Preparation and characterization of foxtail millet bran oil using subcritical propane and supercritical carbon dioxide extraction

Yuzhong Shi · Yuxiang Ma · Ruitin Zhang ·
Hanjun Ma · Benguo Liu

Revised: 10 February 2014 / Accepted: 23 February 2014 / Published online: 6 March 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract The foxtail millet (*Setaria italica* Beauv) bran oil was extracted with traditional solvent extraction (SE), supercritical carbon dioxide extraction (SCE) and subcritical propane extraction (SPE) and analyzed the yield, physicochemical property, fatty acid profile, tocopherol composition, oil oxidative stability in this study. The yields of foxtail millet bran oil by SE, SCE and SPE were 17.14 %, 19.65 %, 21.79 % of raw material weight (corresponded to 75.54 %, 86.60 %, 96.03 % of the total amount of the oil measured by using Soxhlet extraction), respectively. The effect of the extraction methods on the physicochemical properties (peroxide value, saponification value and color) was significant while the difference in fatty acid profile was negligible based on GC analysis. The major components of vitamin E in the obtained oils were identified as α - and β -tocopherols by HPLC, and SPE was superior to SE and SCE in the extraction of tocopherols. In Rancimat test, the oil obtained by SPE showed the highest oil oxidative stability, which could attribute to its high tocopherol content and low peroxide value. In view of oil quality, SPE employed smaller times and lower pressures compared to SE and SCE. SPE was a suitable and selective method for the extraction of the foxtail millet bran oil.

Keywords Foxtail millet bran oil · Supercritical carbon dioxide extraction · Subcritical propane extraction · Fatty acid profile · Tocopherol composition · Oil oxidative stability

Introduction

Foxtail millet (*Setaria italica* Beauv), one of the oldest cultivated crops, originated from China, and is now planted in India, China, Japan, Australia, North Africa and South America for its excellent drought resistance, high tolerance to poor soil and good nutrient value (Sreenivasulu et al. 2004; Prashant et al. 2005; Suma and Urooj 2012). It has been reported that Foxtail millet is rich in starch, protein, lipid, vitamins and minerals (Usha et al. 1996; Bai et al. 2009). Foxtail millet porridge is often used as weaning food in developing countries due to its flavor and high nutritional value. In China, this porridge has been widely used as a nourishing gruel or soup for pregnant and nursing women, and has been applied to food therapy (Bai et al. 2008). Before food use, foxtail millet is dehulled in China, and the produced bran as waste fraction is mainly used as animal feed. Recent years, many reports have found the cereal bran is rich in lipid (Zigoneanu et al. 2008; Nantiyakul et al. 2013). So it is expected that the high added value product could be obtained by oil extraction from foxtail millet bran.

Today, the enhanced concern over the quality and safety of food products, increased preference for natural products, and stricter regulations related to the residual levels of solvents have driven supercritical fluid technology to be a primary alternative to traditional solvents for extraction, fractionation, and isolation of active ingredients in food industry (Ahangari and Sargolzaei 2012). Although supercritical carbon dioxide extraction (SCE) has been widely used and accepted (Herrero et al. 2006), subcritical propane extraction (SPE) also has several advantages besides the merits of both traditional solvent extraction with *n*-hexane and SCE. First, SPE is relatively inexpensive technology without a toxic residue. Second, the pressures of SPE for oil extraction is lower at least an order of magnitude (tens of bar compared with hundreds of bar) than SCE (Dos Santos et al. 2008). However, there are few reports

Y. Shi · H. Ma · B. Liu (✉)
School of Food Science, Henan Institute of Science and Technology,
Xinxiang 453003, China
e-mail: zzgclbg@126.com

Y. Ma · R. Zhang
College of Grain and Food, Henan University of Technology,
Zhengzhou 450001, China

regarding the comparison between extractions using SCE and SPE on aspects related to the yield, physicochemical properties, fatty acid composition tocopherol composition, oxidative stability of the extracted oil.

The purpose of this research was to assess the feasibility of using supercritical carbon dioxide and subcritical propane to extract the oil from foxtail millet bran obtained from waste product of the cereal industry. The fatty acid composition, tocopherol composition and oxidative stability of the obtained oils were also compared.

Materials and methods

Materials and chemicals

The foxtail millet bran was from Jinzhuzi Grain Co. Ltd (Xinxiang, China). The fatty acid methyl esters used as standards and tocopherols (α -, β -, γ -, and δ -isomers) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

Approximate composition analysis

The moisture, lipid content, crude protein and crude fiber of millet bran were determined by the AOCS Official Methods Ba 2a-38, Ba 3-38, Ba 4a-38 and Ba 6-84, respectively (Firestone 1998). And the above results were expressed on a wet basis. The lipid in millet bran was extracted by ethyl ether in a Soxhlet apparatus. The measurement of the nitrogen content was performed on a Foss 2006 digester and Foss 2300 Kjeltex Analyzer Unit (Foss, Sweden). A factor of 6.25 was adopted for protein content estimation. The crude fiber content was measured by a Fibertec M6 fibre analysis system (Foss, Sweden).

Solvent extraction (SE)

The solvent extraction of the foxtail millet bran powder (1,500 g) involved two steps of extraction under gentle stirring at room temperature, respectively, each using 4,500 ml of n-hexane for 3 h and filtered to obtain the supernatant. The supernatant from both steps was combined, concentrated under vacuum at 40 °C to remove n-hexane. The obtained oil was collected, weighed and stored in sealed containers at 4 °C for further analysis.

Supercritical carbon dioxide extraction (SCE)

SCE extraction was carried out using a 5 L-SFE system (Masson Nem, Guangzhou, China), consisting of an extraction vessel equipped with an internal basket (5 L), two separators in series. The extraction medium was carbon dioxide

with a purity of more than 99.5 %. The foxtail millet bran powder (1,500 g) was loaded into the extractor and extracted at 28 MPa pressure and 40 °C temperature for 2.5 h. The operating conditions of the separators were set at 9 MPa and 45 °C, and the liquid CO₂ flow rate was set at 35 L/h. After the extraction was finished, the obtained oil in the separators was collected, weighed and stored in sealed containers at 4 °C for further analysis.

Subcritical propane extraction (SPE)

SPE extraction was carried out using a CBE-5 L subcritical fluid extraction system (Henan Kunhua Biological Technology Co. Ltd, Anyang, China). The foxtail millet bran powder (1,500 g) was loaded into the extractor and extracted at 0.5 MPa pressure and 40 °C temperature for 1.5 h. The evaporating temperature was set at 50 °C. The obtained oil in the separators was collected, weighed and stored in sealed containers at 4 °C for further analysis.

Physicochemical property assays

The density, refractive index, saponification value and peroxide value of the oil were measured by the IUPAC Methods 2.101, 2.102, 2.202 and 2.501 respectively (Paquot and Hauntfenne 1987). The color of the oil was determined according to the AOCS Official Method Cc 13e-92 (Firestone 1998).

Determination of fatty acid composition

The fatty acid composition of the oil was analyzed according to the IUPAC method 2.302 (Paquot and Hauntfenne 1987). And the analysis of fatty acid methyl esters was performed on an Agilent 6,890 N gas chromatograph (Agilent Technologies Co., Ltd.) equipped with a flame ionization detector and a DB-FFAP capillary column (30 m×0.32 mm, 0.25 μ m of film thickness). The column, injector, and detector temperatures were set at 180, 230, and 230 °C, respectively. The flow rate of the carrier gas N₂ with a split ratio of 1:20 was set at 70 mL/min. The fatty acids were identified according to the retention times of standard fatty acid methyl ester performed at the same conditions.

Determination of tocopherol composition

The tocopherol (α -, β -, γ -, and δ -isomers) contents of the oil were determined according to a previous report (Liang et al. 2010) with a few modifications. The samples were analyzed on a LC-10Avp HPLC (Shimadzu, Japan) with a silica column (250×4.6 mm, 5 μ m) (Dalian Yilite, Dalian, China) and a RF-10AXL fluorescence detector (Shimadzu, Kyoto, Japan). The column temperature was set at 40 °C. The excitation and

emission wavelengths were set at 298 and 325 nm, respectively. The oil was dissolved in *n*-hexane at 30 mg/mL. A 5- μ L volume of the loaded sample was isocratically eluted with *n*-hexane/isopropyl ether (99/1, v/v) at 0.8 mL/min. The absolute contents of tocopherols were determined according to the calibrated standard curves.

Determination of oil oxidative stability

The oxidative stability of the oil was determined on a 743 Rancimat analyzer (Metrohm, Switzerland) according to a reported method (Proestos et al. 2006). The oil (5 g) was subjected to oxidation at 110 °C (air flow 20 L/h). Induction time was recorded automatically.

Statistical analysis

The whole experiment was repeated twice and each analysis was done in triplicate. The data obtained were expressed as means with standard deviations. Where applicable, data were subjected to analysis of variance and Fisher's least significant difference tests were used to separate means with significant differences ($P < 0.05$).

Results and discussion

Composition of foxtail millet bran

The composition of millet bran was shown in Table 1. The crude lipid, crude protein and crude fiber contents were 22.69 %, 13.67 % and 5.73 %, respectively. In the previous report, the crude fiber of foxtail millet bran was as high as 51.69 % (Liang et al. 2010), but in this study, the crude fiber was only 5.73 %. Because the bran in their study included the shell. In this study, the shell has been removed from the bran. The crude lipid and crude protein content of millet bran could be equivalent to those of rice bran (Gunstone and Harwood 2007) while its crude fiber could be lower than that of rice bran (7.0–11.4 %) (Orthoefer 2005). The results showed that foxtail millet bran was rich in oil and protein. The cultivated area of foxtail millet in China is approximate 1,400 km², and total production is in the range of 3,700–4,500 thousand ton (Xue et al. 2008), so foxtail millet bran could become a new source of oil and protein in food industry.

Table 1 Composition of millet bran ($n=3$)

	Moisture	Crude lipid	Crude protein	Crude fiber
Content (%)	7.93 $\pm$ 0.12	22.69 $\pm$ 0.35	13.67 $\pm$ 0.23	5.73 $\pm$ 0.16

Table 2 Physicochemical properties of foxtail millet bran oils obtained by different methods

	SE	SCE	SPE
Oil yield (%)	17.14	19.65	21.79
Density	0.899281	0.91942	0.921581
Refractive index	1.47642	1.45057	1.48007
Saponification value	170.95	188.10	185.05
Peroxide value (meq/kg)	4.45	7.05	1.40
Color (Lovibond, 1 in.)	Y35.0, R2.7	Y35.0, R2.2	Y30.0, R4.3

Oil yield

The yields of the foxtail millet bran oils obtained by SE, SCE and SPE were presented in Table 2. The traditional extraction with *n*-hexane at room temperature for 6 h obtained a yield of 17.14 % (w/w), which corresponded to 75.54 % of the total amount of the oil as measured using Soxhlet extraction. Supercritical carbon dioxide at 28 MPa pressure and 40 °C for 2.5 h and subcritical propane at 0.5 MPa pressure and 40 °C for 1.5 h extracted up to 19.65 % (w/w, corresponding to 86.60 % of the total amount of the oil as measured using Soxhlet extraction) and 21.79 % (w/w, corresponding to 96.03 % of the total amount of the oil as measured using Soxhlet extraction) of foxtail millet bran, respectively, which suggested that SPE was much faster than SE and SCE. This aspect is related to the fact that subcritical propane is a better solvent for triacylglycerols than supercritical carbon dioxide (Lanza et al. 2005; Hamdan et al. 2008).

Physicochemical properties

The effects of the extraction methods on the physicochemical properties of the oils were also showed in Table 2. It

Table 3 Fatty acid composition of foxtail millet bran oils obtained by different methods (%) ($n=3$)

	SE	SCE	SPE
C16:0	7.34 $\pm$ 0.03	7.20 $\pm$ 0.03	7.31 $\pm$ 0.02
C16:1	0.10 $\pm$ 0.01	0.07 $\pm$ 0.05	0.11 $\pm$ 0.00
C17:0	0.12 $\pm$ 0.00	0.12 $\pm$ 0.00	0.12 $\pm$ 0.00
C18:0	2.77 $\pm$ 0.01	2.75 $\pm$ 0.01	2.562 $\pm$ 0.02
C18:1	17.53 $\pm$ 0.01	17.62 $\pm$ 0.04	17.62 $\pm$ 0.02
C18:2	68.05 $\pm$ 0.09	67.96 $\pm$ 0.15	68.06 $\pm$ 0.14
C18:3	2.21 $\pm$ 0.02	2.19 $\pm$ 0.02	2.19 $\pm$ 0.01
C20:0	1.14 $\pm$ 0.02	1.14 $\pm$ 0.04	1.07 $\pm$ 0.01
C20:1	0.51 $\pm$ 0.01	0.53 $\pm$ 0.01	0.49 $\pm$ 0.01
C22:0	0.46 $\pm$ 0.02	0.43 $\pm$ 0.05	0.43 $\pm$ 0.04
Total	100 $\pm$ 0.33	100 $\pm$ 0.40	99.96 $\pm$ 0.26

Table 4 Tocopherol composition of foxtail millet bran oils obtained by different methods (mg/100 g) ($n=3$)

	SE	SCE	SPE
α -tocopherol	12.42 $\pm$ 1.22	8.07 $\pm$ 1.15	12.98 $\pm$ 1.64
β -tocopherol	80.16 $\pm$ 3.57	71.88 $\pm$ 6.65	89.47 $\pm$ 1.53
γ -tocopherol	–	–	–
δ -tocopherol	–	–	–

could be found that the oils obtained by SE, SCE and SPE had the similar density and refractive index. But their differences in other physicochemical properties were significant. The saponification values of SE, SCE and SPE were 170.95, 188.10 and 185.05, respectively, which were high and comparable to those of common vegetable oils indicating very high content of low molecular weight triacylglycerols. All the peroxide values were lower than those generally recommended for commercial vegetable oils. The highest peroxide value was found by SCE (7.05 meq/kg oil), followed by SE (4.45 meq/kg oil) and, SPE (1.40 meq/kg oil) which mean that the oil could be prone to peroxide formation in the processing of SCE for its high extraction and separation temperatures (40 °C and 45 °C, respectively). The foxtail millet bran is rich in carotenoids, whose different fatty acid moieties and polarities could lead to their different solubilities in *n*-hexane, supercritical carbon dioxide and subcritical propane. As a result, the significant difference in color could be found. The *Y* values of SE and SFE were same, which was higher than that of SPE. The *R* value of SPE was higher than those of SE and SFE. The oil obtained by SPE was of highly intense red color, the color measure also revealed the possible high content of carotenoids.

Fatty acid composition

Table 3 showed the fatty acid composition of the foxtail millet bran oil obtained by SE, SCE and SPE. Ten fatty acids (C16:0, C16:1, C17:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1 and C22:0) at different quantities were detected in all samples. Although the oils were extracted by different methods, no significant differences were observed in fatty acid composition. Linoleic, oleic and linolenic acids were the main unsaturated fatty acids while palmitic and stearic acids were the saturated fatty acids, whose contents followed the order: linoleic acid > oleic acid > palmitic acid > stearic acid > linolenic acid. Polyunsaturated fatty acids such as linoleic acid (18:2), linolenic acid (18:3), and arachidonic acid (20:4) are called essential fatty acids (EFA) because of their necessity in the human body (Kim et al. 2004), which are very important for human health. The foxtail millet bran oil with high content of linoleic acid may be used as functional food.

Tocopherol composition

Tocopherols are a class of chemical compounds consisting of various methylated phenols, which have vitamin E activity (Betti et al. 2006). It is a series of organic compounds. The tocopherol composition of the oils obtained by SE, SCE and SPE were showed in Table 4. The extracts contained α - and β -tocopherols as the major components of vitamin E. The α -tocopherol contents of SE and SPE were significantly higher than that of SCE. Their difference in β -tocopherol content could be also found. The β -tocopherol contents followed the following order: SPE > SE > SCE. The results showed that the subcritical propane could be a suitable and selective solvent

Fig 1 The oil oxidative stability of SE, SCE, SPE in *Rancimat* test (black down-pointing triangle represented induction time)

for the extraction of the foxtail millet bran oil with high tocopherol content.

Oil oxidative stability

In the *Rancimat* method, the sample is exposed to a stream of air at temperatures from 50 to 220 °C. The volatile oxidation products (chiefly formic acid) are transferred to the measuring vessel by the air stream and absorbed there in the measuring solution (distilled water). When the conductivity of this measuring solution is recorded continuously, an oxidation curve is obtained whose point of inflection is known as the induction time, which provides a good characteristic value for the oxidation stability (Liu et al. 2009). The high amounts of linoleic acid in millet bran oil may have favorable nutritional implications and beneficial physiological effect in the prevention of coronary heart disease and cancer (Reyes et al. 2004; Harris 2008), however, it also makes them especially prone to oxidation. As shown in Fig. 1, the induction periods of the oils obtained by SE, SCE and SPE were 4.49, 1.18 and 4.70 h. That is to say, the oil oxidative stability of SE and SPE were significant higher than that of SCE, which could be attributed to the differences of antioxidant components and peroxide value. In above analysis, it was found the tocopherol contents of SCE were significantly lower than those of SE and SPE while its peroxide value was higher than those of SE and SPE.

Conclusion

The oil from foxtail millet bran as a waste product of the cereal industry were extracted with n-hexane, supercritical carbon dioxide and subcritical propane. The influence of the three methods on the extraction yield and oil composition were studied. The maximum oil yield was obtained by SPE (21.79 %), followed by SE (19.65 %) and SCE (17.14 %). The effect of extraction methods on the acid value, refractive index, saponification value, peroxide value and color of was significant while the effect on fatty acid profile was negligible. The highest tocopherol content and oxidative stability was found in the oil samples by SPE. The results showed that the SPE is a suitable and selective method for the extraction of the foxtail millet bran oil in view of smaller times and lower pressures employed compared to SCE and traditional SE. The production of oil from foxtail millet bran provides a kind of alternative oil resource for vegetable oil, adds value to agricultural products and improves the environment.

Acknowledgments The financial support provided by the National Natural Science Foundation of China (31101232), the Program for Innovative Research Team (in Science and Technology) in University of Henan Province (13IRTSTHN006) and the Program for Young Key Teacher in University of Henan Province (2012GGJS-139) were greatly appreciated.

References

- Ahangari B, Sargolzaei J (2012) Extraction of pomegranate seed oil using subcritical propane and supercritical carbon dioxide. *Theor Found Chem Eng* 46:258–265
- Bai Q, Fan G, Gu Z, Cao X, Gu F (2008) Effects of culture conditions on γ -aminobutyric acid accumulation during germination of foxtail millet (*Setaria italica* L.). *Eur Food Res Technol* 228:169–175
- Bai Q, Chai M, Gu Z, Cao X, Li Y, Liu K (2009) Effects of components in culture medium on glutamate decarboxylase activity and γ -aminobutyric acid accumulation in foxtail millet (*Setaria italica* L.) during germination. *Food Chem* 116: 152–157
- Betti M, Minelli A, Canonico B, Castaldo P, Magi S, Aisa MC, Piroddi M, Di Tomaso V, Galli F (2006) Antiproliferative effects of tocopherols (vitamin E) on murine glioma C6 cells: homologue-specific control of PKC/ERK and cyclin signaling. *Free Radic Biol Med* 41: 464–472
- Dos Santos FL, de Oliveira JV, Dariva C, Jacques RA, Caramão EB (2008) Extraction of grape seed oil using compressed carbon dioxide and propane: extraction yields and characterization of free glycerol compounds. *J Agric Food Chem* 56: 2558–2564
- Firestone D (1998) Official method and recommended practices of the AOCS, 5th edn. AOCS Press, Champaign
- Gunstone FD, Harwood JL (2007) Occurrence and characterization of oils and fats. In: Gunstone FD, Harwood JL, Dijkstra AJ (eds) *The lipid handbook with CD-ROM*, 3rd edn. CRC Press, Boca Raton, pp 37–142
- Hamdan S, Daood HG, Toth MV (2008) Extraction of cardamom oil by supercritical carbon dioxide and sub-critical propane. *J Supercrit Fluids* 44:25–30
- Harris WS (2008) Linoleic acid and coronary heart disease. *Prostaglandin Leukotr Essent Fat Acids* 79:169–171
- Herrero M, Cifuentes A, Ibañez E (2006) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review. *Food Chem* 98:136–148
- Kim SL, Kim SK, Park CH (2004) Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Res Int* 37: 319–327
- Lanza M, Ndiaye PM, Tavares FW, Oliveira D, Dariva C, Oliveira JV (2005) Phase behavior of castor oil in compressed propane and *n*-butane. *J Supercrit Fluids* 34:215–221
- Liang S, Yang G, Ma Y (2010) Chemical characteristics and fatty acid profile of foxtail millet bran oil. *J Am Oil Chem Soc* 87:63–67
- Liu B, Du J, Zeng J, Chen C, Niu S (2009) Characterization and antioxidant activity of dihydromyricetin-lecithin complex. *Eur Food Res Technol* 230:325–331
- Nantiyakul N, Furse S, Fisk ID, Tucker G, Gray DA (2013) Isolation and characterization of oil bodies from *Oryza sativa* bran and studies of their physical properties. *J Cereal Sci* 57:141–145
- Orthoefer FT (2005) Rice bran oil. In: Shahidi F (ed) *Bailey's industrial oil and fat products, edible oil and fat products: edible oils*, vol. 2, 6th edn. Wiley, New Jersey, pp 465–490
- Paquot C, Hauntenne A (1987) IUPAC standard methods for the analysis of oils, fats and derivatives. Blackwell, London
- Prashant SH, Namakkal SR, Chandra TS (2005) Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats. *Nutr Res* 25:1109–1120
- Proestos C, Boziaris IS, Nychas GJE, Komaitis M (2006) Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. *Food Chem* 95:664–671

- Reyes N, Reyes I, Tiwari R, Geliebter J (2004) Effect of linoleic acid on proliferation and gene expression in the breast cancer cell line T47D. *Cancer Lett* 209:25–35
- Sreenivasulu N, Miranda M, Prakash HS, Wobus U, Weschke W (2004) Transcriptome changes in foxtail millet genotypes at high salinity: identification and characterization of a PHGPX gene specifically up-regulated by NaCl in a salt-tolerant line. *J Plant Physiol* 161:467–477
- Suma PF, Urooj A (2012) Antioxidant activity of extracts from foxtail millet (*Setaria italica*). *J Food Sci Technol* 49:500–504
- Usha A, Sripriya G, Chandra TS (1996) The effect of fermentation on the primary nutrients in foxtail millet (*Setaria italica*). *Food Chem* 56: 381–384
- Xue YY, Li P, Lin QB (2008) Research evolution on chemical component and physical character of foxtail millet. *J Chin Cereal Oil Assoc* 22: 51–56
- Zigoneanu IG, Williams L, Xu Z, Sabliov CM (2008) Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. *Bioresource Technol* 99:4910–4918