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Characterization of the Chemical Composition of Lotus Plumule Oil

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Characterizations of lotus plumule and plumule oil, focusing on approximate composition analysis of lotus plumule powder and fatty acid composition, lipid classes, triglyceride (TG) profiles, and sterol analysis of the plumule oil, were conducted in this work. The results revealed that the lotus plumule constitutes 7.8% moisture, 4.2% ash, and 12.5% crude oil and 26.3% protein on the dry base. Lotus plumule oil is rich in linoleic acid (50.4%) and oleic acid (13.5%), and the dominating saturated fatty acids are palmitic acid (18.0%) and behenic acid (6.8%). The principal components of TG in lotus plumule oil are LLL (12.80%), β-PLL (11.27%), β-POL (8.28%), β-PLO (8.58%), and β-BeLL (8.32%). Lipid class assay of the crude oil gave the saponification value of 153.4 KOH mg/g and tocopherol content 390 mg/100 g. A distinct characteristic of lotus plumule oil is that its unsaponifiable matter is incredibly high, up to 14−19%, which consists mainly of β-sitosterol (32%), Δ^5-avenasterol (20%), and campesterol (6.3%). The major occurring form of sterols was found to be steryl ester. This work might be useful to develop innovative applications of lotus plumule oil.

KEYWORDS: Lotus plumule oil; unsaponifiable matter; phytosterol; β-sitosterol; tocopherol; physicochemical property

INTRODUCTION

Nelumbo nucifera is a perennial aquatic plant, widely occurring in the eastern Asian countries. Almost all parts of *N. nucifera*, such as leaves, flowers, seeds, and rhizomes, could find applications as either traditional herbal medicine or healthcare food. Lotus seeds, belonging to the *Nymphaea* genus, have long been a popular food in many Asian states and are believed to be capable of antidepression, inhibiting inflammation, "fire-reducing", etc. (*1*, *2*). Lotus plumule, also known as Lian fang, Lien Tze Hsin, and Lian xu, is the green germ of a mature lotus seed, located between of two cotyledons (*3*). Lotus plumule is 1−1.4 cm long, from its radicle stretching two unequal clavate arms forming an arrow-shaped structure. More attention has been given to the investigation of the occurring water- or alcohol-soluble compounds with physiological activities in lotus plumule (*4*, *5*). The identified biologically active substances includes alkaloids, such as liensinine, isoliensinine, neferine, nuciferine, pronuciferine, lotusine, methylocrypalline, and demethylcolaurine (*4*), and flavonoids, e.g., galuteolin, hyperine, rutin, amylose, etc. (*5*). Substantive occurrence of microelements (Zn, Fe, Ca, Mg, etc.) was also identified (*6*).

Much is known about the traditional use of lotus seeds; however, little information of lotus plumule, regarding its lipid composition characteristic and the lipophilic concomitants, is available (*7*, *8*). Apart from the benefits of naturally occurring lipophilic vitamins, recently phytosterols have also received much attention in the lipids area because the related studies have demonstrated that the dietary intake of plant sterols could inhibit cholesterol absorption and, as a result, might reduce the risk of atherosclerosis and gallstones (*9*, *10*). To explore the innovative application of lotus seeds, also as part of the ongoing project in our group for the characterization of the lipids from Chinese herbal medicines used as healthcare food, this work presents a general protocol for lipid processing and characterization of lotus plumule oil. The study focused on physicochemical property assaying, unsaponifiable matter analysis and identification, and triglyceride profile determination.

MATERIALS AND METHODS

Materials. Lotus plumule was purchased from the filiale of 999 Medicinal Materials Corp. (Zhengzhou, China). Silica G used for TLC plate preparation was obtained from Qingdao Ocean Chemical Factory (Qingdao, China). Fatty acid methyl esters used as standards and pancreatic lipase for Sn-2 position analysis were procured from Sigma Chemical Co. (St. Louis, MO). Tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin used as the references for ECN determination were from Nu-chek Prep, Inc. (Elysian, MN), and with a minimum purity of 99%. β-Sitosterol, campesterol, Δ^5-avenasterol, and stigmasterol are from Fluka Chemie AG (Buchs, Switzerland). Standard tocopherols (α-, β-, γ-, and δ-isomers) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents from local sources are of analytical grade and purified before use.

General Processing Procedure. A general lotus plumule oil processing and analysis procedure conducted in this work is described

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as follows. After removal of the impurity by screening, the plumule was grinded into powder. The obtained powder was subjected to Soxhlet extraction with diethyl ether for 4 h. The crude lotus plumule oil was thus prepared from the diethyl ether extract after the removal of solvent under vacuum. To determine the residual oil and extraction efficiency, a further extraction using the resulting defatted plumule powder was conducted according to the IUPAC method (*11*). The occurrence of pigments and polar lipids not only gives unfavorable color but also generates undesirable effects on the stability, e.g., promotes hydrolysis, rancidification, and oxidation, which do not facilitate the storage of oil. Chromatographic adsorption is a widely used physical refine method for small scale in the laboratory. To examine the effect of physical adsorption and obtain customer-acceptable product, the crude plumule oil was processed by column chromatographic adsorption. The bleaching was performed on a basic aluminum oxide-filled column (18.5 × 2.5 cm). The crude plumule oil dissolved in petroleum ether (1:4, v/v) was loaded on the column and eluted with petroleum ether at the rate of 3 mL/min. The bleached plumule oil was obtained after removing the solvent from the collected fraction by evaporation under vacuum.

Approximate Composition Analysis for Lotus Plumule. AOCS methods with a few modifications were used to determine the moisture and volatiles, ash, nitrogen content (for protein content estimation), and phosphorus content of both plumule powder and defatted plumule powder (*12*). The moisture and volatiles and ash were calculated by mass balance. The absorbance of the digested phosphorus was measured at 650 nm, and a standard curve was used for calibration. The measurement of nitrogen content of samples was performed on a Foss 2006 digestor and Foss 2300 Kjeltec Analyzer Unit (Foss Technologies Co., Ltd., Höganäs, Sweden). A factor of 6.25 was adopted for protein content estimation.

Lipid Class Assays for the Crude and Bleached Plumule Oils. Important physicochemical properties of the crude and bleached oil, concerning acid value, saponification value, peroxide value, phospholipid and unsaponifiable matter content, etc., were characterized according to the AOCS recommended methods (*12*). The phospholipids content of samples was estimated by their absolute content of phosphorus, and the factor used was 30 (*12*).

The contents of tocopherol in crude plumule oil (α-, *β*-, *γ*-, and *δ*-isomers) were determined by high-performance liquid chromatography (HPLC) following the method of Oomah et al. with some modification (*13*) and employing soybean oil, rich in tocopherols, as a comparison. The analysis was conducted on a 10Avp series Shimadzu system (Shimadzu, Japan) with a silico 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 held at 40 °C. The emission and excitation wavelength were set at 325 and 298 nm, respectively. The oil samples were dissolved in hexane at 100 mg/mL. A 5 *μ*L volume of the loaded sample on the column was isocratically eluted with *n*-hexane/diisopropyl ether (90/10, v/v) at 1.5 mL/min. The absolute contents of tocopherols were determined by comparison with the calibrated standard curves.

To confirm the substantive occurrence of sterol ester in plumule oil, the bleached plumule oil, using *β*-sitosteryl oleate, *β*-sitosterol, and refined sunflower oil (from Aahus United, Aahus, Denmark) as references, was subjected to TLC-FID analysis with an Iatroscan MK-6s (Bechenheim, Germany). The developing solvent mixture consists of hexane/diethyl ether/acetic acid (55:15:2, v/v/v). Area percentage was used as weight for the estimation of relative content of individual components.

Preparation and Identification of Unsaponifiable Matters. The unsaponifiable matters of the crude and the bleached lotus plumule oil were prepared according to AOCS method (*12*) with some modification. The mixture of 5.0 g of crude or bleached plumule oil and 50 mL of 1.0 M ethanolic potassium hydroxide was refluxed in a water bath for about 60 min, and then the mixture was extracted three times using 50 mL of petroleum ether. The petroleum ether phase was combined and sequentially washed with 20 mL of 0.5 M KOH solution and distilled water. The resulting petroleum ether phase was washed until neutral with distilled water and dried with anhydrous sodium sulfate. The unsaponifiable matters were obtained by evaporating the anhydrous extracts with a rotary evaporator at 40 °C under vacuum.

The composition of unsaponifiable matters of the lotus plumule lipid was analyzed by gas chromatography−mass spectrometry (GC-MS) (HP 6890 Series GC System) with an HP-5MS column (30 m × 0.25 mm, 0.25 *μ*m of film thickness, Agilent Technologies Co., Ltd., Southfield, MI). The temperature was increased from 180 to 260 °C at 5 °C/min and then from 260 to 280 °C at 25 °C/min. The carrier gas was helium (1 mL/min), and the split ratio was 15:1. The injection and detection temperatures were both set at 280 °C. The parameters were as follows: scan mode, 10−550 amu; electronic ionization voltage, 70 eV; EM voltage, 1871 mV.

To separate sterols from other unsaponifiable components, the unsaponifiable matters were loaded on a TLC plate of Silica G (20 × 20 cm) for fractionation. The sterol band was located with phytosterol as a marker. The developing solvent was the mixture of petroleum ether and diethyl ether (70/30, v/v). A UV lamp was employed to visualize the TLC band after spraying with a 0.2% ethanolic solution of 2′,7′-dichlorofluorescein and drying. The sterol band was scraped off and extracted with hexane. The resulting sterol fraction after removal of solvent was used for further GC-MS analysis to identify the molecule structure of individual sterol species with the method similar to that in the above description.

Fatty Acid Composition and Triglyceride Profile Analysis. Lotus plumule oil was methylated according to the IUPAC method (*11*). And the analysis of fatty acid methyl esters was performed on a gas chromatograph (GC) (Agilent 6890N) equipped with a flame ionization detector (FID) and a DB-FFAP capillary column (30 m × 0.32 mm, 0.25 *μ*m 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 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.

1,3-Specific pancreatic lipase was employed for Sn-2 fatty acid composition analysis according to AOCS methods (*12*). The hydrolyzate of the bleached lotus plumule oil catalyzed by pancreatic lipase was separated by TLC using the mixture of hexane:diethyl ether:acetic acid (70:30:1, v/v/v) as developing solvents. The TLC band of hydrolyzate was visualized under UV light after spraying with a 0.2% ethanolic solution of 2′,7′-dichlorofluorescein sodium salt. Then, the silica gel containing monoglyceride (MG) was collected and extracted with diethyl ether. The obtained MG was methylated, and the resulting fatty acid methyl ester was subjected to GC analysis by employing a similar method for total fatty acid analysis.

The triglyceride composition of lotus plumule oil was analyzed with HPLC (Merck Hitachi, Darmstadt, Germany), conjuncted with an evaporative light scattering detector (ELSD) (Polymer Laboratories, Shropshire, U.K.). The ELSD was operated at an evaporating temperature of 70 °C and a nebulizing temperature of 50 °C with nitrogen as the nebulizing gas. The column used was Supelcosil LC-18 (250 × 4.6 mm, Supelcosil Inc., Bellefonte, PA) packed with a particle size of 5 *μ*m, and column temperature was kept at 30 °C. The mobile phase was a binary solvent system of acetonitrile (solvent A) and acetone (solvent B). The applied flow rate was 1.5 mL/min, and the employed gradient program was a multiple-step gradient elution of solvent A, reducing from 50% to 47% over 6 min and then from 47% to 40% over 9 min, from 40% to 35% over 10 min, and from 40% to 35% over 10 min and finally held at 30% for 25 min. The samples were dissolved in the mixture of chloroform and acetone (3:1, v/v) at the concentration of 10 mg/mL, and 10 *μ*L was injected. Triacylglycerol peaks were determined by comparison with triglyceride standards with reference to retention time and by calculation of the theoretical carbon number (TCN) of respective peaks with reference to the ECN of standards according to the method developed by El-Hamdy and Perkins (*14*). Three determinations were conducted for the analysis (mixture of three parallel extracts from the same sample), and the means were used for result evaluation.

Solvent Extraction from the Defatted Plumule Powder and the Antioxidative Ability of the Extracts. To further explore the possible application of the defatted plumule powder, extractions of the defatted powder with different solvents were performed in a Soxhlet apparatus. The solvents employed were, in order of increasing polarity, ethyl

Table 1. Physicochemical Characteristics of Lotus Plumule Oils

determination	crude diethyl ether extract (plumule oil)	chromatographic bleached plumule oil
color	dark green	yellow
acid value (KOH mg/kg)[a]	0.63 ± 0.02	0.20 ± 0.00
peroxide value (mequiv/kg)[a]	5.60 ± 0.03	4.30 ± 0.03
saponification value (KOH mg/g of oil)[a]	153.41 ± 0.54	123.80 ± 1.72
unsaponifiable matter (%)[a]	14.24 ± 0.32	18.77 ± 0.49
phosphorus (mg/kg)[a]	567.0 ± 9.9	103.0 ± 2.8
phospholipids (%)[a]	(1.47 ± 0.03)	(0.27 ± 0.01)
tocopherol (mg/100 g of oil)[a]		
α	6.61 ± 0.84	
β	4.96 ± 0.00	
γ	323.98 ± 1.31	
δ	58.29 ± 4.41	

[a] Values are mean ± standard deviation of triplicate determinations.

acetate, acetone, and 90% and 70% ethanol. To examine the relationship of extraction efficiency and reaction time, three parallel extractions were done at varied time (4, 6, and 8 h) for each solvent. Solvents were evaporated from the resultant extraction solutions using a rotary evaporator to produce the extracts. The yields of extracts were calculated by mass balance.

The soybean oil was employed as model oil to test the antioxidative ability of each extract. Typically 30 g of soybean oil with 0.02% (wt %) addition of the aforementioned extracts was added to a 50 mL beaker. The bakers with the test oils were then placed into a thermostatic oven which was kept at 45 ± 1 °C. The soybean oil without the addition of extract was used as a control. To monitor the progress of oxidation of soybean oil, aliquots of 0.5 g were periodically withdrawn for peroxide value measurement.

All analysis and measurements in this work were done in triplicate. The adopted values are the means at the 95% confidence limit.

RESULTS AND DISCUSSION

Approximate Composition of Lotus Plumule. Approximate composition analysis of the lotus plumule before and after ether extraction showed that the oil was almost harvested by 4 h extraction, of which only 6.3% residue remained. The crude and defatted powders have a similar moisture content of 8%; however, a slight increase of the mineral content in the defatted plumule powder (from 4.1% of undefatted powder to 4.6%) was observed due to the removal of lipids. An interesting result is that the defatting processing does not result in any increase but a decrease of nitrogen content (from 4.2% to 3.2%) and the corresponding decrease of the calculated protein content (from 26.2% to 19.7%). This probably resulted from that much non-protein nitrogen compounds, which possess higher nitrogen density, were removed from the lotus plumule with extraction. This result suggested the substantive occurrence of alkaloids in lotus plumule, which is consistent with the previous report (*5*). The oil content (13%) of lotus plumule is not high; however, the protein content (26%) could be highly comparable to those of protein-rich foods (*15*, *16*). The amount of crude fiber of lotus plumule could be estimated to be 45−49% by mass balance (*17*). The fiber of lotus plumule is fresh and tender, which indicates the lotus plumule might be a good source of dietary fiber.

Characteristics of the Lipid Class of Lotus Plumule Oil. **Table 1** presented the physicochemical properties of the crude and chromatographically bleached lotus oil. A yellow and transparent oil preparation could be obtained after chromatographic processing to remove chlorophyll and other pigments, which could also provide a customer-acceptable alternative. Inspection of the data in **Table 1** reveals the significant changes of phospholipids content, acid, and peroxide values after chromatographic processing, which might be attributed to the efficient adsorption of Al_2O_3 for polar compounds. It is noteworthy that either crude or bleached oil still possesses very low AV and acceptable PV after multistep processings without extra protection, indicating their good quality as edible oils.

Table 2. Total and Sn-2 Main Fatty Acid Composition of the Bleached Lotus Plumule Oil[a]

fatty acid	Sn-1,2,3 (%)	Sn-1,3 posn (%)	Sn-2 posn (%)
palmtic acid	18.03	25.72	2.65
stearic acid	2.90	4.35	
oleic acid	13.53	12.93	14.73
linoleic acid	50.44	38.11	75.11
linolenic acid	3.00	2.64	3.72
arachidic acid	3.30	4.95	
behenic acid	6.75	10.13	

[a] Means of triplicated determinations.

As indicated in **Table 1**, unanticipated high content of unsaponifiable matters and remarkable high tocopherol concentration constitute two distinguished characteristics of lotus plumule oils. As a comparison, crude soybean oil was employed for quantitative determination of tocopherols. The results showed that crude lotus plumule oil has a total tocopherol content of 393 mg/100 g oil, an almost doubled abundance as in crude soybean oil (186 mg/100 g oil), among which the absolute concentrations of α- and δ-isomers are similar (data not shown). However, crude plumule oil is particularly abundant in γ-tocopherol (up to 324 mg/100 g of oil). This might be one of the leading reasons accounting for the lower PV value of lotus oil after multistep processings.

To validate the substantive occurrence of unsaponifiable matters and also to envisage the major compositions of lipids in lotus plumule oil, TLC-FID was employed for the lipid class analysis. The results depicted that dominant constituents are triglyceride (61−64%) and sterol ester (25−36%) (data no shown). TLC-FID analysis results showed that the crude oil consisted of 61.38% TG, 25.7% sterol ester, 1.61% free sterol, 1.47% phospholipid, and 6.76% pigments (chlorophyll, etc.), and the bleached oil consisted mainly of 63.3% TG and 35.9% sterol ester. If one considers 1 mol of sterol ester reacts with 1 mol of KOH (treated as sitosterol oleate), 1 mol of chlorophyll with 2 mol of KOH, and 1 mol of phospholipid with 2 mol of KOH, on the basis of their compositions, the saponification value of the crude and bleached oil could be estimated to be 148.96 and 152.98 KOH mg/g, respectively. The corresponding calculated unsaponifiable matter content was 19.48% and 21.83%, respectively. Compared with the measured data in **Table 1**, the calculated values were slightly higher but still gave an acceptable agreement. The value differences were possibly related to unsaponifiable matter loss during operation or differring TLC-FID responses of the different components in plumule oil without calibration. However, this analysis presented a basic information that plumule oil has a markedly high content of non-triglyceride components and steryl ester is the major occurring form. The obtained TLC chromatograms also showed that the crude plumule oil contains more polar lipids and complex lipids, the majority of which could be effectively removed by chromatographic processing (data not shown).

Fatty Acid Composition and Triglyceride Profile. The fatty acid composition of lotus plumule oil and the positional distribution were reported in **Table 2**. The major fatty acid was linoleic acid at the concentration of 50.4%, followed by palmitic acid (18.0%) and oleic acid (13.5%). Behenic acid ($C_{22:0}$) was

Table 3. Triglyceride (TG) Composition of the Bleached Lotus Plumule Oil[a]

	ECN		content (%)			ECN		content (%)	
TG	calcd[b]	expt[c]	expt[d]	calcd[e]	TG	calcd[b]	expt[c]	expt[d]	calcd[e]
β-LLLn	40	38.65	1.49	1.51	β-POO	48	47.19	0.98	0.98
LLL	42	40.67	12.80	10.91	β-LLAd	48	47.39	1.67	2.80
β-PLLn	42	41.46	1.35	1.02	β-StLP	48	47.68	1.99	1.68
β-LOL	44	42.95	7.13	2.14	β-POP	48	47.93	0.07	0.97
β-OLL	44	43.40	9.43	7.40	β-PLAd	50	48.34	7.99	1.91
β-PLL	44	43.44	11.27	14.72	β-BeLL	50	48.36	8.32	5.80
β-LPL	44	44.24	0.27	0.38	β-LOBe	52	49.18	2.90	1.14
β-OOL	46	45.13	2.06	2.71	β-OLBe	52	49.72	0.47	1.97
β-PLO	46	45.59	8.58	5.00	β-PLBe	52	50.47	0.94	3.91
β-StLL	46	46.04	2.64	2.49	β-POBe	54	51.03	0.82	0.77
β-POL	46	46.24	8.28	2.89	β-OOBe	54	51.48	0.85	0.39
β-PLP	46	46.27	7.71	4.96					

[a] Abbreviations: P, palmitic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; Be, behenic acid; Ad, Arachidic acid; CN, carbon number; ECN, equivalent carbon number; DBN, double bond number. [b] ECN(calcd) = CN − (2 × DBN). [c] ECN(expt) was determined by the interpolation of the logarithmic of the retention time (R_t) of the individual profiles into the plot of the ECN of standards against their R_t logarithmic. [d] The experimental TG content was calculated by area percentage. [e] The calculated TG content was based on the 1,3-random–2-random hypothesis and the fatty acid analysis results.

found to be occurred at high concentration (6.75%). The total content of unsaturated fatty acids amounted up to 70%. This result was similar to the previous report (*8*). In compliance with the general law for fatty acid distribution of natural triglycerides, unsaturated fatty acids occupied almost 96% of the Sn-2 position of glycerol backbone, in which only very small amount of thermodynamically unfavorable behenic acid and arachidic acid was detected.

Table 3 presents the HPLC analysis results of the triglyceride composition of lotus plumule oil, the calculated values according to the hypothesis of 1,3-random−2-random distribution, and the analysis results of fatty acid distribution (**Table 2**) (*18*). The HPLC-ELSD chromatogram of bleached plumule oil presented that there are altogether 23 identifiable triglycerides found in lotus plumule oil (data not shown). Examination on the comparison of calculated and experimental data (**Table 3**) revealed that the 1,3-random−2-random hypothesis could give not very good but still acceptable predicted results. No dominant triglyceride profile was found; no components have a concentration of more than 15%. LLL (12.8%), β-PLL (11.27%), β-OLL (9.43%), β-PLO (8.58%), β-BeLL (8.32%), β-POL (8.28%), β-PLAd (7.99%), β-PLP (7.71%), and β-LOL (7.13%), fallen in the concentration range 5−15%, were principal components. Other important minor triglycerides include β-LOBe (2.9%), β-StLL (2.64%), β-OOL (2.06%), β-StLP (1.99%), β-LLAd (1.67%), etc.

Unsaponifiable Matters and Sterol Composition. As mentioned above, a high percentage of unsaponifiable matters occurring in lotus plumule oil was found, even markedly higher than those oils rich in unsaponifiable matters, such as groundnut seeds oil (7.8%) (*19*) and *Monodora tenuifolia* seeds oil (7.9%) (*20*). To our knowledge, for such a high concentration of unsaponifiable matters, comparable data (23.3%) were only reported in the ripening high oleic sunflower cultivar (*21*). Whether there are some intrinsic correlations between ripening degree of plumule and unsaponifiable matter percentage, or just an exception, remains unclear, even though a significant decrease of the content of unsaponifiable matters during the ripening study of this genetic modified seed cultivar was observed (*21*).

Phytosterols naturally exist as free sterol, sterol ester, sterol glycosides, and acylated glycosides (*22*, *23*). The relative abundance of sterol forms varied depending on their species. However, sterol ester and free sterol are dominating occurring form in many plants (*22*). The relative contents of different sterol forms in extracted oils also depend on the extraction solvents used (*24*). The main occurring forms in low polar solvent extractions are ester and free forms due to the high polarity of sterol glycosides. It has been reported that in both crude corn and rapeseed oils a major part of the sterols occurs as steryl esters (*25*, *26*). TLC-FID analysis in this work identified that the major occurring form of sterol in lotus plumule oil is also steryl ester.

Although sterols represent only a small component of the total lipid fraction in normal plant oils, they comprise a major portion of the unsaponifiable matters (*27*, *28*). The characteristic abundance and sterol profiles were also suggested to serve as the fingerprint to authenticate specific oils (*29*). To examine the composition and the concentration of individual ingredients of the unsaponifiable matters from lotus plumule oil, the unsaponifiable matters were directly subjected to GC-FID and to the conjugated mass chromatography to identify the individual peaks. In accordance with the TLC analysis results (data not shown), GC-FID analysis together with MS identification also demonstrated that sterols constitute the major portion of the unsaponifiable fraction. A summary of the major identifiable components in the unsaponifiable matters of plumule oil is listed in **Table 4**. It was estimated that sterols consisted of more than 80% of the total unsaponifiable matters, phytol and other plant polyenoic alcohols occupying about 15%, and hydrocarbon and tocopherol representing around 1%, respectively. The sterol fraction of plumule oil consisted mainly of β-sitosterol, Δ^5-avenasterol, campesterol, stigmasta-7,25-dien-3-ol, 24-methylene-9,19-cyclolanostan-3β-ol, and α-sitosterol, among which β-sitosterol and Δ^5-avenasterol were most predominant, representing 31.75% and 19.66% of the total unsaponifiable matters. Other important sterol portions include stigmasterol (2.67%), ergosta-8,24(28)-dien-3-ol (2.5%), ergosta-5,24-dien-3-ol (1.71%), stigmasta-7-en-3-ol (0.96%), etc. β-Sitosterol has been identified as the major sterol subclass of many plant oils (*22*, *30*). It was worth noting that the concentration of Δ^5-avenasterol is at a higher level (19.66%). The interest in the substantial occurrence of Δ^5-avenasterol lies in that this compound is known to act as an antioxidant and as an antipolymerization agent in frying oils (*31*, *32*). A detailed analysis of the compositions of individual portions from TLC fractionation is in progress in our group.

Clinical studies have shown that phytosterols exhibit effective cholesterol-lowering activities by inhibiting the absorption of cholesterol and reducing LDL cholesterol level (*9*, *33*). Therefore, they are recommended as dietary supplements, or supple-

Table 4. Composition of the Unsaponifiable Matter from the Bleached Lotus Plumule Oil

retentn time (min)	compd[a]	rel content[b] (%)
8.33	phytol	5.81 ± 0.25
9.10	hexadeca-2,6,10,14-tetraen-1-ol	5.10 ± 0.17
14.62	2,6,10,14,18,22-tetracosahexaene	0.86 ± 0.01
17.37	γ-tocopherol	1.13 ± 0.02
20.35	ergosta-5,24-dien-3-ol	1.71 ± 0.02
20.49	campesterol	6.28 ± 0.16
21.88	ergosta-8,24(28)-dien-3-ol	2.50 ± 0.02
22.16	stigmasterol	2.67 ± 0.03
22.61	β-sitosterol	31.75 ± 0.33
23.01	Δ^5-avenasterol	19.66 ± 0.27
23.41	stigmasta-7,25-dien-3-ol	3.49 ± 0.02
23.83	stigmasta-7-en-3-ol	0.96 ± 0.01
24.29	unknown	3.27 ± 0.03
25.10	lanost-7-en-3-one	1.51 ± 0.01
25.98	24-methylene-9,19-cyclolanostan-3β-ol	3.79 ± 0.04
26.17	α-sitosterol	3.41 ± 0.03

[a] Compounds were identified by the comparison of each retention time with authentic standards or diagnosed by the mass fragment spectrums from the database of NIST, at least with a library fit factor >90%. [b] Area percentage of individual profiles was used as the relative content of the corresponding components. Values are mean ± standard deviation of triplicate determinations.

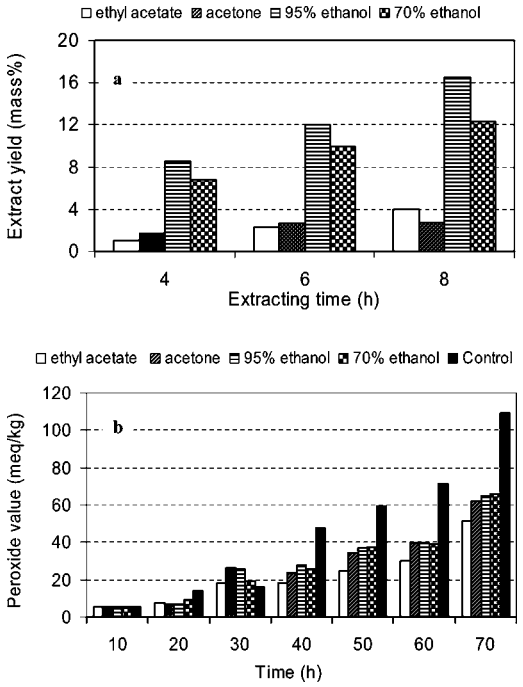

Figure 1. (**a**) Solvent-dependency of the extraction from defatted lotus plumule powder. (**b**) Antioxidative ability of the solvent extracts from the defatted lotus plumule powder.

mental ingredients in foods offering cardiologic health benefits (*34*). Besides the cholesterol-lowering effect, phytosterols were also suggested to possess other beneficial properties such as antiinflammatory and antitumor activities (*35*, *36*). Chemically phytosterols are useful as raw materials for hormone synthesis and cosmetics application (*37*). There is increasing interest in acquiring and isolating these biologically active components (*38*). Since lotus plumule oil has such a high content of sterols, it can be a good source of phytosterols, either as nutraceutical supplements or ingredients for functional foods.

Antioxidative Activity of Solvent Extracts. After ether or hexane extraction, there still are many extractable polar compounds residing in the plumule powders. To fully utilize these components, a few low-toxic solvents were employed for the extraction. In the order of polarity increasing, the solvents are ethyl acetate (log $P = 0.68$), acetone (log $P = -0.23$), 95% ethanol (log P of absolute ethanol $= -0.24$), and 70% ethanol (log P, a indicator of polarity, represents the logarithm of the partition coefficient of a given compound in the octanol−water two-phase system, and solvents with log $P < 2$ served as polar solvents) (*39*). The results in **Figure 1a** depicted that the yield of extracts for all test solvents were positively correlated to the extraction time. And also the effects of time are more significant for polar solvents, which mean that, for higher polar solvents, it will take longer time to attain the extracting equilibrium. For instance, from 6 to 8 h, the extract yield by acetone increased only 5.7%, contrary to 36.7% of the yield by 95% ethanol. The extraction yield was also solvent-dependent. The extraction with 95% ethanol gave the maximum yield whatever the reaction time is. Therefore, 95% ethanol is a recommended solvent for the extraction if judged by the yield of the extract.

To examine the antioxidation activities of the extracted compounds, the oxidation stability of the soybean oil with specific addition of desolvented extracts was tested under controlled conditions (**Figure 1b**). The results showed that all extracts have antioxidation ability, which became more significant after incubation for 40 h. It was also observed that the extract with lower polar solvents possessed better antioxidation activity than the extract with higher polar solvents. For example, after incubation of 70 h, the PV of the sample with ethyl acetate extract addition increased to only 51.3 mequiv/kg other than the 65.8 mequiv/kg with 70% ethanol addition. Previous work has confirmed that the active ingredients of polar herbal extracts for antioxidation are phenolic compounds and the antioxidation activities of extracts strongly depend on the presence of the variety of hydrophilic and lipophilic compounds in them (*40*). In agreement with this observation, it is not surprising that ethyl acetate extract could give best activity and the extract by 70% ethanol yielded poor activity (**Figure 1b**), since ethyl acetate extract contains a higher concentration of lipophilic antioxidants which could provide greater protection against the peroxyl radicals formed in lipid (*41*).

In conclusion, this work has presented a first systemic characterization of lotus plumule oil and its lipophilic concomitants. It turned out that the plumule oil possesses distinct characteristics in approximate composition and lipid class, namely, significantly abundant in phytosterols and tocopherols. The results indicated that lotus plumule could be a good source of naturally biological activity compounds, especially sterols, either for dietary supplements or for supplemental ingredients in functional foods. This work might be useful to explore the innovative application of lotus plumule and plumule oil.

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