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Extraction of Natural Vitamin E from Wheat Germ by Supercritical Carbon Dioxide

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An efficient supercritical fluid extraction (SFE) process with carbon dioxide (SFE-CO_2) was developed for the extraction of natural vitamin E (V_E) from wheat germ. Both the pretreatment of extracted wheat germ and extraction conditions were optimized to ensure maximal V_E yield. The extraction was undertaken at the extracting pressure of 4000−5000 psi, the extracting temperature of 40−45 °C, and the carbon dioxide flow rate of 2.0 mL/min for 90 min. An optimized pretreatment of wheat germ was usually necessary with a particle size of 30 mesh and a moisture content of 5.1%. A yield comparison of V_E and its isomers extracted by supercritical CO_2 with those by conventional solvent extraction suggested that this SFE process was a practical process prospectively superior to conventional solvent extraction to prepare V_E from wheat germ.

KEYWORDS: Supercritical fluid extraction; vitamin E; wheat germ

INTRODUCTION

It has been proven that vitamin E (V_E) exerts several beneficial effects on human health such as free-radical quenching, cancer prevention, resistance against aging, immune enhancement, and antisterility (*1*). According to its source, V_E can be categorized as a synthetic or natural product. It is generally accepted that the natural product is superior to the synthetic one not only for its biological function but also with respect to its dietary safety and public acceptance. An increased demand for the natural product has therefore been observed. Consequently, interest in novel natural V_E sources and the development of efficient extraction techniques is growing (*2*).

Wheat germ, one of the major byproducts of the flour milling industry, is a relatively cheap source of vitamins, minerals, dietary fibers, proteins, and several micronutrients. It is currently applied in the feed industry in a large amount to formulate various animal feeds, although it has been well recognized as one of the richest natural sources of V_E (*3*) and would be therefore considered as an ideal candidate for the large-scale preparation of V_E (*4*, *5*).

Recently, interest in the application of supercritical fluid extraction (SFE) with carbon dioxide (SFE-CO_2) has grown continuously (*6*, *7*) because SFE showed several advantages over classical extraction processes with organic solvents. In summary, supercritical fluid (SF) has high solvent power and solute selectivity. The most commonly used SF, supercritical CO_2, is nontoxic, nonflammable, and noncorrosive, inert to most materials, cheap, and readily available in bulk quantity with satisfied purity. In addition, CO_2 has a low critical temperature (31.1 °C) and pressure (73.8 atm). Supercritical phase CO_2 can therefore be formed economically. SFE can be an efficient extraction process. It allows processing at low temperature (near the critical temperature of SF) to prevent the decomposition of thermal-sensitive products and leaves no solvent residues in the products. The SFE product is therefore safe for the food industry and human health. For these reasons, SFE-CO_2 would be considered to be an ideal technique to extract and prepare various products from natural sources (*6*, *8*).

SFE-CO_2 has been applied for the extraction of V_E from soybean sludge (*9*). Apart from some fundamental studies on the SFE-CO_2 of wheat germ oil (*7*, *10*), very little work has been done on the application of this technique to extract V_E from wheat germ. This paper deals with the optimization of SFE conditions such as pressure, temperature, flow rate, extraction time, and wheat germ pretreatment including its particle size and water content for the extraction of V_E from wheat germ by SFE-CO_2.

MATERIALS AND METHODS

Materials and Chemicals. Raw wheat (*Triricum aestivum*) germ purchased from a local flour mill was carefully cleaned to remove contaminates. All solvents were obtained locally and of analytical grade. Methanol used for V_E analysis was of HPLC grade (Fisons). Liquefied CO_2 with the purity of 99.99% was supplied by a local manufacturer and used without further purification.

Instrumentation. An ISCO-100-DX SFE apparatus (Lincoln, NE, shown in **Figure 1**) was employed for V_E extraction. Wheat germ (up to 5.0 g) was packed in a sample cartridge with the volume of 10 mL. The filled cartridge was inserted into the thermal-controlled extraction chamber. Liquefied CO_2 was introduced into the sample cartridge

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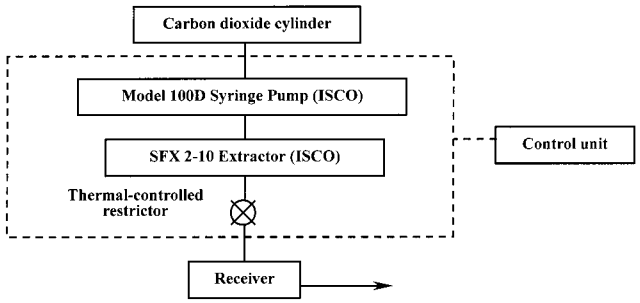

Figure 1. Schematic diagram of ISCO-100-DX SFE apparatus.

through a piston pump with a cooling jacket. Both the pressure and temperature of the cartridge were automatically reached and maintained by a control unit according to settings. After both desired pressure and temperature were reached, opening the pressure-releasing valve located after the cartridge started extraction. The flow rate of CO_2 was regulated by both the pressure-releasing valve and a thermal-controlled restrictor and monitored by a flow meter. Extracts were finally separated from CO_2 phase and collected in 20 mL brown glass tubes at ambient temperature and atmospheric pressure.

Sample Pretreatment. Raw wheat germ was heated by far-infrared rays for 8 min at 105 °C to inactivate enzymes. After 8 min, an additional heating period was necessary to reduce the water content of the material. Variation in this heating period resulted in varied water content (from 4.30 to 11.50%) of the material. The particle of extracted wheat germ was sized from 0.13 to 2.1 mm by grinding and sieving.

Optimization of Extraction Conditions. As described above, both pressure and temperature were set, varied, and maintained through the control unit of the SFE apparatus. The flow rate of CO_2 was regulated by the pressure-releasing valve and restrictor. A significant effort was made in this study to optimize pressure, temperature, and the flow rate of CO_2 to improve the yield of V_E and its isomers.

Yield Determination of V_E and Its Isomers. The amount of total V_E and its isomers including α-, β-, γ-, and δ-tocopherol was determined by HPLC (*11*) with a reversed-phase C18 column (25 cm × 4.0 mm). The mobile phase consisted of methanol and water (98.5:1.5). An isocratic elution was performed at the flow rate of 1.0 mL/min and monitored at 296 nm. Saponification and iodine values of extracts were examined according to the method described by Yu et al. (*12*).

Assay on the Water Content of Wheat Germ. The water content of wheat germ subjected to various pretreatments was assessed according to the method described by Yu et al. (*12*).

Reproducibility. Data from all investigations were represented as the mean value of three replicates and statistically evaluated (*13*).

RESULTS AND DISCUSSION

Effect of Variation in the Water Content of Wheat Germ on V_E Yield. Raw wheat germ was treated by far-infrared rays at 105 °C for 8 min to inactivate enzymes and then for an additional 9, 12, 15, and 18 min to yield pretreated germ with water contents of 11.5, 8.2, 5.1, and 4.3%, respectively. Extraction of those samples allowed the effect of the water content of the germ on V_E yield to be examined. As shown in **Table 1**, V_E yield increased with reduced water content down to 5.1%. However, when the water content was further reduced to 4.3%, V_E yield decreased. Data from **Table 1** suggested that a proper drying process facilitated the penetration of supercritical CO_2 into the tissues of wheat germ and increased the transfer of V_E from the tissues into CO_2 phase. Most probably, a thin film of water was formed between the sample particles and supercritical phase when the water content was high. This film prevented contact between extracted V_E and the supercritical phase. Additionally, the free water from the tissues often resulted in a blocking of the SFE device, especially when the SF is depressed. Fattori et al. (*14*), Liu et al. (*10*), and Ma et al. (*15*) also reported similar observations. It was observed in this study that further reduced water content (<5.1%) resulted in the shrinking of germ particles. V_E transfer was therefore more difficult. Maximal V_E yield was eventually obtained with a water content of 5.1% in this study.

Table 1. Effect of Variation in the Water Content of Wheat Germ on V_E Yield[a]

water content of wheat germ (% w/w)	V_E yield[b] (mg/100 g)
4.3	1470 ± 49^b
5.1	1678 ± 58^a
8.2	1352 ± 46^b
11.5	1290 ± 43^c

[a] SFE conditions: pressure = 3000 psi; temperature = 40 °C; flow rate = 2.0 mL/min; time = 90 min; restrictor temperature = 55 °C; sample amount = 5 g. [b] Each value indicates the mean value of three replicates; values followed by the same superscript are not significantly different ($p < 0.05$).

Table 2. Effect of Variation in the Particle Size of Wheat Germ on V_E Yield[a]

sieving[b] (mesh)	particle size (mm)	V_E yield[c] (mg/100 g)
no grinding	2.1	1610 ± 56^b
20	0.860	1710 ± 59^b
30	0.505	1838 ± 64^a
40	0.390	1550 ± 54^c
60	0.223	1070 ± 37^d
80	0.183	890 ± 31^e
100	0.130	742 ± 26^e

[a] SFE conditions: pressure = 3000 psi; temperature = 40 °C; flow rate = 2.0 mL/min; time = 90 min; restrictor temperature = 55 °C; sample amount = 5 g. [b] Grinding and sieving. [c] Each value indicates the average of three replicates; values followed by the same superscript are not significantly different ($p < 0.05$).

Effect of Variation in the Particle Size of Wheat Germ on V_E Yield. Wheat germ with the water content of 5.1% was ground and fractionated into different particle sizes prepared by sieving and subsequently extracted by SFE-CO_2. The effect of variation in the particle size on V_E yield was summarized in **Table 2**. Maximal V_E yield (1838 ± 64 mg/100 g) was obtained with a particle size of 0.505 mm. Both larger and smaller particles gave lower extraction yields. A properly reduced particle size increased the surface area and accordingly enhanced the contact of extracted V_E with SF. An extensive grinding of the germ may lead to cell wall overbreaking and the formation of V_E microdrops that showed a negative influence on V_E yield, although the formation of such microdrops was expected to facilitate the extraction process. The apparently reduced V_E transfer despite the increased interface between the particles and SF was due to an increased pile density of the raw material, which hampered the penetration of the microdrops into SF. Additionally, very fine particles often formed a hard sample "flake" when a high pressure was applied. As a result, SF preferentially passed through the space between the wall of the extraction cartridge and the flake. This is usually termed a "CO_2 short circuit" (*15*).

Optimization of SFE Conditions. *Pressure.* It is well-known that the extraction pressure is one of the most important parameters in the SFE process because it is the major determinant of solvent power of SF that may have a strong influence on extraction efficiency (*16*).

To determine an optimal pressure for V_E extraction, wheat germ was extracted at a constant 40 °C with varied pressure,

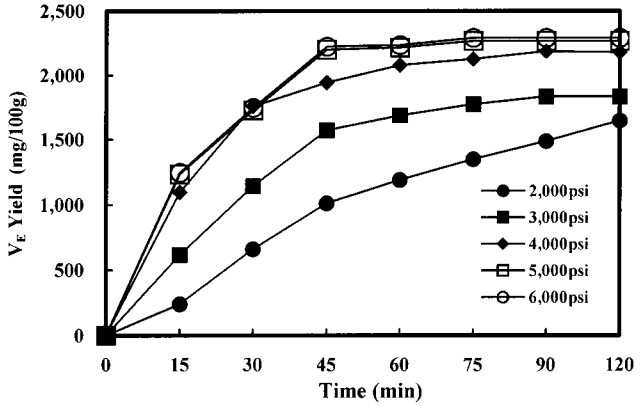

Figure 2. Effect of variation in pressure on V_E yield as a function of extraction time (SFE conditions: temperature = 40 °C; flow rate = 2.0 mL/min; restrictor temperature = 55 °C; sample amount = 5 g).

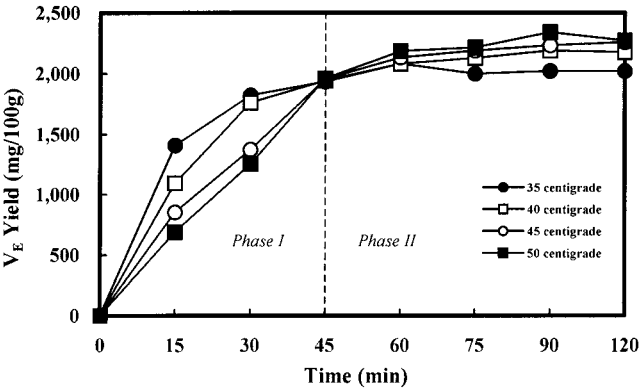

Figure 3. Effect of variation in temperature on V_E yield as a function of extraction time (SFE conditions: pressure = 4000 psi; flow rate = 2.0 mL/min; restrictor temperature = 55 °C; sample amount = 5 g).

and the V_E yield of each extraction was measured. As shown in **Figure 2**, V_E yield increased as a function of pressure. Data from **Figure 2** suggested there was no linear relationship between pressure and V_E yield. An increase in pressure from 2000 to 4000 psi resulted in a slow and insignificant increase in V_E yield. Further increase in pressure from 5000 to 6000 psi did not improve the yield significantly. An extraction pressure in the range between 4000 and 5000 psi would therefore be considered to be optimal for the extraction of V_E from wheat germ in this investigation. It was also observed in practice that an extraction pressure in this range reduced the amount of impurities, such as pigments, from the extracts significantly.

Temperature. The extraction temperature is also important for an SFE process (*17*) and therefore needs to be optimized for any particular sample. To determine an optimal temperature for V_E extraction, wheat germ was extracted at 5000 psi constantly with varied temperature and the V_E yield of each extraction was assessed. **Figure 3** illustrated the effect of variation in temperature on V_E yield as a function of extraction time and suggested that V_E yield decreased with an increase in extraction temperature during the first 45 min. After 50 min, the yields were nearly identical at each temperature and slightly grew with temperature increase. At the end of the extraction, the difference in V_E yield between the extractions was rather small.

In the SFE process, variation in temperature may have an influence on both extract and SF with multiple aspects. For example, increased temperature enhanced the diffusion coefficient of the extracted molecule in SF but reduced the density

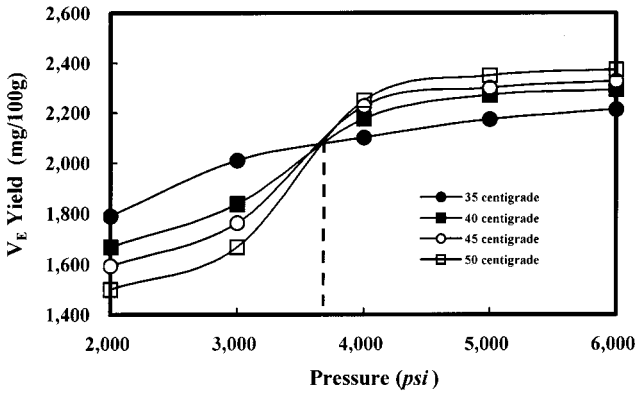

Figure 4. Dependence of V_E yield on pressure and temperature (SFE conditions: flow rate = 2.0 mL/min; restrictor temperature = 55 °C; sample amount = 5 g; extraction time = 90 min).

of the SF and therefore the saturated solubility of the extract in SF. The influence of variation in temperature on V_E yield eventually depended on the relative contribution of each aspect. In this investigation, the extractions would be divided into two phases as shown in **Figure 3**. In phase I (extraction time < 45 min), the reduced density of SF played a major role. In phase II (extraction time > 45 min), the increased diffusion coefficient of the extract exhibited a stronger influence. In practice, 40−45 °C would be preferred to perform an efficient extraction.

When both pressure and temperature were optimized, the dependence of V_E yield on both pressure and temperature should be apparent (see **Figure 4**). It can be observed from **Figure 4** that the curves determined at four different temperatures crossed at a single point close to 3800 psi. This particular intersection and the corresponding pressure are often named the "transformation point" and "transformation pressure", respectively, in the SFE technique (*18*). At pressures below 3800 psi, V_E yield decreased with increased temperature. In contrast, at pressures >3800 psi, V_E yield increased with temperature growth. The main reason for this observation was that the saturated solubility was determined by the vapor pressure of the extract and the density of the SF. At pressures >3800 psi, the density of the supercritical CO_2 was high and its compressibility low. When the temperature increased, the solvent power of supercritical CO_2 decreased. However, at the same time, the vapor pressure, diffusion coefficient, and molecular transfer of extract greatly increased. As a result, solvent−solute affinity increased and the saturated solubility of extract eventually increased as well. In contrast, at pressures <3800 psi, the compressibility of supercritical CO_2 was high, and increased temperature resulted in a rapid reduction in the density of supercritical CO_2. As a result, the enhanced volatility and diffusion coefficient of extract did not fully counteract the reduced density of SF. Apart from those, the thermodynamics of the extraction process would also be concerned. Increased temperature may have a positive influence on the extraction efficiency when the extraction was an endothermic process but reduced the yield of extract when the extraction was an exothermic process. It can therefore be concluded that the extraction efficiency eventually depended on the crossed influence of three factors mentioned as above.

In practice, especially in industrial applications, transformation pressure was preferable because extraction efficiency was only slightly influenced by temperature at this pressure. In this study, 3800 psi and a temperature between 40 and 45 °C would be considered as proper conditions to extract V_E from wheat germ with less energy consumption.

Table 3. Effect of Variation in Flow Rate of CO_2 on V_E Yield[a]

flow rate (mL/min)	V_E yield[b] (mg/100 g)
1.0	948 ± 45[c]
1.5	1482 ± 61[b]
2.0	1838 ± 64[a]
2.5	1882 ± 87[a]
3.0	1927 ± 92[a]

[a] SFE conditions: pressure = 3000 psi; temperature = 40 °C; flow rate = 2.0 mL/min; extraction time = 90 min; restrictor temperature = 55 °C; sample amount = 5 g. [b] Each value indicates the average of three replicates; values followed by the same superscript are not significantly different ($p < 0.05$).

Table 4. Yield Comparison of V_E and Its Isomers Extracted by SFE-CO_2[a] with Those by Conventional Solvent Extraction

yield (mg/100 g)	SFE-CO_2	hexane[b]	$CHCl_3$/MeOH[b]
α-tocopherol	1329	1275	586
β-tocopherol	458	879	1261
γ-tocopherol	305		
δ-tocopherol	87		
total V_E	2179	2154	1874

[a] SFE conditions: pressure = 4000 psi; temperature = 40 °C; flow rate = 2.0 mL/min; extraction time = 90 min; restrictor temperature = 55 °C; sample amount = 5 g. [b] Mill germ of durum wheat, which was admixed with bran and endosperm, was used in this study (*3*).

Flow Rate of CO_2. The flow rate of CO_2 also showed an influence on V_E yield in this study. To determine the influence of flow rate, wheat germ was extracted at 3000 psi and 40 °C for 90 min with varied flow rate. **Table 3** showed a positive but nonlinear correlation between V_E yield and the flow rate of CO_2.

It can be observed from **Table 3** that increased flow rate of CO_2 from 1.0 to 2.0 mL/min resulted in a doubled yield. However, at higher flow rates such an increase in V_E yield was quite limited. Obviously, the optimal flow rate of CO_2 for the extraction of V_E from wheat germ should be ~2.0 mL/min.

Extraction Time. The time course of an SFE process usually consists of three stages: (i) equilibrium control, (ii) transformation, and (iii) diffusion control (*6*). **Figures 2** and **3** showed that the V_E extraction from wheat germ followed a similar time course. Both figures clearly demonstrated that V_E yield increased more or less linearly as a function of extraction time during the initial phase of the process. Thereafter, the slope of the curves progressively decreased to reach a maximal value in ~90 min. For maximal V_E yield, 90 min was necessary for the extraction in this study.

Comparison of SFE-CO_2 with Conventional Solvent Extraction. *Yields of V_E and Its Isomers.* The yields of V_E and its isomers from wheat germ extracted by SFE-CO_2 were assessed by HPLC and compared with those prepared by hexane and chloroform/methanol extraction (*3*) in this study (see **Table 4**). **Table 4** suggested that the yields of V_E and α-tocopherol extracted by SFE-CO_2 were much higher than those prepared by chloroform/methanol extraction. However, the solution of chloroform/methanol showed a stronger "solvent power" to extract β-tocopherol form wheat germ.

The difference in the chemical composition of the extracts prepared by SFE-CO_2 and solvent extraction leads to variation in iodine value of the extracts. The iodine values of the extracts prepared by SFE-CO_2, hexane, and chloroform/methanol extraction were 130, 127, and 116, respectively. The saponification value (188) of extract by SFE-CO_2 showed only a slight increase in comparison with those (182 and 183, respectively) by hexane and chloroform/methanol extraction.

Extraction Process. Grela et al. (*3*) reported that the extraction of natural V_E by hexane and chloroform/methanol took 960 and 140 min, respectively, if a satisfied V_E yield was achieved. The SFE-CO_2 process developed in this study took only 90 min to reach the V_E yield shown in **Figures 2** and **3**. Additionally, the lower extraction temperature (40 °C) of this process might be able to reduce the loss of thermally sensitive components from extracts in comparison with that (70 and 65 °C, respectively; *3*) of hexane and chloroform/methanol extraction.

Conclusion. An efficient SFE-CO_2 process was developed to successfully extract V_E including its isomers from wheat germ with a satisfactory yield. The extraction was performed at 4000−5000 psi and 40−45 °C for 90 min with the flow rate of 2.0 mL/min. Extracted wheat germ was pretreated to form a proper particle size (30 mesh) with a water content of 5.1% before the extraction. The yield comparison of V_E and its isomers by this SFE-CO_2 process with those by conventional solvent extraction suggested that the quality of product prepared by this process was better than that by solvent extraction. The possibility to scale up this process in industrial base would therefore be considered.

ABBREVIATIONS USED

CO_2, carbon dioxide; HPLC, high-performance liquid chromatography; SFE, supercritical fluid extraction; SFE-CO_2, supercritical fluid extraction with carbon dioxide; SF, supercritical fluid; V_E, vitamin E.

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