Oleochemical Properties for Different Fractions of Foxtail Millet Bran

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Abstract: Foxtail millet (FM) is one of the oldest cultivated grain crops with a variety of nutrients, and foxtail millet bran (FMB), a by-product of FM milling process, is also rich in variety of nutrient substance. There are four classifications of FMB, namely coarse bran (FMCB), skin bran (FMSB), polished bran (FMPB) and mixed bran (FMMB). Because these nutrients are distributed within the different fractions of FMB, we compared some chemical composition and its oleochemical properties of four FMB samples. Results showed that the oil extracted from FMB is high value-added plant oil. It contains abundant unsaturated fatty acid (UFA), with the main UFAs were linoleic acid (65%~69%) and oleic acid (12~17%), which accounted for more than 80% of the lipids. The main triacylglycerols were trilinolein (LLL) and oleodilinolein (OLL). There were no evident difference on fatty acid, triacylglycerol and sterols profiles for FMSB, FMPB and FMMB, but the contents of amino acids, tocols, squalene and oryzanol were different.

Key words: foxtail millet bran, oleochemical properties, fatty acid, triacylglycerol, squalene, oryzanol

1 Introduction

Cereal grains are an important component of the human diet worldwide and play a significant role in human nutrition and health. Millet is an ancient cereal grain besides the major wheat, rice, maize, and is the staple diet in some parts of countries, especially in arid and semiarid areas1~3, owing to its short growing season, excellent resistance to pests and diseases, productivity under drought conditions, strong tolerance to drought and barren soil compared to other major cereals4. It has been reported that millet possesses effective medical and nutritional functions such as anti-oxidant, anti-cancer and anti-inflammatory5~6. Millet also has the ability to lower the level of serum cholesterol for its highest amount of sitostanol among cereal grains7. Major varieties of millet include sorghum, pearl millet, finger millet, proso millet, foxtail millet, barnyard millet, kodo millet and little millet1~4,8.

Foxtail millet (Setaria italic L., abbreviated as FM) is a small kernel, thin and long stem crop, and originated in North China, and is currently mostly cultivated in Japan, Australia, India, South America, Africa and China9~10. India and China are the most important growing countries among them.

FM is consumed with dehulling (dehusking), milling and polishing, and produced pericarp, skin bran (abbreviated as FMSB) and polished bran (abbreviated as FMPB) as the by-product, respectively (Fig. 1). FM is processed by dehulling to remove pericarp (also called as coarse bran, abbreviated as FMCB), a non-edible ingredient of the grain, traditionally been used to raise livestock, or returned to the field, even discarded due to its limited nutritional value11. FMSB, also known as “foxtail millet fine bran”, is the by-product of the milling process, mainly consisting of testa, nucellar layer, aleurone layer, embryo (germ), and a small number of starchy endosperm. FMPB is the by-product from polishing process of brown millet to polished millet. However, commercial foxtail millet bran (abbreviated as FMB), commonly, is a mixture of FMSB and FMPB, or a mixture of FMCB, FMSB and FMPB, and they are uniformly called ”mixed bran (abbreviated as FMB)”11. Current review of literature shows that FMB involves nutritious elements such as lipid, protein, dietary fiber, phytosterol, γ-oryzanol, polyphenol compounds and squalene. In terms of these multiple phytochemicals, FMB was endowed with abundant physiological activities, including antioxidative12, antitumor13, and immunomodulating activities14. In this regard, FMB may act as a natural source of antioxidants in food industry, as well as a nutraceutical in reducing disease risks and promoting health. Unfortunately, lots of FMB are not fully utilized, it is extensively used as animal feed and agricultural fertilizers in China, which results in great waste of valuable resources. Recent years, many reports have found

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that FMB is rich in lipids\textsuperscript{[50]}. Therefore, it is expected that high value-added products can be obtained by oil extraction from FMB.

Foxtail millet oil (abbreviated as FMO) is a kind of high-grade oil with nutritional value and added value. Besides eating, it can also be used in medicine, health care products, cosmetics and other industries. According to the data, the cosmetic function of FMO is very good, it also has the prevent effect of hurricane, itching and astringency. It is commonly used in traditional Chinese medicine for the treatment of psoriasis, ulceration, chronic eczema, neurodermatitis and other diseases. It can also be used to treat skin diseases with other drugs. It can be seen that the development of research on FMO has important practical significance for improving the comprehensive utilization of millet grain oil, however, there have been limited studies reporting on FMB. On the other hand, information on the amino acid composition of FMSB, FMPB and FMMB is very scarce. Therefore, the current work were carried out to evaluate the disparity of fatty acid profile and bioactive component differences, and triglyceride (TAG) composition of the oil extracted from them.

2 Experimental Procedures

2.1 Chemicals and standards

FMSB, FMPB and FMMB (mixture of FMBs) used in this research were supplied by the market of Qinshui Country (Shanxi Province, China). Prior to analysis, all samples were dried, ground to powder, passed through a 60-mesh screen, and then stored in a refrigerator until analyzed.

Individual standards of fatty acid methyl esters (FAMEs, purity ≥ 99%) including methyl ester of lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eicosenoic (20:1), behenic (22:0), lignoceric (24:0), 17 amino acids, 8 isomers of tocopherols (T\(_3\)s) and tocotrienols (T\(_3\)s), 5-α cholesterol standard (purity ≥ 95%), campesterol, stigmasterol, β-sitosterol, sitostanol, squalene (purity ≥ 98%), acetonitrile, \(n\)-hexane and methanol of chromatographic grade were all obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Other reagents were of analytical pure grade and directly used without further purification.

2.2 Proximal composition analysis for FMB

Proximal composition (moisture, ash, crude lipid, crude protein and crude fiber) of FMB fraction was determined in agreement with AOCS official methods: Ba 2a-38, Ba 5a-49, Ba 3-38, Ba 4a-38, Ba 6-84\textsuperscript{[78]}. Lipids in FMB were extracted by a Soxhlet apparatus with \(n\)-hexane. The protein content was determined in a Foss 8400 automatic Kjeltec Analyzer (Foss Technologies Co., Ltd., Denmark) and estimated by a nitrogen conversion factor of 6.25. The fiber was measured by a Fibertec\textsuperscript{TM} 2010 automatic fiber analysis system (Foss Technologies Co., Ltd., Denmark). And the results were recorded on a dry basis.

2.3 Extraction of FMO

Triplicate samples of the test milled FMB (300 g) were extracted in a Soxhlet apparatus with \(n\)-hexane for 8 h at 75 ± 5°C. The solvent was removed by rotary evaporation and a stream of \(N_2\), afterward, the oils were heated at 105°C for 30 mins. The oil was purged with \(N_2\) and kept at −4°C until analysis. The oils extracted from FMSB, FMPB and FMMB were named FMO, FMPO and FMMO, respectively.

2.4 Amino acid analysis

Amino acid composition of FMSB, FMPB and FMMB were determined following the procedure by ISO13903-2005\textsuperscript{[77]}. In brief, an aliquot of the dried and fully defatted sample powder (200 mg) was homogenized with 10 mL of 6 mol/L hydrochloric acid (chromatographic purity) and 3–4 drops freshly distilled phenol in an individual 25 mL ampulla tube. The tube was capped, vortexed, and then placed in an oven at 110 ± 1°C for 22 h for full hydrolysis. The mixture was again vortexed, and passed through a 0.45 μm filter to remove solid particulates. The hydrolyzed solu-
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2.5 Physicochemical properties assays of FMO

The acid value (AV), peroxide value (PV), iodine value (IV), color, saponification value and unsaponifiable matter of the FMO were evaluated by the AOCS Methods Cd 3d-63, Cd 8-53, Cd 1-25, Cc 13e-92, Cd 3a-94 and Ca 6a-40, respectively. The amino acid composition was monitored at 570 nm and 440 nm, and the amount of each amino acid was given in mg/g of dry sample.

2.6 Fatty acid (FA) and triacylglycerols (TAGs) composition of FMO

FA composition was analyzed according to the AOCS Ce1-62, and based on the purpose, they were converted to their corresponding volatile FAMEs by direct esterification with boron trifluoride-methanol solution. And FAMEs analysis were carried out on an Agilent 7890 B gas chromatograph (GC, Agilent, USA) consisting of a flame ionization detector (FID), a Supelco HP-88 capillary column (100 m x 0.25 mm inner diameter, 0.20 μm film thickness) and an auto-sampler. Helium was used as the carrier gas (flow rate of 1 mL/min) with a split ratio of 1:50. FA was identified by comparison of the retention times with those of FAME mixture standards performed at the same GC conditions, and the results were reported as peak area percent. The separations of sterols and squalene were both carried out by an Agilent 1200 HPLC-MS (Agilent, USA) based on reference. The oil was dissolved in hexane at about 48 mg/mL, and this solution was then diluted with acetonitrile/isopropanol containing 0.5% ammonia (7/3, v/v) to 4.8 mg/mL. HPLC-MS separation was achieved by an Agilent ZORBAX SB-C18 column (4.6 mm x 250 mm, inner diameter, 5 μm particle size) at 30°C.

2.7 Analysis of Vitamin E, plant sterol, squalene and γ-oryzanol

Vitamin E is comprised of tocopherols (Ts) along with tocotrienols (T3s). The α-, β-, γ-, and δ- forms of Ts and T3s were analyzed using HPLC system according to the procedure of AOCS Ce 8-89 and reference. Oil samples (0.5 g) were dissolved in hexane at 50 mg/mL and filtered through a 0.22 μm pore size nylon syringe filter. The analysis were performed on a Waters 2695 HPLC (Waters, USA) with a Spherisorb® Si5 NH2 column (250 mm x 4.6 mm, 5 μm, Waters, USA) and a Waters 2475 FLD detector. The detector excitation and emission wavelengths were monitored at 298 and 325 nm, respectively. The contents of individual Ts and T3s were calculated by the calibration curve of the corresponding standard compound and the results are recorded as mg/100 g of dry weight.

Both sterol and squalene fractions are unsaponifiable. The process for measuring contents of sterol and squalene is similar in that it involves sample complete saponification, extraction of the unsaponifiable matter with hexane, and fractionation through GC. Following the procedure of ISO 12228:1999, 2 g of FMO was accurately weighed into a screw-top tube and mixed with 1 mL of 5α-cholestan-3β-o1 (internal standard, 2 mg/mL in n-hexane) for 1 h. For squalene analysis, 2 g of FMO was mixed with 300 μL of squalene (internal standard, 1 mg/mL in n-hexane). Afterwards, the mixture was saponified with 20 mL of KOH 4 mol/L in 95% ethanol at 85°C for 2 h.

Unsaponifiable compounds were extracted with n-hexane of chromatographic purity three times and washed with 30 mL of ethanol/distilled water (v:v, 9:1) until the washing was neutral. The aqueous portion was withdrawn, and the upper organic fraction was dried with dry anhydrous sodium sulfate and then purged to dryness with N2. For squalene, the residues were redissolved with n-hexane to 10 mL and then sealed in an auto sampler injection vial for GC analysis. For sterol, the residues were dissolved with 0.4 mL chloroform. Subsequently, steroid fraction was captured after thin layer chromatography with anhydrous diethyl ether / n-hexane (35:65, v:v) as developing solvent and a methanol spray as fluorescent indicator. Then derivatization was carried out with 200 μL of N,O-bis (trimethylsilyl)– trifluoro-acetamide in 1% trimethylchlorosilane and 200 μL of pyridine at 85°C for 50 min, the derivatization fluid was evaporated to dryness. Finally, 1 mL of n-hexane was added and the solution was passed through a 0.22 μm syringe filter, then was transferred into a vial, and 1 μL portion for GC analysis.

The separations of sterols and squalene were both carried out by an Agilent 7890A GC equipped with an HP-5 capillary column (30 m x 0.25 mm) coated with a 0.25 μm-thick of 5% phenylmethylsiloxane film and a FID. N2 (99.999%) carrier gas with a flow rate of 1.0 mL/min was used. For sterols, the split ratio was 1:20; the temperatures of injector and FID were set at 300°C and 360°C, respectively. For squalene, the split ratio was 1:10; the inlet and detector temperatures were 250°C and 300°C, respectively. The following temperature program was used for the column: from initial 130°C to 230°C with a ramp rate of 10°C/min and kept for 5 min, and then to the final 270°C at a rate of 3°C/min. Squalene was eluted at 15.06 min.
3 Results and Discussion

3.1 Proximal composition of FMB

Results of the investigations of the proximal composition of FMB are summarized in Table 1. The crude lipid, protein and moisture content of FMB is basically equivalent to those of rice bran\(^\text{20}\), and the polished bran contains more broken millet, so its oil content is lower. The crude fiber contents of polished bran (only 2.06\%) were lower than skin bran (18.13\%). The main reason is that skin bran contains much skin layer and shell, and the polished bran has a large amount of broken millet, resulting in low cellulose, and the corresponding protein content will be as high as 12.46\%. The crude fiber of FMMB (28.03\%) was obviously lower than FMCB because of the FMMB included the pericarp and the pericarp contains a lot of fiber. However, compared with the previous literature\(^\text{21}\), the crude fiber content in this experiment is also lower, which may be caused by the difference of varieties. Considering the lipid content in FMCB is only a handful, the oil in FMCB was not extracted and further studied.

3.2 Amino acid composition

Amino acids, as building modules of proteins and polypeptides, play essential roles in metabolism. The metabolism of amino acids (AA) has been linked to periodontal disease\(^\text{22}\), chronic obstructive pulmonary disease\(^\text{23}\), and Alzheimer’s disease\(^\text{24}\). In addition to clinical and nutritional properties, some free amino acids taste sweet to humans\(^\text{25}\), and contribute to food flavor. As Table 2 shows, FMB is rich in total amino acids (TAA) including seven of the essential amino acids (EAA), namely, lysine, threonine, leucine, isoleucine, valine, methionine, phenylalanine, as well as ten non-essential amino acids (NEAA), namely, alanine, glycine, glutamic acid, aspartic acid, serine, proline, valine, tyrosine, cysteine, arginine and histidine. The concentration of EAA was 3.96\~5.17 g/100 g dry weight, of EAAs/TAAs was 35.48\~37.51\% and EAAs/NEAs was 54.99\~60.03\% which is close to the composition of soybean\(^\text{26}\) (about 30\% and 50\%, respectively). The result also indicates that glutamic acid was the highest amino acid in FMB, representing 1.84 g/100 g DW for skin bran, 2.38 g/100 g DW for polished bran and 2.35 g/100 g DW for mixed bran, and followed by aspartic acid. The most abundant EAAs were leucine, representing 28\%~30\% of the total EAAs, respectively. The content of each amino acid in the polished bran is higher than that in the skin bran. The concentrations of glutamic acid, leucine and aspartic were found to be large. According to the literature, aspartic and glutamic acid are responsible for the specific flavors of oilseeds\(^\text{27}\). Cysteine and methionine were present in only small amounts.

3.3 Physicochemical characteristics

The physicochemical parameters of oil are important to chemists because they express chemical composition and are important to industry because they determine suitability for applications. Table 3 for the physicochemical properties showed no difference between the oils extracted from skin bran, polished bran and mixed bran. The average values were acid value (AV) of 17.26\~0.18 mgKOH/g (FMSO), 37.33\~0.41 mgKOH/g (FMPO) and 29.82\~0.33 mgKOH/g (FMMO), peroxide of 3.32\~0.13 g I\(_2/100\) g (FMSO), 4.41\~0.17 g I\(_2/100\) g (FMPO) and 3.71\~0.22 g I\(_2/100\) g (FMMO) and a color expressed as Y43 R4 (FMSO), Y46 R5.2 (FMPO) and Y36 R4.4 (FMMO), corresponding to yellow (Y) and red (R), respectively. Both AV and PV are high and need to be further refined before consumption. The saponification value of the crude oil (178~186 mg KOH/g) clearly indicates that the FMO consists mainly of medium-chain fatty acids and, as such, is easily absorbed by the human body. The iodine values of FMO were 165~

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**Table 1** Proximate composition of FMB (DW\%).

| Composition       | FMCB          | FMSB          | FMPB          | FMMB          | FMMB²          |
|-------------------|---------------|---------------|---------------|---------------|---------------|
| Crude protein\(^1\) | 5.87 ± 0.19\(^a\) | 11.04 ± 0.36\(^b\) | 12.46 ± 0.27\(^c\) | 12.13 ± 0.32\(^d\) | 12.48 ± 0.41\(^e\) |
| Crude lipids\(^1\) | 2.28 ± 0.08\(^a\) | 12.67 ± 0.38\(^b\) | 8.60 ± 0.12\(^c\) | 10.01 ± 0.23\(^d\) | 9.39 ± 0.17\(^e\) |
| Ash\(^1\)          | 9.93 ± 0.07\(^d\) | 5.96 ± 0.03\(^b\) | 3.34 ± 0.04\(^c\) | 7.34 ± 0.04\(^d\) | 7.50 ± 0.18\(^e\) |
| Moisture\(^1\)      | 7.01 ± 0.05\(^a\) | 7.41 ± 0.02\(^b\) | 11.59 ± 0.06\(^c\) | 8.98 ± 0.14\(^d\) | 8.29 ± 0.16\(^e\) |
| Crude fiber\(^1\)   | 43.44 ± 1.32\(^d\) | 18.13 ± 0.49\(^b\) | 2.06 ± 0.01\(^c\) | 28.03 ± 0.84\(^e\) | 51.69 ± 2.14\(^e\) |

\(^1\) Values are expressed as means lever ± standard deviation in triplicate (n=3). Values in the same row with different superscript letters are significantly different (p < 0.05).

\(^2\) Data from the reference 21.
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172 g L/100 g, indicating that FMO has a high degree of unsaturation and can be considered a drying oil. Therefore, attention must be paid in the processing, storage, transportation and heating of FMO to prevent the loss of nutrients caused by oxidation and rancidity. The unsaponifiable matter content (above 2.8~3.1%) was higher in FMO compared to the oil of sesame (1.2%), and corn (1.3~2.3%), while slightly lower than white camellia oil (3.57%), shea butter Oil (3~10%), and rice bran (4~6%). Unsaponifiable compounds in FMO mainly included sterols, big-molecule fatty alcohols, terpenes, pigments, tocopherols and squalene.

3.4 Fatty acid (FA) and triglyceride (TGA) profile

FA profile is an essential characteristic of every vegetable oil and has traditionally been used as an indicator of oil purity. Eight fatty acids were identified and quantified in FMO analyzed (Table 4). There are similar FA profiles where linoleic acid (more than 65%) is the major fatty acid, followed by oleic acid and palmitic acid. Monounsaturated fatty acids (MUFAs) were extremely represented by oleic acid and palmitoleic acid in three lipid fractions, which accounted a higher proportion in FMMO than in FMPO and FMSO. It has long been recognized that there is an evidently lower risk of coronary heart disease (CHD) in Mediterranean populations owing to the higher intake of MUFAs. Linoleic acid is an essential fatty acid for humans as it is essential for the formation of vitamin D, cellular membrane and various hormones. Saturated fatty acids (SFAs) were expressed by high levels of palmitic and stearic acid in three samples. Palmitoleic (16:1), arachidic (20:0) and behenic (22:0) acids had a minimal presence in FMO. From Table 4, it could be deduced that the FA profile of FMO presented the oils as a good source of the essential

| Amino acid | FMSB | FMPB | FMB | FMMB |
|------------|------|------|-----|------|
| ΣEAs       | 3.96 | 4.28 | 5.17 | 4.35 |
| Lysine     | 0.52 | 0.48 | 0.70 | 0.53 |
| Threonine  | 0.48 | 0.53 | 0.58 | 0.40 |
| Ieucine    | 1.12 | 1.25 | 1.47 | 1.31 |
| Isoleucine | 0.47 | 0.48 | 0.49 | 0.51 |
| Valine     | 0.66 | 0.67 | 0.74 | 0.71 |
| Methionine | 0.18 | 0.26 | 0.31 | 0.24 |
| Phenylalanine | 0.53 | 0.61 | 0.88 | 0.65 |
| ΣNEAs      | 7.10 | 7.13 | 9.04 | 7.91 |
| Glycine    | 0.59 | 0.51 | 0.65 | 0.62 |
| Alanine    | 0.92 | 1.04 | 1.39 | 1.10 |
| Serine     | 0.47 | 0.53 | 0.69 | 0.58 |
| Aspartic   | 1.08 | 0.87 | 1.24 | 1.15 |
| Glutamic   | 1.84 | 2.38 | 2.35 | 2.22 |
| Proline    | 0.62 | 0.55 | 0.76 | 0.72 |
| Tyrosine   | 0.35 | 0.28 | 0.59 | 0.21 |
| Cystine    | 0.07 | 0.08 | 0.12 | 0.09 |
| Arginine   | 0.72 | 0.49 | 0.84 | 0.74 |
| Histidine  | 0.44 | 0.40 | 0.41 | 0.48 |
| Total AAs  | 11.06| 11.41| 14.21| 12.26|
| (EAAs/TAAs) | 35.80 | 37.51 | 36.38 | 35.48 |
| (EAAs/NEAs) | 55.77 | 60.03 | 57.19 | 54.99 |

FMB, foxtail millet coarse bran; DW, Dry weight; FMSB, foxtail millet skin bran; FMPB, foxtail millet polished bran; FMMB, foxtail millet mixed bran.

AAs, amino acids; EAAs, essential amino acids; NEAAs, non-essential amino acids.

Values are means ± standard deviation of triplicate determinations. Values in the same row with different superscript letters are significantly different (p < 0.05).

1 Data from the reference 21.
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Table 3  Some characteristics, tocol, sterol, squalene and γ-oryzanol contents of FMO.

| Characteristic          | FMSO      | FMPO      | FMMO      | FMOO |
|-------------------------|-----------|-----------|-----------|------|
| Acid value (mg KOH/g oil) | 17.26 ± 0.18<sup>a</sup> | 37.33 ± 0.41<sup>c</sup> | 29.82 ± 0.33<sup>b</sup> | NR   |
| Peroxide value (mmol O₂/kg oil) | 3.32 ± 0.13<sup>a</sup> | 4.41 ± 0.17<sup>c</sup> | 3.71 ± 0.22<sup>b</sup> | NR   |
| Color (Lovibond, 25.4 mm) | Y43 R4.0 | Y46 R5.2 | Y36 R4.4 | Y73 R10 |
| Iodine value (g I/100 g) | 171.3 ± 1.23<sup>a</sup> | 168.6 ± 0.97<sup>c</sup> | 165.9 ± 1.01<sup>c</sup> | NR   |
| Saponification value (mg KOH/g) | 178.9 ± 0.74<sup>a</sup> | 181.4 ± 0.63<sup>b</sup> | 179.7 ± 0.39<sup>c</sup> | 186.29 ± 0.51 |
| Unsaponifiables (%) | 3.06 ± 0.13<sup>b</sup> | 2.83 ± 0.09<sup>b</sup> | 2.88 ± 0.09<sup>c</sup> | 3.62 ± 0.19 |
| ΣTocol (mg/100g oil) | 122.88 ± 3.13<sup>c</sup> | 98.67 ± 2.22<sup>c</sup> | 56.78 ± 1.21<sup>c</sup> | 64.83 ± 0.83 |
| α-T<sup>1</sup> | 56.87 ± 1.01<sup>c</sup> | 25.48 ± 0.58<sup>b</sup> | 21.93 ± 0.41<sup>c</sup> | 15.53 ± 0.31 |
| α-T3<sup>1</sup> | 11.56 ± 0.43<sup>a</sup> | 3.99 ± 0.13<sup>c</sup> | 9.09 ± 0.16<sup>b</sup> | NR   |
| β-T<sup>1</sup> | 2.70 ± 0.16<sup>c</sup> | 3.15 ± 0.11<sup>b</sup> | 7.20 ± 0.12<sup>c</sup> | NR   |
| γ-T<sup>1</sup> | 34.53 ± 1.15<sup>b</sup> | 34.88 ± 0.72<sup>b</sup> | 11.96 ± 0.47<sup>c</sup> | 48.79 ± 0.46 |
| (β + γ)-T3<sup>1</sup> | 12.92 ± 0.39<sup>c</sup> | 18.27 ± 0.31<sup>c</sup> | NR   |
| δ-T<sup>1</sup> | 0.78 ± 0.05<sup>c</sup> | 9.44 ± 0.23<sup>c</sup> | 6.61 ± 0.05<sup>b</sup> | 0.51 ± 0.06 |
| δ-T3<sup>1</sup> | 3.52 ± 0.12<sup>c</sup> | 3.46 ± 0.14<sup>c</sup> | NR   |
| ΣSterol (mg/100 g oil)<sup>2</sup> | 2060.79 ± 6.18<sup>c</sup> | 2026.93 ± 6.70<sup>c</sup> | 1963.35 ± 5.85<sup>c</sup> | NR   |
| Campesterol | 263.15 ± 1.46<sup>a</sup> | 271.49 ± 1.77<sup>c</sup> | 269.53 ± 2.01<sup>b</sup> | NR   |
| Stigmasterol | 81.17 ± 0.53<sup>b</sup> | 73.44 ± 0.65<sup>c</sup> | 89.85 ± 0.48<sup>c</sup> | NR   |
| β-Sitosterol | 887.49 ± 2.03<sup>b</sup> | 916.84 ± 1.97<sup>c</sup> | 869.58 ± 1.52<sup>c</sup> | NR   |
| Sitostanol | 828.98 ± 2.16<sup>c</sup> | 765.16 ± 2.31<sup>b</sup> | 734.39 ± 1.84<sup>c</sup> | NR   |
| Squalene (mg/100 g oil) | 12.59 ± 0.37<sup>b</sup> | 10.52 ± 0.65<sup>c</sup> | 12.87 ± 0.21<sup>b</sup> | NR   |
| γ-oryzanol (%) | 1.77 ± 0.04<sup>c</sup> | 1.65 ± 0.03<sup>c</sup> | 1.72 ± 0.04<sup>b</sup> | NR   |

FMO, foxtail millet bran oil; FMSO, foxtail millet skin bran oil; FMPO, foxtail millet polished bran oil; FMMO, foxtail millet mixed bran oil, "NR", not reported; "-", not detected.

Values are means ± standard deviation of triplicate determinations. Values in the same row with different superscript letters are significantly different (∆p < 0.05).

<sup>1</sup> T, tocopherol; T3, tocotrienol.

<sup>2</sup> Only the major sterols are listed in this table.

<sup>3</sup> Data from the reference 21.

fatty acids.

TAG general follow a unique and typical pattern in different oils. Table 5 sketched the predicted TAG composition of FMSO, FMPO and FMMO, which were detected by reversed phase HPLC and MS instrumentation. The main constituent TAGs of the three FMO were trilinolein (LLL), oleodilinolein (OLL), palmitodilinolein (PLL), stearodilinolein (SLL), dioleodilinolein (OLL), palmitoleodilinolein (POL) and linolenodilinolein (LNLL) and linoleoleostearin (LOS).

3.5 Tocol (tocopherol and tocotrienol) contents

Tocols are considerably important non-glyceride ingredients of vegetable oils, and they are comprised of eight distinct isomers (α-, β-, γ-, δ-T3s and α-, β-, γ-, δ-T3s) which are well recognized for their biological activities<sup>11</sup>. Biological activities of tocols are generally regarded to be due to their antioxidant activity, which restrains lipid peroxidation in biological membranes. Alpha-tocopherol is generally considered to be one of the most effective antioxidants.

A quantitative detection of tocols in FMO by HPLC shows the presence of tocols (Table 3). Among them, α-T and γ-T were found to predominate which accounts for about 60% of the total content, while β-T, δ-T3 and δ-T being the minor components, and the total T3 content was higher than T3s. In general, high α-T indicates propensity for high antioxidant activity. It is well known that wheat germ oil has the highest tocol content up to about 250 mg/100 g oil of all the vegetable oils. By comparison, the tocol contents in FMO is parallel to rice bran oil (40 ~130 mg/100 g oil), and FMMO is lower than that of FMSO and FMPO.
Table 4 Major fatty acid compositions of FMO.

| Fatty acid (%) | FMSO | FMPO | FMMO | FMMO |
|----------------|------|------|------|------|
| palmitic (16:0, P) | 7.81 | 7.43 | 8.51 | 6.44 |
| stearic (18:0, S) | 4.64 | 5.04 | 4.59 | 6.27 |
| arachidic (20:0, A) | 1.50 | 1.54 | 0.87 | 2.53 |
| behenic acid (22:0, Be) | 0.65 | 0.63 | 0.69 | 1.29 |
| ΣSFA | 14.60 | 14.64 | 14.66 | 16.53 |
| palmitoleic (16:1, Po) | 0.10 | 0.09 | 0.10 | – |
| oleic (18:1 n-9, O) | 12.86 | 14.32 | 17.08 | 13.05 |
| gadoleic acid (20:1) | – | – | – | 0.93 |
| ΣMUFA | 12.96 | 14.41 | 17.18 | 13.98 |
| linoleic (18:2 n-6, L) | 68.81 | 67.79 | 65.21 | 66.49 |
| linolenic (18:3 n-3, Ln) | 3.65 | 3.16 | 2.94 | 3.00 |
| ΣUFA | 72.46 | 70.95 | 68.15 | 69.49 |
| ΣPUFA | 72.46 | 70.95 | 68.15 | 69.49 |

ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids.

FMO, foxtail millet bran oil; FMSO, foxtail millet skin bran oil; FMPO, foxtail millet polished bran oil; FMMO, foxtail millet mixed bran oil.

1 Only the major fatty acids are listed in this table.
2 Values are means of three parallel tests.
3 Data from the reference 21.

3.6 Sterols, squalene and γ-oryzanol contents

Sterols are the major unsaponifiable fractions and considered as important biological constituents in oils because they are related to the oil quality. Biological effects associated with sterols intake are their potent antioxidant action and impact on health. The sterols compositions of FMO are listed in Table 3. From the data, the FMO is rich in β-sitosterol (869~916 mg/100 g, 44% ~45% of total sterols) and sitostanol (734~828 mg/100 g, 37% ~40% of total sterols). High β-sitosterol content is found in most vegetable oils, such as groundnut oil, sunflower oil, grape seed oil, olive oil and date seed oil, in which the mean relative contents are 62.3%, 61.9%, 69.2%, 84.3% and 81.0% of total sterols, respectively. In FMO, total sterols were found to be at a relatively high level of 1958~2066 mg/100 g, more than commercial peanut oil (147~171 mg/100 g) and soybean oil (205~287 mg/100 g). Squalene is a highly unsaturated, long-chained triterpene compound (2,6,10,15,19,23-hexamethyldotetracos-2,6,10,14,18,22-hexaene, C30H50), and belongs to the unsaponifiable lipid category. It has a variety of applications for food, medicine, cosmetics and chemical engineering because of the powerful physiological, anti-carcinogenic, anti-oxidative and anti-inflammatory effects. Squalene was originally extracted from deep-sea sharks and whales; however, in consideration of species protection and ecological balance, attention has been focused on identifying plant resources of squalene. The reasonable amounts are found in virgin olive oil (200~700 mg/100 g), amaranth seed oil (2400~8000 mg/100 g), camellia oil (12 mg/100 g) and rice bran oil (8~28 mg/100 g). The content of squalene in FMO was 12.59±0.37 mg/100 g (FMSO), 10.52±0.65 mg/100 g (FMPO) and 12.87±0.21 mg/100 g (FMMO).

Oryzanol, a group of ferulate esters of triterpene alcohols and phytosterols, exhibits anti-hyperlipemia, anti-diabetic, anti-inflammatory, avoids oxidation of lipids, prevents platelet aggregation, repressing cholesterol and reduces the absorption of cholesterol by the human body. Oryzanol has also been widely used to cure nerve imbalance and menopausal disorders. In addition, it also promotes the regulation of neurosis and promotes the growth and development of the human body. Crude FMO contains about 1.6~1.8% γ-oryzanol which equal to rice bran oil (1.6~3.0%).

4 Conclusion

Chemical characteristics, particularly lipid compositions, of foxtail millet oil (FMO) which involves three samples (FMSB, FMPB and FMMB) were analyzed in this study. The crude protein and lipid contents in foxtail millet were about 5.6~12.6% and 2.2~13.1% on a wet weight basis, respectively. FMO contains relatively large amounts of...
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polyunsaturated fatty acids and essential amino acids. The main unsaturated fatty acids in FMO are linoleic and oleic acid, which account for above 80% of the oil. SFA is a minor component and accounts for only 14% of the total FA. In addition, FMO was found to have relatively abundant tocols, sterols, γ-oryzanol and squalene, all of which are potentially valuable. There were no evident difference on fatty acid, triacylglycerol and sterols profiles for FMSB, FMPB and FMMB, but the contents of amino acids, tocols, squalene and γ-oryzanol were different. The proportion of total tocols, sterols, squalene and γ-oryzanol in FMPO was lower than in FMSO. The main triacylglycerols were trilinolein (LLL) and oleodilinolein (OLL). The utilization of millet bran resource, not only can realize the effective utilization of subsidiary agricultural products, decrease agricultural wastes, but increase farmers income, development of living standard.

Table 5 Major triacylglycerol (TAG) compositions of FMO.

| TAG composition¹ | ACN:n | ECN | FMSO (%) | FMPO (%) | FMMO (%) | FMMO² (%) |
|------------------|-------|-----|----------|----------|----------|-----------|
| LLnLn 18:2/18:3/18:3 | 54:8  | 38  | 0.13     | 0.18     | 0.1      | NR        |
| LLLn 18:2/18:2/18:3 | 54:7  | 40  | 3.96     | 3.17     | 3.78     | 4.01      |
| LLL 18:2/18:2/18:2  | 54:6  | 42  | 30.62    | 29.04    | 27.21    | 29.29     |
| OLLn 18:1/18:2/18:3 | 54:6  | 42  | 1.89     | 1.93     | 1.97     | 1.58      |
| PLLn 16:0/18:2/18:3 | 52:5  | 40  | 1.03     | 1.24     | 0.98     | NR        |
| OLL 18:1/18:2/18:2 | 54:5  | 44  | 20.77    | 18.78    | 21.48    | 17.20     |
| PLL 16:0/18:2/18:2 | 52:4  | 44  | 10.52    | 11.55    | 11.44    | 8.62      |
| SLLn 18:0/18:2/18:3 | 54:5  | 44  | 0.60     | 0.37     | 0.54     | NR        |
| OOLn 18:1/18:1/18:2 | 54:5  | 44  | 0.25     | 0.31     | 0.26     | NR        |
| SLL 18:0/18:2/18:2 | 54:4  | 46  | 7.48     | 6.26     | 6.17     | 8.39      |
| LOO 18:2/18:1/18:1 | 54:4  | 46  | 4.11     | 5.33     | 5.65     | 3.36      |
| POL 16:0/18:1/18:2 | 52:3  | 46  | 4.84     | 4.73     | 5.96     | 3.40      |
| PPL 16:0/16:0/18:2 | 50:2  | 46  | 1.19     | 1.81     | 1.32     | 0.82      |
| SOL 18:0/18:1/18:2 | 54:3  | 48  | 2.92     | 2.83     | 3.2      | 3.30      |
| OOO 18:1/18:1/18:1 | 54:3  | 48  | 0.38     | 0.54     | 0.49     | NR        |
| POO 16:0/18:1/18:1 | 52:2  | 48  | 0.71     | 0.83     | 0.76     | NR        |
| PPO 16:0/16:0/18:1 | 50:1  | 48  | 0.36     | 0.25     | 0.33     | NR        |
| ALL 20:0/18:2/18:2 | 56:4  | 48  | 1.15     | 1.43     | –        | 3.41      |
| BLL 22:0/18:2/18:2 | 58:4  | 50  | 1.04     | 0.89     | 0.94     | NR        |
| SSL 18:0/18:0/18:2 | 54:2  | 50  | 0.27     | 0.19     | 0.38     | NR        |
| SOO 18:0/18:1/18:1 | 54:2  | 50  | 0.29     | 0.34     | 0.42     | NR        |
| PSO 16:0/18:0/18:1 | 52:1  | 50  | 0.40     | 0.22     | 0.34     | NR        |
| AOL 20:0/18:1/18:2 | 56:3  | 50  | 0.71     | 0.55     | 0.81     | 1.24      |
| PSL 16:0/18:0/18:2 | 52:1  | 50  | 1.27     | 1.08     | 1.40     | 1.59      |
| APL 20:0/16:0/18:2 | 54:2  | 50  | 0.25     | 0.19     | 0.29     | NR        |
| PSL 16:0/18:0/18:2 | 54:2  | 50  | 1.33     | 1.41     | 1.38     | NR        |
| BLL 22:0/18:2/18:2 | 58:4  | 50  | 1.02     | 0.96     | 0.94     | 1.74      |
| BOL 22:0/18:1/18:2 | 58:3  | 52  | 0.39     | 0.47     | 0.51     | NR        |

CAN, total carbon numbers of fatty acid; n, double bond numbers; ECN, equivalent carbon numbers; ECN = ACN - 2n.

FMO, foxtail millet bran oil; FMSO, foxtail millet skin bran oil; FMPO, foxtail millet polished bran oil; FMMO, foxtail millet mixed bran oil; “-”, not detected.

¹ Only the major triacylglycerols are listed in this table.

² Data from the reference 21.
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Conflict of interest
The authors declare no conflict of interest.

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