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Supercritical Carbon Dioxide Extraction of Phenolics and Tocopherols Enriched Oil from Wheat Bran

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***Abstract*—**Supercritical carbon dioxide (SC-CO_2) was used as a solvent to extract oil from wheat bran. Extractions were carried out in a semi-batch process at temperatures ranging from 40 to 60ºC and pressures ranging from 10 to 30 MPa, with a carbon dioxide (CO_2) flow rate of 26.81 g/min. The oil obtained from wheat bran at different extraction conditions was quantitatively measured to investigate the solubility of oil in SC-CO_2. The solubility of wheat bran oil was found to be enhanced in high temperature and pressure. The composition of fatty acids in wheat bran oil was measured by gas chromatography (GC). Linoleic, palmitic, oleic and γ-linolenic acid were the major fatty acids of wheat bran oil. Tocopherol contents in oil were analyzed by high performance liquid chromatography (HPLC). The highest amount of phenolics and tocopherols (α and β) were found at temperature of 60ºC and pressure of 30 MPa.

***Keywords*—**Supercritical carbon dioxide, Tocopherols, Total phenolic content, Wheat bran oil

I. Introduction

WHEAT is a significant crop in many countries and worldwide cultivated grass. The milling process of wheat produces large amounts of bran as by product consisting of 14.5 wt% of rough wheat grain [1]. Wheat bran is not only a good source of dietary fibres [2], [3] but also a rich source of various natural antioxidants including tocopherols, phenolic acids etc. [4], [5]. Antioxidants modulate cellular oxidative status and prevent biologically important molecules such as DNA, proteins, and membrane lipids from oxidative damage and consequently reduce the risk of several chronic diseases including cancer and cardiovascular disease [6]-[8]. Tocopherols, phenolic acids and other antioxidants in wheat bran are generally believed to be primarily responsible for its positive effects on cardiovascular disease [2], [3], [9]. It has been suggested that these compounds of wheat bran exhibited significant capabilities in scavenging free radicals, chelating metal ion oxidants, and reducing lipid oxidation at different conditions [10], [11].

Conventionally, different antioxidants obtained from plant sources by organic solvent extraction. But the use of organic solvent is not environmental friendly. Beside this, the production of plant extracts is currently limited by safety and regulatory constraints to the concentration of toxic residues of conventional organic solvents [12]. Supercritical fluid extraction (SFE) has been widely employed as alternative of organic solvent extraction. The application of SFE has grown continuously because it showed several advantages over classical extraction processes with organic solvents. CO_2 is probably the most widely used supercritical fluid because it is nontoxic, nonflammable, and noncorrosive, inert to most materials, cheap, and readily available in bulk quantity with high purity [13]. Due to low critical temperature (31.1ºC), CO_2 is applied in SFE processes at near-environmental temperatures thus minimizing heat requirement and thermal damage to bioactive compounds [14]. SC-CO_2 can achieve extraction yields for tocopherols and phenolic acids similar to traditional hexane extraction. The production of oil from rice bran and wheat germ with high contents of tocopherols using SC-CO_2 has been reported in several reports [13], [15]-[17]. Although phenolic acids and their esters [18], [19] are soluble in SC-CO_2 without the addition of a co-solvent it has been given less importance on SC-CO_2 extraction of wheat bran oil with high content of phenolics as well as tocopherols.

Therefore, the aim of this study was to extract wheat bran oil with high content of phenolics and tocopherols by SC-CO_2 and also to compare the yield at different extraction conditions. The solubility of oil in SC-CO_2 was also measured at different extraction temperatures and pressures.

II. Materials and Methods

A. Materials

Wheat bran was provided by Young Nam Flour Mills Company, Busan, Korea. Pure CO_2 (99.99%) was supplied by KOSEM, Korea. α- and β-tocopherols were purchased from Sigma-Aldrich, St. Luis, Mo., USA and Supleco, USA, respectively. All other chemicals used in different analysis were of analytical or HPLC grade.

B. Sample Preparation

After drying in oven at low temperature, wheat bran samples were crushed in a mechanical blender and sieved (500 µm) by mesh. The sieved samples were then stored at 2ºC and used for SC-CO_2 extraction.

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C. SC-CO_2 Extraction

The set up of a laboratory scale of SFE process is shown in Fig. 1. This apparatus can be operated at pressure up to 30 MPa. Twelve gram of wheat bran samples was loaded into the stainless steel extraction vessel which was 50 mL in volume. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with cap another layer of cotton was used at the top of the sample. CO_2 was pumped at constant pressure into the extraction vessel by high pressure pump up to the desired pressure for 2 hrs. The pressure of CO_2 was maintained by a digital pressure controller. An electric oven was used for maintaining the temperature of extractor. The flow rate of CO_2 was constant at 26.81 g/min for all extraction conditions and CO_2 volume passing through the apparatus were measured using a gas flow meter. The oil extracted by SC-CO_2 was collected by a glass separating vessel. The amount of extract obtained at regular intervals of time was established by weight using a balance with a precision of ±0.001 g. The extracted oil was then stored at -40°C until further analysis.

The effects of temperature and pressure on SC-CO_2 extraction of wheat bran oil were studied at temperature ranging from 40 to 60°C and pressure ranging from 10 to 30 MPa.

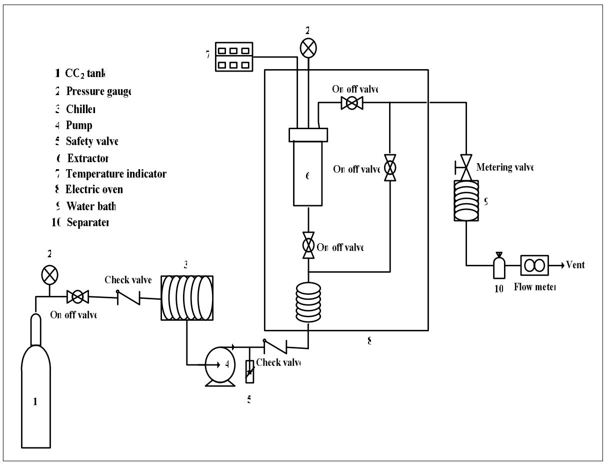


Fig. 1 Schematic diagram of SC-CO_2 extraction process

D. GC Analysis

The fatty acid compositions of wheat bran oil extracted by SC-CO_2 were determined by GC using a Hewlett Packard gas chromatograph (5890 Series II GC system). The fatty acid methyl esters were prepared firstly according to AOCS official method Ce 2-66 [20] and then separated using an Agilent DB-Wax capillary column (30 m length x 0.250 mm internal diameter, 0.25 µm of film). Nitrogen was used as a carrier gas (1.0 mL/min) of fatty acid methyl esters. The oven temperature was programmed starting at a constant temperature of 130ºC for 3 min, and then increased to 240ºC at a rate of 4ºC/min and hold at 240ºC for 10 min. Injector and detector temperatures were 250ºC. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supleco, USA).

E. Total Phenolic Content (TPC) of the Extracts

The TPC was determined by spectrophotometry according to the method described by the International Organization for Standardization (ISO) 14502-1 [21] using gallic acid as standard, Briefly, 0.1 mL of the SC-CO_2 extracted oil was mixed with 5.9 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The mixture was then allowed to stand at room temperature for an hour and absorbance was measured at 765 nm (UVIKON 933, Kontron Instruments) against blank. A calibration curve was made using gallic acid and the TPC was expressed as gallic acid equivalents (GAE) in mg/g wheat bran oil.

F. Tocopherols Analysis by HPLC

Tocopherols analysis was carried out using a Waters HPLC equipped with a model 600E system controller, an evaporative light scattering detector (ELSD, Softa Corporation) and a Eclipse Plus C18 column (5µm, 4.6 x 250 mm, Agilent, USA). Tocopherols were quantified by an isocratic method reported by Leray et al. [22]. A mobile phase consisting of methanol and water (98:2) was eluted 1.5 mL/min. The drift tube temperature of ELSD was fixed at 60ºC and the pressure of N_2 used as spray gas was 50 psi. The amount of α- and β-tocopherols in the extract was measured based on the peak area of the standard tocopherols.

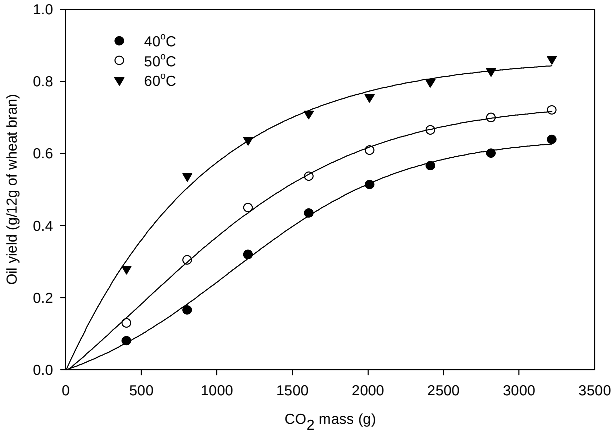
III. Results and Discussion

A. SC-CO_2 Extraction

SFE curves of wheat bran oil at different temperatures (40-60°C) and pressure (10-30 MPa) are shown in Fig. 2(a)-(e). The highest yield obtained at pressure of 30 MPa and temperature of 60°C was 2.69 g/12 g of wheat bran. The solvating power of SC-CO_2 was significantly changes by the applied pressure and temperature variation. Depending on the pressure and temperature, the oil yield was increased with the increasing of CO_2 mass. At constant temperature, the amount of oil extracted from wheat bran was increased with increasing pressure. This happened due to direct increase of density and hence the solvating power of SC-CO_2. The increased solvating power and the strength of intermolecular physical interactions considered as belonging to the effect of pressure [23], [24]. Similar pressure effect was reported in SC-CO_2 extraction of green coffee oil [25].

At a constant pressure, the oil yield increased with the temperature from 40 to 60ºC. The solvent density was decreased with the increasing temperature. However, despite of the decreasing of solvent density, the oil yield was increased with the temperature which can be attributed to the increase of the oil components vapour pressure. Herein, the increase of solute vapour pressure was dominated over solvent density. Azevedo et al. [25] reported the similar effect of vapour pressure on SC-CO_2 extraction of green coffee oil.

(a)



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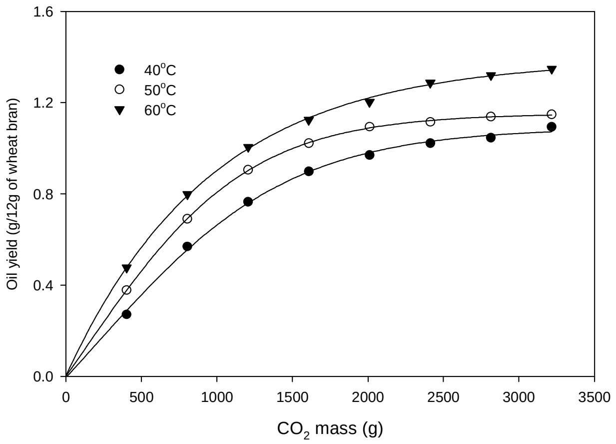

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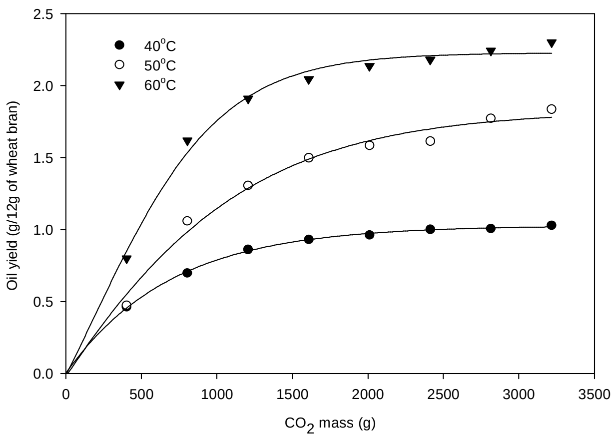

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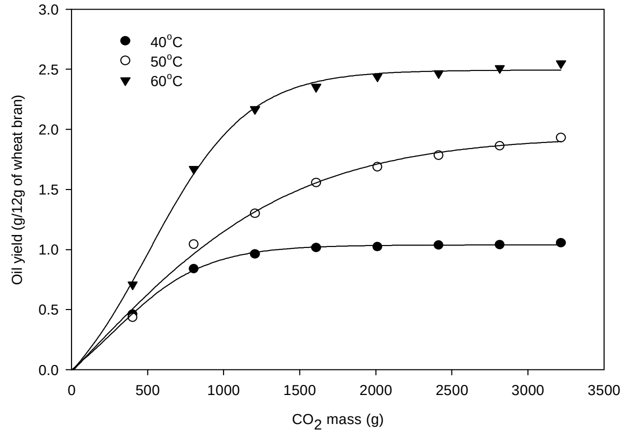

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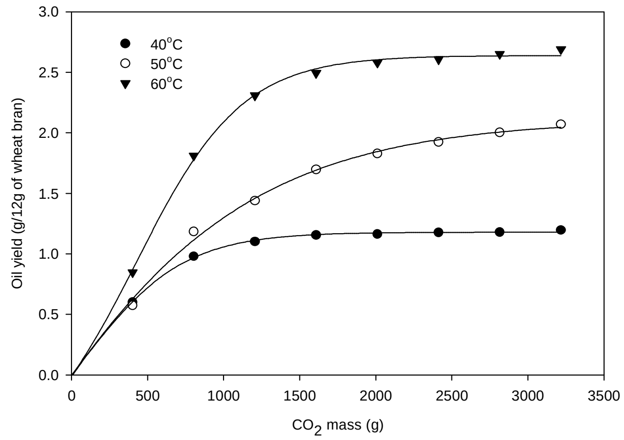

Fig. 2 SC-CO_2 extraction of wheat bran oil at different pressures and temperatures. (a) 10 MPa (b) 15 MPa (c) 20 MPa (d) 25 MPa (e) 30 MPa

B. Solubility Measurement of Wheat Bran Oil

The solubility of wheat bran oil at each extraction condition of temperatures and pressures were calculated from the slope of the linear sections of the extraction curves shown in Fig. 2(a)-(e). Table I shows the oil solubility at different extraction conditions. It was found that the highest value of wheat bran oil solubility was 2.26 mg/g CO_2 at 30 MPa and 60ºC. Solubility of oil depends on density of CO_2 and vapour pressure of oil components as described above in SC-CO_2 extraction section. At similar temperatures and pressures, Sovova et al. [26] and Ozkal et al. [27] reported higher solubility of oil from black currant and apricot seeds, respectively. The variation of solubility might be happened due to variation of sample, extraction unit, sample size, flow rate of CO_2 etc.

C. Solubility Correlation

The solubility of wheat bran oil in SC-CO_2 was correlated by Chrastil [28] model. It was revealed that Chrastil model is useful to correlate vegetable oil solubility [26], [27]. This model was based on the direct relationship between solubility of solute and density of a solvent. The correlations based on empirical density are very useful to determine the solubility of solids and liquids in compressed fluids, as they are both simple and do not require physicochemical properties of the solute. Despite its limitations, the usefulness of equation has proved in SFE work and is easy to use. The experimental values represented by points and the calculated solubilities by lines are shown in Fig. 3. It clearly showed the isotherms and the effects of temperature and solvent density. The correlation of oil solubility with solvent density was obtained from the equation (1).

$$y = \rho_{CO_2}^{k} \exp\left(\frac{a}{T} + b\right) \quad (1)$$

where y is the solubility of wheat bran oil (mol/mol), ρ_{CO_2} is the density of CO_2, T is experimental temperature (K) and a, b and k are empirical fitting parameters. The solubility data of wheat bran oil were fitted well in Chrastil model because at a given temperature, almost a linear relation between the solubility of oil and solvent density was obtained.

TABLE I
SOLUBILITY OF WHEAT BRAN OIL IN SC-CO_2 AT DIFFERENT TEMPERATURES AND PRESSURES

Temperature (°C)	Pressure (MPa)	Solubility (mg oil/g of CO_2)		
		Wheat bran	Black currant seed[1]	Apricot seed[2]
40	10	0.21		
	15	0.71	1.3	1.1
	20	0.87	3.0	
	25	1.04		
	30	1.22		
50	10	0.38		
	15	0.86	0.59	0.9
	20	1.28	2.4	
	25	1.30		
	30	1.47		
60	10	0.67		
	15	0.99		
	20	2.01		
	25	2.08		
	30	2.26		

[1]Sovova et al. [26]; [2]Ozkal et al. [27]

D. Fatty Acid Compositions

Wheat bran oil was characterized by a yellowish colour and a light odour. The fatty acid compositions of wheat bran oil obtained SC-CO_2 extraction are shown in Table II. The major fatty acids of wheat bran oil were linoleic, palmitic, oleic and γ-linolenic acids. Linoleic acid was present in highest amount and it was present in the ranging from 45.4 to 57.3% of total identified fatty acids. Within saturated fatty acids, palmitic acid was present in the highest concentration ranging from 15.53 to 22.0% of total identified fatty acids. The fatty acid compositions were remarkably changed at different extraction conditions. The solubility of fatty acids in SC-CO_2 might be affected by extraction temperatures and pressures.

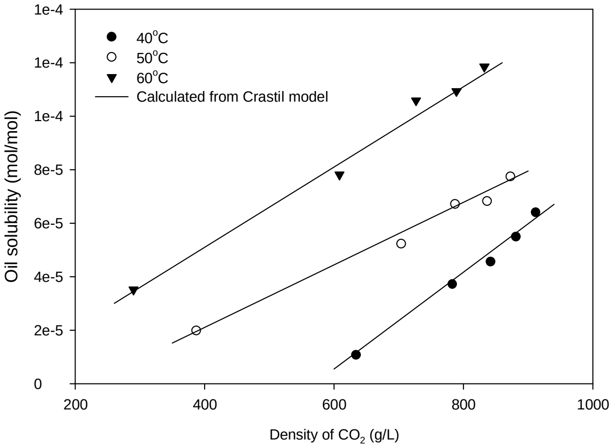

Fig. 3 Experimental data of wheat bran oil solubility as a function of CO_2 density and calculated results from Chrastil model

TABLE II
MAJOR FATTY ACID COMPOSITIONS OF WHEAT BRAN OIL AT DIFFERENT EXTRACTION CONDITIONS

Pressure (MPa)	Temperature (°C)	Fatty acids (%)			
		Palmitic	Oleic	Linoleic	γ-linolenic
10	40	22.0	12.6	56.4	7.1
	50	18.8	14.3	55.8	7.0
	60	18.6	14.1	56.2	7.6
15	40	16.0	12.6	48.4	6.0
	50	17.4	15.1	56.5	7.4
	60	18.3	14.6	57.3	8.0
20	40	18.3	15.9	56.8	7.0
	50	17.4	15.5	56.8	7.3
	60	15.53	11.9	45.4	5.7
25	40	17.73	15.2	57.1	7.2
	50	17.83	11.8	56.8	7.1
	60	20.03	14.9	56.2	6.7
30	40	17.3	15.3	57.1	7.1
	50	17.8	15.3	57.1	7.2
	60	17.8	15.2	57.3	7.2

E. TPC of the Oil

TPC of the extract obtained by SC-CO_2 are shown in Table III. The highest TPC (3.57 mg/g of wheat bran oil) was observed at temperature of 60ºC and pressure of 30 MPa. TPC of the oil obtained by SC-CO_2 extraction increased with increasing pressure. It is reported that solubility of phenolics increases with increasing pressure [18], [19] resulting an increase in phenolic yield [29], [30]. The effect of pressure on the solubility of TPC was similar with the solubility of wheat bran oil.

At constant pressure of 15, 20, 25 and 30 MPa, the solubility of TPC increased with increasing temperature. Since increasing the temperature decreases the viscosity and increases the diffusivity resulting in an increased extraction rate. But at 10 MPa, the solubility of TPC decreased with increasing temperature. Similar results were reported that the solubility of phenolic acids and their esters in SC-CO_2 decreased with increasing temperature at below the cross over pressure (<15 MPa) [18], [19].

F. Tocopherol Contents

□- and β-tocopherol contents of wheat bran oil obtained by SC-CO_2 extraction are given in Table III. The highest amounts of α- and β-tocopherols were 1481.05 and 735.09 µg/g oil, respectively at 30 MPa and 60ºC. High tocopherol contents of roasted wheat germ oil obtained by SC-CO_2 extraction at higher pressure was reported by Gelmez et al. [31]. It was found that both α- and β-tocopherol contents increased while the pressure was increased. This effect is expected because the pressure increased the density and hence the solvating power of SC-CO_2 which allowed it to dissolve more solutes. At constant pressure, α- and β-tocopherol contents increased with increasing temperature. Increasing the temperature decreases the viscosity and increases the diffusivity resulting in an increased solubility of solute. Imsanguan et al. [17] also reported similar trends of solubility for α-tocopherol in SC-CO_2 from rice bran.

TABLE III
TPC AND α- AND β-TOCOPHEROL CONTENTS OF WHEAT BRAN OIL AT DIFFERENT EXTRACTION CONDITIONS

Temperature (ºC)	Pressure (MPa)	TPC (mg/g oil)	Tocopherols (µg/g oil)	
			α-tocopherol	β-tocopherol
40ºC	10	1.23	377.15	252.98
	15	1.46	515.39	304.75
	20	1.81	605.63	345.28
	25	2.06	774.49	420.45
	30	2.39	967.81	621.79
50ºC	10	1.08	514.3	344.2
	15	1.66	868.03	411.79
	20	1.98	993.96	588.75
	25	2.36	1290.48	623.27
	30	2.89	1143.93	647.2
60ºC	10	0.98	713.66	370.26
	15	1.87	943.27	415.22
	20	2.29	1143.93	629.37
	25	2.92	1222.76	689.21
	30	3.57	1481.05	735.09

IV. Conclusions

Wheat bran oil was extracted in a high pressure apparatus using SC-CO_2 at different temperatures and pressures. In the conditions performed in this study the highest yield of oil was found at 60ºC and 30 MPa. The solubility of oil calculated from extraction curve was also higher at high temperature and pressure. Wheat bran oil contained highest percentage of linoleic acid in all extraction conditions. The TPC, and α- and β-tocopherols were found significant amount in wheat bran oil obtained by SC-CO_2 extraction. The identification of species cultivars with high level of antioxidants, as well as in tracing of biological activities is important for human and other living organisms. Therefore, antioxidants in wheat bran might be quantified by SC-CO_2 extraction for proper using in biological purposes.

Acknowledgment

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