

## Quality of Wheat Germ Oil Obtained by Cold Pressing and Supercritical Carbon Dioxide Extraction

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### Abstract

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Laboratory-prepared wheat germ oil was obtained by cold pressing and supercritical CO<sub>2</sub> extraction. The main objective was to compare the quality of both oil samples obtained, with emphasis on their fatty acids compositions and tocopherol contents. The percentages of palmitic, oleic, linoleic, and linolenic acids determined in the cold-pressed oil were 15.89, 15.48, 54.88, and 7.34% of total fatty acids, respectively, and those in the oil extracted by supercritical CO<sub>2</sub> were 16.50, 15.05, 54.79, and 7.29% of total fatty acids, respectively. The average proportions of saturated, mono- and polyunsaturated fatty acids calculated for wheat germ oil obtained by cold pressing accounted for 17.15, 17.63, and 62.22% of total fatty acids, respectively, and those calculated for wheat germ oil extracted by supercritical CO<sub>2</sub> were very similar, accounting for 18.14, 17.58, and 62.08% of total fatty acids, respectively. As expected, the fatty acid profiles determined in both oils studied were observed to be almost identical. In contrast, the level of  $\alpha$ -tocopherol in the oil extracted by supercritical CO<sub>2</sub> was found to be considerably higher (1.27 mg/g) than that in the oil obtained by the cold pressing procedure (0.79 mg/g).

**Keywords:** fatty acid profile;  $\alpha$ -tocopherol

Oils are generally obtained from the oil-bearing seeds or fruits where they occur in great abundance. Three different methods are commonly used for the production of oils: pressing, solvent extraction, and a combination of pre-pressing and solvent extraction. The efficiency of these methods can be improved with the assistance of enzymes or supercritical carbon dioxide. The oils are typically used in snack foods and as ingredients in a variety of processed foods, especially in bakery and confectionery products (GOMEZ & DE LA

OSSA 2000). Wheat is an excellent source of polyunsaturated fatty acids and vitamin E. The wheat germ constitutes only about 2% of the whole wheat grain and contains about 8–14% oil (ZACCHI *et al.* 2006). Wheat germ oil has been used as a fertility agent, an antioxidant, and an additive in natural food and health and cosmetic products (WANG & JOHNSON 2001). Wheat germ oil has the highest tocopherol content of all vegetable oils, up to about 2500 mg/kg (SHULER 1990), and also the highest content of  $\alpha$ -tocopherol, which represents around

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60% of the total content. Also, wheat germ oil is highly valued due to its high content of unsaturated fatty acids of which it contains about 80%, mostly linoleic (18:2) and linolenic (18:3) (WANG & JOHNSON 2001). These two fatty acids are of great importance in human metabolism and can not be synthesised by the organism. They are precursors of a group of hormones called prostaglandins, which play an important role in muscle contractions and in the proper healing of inflammatory processes (COULTAE 1989). In recent years, supercritical fluid extraction (SFE) has received increased attention as an important alternative to the conventional separation methods. Its critical temperature and pressure of supercritical CO<sub>2</sub> extraction system are not high (DE REUCK *et al.* 1983). It has been demonstrated that SFE can produce superior quality products characterised by the absence of artifacts (MARZOUKI *et al.* 2008). Vegetable oils are traditionally produced by hexane extraction from ground seeds. The process is very efficient, but hexane elimination after the extraction is a major problem. Consequently, several authors have proposed the substitution of the traditional process by supercritical CO<sub>2</sub> extraction of oil from seeds (MARZOUKI *et al.* 2008). The aim of this study was to determine the fatty acid compositions, tocopherol contents, and SEA, MUFA, and PUFA of cold pressed and supercritical CO<sub>2</sub> extracted wheat germ oils.

## MATERIAL AND METHODS

**Material.** Wheat germ was provided from a flour company located in Konya, Turkey. The material was ground in a mill and the particle sizes ranged from 0.5 to 0.7 mesh.

**Reagents.** Triolein, trilinolein, fatty acids, fatty acid methyl esters,  $\alpha$ -tocopherol, and desferal (desferoxamine mesylate salt) were purchased from Sigma-Aldrich (Milan, Italy). All solvents used, of the highest available purity, were also purchased from Sigma-Aldrich (Milan, Italy). Methanolic HCl (3N) was purchased from Supelco (Bellefonte, USA), *cis*, *trans*-13-hydroperoxyoctadecadienoic acid (*c,t*-13-HPODE) and *cis*, *trans*-9-hydroperoxyoctadecadienoic acid (*c,t*-9-HPODE) were obtained from Cascade (Cascade Biochem. Ltd., London, UK). All the other chemicals used in this study were of analytical grade.

**Cold pressing.** Cold pressing was performed in a laboratory prototype apparatus in series in Zade

Oil Company (Konya, Turkey). The extraction was carried out in a continuous system. The extraction of wheat germ oil was carried out working at 1.1–4 kW.

**Supercritical CO<sub>2</sub> extraction (SFE).** Supercritical CO<sub>2</sub> (purity 99%; Air Liquide Italia, Cagliari, Italy) extractions were performed in a laboratory apparatus (PIRASA *et al.* 2009), equipped with a 400 cm<sup>3</sup> extraction vessel and two separator vessels of 300 and 200 cm<sup>3</sup>, respectively, connected in series. The extraction was carried out in a semi batch mode: batch charging of vegetable matter and continuous flow solvent. The extraction of wheat germ oil was carried out working at 40°C and 250 bar in the extraction vessel and using only one separator (at 20 bar and 15°C) to recover the extract.

**Fatty acid and  $\alpha$ -tocopherol analysis.** The separation of fatty acids and  $\alpha$ -tocopherol was obtained by mild saponification (ROSA *et al.* 2011) as follows: 3 mg of each wheat germ oil were dissolved in 5 ml of EtOH and 100  $\mu$ l of Desferal solution (25 mg/ml of H<sub>2</sub>O), 1 ml of a water solution of ascorbic acid (25% w/v), and 0.5 ml of 10N KOH were added. The mixture was left in the dark at room temperature for 14 hours. After the addition of 10 ml of *n*-hexane and 7 ml of H<sub>2</sub>O, the sample was centrifuged at 900 g for 1 hour. The hexane phase (unsaponifiable fraction) with  $\alpha$ -tocopherol was collected, the solvent was evaporated in a rotary evaporator (Rotavapor R-114; Büchi Labortechnik, Flawil, Switzerland) under reduced pressure, the residue was dissolved in MeOH and aliquots of the sample were injected into the HPLC system. After the addition of further *n*-hexane to the mixture, the sample was acidified with 37% HCl to pH 3–4 and then centrifuged at 900 g for 1 hour. The hexane phase (saponifiable fraction) with free fatty acids and conjugated diene fatty acids hydroperoxides (HP) was collected and the solvent was evaporated. A portion of the dried residue was dissolved in CH<sub>3</sub>CN with 0.14% CH<sub>3</sub>COOH (v/v) and aliquots of the sample were injected into the HPLC system.

An aliquot of dried fatty acids was methylated with 1 ml of methanolic HCl (3N) (CHRISTIE 1993) for 30 min at room temperature. After the addition of 4 ml of *n*-hexane and 2 ml of H<sub>2</sub>O, the sample was centrifuged for 20 min at 900 g. The hexane phase with fatty acid methyl esters was collected, the solvent was evaporated, the residue was dissolved in *n*-hexane, and aliquots of the sample were injected into the GC system. The recovery of fatty acids during saponification was calculated

by using an external standard mixture prepared by dissolving 1 mg of triolein and trilinolein in EtOH and processing as the samples. All solvents evaporation was performed under vacuum.

**HPLC analysis.** The analyses of unsaturated fatty acids,  $\alpha$ -tocopherol, and HP were carried out with an Agilent Technologies 1100 liquid chromatograph (Agilent Technologies, Palo Alto, USA) equipped with a diode array detector (DAD).  $\alpha$ -tocopherol, detected at 292 nm, was measured with the use of a Chrompack column (Chrompack, Middelburg, the Netherlands), Inertsil 5 ODS-3, 150 mm  $\times$  3 mm, and MeOH as mobile phase, at a flow rate of 0.4 ml/minute. The analyses of unsaturated fatty acids and HP, detected at 200 nm and 234 nm, respectively, were carried out with a XDB-C18 Eclipse (150 mm  $\times$  4.6 mm, 3.5  $\mu$ m particle size) (Agilent Technologies Palo Alto, USA) equipped with a Zorbax XDB-C18 Eclipse (12.5 mm  $\times$  4.6 mm, 5  $\mu$ m particle size) guard column (Agilent Technologies, Palo Alto, USA), with a mobile phase of CH<sub>3</sub>CN/H<sub>2</sub>O/CH<sub>3</sub>COOH (75/25/0.12, v/v/v), at a flow rate of 2.3 ml/min (ROSA *et al.* 2011). The temperature of the column was maintained at 37°C. The identification of fatty acids and HP was made using standard compounds and the second derivative, as well as conventional UV spectra, generated with the Agilent Chemstation A.10.02 software (Agilent Technologies, Palo Alto, USA). The calibration curves of all of the compounds were constructed using standards and were found to be linear with correlation coefficients > 0.995.

**GC analysis.** Fatty acid methyl esters were determined on a gas chromatograph Hewlett-Packard HP-6890 with a flame ionisation detector (FID) and equipped with a cyanopropyl methyl-polysiloxane HP-23 FAME column (30 m  $\times$  0.32 mm  $\times$  0.25  $\mu$ m) (Hewlett-Packard, Palo Alto, USA). Nitrogen was used as carrier gas at a flow rate of 2 ml/minute. The oven temperature was set at 175°C; the injector temperature was set at 250°C; and the detector temperature was set at 300°C. The fatty acid methyl esters were identified by comparing the retention times to those of the standard compounds. The composition of individual fatty acids was calculated as a percentage of the total amount of fatty acids, using the Hewlett-Packard A.05.02 software. (Hewlett-Packard, Palo Alto, USA)

**Statistical analyses.** The results of the research were analysed for statistical significance by analysis of variance (PUSKULCU & İKİZ 1989).

## RESULTS AND DISCUSSION

The composition of fatty acids determined in wheat germ oils obtained by cold pressing and supercritical CO<sub>2</sub> extraction are shown in Table 1. The analysed oil exhibited a concentration of approximately 18.14% saturated, 17.58% monounsaturated, and 62.08% polyunsaturated fatty acids in wheat oil obtained by CO<sub>2</sub> extraction. In addition, with this system, wheat germ oil contained mainly 16.50% palmitic, 15.05% oleic, 54.79% linoleic, and 7.29% linolenic acids. The most represented fatty acids of cold pressed wheat germ oil were palmitic (15.89%), oleic (15.48%), linoleic (54.88%), and linolenic (7.34%) acids. Saturated, monounsaturated, and polyunsaturated fatty acids contents of cold pressed wheat oil were found to be 17.15%, 17.63%, and 62.22%, respectively. Furthermore, the unsaturated fatty acids contents in the cold pressing and supercritical CO<sub>2</sub> extracted oils were detected by HPLC (Table 2) as follows: 142.08  $\mu$ g/mg of oleic, 390.26  $\mu$ g/mg of linoleic, 71.23  $\mu$ g/mg of linolenic, 111.01  $\mu$ g/mg of oleic, 339.74  $\mu$ g/mg of linoleic, and 56.50  $\mu$ g/mg of linolenic acids, respectively. The  $\alpha$ -tocopherol levels in both oils (the cold pressed and supercritical CO<sub>2</sub> extracted oils) were

Table 1. Fatty acid composition (% of total fatty acids) determined by GC in wheat germ oil obtained by cold pressing and supercritical CO<sub>2</sub> extraction

Fatty acids	Cold pressed oil	Supercritical CO <sub>2</sub> oil
12:0	0.08 $\pm$ 0.03	0.02 $\pm$ 0.00
14:0	0.25 $\pm$ 0.15	0.15 $\pm$ 0.03
16:0	15.89 $\pm$ 0.55	16.50 $\pm$ 0.42
16:1 (n-7)	0.16 $\pm$ 0.01	0.20 $\pm$ 0.03
18:0	0.75 $\pm$ 0.03	0.79 $\pm$ 0.03
18:1 (n-7)	0.74 $\pm$ 0.05	0.81 $\pm$ 0.10
18:1 (n-9)	15.48 $\pm$ 0.36	15.05 $\pm$ 0.19
18:2 (n-6)	54.88 $\pm$ 2.27	54.79 $\pm$ 0.72
18:3 (n-3)	7.34 $\pm$ 0.29	7.29 $\pm$ 0.05
20:0	0.18 $\pm$ 0.04	0.68 $\pm$ 0.04
20:1 (n-9)	1.34 $\pm$ 0.09	1.52 $\pm$ 0.19
SFA	17.15 $\pm$ 0.29	18.14 $\pm$ 0.39
MUFA	17.63 $\pm$ 0.51	17.58 $\pm$ 0.13
PUFA	62.22 $\pm$ 2.57	62.08 $\pm$ 0.76

Mean and standard deviation of two samples ( $n = 2$ ); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

Table 2. Composition of unsaturated fatty acids ( $\mu\text{g}/\text{mg}$ ) determined by HPLC in wheat germ oil obtained by cold pressing and supercritical  $\text{CO}_2$  extraction

Fatty acids	Cold pressed oil	Supercritical $\text{CO}_2$ oil
16:1 (n-7)	$0.88 \pm 0.06^a$	$0.80 \pm 0.28$
18:1 (n-9)	$142.08 \pm 3.63$	$111.01 \pm 1.84$
18:2 (n-6)	$390.26 \pm 5.97$	$339.74 \pm 5.95$
18:3 (n-3)	$71.23 \pm 1.55$	$56.50 \pm 2.02$
18:3 (n-6)	$0.27 \pm 0.00$	nd

Mean and standard deviation of two samples ( $n = 2$ ); nd – non determined

measured as the mean content of 0.79 mg/g oil and 1.27 mg/g oil, respectively (Table 3). GOMEZ and DE LA OSSA (2000) determined 166.0–319.2 mg/g  $\alpha$ -tocopherol and 66.6–121.0 mg/g  $\beta$ -tocopherol in wheat germ oil. WANG and JOHNSON (2001) established 1817 mg/kg  $\alpha$ -tocopherol and 864 mg/kg  $\beta$ -tocopherol in crude wheat germ oil. MAHMOUD *et al.* (2009) reported that wheat germ oil contained 70.0%  $\alpha$ -tocopherol, 19.0%  $\beta$ -tocopherol, 7.0%  $\gamma$ -tocopherol, 2.0%  $\alpha$ -tocotrienol, and 2.0%  $\gamma$ -tocotrienol. Conjugated diene fatty acid hydroperoxide (HP) levels measured by HPLC in wheat germ oil by cold pressing and supercritical  $\text{CO}_2$  extraction were found to be 10.35  $\mu\text{moles}/\text{g}$  oil and 21.34  $\mu\text{mol}/\text{g}$ , respectively (Table 3). Wheat germ represents approximately 3% of the grain and it contains 8–14% oil, which is a rich source of tocopherols (vitamin E) and polyunsaturated fatty acids, mainly linoleic acid. The present work shows the influence of temperature (27 and 45°C) and storage time (maximum 35 days) of the wheat germ on the concentration of tocopherol in the oil (CAPITANI *et al.* 2011). A Box-Behnken design combined with response surface methodology (RSM) was used to optimise the parameters of supercritical  $\text{CO}_2$  extraction (SFE) of wheat germ oil. The quality of the oil and residual meal obtained by

Table 3.  $\alpha$ -Tocopherol and conjugated diene fatty acid hydroperoxide (HP) levels measured by HPLC in wheat germ oil by cold press and supercritical  $\text{CO}_2$  extractions (mg/g oil)

Oils	$\alpha$ -Tocopherol	HP
Cold pressed oil	$0.79 \pm 0.03$	$10.35 \pm 0.42$
Supercritical $\text{CO}_2$ oil	$1.27 \pm 0.08$	$21.34 \pm 0.74$

Mean and standard deviation of two samples ( $n = 2$ )

SFE and solvent extraction (SE) were evaluated from proximate analysis, fatty acid composition, and antioxidant activity. A maximum oil yield of 10.46% was achieved under the optimal conditions of wheat germ particle size 60–80 mesh; water content 4.37%; pressure 30 MPa; temperature 40°C; extraction time 1.7 hour. The oil obtained by SFE showed stronger DPPH radical scavenging ability than SE oil at the same concentration. The fatty acid composition of SFE oil was similar to that of SE oil. In this paper, the working conditions for the extraction of wheat germ oil in a supercritical  $\text{CO}_2$  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,  $\text{CO}_2$  flow rate 1.5 l/minute. 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 colour of the by product). Moreover, the oil quality was evaluated in relation to the progress of the supercritical extraction (PANFILI *et al.* 2003). The higher contents of tocopherol and total phenolic compounds in the wheat germ oil, as well as the higher content of unsaturated fatty acids, could account for the stronger antioxidant activity of the wheat germ oil extracted by SFE (EISENMENGER & DUNFORD 2008). The wheat germ oil was rich in polyunsaturated fatty acids, especially in linoleic acid. As can be observed, the fatty acid contents of the wheat germ oil obtained from SFE were similar to those from SE. Similar results were also reported in these studies (EISENMENGER *et al.* 2006; SHAO *et al.* 2008; PIRASA *et al.* 2009). This is because the proper amount of water can facilitate the penetration and diffusion of supercritical  $\text{CO}_2$  into the tissues of wheat germ and promote oil extraction. A similar phenomenon was found by GE *et al.* (2002).

## CONCLUSION

The overall results indicated that the extraction of wheat germ oil can be successfully performed by using supercritical  $\text{CO}_2$ . The composition of oil obtained by this technique is largely influenced by solvent density. Also, oil composition is comparable with the composition of the products obtained by conventional processes of extraction (MARZOUKI

*et al.* 2008). By increasing the extraction pressure, it is possible to increase the yield of the process. The qualitative and quantitative analysis differences between our results and literature may depend on ambient and climatic conditions and different vegetative stages (MARONGIU *et al.* 2005). The differences in the fatty acid and tocopherol contents could depend on the geographical location, harvesting period, environmental conditions, and analytical conditions. In conclusion, the present study indicates that wheat germ oil is a good natural source of both essential fatty acid and  $\alpha$ -tocopherol.

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