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Analysis of Fatty Acids and Phytosterols in Ethanol Extracts of *Nelumbo nucifera* Seeds and Rhizomes by GC-MS

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ABSTRACT: The purpose of this study was to investigate the fatty acid and phytosterol contents in ethanol extracts of lotus seeds and rhizomes. These ethanol extracts were extracted with hexane. The hexane extracts were hydrolyzed in a microwave reactor, and total fatty acids and phytosterols were analyzed. The hexane extracts were also subjected to silica gel column chromatography. Nonpolar components (triglycerides and steryl-fatty acid esters) were hydrolyzed, and then the contents were analyzed. Polar components (diglycerides, monoglycerides, fatty acids, and phytosterols) were analyzed directly. Seeds contained higher concentrations of fatty acids and phytosterols compared to rhizomes. Linoleic acid, palmitic acid, and oleic acid were the main fatty acid components in seeds and rhizomes, and most of them in seeds were in the ester form. In seeds, phytosterols existed mainly in the free form rather than in steryl-fatty acid ester form. β-Sitosterol was the most abundant phytosterol in seeds and rhizomes.

KEYWORDS: *Nelumbo nucifera (lotus) seeds, Nelumbo nucifera rhizomes, fatty acids, phytosterols, ethanol extracts*

INTRODUCTION

Lotus (*Nelumbo nucifera* Gaertn.) is widely cultivated in Asia. Lotus leaves, flowers, seeds, and rhizomes are staples of the Eastern Asian diet, particularly in China, Japan, and Korea. All parts of the lotus have been used as medicines in the traditional medicine of South Korea. Lotus seeds and rhizomes have been consumed as common edible vegetables for hundreds of years in South Korea. Several studies have shown that the lotus seeds and rhizomes possess antipyretic, antidiarrheal,[1] hepatoprotective,[2−4] immunomodulatory,[5,6] and antioxidant effects.[7−10] In addition, lotus seeds possess sedative,[11] antidiabetic,[12] and anti-inflammatory effects,[13,14] and lotus rhizomes can improve sleep,[15] learning, memory,[16] and blood sugar levels.[17,18]

Lipids are important components of the body with many biological functions, including energy storage, as components of cell membranes, and as signaling molecules. The major dietary lipids in plants are free fatty acids and phytosterols (plant sterols), glycerides, and other esters. The biological functions of fatty acids, which are usually derived from glycerides and other esters, have been studied extensively. In previous papers, the ω-3 polyunsaturated fatty acid intake of humans was found to be at least 250 mg/day, and this class of fatty acids can reduce the risk of cardiovascular disease.[19,20] In other papers, fatty acids possess antioxidant, hypotensive, hypolipidemic,[21,22] anticancer, and anti-inflammatory effects.[23] Most of the fatty acids can be produced by the human body, when needed, except α-linolenic acid and linoleic acid. Therefore, they have to be obtained from food sources. The Agricultural Research Service of the U.S. Department of Agriculture (USDA) has reported that the percentages of total fatty acids in raw lotus seeds and rhizomes are 0.50%[24] and 0.07%,[25] respectively. Phytosterols are steroid compounds similar in structure to cholesterol and are found in all plant foods. Human foods generally contain free phytosterols, steryl-fatty acid esters (mainly, esterified with linoleic and oleic acid), phytosterol−sugar adducts, and phytosterol−phospholipid adducts, and the principal phytosterols are stigmasterol, sitosterol, and campesterol.[26] These phytosterols act as precursors of brassinolide in the germinative seed[27] and are present in large amounts in plant seeds and in small amounts in rhizomes. The phytosterol intake of humans is approximately 250 mg/day from plant foods.[26] The first study showing that phytosterols can reduce cholesterol levels in human was published in 1953.[28] Thereafter, many studies have shown that phytosterols possess hypocholesterolemic, LDL-cholesterol-reducing,[29,30] anti-colon cancer,[31] and anti-inflammatory effects.[32]

Xu et al.,[33] Huang et al.,[34] and Bhat and Sridhar[35] analyzed the quantities of fatty acids and phytosterols in lotus bee pollen, leaves, and seeds, respectively. However, there are few reports of the analysis of fatty acids and phytosterols in lotus seeds and rhizomes. The extracts of lotus seeds and rhizomes, which may have better health-promoting effects, are of interest to us and other researchers focused on the utility of the lotus in the development of functional foods or food additives. In this study, as a nontoxic, safe, and effective solvent, ethanol was used for the extraction of lotus seeds and rhizomes. We analyzed the qualitative and quantitative characteristics of lipid components, such as esters, free fatty acids, and free phytosterols, in the ethanol extracts of lotus seeds and rhizomes.

MATERIALS AND METHODS

Materials and Chemicals. Raw red lotus seeds and rhizomes were purchased from a lotus farm in the Siheung region in South Korea.

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Table 1. Retention Times and Mass Spectrometric Data for TMS Derivatives of Lipids by GC-MS

common name	retention time (min)	major fragmentation ions (m/z)
fatty acids		
myristic (C14:0)	12.95	300 (M^+, 6) , 285 (96), 207 (21), 132 (54), 129 (36), 117 (100), 75 (55), 73 (83)
palmitic (C16:0)	16.45	328 (M^+, 4) , 313 (51), 132 (29), 129 (42), 117 (100), 75 (52), 73 (59)
linoleic (C18:2)	21.22	352 (M^+, 3), 337 (20), 129 (48), 117 (39), 109 (30), 95 (52), 81 (71), 75 (100), 73 (84)
linolenic (C18:3)	21.27	350 (M^+, 3), 335 (14), 129 (42), 117 (37), 108 (48), 95 (68), 79 (100),75 (92), 73 (81)
oleic (*cis*-C18:1)	21.38	354 (M^+, 4), 339 (51), 199 (13), 129 (77), 117 (100), 109 (15), 96 (33), 73 (79)
elaidic (*trans*-C18:1)	21.50	354 (M^+, 11), 339 (68), 145 (41), 129 (62), 117 (85), 96 (32), 75 (85), 73 (100)
stearic (C18:0)	22.03	356 (M^+, 3), 341 (46), 201 (72), 145 (23), 129 (46), 118 (11), 117 (100), 73 (57)
gondoic (C20:1)	25.26	382 (M^+, 11), 367 (92), 207 (65), 145 (36), 129 (63), 117 (80), 96 (50), 73 (100)
arachidic (C20:0)	25.80	384 (M^+, 4), 369 (41), 145 (22), 132 (30), 129 (42), 117 (100), 73 (49)
heneicosylic (C21:0)	27.48	398 (M^+, 25), 383 (81), 145 (42), 132 (73), 129 (42), 117 (100), 73 (93)
behenic (C22:0)	29.11	412 (M^+, 9), 397 (42), 145 (26), 129 (43), 117 (100), 73 (49)
tricosylic (C23:0)	30.58	426 (M^+, 39), 411 (97), 207 (100), 145 (44), 132 (70), 129 (40), 117 (87), 73 (70)
lignoceric (C24:0)	32.04	440 (M^+, 42), 425 (93), 207 (64), 145 (50), 132 (83), 117 (100), 73 (71)
phytosterols		
campesterol	37.70	472 (M^+, 40), 457 (12), 382 (78), 367 (29), 343 (74), 207 (75), 129 (100), 95 (32)
isofucosterol	38.58	484 (M^+, 6), 394 (4), 355 (3), 281 (12), 207 (100), 190 (8), 96 (11), 73 (12)
stigmasterol	38.71	484 (M^+, 1), 393 (2), 386 (2), 355 (2), 281 (11), 207 (100), 133 (9), 96 (12), 73 (7)
β-sitosterol	38.86	486 (M^+, 43), 471 (10), 396 (79), 381 (27), 357 (69), 145 (21), 129 (100), 73 (48)
β-amyrin	38.97	498 (M^+, 1), 483 (3), 393 (8), 281 (10), 218 (100), 190 (14), 189 (18), 135 (17), 73 (43)
α-amyrin	39.32	498 (M^+, 6), 483 (3), 327 (1), 279 (6), 218 (100), 190 (17), 189 (19), 135 (16), 73 (34)
lanosterol	39.45	498 (M^+, 17), 483 (33), 393 (66), 271 (7), 227 (8), 189 (16), 147 (16), 69 (100)
monoglycerides		
1-palmitoylglycerol	28.07	474 (M^+, 0.07), 459 (12), 403 (8), 329 (6), 313 (33), 218 (93), 207 (32), 191 (25), 147 (49), 129 (100)
2-palmitoylglycerol	28.61	474 (M^+, 0.13), 459 (10), 371 (100), 313 (4), 239 (13), 203 (11), 147 (20), 129 (13)
1-linoleoylglycerol	31.05	498 (M^+, 5), 483 (16), 408 (28), 395 (148), 305 (12), 262 (15), 237 (5), 147 (43), 129 (100)
1-oleoylglycerol	31.14	500 (M^+, 8), 485 (18), 410 (15), 397 (100), 307 (5), 265 (13), 257 (13), 207 (25), 147 (44), 133 (13), 129 (85)
2-behenoylglycerol	36.78	558 (M^+, 0.66), 543 (4), 455 (39), 281 (13), 207 (100), 147 (7), 133 (8), 129 (7)
diglycerides		
1,3-dipalmitoylglycerol	46.33	640 (M^+, 0.19), 625 (2), 385 (1), 371 (7), 355 (1), 313 (1), 281 (11), 265 (1), 207 (100), 147 (2), 133 (6)
1-palmitoyl-2-linoleoylglycerol	49.07	664 (M^+, 0.24), 649 (15), 408 (23), 385 (65), 337 (7), 313 (22), 262 (18), 207 (80), 145 (18), 129 (100)
1-palmitoyl-3-linoleoylglycerol	50.04	664 (M^+, 2), 649 (33), 408 (23), 395 (36), 385 (80), 371 (97), 313 (32), 262 (77), 207 (100), 146 (20), 129 (74)
1-oleoyl-2-linolenoylglycerol	53.44	688 (M^+,1), 411 (17), 409 (13), 281 (11), 262 (6), 207 (100), 191 (8), 129 (42)
1-oleoyl-3-linolenoylglycerol	54.74	688 (M^+, 3), 673 (4), 600 (4), 411 (20), 395 (13), 339 (5), 281 (11), 262 (20), 207 (100), 147 (5), 129 (28)

The seed and rhizome samples were frozen rapidly in liquid nitrogen and stored at −20 °C until use.

Oleic acid and linoleic acid were purchased from Sigma-Aldrich. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide, palmitic acid, linolenic acid, arachidic acid, and behenic acid were obtained from Tokyo Chemical Industry Co., Ltd. Silica gel 100 was purchased from Merck.

Extraction of Lipid Components from Lotus Seeds and Rhizomes. The frozen lotus seeds (1.2 kg) were blended and extracted with 95% ethanol (6 L) four times at 50 °C for 2 h. The combined ethanol extracts were filtered over a short pad of Celite and evaporated to complete dryness under reduced pressure. The lotus rhizomes (1.2 kg) were extracted and dried following the same procedure. The ethanol extracts from the seeds and rhizomes are referred to hereafter as LS95E (69.79 g) and LR95E (44.00 g), respectively. The crushed ethanol extracts were also continuously extracted with hexane (400 mL) five times at room temperature, and the combined hexane extracts were evaporated to dryness under reduced pressure. The hexane extracts are referred to hereafter as LS95EH (16.97 g) and LR95EH (2.07 g), respectively.

Silica Gel Column Chromatography. LS95EH (0.5 g) and LR95EH (0.4 g) were each subjected to silica gel column chromatography (140 mm × 20 mm i.d.) and eluted with hexane/diethyl ether (from 1:0, 8:1, 6:1, 1:1, to 0:1) followed by hexane/ethyl acetate (from 1:1 to 0:1) to afford fractions 1−3. Fraction 1 contained a small amount of plant waxes. Fraction 2 contained nonpolar compounds such as triglycerides and steryl-fatty acid esters. Fraction 3 contained polar compounds such as diglycerides, monoglycerides, free phytosterols, and free fatty acids. Each component could be identified by thin-layer chromatography (TLC) with a solvent system of hexane/ethyl acetate (4:1). Fraction 2 was subjected to hydrolysis followed by silylation. Fraction 3 was directly converted to trimethylsilyl (TMS) derivatives for GC-MS analysis.

Hydrolysis. Aliquots of LS95EH (1.1 g) and LR95EH (1.0 g) were mixed with potassium hydroxide (1.0 g), tetrahydrofuran (30 mL), and distilled water (about 20 drops). The mixture was hydrolyzed in a microwave reactor (CEM Focused Microwave Synthesis System; Discover, CEM, USA) at 73 °C, 70 W, for 10 min, and then at 76 °C, 70 W, for 15 min.[36] Hydrolysis progress was monitored by TLC. Phytosterols were separated directly from the strongly basic solution with hexane. The residual aqueous solution was acidified with 35% hydrochloric acid (2 mL), and then fatty acids were separated from the acidic solution with hexane. The dry samples containing phytosterols and fatty acids were silylated and then analyzed by GC-MS. The mixture of triglycerides and steryl-fatty acid esters separated from silica gel column chromatography (fraction 2) was hydrolyzed and silylated using the same method as above.

Table 2. Concentrations of Total Fatty Acids and Total Phytosterols from Hydrolysis

item	LS95EH[a] (g/kg of hexane extract)	LR95EH[a] (g/kg of hexane extract)	*t* value[b]
fatty acids			
myristic (C14:0)	2.89 ± 0.23	1.49 ± 0.12	9.27**
palmitic (C16:0)	183.19 ± 6.94	187.42 ± 10.16	−0.60
linoleic (C18:2)	354.47 ± 3.77	211.15 ± 3.84	46.13***
linolenic (C18:3)	26.17 ± 1.88	31.48 ± 2.55	−2.91*
oleic (*cis*-C18:1)	80.12 ± 0.56	61.61 ± 3.47	9.10**
elaidic (*trans*-C18:1)	5.40 ± 0.47	4.80 ± 0.07	2.19
stearic (C18:0)	6.88 ± 0.81	7.06 ± 0.94	−0.25
gondoic (C20:1)	1.08 ± 0.20	1.27 ± 0.24	−1.10
arachidic (C20:0)	7.20 ± 0.95	1.51 ± 0.09	10.34***
heneicosylic (C21:0)	0.54 ± 0.07	0.90 ± 0.09	−5.60**
behenic (C22:0)	27.84 ± 1.86	4.86 ± 0.60	20.38***
tricosylic (C23:0)	1.32 ± 0.54	2.37 ± 0.35	−2.80*
lignoceric (C24:0)	4.21 ± 0.69	2.64 ± 0.31	3.62*
saturated fatty acids	234.52 ± 7.70	210.07 ± 9.60	3.31*
monounsaturated fatty acids	86.60 ± 1.00	67.68 ± 3.17	9.85**
polyunsaturated fatty acids	380.64 ± 3.34	242.63 ± 2.35	58.51***
total fatty acids	701.75 ± 10.49	520.39 ± 14.57	17.33***
phytosterols			
campesterol	4.81 ± 0.14	4.97 ± 0.78	−0.35
isofucosterol	0.26 ± 0.05		
stigmasterol	0.10 ± 0.01		
β-sitosterol	81.20 ± 1.13	72.58 ± 1.83	6.95**
β-amyrin	7.24 ± 0.16	6.98 ± 0.89	0.50
α-amyrin		8.02 ± 0.68	
lanosterol		11.60 ± 0.65	
total phytosterols	93.59 ± 1.27	104.14 ± 0.90	−11.72***

[a]Data shown are the mean ± SD from SPSS; each data point was derived from three independent repetitions. Fatty acids and phytosterols were quantitatively analyzed as TMS derivatives. [b]Values with * are significantly different between LS95EH and LR95EH by Student's *t* test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Silylation. Each sample containing analytes (about 0.5 mg) was mixed with trimethylsilylation reagent (*N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane, 100 μL), pyridine (50 μL), and acetonitrile (350 μL), and then the mixture was microwave-heated at 70 °C, 50 W, for 10 min.[37] After completion of the silylation reaction, 1 μL of the solution was used for GC-MS analysis.

GC-MS Analysis. GC-MS was performed with a Shimadzu QP-5000 gas chromatograph 17A instrument coupled to a QP5050A mass spectrometer and Class-5000 software (Shimadzu Co. Ltd., Kyoto, Japan). An Rtx-1 capillary column (30 m × 0.25 mm i.d.) was coated with a 0.25 μm film. The column temperature was initially set at 100 °C and held at that temperature for 5 min after injection and then increased at a rate of 20 °C/min to 200 °C and held for 10 min. The temperature was continually increased at a rate of 10 °C/min to 230 °C and, then, finally increased at a rate of 5 °C/min to 320 °C and held for 35 min. Split injection (1 μL) was conducted with a split ratio of 1:30, and the injection temperature was set at 260 °C. Helium was used as the carrier gas at a flow rate of 1 mL/min, and the interface temperature was set at 280 °C. The electron energy was 70 eV, and the scan range was from m/z 30 to 1050 in mass detection conditions.

The concentrations of each composition were calculated from the weights of samples and area percentages of each component in the GC-MS results. Most of the concentration data were used without calibration. The validity of some of the data was checked using standard samples (oleic acid, linoleic acid, palmitic acid, linolenic acid, arachidic acid, behenic acid, and *β*-sitosterol). Monoglycerides and diglycerides were identified using the NIST62 library spectra and on the basis of the results of Isidorov et al.[38,39]

Statistical Analysis. Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) version 17.0. Results from three independent GC-MS analyses for each sample were used to calculate the mean ± SD. The differences between the lotus seeds and rhizomes were analyzed using Student's *t* test.

RESULTS AND DISCUSSION

In this study, the lipid components such as triglycerides, steryl-fatty acids esters, diglycerides, monoglycerides, free fatty acids, and free phytosterols of hexane extracts of lotus seeds and rhizomes, extracted from the 95% ethanol extracts, were investigated. Three methods were used for analysis of lipid components in LS95EH and LR95EH. First, crude hexane extract (containing glycerides, steryl-fatty acid esters, free fatty acids, free phytosterols, phospholipids, phytosterol−sugar adducts, phytosterol−phospholipid adducts, and fatty acid−sugar adducts) was hydrolyzed, and then total fatty acids and total phytosterols were analyzed. Second, the nonpolar fraction containing triglycerides and steryl-fatty acid esters from silica gel column chromatography was hydrolyzed, and then fatty acids and phytosterols were analyzed. Third, the polar fraction containing diglycerides, monoglycerides, free fatty acids, and free phytosterols from silica gel column chromatography was analyzed. The retention time, mass of molecular M^+, and m/z of major fragmentation ions in the mass spectra of the TMS derivatives of the fatty acids, phytosterols, monoglycerides, and diglycerides are listed in Table 1.

The saponification reaction has been used frequently for free fatty acid analysis. However, the conventional saponification reaction requires a long reaction time,[40] and the reaction is reversible in nature, which is unsuitable for accurate total fatty acid and total phytosterol analysis. A new and facile hydrolysis

Table 3. Concentrations of Fatty Acid and Phytosterol Contents in Triglycerides and Steryl-Fatty Acid Esters

	LS95EH[a] (g/kg of hexane extract)		LR95EH[a] (g/kg of hexane extract)		
item	fatty acids	phytosterols	fatty acids	phytosterols	*t* value[b]
fatty acids					
myristic (C14:0)	0.86 ± 0.01				
palmitic (C16:0)	59.47 ± 1.58		43.64 ± 1.94		10.94***
linoleic (C18:2)	176.74 ± 5.38		53.68 ± 0.47		39.48***
linolenic (C18:3)	6.66 ± 0.34		7.91 ± 0.35		−0.92
oleic (*cis*-C18:1)	43.19 ± 0.68		14.27 ± 0.96		42.71***
elaidic (*trans*-C18:1)	3.87 ± 0.04		1.17 ± 0.19		24.16***
stearic (C18:0)	5.40 ± 0.10		8.34 ± 0.15		−28.75***
gondoic (C20:1)	1.47 ± 0.10				
arachidic (C20:0)	7.77 ± 0.34		0.43 ± 0.13		35.18***
heneicosylic (C21:0)	1.04 ± 0.05				
behenic (C22:0)	28.94 ± 1.62		1.56 ± 0.23		29.10**
tricosylic (C23:0)	1.64 ± 0.15				
lignoceric (C24:0)	8.34 ± 0.52				
total fatty acids	345.97 ± 3.75		131.01 ± 2.77		79.94***
phytosterols					
campesterol		1.55 ± 0.07			
isofucosterol		0.12 ± 0.10			
β-sitosterol		10.57 ± 0.29			
β-amyrin		5.34 ± 0.18			
total phytosterols		17.58 ± 0.64			

[a]Data shown are the mean ± SD from SPSS; each data point was derived from three independent repetitions. Fatty acid and phytosterol contents were quantitatively analyzed as TMS derivatives. [b]Values with * are significantly different between LS95EH and LR95EH by Student's *t* test (**, $P < 0.01$; ***, $P < 0.001$).

reaction was used in this study for the analysis of total fatty acids and total phytosterols. The use of tetrahydrofuran as a new solvent and microwave technology decrease the reaction time to 25 min. The concentrations of total fatty acids and total phytosterols after hydrolysis are shown in Table 2. Linoleic acid predominated in both LS95EH and LR95EH, followed by palmitic acid, oleic acid, behenic acid, and linolenic acid. The concentrations of fatty acids in LS95EH were significantly different from the concentrations of fatty acids in LR95EH, except palmitic acid, elaidic acid, stearic acid, and gondoic acid. The concentration of total fatty acids was significantly higher in LS95EH than in LR95EH. In this study, the percentages of total fatty acids were 0.99% and 0.09% in raw lotus seeds and rhizomes, respectively, which were slightly higher than those in data from the USDA.[24,25] β-Sitosterol predominated in LS95EH, followed by β-amyrin and campesterol. Lanosterol and α-amyrin were found only in LR95EH. It is reported that the typical plant sterols act in membranes to restrict the motion of fatty acid chains.[41] Although the concentration of total phytosterols was significantly lower in LS95EH (93.59 g/kg hexane extract) than in LR95EH (104.14 g/kg hexane extract), the total amount of phytosterols was larger in raw lotus seeds (1.32 g/kg raw seed) than in raw lotus rhizomes (0.18 g/kg raw rhizomes). For the other parts of lotus, a relatively low concentration of phytosterols (10.71 g/kg extract) was reported in lotus bee pollen oil extracted by supercritical CO_2.[33] The differences in the fatty acid and phytosterol contents between previous studies and this study may be explained by the differences in lotus parts used and in the methods of extraction and analysis. The likely reason for the highest concentration of total fatty acids in lotus seeds, compared to that in the other lotus parts, is that fatty acids are prepared as fuel for embryonic development and plant growth. The large amount of total phytosterols in lotus seeds may be explained by the fact that phytosterols regulate membrane fluidity and play roles in cellular differentiation as plant hormone precursors of brassinolides in the germinative seed.[27,41−43] The concentrations of fatty acids and phytosterols will gradually decrease after the seed germinates.[27] According to our studies, the hexane extracts of lotus seeds and rhizomes contained relatively less unsaturated fatty acids (66.6 and 59.6%) and more phytosterols (93.59 and 104.14 g/kg) than common edible oils.[44,45] Therefore, LS95EH and LR95EH, which contained higher concentrations of phytosterols, may be of benefit for people with particular conditions, such as hypercholesterolemia.[29] The physiological functions of LS95EH and LR95EH in vivo need to be studied in the future.

Triglycerides and steryl-fatty acid esters as nonpolar components were separated by silica gel column chromatography. Triglycerides have high boiling points, so they cannot be analyzed directly by GC-MS. For this reason, the triglycerides and steryl-fatty acid esters were hydrolyzed and then converted to TMS derivatives for GC-MS. Linoleic acid, palmitic acid, oleic acid, β-sitosterol, and β-amyrin were the main components of fatty acids and phytosterols in triglycerides and steryl-fatty acid esters of LS95EH (Table 3), respectively. Linoleic acid, palmitic acid, and oleic acid were also the main components of fatty acids in triglycerides of LR95EH, but the amount of phytosterols in steryl-fatty acid esters was under the detection limit. The concentrations of fatty acids and phytosterols in triglycerides and steryl-fatty acid esters were significantly higher in LS95EH (354.97 and 17.58 g/kg) than in LR95EH (131.01 and ∼0 g/kg), respectively. In addition, about half of the total fatty acids in LS95EH existed in the triglyceride form. Triglycerides are regarded as efficient energy-storing compounds, and steryl-fatty acid esters (about 29.5 g/kg) are regarded as reservoirs for the phytosterols.[46] The reasons for the different concentrations of ester contents between LS95EH

Table 4. Concentrations of Diglycerides, Monoglycerides, Free Fatty Acids, and Free Phytosterols

item	LS95EH[a] (g/kg of hexane extract)	LR95EH[a] (g/kg of hexane extract)	*t* value[b]
diglycerides			
1,3-dipalmitoylglycerol	0.27 ± 0.07		
1-palmitoyl-2-linoleoylglycerol	13.17 ± 0.43	0.27 ± 0.07	50.82***
1-palmitoyl-3-linoleoylglycerol	32.56 ± 0.33	0.45 ± 0.13	157.21***
1-oleoyl-2-linolenoylglycerol	4.91 ± 0.42		
1-oleoyl-3-linolenoylglycerol	14.44 ± 0.30		
monoglycerides			
1-palmitoylglycerol	0.80 ± 0.06		
2-palmitoylglycerol	12.64 ± 1.13	8.13 ± 0.98	5.24**
1-linoleoylglycerol	3.60 ± 0.25	7.11 ± 0.10	−22.73***
1-oleoylglycerol	2.06 ± 0.13		
2-behenoylglycerol	0.26 ± 0.03		
free fatty acids			
myristic (C14:0)	0.31 ± 0.06	0.11 ± 0.00	5.50*
palmitic (C16:0)	43.26 ± 1.17	6.80 ± 0.21	37.55***
linoleic (C18:2)	26.06 ± 1.49	5.21 ± 0.06	24.09**
pinolenic (C18:3)	1.26 ± 0.17	1.51 ± 0.12	−2.43
oleic (*cis*-C18:1)	13.20 ± 0.27	1.51 ± 0.08	70.00***
elaidic (*trans*-C18:1)	0.74 ± 0.15	0.15 ± 0.02	6.81*
stearic (C18:0)	1.10 ± 0.01	0.41 ± 0.03	42.47***
gondoic (C20:1)	0.45 ± 0.07	0.02 ± 0.00	10.87**
arachidic (C20:0)	0.37 ± 0.03	0.09 ± 0.01	17.28***
heneicosylic (C21:0)		0.02 ± 0.01	
behenic (C22:0)	2.24 ± 0.07	0.34 ± 0.04	44.14***
tricosylic (C23:0)	0.38 ± 0.03	0.08 ± 0.01	17.50***
lignoceric (C24:0)	0.85 ± 0.03	0.45 ± 0.07	9.61**
total free fatty acids	83.91 ± 3.20	16.02 ± 0.46	36.43**
free phytosterols			
campesterol	5.25 ± 0.13	4.51 ± 0.16	6.17**
isofucosterol		0.65 ± 0.03	
stigmasterol	0.31 ± 0.02		
β-sitosterol	56.77 ± 2.27	76.27 ± 0.24	−14.80**
β-amyrin	1.61 ± 0.02	7.34 ± 0.14	−69.92***
α-amyrin		7.78 ± 0.10	
lanosterol		12.68 ± 0.38	
total free phytosterols	63.94 ± 2.42	109.22 ± 0.64	−31.36***

[a]Data shown are the mean ± SD from SPSS; each data point was derived from three independent repetitions. Diglycerides, monoglycerides, free fatty acids, and free phytosterols were quantitatively analyzed as TMS derivatives. [b]Values with * are significantly different between LS95EH and LR95EH by Student's *t* test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

and LR95EH may be explained by the fact that the seed must contain a large amount of energy and phytosterols in a small space.

Diglycerides, monoglycerides, free phytosterols, and free fatty acids as polar components were separated by silica gel column chromatography. With regard to the diglycerides, 1-palmitoyl-3-linoleoylglycerol, 1-oleoyl-3-linolenoylglycerol, and 1-palmitoyl-2-linoleoylglycerol were the main components in LS95EH (Table 4). 1-Palmitoyl-3-linoleoylglycerol and 1-palmitoyl-2-linoleoylglycerol were present in significantly smaller amounts in LR95EH. With regard to the monoglycerides, 2-palmitoylglycerol predominated in LS95EH, followed by 1-linoleoylglycerol and 1-oleoylglycerol. LR95EH contained only 2-palmitoylglycerol and 1-linoleoylglycerol. Palmitic acid was the predominant compound in free fatty acids of LS95EH and LR95EH (Table 4). The concentration of total free fatty acids was significantly higher in LS95EH (83.91 g/kg) than in LR95EH (16.02 g/kg). A large proportion of phytosterols in LS95EH and all of the phytosterols in LR95EH were in the free form. *β*-Sitosterol was the predominant compound in free phytosterols of LS95EH and LR95EH. These results are consistent with those of previous studies.[43,47]

In conclusion, the ethanol extract of lotus seeds contained more total fatty acids and total phytosterols than did the ethanol extract of lotus rhizomes. Most of the fatty acids were in the ester form in LS95EH, especially in the triglyceride form. Linoleic acid was the most abundant fatty acid compound in both LS95EH and LR95EH. Fatty acids in LS95EH exist in the glycerides form (59.8%) and to a lesser extent in their free acid form (12.0%). The remaining portion (28.2%) can be explained as chromatographic loss due to the existence of nonisolated fatty acid−sugar adducts and phospholipids. Phytosterols exist mainly in the free form (68.3%) and to a lesser extent in the steryl-fatty acid ester form (18.8%) in LS95EH. The remaining portion (12.9%) can be explained as chromatographic loss due to the existence of nonisolated phytosterol−sugar adducts and phytosterol−phospholipid adducts. In this study, we identified the lipid components of the ethanol extracts of lotus seeds and rhizomes. The results of this study also provide a basis for further investigation of the physiological functions, including

antiobesity and antioxidative effects, of lotus seed and rhizome extracts in vivo.

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Notes

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