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Quality characteristics of soybean germ oil obtained by innovative subcritical butane experimental equipment

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RESEARCH ARTICLE

Abstract

Subcritical butane extraction (SBE) was used to extract oil from soybean germ and was compared with conventional n-hexane extraction (CHE) and supercritical CO_2 extraction (SC-CO_2E) methods. By analysing the extraction yield, composition of fatty acids and characteristic functional contents such as tocopherol, phytosterols and phospholipids in soybean germ oil (SGO), the selective extraction characteristics of subcritical butane were evaluated. The results indicate that compared to CHE and SC-CO_2E, SBE had no significant difference on oil yield, fatty acid compositions, acid value and peroxide value of SGO. However, the contents of phytosterol and tocopherol in the oil extracted by SBE (2,772.9 and 188.9 mg/100 g) were evidently higher than those extracted by CHE (1,437.2 and 171.3 mg/100 g), with both being lower than those of supercritical CO_2 extracted oil (3,982.4 and 227.6 mg/100 g). And, the content of phospholipids in SGO extracted by SBE (23.92 mg/g) was lower than that extracted CHE (38.47 mg/g). In conclusion, SBE was able to retain the bioactive compounds in SGO when compared with CHE.

Keywords: extraction, phytosterol, quality, soybean germ oil, subcritical butane

1. Introduction

Soybean germ (SG) offers fat (10%), protein (40%), oligosaccharides (14%) and active ingredients, such as phytosterols, tocopherols, isoflavones, etc. (Gu and Gu, 2000). Soybean germ oil (SGO) has a number of health benefits such as reducing plasma and liver cholesterol levels, delaying aging and so on. The health benefits of SGO are attributed to its high content of phytosterols, tocopherols and polyunsaturated fatty acid (Bonina *et al.*, 2005; Ozawa *et al.*, 2001). With the improvement of people's living, the need for nutritional oils enriched in bioactive compounds is increasing. SGO is a type of functional oil which has a great potential for further exploitation.

Recently, the main use of SG has been the extraction of SGO, obtained using solvent extraction and supercritical CO_2 extraction (SC-CO_2E) methods (Song *et al.*, 2007; Yu *et al.*, 2007). Hexane is commonly used as the extraction solvent (Bonina *et al.*, 2005; Ozawa *et al.*, 2001). However, when the residual solvent is separated from the miscella and wet meal by means of steam heating, conventional hexane extraction (CHE) methods not only result in oxidation and degradation of heat-sensitive components but also limit further development and use of the residual germ (Panfili *et al.*, 2003; Pradhan *et al.*, 2010). Extraction with hexane also leads to high utility costs due to high energy and water consumption (Wang and Liu, 2005). The SC-CO_2E method can ensure the high-value-added quality of the oil, and CO_2 is cheaper than other solvents (Pereira and Meireles, 2010). However, it has shortcomings such as relatively high equipment investment, low processing capacity and high cost of production (Sahena *et al.*, 2009). These shortcomings have kept this technology from being widely considered as an alternative. Compared with SC-CO_2E, subcritical fluid extraction is an extraction method that guarantees quality, has higher productivity and is easier to industrialise. Thus, for the grain germs (soybean germ, corn germ, etc.), the subcritical fluid extraction technique is a better choice to maintain the quality of the products. Several solvents can be used in subcritical fluid extraction of added oil or active components from natural products, such as butane, propane, liquefied petroleum gas, 1,1,1,2-tetrafluoroethane alkyl, and dimethyl ether (Catchpole *et al.*, 2010). Rosenthal and Trevithlek (1934) investigated the extraction of cottonseed oil with a liquefied butane and propane mixture and obtained 97% oil with light colour and low refining losses. Wang (2012) studied sesame oil using subcritical butane extraction (SBE). The results showed that most sesamin was obtained and remained in the sesame oil after extraction and more than 90% of the soluble protein was preserved in the defatted sesame meal. Moreover, compared to CO_2, propane has higher extraction efficiency and, as a result, requires less solvent (Corso *et al.*, 2010; Pederssetti *et al.*, 2011). In 1993, Qi and Wang (1995) built the first industrial line for oil extraction based on liquefied petroleum gas extraction techniques and developed high-quality products such as low-temperature soybean protein, wheat germ oil, grape seed oil and others.

Many researchers (Corso *et al.*, 2010; Hamdan *et al.*, 2008) have used the same equipment or design ideas as those for SC-CO_2E in studies of subcritical propane extraction. However, these extractors are still constructed as a small-diameter and limited-height cylindrical vessel to maintain the efficiency of extraction occurring through a fixed bed of material. In this context, we designed subcritical fluid extraction equipment using a single or mixed solvent. A batch and stirring system were adopted for extraction. The difficulties of enlarging capacity were overcome by the design of these devices. The objective of this research was to study the SBE of SGO obtained by our self-designed subcritical fluid extraction equipment and the quality characteristics of the extracted oil compared with those extracted by n-hexane and supercritical CO_2.

2 Materials and methods

Chemicals and materials

SG (≥95% purity) was provided by Jiusan Oil & Fat Co., Ltd. (Heilongjiang, China P.R.) and stored, sealed, at -20 °C until use. Standards of α-tocopherol (≥95% purity), β-tocopherol (≥95% purity), γ-tocopherol (≥96% purity), and δ-tocopherol (≥95% purity) came from Supelco (Bellefonte, PA, USA). Plant sterol mixture (13% brassicasterol, 26% campesterol, 7% stigmasterol, and 53% beta-sitosterol) was obtained from Matreya LLC (Pleasant Gap, PA, USA). Bis(trimethylsilyl) trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS) (99:1) was purchased from Tokyo Chemical Industry Co., Ltd. (Shanghai, China P.R.). A 5-component fatty acid methyl ester mix (methyl palmitate, methyl stearate, methyl oleate, methyl linoleic acid and linolenic acid methyl ester standard) was purchased from Supelco. All of the other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China P.R.) and were analytical or chromatographic grade.

Sample preparation

SG (9.34±0.21% raw moisture content, wet basis, w/w) was pre-treated under hygrothermal conditions (temperature 70 °C; humidity 85%) until reaching a moisture content of 13-15% (wet basis, w/w), then flaked (0.2-0.25 mm) and dried in a vacuum oven at 45 °C for 3 h until reaching a moisture content of 7-8% (wet basis, w/w). The moisture content of raw SG was measured using a halogen moisture analyser (model HB43-S, Mettler Toledo International Inc., Shanghai, China P.R.).

Subcritical butane extraction

The experiments were performed using self-designed subcritical fluid extraction equipment and the schematic structure of this equipment and the experiment method were described in detail in previously published literature (Xu *et al.*, 2016).

Flaked and dried SG (500 g) was loaded into a 5-l extraction vessel. The air in the extraction vessel was evacuated by a vacuum pump. The butane was injected into the extraction vessel with the assistance of a pressure difference. During the extraction process, the ratio of material to liquid was 1:2 (SG : solvent, w/v), and the extraction temperature was controlled at 45 °C through heating using recycled hot water, and the pressure was 0.34 Mpa. After extraction (30 min), the miscella was moved from the bottom of the extraction vessel to the evaporation vessel. The raw material sample was extracted three times to ensure maximum extraction yield. Finally, the miscella and the defatted SG residue were evaporated with reduced pressure (45 °C for 0.5 h). The butane gas was compressed through the compressor and condensed at the condenser for recycling. The vacuum pump was turned on to produce further evaporation of the butane at a pressure of -0.1 Mpa. The SGO was taken out from the bottom of the evaporation vessel. Next, hexane (25 ml) was used to wash the evaporation vessel to collect the residual oil. Then, the hexane was distilled and oil yields were determined using a gravimetric method.

Supercritical CO_2 extraction

The SC-CO_2E was performed using a laboratory scale supercritical fluid extraction unit (model HA121-50-01; Hua'an Supercritical Fluid Extraction Corp., Nantong, China P.R.). For each extraction, 500 g of SG were placed in the 1-l sample vessel. The high-purity CO_2 used for supercritical fluid extraction was supplied by Wuxi Gas Inc. (Wuxi, China P.R.).

In this study, the SC-CO_2E experiments were performed at 45 °C under a pressure of 35 MPa for 2 h with a constant flow rate of CO_2 at 26 l/h. The expanded CO_2 was measured by a flow totaliser and the mass flow rate of 2.95 kg/h was calculated. The temperature and pressure in the separation tank were 45 °C and 5 Mpa, respectively. The yield of SGO reached its maximum value after 2 h and SGO was collected from the bottom of the separation tank. Those experimental conditions were controlled by adjusting the valves on the front panel. Finally, hexane (25 ml) was used to wash the separation tank to ensure collection of the residual oil and the oil yields were determined using a gravimetric method.

Conventional n-hexane extraction

In order to obtain maximum oil yield of the samples by solvent extraction, SG (50 g) was extracted with n-hexane each time in a Soxhlet apparatus for 6 h following the ISO 734-1:2006 (ISO, 2006). The miscellas were combined and separated by vacuum evaporation in a water bath heated by vacuum rotary evaporator (Mode RE-52A, Shanghai Yarong Biochemical Instrument Factory, Shanghai, China P.R.) at 45 °C for 1 h. The extraction yield was determined gravimetrically and reported as percentage: (g extract / g dry germ) × 100.

Analysis of fatty acid compositions

To obtain fatty acid composition of the oil samples, the SGO was derivatised into fatty acid methyl esters and analysed by gas chromatography (GC). Oil samples (0.1 g) were esterified with a 0.5 N NaOH-methanol solution catalysed by 14% BF3/MeOH solution explained in the same method. GC was performed with a Hewlett-Packard model 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with an HP-INNOWax capillary column (30 m, 0.25-mm i.d., and 0.25-µm film thickness; Agilent Corp., Palo Alto, CA, USA) and a flame ionisation detector. The injection and detector temperatures were 250 and 260 °C, respectively. The nitrogen carrier gas flow rate was 1.0 ml/min. The oven temperature was held at 180 °C for 4 min and increased to 240 °C at the rate of 3 °C/min. The temperature was held at 240 °C for 15 min. The sample (1 µl) was injected into the gas chromatograph with a split ratio of 40:1. The peaks in the GC graph were identified based on their retention times using authentic standard fatty acid methyl esters. All samples were run in triplicate.

Analysis of phytosterols

Oil samples (0.5 g) were saponified by refluxing with 1.0 M KOH (10 ml) in methanol for 45 min. The unsaponifiable matter was extracted by hexane three times. The hexane phase collected from three extractions was combined and washed with deionised water until neutrality and then dried with anhydrous sodium sulfate. The solvent was evaporated to dryness in a vacuum at 40 °C. 2 ml acetone was added to the residue, and the solution was transferred to a 2.5-ml Eppendorf tube. The solvent was evaporated to dryness under the protection of nitrogen, then 200 µl anhydrous pyridine and 200 µl BSTFA + TMCS (99:1) were added into the 2.5-ml Eppendorf tube. The solution was sealed and heated at 70 °C for 30 min for derivatisation. Chloroform was added to reach a total sample volume of 1 ml before analysis.

Trimethylsilyl derivatives of phytosterols were analysed with an Agilent 7890A gas chromatography system (Agilent Technologies, Inc., Palo Alto, CA, USA) equipped with a flame ionisation detector. An HP-5 capillary column (30 m, 0.25-mm i.d., 0.32-µm film thickness; Agilent Technologies, Inc.) was used for the analysis. The oven temperature was held at 190 °C for 2 min and ramped to 230 °C at the rate of 20 °C/min. The temperature was held at 230 °C for 5 min. The oven was then ramped a second time to 255 °C at the rate of 40 °C/min and held for 25 min at 255 °C. Nitrogen was used as the carrier gas at a flow rate of 1.0 ml/min. The injection and detector temperatures were both 300 °C. The sample (1 µl) was injected into the gas chromatograph with a split ratio of 50:1. The phytosterol compositions of the samples were identified by direct comparison of their chromatographic retention times with known standards. The corresponding standards were quantified using external calibration curves. The content of campesterol, stigmasterol and β-sitosterol (mg) and area of peaks is well linearly related: $y = 976.7x - 3.2632$ ($R^2=0.9991$), $y = 1{,}026.1x - 6.5295$ ($R^2=0.9924$) and $y = 1{,}144.8x - 10.499$ ($R^2=0.9964$), respectively (x = the content of campesterol, stigmasterol or β-sitosterol; y = area of peaks).

Analysis of tocopherols

Tocopherol content of the oil samples was analysed by a high-performance liquid chromatography (HPLC) method. The oil samples were dissolved in hexane (about 10.0 mg/ml) and filtered through a 0.45-µm PTFE membrane filter. The samples were analysed with a Shimadzu LC-20A HPLC system (Shimadzu Corp., Kyoto, Japan) equipped with an ultraviolet detector. A normal-phase HPLC column, Zorbax RX-SIL (250 mm, 4.6 mm i.d., and 5-µm particle size; Agilent Technologies, Santa Clara, CA, USA), was used for separation of the tocopherol isomers. Analytical separation of the oil components on the column was performed using a mobile phase consisting of hexane : isopropyl alcohol (98.5:1.5, v/v). Total run time, column temperature and flow rate were 25 min, 27 °C and 0.9 ml/min, respectively. The ultraviolet detector was set at 292 nm absorption wavelength. Peaks were quantified by area and standards of α-, β-, γ- and δ-tocopherol were used to elaborate calibration curves, respectively. The correlation coefficient was higher than 0.99 in all of the cases. The content of α-, γ- and δ-tocopherol (µg) and area of peaks is well linearly related: $y = 491{,}737x - 18{,}841$ ($R^2=0.9999$), $y = 414{,}375x + 101{,}906$ ($R^2=0.9996$) and $y = 375{,}765x$ ($R^2=0.9999$), respectively (x = the content of α-, γ- or δ-tocopherol; y = area of peaks).

Analysis of phospholipids

The content of phospholipids was determined using a colorimetric method according to the recommended methods of the Standardization Administration of the People's Republic of China (SAC, 2008; GB/T5537-2008). 0.5 g oil sample was weighed to the crucible, and 0.1 g of zinc hydroxide was added. The oil sample was heated to carbonisation before being transferred to a muffle furnace for burning to gray completely. The ash was dissolved in 5 ml HCl solutions (1:1, v/v) and boiled for 5 min before cooling to room temperature. The solution was filtered and injected into a volumetric flask (100 ml). 50% (w/v) KOH solution was added until the mixture became turbid. HCl solution (1:1, v/v) was slowly added until the zinc hydroxide completely dissolved. Finally, the solution was diluted with distilled water to 100 ml. Control was prepared simultaneously.

10 ml sample solutions were transferred into 50 ml colorimetric tubes with pipettes. 8 ml hydrazine sulfate solution (mass fraction 0.015%) and 2 ml dilute sulphuric acid solution of sodium molybdate (mass fraction 2.5%) were added sequentially. The colorimetric tube was put into a boiling water bath for 10 min after vortex mixing. The sample was cooled to room temperature and diluted to 50 ml with distilled water. The solution was measured at 650 nm by spectrophotometer (model UV-9600; Rayleigh, Beijing, China P.R.). A standard curve of standard phosphorus solution was drawn. The absorbance and content of phosphorus (mg) is well linearly related: $y = 5.7837x + 0.0003$, $R^2=0.9999$ (x = content of phosphorus (mg); y = absorbance). The content of phosphorus was calculated according to the standard curve and the content of phospholipids in the oil sample was calculated.

Determination of free fatty acid

The acid value measures the content of free fatty acids in oil samples. Analyses of the acid value (mg KOH/g) were carried out using a standard titration method according to the official methods described in the method for analysis of hygienic standard of edible oils of the Standardization Administration of the People's Republic of China (SAC, 2003; GB/T5009.37-2003). The extracted oil was dissolved in neutral diethyl ether-ethanol medium (2:1, v/v) and titrated with 0.05 N KOH solution using phenolphthalein as indicator.

Determination of peroxide value

The peroxide value of the oils was determined according to the recommended methods of the Standardization Administration of the People's Republic of China (SAC, 2005; GB/T5538-2005). The extracted oil was dissolved in acetic acid-isooctane medium (3:2, v/v) and titrated with 0.01 N $Na_2S_2O_3$ using starch solution (5 g/l) as indicator.

Statistical analysis

The results reported are the averages of three measurements. The data are reported as mean value ± standard deviation. An analysis of variance (ANOVA) was performed using SPSS Statistics software version 17.0 (SPSS, Chicago, IL, USA). The least significant difference test was used to evaluate the differences between the values ($P<0.05$).

3. Results and discussion

Extraction procedure and yield

Table 1 shows the experimental conditions and extraction yield of SGO from SBE, SC-CO_2E and CHE methods. The extraction yield was calculated with the mass of oil extracted as a percentage of the mass of raw material (dry basis) fed into the extractor. To make the study comparable, each extraction method used an optimised extraction process to maximise the extraction of oil. It is worth noting that unlike the SC-CO_2E and CHE methods, SBE is a semi-continuous extraction method. Therefore, the sample was extracted three times to optimise the oil yield. Experimental conditions for SC-CO_2E, experimental conditions were

Table 1. Experimental conditions and extraction yield of soybean germ oil samples extracted by three methods.[1]

Extraction method[2]	Extraction pressure (Mpa)	Extraction temperature (°C)	Solid-liquid ratio or flow rate	Extraction time (h)	Desolventizing pressure (Mpa)	Desolventizing temperature (°C)	Yield (%, w/w) (dry basis)[3]
SBE	0.34	45	1:2 (w/v)	1.5	-0.1	45	9.3a±0.10
SC-CO_2E	35	45	2.95 (kg/h)	2	5	45	9.0a±0.14
CHE	0.10	–	–	6	-0.09	45	9.2a±0.06

[1] Values are reported as the mean ± standard deviation based on triplicate extractions and analyses. Means with different superscript letters in the same column indicate significant differences ($P<0.05$).

[2] CHE = conventional hexane extraction; SBE = subcritical butane extraction; SC-CO_2E = supercritical CO_2 extraction.

[3] The extraction yield was calculated as the mass of oil extracted as a percentage of the mass of raw material fed (dry basis) into the extractor.

selected according to the procedure which was described in previous studies (Shao *et al.*, 2008; Sparks *et al.*, 2006). The SC-CO_2E SGO was centrifuged to remove water. From Table 1, it can be noted that the SBE method produced an 9.3% yield (dry basis, w/w), which was slightly higher than that of the SC-CO_2E (9.0%) or CHE (9.2%) methods. However, there were no significant differences ($P>0.05$) in the yields of oil that were obtained by these three methods

Compared with SC-CO_2E, SBE had a lower operation pressure (0.34 MPa) which was 1/100 that of SC-CO_2E under the chosen extraction processing (Table 1). In this study, we can infer that SBE had a lower equipment cost and higher safety production. This viewpoint agrees with the research of Spark *et al.* (2006). However, due to the complexity of assessing economic feasibility, further research is recommended using an industrial- scale process. As shown in Table 1, when dealing with the same amount of raw material, the duration of SBE (1.5 h) was shorter than SC-CO_2E (2 h). However, the extraction yield of the former was slightly higher than that of the latter. These results indicated that the efficiency of SBE was higher than SC-CO_2E. Many researchers have pointed out that the solubility of vegetable oils in propane is higher than that in CO_2 (Corso *et al.*, 2010; Hamdan *et al.* 2008; Pederssetti *et al.*, 2011) because the oil has a high percentage of triacylglycerols and their fatty acid esters have lowest solubility in CO_2 (Sovova *et al.*, 2001; Yu *et al.*, 1994). Tres *et al.* (2009) and Lanza *et al.* (2005) indicated that soybean oil and castor oil, respectively, are much more soluble in n-butane than in propane. In contrast to hexane, the use of butane offers the advantages of lower separation costs and solvent-free products.

Fatty acid compositions

Table 2 shows the content and composition of major fatty acids at the three different SGO extraction conditions. The results showed that the major fatty acids in SGO included palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). The total contents of unsaturated fatty acid and polyunsaturated fatty acid in the SGO was more than 80% and 70%, respectively. The most abundant unsaturated fatty acid was linoleic acid (C18: 2) (>50%), followed by linolenic acid (C18: 3) about 20%, which agreed to the results of Gu and Gu (2000) (C16:0, 13.2%; C18:0, 3.0%; C18:1, 6.7%; C18:2, 52.7%; C18:3, 23.7%). The composition of fatty acid of SGO obtained by these three methods had no significant difference ($P>0.05$) (Table 2). Researches (Corso *et al.*, 2010; Pederssetti *et al.*, 2011) have reported that the sesame seed oil and canola seed oil both can be extracted using subcritical propane, supercritical CO_2 and hexane as solvents. Researchers also found that these methods do not influence the fatty acid distribution significantly in the extracted oil (Corso *et al.*, 2010; Pederssetti *et al.*, 2011). These results indicated that the triglycerides and fatty acids were non-selectively extracted and that isomerisation and/or oxidation of fatty acids did not occur under the extraction conditions (Dunford and Martinez, 2003).

Table 2. Fatty acid composition of soybean germ oil (SGO) samples extracted by three methods.[1,2]

Fatty acid (%)	SBE SGO	SC-CO_2E SGO	CHE SGO
C16:0	13.13a±0.07	12.95a±0.23	12.99a±0.12
C18:0	3.48a±0.01	3.50a±0.02	3.48a±0.01
C18:1	9.50b±0.03	9.79a±0.14	9.34b±0.06
C18:2	51.36a±0.02	51.29a±0.15	51.47a±0.14
C18:3	22.53a±0.06	22.93a±0.36	22.71a±0.11
SFA	16.61a±0.08	16.45a±0.25	16.47a±0.13
UFA	83.39a±0.06	83.51a±0.23	83.52a±0.12
PUFA	73.89a±0.08	73.52a±0.35	73.68a±0.10

[1] Gas-chormatogram area percentage. Values are reported as the mean ± standard deviation based on triplicate analyses. Means with different letters in the same row indicate significant differences ($P<0.05$).

[2] CHE = conventional hexane extraction; PUFA = polyunsaturated fatty acid; SBE = subcritical butane extraction; SC-CO_2E = supercritical CO_2 extraction; SFA = saturated fatty acid; UFA = unsaturated fatty acid.

Phytosterols

Table 3 presents the phytosterol compositions of SGO samples extracted by the three methods. From Table 3, it can be clearly noted that β-sitosterol, campesterol and stigmasterol are the main phytosterols in the SGO, with β-sitosterol as the most prominent (over 80% of the total phytosterols), which agrees with the viewpoints of Gu and Gu (2000). The total phytosterol content of CHE SGO is similar to the result of Ozawa *et al.* (2001). Cravotto *et al.* (2005) found that soybean germ oil had 44.35 mg/kg total sterols. The total phytosterol content of the SBE SGO was 2,772.9 mg/100 g, which was significantly higher than that of the CHE SGO (1,437.2 mg/100 g) and lower than SC-CO_2E SGO (3,982.4 mg/100 g). These results showed that these three methods of extraction had a significant effect on the content of phytosterol in the extracted oil ($P<0.05$). Dunford *et al.* (2009) also demonstrated that the solvent type and temperature had significant effects on both phytosterol content and composition of extracts collected from wheat germ oil fractions. The reason is that physicochemical properties (dielectric constant, density, viscosity and diffusion coefficient in oil) of extraction solvents are significantly different (Pronyk and Mazza, 2009). Compared with hexane and supercritical CO_2, subcritical butane has a lower viscosity and higher diffusion coefficient in oil. Comparing these three methods, the phytosterols were extracted selectively by subcritical butane.

Table 3. Phytosterol compositions (mg/100 g) of soybean germ oil (SGO) samples extracted by three methods.[1]

Samples[2]	Campesterol	Stigmasterol	β-sitosterol	Total phytosterol
SBE SGO	269.1b±15.6	181.3b±12.2	2,322.5b±51.2	2,772.9b±49.8
SC-CO_2E SGO	424.1a±27.3	276.1a±14.2	3,282.2a±63.4	3,982.4a±51.2
CHE SGO	87.5c±12.3	81.4c±11.2	1,267.3c±28.6	1,437.2c±18.2

[1] Values are reported as the mean ± standard deviation based on triplicate analyses. Means with different letters in the same column indicate significant differences (*P*<0.05).

[2] CHE = conventional hexane extraction; SBE = subcritical butane extraction; SC-CO_2E = supercritical CO_2 extraction.

Although the selective extraction power of subcritical butane was weaker than that of supercritical CO_2, it was much more intense than that of n-hexane.

Tocopherols

Tocopherol content and compositions of the SGO samples are shown in Table 4. As can be seen, SGO was rich in tocopherols (171.3-227.6 mg/100 g), which is close to wheat germ oil and corn germ oil (50-250 mg/100 g) (Dunford *et al.*, 2009). The main tocopherols in SGO were γ-tocopherol (about 64% of the total tocopherols), α-tocopherol (about 25%) and δ-tocopherol (about 11%). These results showed the significant difference with Cravotto *et al.* (2005), who reported the contents of α-tocopherol, β- plus γ-tocopherol, δ-tocopherol and total tocopherols in SGO were 1,962.0, 2,156.0, 235.0 and 4,354.0 mg/kg, respectively. The main reason was probably that the source of raw materials were (soybean germ) from different varieties of soybean. The explanation can be proved by Yoshida *et al.* (2006) who working with characterisation of tocopherols within the different varieties of soybean and different parts of soybean parts.

Table 4, the SBE SGO (188.9 mg/100 g) contained a higher amount of tocopherols than the CHE SGO sample (171.3 mg/100 g) and lower than the SC-CO_2E SGO sample (227.6 mg/100 g). These results showed that these three methods also had significant effect on the content of tocopherol in the extracted oil ($P<0.05$).

Nimet *et al.* (2011) studied the α-tocopherol content in sunflower oil extracted by subcritical propane (30 °C, 60 °C, 8 MPa, 12 MPa), supercritical CO_2 (40 °C, 60 °C, 19 MPa, 25 MPa) and hexane (Soxhlet, 69 °C). They found that the α-tocopherol content of extracted oil decreased in the following order: subcritical propane extraction > SC-CO_2E > CHE under the experimental conditions. According to this result, we might infer that the content of tocopherols in SBE SGO should be the highest. Hadolin *et al.* (2001) reported that the content of vitamin E in propane extracts was generally lower than that of supercritical CO_2 extracts. In addition, SBE SGO contained a higher amount of tocopherols than the CHE SGO, which might be due to effective limitation on oxidation reactions and low temperature when SBE was used to extract soybean germ. These results suggested that the SBE was able to preserve the tocopherols in SGO.

Phospholipid

Phospholipids are regarded as an antioxidant synergist and a bioactive component. However, phospholipids are detrimental to colour, flavour, foaming and smoke stability of the finished product. In addition, phospholipids pose many problems for the storage and processing of the crude oil and must be removed by purification (Krings and Berger, 2001). Therefore, a method that extracts less phospholipid in oil would be more beneficial to oil refining.

As shown in Table 5, CHE SGO (38.47 mg/g) contained the highest amount of phospholipids of the samples in this study. The numeric value is close to the result of former literature (43.5 mg/g) Gu and Gu (2000). The SC-CO_2E SGO (0.45 mg/g) displayed the lowest level of phospholipids content of all of the SGO samples. The SBE SGO (23.92 mg/g) fell within the middle level. Phospholipids are more easily extracted in hexane than in subcritical butane, whereas the solubility of phospholipids is very low in pure supercritical CO_2 (Dunford and Temelli, 1995; Eisenmenger *et al.*, 2006). These results indicated that subcritical butane offered selective extraction for phospholipids compared with n-hexane, thus lowering the extraction yield of phospholipids and making the oil easier to refine, whilst also decreasing the loss of other active components.

Acid value and peroxide value

Preferences for fat and oil products with fresh bland flavours and odours require evaluations of quality and rancidity both during development and after processing. The acid value and peroxide value are widely used chemical tests for fat and oil quality determinations. The peroxide value measures the amount of primary oxidation that has taken

Table 4. Tocopherol compositions (mg/100 g) of soybean germ oil (SGO) samples extracted by three methods.[1]

Samples[2]	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherol
SBE SGO	46.1b±3.6	ND[3]	121.6b±0.5	21.2b±0.4	188.9b±3.1
SC-CO_2E SGO	57.0a±0.8	ND	145.0a±4.5	25.1a±1.6	227.6a±4.5
CHE SGO	43.3b±1.4	ND	109.1c±1.7	19.0c±0.8	171.3c±0.9

[1] Values are reported as the mean ± standard deviation based on triplicate analyses. Means with different letters in the same column indicate significant differences ($P<0.05$).

[2] CHE = conventional hexane extraction; SBE = subcritical butane extraction; SC-CO_2E = supercritical CO_2 extraction.

[3] ND = not detected.

Table 5. Phospholipid content of soybean germ oil (SGO) samples extracted by three methods.[1]

Samples[2]	Phospholipid (mg/g)
SBE SGO	23.92b±0.52
SC-CO_2E SGO	0.45c±0.08
CHE SGO	38.47a±0.31

[1] Values are reported as the mean ± standard deviation based on triplicate analyses. Means with different letters in the same column indicate significant differences ($P<0.05$).

[2] CHE = conventional hexane extraction; SBE = subcritical butane extraction; SC-CO_2E = supercritical CO_2 extraction.

Table 6. Acid value and peroxide value of soybean germ oil (SGO)extracted by three methods.[1]

Samples[2]	Acid value (mg KOH/g)	Peroxide value (mmol/kg)
SBE SGO	3.57a±0.02	2.41a±0.02
SC-CO_2E SGO	3.56a±0.00	2.35a±0.02
CHE SGO	3.51a±0.16	2.43a±0.12

[1] Values are reported as the mean ± standard deviation based on triplicate analyses. Means with different letters in the same column indicate significant differences ($P<0.05$).

[2] CHE = conventional hexane extraction; SBE = subcritical butane extraction; SC-CO_2E = supercritical CO_2 extraction.

place in the oil and has resulted in the production of allylic hydroperoxides. The acid value is used to measure the amount of free fatty acids present. These two parameters show good correlation with sensory flavour scores. Storage time has a significant effect on the acid value and peroxide value of oil. Thus, the acid value and peroxide value of all of the oil samples obtained from different extraction methods were determined immediately. As Table 6 showed the acid value and peroxide value of SBE SGO were 3.57 KOH mg/g and 2.41 mmol/kg oil, respectively, similar to the values of CHE SGO and SC-CO_2E SGO. Table 6 also indicated that the methods used for oil extraction had an insignificant effect on the acid value and peroxide value of oil ($P>0.05$). The results also showed that use of SBE had no influence on the initial acid value and peroxide value of SGO. Therefore, we can conclude that the initial acid value and peroxide value of the oil were not related to the extraction process under the experimental conditions in this study but were affected mainly by the quality of materials used.

4. Conclusions

Although SBE provided a slightly higher yield of oil than either SC-CO_2E or CHE, there were no significant difference in yield between the three methods. Moreover, the fatty acid composition, acid value and peroxide value of the oil obtained by these three methods also showed insignificant differences under the experimental conditions in this study. However, the results showed that the extraction methods had significant effect on the content of bioactive compounds (phytosterol, tocopherol and phospholipid) in the extracted SGO. Overall, although the bioactive compounds of SGO extracted by the subcritical butane method were poorer than those by the supercritical CO_2, SBE was still able to retain these compounds in the SGO. The SBE method appeared to be preferably suitable for SGO extraction.

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References

Bonina, F., Puglia, C., Avogadro, M., Baranell, I.E. and Cravotto, G., 2005. The topical protective effect of soybean germ oil against UVB-induced cutaneous erythema: an *in vivo* evaluation. Archiv Der Pharmazie 338: 598-601.

Catchpole, O., Ryan, J., Zhu, Y., Fletcher, K., Grey, J., Vyssotski, M., Mackenzie, A., Nekrasov, E. and Mitchell, K., 2010. Extraction of lipids from fermentation biomass using near-critical dimethylether. Journal of Supercritical Fluids 53: 34-41.

Corso, M.P., Fagundes-Klen, M.R., Silva, E.A., Cardozo Filho, L., Santos, J.N., Freitas, L.S. and Dariva, C., 2010. Extraction of sesame seed (*Sesamun indicum* L.) oil using compressed propane and supercritical carbon dioxide. Journal of Supercritical Fluids 52: 56-61.

Cravotto, G., Robaldo, B., Binello, A., Viola, F., Oliaro Bosso, S. and Avogadro, M., 2005. Soybean germ oil inhibits oxidosqualene cyclase in 3T3 fibroblasts. European Journal of Lipid Science and Technology 107: 701-705.

Dunford, N.T. and Martinez, J., 2003. Nutritional components of supercritical carbon dioxide extracted wheat germ oil. In: 6th International Symposium on Supercritical Fluids, Versailles, France, pp. 273-278.

Dunford, N.T. and Temelli, F., 1995. Extraction of phospholipids from canola with supercritical carbon dioxide and ethanol. Journal of the American Oil Chemists' Society 72: 1009-1015.

Dunford, N.T., Irmak, S. and Jonnala, R., 2009. Effect of the solvent type and temperature on phytosterol contents and compositions of wheat straw, bran, and germ extracts. Journal of Agricultural and Food Chemistry 57: 10608-10611.

Eisenmenger, M., Dunford, N.T., Eller, F., Taylor, S. and Martinez, J., 2006. Pilot-scale supercritical carbon dioxide extraction and fractionation of wheat germ oil. Journal of the American Oil Chemists' Society 83: 863-868.

Gu, L.W. and Gu,W.Y., 2000. Analysis of composition in soy germ [in Chinese]. China Oils Fats 25: 137-140.

Hadolin, M., Skerget, M., Knez, Z. and Bauman, D., 2001. High pressure extraction of vitamin E-rich oil from *Silybum marianum*. Food Chemistry 74: 355-364.

Hamdan, S., Daood, H.G., Toth-Markus, M. and Illés, V., 2008. Extraction of cardamom oil by supercritical carbon dioxide and sub-critical propane. Journal of Supercritical Fluids 44: 25-30.

International Organization for Standardization (ISO), 2006, Oilseed meals – Determination of oil content – Part 1: extraction method with hexane (or light petroleum), ISO 734-1:2006. ISO, Genève, Zwitserland.

Krings, U. and Berger, R.G., 2001. Antioxidant activity of some roasted foods. Food Chemistry 72: 223-229.

Lanza, M., Ndiaye, P.M., Tavares, F.W., Oliveira, D., Dariva, C. and Oliveira, J.V., 2005. Phase behavior of castor oil in compressed propane and n-butane. Journal of Supercritical Fluids 34: 215-221.

Nimet, G., Da Silva, E.A., Palú, F., Dariva, C., Dos Santos Freitas, L., Neto, A.M. and Cardozo Filho, L., 2011. Extraction of sunflower (*Heliantus annuus* L.) oil with supercritical CO_2 and subcritical propane: experimental and modeling. Chemical Engineering Journal 168: 262-268.

Ozawa, Y., Sato, H., Nakatani, A. and Mori, O., 2001. Chemical composition of soybean oil extracted from hypocotyl-enriched soybean raw material and its cholesterol lowering effects in rats. Journal of Oleo Science 50: 217-223.

Panfili, G., Cinquanta, L., Fratianni, A. and Cubadda, R., 2003. Extraction of wheat germ oil by supercritical CO_2: oil and defatted cake characterization. Journal of the American Oil Chemists' Society 80: 157-161.

Pederssetti, M.M., Palú, F., Da Silva, E.A., Rohling, J.H., Cardozo-Filho, L. and Dariva, C., 2011. Extraction of canola seed (*Brassica napus*) oil using compressed propane and supercritical carbon dioxide. Journal of Food Engineering 102: 189-196.

Pereira, C.G. and Meireles, M.A.A., 2010. Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food and Bioprocess Technology 3: 340-372.

Pradhan, R.C., Meda, V., Rout, P.K., Naik, S. and Dalai, A.K., 2010. Supercritical CO_2 extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes. Journal of Food Engineering 98: 393-397.

Pronyk, C. and Mazza, G., 2009. Design and scale-up of pressurized fluid extractors for food and bioproducts. Journal of Food Engineering 95: 215-226.

Qi, K. and Wang, X.D., 1995. Research on extraction of oil with liquefied petroleum gas [in Chinese]. China Oils and Fats 20: 16-22.

Rosenthal, H. and Trevithlek, H.P., 1934. Low boiling hydrocarbons as oil extraction media. Oil and Soap 77: 133-134.

Sahena, F., Zaidul, I.S.M., Jinap, S., Karim, A.A., Abbas, K.A., Norulaini, N.A.N. and Omar, A.K.M., 2009. Application of supercritical CO_2 in lipid extraction – a review. Journal of Food Engineering 95: 240-253.

Shao, P., Sun, P. and Ying, Y., 2008. Response surface optimization of wheat germ oil yield by supercritical carbon dioxide extraction. Food and Bioprocess Technology 86: 227-231.

Song, Y.Q., Yu, D.Y., Zhang, X.H. and Zhang, Z., 2007. Study on extraction of soy germ oil with supercritical carbon dioxide [in Chinese]. Journal of Food Science 10: 293-297.

Sovova, H., Zarevucka, M., Vacek, M. and Stránský, K., 2001. Solubility of two vegetable oils in supercritical CO_2. Journal of Supercritical Fluids 20: 15-28.

Sparks, D., Hernandez, R., Zappi, M., Blackwell, D. and Fleming, T., 2006. Extraction of rice bran oil using supercritical carbon dioxide and propane. Journal of the American Oil Chemists' Society 83: 885-891.

Standardization Administration of the People's Republic of China (SAC), 2003. GB/T5009.37-2003. Analytical methods for hygienic standard of edible vegetable oil. SAC, Beijing, China P.R.

Standardization Administration of the People's Republic of China (SAC), 2005. GB/T5538-2005. Measurment of the peroxide value for animal and vegetable fats. SAC, Beijing, China P.R.

Standardization Administration of the People's Republic of China (SAC), 2008. GB/T5537-2008. Inspection of grain and oils – Determination of phosphatide content. SAC, Beijing, China P.R.

Tres, M.V., MohR, S., Di Luccio, M. and Vladimir Oliveira, J., 2009. Low-pressure solubility of propane and n-butane in refined soybean oil. Journal of Chemical Thermodynamics 41: 1378-1381.

Wang, X.D., 2012. Oil crops: research on extracting sesame oil by subcritical extraction technology. In: Book of abstracts of the 14th ICC Cereal and Bread Congress and Forum on Fats and Oils. ICC, Vienna, Austria.

Wang, X.D. and Liu, K.S., 2005. Extraction with compressed petroleum gases for specialty oil and meal products. INFORM April 1, 2005.

Xu, B., Han, J.H., Zhou, S.L., Wu, Q.F. and Ding, F., 2016. Quality characteristics of wheat germ oil obtained by innovative subcritical butane experimental equipment. Journal of Food Process Engineering 39: 79-87.

Yoshida, H., Tomiyama, Y., Kanrei, S. and Mizushina, Y., 2006. Regional characterization of tocopherols and distribution of fatty acids within soybean seeds (*Glycine max* L.). Journal of Food Lipids 13: 12-26.

Yu, J., Liu, Y.F., Qiu, A.Y. and Wang, X.G., 2007. Preparation of isoflavones enriched soy protein isolate from defatted soy hypocotyls by supercritical CO_2. LWT-Food Science and Technology 40: 800-806.

Yu, Z.R., Singh, B., Rizvi, S.S. and Zollweg, J.A., 1994. Solubilities of fatty acids, fatty acid esters, triglycerides, and fats and oils in supercritical carbon dioxide. Journal of Supercritical Fluids 7: 51-59.