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Chemical Characteristics and Fatty Acid Profile of Foxtail Millet Bran Oil

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Abstract Chemical characteristics of a sample of foxtail millet bran and its oil, focusing on the approximate composition of foxtail millet bran and the fatty acid profile, physicochemical properties and tocopherol composition of foxtail millet bran oil, are presented in this work. The results indicate that the millet bran constituted 9.39 ± 0.17% crude oil, 12.48 ± 0.41% crude protein, and 51.69 ± 2.14% crude fiber. The specific gravity, refractive index, saponification value, and unsaponifiable matter content of millet bran oil were 0.9185 ± 0.0003 g/cm^3 $\left(d_{20}^{20}\right)$, 1.4676 ± 0.0002 $\left(n_D^{40}\right)$, 186.29 ± 0.51 mg KOH/g, and 3.62 ± 0.19 g/100 g, respectively. The tocopherol content was 64.83 ± 0.83 mg/100 g oil, which consisted mainly of γ-tocopherol (48.79 ± 0.46 mg/100 g oil) and α-tocopherol (15.53 ± 0.31 mg/100 g oil). The millet bran oil was rich in linoleic acid (66.5%) and oleic acid (13.0%). The saturated fatty acids included palmitic acid (6.4%) and stearic acid (6.3%). The major fatty acid in the *sn*-2 position of the millet oil was linoleic acid (71.2%). The dominant triacylglycerols, calculated according to the 1,3-random-2-random hypothesis, were trilinoleate (LLL, 29.3%) and dilinoleoyl-monoolein (LLO, 17.2%). This work might be useful for developing applications for millet bran and its oil.

Keywords Foxtail millet bran oil · Chemical characteristics · Fatty acid profile-Tocopherol

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Introduction

Foxtail millet (*Setaria italica Beauv*), one of the oldest cultivated crops, originated from China, and is now planted all over the world. It has been recorded that millet has many nutritious and medical functions [1–3]. In China, the foxtail millet, as one of the major grain sources, was cultivated mainly in Intermongolia, Jilin, Heilongjiang, Liaoning, Hebei, Shandong, Henan, Shanxi, and Shaanxi. The cultivated area of foxtail millet in China is approximate 1,400 km^2, and total production is in the range of 3,700–4,500 thousand ton [3]. The millet bran is a by-product of the foxtail millet production, and is used as animal feed in China extensively [4].

The millet is rich in carbohydrate [5] and protein [6], and also contains oils [7], and vitamins [8]. There are many reports on the composition of millet bran and millet lipids [9–14]. Lorenz and Hwang [9] analyzed the composition of free and bound lipids in proso millet (*Panicum miliaceum*) flours and brans. In the free lipids, hydrocarbons, sterol esters, triacylglycerols, diacylglycerols, and free fatty acids were present. The predominant fatty acids in the free lipids were linoleic, oleic, and palmitic acids. In the bound lipids, monogalactosyl diacylglycerols, digalactosyl diacylglycerols, phosphatidylethanolamine, phosphatidyl serine, and phosphatidyl choline were tentatively identified [9]. Osagie and Kates [10] evaluated the lipid composition including neutral lipids, phospholipids and glycolipids of millet seeds by silicic column, thin layer chromatography and gas chromatography. The major fatty acid composition of neutral lipids were linoleic (L, 45.7%), oleic (O, 24.7%), palmitic (P, 16.7%), and stearic (St, 8.2%) [10]. Taira [11] analyzed the lipid content and fatty acid composition of different foxtail millets. The dominant fatty acid of foxtail millet oil was linoleic acid (about 70% of total acids) for

both nonglutinous and glutinous varieties, and the major differences between the two types were stearic and arachidic acid contents [11]. Sridhar and Lakshinarayana [12] studied the content of total lipids, lipid classes and fatty acids composition in small millets, such as foxtail (*Setaria italica*), proso (*Panicum miliaceum*), and finger (*Eleusine coracana*). Ibrahima et al. [13] reported the lipid content and fatty acids composition of some millet cultivars from Tunisia and Mauritania. Huo et al. [14] evaluated the saponification value, unsaponifiable matter, color and fatty acids composition of millet oils. However, little information on fatty acid distribution of triacylglycerol, triacylglycerol composition, and tocopherol composition of millet bran oil is available.

To explore the application of millet bran, this work presented the general composition of millet bran, the physiochemical properties and fatty acid profile of its oil. We focused on the fatty acid profile of millet oil triacylglycerols and the tocopherol composition of millet oil.

Materials and Methods

Materials

Millet bran was obtained from an Oil Plant in Shijiazhuang (Hebei, China). Fatty acid methyl esters used as standards [P, St, O, L, linolenic (Ln), arachidic (Ad), and behenic (Be)] and pancreatic lipase for *sn*-2 position analysis were purchased from Sigma Chemical Co. (St. Louis, MO). Standard tocopherols (α-, β-, δ-, and γ-isomers) were obtained from Sigma Chemical Co. (St. Louis, MO). Silica G used for TLC plate preparation was obtained from Qingdao Ocean Chemical Factory (Qingdao, China). All other reagents were of analytical grade and purified before use.

Approximate Composition Analysis for Millet Bran

AOCS Official Methods with a few modifications were used to determine the moisture (Ba 2a-38), ash (Ba 5a-49), lipid content (Ba 3-38), crude protein (Ba 4a-38), and crude fiber (Ba 6-84) of millet bran powder [15]. The lipid content was measured by gravimetry, the lipid was extracted from 10 g millet bran by ethyl ether in a Soxhlet apparatus, and the lipid content, expressed as a percentage by mass, was the ratio of lipid extracted by ethyl ether and original bran. The measurement of nitrogen content of samples was performed on a Foss 2006 digestor and Foss 2300 Kjeltec Analyzer Unit (Foss Technologies Co., Ltd., Höganaös, Sweden). A factor of 6.25 was adopted for protein content estimation. All the data were expressed on a wet basis.

Extraction of Oils from Millet Bran

The millet bran was subjected to Soxhlet extraction with diethyl ether for 8 h. After the removal of solvent from the diethyl ether extract under vacuum, the crude millet oil was obtained.

Physicochemical Property Assays for the Crude Millet Oils

Important physicochemical properties of the crude oil, concerning specific gravity, refractive index, saponification value, and unsaponifiable matter content, were characterized according to the IUPAC Methods 2.101, 2.102, 2.202, and 2.401, respectively [16]. The color of the crude oil was determined according to the AOCS Official Method Cc 13e-92 [15].

The tocopherol (α-, β-, δ-, and γ-isomers) contents of crude millet oil were determined according to a previous report [17] with a few modifications. The samples were analyzed by HPLC using a 10Avp series Shimadzu system (Shimadzu, Japan) with a silica column (250 × 4.6 mm, 5 μm) (Dalian Yilite, Dalian, China) and a RF-10AXL fluorescence detector (Shimadzu, Kyoto, Japan). The column temperature was maintained at 40 °C. The excitation and emission wavelength were 298 and 325 nm, respectively. The oil samples were dissolved in hexane at 100 mg/mL. A 5-μL volume of the loaded sample was isocratically eluted with *n*-hexane/isopropyl ether (90/10, v/v) at 1.5 mL/min. The absolute contents of tocopherols were determined according to the calibrated standard curves.

Fatty Acid Composition of Millet Bran Oil

The fatty acid composition of millet bran oil was analyzed according to IUPAC method 2.302 [16]. And the analysis of fatty acid methyl esters was performed on a gas chromatograph (GC) (Agilent 6890 N) equipped with a flame ionization detector (FID) and a DB-FFAP capillary column (30 m × 0.32 mm, 0.25 μm of film thickness) (Agilent Technologies Co., Ltd.). The column, injector, and detector temperatures were set at 180, 230, and 230 °C, respectively. The flow rate of carrier gas N_2 with a split ratio of 1:20 was set at 70 mL/min. The fatty acids were identified with reference to the retention times of standard fatty acid methyl ester performed at the same conditions.

sn-2 Fatty Acid Distribution of the Triacylglycerols of Millet Bran Oil

The millet oil triacylglycerols were separated from crude millet oil by thin layer chromatography (TLC) using a

Table 1 Composition of millet bran

	Crude protein[a]	Lipids[a]	Crude fiber[a]	Ash[a]	Moisture[a]
Content, % (m/m)	12.48 ± 0.41	9.39 ± 0.17	51.69 ± 2.14	7.50 ± 0.18	8.29 ± 0.16

[a] Values are means ± standard deviation of triplicate determinations

mixture of hexane:diethyl ether:acetic acid (70:30:1, v/v/v) as developing solvents. The TLC plate was visualized under UV light after spraying with a 0.2% ethanolic solution of 2′,7′-dichlorofluorescein sodium salt. Then, the triacylglycerol band was collected and extracted with diethyl ether, the triacylglycerols obtained were used for the *sn*-2 fatty acid composition analysis. 1,3-Specific pancreatic lipase was employed for the chromatography purified triacylglycerol hydrolyzation according to IUPAC method 2.210 [16]. The hydrolyzate of the millet bran oil was separated on TLC using mixture of *n*-hexane:diethyl ether:acetic acid (70:30:1, v/v/v) as developing solvents. The TLC bands of the hydrolyzate were visualized under UV light after spraying with a 0.2% ethanolic solution of 2′,7′-dichlorofluorescein sodium salt. Then, the band containing monoacylglycerol (MAG) was collected and extracted with diethyl ether. The obtained MAG was methylated, and the resulting fatty acid methyl ester was subjected to GC analysis.

Results and Discussion

Composition of Millet Bran

Table 1 shows the composition of millet bran. The crude protein, lipid, and crude fiber contents were 12.48 ± 0.41%, 9.39 ± 0.17%, and 51.69 ± 2.14%, respectively. The lipid content of the millet bran was lower than those of rice bran (18–24% oil and 4–6% free fatty acids [18], and 15–19.7% crude fat [19]). Crude protein content of millet bran was similar to that of rice bran (12–15.6%) [19]. The fiber content of foxtail millet bran was higher than that of rice bran (7.0–11.4%) [19]. The fiber content of foxtail millet bran was very high, which indicated that it was a good material for edible fiber production. The ash content of millet bran was lower than that of millet bran (10.5% on 14% mb) [19]. The data on Table 1 and the lipid results are only based on the millet bran from Shijiazhuang Oil Plant (Hebei, China).

Physicochemical Properties of Millet Bran Oil

The physicochemical properties of crude millet bran oil are presented in Table 2. The specific gravity (20 °C), refractive index at 40 °C, saponification value, and unsaponifiable matter were 0.9185 ± 0.0003 g/cm^3, 1.4676 ± 0.0002, 186.29 ± 0.51 mg KOH/g, and 3.62 ± 0.19 g/100 g, respectively. Previous work showed that the saponification value and the unsaponifiable matter of millet oil were 192–197 mg KOH/g, and 1.9–4.0%, respectively [14]. As a comparison, the ranges of specific gravity (20 °C), refractive index (20 °C), and saponification value of rice bran oil were 0.916–0.922 g/cm^3, 1.470–1.474, 180–195 mg KOH/g, respectively [20].

Table 2 Physicochemical properties of millet bran oil

Characteristic	Crude millet bran oil
Specific gravity $\left(d_{20}^{20}\right)$[a]	0.9185 ± 0.0003
Refractive index (n^{40})[a]	1.4676 ± 0.0002
Saponification value (mg KOH/g)[a]	186.29 ± 0.51
Color (Lovibond, 1 in.)	Y73.0, R10.0
Unsaponifiable matter (%)[a]	3.62 ± 0.19
Tocopherol (mg/100 g)[a]	
α	15.53 ± 0.31
β	–
γ	48.79 ± 0.46
δ	0.51 ± 0.06

[a] Values are means ± standard deviation of triplicate determinations

The α-tocopherol, γ-tocopherol, and δ-tocopherol contents of crude millet oil were 15.53 ± 0.31 mg/100 g oil, 48.79 ± 0.46 mg/100 g oil, and 0.51 ± 0.06 mg/100 g oil, respectively. However, β-tocopherol was not detected. As a comparison, crude rice bran oil was found to contain 19–46 mg of α-tocopherol, 1–3 mg of β-tocopherol, 1–10 mg of γ-tocopherol, and 0.4–0.9 mg of δ-tocopherol per 100 g of oil [19]. The α-tocopherol in crude millet oil was lower than that of crude rice bran oil. However, γ-tocopherol in crude millet oil was higher than that of crude rice bran oil.

Fatty Acids Composition and Positional Distribution in Millet Bran Oil

Fatty acids composition and their distribution of the oil could affect its physicochemical and physiochemical properties. The major fatty acid of millet bran oil was L (66.5%), followed by O (13.0%). The contents of P and St were 6.4 and 6.3%, respectively. The contents of Ln, Ad, Be, and gadoleic acid (G) were lower than 5% (Fig. 1; Table 3). The total unsaturated fatty acids amounted up to 83%, and the ratio of total unsaturated fatty acids and saturated fatty acids was about 5. The results were similar

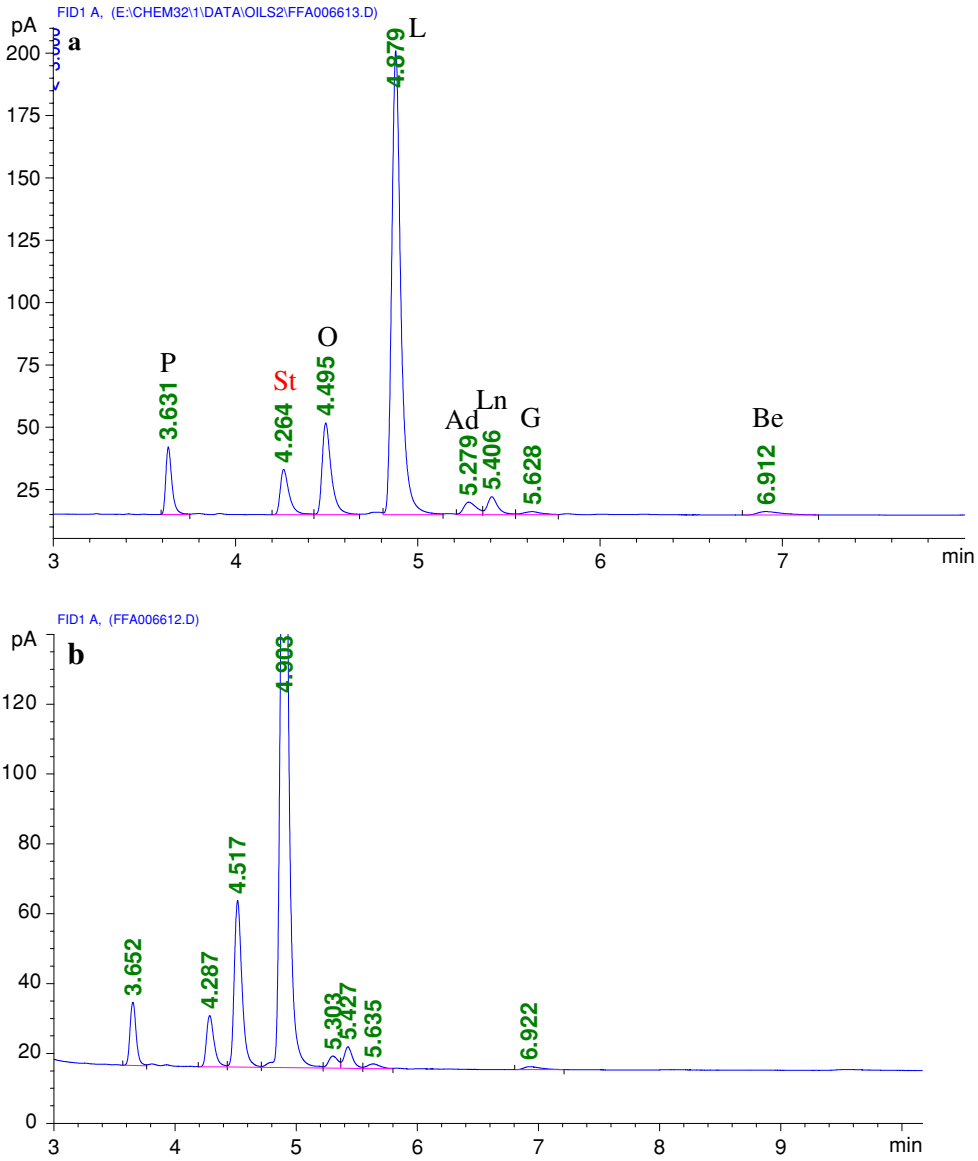

Fig. 1 Gas chromatograms of total (**a**) and *sn*-2 position (**b**) fatty acid profile of millet bran oil. *P* palmitic acid, *St* stearic acid, *O* oleic acid, *L* linoleic acid, *Ad* arachidic acid, *Ln* linolenic acid, *G* gadoleic acid, *Be* behenic acid

to previous reports [7, 11–14, 21, 22]. As a comparison, the major fatty acids in rice bran oil were P (12–28%, typically 20%), O (35–50%, typically 42%), and L (29–45%, typically 32%) [19]. The major fatty acid in the *sn*-2 position of triacylglycerol in millet bran oil was L (71.2%), followed by O (15.0%). The contents of P and St were 4.5 and 4.6%, respectively. The Ln and Ad contents were lower than 2.5%, while G and Ad were lower than 1%. In compliance with the general law for fatty acid distribution of natural triacylglycerols, unsaturated fatty acids occupied almost 89% of the *sn*-2 position of glycerol backbone, in which only very small amount of thermodynamically unfavorable behenic acid and arachidic acid were detected.

Table 4 presents the millet oil triacylglycerol composition, which is calculated according to the 1,3-random-2-random distribution hypothesis, proposed almost simultaneously by R. Van der Waal and Coleman and Fulton, assumed that the fatty acids distributed in *sn*-1,3 and *sn*-2 position of the triacylglycerols at random and independently [23]. Previous works had revealed that the calculated method based on the 1,3-random-2-random hypothesis was acceptable compared to the value determined by HPLC [24, 25]. The dominant triacylglycerols of millet oil were LLL (29.29%) and LLO (17.20%), which are different from the major triacylglycerols (PLO, PLL, and OOO) of the rice bran oil [20]. The other important minor triacylglycerols of millet bran oil were LLnL (4.01%), LLAd (3.41%), PLO (3.40%), OLO (3.36%), StOL (3.30%), LLBe (1.74%), StPL (1.59%), LOLn (1.58%), and LOAd (1.24%).

Table 3 Total and *sn*-2 fatty acid composition of millet bran oil

Fatty acid	*sn*-1,2,3 Position (%)[a]	*sn*-2 Position (%)[a]	*sn*-1,3 Position (%)[b]
Palmitic acid (P)	6.44	4.48	7.42
Stearic acid (St)	6.27	4.56	7.12
Oleic acid (O)	13.05	15.00	12.08
Linoleic acid (L)	66.49	71.17	64.15
Linolenic acid (Ln)	3.00	2.16	3.42
Arachidic acid (Ad)	2.53	1.33	3.13
Gadoleic acid	0.93	0.71	1.04
Behenic acid (Be)	1.29	0.59	1.64
Saturated fatty acid (S)	16.53	10.96	19.31
Unsaturated fatty acid (U)	83.47	89.04	80.69
U/S	5.05	8.12	4.18

[a] Mean of triplicated determinations

[b] Value calculated according to the 1,3-random-2-random hypothesis

Table 4 Predicted major triacylglycerol molecular species in the foxtail millet oil

TG composition	Content/%	TG composition	Content/%
LLnL	4.01	OLO	3.36
LLL	29.29	LStL	8.39
LOLn	1.58	StOL	3.30
LLO	17.20	LLAd	3.41
LPL	8.62	StPL	1.59
PLP	0.82	LLBe	1.74
PLO	3.40	LOAd	1.24

Calculated on the base of the 1,3-random-2-random hypothesis and the fatty acid analysis results shown in Table 3

P palmitic acid, *St* stearic acid, *O* oleic acid, *L* linoleic acid, *Ln* linolenic acid, *Be* behenic acid, *Ad* arachidic acid

In conclusion, this work has presented the general properties of foxtail millet oil and its fatty acid profile. It turned out that millet oil could be a good source of natural oil rich in linoleic acid and tocopherols. The foxtail bran may be a promising material for edible fiber production. This work might be useful for exploring the applications of millet bran and its oil.

References

1. 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
2. Park K-O, Ito Y, Nagasawa T, Choi M-R, Nishizawa N (2008) Effects of dietary Korean proso-millet protein on plasma adiponectin, HDL cholesterol, insulin levels, and gene expression in obese type 2 diabetic mice. Biosci Biotechnol Biochem 72:2918–2925
3. 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
4. >En H, Pang ZH, Xiong BH (2008) Comparative analysis of composition and nutrition value of millet bran feed. China Feed 18:39–41
5. Malleshi NG, Desikachar HSR, Tharanathan RN (1986) Free sugars and non-starchy polysaccharides of finger millet (*Eleusine coracana*), pearl millet (*Pennisetum typhoideum*), foxtail millet (*Setaria italica*) and their malts. Food Chem 20:253–261
6. Ravindran G (1992) Seed protein of millets: amino acid composition, proteinase inhibitors and in vitro protein digestibility. Food Chem 44:13–17
7. Becker R (2008) Fatty acids in food cereal grains and grain products. In: Chow CK (ed) Fatty acids in foods and their health implications, 3rd edn. CRC Press, Taylor & Francis, Boca Raton, pp 303–316
8. Xiong FL, Yuan LJ (1992) Study on determination of vitamin E content in millets by high performance liquid chromatography. J Southwest Agric Univ 14:525–527
9. Lorenz K, Hwang YS (1984) Lipids in proso millet (*Panicum miliaceum*) flours and brans. Cereal Chem 63:387–390
10. Osagie AU, Kates M (1984) Lipid composition of millet (*Pennisetum americanum*) seeds. Lipids 19:958–965
11. Taira H (1984) Lipid content and fatty acid composition of nonglutinous and glutinous varieties of foxtail millet. J Agric Food Chem 32:369–371
12. Sridhar R, Lakshinarayana G (1994) Contents of total lipids and lipid classes and composition of fatty acids in small millets: foxtail (*Setaria italica*), proso (*Panicum miliaceum*), and finger (*Eleusine coracana*). Cereal Chem 71:355–359
13. Ibrahima O, Dhifi W, Raies A, Marzouk B (2004) Study of the variability of lipids in some millet cultivars from Tunisia and Mauritania. Riv Ital Sostanze Grasse 81:112–116
14. Huo QG, Fan L, Bi YL, Zhu XP, Wang MM (2006) Study on components of millet oil. J China Oil 31:63–64
15. Firestone D (1998) Official method and recommended practices of the AOCS, 5th edn. AOCS Press, Champaign
16. Paquot C, Hauntfenne A (1987) IUPAC standard methods for the analysis of oils, fats and derivatives. Blackwell, London
17. Oomah BD, Ladet S, Godfrey DV, Liang J, Girard B (2000) Characteristics of raspberry (*Rubus idaeus* L.) seed oil. Food Chem 68:187–193
18. Gunstone FD, Harwood JL (2007) Occurrence and characterisation 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
19. 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
20. Gunstone FD (2002) Vegetable oils in food technology: composition, properties and uses. CRC Press, Boca Raton, pp 309–319
21. Liu FM, Yu SQ, Tang ZL, Ye M (1997) Study on the contents of fatty acids in millets of China. J Southwest Agric Univ 19:371–374
22. Devittori D, Gumy D, Kusy A, Colarow L, Bertoli C, Lambelet P (2000) Supercritical fluid extraction of oil from millet bran. J Am Oil Chem 77:573–579
23. Lichfield C (1972) Analysis of triglycerides. Academic Press, New York, pp 250–251
24. Xu XB, Fomuso LB, Akoh CC (2000) Synthesis of structured triglycerols by lipase-catalyzed acidolysis in a packed bed reactor. J Agric Food Chem 48:3–10
25. Bi YL, Yang GL, Li H, Zhang GW, Guo Z (2006) Characterization of the chemical composition of lotus plumule oil. J Agric Food Chem 54:7672–7677