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*)[1]

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ABSTRACT

Grain samples of small millets, namely foxtail (*Setaria italica*), proso (*Panicum miliaceum*), and finger (*Eleusine coracana*), 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 lipid content (dwb), comprising free, bound, and structural lipids was: 11.0% (45.4, 47.3, and 7.3%) in foxtail, 9.0% (62.2, 27.8, and 10.0%) in proso, and 5.2% (42.3, 46.2, and 11.5%) in finger millets. The nonpolar lipids (NL), glycolipids (GL), and phospholipids (PL), separated by silicic acid column chromatography, constituted 80–83%, 6–14%, and 5–14%, respectively, of the total lipids. The subclasses, separated by thin-layer chromatography, consisted chiefly of triacylglycerols in NL; esterified sterylglycosides, monogalactosyldiglycerides, and digalactosyldiglycerides in GL; and phosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine in PL. Linoleic, oleic, and palmitic acids were the chief constituents in all the lipid classes. Linolenic acid was present in appreciable proportions in the PL classes.

Lipids are relatively minor constituents in cereal grains. However, they contribute significantly to diet as a source of invisible fat and essential fatty acids (Achaya 1987). The lipids also have an important role in storage quality and processing of cereals. Among cereals, small millets (minor millets) account for about 1% of food grains produced in the world, and they are useful as food crops in their respective agro-eco systems (de Wet 1989). The content and composition of lipids determined in cereals depend largely on extraction and purification procedures. Several reviews covered the lipid content and fatty acid composition of ether extractables of small millets, especially foxtail (*Setaria italica*), proso (*Panicum miliaceum*), and finger (*Eleusine coracana*) millets (Aykroyd et al 1963, Morrison 1978, Rooney 1978, Hulse et al 1980, Chung 1991). However, information on the bound lipids and total lipids is meager. We recently reported contents of total lipids and lipid classes and fatty acid compositions of major lipid classes and their subclasses in three small millets: kodo (*Paspalum scrobiculatum*), little (*Panicum sumatrense*), and barnyard (*Echinocloa colona*) millets (Sridhar and Lakshminarayana 1992). We now report the contents of total lipids and lipid classes and the composition of constituent fatty acids in the whole grains of foxtail, proso, and finger millets.

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MATERIALS AND METHODS

Grain samples of small millet accessions of foxtail (ISe 1541), proso (IPm 2612), and finger (IE 2214) were obtained from Genetic Resource Unit, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India.

Extraction of Total Lipids

A representative sample (10–15 g), in duplicate, of clean grains was ground to a fine powder and extracted in a Soxhlet apparatus with *n*-hexane by refluxing for 8 hr on a water bath. Free lipids (FL) easily extractable by a nonpolar solvent were estimated. To estimate the bound lipids (BL), the extracted flour was reextracted three times in a screw-cap vial with hot water-saturated butanol (WSB) (1:5, w/v) for 1 hr each time, using vigorous shaking with a mechanical shaker (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 reextraction with a mixture of chloroform, methanol, and water (1:2:0.8, 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 hr to estimate the structural lipids (Hoseney 1986). FL, BL, 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 nonpolar lipids (NL), glycolipids (GL), and phospholipids (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, and acetic acid (80:20:1, v/v) (Mangold 1969) separated the total NL into various subclasses. The GL subclasses were separated using a mixture of chloroform, methanol, acetic acid, and water (170:24:25:4, v/v) as a developer (Nichols 1970). The PL subclasses were separated using a mixture of chloroform, methanol, and water (65:25:4, v/v) as a developer (Lepage 1964, Rouser et al 1976). The lipid subclasses were estimated by gravimetry. The reagents used for detection of the lipids were as reported earlier (Sridhar and Lakshminarayana 1992).

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 gas chromatography according to the previously described method (Sridhar and Lakshminarayana 1992).

TABLE I
Contents of Free, Bound, and Structural Lipids (%, dwb) in Foxtail, Proso, and Finger Millets[a]

	Foxtail	Proso	Finger
Hexane extract (free lipids)	5.0	5.6	2.2
Hot water-saturated butanol extract (bound lipids)	5.2	2.5	2.4
Hexane extract of acid hydrolyzate (structural lipids)	0.8	0.9	0.6
Total lipids	11.0	9.0	5.2

[a] Obtained by sequential extraction procedure. Values represent means of duplicate determinations.

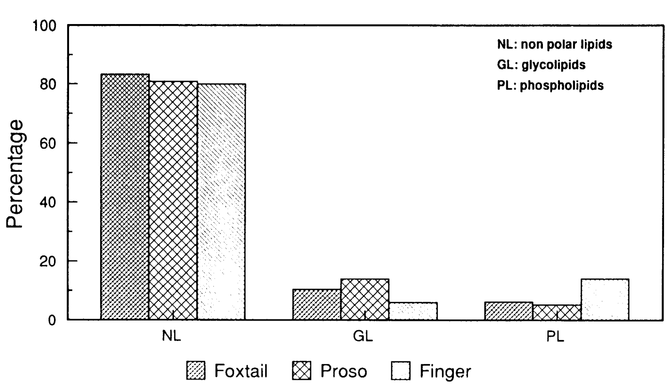

Fig. 1. Comparison of major lipid fractions of total lipids in three species of small millets.

RESULTS

Total Lipid Contents

FL are easily extractable with nonpolar solvents such as petroleum ether, hexane, or diethyl ether by using a Soxhlet apparatus, or a Goldfish extractor, or by shaking. BL are then extracted at room temperature with more polar solvents, generally alcohol alone or mixed with a small portion of another solvent, most commonly with water (Chung 1991). In the present investigation, FL were extracted with hexane by Soxhlet method. The residue was extracted by hot WSB, followed by hexane extraction after acid hydrolysis. Hot WSB was chosen because it is most efficient in extracting the polar lipids as well as NL, more so than WSB at room temperature, and it gives the highest yield of true total lipids (Osagie and Kates 1984). The total lipid content in the foxtail, proso, and finger millets ranged from 5.2 to 11.0% (db) (Table I); in little, kodo, and barnyard millets, it ranged from 5.1 to 8.3% (Sridhar and Lakshminarayana 1992). Foxtail contained the highest amount of total lipids, and kodo contained the least. Jiaju (1989) analyzed 2,684 accessions of foxtail and reported that some accessions contained lipid content of >5%. Seetharam et al (1983) reported 5–6% oil in whole seeds of foxtail using nuclear magnetic resonance, whereas Obara and Kihara (1973) obtained 13.5% lipid. Wankhede et al (1979) also found foxtail to be quite high in total lipid, up to 15%. Higher amounts of total lipids were extracted in the finger sample (4.6%, wb) as compared to the total lipid content of 3.1% (Mahadevappa and Raina 1978a). The FL was 42, 46, and 62% in foxtail, finger, and proso millets, respectively. The FL contents are similar to those reported for foxtail and proso (Rooney 1978). Lorenz and Hwang (1986) reported that the hexane-extracted FL content varied between 3.2 and 4.1% in flour and between 3.5 and 6.8% in bran. The WSB-extracted BL content was between 0.5 and 0.9% in flour and between 0.3 and 0.7% in the bran of nine proso millets. In the present investigation, slightly higher amounts of FL and BL were found in the whole grains of foxtail, proso, and finger millets. The FL also predominated in other small millets (kodo, little, and barnyard) (Sridhar and Lakshminarayana 1992). BL and FL were present in about the same quantity in foxtail and finger millets. The quantity of lipids extracted by acid hydrolysis (structural or membrane-bound lipids) was 0.6–0.9%. This amount is nearly the same as that reported (1%) in cereal grains, even after extraction of BL with polar solvents (Achaya 1986).

Contents of Major Lipid Classes

The major lipid classes were quantified after separating the total lipids by silicic acid column chromatography. The NL constituted the major portion of total lipids ranging from 80 to 83%, whereas the GL and PL contents varied at 6–14% and 5–14%, respectively (Fig. 1). The GL content was higher than the PL content in all except finger millet. Mahadevappa and Raina (1978a) reported 10–12% of GL, 5–6% of PL, and 70–72% of NL in total lipids of finger millet. Obara and Kihara (1973) reported 17.5% of GL in foxtail millet. The present investigation showed higher amounts of PL in finger millet and lower amounts of GL than the reported values because WSB extracts polar lipids to a greater extent than do the chloroform-methanol and nonpolar solvents (Rogols et al 1969, Finney et al 1976). The quantity of PL or GL is related to the functional properties of good and poor varieties of wheat (Chung et al 1982). Finger contained more PL, and proso contained more GL as compared to others.

Contents of NL Subclasses

The NL subclasses of small millets consisted of steryl esters (SE), triacylglycerols (TAG), free fatty acids (FFA), free sterols (FS), diacylglycerols (DAG), and monoacylglycerols (MAG). The TAG constituted the major portion of NL, ranging from 81 to 85% in small millets (Table II). Foxtail millet contained highest amount of SE. The FFA ranged from 3.5 to 4.0% in the small millets. Small amounts of MAG were present in all the small millets. The DAG contents were similar in most of the varieties.

Contents of GL Subclasses

Esterified sterylglycosides (ESG), MGDG, and DGDG were the major species of GL, followed by sterylglycosides (SG) and cerebrosides (CS). Very small or trace amounts of digalactosylmonoglycerides (DGMG) and monogalactosylmonoglycerides (MGMG) were also present in the small millets. Foxtail contained a high amount of MGDG (47.6%), while little was reported to contain an even higher amount (55.3%) (Sridhar and Lakshminarayana 1992). Finger contained a high amount of DGDG (30.7%); this was similar to that of kodo (28.0%).

Contents of PL Subclasses

Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and lysophosphatidyl choline (LPC) were the major constituents; small amounts of phosphatidic acid (PA), PG, phosphatidylserine (PS), and PI were also present in the small millets. Earlier workers did not report LPC in the foxtail, finger, and proso millets. This could be because of the solvent used for the extraction of lipids. LPC is well extracted by solvent systems of ethanol and water (80:20, v/v), hot WSB, cold WSB, and methanol and water (85:15, v/v) but not by other solvents (Osagie and Kates 1984). Proso contained higher amounts of PS and PE than did the other small millets.

TABLE II
Contents of Lipid Subclasses (wt%) in Foxtail, Proso, and Finger Millets[a]

	Foxtail	Proso	Finger
Nonpolar lipid subclasses			
Triacylglycerols	83.2	81.2	84.8
Diacylglycerols	2.2	2.9	2.9
Monoacylglycerols	Trace	1.1	1.7
Free fatty acids	3.5	4.0	3.6
Free sterols	6.0	7.8	5.8
Steryl esters	4.8	3.0	1.2
Glycolipid subclasses			
Sterylglycosides	8.5	12.5	6.9
Esterified steryl glycosides	23.4	29.5	13.2
Cerebrosides	1.5	2.0	8.3
Digalactosyldiglycerides	15.7	11.5	30.7
Digalactosylmonoglycerides	2.0	...	...
Monogalactosyldiglycerides	47.6	40.4	40.9
Monogalactosylmonoglycerides	1.3	4.1	...
Phospholipid subclasses			
Phosphatidic acid	5.5	1.5	2.5
Phosphatidylglycerol	8.0	Trace	9.2
Phosphatidylethanolamine	21.0	30.0	23.0
Phosphatidylcholine	36.5	36.8	26.5
Lysophosphatidylcholine	28.0	22.0	30.0
Phosphatidylserine	Trace	8.5	Trace
Phosphatidylinositol	Trace	Trace	8.0

[a] Values represent means of duplicate determinations. Trace values are <0.5%.

Fatty Acid Compositions of Major Lipid Classes

Proso and foxtail lipids were very similar in the fatty acid compositions of their major lipid classes, and they resemble wheat, barley, and rye in this respect. The major fatty acid was linoleic acid (18:2) in NL, GL, and PL of foxtail and proso, and oleic acid (18:1) in finger (Tables III–V). Finger millet resembles oats in this respect. Also present in very small amounts in foxtail and proso were arachidic (20:0), behenic (22:0), erucic (22:1), and arachidonic (20:4) acids. Small amounts (1.3–4.4%) of linolenic acid (18:3) were present in the NL. The PL contained higher amounts of 18:3 as compared to NL and GL. Mahadevappa and Raina (1978a) reported lower amounts of 18:3 in the finger varieties, ranging from traces in GL to 1.4% in the PL, and they did not find any significant variation among the fatty acid compositions of major lipid classes. Even in pearl millet, distinct differences in fatty acid composition were reported for the NL, GL, and PL fractions. The highest and lowest contents of 18:2 and palmitic acid (16:0) were in NL (the reverse was found in the PL fraction); the highest content of 18:3 was found in GL (Osagie and Kates 1984). The other small millets (little, kodo, and barnyard) also contained 18:2 in high amounts (Sridhar and Lakshminarayana 1992).

Fatty Acid Compositions of NL Subclasses

The major fatty acids were 16:0, 18:1, and 18:2 in most of the NL subclasses; their proportions varied with the class. DAG of proso contained 16:0 as the major constituent, whereas other NL classes of foxtail and proso contained 18:2 as the major constituent. Finger contained 18:1 as its major fatty acid constituent in all the NL. DAG of proso contained stearic acid (18:0) in higher proportion than that found in the other NL classes. Trace amounts of 20:0, 20:4, 22:0, and 22:1 were found in TAG of foxtail and proso (Table III).

Fatty Acid Compositions of GL Subclasses

The major fatty acid of GL subclasses was 18:1 in finger and 18:2 in foxtail and proso. Minor classes of GL (CS and DGMG) contained lower amounts of 18:2 and higher amounts of 18:0, whereas the major classes (DGDG and MGDG) contained similar amounts of 18:2 compared to that of other GL subclasses in foxtail lipids. In particular, very high 16:0 content was noticed in CS, followed by 18:0 in foxtail and finger (Table IV). In contrast to reports of Mahadevappa and Raina (1978b, 1984), higher

TABLE III
Fatty Acid Compositions (wt%) of Nonpolar Lipid Subclasses of Foxtail, Proso, and Finger Millets[a]

	16:0	18:0	18:1	18:2	18:3	20:0	20:4	22:0	22:1
Foxtail									
Total NL[b]	8.7	1.3	20.0	66.3	2.5	0.4	0.2	0.5	0.1
TAG	8.5	1.3	20.4	66.3	2.5	0.3	0.2	0.4	0.1
DAG	19.4	3.3	17.0	53.8	6.0	...	...	0.5	...
FFA	20.5	5.9	15.6	54.0	4.0	...	...	...	...
SE	11.2	2.5	20.6	60.7	0.5	...	1.0	2.2	1.3
Proso									
Total NL	6.3	1.2	24.6	65.5	1.3	0.5	0.1	0.4	0.1
TAG	6.4	1.0	23.8	64.7	1.8	0.8	0.4	0.7	0.4
DAG	36.5	12.1	13.8	33.1	3.8	...	...	0.7	...
FFA	11.9	3.2	22.0	59.9	2.8	...	...	0.2	...
SE	11.1	2.2	22.2	62.4	1.2	...	0.1	0.2	0.6
Finger									
Total NL	23.3	1.8	47.6	22.4	4.4	0.5	...	...	...
TAG	23.7	2.1	47.4	22.5	3.8	0.5	...	...	...
DAG	33.0	1.6	42.4	16.4	6.6	...	...	...	...
FFA	28.9	1.6	51.6	13.9	4.0	...	...	...	...
SE	25.1	2.5	45.1	25.3	2.0	...	...	...	...

[a] Figure before colon indicates the number of carbon atoms and the figure after colon the number of double bonds in the fatty acid chain.
[b] NL = nonpolar lipids, TAG = triacylglycerols, DAG = diacylglycerols, FFA = free fatty acids, SE = steryl esters.

amounts of 16:0, 18:2, and 18:3 were found in MGDG and DGDG, and lower amounts of 16:0 were found in ESG.

Fatty Acid Compositions of PL Subclasses

All the PL classes contained 18:2 as the predominant acid, except finger, which contained 18:1 as the major constituent, followed by 16:0. The contents of 16:0 and 18:1 were similar; the content of 18:2 was lower; and the content of 18:3 was slightly higher than those reported by Mahadevappa and Raina (1984) for PL classes of finger millet. Foxtail, among the three small millets presently studied, contained the highest amount of 18:3 (Table V).

TABLE IV
Fatty Acid Compositions (wt%) of Glycolipid Subclasses of Foxtail, Proso, and Finger Millets[a]

	16:0	18:0	18:1	18:2	18:3	22:0
Foxtail						
Total GL[b]	27.5	2.4	14.3	50.9	4.3	0.6
ESG	32.3	7.8	9.2	47.1	3.0	0.6
CS	42.4	26.5	16.6	9.8	4.5	0.2
DGDG	22.2	9.6	11.6	50.6	6.0	...
DGMG	27.5	15.2	13.7	38.4	5.2	...
MGDG	13.3	3.8	14.9	62.8	5.0	0.2
MGMG	34.5	9.3	10.6	40.8	4.0	0.8
Proso						
Total GL	21.9	4.0	17.0	53.2	3.9	...
ESG	20.5	4.9	13.8	56.6	4.0	0.2
CS	23.4	7.6	13.3	52.5	3.2	...
DGDG	26.8	4.5	14.3	49.0	5.4	...
MGDG	12.8	3.4	15.4	63.6	4.8	...
MGMG	23.6	7.2	12.1	53.6	3.5	...
Finger						
Total GL	25.4	1.3	50.7	18.0	4.6	...
ESG	28.6	3.4	42.0	22.0	4.0	...
CS	50.3	10.8	26.6	8.5	3.8	...
DGDG	28.2	2.5	48.5	16.2	4.6	...
MGDG	25.3	2.0	49.3	19.2	4.2	...

[a] Figure before colon indicates the number of carbon atoms and the figure after the colon is the number of double bonds in the fatty acid chain.
[b] GL = glycolipids, ESG = esterified sterylglycosides, CS = cerebrosides, DGDG = digalactosyldiglycerides, DGMG = digalactosylmonoglycerides, MGDG = monogalactosyldiglycerides, MGMG = monogalactosylmonoglycerides.

TABLE V
Fatty Acid Compositions (wt%) of Phospholipid Subclasses of Foxtail, Proso, and Finger Millets[a]

	16:0	16:1	18:0	18:1	18:2	18:3
Foxtail						
Total PL[b]	16.6	1.0	3.2	15.7	47.7	15.8
PA	15.0	0.8	7.0	19.1	47.6	10.5
PG	23.4	0.2	4.1	16.1	45.2	11.0
PE	22.2	0.8	3.7	16.8	42.5	14.0
PC	20.0	1.6	2.8	12.4	47.0	16.2
LPC	20.4	0.4	3.2	14.2	45.4	16.4
Proso						
Total PL	22.5	...	3.8	18.2	50.3	5.2
PA	40.2	...	10.2	12.8	31.5	5.3
PE	20.7	...	4.0	23.3	47.5	4.5
PC	21.4	...	3.5	20.6	48.3	6.2
PS	34.3	...	5.3	8.6	46.4	5.4
LPC	23.8	...	3.8	17.3	49.4	5.7
Finger						
Total PL	26.7	...	1.8	46.4	17.3	7.8
PA	25.8	...	6.0	50.2	11.9	6.1
PG	26.4	...	3.3	46.0	17.3	7.0
PE	20.7	...	2.8	47.1	21.8	7.6
PC	27.2	...	2.3	46.4	15.9	8.2
PI	21.6	...	4.0	47.7	18.9	7.8
LPC	31.3	...	3.7	41.1	15.3	8.6

[a] Figure before colon indicates tthe number of carbon atoms and the figure after the colon is the number of double bonds in the fatty acid chain.
[b] PL = phospholipid, PA = phosphatidic acid, PG = phosphatidylglycerol, PE = phosphatidylethanolamine, PC = phosphatidylcholine, LPC = lysophosphatidylcholine, PS = phosphatidylserine, PI = phosphatidylinositol.

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