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## Optimization and evaluation of foxtail millet (*Setaria italica*) bran oil by supercritical carbon dioxide extraction

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**SUMMARY:** A Box-Behnken central composite design combined with the response surface methodology (RSM) was used to optimize the parameters of a supercritical fluid extraction (SFE) of foxtail millet bran oil (FMBO). Results showed that a maximum oil yield of 7.97% was achieved under the optimal conditions with an extracting pressure of 30.03MPa, extracting temperature of 47.93 °C; and an extraction time of 2.3 h. The quality of the oil obtained from SFE and solvent extraction (SE) was evaluated by proximate analysis to include physicochemical properties, fatty acids and sterol compounds. The FBMO obtained from SFE showed a much lower phospholipid (0.188 mg/g) content and a preferable color compared to the oil from SE, while it contained a higher content of total sterols, 1.55%. The thermal gravimetric analysis results showed one major regime of weight loss over a temperature range of 300–500 °C. The results show that FBMO obtained by SFE can be a promising nutritional source for food fortification and is understood to have more potentially healthy biological properties.

**KEYWORDS:** Fatty acids; Foxtail millet bran oil; Physicochemical properties; Phytosterols; Reponses surface methodology; Supercritical carbon dioxide extraction

**RESUMEN:** Optimización y evaluación de aceite de salvado de mijo (*Setaria italica*) mediante extracción supercrítica con dióxido de carbono. Un diseño Box-Behnken combinado con la metodología de superficie de respuesta (RSM) se usó para optimizar los parámetros de extracción mediante fluido supercrítico (SFE) de aceite de salvado de mijo (FMBO). Los resultados mostraron que un rendimiento máximo de extracción de aceite del 7,97% se logró en las condiciones óptimas correspondientes a una presión de 30.03MPa, una temperatura 47.93 °C y un tiempo 2,3H. Además, se evaluó la calidad del aceite obtenido por SFE y mediante extracción con disolvente (SE) a partir de un análisis proximal que incluye propiedades fisicoquímicas, ácidos grasos y esteroides. El aceite de FBMO obtenido mediante SFE mostró un contenido mucho menor de fosfolípidos (0.188 mg/g) y un color mas aceptable que el aceite de la SE, mientras que contenía un mayor contenido de esteroides totales: 1,55%. El resultado del análisis térmico gravimétrico mostró un régimen importante de pérdida de peso durante un intervalo de temperatura de 300–500 °C. Los resultados muestran que FBMO obtenido por SFE puede ser una fuente nutricional prometedora para la fortificación de alimentos y se supone potencialmente que tiene mejores propiedades biológicas saludables.

**PALABRAS CLAVE:** Aceite de salvado de mijo; Ácidos grasos; Extracción supercrítica con dióxido de carbono; Fitoesteroides; Metodología de superficie de respuesta; Propiedades fisicoquímicas

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## 1. INTRODUCTION

Foxtail millet (*Setaria italica*) is one of the most important cereal crops in semi-arid areas all over the world (Zohary *et al.*, 2012). In China, foxtail millet is planted as one of the major grain sources. Foxtail millet bran, which consists of an anatomical pericarp layer, an aleurone layer and cereal germ, makes up about 8%–10% of millet quality. As a by-product of the millet processing from foxtail millet seed, foxtail millet bran has been extensively used as animal feed in China in the preceding years. Recently, several studies regarding foxtail millet have been reported. The potential effects of antioxidant activity estimations for foxtail millet insoluble fibers were confirmed by using different *in vitro* tests (Bangoura *et al.*, 2013). The protein, total carbohydrates and crude fat contents of foxtail millet were rapidly and accurately investigated by near infrared spectroscopy (Chen *et al.*, 2013). The purification and characterization of foxtail millet-derived peptides with antioxidant and antimicrobial activities were investigated (Amadou *et al.*, 2013). With respect to foxtail millet bran, there are still few reports in spite of the fact that the oil from foxtail millet bran has proved to be rich in polyunsaturated fatty acids (PUFA), especially linoleic acid and several lipid bioactive compounds (Liang *et al.*, 2010; Pang M, 2014).

Because it is rich in PUFA and bioactive constituents, the oil from foxtail millet bran is supposed to have potentially healthy biological properties similar to other edible oils such as olive oil, tea seed oil and rice bran oil, which are rich in unsaturated fatty acids and special antioxidants which are well established as having functional effects against several degenerative pathologies, including cardiovascular diseases, hepatic injury and cancer (Chen and Cheng, 2006; Martinez-Gonzalez and Estruch, 2004; Panagiotakos *et al.*, 2006). The mechanism seems to indicate that the antioxidants and phytochemicals in these edible oils are used as oxygen radical scavengers due to their ability to neutralize the actions of free radicals.

Over the past decades, supercritical fluid extraction (SFE) technology has made a great deal of progress in oil extraction from plant materials (Kim *et al.*, 1999; Shao *et al.*, 2008; Zacchi *et al.*, 2006). With low extraction temperatures and high extraction efficiency, supercritical fluid extraction has also become a widely used type of extraction separation technology in the oil industrial field (Chen *et al.*, 2008; Ge *et al.*, 2002). When oils are extracted using SFE, there is no risk of solvent contamination, thermal ability, or chemical deformation which may often occur in solvent extraction (SE) and the following refining process.

In our previous study, we observed that foxtail millet bran oil (FMBO) is capable of attenuating

ethanol-induced hepatic injury in mice (Pang M, 2014). However, the relative research on FMBO is still limited especially concerning the processing of the oil extract from foxtail millet bran. In the present study, the SFE of oil from foxtail millet bran was examined by adopting a three-variable, three-level Box-Behnken design to determine, via response surface methodology, the optimum specifications for extraction pressure, extraction temperature, and time by supercritical CO<sub>2</sub>. Not only did we establish an economical, convenient and no risk of solvent contamination extraction process for foxtail millet (*Setaria italica*) bran, but also we evaluated the physicochemical properties, fatty acid compositions and sterols of FMBO obtained by SFE and SE. This report was proposed to provide the theoretical basis for the development and utilization of foxtail millet bran oil.

## 2. MATERIALS AND METHODS

### 2.1. Materials and Reagents

Foxtail millet bran donated from an oil plant in Baishui County (Shaanxi, China) was carefully selected and sieved to remove contaminants. Carbon dioxide (purity 99.9%) was purchased from Henglong Gas Corp. (Hefei, China). The methanol used for fatty acid analysis and the hexane used for GC-MS analysis were of HPLC grade purchased from Guoyao Chemical Reagent Co., Ltd. (Chengdu, China). All other reagents were of analytical grade.

### 2.2 Analysis of Components of Raw Material

Moisture, ash, protein, crude fiber and lipid contents were determined according to the protocols established by the Association of Official Analytical Chemists (AOAC). Moisture was 7.39% which was determined gravimetrically after drying the sample overnight at 105 °C. Total protein content was about 12.2% when it was established by the Kjeldahl method. Ash was quantified after incinerating the sample overnight at 550 °C and the value was about 7.5%. The crude fiber content was about 52.3%. The lipid content was about 9.12% which was expressed as the ratio of lipids extracted by ethyl ether from the original bran.

### 2.3. Supercritical Fluid Extraction (SFE) of Oil

Foxtail millet bran oil was extracted using an HA121-50-01C device (Hua'an Supercritical Fluid Extraction corp., Nantong, China), described in detail by Wei *et al.*, 2009, using carbon dioxide as solvent. Foxtail millet bran samples (150 g), with the chosen particle size and water content, were loaded into the extraction vessel. Carbon dioxide from a cylinder was passed through a chiller kept

at 2 °C and pumped into the extractor by a high pressure pump. The pressure and temperature were controlled to an accuracy of  $\pm 0.5$  MPa and  $\pm 0.5$  °C, respectively. The flow rate of CO<sub>2</sub> was controlled at 20 kg/h for all experiments. After each extraction, the oil was collected in the first separator while water and volatile components were recovered in the second one. The amount of extracted oil was determined gravimetrically after collection and the oil yield percentage was calculated according to the following equation:

$$\text{extraction rate (ER)(\%)} = \frac{\text{Amount of extracts oil (g)} \times 100}{\text{Amount of initial bran powder (g)}}$$

#### 2.4. Experimental Design for RSM of SFE

RSM with three variables and three levels was applied to optimize the extraction rate of millet bran oil. The separating pressure (X<sub>1</sub>), extraction temperature (X<sub>2</sub>) and extraction time (X<sub>3</sub>) were independent variables studied to optimize the RE. The uncoded and coded values of the variables are given in Table 1. The levels of the parameters were based on preliminary experimental results. All experiments were carried out in randomized order. The quadratic polynomial regression model was used to express the Y variable (ER= the extract rate of millet bran oil) as a function of the independent variables as follows:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j$$

$\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$ , and  $\beta_{ij}$  are constant coefficients of intercept, linear, quadratic and interaction terms, respectively. The data collected from the extraction tests were analyzed using a response surface analysis procedure (Design-Expert 8.0 Trial, State-Ease, Inc., Minneapolis MN, USA).

#### 2.5. Soxhlet extraction procedure

A conventional method of Soxhlet extraction (SE) was performed in a Soxhlet apparatus to compare the extraction performances with SFE. Foxtail millet bran samples (5 g) were extracted using petroleum ether (v/w=10:1) as the solvent at room temperature

TABLE 1. Uncoded and coded levels of independent variables used in the RSM design

Coded variables levels	Extracting pressure (X <sub>1</sub> , MPa)	Temperature (X <sub>2</sub> , °C)	Time (X <sub>3</sub> , h)
-1	25	40	1.5
0	30	45	2
+1	35	50	2.5

for 24 h, evaporated at 60 °C to remove the solvent and then the oil was obtained.

#### 2.6. Assay for physicochemical characterization of FMBO

##### 2.6.1. Physicochemical property assays for FMBO

Important physicochemical properties of the crude FMBO, concerning specific gravity, refractive index, saponification value, acid value, peroxide value, and phospholipid content were characterized according to the AOCS methods (Firestone, 1998). The color of the oils was determined by the Lovibond tintometer (Shanghai Technologies, China). Three replicates were carried out for each analysis.

##### 2.6.2. Fatty acid composition of FMBO

The fatty acid composition of FMBO was analyzed according to the AOCS method. A Shimada GC-MS-QP2010 (Shimadzu Co., Kyoto, Japan) equipped with a capillary column (DB-wax, 30.0 m×0.25mm, 0.25 μm film thickness, Agilent Technologies Co., Ltd.) was used. The GC parameters were: the carrier gas, high purity helium; injector temperature, 250 °C; detector temperature, 320 °C; split ratio, 20:1; column flow rate, 1 mL·min<sup>-1</sup>; injection volume, 1 μL. The column temperature was programmed to increase to 230 °C from the initial 180 °C at the rate of 2 °C·min<sup>-1</sup>. The mass spectrometer was operated in electron impact ionization (70eV), full scan (40–800 m/z) mode. The MS parameters were: scan speed, 1666; inter scan, 0.5s; source temperature, 250 °C; interface temperature 285 °C. Compounds were identified by comparison of their retention indices and mass spectra with the mass spectra library. The relative content of each fatty acid was detected by the normalization method of peak areas.

##### 2.6.3. Determination of unsaponifiable matter of FMBO

An amount of 1.0 g of FMBO was saponified with 10 mL 0.5 M KOH-C<sub>2</sub>H<sub>5</sub>OH solution and the unsaponifiable matter was recovered and then diluted with 2 mL of hexane and injected into the GC-MS instrument. A Shimada GC-MS-QP2010 (Shimadzu Co., Kyoto, Japan) equipped with a capillary column (HP-5, 30.0 m×0.32mm, 0.25 μm film thickness, Agilent) was used. The GC parameters were: the carrier gas, high purity helium; injector temperature, 250 °C; detector temperature, 320 °C; the split ratio, 20:1; column flow rate, 1 mL·min<sup>-1</sup>; injection volume, 1 μL. The column temperature was programmed to increase to 285 °C from the initial 200 °C at the rate of 5 °C·min<sup>-1</sup> and then maintained for 10 min. The mass spectrometer was operated in electron impact

ionization (70 eV), full scan (40–800 m/z) mode. The MS parameters were: scan speed, 1666; inter scan, 0.5s; source temperature, 250 °C; interface temperature 285 °C. Compounds were identified by comparison of their retention indices and mass spectra with the mass spectra library. The relative content of sterols (or stanols) was determined by the normalization method of peak areas.

#### 2.6.4. Thermal analysis

The thermal decomposition reaction kinetics of FMBO were investigated with STA 449 F3 Jupiter simultaneous thermal analyzer (Netzsch, Germany). The sample was analyzed at 10 °C·min<sup>-1</sup> heating rates in the temperature range between 30 °C and 1200 °C. All thermal analysis runs were performed on 3–5 mg of sample under a 100 cm<sup>3</sup>·min<sup>-1</sup> stream of air. Three replicates were analyzed for each sample. Curves of simultaneous thermo gravimetric (TG) and differential thermal analysis (DTA) were performed using an origin spreadsheet suitably designed to approximate as closely as possible the experimental values to the predicted ones.

### 3. RESULTS AND DISCUSSION

#### 3.1. Fitting the model

The statistical combination of the independent variables in coded and the natural values along with the predicted values are presented in Table 2 according to RSM design. The good agreement between the predicted and experimental values confirms the validity of the model.

Variance analyses of the factors studied for the response surface model are shown in Table 3. From the statistical analysis, the model with the *P*-value was highly significant at 0.0014, which implied that the model was suitable for this experiment. Meanwhile, the “lack of fit” was insignificant, the *R*-squared was 0.9944, which indicated that the model was in good agreement with the experimental results.

The regression coefficients and the corresponding *P*-values are presented in Table 4. From the *P*-values of each model term, it can be concluded that the separating time (*X*<sub>3</sub>) and separating temperature (*X*<sub>2</sub>) affected the extraction rate (ER) with high significance, while the effect of separating pressure (*X*<sub>1</sub>) on the ER was not significant. The interaction between temperature and time had a significant effect on the ER. However, the interaction between the other parameters was not significant. Some of the second-order terms (*X*<sub>2</sub><sup>2</sup>, *X*<sub>3</sub><sup>2</sup>) highly significantly affected the ER, while the effect of the second-order term of *X*<sub>1</sub><sup>2</sup> on the ER was not significant. The coefficients of independent variables determined for the second-order polynomial model for the ER is given below:

TABLE 2. Experimental scheme and results obtained from RSM

Runs	Factors			Response ER	
	Extracting pressure ( <i>X</i> <sub>1</sub> , MPa)	Temperature ( <i>X</i> <sub>2</sub> , °C)	Time ( <i>X</i> <sub>3</sub> , h)	Exp. (%)	Pred. (%)
1	30	45	2	7.75	7.70
2	30	50	1.5	6.19	6.22
3	30	45	2	7.75	7.70
4	30	40	2.5	7.69	7.61
5	30	45	2	7.73	7.7
6	30	45	2	7.61	7.70
7	30	50	2.5	7.82	7.89
8	35	50	2	7.62	7.55
9	25	45	1.5	5.82	5.82
10	30	45	2	7.65	7.7
11	30	40	1.5	5.46	5.39
12	25	50	2	7.68	7.6
13	35	45	1.5	5.7	5.69
14	25	45	2.5	7.65	7.70
15	35	40	2	6.91	6.99
16	35	45	2.5	7.71	7.71
17	25	40	2.0	6.93	7.0

$$Y = 7.70 - 0.017X_1 + 0.29X_2 + 0.96X_3 - 0.01X_1X_2 + 0.045X_1X_3 - 0.15X_2X_3 - 0.24X_1^2 - 0.17X_2^2 - 0.74X_3^2$$

where *Y* is the ER (%), *X*<sub>1</sub> is the extracting pressure, *X*<sub>2</sub> is the extracting temperature, *X*<sub>3</sub> is the extracting time.

#### 3.2. Optimization of reaction conditions by RSM

Contour plots were employed to determine the effect of the interaction of parameters on the extraction rate (ER) of foxtail millet bran oil by SFE. From the shape of the contour plots in Fig. 1, the significance of the mutual interactions between the independent variables can be estimated. Fig. 1 (a) shows the interaction of the extraction pressure (*X*<sub>1</sub>) and temperature (*X*<sub>2</sub>) at the fixed extraction time at 2.0 hours. It was observed that the response value of the extraction rate increased with the rising of pressure when the temperature was lower than 47.5 °C; when the temperature was higher than 47.5 °C, the response value decreased. This phenomenon was in consistence with other previous reports and is explained by the fact that the increased pressure would also lead to an increase in the density of supercritical CO<sub>2</sub> (Jiang and Niu, 2011; Shao *et al.*, 2008). Fig. 1 (b) shows the interaction between the extraction pressure (*X*<sub>1</sub>) and time (*X*<sub>3</sub>) at the fixed temperature at 45 °C. The extraction rate was steadily increased by the extraction time but not significantly increased with the rise in extraction pressure. This is probably attributed



TABLE 3. Analysis of variance of regression parameters for the response surface model

Source	Sum of squares	Degree of freedom	Mean Square	F-value	P-value <sup>a</sup>
Model	11.02	9	1.22	139.14	0.0014
Linear	8.09	3	2.70	11.71	0.005
Quadratic	2.83	3	0.94	548.98	0.0011
Lack of Fit	0.045	3	0.015	3.65	0.1216
Pure Error	0.016	4	0.0042	0.0011	
R-Squared	0.9944				

<sup>a</sup>p<0.01 highly significant; 0.01≤p<0.05 significant; p≥0.05 not significant.

TABLE 4. Regression analysis of a full second-order polynomial model

Factor <sup>a</sup>	Coefficient Estimated	P-value <sup>b</sup>
X <sub>1</sub>	-0.017	0.614
X <sub>2</sub>	0.29	<0.0001
X <sub>3</sub>	0.96	<0.0001
X <sub>1</sub> ×X <sub>2</sub>	-0.01	0.8372
X <sub>1</sub> ×X <sub>3</sub>	0.045	0.3692
X <sub>2</sub> ×X <sub>3</sub>	-0.15	0.0151
X <sub>1</sub> ×X <sub>1</sub>	-0.24	0.0011
X <sub>2</sub> ×X <sub>2</sub>	-0.17	0.0071
X <sub>3</sub> ×X <sub>3</sub>	-0.74	<0.0001

<sup>a</sup>X<sub>1</sub>:extraction pressure (MPa); X<sub>2</sub>: temperature(°C); X<sub>3</sub>: time (h).  
<sup>b</sup>p<0.01 highly significant; 0.01≤p<0.05 significant; p≥0.05 not significant.

to the fact that the temperature in these cases was fixed at 45 °C, and has decreased the density of supercritical CO<sub>2</sub>. A similar result can also be seen in a previous report (Shao *et al.*, 2008). Fig.1 (c) shows the interaction between the extraction temperature (X<sub>2</sub>) and time (X<sub>3</sub>) at the fixed pressure at 30 MPa. Since the oil content of foxtail millet bran is lower, even less than 10%, extraction time was also an essential factor affecting oil yield. It can be observed that the extraction rate continued slowly with the extraction time and did not significantly increase until the time was at about 2.1h. However, the subsequent time from 2.1h to the end time of 2.5h only produced a slight change in the oil yield.

From the results, the optimum conditions for foxtail millet bran oil extracted by SFE were predicted (X<sub>1</sub>=30.03, X<sub>2</sub>=47.93, X<sub>3</sub>=2.3, Y=8.07) by the regression model according to the limit criterion of maximum response Y and an appropriate range level for each parameter. An ER of 8.01% was achieved under optimal conditions. Additional experiments in triplicate under these optimized extraction conditions were carried out. These triplicate experiments obtained an average RE of 7.97%, which concurred with the model prediction and as a result, the model was considered to be accurate and reliable.

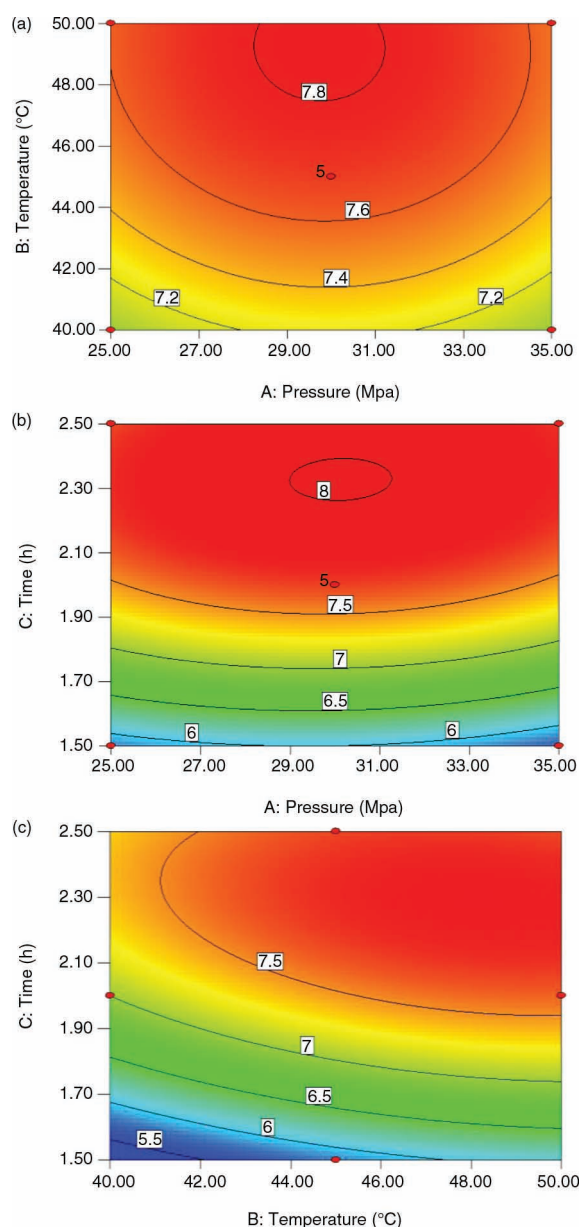


FIGURE 1. Contour plots between two parameters of extracting rate foxtail millet bran oil extracted by supercritical carbon dioxide; (a), pressure (Mpa) and temperature (°C); (b) pressure and time (h); (c) temperature and time.

### 3.3. Physicochemical properties of FMBO

The physical and chemical properties of crude FMBO by SFE and SE methods are shown in Table 5. The specific gravity (20 °C), refractive index at 20 °C, and iodine value were slightly different between two samples. The saponification value (189.58±0.41) of FMBO by SE showed a significant difference ( $p<0.05$ ) from oil by SFE, while it was consistent with previous research in that the saponification value of millet oil was 192–197 mg KOH/g (Huo QG, 2006) and 186.29±0.51 (Liang *et al.*, 2010), respectively. Another significant difference between the FMBO by SE and SFE is the phospholipid content. The phospholipid content by SFE was 0.188 mg/g lower than 44.22 mg/g by SE, which indicated that SFE was superior to SE in phospholipid extraction and the subsequent refining step of degumming is not necessarily required for FMBO by SFE during the industrial process. In addition, the unsaponifiable matter content of FMBO by SFE (3.58±0.23%) showed significant differences compared to the FMBO by SE (2.89±0.21%). These results demonstrate that SFE can improve the FMBO quality, especially by decreasing the acid value, and peroxide value as well as color. The SFE method could better protect the bioactive components than the petroleum ether in terms of low pressure (30.03MPa), short extraction time (2.3 h) and low temperature (47.93 °C) without any toxic solvent.

### 3.4. Fatty acid composition of FMBO

The major fatty acid composition of FMBO by different extraction methods is shown in Table 6. For both SFE and SE methods, eight main components of fatty acids including four saturated fatty acids (SAFA), two monounsaturated fatty acids (MUFA) and two polyunsaturated fatty acids (PUFA) were

identified. The amount of unsaturated fatty acids especially the linoleic acid C18:2, (58.03%±0.66 by SE and 58.85%±0.55 by SFE, respectively) was high, which is consistent with our previous results (Pang M, 2014). As can be observed from Table 5, the fatty acid contents of FMBO obtained by SFE were similar to those by SE. Palmitic acid C16:0 and stearic acid C18:0 were the most predominant SAFA, oleic acid C18:1 was the principal MUFA and linoleic acid C18:2 as well as the linolenic acid C18:3 were the main PUFA (Table 5). Minor differences in some fatty acid contents were recorded when FMBO were extracted by SE versus SFE ( $p<0.05$ ), indicating that SE did not change the fatty acid composition. Both values of fatty acid composition of FMBO by SFE and SE are close to the previous report (Liang *et al.*, 2010). FMBO was characterized by relatively high levels of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). Both of them have been shown to lower “bad” LDL cholesterol (low density lipoproteins) and retain “good” HDL cholesterol (high density lipoproteins) levels in the blood (Ramadan *et al.*, 2010; Wang *et al.*, 2011). The fatty acid profile and high amounts of UFA confirmed that FMBO can be considered to have a similar beneficial effect as other nutritious and healthy vegetable oils (Boskou, 2006; Innis and Dyer, 1997; Sahari *et al.*, 2004).

### 3.5. Analysis of steroid compounds for FMBO

Plant sterols play a major role in pharmaceutical and nutrition since they are known to be cholesterol lowering agents as well as having anti-inflammatory, anti-oxidant, and anti-cancer functions (Bohn *et al.*, 2007; Moreau *et al.*, 2002). The levels of sterol in vegetable oils are recognized as the identification of oils, oil derivatives and used to determine the oil quality.

TABLE 5. Physical and chemical properties of FMBO by SE and SFE<sup>a</sup>

Characteristic	SE	SFE
Specific gravity	0.9288±0.0002 <sup>a</sup>	0.9199±0.0002 <sup>a</sup>
Refractive index (20 °C)	1.4693±0.0001 <sup>a</sup>	1.4706±0.0001 <sup>a</sup>
Color (Lovibond, 25.4 mm)	Y65, R12 <sup>a</sup>	Y35, R5.0 <sup>b</sup>
Acid value (mg KOH·g <sup>-1</sup> )	10.5±0.23 <sup>a</sup>	9.2±0.35 <sup>b</sup>
Peroxide value (mmol·kg <sup>-1</sup> )	3.12±0.11 <sup>a</sup>	2.33±0.03 <sup>b</sup>
Iodine value (gI <sub>2</sub> ·100g <sup>-1</sup> )	101.23±1.43 <sup>a</sup>	103.16±1.90 <sup>a</sup>
Saponification value (mg KOH·g <sup>-1</sup> )	189.58±0.41 <sup>a</sup>	176.89±0.33 <sup>b</sup>
Unsaponifiable matter (%)	2.89±0.21 <sup>a</sup>	3.58±0.23 <sup>b</sup>
Phospholipid (mg·g)	44.22 <sup>a</sup>	0.188 <sup>b</sup>

<sup>a</sup>Data are presented as means±standard deviation of triplicate determinations. Within each row, means not followed by the same letter are significantly different at  $p\leq0.05$ .

TABLE 6. Analysis of fatty acid composition for FMBO<sup>a</sup>

Fatty acids <sup>a</sup>	SE (%)	SFE (%)
Palmitic acid (C16:0)	10.83±0.10 <sup>a</sup>	10.23±0.12 <sup>b</sup>
Stearic acid (C18:0)	3.96±0.05 <sup>a</sup>	3.73±0.03 <sup>b</sup>
Oleic acid (C18:1)	19.43±0.21 <sup>a</sup>	19.65±0.16 <sup>a</sup>
Linoleic acid (C18:2)	58.03±0.66 <sup>a</sup>	58.85±0.55 <sup>a</sup>
Linolenic acid (C18:3)	3.90±0.08 <sup>a</sup>	3.96±0.04 <sup>a</sup>
Arachidic acid (C20:0)	1.69±0.02 <sup>a</sup>	1.52±0.01 <sup>b</sup>
Behenic acid (C20:1)	1.38±0.01 <sup>a</sup>	1.30±0.01 <sup>b</sup>
Docosenoic acid (C22:0)	0.78±0.01 <sup>a</sup>	0.76±0.01 <sup>a</sup>
Saturated fatty acid (SAFA)	17.26	16.24
Unsaturated fatty acid (UFA)	82.74	83.76

<sup>a</sup>Results are expressed as % over the total content (i.e. relative content); data are presented as means±standard deviation of triplicate determinations. Within each row, means not followed by the same letter are significantly different at  $p\leq0.05$ . SE, Solvent extraction by petroleum ether; SFE, supercritical carbon dioxide extraction.

TABLE 7. Analysis of steroid compounds for FMBO by SE and SFE<sup>a</sup>

<sup>a</sup> Steroids	SE(%)	SFE(%)
Campesterol	15.22±0.28 <sup>a</sup>	15.91±0.19 <sup>a</sup>
Ergosterol	2.77±0.04 <sup>a</sup>	2.97±0.03 <sup>b</sup>
Stigmasterol	5.85±0.07 <sup>a</sup>	5.58±0.05 <sup>b</sup>
β-Sitosterol	56.58±0.77 <sup>a</sup>	56.26±0.82 <sup>a</sup>
Stigmastanol	15.33±0.11 <sup>a</sup>	15.12±0.24 <sup>a</sup>
Fucosterol	4.25±0.02 <sup>a</sup>	4.16±0.05 <sup>b</sup>

<sup>a</sup>Results are expressed as % over the total content (i.e. relative content); data are presented as means±standard deviation of triplicate determinations. Within each row, means not followed by the same letter are significantly different at  $p \leq 0.05$ . SE, Solvent extraction by petroleum ether; SFE, supercritical carbon dioxide extraction.

In this study, the sterol levels of the oils by both SFE and SE are evaluated and the value obtained for sterol composition are listed in Table 7. The total sterols of FMBO amounted to 1.55% by SFE and 1.09% by SE, which indicates that FMBO by SFE can contain 1.422 times higher sterols than FMBO by SE. This result is in agreement with other previous research where the SFE were used to extract oils from plant seeds (Jiang and Niu, 2011; Shao *et al.*, 2008). β-sitosterol was found to be the major sterol (56.26% of the total sterols by SFE), followed by campesterol and stigmastanol, at about 15.91% and 15.1%, respectively. Other chemical types of phytosterols (or phytostanols) were also identified such as ergosterol, stigmasterol, and fucosterol in both the oil extracted by SFE and SE. The composition of these sterols make FMBO a potentially functional oil to perform healthy biological activities.

### 3.6. Thermal analysis

Vegetable oils are mostly used for cooking foods, so it is important to understand their thermo-physical properties during the heating processes. Thermal

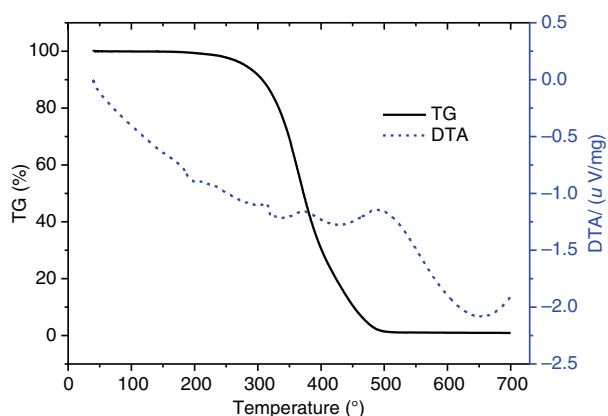


FIGURE 2. TG-DTA curves of FMBO.

gravimetric (TG) and differential thermal analysis (DTA) analysis can show the thermal behavior of oils for their identification since thermal properties are related to major (triacylglycerols, total fatty acids) and minor (diacylglycerols, lipid oxidation products) chemical components (Chiavaro *et al.*, 2008). Fig. 2 shows the TG-DTA curves of FMBO in an open air atmosphere. The essential weight loss occurred mainly in the 300–500 °C range. At 512.2 °C, the weight was lost completely. In the DTA curve, there are decalescence peaks near the decomposition temperature which correspond with the weight loss stages.

## 4. CONCLUSIONS

A second-order polynomial model in our experiment was determined to be sufficient to describe and predict the response variable of the oil yield for the SFE of foxtail millet bran. We also investigated the mechanism involved in that process, and analyzed the physicochemical properties, fatty acid composition and steroids. Compared with the solvent extraction method using petroleum ether, FBMO obtained from SFE showed much lower phospholipid (0.188 mg/g) content and a preferable color, while it also contained more important bioactive components in that the content of total sterols reached 1.55%. The results from the thermal gravimetric analysis showed one major regime of weight loss over a temperature range of 300–500 °C. With the increasing demand for natural, nutritive foxtail millet bran oil, which is rich in unsaturated fatty acids and other lipid accompaniments as well as the physicochemical properties which are close to values of other edible oils, making it a natural source of food fortification is promising as it has been determined to have many potentially healthy biological properties.

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