NUTRITIONAL COMPONENTS OF SUPERCRITICAL CARBON DIOXIDE EXTRACTED WHEAT GERM OIL

Nurhan Turgut Dunford[1*] and Jose L. Martinez[2]

[1]Oklahoma State University, Department of Plant and Soil Sciences and Food and Agricultural Products Research and Technology Center, FAPC Room 103, Stillwater, OK 74078, Phone: 405-744-7062, Fax: 405-744-6313, E-mail: dunford@okstate.edu

[2] Thar Technologies, Inc., 730 William Pitt Way Pittsburgh, PA 15238

ABSTRACT

Wheat germ is one of the richest natural sources of alpha tocopherol, which possesses vitamin E activity. Wheat germ oil has a number of health benefits such as reducing plasma and liver cholesterol levels, improving physical endurance/fitness and delaying aging. The health benefits of wheat germ oil are attributed to its high vitamin E, polyunsaturated fatty acid and long chain n-alkanol (i.e. octacosanol and polycosanols) content. Wheat germ oil has been used as a fertility agent, and as an additive in natural food, health and cosmetic products.

Wheat germ is a by-product of the wheat milling industry. Wheat germ processing presents challenges due to its high content of polyunsaturated fatty acids and bioactive compounds. These compounds are prone to oxidation and degradation under the conditions used for conventional edible oil extraction and refining methods. In this study supercritical fluid extraction technology is examined as an alternative technique to obtain wheat germ oil of high quality and purity. Extraction yields will be discussed as a function of temperature (40-80^{o}C), pressure (100-550 Bar) and solvent/feed ratio. The focus of this presentation will be the evaluation of bioactive components of supercritical carbon dioxide (SC-CO_2) extracted wheat germ oil. The effects of SC-CO_2 extraction conditions on omega-3 and omega-6 fatty acids, tocopherol and tocotrienol content of the wheat germ oil will be presented. Nutritional composition of SC-CO_2 extracted oils will be compared with those of the hexane extracted wheat germ oil.

INTRODUCTION

Wheat germ contains about 11 % oil [1]. Wheat germ oil is used in foods, biological insect control agents, pharmaceuticals and cosmetic formulations [2]. Wheat germ processing presents challenges due to its high content of polyunsaturated fatty acids and bioactive compounds. These compounds are prone to oxidation and degradation under the conditions used for conventional edible oil extraction and refining methods. Furthermore, a significant portion of the nutritionally beneficial oil components, such as phytosterols and tocopherols are lost during the conventional oil processing operations [3].

Supercritical fluid extraction technology is an alternative method to conventional hexane extraction. Liquid and supercritical carbon dioxide (SC-CO_2) extraction of wheat germ oil has

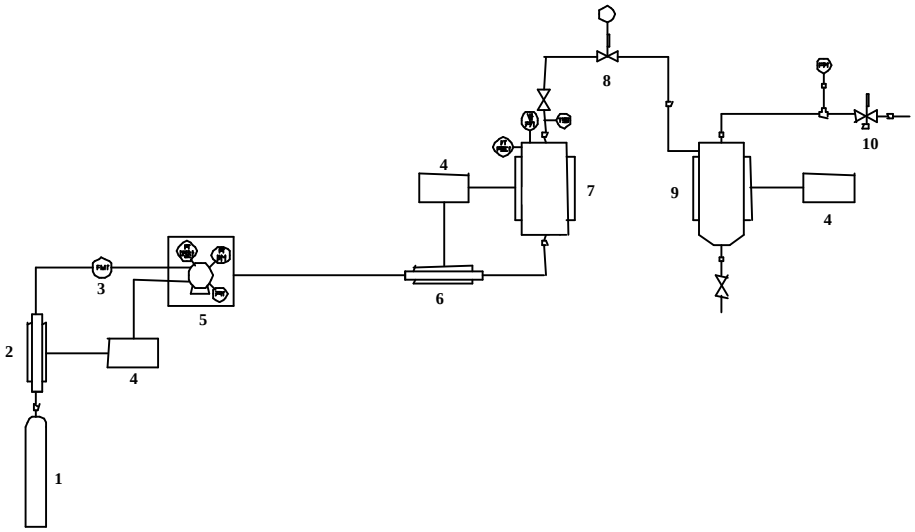
been studied at relatively low pressure and temperature ranges, 50-300 bar and 10-60^{o}C, respectively [4, 5]. Taniguchi et al. [4] reported that pressure had a significant effect on the supercritical carbon dioxide extraction of wheat germ oil. Oil solubility in supercritical carbon dioxide at 40^{o}C and 200 bar was 0.35 % (w/w). To our knowledge SC-CO_2 extraction of wheat germ oil at pressure above 300 bar and 60^{o}C has not been reported.

The objective of this research study is to examine the supercritical fluid extraction of wheat germ oil and compare the composition of bioactive components in supercritical carbon dioxide extracted oil to that of the hexane extracts.

MATERIALS AND METHODS

Wheat germ samples were supplied by ADM Milling Co. (Enid, OK, U.S.A.). Germ was obtained from milling of winter wheat (20% Kansas, 80% Oklahoma-grown winter wheat). Samples were used as is with no pretreatment. Hexane extraction of wheat germ oil was carried out according to the AOCS Official methods Ba 3-38 using *n*-hexane [6]. Solvent, *n*-hexane, (Pharmca, Brockfield, CT, U.S.A.) was reagent grade. Oil samples were prepared in HPLC-grade hexane (Pharmca, Brockfield, CT) for analytical tests. Fatty acid composition of the oil was analyzed by gas chromatography (GC). The GC unit was a HP 6890 Plus system equipped with a flame ionization detector (FID) (HP Company, Wilmington, DE). Methylation of the fatty acids was carried out according to the AOCS Official Method Ce 2-66 [6]. An Omegawax 250 fused silica capillary column, 30 m x 0.25 mm x 0.25 μm film thickness (Supelco, Bellefonte, PA) was used for fatty acid analysis. Fatty acid standards were purchased from Supelco (Supelco 37 component FAME mix, Supelco, Bellefonte, PA). The helium carrier gas flow rate was 30 mL/s. The injector temperature was maintained at 250^{o}C. A temperature program with total run time of 82 min was used. The column temperature, after an initial isothermal period of 2 min at 50^{o}C, was increased to 220^{o}C at a rate of 4^{o}C/min, and maintained at this temperature for 37.5 min. The detector conditions were as follows: temperature 260^{o}C, H_2 flow 40 mL/min, air flow 450 mL/min and make-up gas (He) 45 mL/min. Oil samples (1 μL) were injected by an autosampler (HP 7683, HP Company, Wilmington, DE). Peak areas were calculated and data collection was managed using an HP Chemstation (Revision. A.09.01, Agilent Technologies, Palo Alto, CA). Tocol content of the samples were analyzed by HPLC according to Katsanidis and Addis [7]. A florescence detector was used for quantification.

The supercritical carbon dioxide extraction experiments were carried out in a system manufactured by Thar Technologies (Pittsburgh, PA), as shown in Fig.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 (100-550 bar) at constant flow rate. The high-pressure pump (flow rate: 20-200 g/min) features 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 extraction temperature (40-80°C) was 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 (BPR). 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. Total amount extracted was calculated based on weight difference of the extraction vessel before and after each extraction.



1- CO_2 cylinder, 2– Low pressure heat exchanger, 3– Flow meter, 4- Water bath, 5- CO_2 pump, 6– High pressure heat exchanger, 7– Extraction vessel, 8- Automated BPR, 9- Cyclone, 10- Manual BPR

Figure 1: Schematic diagram of the supercritical fluid extraction equipment.

RESULTS AND DISCUSSION

The effect of pressure and temperature on the SC-CO_2 extraction yield of wheat germ was examined in the range of 100-550 Bar and 40-80^{o}C. Yields of SC-CO_2 extracts [(Weight loss from the sample during the extraction/Initial weight of wheat germ used for extraction)*100] varied significantly with temperature and pressure (Fig. 2) in the 2 to 20 %, w/w range. The actual amount of extract recovered for analytical tests was 0-13 %, w/w due to the losses in the system and CO_2 exhaust. Wheat germ oil yield was 11%, w/w when hot hexane (Soxhlet) was used for extraction. Higher SC-CO_2 extraction yield (>11%) indicates that SC-CO_2 extracted some of the wheat germ components, which are not soluble in hexane. Moisture in the wheat germ might be one of the compounds extracted with SC-CO_2. It was reported that some water was co-extracted with oil especially at high temperatures and pressures during the SC-CO_2 extraction [8, 9]. The highest SC-CO_2 extraction yield was obtained at the highest pressure used (550 Bar). The temperature dependence of the extract yield was more pronounced at higher temperatures (60 and 80^{o}C) and the lowest pressure examined in this study (100 Bar). This is due to the significant change in SC-CO_2 density under those conditions.

The fatty acid composition of the extracts was not affected by temperature, pressure and the extraction method (Table 1). Supercritical carbon dioxide extracted oil samples had similar fatty acid composition to that of the Soxhlet extracted oil (Table 1). These results illustrate that under the extraction conditions used for these experiments triglycerides and fatty acids were non-selectively extracted and isomerization and/or oxidation of fatty acids did not occur. All of the

wheat germ *n*-hexane extracts consisted of about 56 % linoleic acid (18:2 n6), which is an essential fatty acid (Table 1). Total unsaturated and polyunsaturated fatty acid (PUFA) content of the wheat germ oil was about 81 and 64 %, respectively. It has been suggested that unsaturated fatty acid, especially PUFA intake reduces cardiovascular heart disease (CHD). Several scientific studies have shown that n-3 fatty acids have benefits for lowering CHD risk [10]. It has been also suggested that n-3/n-6 ratio of 10 or less results in reduction in fatal CHD risk [11] . Wheat germ oil also has very high unsaturated and polyunsaturated fatty acid content and an excellent n-3/n-6 fatty acid ratio (1/9) (Table 1).

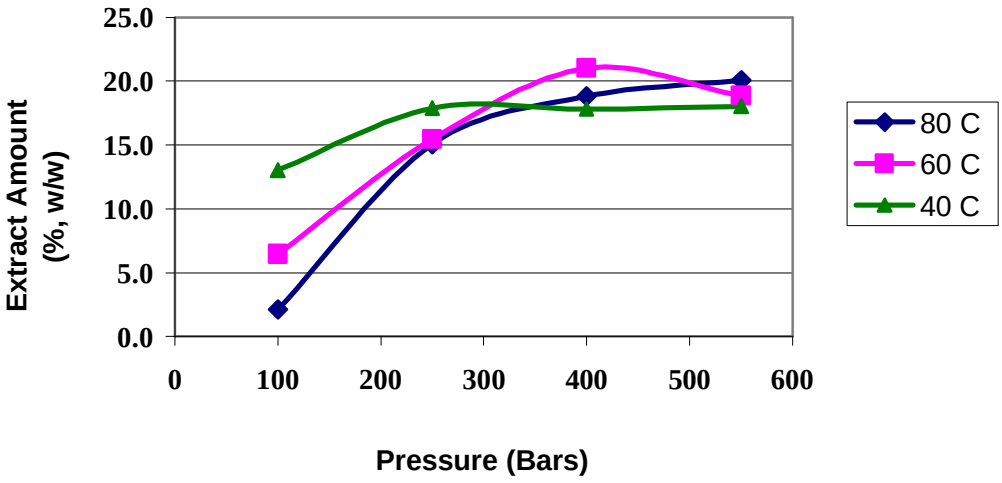


Figure 2: Effect of temperature and pressure on the SC-CO_2 extraction of wheat germ.

TABLE 1: Comparison of fatty acid composition[1] of wheat germ oil extracted with SC-CO_2 [2] and Soxhlet[3] methods.

Fatty Acid	16:0	18:0	18:1n9	18:2n6	18:3n3	20:0	20:1n9	20:2n6	22:1n9	24:1n9
SC-CO_2[2]	16.4	0.6	14.0	56.2	6.1	0.2	1.6	0.2	0.5	0.1
Soxhlet[3]	16.7	0.7	14.6	56.5	6.2	0.2	1.5	0.2	0.4	0.2

[1]GC area percentage
[2]Extracted at 550 Bar and 40^{o}C

[3]Extracted with hexane

Wheat germ is one of the richest sources of tocopherols, specifically α-tocopherol, which possesses vitamin E activity. Hexane-extracted wheat germ oil contains about 1400 and 1200 ppm α- and β-tocopherol, respectively (Table 2). These results are within the range of wheat germ tocopherol content reported in the literature [12]. Trace amount of tocotrienols were detected in some of the wheat germ extracts. Tocol content of Soxhlet and SC-CO_2 extracted wheat germ oil was similar (Table 2). These finding are in agreement with the previous work carried out on SC-CO_2 extraction of wheat germ oil [4, 5]. Our study indicated that α-tocopherol content of the extracts was slightly lower at the 60 min as compared to that of the extracts collected at 15, 30 and 45 min. This might be due to the dilution effect, which could result from the higher extraction yield (21 % at 60 min as compared to 17% at 15 min). More wheat germ components other than tocopherols might be extracted during the later phase of the SC-CO_2 extraction.

TABLE 2: Tocol Content of Wheat Germ Oil.

	SC-CO_2 Extraction (550 Bar, 80°C)				**Soxhlet Extraction (Hexane)**
Extraction time (min)	**15**	**30**	**45**	**60**	
a- **Tocopherol (ppm)**	**1353**	**1320**	**1365**	**1176**	**1377**
a-**Tocotrienol (ppm)**	**n.d.[1]**	**9**	**n.d.[1]**	**n.d.[1]**	**n.d.[1]**
b- **Tocopherol (ppm)**	**1005**	**945**	**998**	**1277**	**1209**
b-**Tocotrienol** + g-**Tocopherol (ppm)**	**23**	**19**	**24**	**31**	**48**
g- **Tocotrienol (ppm)**	**n.d.[1]**	**n.d.[1]**	**n.d.[1]**	**47**	**n.d.[1]**
d- **Tocopherol (ppm)**	**4**	**5**	**5**	**16**	**5**
d- **Tocotrienol (ppm)**	**n.d.[1]**	**n.d.[1]**	**n.d.[1]**	**12**	**7**

[1]not detected

CONCLUSIONS

This study showed that SC-CO_2 extraction of wheat germ resulted in extracts with similar fatty acid and tocopherol compositions to those of the Soxhlet extracts. Supercritical CO_2 extraction yield was significantly higher at higher pressures as compared to that of the Soxhlet extraction. Supercritical CO_2 extracted products could meet the demand for high purity wheat germ oil with no solvent residue.

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