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Full Length Research Paper

Comparison of supercritical and near-critical carbon dioxide extraction of carotenoid enriched wheat bran oil

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Supercritical and near-critical carbon dioxide (CO_2) extraction were carried out to extract oil from wheat bran. The extraction temperatures for supercritical and near-critical CO_2 were 35 - 45°C and 25 - 30°C, respectively. The applied pressure was ranging from 10 to 30 MPa for both supercritical and near-critical CO_2 extraction. The extraction was performed in a semi batch process with a CO_2 flow rate of 26.81 g/min for 1.5 h. The oil obtained from wheat bran at different extraction conditions was quantitatively measured to investigate the solubility of oil at supercritical and near-critical CO_2. The highest solubility was found at near-critical condition. The fatty acid compositions of wheat bran oil were measured by gas chromatography (GC). Linoleic, palmitic, oleic and γ-linolenic acid were the major fatty acids of wheat bran oil. Total carotenoid was measured spectrophotometerically. Highest yield of total carotenoid was found at 45°C and 30 MPa.

Key words: Supercritical and near-critical carbon dioxide, wheat bran oil, total carotenoid.

INTRODUCTION

Wheat is an important agricultural commodity and dietary component across the world. During processing of wheat it produces large amounts of bran as by product consisting of 14.5 wt% of rough wheat grain (Xie et al., 2008). Similar to other grains, wheat bran is a rich source of various natural antioxidants including carotenoids, phytosterols, tocopherols, phenolic acids etc. (Nystrom et al., 2005; Zhou et al., 2005; Halliwell, 1992; Truswell, 2003). Carotenoids are the most widespread pigments in nature. They occur in bacteria, fungi, plants, and animals, comprising a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls). Carotenoids are found in fruits and vegetables in relatively high concentrations and the important are lycopene, α- and β-carotene, lutein, zeaxanthin and β-cryptoxanthin. The main roles of carotenoids in the human diet are as precursors of vitamin A and as antioxidants (Ishida and Bartley, 2005; Ishida and Chapman, 2009; Filho et al., 2008). Antioxidants modulate cellular oxidative status and prevent biologically important molecules such as DNA, proteins, and membrane lipids from oxidative damage by scavenging free radicals, chelating metal ion oxidants, and reducing lipid oxidation at different conditions (Yu et al., 2002a; Adom and Liu, 2005) and consequently reduce the risk of several chronic diseases including cancer and cardiovascular disease (Halliwell et al., 1992; Wang and Zheng, 2001; Yu et al., 2002b). Therefore, carotenoids are gaining interest in the food industry due to their nutritional and antioxidant properties. They are used as colourants in foodstuffs, e.g. they are used as suspensions to give colour to drinks (for instance, β-carotene is used in orange drinks). This colourant is unaffected by ascorbic acid, heating or freezing and is active at very low concentrations (1 ppm) (Macias-Sanchez et al., 2008).

Separation of active ingredients from various materials (plants, food-by-products, algae, etc.) has attracted much attention in food, cosmetics and pharmaceutical industries. Currently, the most common way for extraction is liquid solvent extraction using toluene, hexane, petroleum ether, chloroform, acetone etc. Decomposition or degradation of thermolabile compounds cannot be avoided in a conventional separation method, since relatively high

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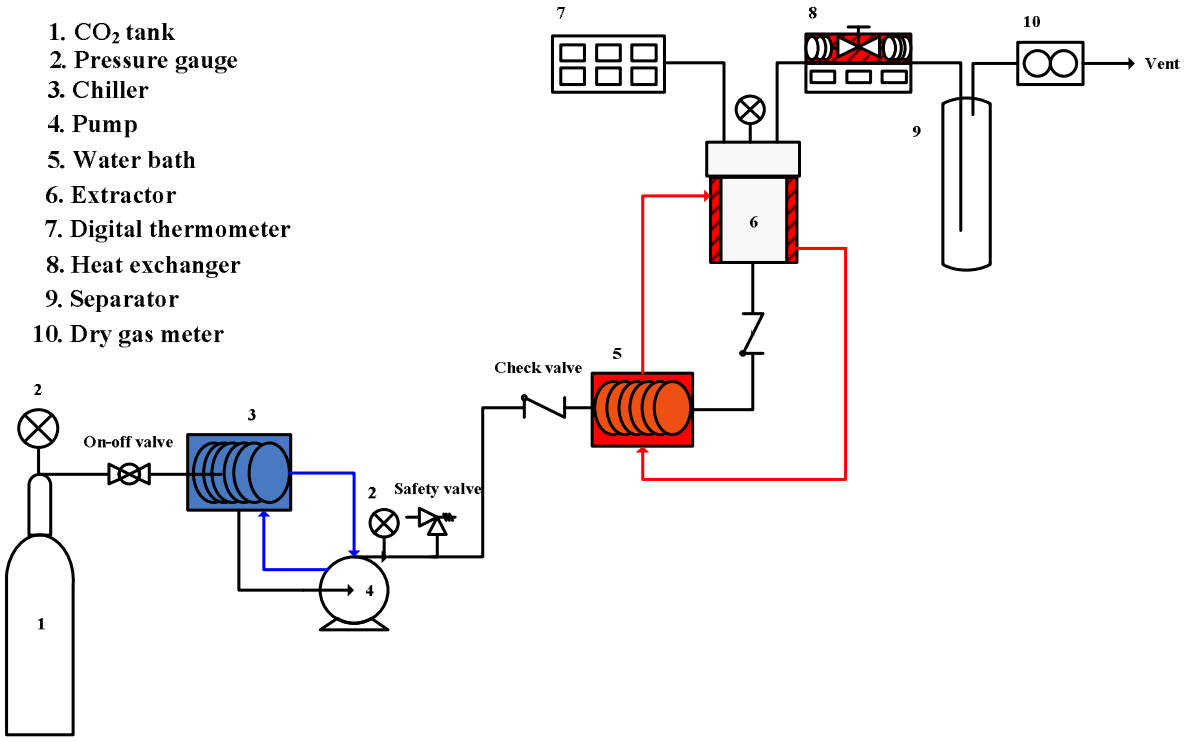

Figure 1. Schematic diagram of supercritical and near-critical CO_2 extraction process.

temperatures are required for these processes. The production of plant extracts is limited by safety and regulatory constraints to the concentration of toxic residues of conventional organic solvents (Sanders, 1993). Organic solvents are also harmful to human health as well as the environment. Supercritical fluid extraction (SFE) has been widely employed as alternative of organic solvent extraction. 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 (Ge et al., 2002). The critical temperature of CO_2 (31.1°C) is also low. Several studies have been carried out in which supercritical CO_2 extraction was an alternative technique to produce high value oil (Chan and Ismail, 2009; Diaaz-Reinoso et al., 2006; Liu et al., 2009). Some authors have also reported the carotenoids extraction by supercritical CO_2 from different sources (Macias-Sanchez et al., 2005; Careri et al., 2001; Rozzi et al., 2002; Spanos et al., 1993; Franc and Meireles, 2000). The equipment cost for supercritical CO_2 extraction at higher conditions is comparatively high. Low cost of product has a great benefit to commercialize in market. Moreover, the quality of oil extract might be better at low temperature. However, to the best of our knowledge no report has been disclosed regarding comparison of yield of wheat bran oil including carotenoids by supercritical and near-critical CO_2 extraction.

Therefore, the aim of this study was to extract wheat bran oil enriched with carotenoids by supercritical and near-critical CO_2 at different extraction conditions and to compare the yield obtained by supercritical and near-critical CO_2 extraction. The solubility of oil was also measured at different extraction temperatures and pressures.

MATERIALS AND METHODS

Materials

Wheat bran was provided by Young Nam Flour Mills Company, Busan, Republic of Korea. Carbon dioxide (99.99% pure) was supplied by KOSEM, Republic of Korea. β-carotene was purchased from Wako Pure Chemical Industries Ltd., Japan. All other chemicals used in different analysis were of analytical or HPLC grade.

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 2°C and used for supercritical and near-critical CO_2 extraction.

Supercritical and near-critical CO_2 extraction

A laboratory scale supercritical and near-critical fluid extraction unit was used for extracting oil from wheat bran (Figure 1). Fifty gram of wheat bran samples was loaded into the stainless steel extraction vessel which was 200 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 preparative CO_2 pump (pu-2088 plus, Jasco) up to the desired pressure for 1.5 h. The pressure of CO_2 was automatically maintained by the pump. Extractor temperature was maintained by connecting with a water bath. 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 dry gas meter. The oil extracted by supercritical and near-critical CO_2 was collected by a glass separating vessel. The amount of extract obtained at regular intervals of time was weighted using a balance with a precision of ±0.001 g. The extracted oil was then stored at -40°C until further analysis.

The effect of temperatures on supercritical and near-critical CO_2 extraction of wheat bran oil were studied at 25 - 30°C and 35 - 45°C, respectively. The pressure range was 10 - 30 MPa for both supercritical and near-critical CO_2 extraction.

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 (AOCS, 1998) and then separated using an Agilent DB-Wax capillary column (30 m length × 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 130°C for 3 min, and then increased to 240°C at a rate of 4°C/min and held at 240°C for 10 min. Injector and detector temperatures were 250°C. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supleco, USA).

Total carotenoid assay

Total carotenoid was assayed according to the method of Ranjith et al. (2006). β-Carotene was used as a standard. Briefly, 1 g of the oil samples was added to 0.5 mL of 5% NaCl. The mixture was vortexed for 30 s and then centrifuged for 10 min at 3000 g. The supernatant was appropriately diluted with hexane and the absorbance was recorded at 460 nm. A calibration curve was constructed using β-carotene and the amount of total carotenoid was expressed as β-carotene equivalent.

Statistical analysis

Experiments were performed in triplicate and each set of yields were averaged. The standard deviations were used as the basis for the error bars shown in the figures. The least significant difference at the 95% confident ($P < 0.05$) level was calculated by Duncan test using Statistical Analysis System (SAS Ver. 9.1, SAS Institute, USA).

RESULTS AND DISCUSSION

Supercritical and near-critical CO_2 extraction

Extraction curves of wheat bran oil at different temperatures (25 - 45°C) and pressure (10 - 30 MPa) are shown in Figures 2A - E. In this study, the highest yield of oil obtained at temperature of 25°C and pressure of 30 MPa was 4.09 g/50 g of wheat bran. The amount of oil yield was significantly changed by the applied temperature and pressure in both supercritical and near-critical conditions. The variation of yield was due to the change of solvent density. At constant temperature, the amount of oil extracted from wheat bran was increased with increasing pressure. It happened because the density of CO_2 was increased and hence the solvating power of supercritical and near-critical CO_2. The increased solvating power and strength of intermolecular physical interactions considered as belonging to the effect of pressure (Morita and Kajimoto, 1990; Bulgarevich et al., 2002). Corso et al. (2010) reported similar pressure effect in the extraction of sesame seed oil.

At supercritical CO_2 extraction conditions, the extract yield is increased with increasing temperature at a fixed pressure. Due to increase of temperature, the solvent density was decreased. However, despite decreasing of solvent density, the oil extraction yield was increased with increasing temperature which can be attributed to the increase of the oil components vapour pressure. The effect of the increase of solute vapour pressure seems to have dominated over solvent density. Similar effects were reported by De Azevedo et al. (2008) on supercritical CO_2 extraction of green coffee oil. On the other hand, at near-critical extraction conditions, the yield decreased with increasing temperature. Therefore, at near-critical conditions there was no significant effect of temperature to increase oil component vapour pressure.

It was also found that the extraction yield was higher at near-critical extraction comparative to supercritical CO_2 extraction. Under critical temperature, the effect of the density of solvent was dominant over oil component vapour pressure.

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 Figures 2A - E. Table 1 shows the oil solubility at different extraction conditions. It was found that the highest value of wheat bran oil solubility was 1.40 mg/g CO_2 at 25°C and 30 MPa. Solubility of oil depends on density of CO_2 and vapour pressure of oil components as described in supercritical and near-critical CO_2 extraction section. At similar temperatures and pressures, Sovova et al. (2001) and Ozkal et al. (2006) reported higher solubility of oil from black currant and apricot seeds, respectively. The variation of oil solubility might have happened due to variation of sample, extraction unit, sample size, flow rate of CO_2 etc.

Solubility correlation

The solubility of wheat bran oil at supercritical and near-

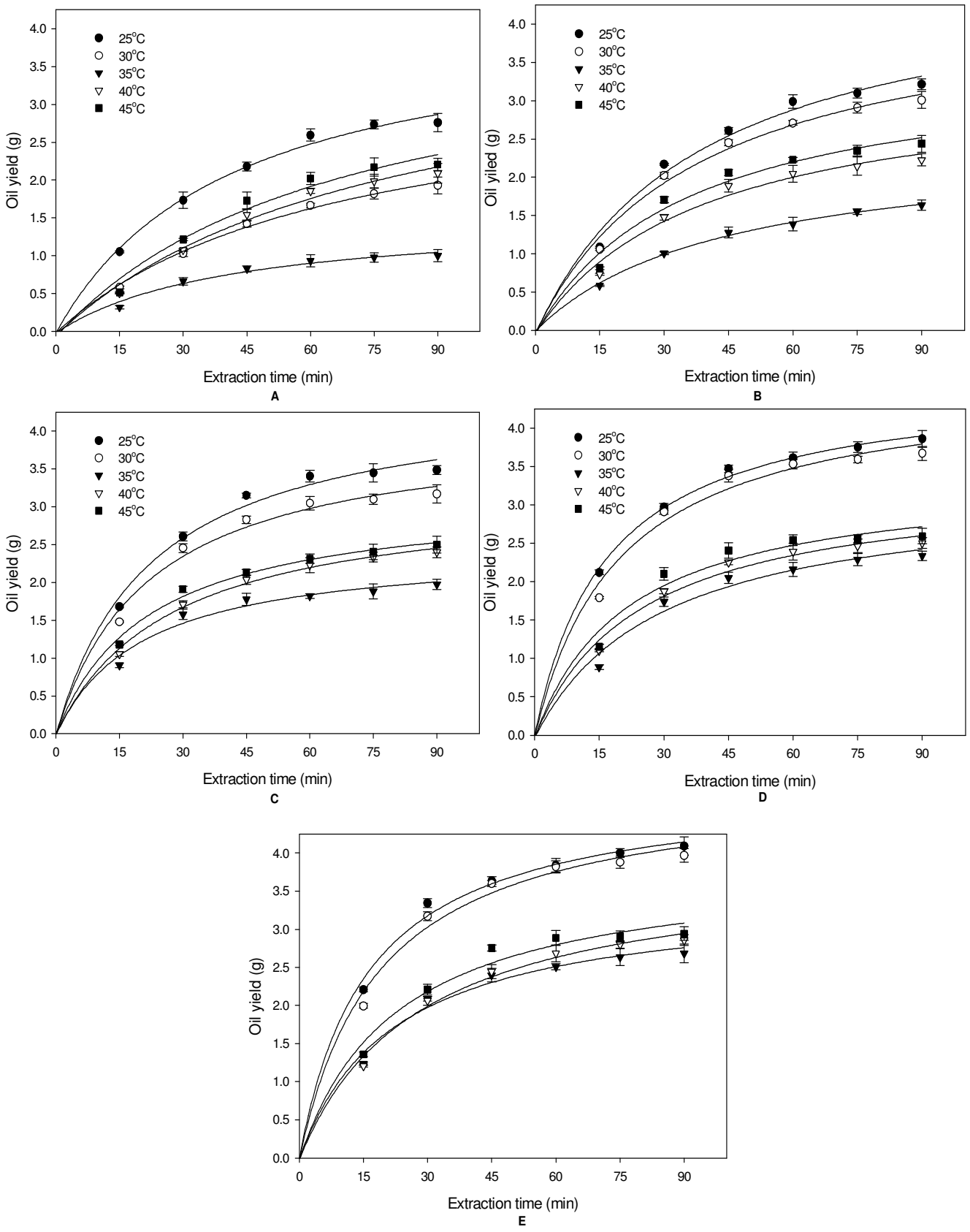

Figure 2. Supercritical and near-critical CO_2 extraction of wheat bran oil at different pressures and temperatures A) 10 MPa B) 15 MPa C) 20 MPa D) 25 MPa and E) 30 MPa.

Table 1. Solubility of wheat bran oil at different extraction temperatures and pressures by supercritical and near-critical CO_2.

Extraction	Temperature (°C)	Pressure (MPa)	Solubility (mg oil/g CO_2)		
			Wheat bran	Black currant seed[1]	Apricot seed[2]
Near-critical CO_2	25	10	0.72		
		15	0.90	1.3	1.1
		20	1.08	3.0	
		25	1.20		
		30	1.40		
	30	10	0.43		
		15	0.84		
		20	1.02		
		25	1.21		
		30	1.32		
Supercritical CO_2	35	10	0.27		
		15	0.49		
		20	0.66		
		25	0.72		
		30	0.87		
	40	10	0.45		
		15	0.61	0.59	0.9
		20	0.71	2.4	
		25	0.78		
		30	0.86		
	45	10	0.50		
		15	0.71		
		20	0.79		
		25	0.87		
		30	0.92		

[1]Sovova et al. (2001); [2]Ozkal et al. (2006).

critical CO_2 was correlated by Chrastil (1982) model. It was revealed that Chrastil model is useful to correlate vegetable oil solubility (Sovova et al., 2001; Ozkal et al., 2006). 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. Figure 3 shows Chrastil model in which the experimental values and the calculated solubilities were represented by points and lines, respectively. It clearly showed the isotherms and the effects of temperature and solvent density. The correlation of oil solubility with solvent density was obtained from 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 obtained by supercritical and near-critical CO_2 extraction 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.

Fatty acid compositions

Wheat bran oil was characterized by a yellowish colour and light odour. The fatty acid compositions of wheat bran oil obtained by supercritical and near-critical CO_2 extraction are shown in Table 2. The major fatty acids of wheat bran oil were palmitic, oleic, linoleic and γ-linolenic acids. Linoleic acid was found in highest amount and it

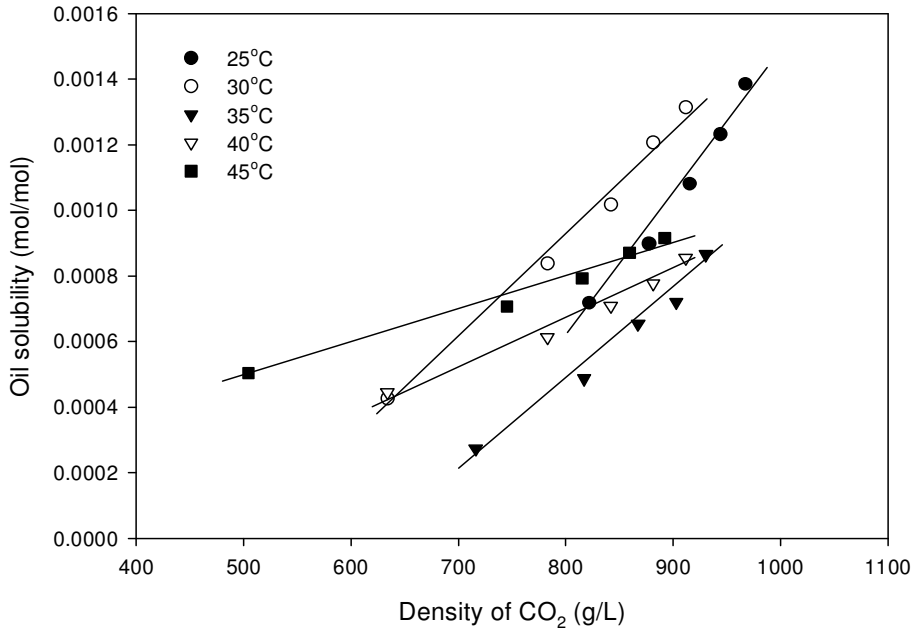

Figure 3. Experimental data of wheat bran oil solubility as a function of CO_2 density and calculated results from Chrastil model both in supercritical and near-critical conditions.

Table 2. Major fatty acid compositions of wheat bran oil obtained by supercritical and near-critical CO_2 extraction.

Pressure (MPa)	Temperature (ºC)	Fatty acids (%)			
		Palmitic	Oleic	Linoleic	γ-linolenic
10	25	17.8±0.12	14.5±0.11	54.8±0.65	6.4±0.16
	30	17.0±0.21	14.6±0.15	54.2±0.42	7.1±0.26
	35	17.1±0.44	14.3±0.37	55.2±1.01	6.8±0.19
	40	17.9±0.32	14.2±0.26	56.4±0.63	7.1±0.29
	45	17.5±0.21	14.6±0.24	56.2±.041	6.9±0.31
15	25	17.5±0.33	15.3±0.19	56.3±0.33	6.9±0.10
	30	17.9±0.16	15.2±0.34	55.7±0.61	6.9±0.17
	35	17.8±0.27	14.2±0.16	54.3±0.88	6.4±0.23
	40	17.2±0.37	14.6±0.26	54.4±1.23	6.2±0.21
	45	17.7±0.24	14.2±0.27	54.4±1.32	6.5±0.34
20	25	18.6±0.28	13.3±0.33	53.2±1.09	6.4±0.14
	30	17.6±0.11	14.2±0.17	52.7±0.75	6.5±0.24
	35	17.1±0.25	15.0±0.12	54.7±1.19	6.7±0.13
	40	18.3±0.26	15.9±0.39	56.8±0.89	7.0±0.29
	45	17.4±0.41	13.3±0.29	60.0±0.99	6.4±0.21
25	25	17.3±0.45	14.7±0.17	53.6±0.56	6.5±0.12
	30	17.5±0.24	15.3±0.20	55.1±1.24	6.8±0.20
	35	18.2±0.17	14.6±0.29	53.3±1.06	6.2±0.18
	40	17.3±0.42	15.2±0.38	57.1±0.95	7.2±0.32
	45	16.8±0.46	15.6±0.28	57.8±1.37	6.7±0.22
30	25	17.2±0.15	15.4±0.27	55.1±1.17	6.7±0.15
	30	17.2±0.35	14.0±0.14	54.7±0.88	7.3±0.26
	35	17.3±0.34	15.3±0.13	55.3±0.81	6.7±0.18
	40	17.3±0.49	15.3±0.34	56.1±0.72	7.2±0.07
	45	17.6±0.23	15.8±0.24	57.4±1.25	6.3±0.12

The results showed mean value ± standard deviation.

Table 3. Total carotenoid content of wheat bran oil obtained by supercritical and near-critical CO_2 extraction

Pressure (MPa)	Total carotenoid (µg/g oil)				
	Near-critical CO_2 extraction		Supercritical CO_2 extraction		
	25°C	30°C	35°C	40°C	45°C
10	27.9±0.91	27.2±1.13	26.5±0.44	29.3±0.72	29.8±0.95
15	29.3±1.01	31.2±0.54	27.8±1.16	31.5±1.32	33.8±1.41
20	34.1±1.25	33.8±1.19	30.7±0.48	34.7±1.47	35.7±0.36
25	36.0±0.73	35.8±0.96	33.3±1.22	36.4±0.55	37.9±1.29
30	36.8±1.27	36.5±1.28	35.1±0.67	37.1±1.16	39.2±1.48

The results showed mean value ± standard deviation.

was present in the range from 52.2 to 60% of total identified fatty acids. Another unsaturated fatty acid, oleic acid was also present in higher percentage, ranging from 13.3 to 15.9. Within saturated fatty acids, palmitic acid was present in the highest concentration, ranging from 16.8 to 18.6% of total identified fatty acids. The fatty acid compositions were changed moderately at different extraction conditions. But no significant differences of fatty acid compositions of wheat bran oil extracted by supercritical and near-critical CO_2 was found.

Total carotenoid content

Total carotenoid content of wheat bran oil are shown in Table 3. The highest carotenoid was 39.2 µg/g wheat bran oil obtained by supercritical CO_2 extraction at 45°C and 30 MPa. For each temperature, it was found that total carotenoid content of wheat bran oil increased with increasing pressure both at supercritical and near-critical extraction conditions. This trend was attributed to an increase in the density of solvent with pressure. Filho et al. (2008) also reported that total carotenoid yield increased upto the pressure of 30 MPa. On the other hand, there was dual effect of temperature on carotenoids extraction by supercritical CO_2. Total carotenoid yield increased with increasing temperature at supercritical extraction conditions. Raising the temperature decreases the fluid density, but can increase the solute vapour pressure, which enhances the yield of extraction process. Macias-Sanchez et al. (2008) also reported that increase in vapour pressure of carotenoids and the diffusion coefficient of the solvent with increasing temperature caused higher extraction yield. But at near-critical extraction conditions, total carotenoid content of wheat bran oil decreased with increasing temperature. Therefore, at near-critical conditions the effect of the density of solvent was dominant over carotenoids vapour pressure.

Conclusions

Wheat bran oil was extracted by supercritical and near-critical CO_2 extraction at different temperatures and pressures. In this study, the highest yield of oil was found at 25°C and 30 MPa. Wheat bran oil contained highest percentage of linoleic acid in all extraction conditions. Total carotenoid was highest at 45°C and 30 MPa. However, total carotenoid yield by near-critical CO_2 extraction was not significantly reduced compared to supercritical CO_2 extraction. By considering higher cost in high extraction conditions, near-critical CO_2 extraction might be useful to extract oil with high level of antioxidant from different cultivars.

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