Comparative of Physico-Chemical Properties of Wheat Germ Oil Extracted with Cold Press and Supercritical CO_2 Extraction

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ABSTRACT: *In this study, moisture content, free fatty acids, peroxide, iodine value, unsapaonifiable matter, saponification value, fatty acid composition and tocopherol contents of wheat germ oil obtained by SC-CO_2 extraction and cold press technology were investigated. Moisture, free fatty acid (FFA), peroxide value, iodine value, unsaponifiable matter and saponification value of cold press and supercritical CO_2 extraction method oils were established as 0.097% and 13.32, 0.84% and 5.9%, 8.9 meq O_2/kg and 15.8, 132 and 128, 6.5 g/kg and 8.04 and 197 and 182mg KOH/g, respectively. Major fatty acids of samples were determined as palmitic, oleic and linoleic acids. Campesterol and β-stosterols of wheat germ oils obtained by cold press and supercritical CO_2 extraction are the major sterols. The germ oil extracted by both methods contained 24.19% and 23.44 % campesterol and 60.98% and 61.56% β-stosterol, respectively. While germ oil obtained supercritical CO_2 extraction contains 50.60% α-tocopherol and 49.39% β-tocopherol, oil obtained by cold press contained 73.12% α-tocopherol and 26.83% β-tocopherol. Supercritical CO_2 extraction being conducted for the process must be decided whether the pilot or industrial scala. Supercritical CO_2 extraction that high oil yield is very high investment costs.*

KEYWORDS: *Wheat germ oil; Cold press; Super critical carbondioxide extraction; Tocopherol; Fatty acids.*

INTRODUCTION

Wheat (*Triticum aestivum*) composed of endosperm, germ and shell, and the respective ratios are given as: 81-84%, 14-16% and 2-3% ratio ranges, respectively [1]. Wheat germ is a byproduct of wheat flour industry, and contains crude ol at different rates depending on wheat varieties [2]. Wheat germ oil (8-14%) is rich in tocopherol phytosterol, polycasanol, thiamin, riboflavin and niacin [1,2]. There are lot of extractions methods. Mechanic extraction (pressing) and/or solvent extraction is one of the most widely used methods for oil extraction. Cold press is an oil extraction method of non-applying heat and chemical treatment, and therefore

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has many health benefits such as natural antioxidants within compounds [3-5]. Supercritical extraction technique is a highly desirable method for fractional extraction of biomass constituents. Most current commercial application of the supercritical extraction involve biologically-produced materials; the technique may be particularly relevant to the extraction of biological compounds in cases where there is a requirement for low-temperature processing, high mass-transfer rates, and negligible carrying over of the solvent into the final product [6-7]. Special applications to food processing include the decaffeination of green coffee beans, the production of hops extracts, the recovery of aromas and flavors from herbs and spices, the extraction and fractionation of edible oils, and the removal of contaminants, among others [6-9]. There is an increasing public awareness of the health, environment and safety hazards associated with the use of organic solvents in food processing and the possible solvent contamination of the final products. The high cost of organic solvents and the increasingly stringent environmental regulations together with the new requirements of the medical and food industries for ultra-pure and high added value products have pointed out the need for the development of new and clean technologies for the processing of food products. Extraction with supercritical fluids is also a unit operation that could be employed for a variety of applications including the extraction and fractionation of edible fats and oils, purification of solid matrices, and separation of tocopherols and other antioxidants [10-11]. The aim of current study is to establish differences among physical and chemical properties, fatty acid composition, tocopherol, and sterol contents of wheat germ oils obtained by using supercritical CO_2 extraction and cold press extraction.

EXPERIMENTAL SECTION

Material

Wheat germ was used as raw material in this study. This material was obtained from Altınapa Milling Com. And Industry in Konya in Turkey. It was kept in cold conditions (-18 ºC) until use.

Method

Production of wheat germ oil with cold press method: Cold pres oil is obtained by using mechanic extraction without heat treatment. Wheat germ oil was obtained by the method of cold press extraction process in Helvacızade Food, Pharmaceutical and Chemistry Industry Com. Inc.. Extracted oils were hermethically closed under the nitrogen.

Supercritical CO_2 extraction of wheat germ oil

Supercritical CO_2 extraction was carried out under laboratory conditions [12,13]. Extraction is semi-continuous system. Wheat germ oil extraction at 40 ºC with 200, 250 and 300 bar pressure of the oil recovery were realized by using only a separator [14].

Physical Analysis

Moisture, Free Fatty Acid (FFA), peroxide value, iodine value, saponification value and unsaponifiable matter were determined according to AOAC [15] methods.

Determination of fatty acid

About 0.5 mL germ oil sample was added into a 10 mL graduated cylinder, and added onto 1 mL of 2N methanolic KOH and 7 mL of n-heptane. Mix was stirred thoroughly, and it was put for centrifugation for 10 min. From the liquid phase on the top, 1 µL was injected to Gas Chromatograph. In analyses, SP 2-4111 column (100 m × 0.25 mm ID × 0.2 µm) was used. The temperature program was as follows: from 155 ºC; heated to 220 °C (1.5 °C/min), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 36 ml/min. hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 µL. The peak areas were computed by the integration software, and percentages of Fatty Acid Methyl Esters (FAME) were obtained as weight percent by direct internal normalization [16].

Working conditions of GC:

Instrument: GC/FID (HP-AGILENT/6890)

Inlet Mode: Split

Dedector: FID

Column: Supelco SP 2560 (100m x 0.25mm ID x 0.2 µm HP-88)

Inlet temperature: 250 ºC

Enjection: 1µL

Split ratio: 1/50

Carrier gas: Hydrogen

Head pressure: 2mL/min.
Oven temperature: 120ºC,1 min.
Dedector temperature: 250ºC
Gas flow (H_2): 36 mL/min.
O_2:300mL/ min.

Determination of sterol

In sterol analyses, α-cholestanol, 2,7-dichlorofluoroscein, betulin and KOH were used as main reactives. KOH solution was prepared with 14 gof KOH dissolved in 100 mL volumetric flasks with 10 mL of water and ethyl alcohol on top wa completed by addition of 100 mL. In experiment, 50 mL of this solution was used directly for preparing the sample solution desired. As a coloumn, SP B5 2-4034 (30mx0.25 mm id.) was used. 1 mL of betulin solution was added into a 250 mL round-bottomed flask including 5 g oil sampleIt was put the end of reflux condenser heater, and allowed for 30 minutes after boling. As they continue to develop fort hin layer chromatography boiling tank, solution, and started to prepare silica gel plate. Round-bottomed flask was cooled in tap water after boling process. Thye cooled sample was first separating funnel. In the same separatory funnel, 100 mL of diethyl ether was added. The phases were separated. After separating the phases the remaining throw bottom lyer of 50 mL of water were added to the upp after doing this operation 4 times taken until the top of the lower phase to 2-3 mL of phenolphthalein was added to a beaker until the pink color. In the meantime, 250 mL round-bottomed flask and weight were recorded. The solvent of the solution was evaporated under vacum to Rotary evaporator. The residue was continued until clear. After rotary, remaining solvent in balon joje was evaporated under nitrogen, and allowed to stand for half an hour in the oven of 105 ºC. After these processing, 90 mL of 99% n-hexane and 65 mL of diethyl ether were added into prepared DEVELOP tank. Dicloroflоresan solution previously prepared was sprayed onto the plate. This process was carried in a small cubic plastic container. The surroinding of alpha-cholestanol and erythrodiol-uvol bands were drawn. Then band was in a beaker with a spatula and scraped from bottom up. 15 mL of chloroform was added on sample and filtered. Filter paper can take the sample was washed 3 times with 10 mL of chloroform. After this operation, chloroform was evaporated with a rotary evaporator under wacuum. After chloroform evaporated, 0.10 mL of pure pyridine and 0.10 mL of Cylon BFTA were added into flask. It was allowed for 25 min., and injected into the device of GC [17].

The mean values were given in the tables, without the standard deviation, because this value would represent only the deviation of the method and not the variation of the appropriate sample.

GC conditions

Column Mode: SP B5 2-4034 (30 mx0.25 mm id.)
Flow : 1 mL
Front inlet mode: split
Front inlet heater: 280ºC
Back inlet: Heater, Pressure “Off” durumunda
Split ratio: 40:1

Determination of Tocopherol

Instrument: HPLC (HP-AGILENT/1100)
Column: Lichrosorb SI60 (5 µm x250 mmx 4.0mm)
Dedector: VWD (Variable Wavelength Dedetor-UV)
Flow rate: 1 mL/min.
Analysis time: 30 min.
Working pressure: 200 bar
Carrier phase: Acetonitril/Methanol/ Water/ Phosphoric acid (480 l/ 480 ml/ 40 ml/ 0.2 ml)
Reactive:
Standard:2 mL stock solution+23 mL acetone
Stock solution: D1-α-tocopherol, 4-7783 Supelco

1 g of wheat germ oil were weighed into 10 mL graduated cylinder, and then weighing was recorded. A sample completed with acetone to 10 mL was prepared in the same way. After injecting, the same for 30 minutes was allowed for completion of the analysis. Then the prepared solution was injected in to the second. After the analyses of samples were injected Standard [18].

Results of the research were evaluated by variance analysis (ANOVA) by using SPPS 10.0 statistical program and differences between types of germ oil were detected by Duncan Multiple Research Test.

RESULTS AND DISCUSSION

Moisture, free fatty acid (FFA), peroxide value, iodine value, unsaponifiable matter and saponification value of wheat germ oils obtained by supercritical CO_2 extraction and cold press methods are given in Table 1. Moisture, free fatty acid (FFA), peroxide value, iodine value,

Table 1: Physico-chemical properties of wheat germ oils obtained by cold press and SC-CO_2 extraction methods.

Parameters	Cold press	SC-CO_2
Moisture (%)	$0.10 \pm 0.01^{*}{}_{d}$	$13.32 \pm 2.27_{c}$
Free fatty Acids (%)	$0.84 \pm 0.07_{d}$**	$5.90 \pm 1.30_{e}$
Peroxide (meqO_2/Kg)	$8.90 \pm 0.14_{c}$	$15.80 \pm 3.10_{c}$
Iodine	$132.00 \pm 11.80_{b}$	$128.00 \pm 10.20_{b}$
Unsaponifiable matter (g)	$6.50 \pm 1.40_{c}$	$8.04 \pm 1.10_{cd}$
Saponification value	$197.00 \pm 12.70_{a}$	$182.00 \pm 9.80_{a}$

**mean ± Standard deviation*

*** Mean values of the same variety and the same crop year with a different superscript nondiffer significantly (P<0.05)*

unsaponifiable matter and saponification value of cold press oil were established as 0.097%, 0.84%, 8.9 meq O_2/kg, 132, 6.5 g/Kg and 197 mg KOH/g, respectively. In addition, moisture, Free Fatty Acid (FFA), peroxide value, iodine value, unsaponifiable matter and saponification value of supercritical CO_2 extraction method were determined as 13.32%, 5.9%, 15.8 meq O_2/kg, 128, 8.04 g/kg and 182 mg KOH/g oil, respectively. Specific gravity, iodine value, saponification values and unsaponifiable values of germ oil ranged from 0.925 to 0.938, 115 to 128, 179 to 190 and 2 to 5%, respectively [20]. While moisture, acid value, peroxide, saponification value, iodine value and unsaponifiable matter contents of germ oil obtained by solvent extraction method, respectively, were established as 0.68%, 12.8 mg KOH/g, 2.95 meqO_2/kg, 12.5, 142.8 and 3.34%, the same parameters of germ oil obtained by SFE were determined as 0.47%, 9.1 mg KOH/g, 2.05 mmol/kg, 169.3 mg KOH/g, 149.1 and 4.16%, respectively. Moisture content of germ oil obtained by cold press is determined as 0.097%, it was 13.32% in germ oil extracted by the supercritical CO_2 extraction. It was shown between moisture contents of both oil samples. This is probably the extraction of raw materials during storage conditions or due to extraction conditons [21]. Moisture of product may be change depending on extraction system and moisture amount of raw material [22,23]. As a result, if the oil will extract by supercritical CO_2 extraction method, it should be removed before recycling. FFA contents of germ oils obtained by supercritical CO_2 extraction and cold press were determined as 5.9% and 0.84%, respectively.

Fatty acid compositions of wheat germ oils obtained by cold press and supercritical CO_2 extractions are presented in Table 2. Fatty acids extracted by both extraction methods were palmitic, oleic and linoleic acids. Fatty acid compositions of wheat germ oils obtained by cold press and supercritical CO_2 extraction methods were determined as 16.751% and 17.208% palmitic, 17.065% and 17.255% oleic, 54.789% and 54.339% linoleic and 7.307% and 7.012% linolenic acids. The reason of this fatty acid diversity may be the variety, growing conditions, storage, preservation conditions, different extraction and analytical conditions [24]. Wheat germ oil obtained by the hexane extraction contained 56% linoleic acid [25].

Sterol contents of wheat germ oils obtained by supercritical CO_2 extraction and cold press extraction methods are given in Table 3. Campesterol and beta-stosterols of wheat germ oils obtained by cold press and supercritical CO_2 extraction are the major sterols. The germ oil extracted by both methods contained 24.19% and 23.44 % campesterol and 60.98% and 61.56% β-sitosterol, respectively.

Tocopherol content of wheat germ oils are given in Table 4. While germ oil obtained supercritical CO_2 extraction contains 50.60% α-tocopherol and 49.39% β-tocopherol, oil obtained by cold press contained 73.12% α-tocopherol and 26.83% β-tocopherol. Wheat germ oil is useful due to tocopherol content and a high content of unsaturated fatty acids have beneficial health effects [26]. Gustone et al., [27]reported that 1g wheat germ oil contains about 2188 mg total tocopherol, consist of 53.9% α-tocopherol, 18.2% β-tocopherol and 22.5% ∞-tocopherol.

The amount of FFA in raw wheat germ oil was considerable higher, and ranges from 5 to 25% [26]. High FFA rate reduces stability of the oil, give the bitter taste

Table 2: Fatty acid compositions of wheat germ oils obtained by cold press and SC-CO_2 extraction methods (%).

Fatty acids	C number	Cold press	SC-CO_2
Caprilic	8:0	0.008	0.002
Lauric	12:0	0.060	0.008
Myristic	14:0	0.108	0.088
Palmitic	16:0	16.751	17.208
Palmitoleic	16:1	0.148	0.153
Stearic	18:0	0.803	0.794
Oleic	18:1	17.065	17.255
Linoleic	18:2	54.789	54.339
Arachidic	20:0	0.173	0.177
Gadoik	20:1	1.508	1.531
Linolenic	18:3	7.307	7.012
Behenic	22:0	0.094	0.089
Erusic	22:1	0.265	0.258

Table 3: Sterol contents of wheat germ oils obtained by cold press and SC-CO2 extraction methods (%).

Sterols (%)	Cold press	SC-CO_2
Colesterol	0.015	0.097
Colestanol	0.752	1.239
Brassicasterol	0.178	0.285
24-Metilen	0.329	0.530
Campesterol	24.193	23.444
Campestanol	0.013	0.068
Stigmasterol	0.775	0.845
δ-7-Campesterol	1.435	1.178
δ-5,23-Stigmasterol	1.457	1.269
Cleresterol	0.398	0.391
β-Sitosterol	60.985	61.567
Sitostanol	1.590	1.990
δ-5-avenastenol	4.777	4.409
δ-5-D24	0.968	0.793
δ-7- stigmastenol	2.123	1.719
Uvaol	0.009	0.172

Table 4: Tocopherol content of wheat germ oils obtained by SC-CO_2 andcold press extraction methods (%).

	SC-CO_2	Cold Press
α- Tocopherol	50.60_a*	73.12_b
β- Tocopherol	49.39_a	26.82_b

* *Mean values of the same variety and the same crop year with a different superscript nondiffer significantly ($p \leq 0.05$)*

and causes a soapy flavor [28]. *Irmak* and *Dunford* [23] reported that the germ oil obtained by SC-CO_2 method contained 7.9% FFA. In previous study, peroxide values of wheat germ oils by SE and SFE methods were determined as 2.95 mmol/kg and 2.05 mmol/kg, respectively. While peroxide value of germ oil obtained by cold press in the applied low temperature (40-50 ^oC) is found as 8.9 meqO_2/kg, in oil obtained by supercritical CO_2 extraction method was found as 15.8 meqO_2/kg oil. *Jiand* and *Niu* [29] reported that iodine values of germ oils obtained by SE and SFE methods were determined as 121 and 169, respectively. In oher study, *Firestone* [20] determined that iodine value ranged from 115 to 128. *Jiang* and *Nui* [29] determined 3.34 and 4.16% unsaponifiable matter and 121.5 and 169.3 saponification values in germ oil extracted by SE and SFE, respectively. Results were found high compared with literature values. But some differences were found. The reason of these differences may be due to storage, processing and analytical conditions [21].

Fatty acid compositions of wheat germ oil obtained with supercritical CO_2 extraction method were found similar to that reported with hexane extraction [30,31]. 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_2 were 16.50, 15.05, 54.79, and 7.29% of total fatty acids, respectively [32]. The highest supercritical CO_2 extraction yield was the highest pressure (550 bar), and fatty acid composition of germ oil had not been effected from pressure, temperature and extraction [33]. Our results shown parallel with literature values and fatty acid composition did not show differences depending on extraction methods.

Phytosterols are the major components of wheat germ oil unsaponifiable [34]. Germ oil contains higher phytosterol according to other commercial vegetable oils. Sitosterol (60-70%) and campesterol (20-30%) are mainly two sterol in wheat germ oil [34,35]. The most of phytosterols in wheat germ oil are found in ester form [36]. Tocopherol consist of 18% of unsaponifiable matter, and wheat germ oil is the richest natural source of tocopherol [37]. As a result, wheat germ oil obtained by both extraction methods have tocopherol in high rate. It contained high α-tocopherol compared with other cold press germ oil. The study shown that, there was no differences between fatty acid compositions of oils obtained from two methods but α-tocopherol content of samples by cold press methods higher than SC-CO_2 extraction samples and β-tocopherol content of samples obtained from SC-CO_2 extraction is higher than the other methods samples.

CONCLUSIONS

Wheat germ oil is removed by cold press and supercritical CO_2 extraction methods, a study of literature has been created. Wheat germ oil contained high tocopherol with both methods. Considering the composition fatty acids, dominated fatty acids linolenic acids, and these results are close to each other depending on extraction methods. In germ oil, stosterol and campesterol were established as major sterols. Wheat germ oil is one of the richest oil due to tocopherol. Wheat germ oil rich in unsaturated fatty acid, sterols and tocopherols. Although cold press method efficient low, oil obtained by cold press has the highest content of α-tocopherol. Supercritical CO_2 extraction being conducted forthe process must be decided whether the pilot or industrial scala. Supercritical CO_2 extraction that high oil yield is very high investment costs.

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