Lipid Class Contents and Fatty Acid Composition of Small Millets: Little (*Panicum sumatrense*), Kodo (*Paspalum scrobiculatum*), and Barnyard (*Echinocloa colona*)†

Rudravarapu Sridhar and Gollamudi Lakshminarayana*

Indian Institute of Chemical Technology, Hyderabad 500 007, India

Grain samples of small millets, namely little (*Panicum sumatrense*), kodo (*Paspalum scrobiculatum*), and barnyard (*Echinocloa colona*), were extracted sequentially with hexane for free lipids, with hot water-saturated butanol for bound lipids, and again with hexane after acid hydrolysis for structural lipids. The total lipids (comprising free, bound, and structural lipids) amounted to 8.3 (65.1, 26.5, and 8.4%), 5.1 (66.7, 25.5, and 7.8%), and 8.0% (71.3, 21.2, and 7.5%) on dry weight basis in little, kodo, and barnyard millets, respectively. The neutral lipids (NL) and glyco- (GL) and phospholipids (PL), separated by silicic acid column chromatography, constituted 84.1–88.1, 8.0–11.0, and 3.9–5.5% of the total lipids, respectively. The subclasses, separated by thin-layer chromatography, consisted chiefly of triacylglycerols in NL, esterified steryl glycosides, monogalactosyl diglycerides, and digalactosyl diglycerides in GL and phosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine in PL. In all of the lipid classes linoleic (18:2), oleic (18:1), and palmitic (16:0) acids were the chief constituents. Linolenic acid (18:3) was present in appreciable proportions in the GL and PL classes.

Cereal lipids contribute significantly to the diet as a source of invisible fat and essential fatty acids (Achaya, 1987). The lipids have also a role in storage stability, breadmaking, and brewing quality of cereals (Morrison, 1978). Among the cereals, small millets (minor millets) account for about 1% of the food grains produced in the world. These cereals, though not important in terms of world food production, are useful as food crops in their respective agro-eco systems (deWet, 1989). By virtue of their composition, small millets are quite comparable to rice or wheat in their nutritive value (Malleshi, 1989). Several reviews cover the literature on lipids of major cereals such as rice, wheat, barley, oat, sorghum, corn, and pearl millets (Weber, 1973; Morrison; 1978). Information on lipids of small millets is available to some extent for foxtail (*Setaria italica*), proso (*Panicum miliaceum*), and finger (*Eleusine coracana*) millets but to a meagre extent for little (*Panicum sumatrense*), kodo (*Paspalum scrobiculatum*), and barnyard (*Echinocloa colona*) millets (Hulse et al., 1980). In the present investigation the total lipids, lipid classes, and fatty acid compositions of little, kodo, and barnyard millets are reported.

EXPERIMENTAL PROCEDURES

Materials. Grain samples of little, kodo, and barnyard millets were obtained from Genetic Resources Unit, International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. Methyl esters of fatty acids [palmitic (16:0), palmitoleic (16:1), heptadecanoic (17:0), stearic (18:0), oleic (18:1), linoleic (18:2), α-linolenic (18:3), arachidic (20:0), arachidonic (20:4), behenic (22:0), and erucic (22:1)] and neutral lipid (NL) standards [triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG), free sterols (FS), and steryl esters (SE)] were purchased from Nuchek Inc., Elysian, MN. γ-Linolenic acid (GLA, 18:3) and phospholipid (PL) standards [phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and lysophosphatidylcholine (LPC)] were purchased from Sigma Chemical Co., St. Louis, MO. Glycolipid (GL) standards were given by Prof. K. Subba Rao, University of Hyderabad, India. Silica gel G was purchased from Acme Synthetic Chemicals Limited, Bombay, India. 2′,7′-Dichlorofluorescein was purchased from Loba Chemie, Bombay. Distilled solvents were used throughout. Column packing for gas–liquid chromatography (GLC) was obtained from Supelco Inc., Bellefonte, PA.

Extraction of Total Lipids. A representative sample, in duplicate, of clean grains (10–15 g) was ground to a fine powder and extracted in a Soxhlet apparatus with *n*-hexane by refluxing for 8 h on a water bath for the estimation of free lipid content. The extracted flour was re-extracted three times in a screw-cap vial with hot water-saturated butanol (WSB) (w/v, 1:5) for 1 h each time by vigorous shaking with a mechanical shaker to estimate the bound lipids (Osagie and Kates, 1984). After each extraction, the mixture was filtered in a sintered glass funnel under suction. The filtrates were pooled, concentrated on a rotavapor at 65–70 °C, and purified after re-extraction with chloroform–methanol–water (1:2:0.8 v/v/v) according to the procedure of Bligh and Dyer (1959). The residue was further subjected to acid hydrolysis (Taylor and Nelson, 1920), filtered in a sintered glass funnel, freed of acid, dried at 50 °C in an air oven, and extracted with *n*-hexane in a Soxhlet apparatus for 3 h to estimate the structural lipids (Hoseney, 1986). Free, bound, and structural lipids were separately estimated and then pooled for analysis of lipid class and fatty acid compositions.

Separation of Lipid Classes. The total lipids were fractionated into major lipid classes, NL, GL, and PL, by silicic acid column chromatography using chloroform, acetone, and methanol, respectively. The lipid classes were further separated by preparative thin-layer chromatography (TLC). Development with a mixture of *n*-hexane–diethyl ether–acetic acid (80:20:1 v/v/v) (Mangold, 1969) separated the total NL into various subclasses. The GL subclasses were separated using chloroform–methanol–acetic acid–water (170:24:25:4 v/v/v/v) as developer (Nichols, 1970). The PL subclasses were separated using chloroform–methanol–water (65:25:4 v/v/v) as developer (Lepage, 1964; Rouser et al., 1976). The lipid subclasses were estimated by gravimetry. The reagents used for identification of the lipids were 2′,7′-dichlorofluorescein for general purpose, α-naphthol for GL (Siakotos and Rouser, 1965), Zinzadze reagent for PL (Dittmer and Lester, 1964), sulfuric acid–acetic acid for sterols (Kates, 1986), periodate–Schiff reagent for PG, PI, monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) (Beiss, 1964), ninhydrin for amino group-containing lipids

* Author to whom correspondence should be addressed.
† Communication No. 3010 of IICT, Hyderabad, India.

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(Marinetti, 1964), and Dragendorff reagent for lipids containing choline (Shaw, 1968).

Fatty Acid Analysis. Fatty acid methyl esters (FAME) of the lipid materials were prepared using 14% (w/v) boron trifluoride in methanol (Morrison and Smith, 1964) and analyzed by using a Hewlett-Packard 5840A gas chromatograph fitted with a hydrogen flame ionization detector and a data processor (Hewlett-Packard Co., Palo Alto, CA). A glass column [1.8 m × 4.8 mm (i.d.)] packed with 10% Silar 10C on Chromosorb W HP (60–80 mesh) was used. The column, detector, and injection port were maintained at 190, 300, and 250 °C, respectively. Nitrogen was used as carrier gas (35 mL/min). Peaks were identified by using standard FAME and quantitated by using methyl heptadecanoate as internal standard. Capillary GLC was also performed to examine the occurrence of GLA. A Tracor 540 gas chromatograph fitted with a flame ionization detector was used. A fused silica capillary column (0.24 mm × 30 m) coated with SP 2330 (film thickness 0.2 μm) was used. The column temperature was programmed from an initial temperature of 160 °C (held for 2 min) to a final temperature of 220 °C (held for 10 min) at a rate of 5 °C/min. The temperatures of injector and detector were maintained at 250 and 300 °C, respectively. Nitrogen was used as carrier gas, and the pressure was maintained at 15 psig.

RESULTS AND DISCUSSION

Contents of Free and Bound Lipids. Accurate quantification of lipids in cereal grains is difficult because substantial quantities of lipids are present inside the impermeable cells and starch granules, where they are inaccessible to solvents under normal extraction conditions. Cereal lipids are often defined as free or bound, depending on their solubility in a nonpolar solvent or a polar solvent, respectively. Extraction of invisible fat from cereals with nonpolar solvents such as petroleum ether–diethyl ether estimates only the free lipids in cereals and gives a gross underestimate of the total lipids (Inkpen and Quackenbush, 1969; Kinsella et al., 1975; Rogols et al., 1969). Several methods for extraction of both free and bound lipids employing solvents such as petroleum ether, *n*-hexane, *n*-heptane, benzene, chloroform, acetone, water-saturated butanol (WSB), methanol, and 95% ethanol have been described (Finney et al., 1976; Mecham, 1971). Hot WSB is efficient in extracting the bound lipids, polar as well as neutral, more so than WSB at room temperature and gives the highest yield of the true total lipids (Osagie and Kates, 1984). Achaya (1987) suggested extraction with petroleum ether for free lipids, chloroform–methanol extraction for bound lipids, and finally acid hydrolysis with concentrated hydrochloric acid followed by hexane extraction for structural lipids, whereas Hoseney (1986) suggested sequential extraction with *n*-hexane, hot WSB, and *n*-hexane again after acid hydrolysis for the estimation of total lipids. Preliminary investigations in the present studies have shown that chloroform–methanol extracts of the small millets contained high amounts of non-lipid material. Hence, hot WSB was used for extraction of bound lipids. Little and barnyard millets contained similar amounts of total lipids (8.3 and 8.0%), while kodo contained a lesser amount (5.1%) on dry weight basis (dwb) (Table I). The reported values of lipid content (dwb) were 4.1–9.0% in little, 6.6% in kodo (Ramanathan et al., 1975), and 2.5–3.6% in barnyard (Indira and Naik, 1971) millets. The quantity of free lipids obtained by hexane extraction varied from 65 to 71%, bound lipids obtained by hot WSB extraction from 21.2 to 26.5%, and structural lipids obtained by hexane extraction after acid hydrolysis from 7.5 to 8.4% of the total lipids.

Contents of Major Classes and Subclasses of Total Lipids. The NL constituted the major portion of total lipids ranging from 84 to 88%, whereas the GL and PL contents varied from 8 to 11% and from 3.9 to 5.5%, respectively (Table II). The NL subclasses consisted of SE, TAG, free fatty acids (FFA), FS, DAG, and MAG. The TAG constituted the major portion of NL ranging from 84.2 to 90.8%. DAG and MAG were present in very small amounts. Barnyard millet contained a significant amount of FS. Esterified steryl glycosides (ESG), MGDG, and DGDG were the major species of GL followed by steryl glycosides (SG) and cerebrosides (CS). Very small amounts of digalactosyl monoglycerides (DGMG) and monogalactosyl monoglycerides (MGMG) were also present in the little millet. Kodo and barnyard millets contained MGDG and DGDG and little millet contained MGDG as major constituents. Barynard millet contained larger amounts of SG and CS than the other two millets. PC, PE, and LPC were the major constituents in the small millets. Among the minor constituents, PG in kodo and PS and PI in barnyard millet were present in significant amounts.

Table I. Contents of Free, Bound, Structural, and Total Lipids (Percent, Dry Weight Basis)[a] of Little, Kodo, and Barnyard Millets Obtained by Sequential Extraction Procedure

	little	kodo	barnyard
moisture, %	10.8	11.4	12.0
hexane extract (free lipids)	5.4	3.4	5.7
hot WSB extract (bound lipids)	2.2	1.3	1.7
hexane extract of acid hydrolysate (structural lipids)	0.7	0.4	0.6
total lipids	8.3	5.1	8.0

[a] Mean of duplicate analyses.

Table II. Contents (Weight Percent)[a] of Major Classes and Subclasses of Little, Kodo, and Barnyard Millet Lipids

lipid subclass	little	kodo	barnyard
neutral lipids	84.1	88.1	85.5
SE	3.3	0.8	2.8
TAG	86.3	90.8	84.2
FFA	1.5	1.8	1.8
FS	5.9	3.8	10.2
DAG	2.3	2.0	0.8
MAG	0.7	0.8	0.2
glycolipids	11.1	8.0	9.0
ESG	24.1	21.4	18.6
MGDG	55.3	29.2	30.7
MGMG	0.9		tr
SG	7.6	16.3	20.2
CS	1.2	5.0	9.1
DGDG	9.0	28.0	21.4
DGMG	1.8	tr	
phospholipids	4.9	3.9	5.5
PA	5.5	9.0	1.8
PG	8.0	18.0	3.8
PE	21.0	30.2	24.0
PC	36.5	21.2	30.7
PS	tr	tr	8.3
PI	tr	tr	6.6
LPC	28.0	20.1	24.8

[a] Mean of duplicate analyses.

Fatty Acid Composition of Major Lipid Classes. The fatty acid compositions of little and kodo millets were similar in having more or less equal amounts of 18:1 and 18:2 in their major lipid classes (Table III); they resemble oat groats in this respect (Aylward and Showler, 1962), while barnyard millet resembles sorghum and pearl millet in containing lower amounts of 18:1 and higher amounts of 18:2 (Freeman and Bocan, 1973). Very small quantities of 20:0, 22:1, and 20:4 were present in the NL of the three small millets. The major fatty acids in GL and PL were 16:0, 18:1, and 18:2. The GL and PL of barnyard millet contained significant amounts of 18:3. The GL of kodo millet contained a significant amount of 16:0.

Table III. Fatty Acid Composition (Weight Percent)[a] of Major Lipid Classes of Little, Kodo, and Barnyard Millets

	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:4	22:0	22:1
					Little					
NL	16.1	0.4	5.2	35.4	40.5	1.4	0.5	0.1	0.2	0.2
GL	20.0		4.0	30.7	40.8	4.0		0.3	0.1	0.1
PL	21.5	0.8	3.7	27.4	35.8	10.8				
					Kodo					
NL	18.1	0.3	2.2	36.6	39.6	1.3	0.7	0.3	0.8	0.1
GL	37.9		3.3	22.0	32.0	4.8				
PL	20.8	0.2	2.9	30.2	36.3	9.2			0.4	
					Barnyard					
NL	17.4	0.4	4.3	27.6	48.1	1.5	0.3	0.1	0.2	0.1
GL	31.4	0.3	3.6	18.6	37.1	9.0				
PL	23.1	0.6	3.4	19.0	40.8	13.1				

[a] Mean of duplicate analyses.

Table IV. Fatty Acid Composition (Weight Percent)[a] of NL Subclasses of Little, Kodo, and Barnyard Millets

	16:0	18:0	18:1	18:2	18:3	20:0	20:4	22:0	22:1
				Little					
SE	19.4	3.5	33.8	42.2	1.1				
TAG	16.0	5.3	35.6	39.4	1.7	0.8	0.3	0.6	0.3
FFA	18.0	5.9	31.9	38.1	4.7			0.8	0.5
DAG	36.7	14.5	19.5	23.8	5.3			0.3	
MAG	38.4	11.9	22.6	21.2	5.0			0.9	
				Kodo					
SE	20.1	4.7	26.3	44.2	1.3	0.4		3.0	
TAG	18.2	2.0	36.9	40.1	0.9	1.0		0.6	0.3
FFA	31.7	8.8	27.3	28.5	1.2	1.4		1.1	
DAG	44.5	16.1	19.1	17.1	1.0	1.5		0.5	0.2
				Barnyard					
SE	16.0	4.4	17.7	56.6	1.2		0.8	2.8	0.5
TAG	19.0	4.5	28.2	46.4	1.7			0.2	
FFA	22.7	5.8	24.3	43.2	2.2			1.0	0.8
DAG	31.1	9.2	26.4	30.3	2.8			0.2	
MAG	42.1	6.5	21.7	25.6	3.5			0.6	

[a] Mean of duplicate analyses.

Table V. Fatty Acid Composition (Weight Percent)[a] of GL Subclasses of Little, Kodo, and Barnyard Millets

	16:0	18:0	18:1	18:2	18:3	22:0	22:1	20:4
				Little				
ESG	21.5	4.6	26.6	41.4	4.0	0.8	0.6	0.5
MGDG	14.5	3.5	30.4	44.9	6.0	0.1	0.2	0.4
MGMG	20.9	8.6	19.0	45.0	5.4	0.2	0.4	0.5
CS	36.9	20.3	14.9	22.7	5.2			
DGDG	26.8	6.0	22.9	36.7	7.6			
DGMG	27.8	12.3	18.0	35.6	6.1	0.2		
				Kodo				
ESG	40.0	5.0	18.0	32.0	5.0			
MGDG	40.1	4.4	19.0	31.3	5.2			
CS	49.1	12.9	11.9	21.3	4.8			
DGDG	40.7	3.9	12.3	37.1	6.0			
				Barnyard				
ESG	33.9	2.3	16.8	38.5	8.5			
MGDG	30.6	3.6	21.4	36.5	7.9			
MGMG	26.0	7.4	13.9	44.0	8.7			
CS	44.3	13.9	10.3	22.4	9.0			
DGDG	32.5	5.0	15.5	38.8	8.2			

[a] Mean of duplicate analyses.

Fatty Acid Compositions of Lipid Subclasses. The major fatty acids were 16:0, 18:1, and 18:2 in most of the NL subclasses, and their proportions varied with the class (Table IV). DAG and MAG contained 16:0 and other NL classes contained 18:2 as the major constituent. Kodo millet contained 20:0 in all of the NL. The major fatty acid of the GL subclasses was 16:0 in kodo and 18:2 in other varieties (Table V). The CS, a minor class of the GL, contained lower amounts of 18:2 and higher amounts of 18:0 than the other GL classes. The CS and ESG of the small millets contained high amounts of 16:0. The PL subclasses contained 16:0 and 18:2 as the major constituents (Table VI). Highest amounts of 18:3 (*cis*-9,12,15) were present in all of the PL of barnyard millet. GLA (18:3, *cis*-6,9,12) was not detected in the lipid components of small millets by either packed or capillary GLC.

Table VI. Fatty Acid Composition (Weight Percent)[a] of PL Subclasses of Little, Kodo, and Barnyard Millets

	16:0	16:1	18:0	18:1	18:2	18:3	22:0
				Little			
PA	38.2		5.5	14.2	33.4	8.7	
PG	29.5	0.2	2.9	21.6	36.6	9.3	
PE	24.2	1.2	2.8	20.2	41.8	9.8	
PC	20.3	0.7	1.9	33.6	34.9	8.6	
LPC	23.7	0.9	3.0	21.9	38.5	12.0	
				Kodo			
PA	26.1	0.7	4.6	18.9	40.7	8.6	0.4
PG	21.2		4.7	32.4	33.5	8.2	
PE	20.7		4.8	29.3	35.8	9.4	
PC	25.3		3.1	25.2	36.9	9.5	
LPC	24.8		3.8	31.1	29.8	10.5	
				Barnyard			
PA	31.0		9.4	15.5	30.7	13.4	
PG	27.4		5.3	18.5	36.0	12.8	
PE	25.5		4.3	17.5	38.7	14.0	
PC	23.6		2.5	19.5	41.2	13.2	
PS	33.0		2.8	15.8	36.2	12.2	
PI	41.8	0.4	3.6	14.0	29.4	10.8	
LPC	24.0	0.6	2.4	19.0	38.5	15.5	

[a] Mean of duplicate analyses.

The results of the present investigation show that little, kodo, and barnyard millets are valuable sources of invisible fats rich in essential fatty acids.

ABBREVIATIONS USED

WSB, water-saturated butanol; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; NL, neutral lipids; GL, glycolipids; PL, phospholipids; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; FS, free sterols; FFA, free fatty acids; SE, steryl esters; SG, steryl glycosides; ESG, esterified steryl glycosides; MGDG, monogalactosyl diglycerides; MGMG, monogalactosyl monoglycerides; DGDG, digalactosyl diglycerides; DGMG, digalactosyl monoglycerides; CS, cerebrosides; PA, phosphatidic acid, PE, phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; GLA, γ-linolenic acid; dwb, dry weight basis. Fatty acids are denoted by the number of carbon atoms followed after a colon by the number of double bonds.

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Registry No. 16:0, 57-10-3; 18:0, 57-11-4; 20:0, 506-30-9; 22:0, 112-85-6; 16:1, 373-49-9; 18:1, 112-80-1; 18:2, 60-33-3; 18:3, 463-40-1; 20:4, 506-32-1; 22:1, 112-86-7.