty acid chain and the second number indicates the number of double bonds on the carbon chain

Table 2 Free fatty acid composition (FFA) of WGO extracted and refined through various methods

Sample	FFA (%, w/w)
HE WGO	7.9a
CR WGO	0.4c
PR WGO	1.1b
TC WGO	0.4c
SFE WGO	6.2a
Soybean	0.03d

Means in the same column with the same letter are not significantly different at $P > 0.05$

WGO. Second, gentler refining is usually preferred for WGO to retain bioactive oil components in the final product. In a previous study it was shown that SC-CO_2 was able to remove FFAs from crude oil when a fractionation column was used for processing [20, 29].

CR WGO (5.7 mg/g oil) and PR WGO C (8 mg/g oil) contained significantly lower tocopherols then the HE WGO (15.1 mg/g oil) indicating that conventional refining techniques cause substantial tocopherol loss (Table 3). As expected, TC WGO had the highest tocopherol levels (117.2 mg/g oil) since it was produced with a propriety process to obtain high tocopherol content in the final product. SFE WGO contained a significantly higher amount of tocopherols than those of the commercial WGO samples. All the WGO samples examined in this research contained significantly higher amounts of tocopherol than that of the refined soybean oil. A previous study reported that SFF extracts contained very low amounts of tocopherols (0.05–0.04 mg/g oil) indicating that tocopherols were retained with the triglycerides in the raffinate fraction [20]. The majority of the tocopherols in WGO were in the form of α-tocopherol (>90% of the total tocopherols). β-Tocopherol was the second most abundant tocopherol in the WGO samples. γ-Tocopherol was the main isomer in soybean oil. Similar tocopherol contents and compositions were reported in the literature for WGO and soybean oil [25].

HE WGO and SFE WGO contained similar amounts of total phytosterols (about 3.7 mg/g oil) (Table 4). Although refined WGO samples contained slightly lower phytosterol content than those of the crude oil, differences were not statistically significant ($P > 0.05$). Tocopherol enriched WGO (TC WGO) and soybean oil had significantly higher and lower total phytosterol content, respectively, than both crude and refined WGO oils. β-Sitosterol was the most prominent (78–85% of the total phytosterols) phytosterol with campesterol being the second and stigmasterol being the least prevalent in all WGO samples. TC WGO showed the highest levels of β-sitosterol (6.63 mg/g oil). SFE WGO also had significantly higher β-sitosterol amount (about 2.94 mg/g oil) than the other WGO samples except TC WGO. High phytosterol contents of TC WGO indicate that the process used for tocopherol concentration also results in phytosterol enrichment in the final product. Campesterol (0.61 mg/g oil) concentration of SFE WGO was similar to that of hexane extracted crude HE WGO (0.673 mg/g oil). However, SFE WGO did display the lowest level of stigmasterol of all the WGO samples. All WGO samples tested in this study showed greater levels of phytosterols than soybean oil. Eisenmenger et al. [20] reported that phytosterol contents (both free and fatty acid esters of phytosterols) of SFF extracts were lower (2–3%) than that of the feed material (8–10%) indicating that phytosterols were retained with triglycerides during SFF of WGO.

Hexane extracted crude WGO (HE WGO) contained the highest amount of phospholipids among the samples tested in this study (Table 5). However, total phospholipids content of crude WGO was lower than the literature values, about 45–50 mg/g oil [12]. This is due to the fact that HE WGO was stored in a cold room until the chemical tests. Significant amount of precipitate were formed during the cold storage. HE WGO was centrifuged and filtered prior to

Table 3 Tocopherol compositions (mg/g oil) of WGO extracted and refined through various methods

Sample	α-Tocopherol	β-Tocopherol	γ-Tocopherol
HE WGO	13.9c	1.1b	0.08cd
CR WGO	6.9d	0.7c	0.1c
PR WGO	7.3d	0.6c	0.07dc
TC WGO	109.7a	6.8a	0.7a
SFE WGO	25.6b	1.2b	0.06d
Soybean	ND	ND	0.2b

Mean in the same column with the same letter are not significantly different at $P > 0.05$

ND Not detected

Table 4 Phytosterol compositions (mg/g oil) of WGO extracted and refined through various methods

Samples	Campesterol	Stigmasterol	β-Sitosterol	Total phytosterol
HE WGO	0.67b	0.25a	2.77b	3.70b
CR WGO	0.63b	0.21b	2.59b	3.05b
PR WGO	0.52c	0.21b	2.70b	3.44b
TC WGO	1.74a	ND	6.27a	8.01a
SFE WGO	0.61bc	0.20b	2.94b	3.75b
Soybean	0.034d	0.28a	0.29c	0.60c

Means in the same column with the same letter are not significantly different at $P > 0.05$

ND Not detected

Table 5 Phospholipid compositions (mg/g oil) of WGO extracted and refined through various methods

Samples	PE	PI + PA	PS	PC
HE WGO	3.5a	12.1a	3.3	0.9
CR WGO	1.9c	0.6b	ND	ND
PR WGO	2.1b	ND	ND	ND
TC WGO	ND	ND	ND	ND
SFE WGO	ND	ND	ND	ND
Soybean	ND	ND	ND	ND

Means in the same column with the same letter are not significantly different at $P > 0.05$

PE phosphatidylethanolamine, *PI + PA* phosphatidylinositol and phosphatic acid, *PS* phosphatidylserine, *PC* phosphatidylcholine *ND* Not detected

Table 6 Water content of WGO extracted and refined through various methods

Sample	Water content (%, w/w)
HE WGO	0.49b
CR WGO	0.06c
PR WGO	0.07c
TC WGO	0.04c
SFE WGO	4.4a
Soybean	0.05c

Means in the same column with the same letter are not significantly different at $P > 0.05$

chemical characterization. Hence a significant portion of the phospholipids and wax components were removed with the precipitate. SFE WGO did not contain any detectable amount of phospholipids. Similar results have been reported in the literature for oils extracted with SC-CO_2 [14]. This is due to the low solubility of phospholipids in SC-CO_2. Phospholipid contents of refined oils were either very low or below the detection levels because these compounds are removed from the crude oil during the degumming step of the refining process. Extraction of vegetable oils by SFE eliminates the need for a degumming step. Total phosphatidylinositol (PI) + phosphatic acid (PA) contents of oil samples were given in this study because the HPLC method used for the analysis of phospholipids did not separate these two components on the analytical column. Commercial WGO contained high amounts of PI + PA (>60% of total phospholipids). It has been reported in the literature that high PA content in crude vegetable oils may be an indication of poor seed handling and extraction conditions [12]. PA is a nonhydratable phospholipid and separation of this compound by water degumming is very difficult during the refining process. Although crude WGO contained PC (about 20% of total phospholipids) refined WGO samples did not have detectable amount of PC indicating that refining process was very effective at removing this compound.

Supercritical carbon dioxide may extract significant amounts of water depending on the extraction conditions and the moisture content of the feed material [30, 31]. The main concerns regarding the moisture content in the oil are the following: a high moisture content in oil promotes microbial growth, hydrolysis during high temperature applications, phase separation and cloudiness in the oil. SFE WGO had significantly higher moisture content than those of the commercial WGO including hexane extracted crude WGO (Table 6). These results are in agreement with the literature data. During the industrial-scale SFE extraction of vegetable oils water would be separated in a high-pressure separator prior to precipitation of lipids from the supercritical fluid.

In a previous study we reported POC content and composition of wheat germ extracts [7]. There was no detectable amount of POC in WGO samples examined in this study. This was due to refining and cold storage of the oil samples. POCs in crude oil precipitate out during cold storage and the precipitate was filtered out before analytical tests. This study indicated that although conventional edible oil refining causes loss of some bioactive compounds naturally present in WGO, it is possible to preserve and enrich WGO in bioactive compounds by choosing the right extraction and refining methods.

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