Total Lipids of Sorghum Grain

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The composition of lipids extracted from two improved sorghum varieties SSH_3 and L187 were analyzed. The lipids were extracted with hexane–ether (4:1), chloroform–methanol (2:1), and hot-water-saturated butanol. The seed lipids were then fractionated by column chromatography into three lipid fractions. The lipid fractions were further separated by thin-layer chromatography (TLC) and the individual lipid classes quantified by analysis of fatty acid methyl esters by gas–liquid chromatography (GLC) with heptadecanoic acid as internal standard. Water-saturated butanol was most efficient in extracting the polar lipids. Total lipid content of the grains was 5.28% for SSH_3 variety and 3.68% for L187 variety. Eight glycolipid and six phospholipid classes were separated and characterized. Lysophospholipids constitute over 50% of the phospholipids. Fatty acid composition of the total lipids was similar for all extraction procedures with linoleic acid (18:2) being the predominant fatty acid.

Sorghum is one of the world's four leading sources of food from cereal crops. It provides the staple diet for low-income groups of India and Africa and accounts for about 50% of the total cereal production in Nigeria (Oke, 1977). In addition to the low quality of the proteins, sorghum seeds contain tannins that decrease the digestibility of the proteins. Much effort has been put into improving the yield of sorghum varieties and at the same time reducing the level of tannin. L187 and SSH_3 are two such improved sorghum varieties developed at the Institute of Agricultural Research and Training (IART), Zaria, Nigeria.

There have been several reports on the lipid composition of sorghum seeds (Kummerow, 1946; Baldwin and Sniegowski, 1954; Anderson et al., 1969; Wall and Blessin, 1969; Rooney, 1973, 1978; Price and Parsons, 1975; Badi et al., 1976; Weihrauch et al., 1976; Neucere and Sumrell, 1980). With the exception of the study by Price and Parsons (1975), all previous studies were carried out with nonpolar solvents that extracted only the neutral and unbound lipids. Since sorghum lipids are predominantly nonpolar, the characteristics of polar lipids were obscured by those of the nonpolar lipids. According to Boissy and Perles (1965), phospholipids represent about 5% of the total sorghum lipids with about 1:1 distribution between the lecithin and cephalin fractions. They identified phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) in the cephalin fraction. Badi et al. (1976) indicated that the polar lipids of sorghum seed include phosphatidylcholine (PC) and lysophosphatidylcholine (LPC). Overall, the polar lipids in sorghum seeds have been poorly characterized.

In the present paper, comparative studies of three solvent mixtures for the extraction of sorghum lipids have been carried out. Detailed information on the lipid components has been presented. The information will provide a basis for monitoring changes that occur in sorghum lipids during storage and germination.

EXPERIMENTAL SECTION

The sorghum varieties L187 and SSH_3 are long-season, high-yielding varieties developed for cultivation in the northern Guinea Savannah (Okoh et al., 1982). SSH_3 is a single-cross hybrid. Samples of the sorghum varieties were obtained from IART, Zaria, Nigeria, immediately after harvest. They were sun-dried until a moisture content at about 12% was attained. The whole seeds were stored at 4 °C in plastic screw-capped bottles until analysis. The seeds were ground in a Phillips coffee grinder and analyzed for moisture by the standard AOAC (1970) procedure. All organic solvents were of analytical grade and were redistilled before use.

Three extraction procedures (Osagie and Kates, 1984) were tested as to their efficiency in extracting total lipids from sorghum seeds: (1) Soxhlet extraction with hexane–diethyl ether (4:1, v/v) for 16 h; (2) extraction with boiling 2-propanol followed by extraction three times with methanol–chloroform (2:1); (3) extraction three times with boiling, water-saturated butanol. The lipid extracts were evaporated to dryness in a rotary evaporator. The residues were taken up in chloroform–methanol–water (1:2:0.8) and purified by the Bligh and Dyer (1959) procedure.

Total lipids were fractionated into neutral lipid (NL), glycolipid (GL), and phospholipid (PL) fractions by column chromatography on silicic acid (Kates, 1972). Neutral lipids were separated further by TLC using hexane–diethyl ether–formic acid (80:20:1) as the developing solvent system. Glycolipids and phospholipids were separated by TLC using several solvent systems previously described (Osagie and Kates, 1984). The lipid components separated by TLC were identified by cochromatography with authentic compounds and by comparing R_f values with those of the literature (Clayton et al., 1970). Fatty acid methyl esters (FAME) were prepared and analyzed as described by Osagie and Kates (1984).

RESULTS AND DISCUSSION

Table I shows that all three extraction methods yielded comparable amounts of neutral lipids from the seeds. However, the hexane–diethyl ether system extracted much less phospholipid than did chloroform–methanol or water–saturated butanol. This observation is in agreement with previous reports that hot-water-saturated butanol is the most efficient solvent system for extracting polar lipids as well as nonpolar lipids from cereals (MacMurray and Morrison, 1970; Sahasrabudhe, 1979; Osagie and Kates, 1984). Seed lipids bound to proteins require the addition of water to an extracting organic solvent to disrupt lipoprotein complexes and increase lipid extractibility. On the other hand, seed lipids bound to starch require an elevated temperature for extraction because such lipids can be extracted only when starch granules are gelatinized. Hot-water-saturated butanol meets the requirements for extraction of lipids bound to protein and starch.

The improved varieties L187 and SSH_3 were found to contain 3.68% and 5.28% lipids, respectively. The oil

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Table I. Composition of Lipids Extracted from Two Varieties of Sorghum Seeds by Three Solvent Systems

extraction method[a]	total lipids[b]	neutral lipids[b]	glycolipids[b]	phospholipids[b]	fatty acid compn of total lipids (area, %) 16:0	18:0	18:1	18:2	18:3
				SSH_3 Variety					
hex/ether	4.43	4.13	0.26	0.04	13.0	1.1	27.3	56.5	2.1
$CHCl_3$/MeOH	4.99	4.25	0.10	0.64	14.8	1.2	27.4	54.5	2.1
hot WSB	5.28	4.22	0.13	0.93	15.6	1.3	25.8	54.8	2.5
				L187 Variety					
hex/ether	2.81	2.65	0.13	0.03	15.3	0.7	21.0	59.0	4.0
$CHCl_3$/MeOH	3.58	2.77	0.20	0.61	16.9	0.9	21.4	57.8	3.0
hot WSB	3.68	2.54	0.23	0.91	21.3	1.3	21.0	52.1	4.3

[a] See text for extraction and purification procedure. [b] Each value is the average of three separate analyses. In grams/100 g of dry seed.

Table II. Fatty Acid Composition of Lipid Fractions Extracted by Hexane–Ether (4:1)

lipid fraction	total lipid, %	fatty acid compn (area, %) 16:0	18:0	18:1	18:2	18:3
		SSH_3				
neutral lipids	93.22	12.9	1.1	26.5	56.7	2.7
glycolipids	5.87	26.7	6.4	23.9	38.8	4.2
phospholipids	0.90	23.2	0.7	20.7	51.9	3.5
		L187				
neutral lipids	94.30	15.6	1.1	21.4	57.6	4.3
glycolipids	4.62	24.0	5.0	23.3	42.8	4.9
phospholipids	1.07	24.0	0.6	21.6	50.7	3.0

Table III. Fatty Acid Composition of Lipid Fractions Extracted by Chloroform–Methanol (2:1)

lipid fraction	total lipid, %	fatty acid compn (area, %) 16:0	18:0	18:1	18:2	18:3
		SSH_3				
neutral lipids	85.17	13.0	1.3	26.8	56.9	2.0
glycolipids	2.00	31.0	7.2	18.9	32.7	10.2
phospholipids	12.83	24.1	1.2	27.2	44.6	2.9
		L187				
neutral lipids	77.38	15.2	1.2	20.3	58.4	4.8
glycolipids	5.58	26.4	6.3	18.6	39.5	9.1
phospholipids	17.04	23.2	1.1	20.2	51.9	3.5

Table IV. Fatty Acid Composition of Lipid Fractions Extracted by Hot-Water-Saturated Butanol

lipid fraction	total lipid, %	fatty acid compn (area, %) 16:0	18:0	18:1	18:2	18:3
		SSH_3				
neutral lipids	79.92	14.5	1.2	25.8	56.2	2.3
glycolipids	2.46	30.2	4.6	21.1	37.8	6.3
phospholipids	17.61	39.7	1.3	22.1	35.5	1.4
		L187				
neutral lipids	69.02	21.7	1.3	20.2	52.9	3.9
glycolipids	6.25	28.2	2.5	19.6	45.1	4.5
phospholipids	24.73	24.6	2.4	20.4	47.8	4.8

Table V. Composition of Sorghum Seed Nonpolar Lipids[a]

lipid component	wt, %	fatty acid compn (area, %) 14:0	16:0	18:0	18:1	18:2	18:3
steryl ester	2.8	8.1	17.9	5.0	17.1	51.9	tr
triacylglycerols	85.3	tr	17.1	0.8	21.7	56.8	3.6
free fatty acids	1.4	tr	32.6	1.8	20.8	42.4	2.4
diacylglycerols	4.0	3.1	30.9	2.6	29.8	31.8	1.8
monoacylglycerols	2.4	2.1	35.0	2.5	20.0	36.4	4.0
free sterols	4.1						

[a] Nonpolar lipids were separated on TLC on silica gel G with hexane–diethyl ether–formic acid (80:20:2, v/v/v) as the developing solvent system.

content of commercial varieties of sorghum ranges from 2.1 to 5.3% with a mean of 3.6% in U.S. varieties (Morrison, 1978; Rooney, 1978). The oil content of sorghum seeds can be improved by breeding, but the results are not reliably predictable (Mukuru, 1974; Neucere and Sumrell, 1980).

Analysis of the fatty acid composition of the total lipids obtained by the different extraction methods by GLC showed no major difference in the fatty acid profiles (Tables I–IV). Linoleic (18:2) was the predominant fatty acid in all extracts, followed by oleic (18:1) acid and palmitic (16:0) acid. The percentage of palmitic acid in the hot-water-saturated butanol extracts was increased probably as a result of the efficient extraction of phospholipids. As previously established for millet seed lipids (Osagie and Kates, 1984), the NL fractions had the highest and lowest contents of 18:2 and 16:0 acids, respectively, while the PL fractions had the lowest and highest contents of these acids; the GL fractions had the highest content of 18:3 and 18:0. The total lipids and lipid fractions for both sorghum varieties were qualitatively and quantitatively similar so further results are presented only for the SSH_3 variety.

Wall and Blessin (1969) reported that most of the NL in sorghum seeds is located in the germ. The major component of the NL fraction was found to be triacylglycerol (TAG) followed by free and esterified sterols (Table V). The major sterols are sitosterol, stigmasterol, campesterol, and lophenol (Palmer and Bowden, 1975). The fatty acid composition of the neutral lipids largely reflects that of the triacylglycerols. Although the TAGs of the cotyledons serve mainly as reserve energy sources, we are currently pursuing studies on the biological properties of the molecular species during germination in regard to the fatty acid distribution.

The GL fraction was further resolved by TLC into eight components identified by differential staining properties and identification of deacylation products (Kates, 1972). The major glycolipids were esterified sterol glycoside (ESG), cerebrosides (CER), digalactosyldiacylglycerol (DGDG), and sterolglycoside (SG). Other components were monogalactosyldiacylglycerol (MGDG), monogalactosylmonoacylglycerol (MGMG), digalactosylmonoacylglycerol (DGMG) and an unidentified component (Table VI). In contrast to the glycolipid composition in wheat (Morrison et al., 1975) and corn (Weber, 1979), sterol glycosides greatly predominate over glycosyldiacylglycerols. Although sterol glycosides and cerebrosides are usually associated with photosynthetic tissues, they have now been identified in several cereals. The pattern of distribution of glycolipid components in sorghum is similar to that in millet (Osagie and Kates, 1984) and other cereals (Price and Parsons, 1975).

The individual phospholipids in sorghum seeds have not been previously quantified. The phospholipid composition of sorghum was similar to that in millet (Osagie and Kates, 1984). PC and LPC were the predominant components,

Table VI. Composition of Sorghum Seed Glycolipids[a] (Variety SSH_3)

glycolipids	wt, %	fatty acid compn (area, %)					
		14:0	16:0	18:0	18:1	18:2	18:3
esterified sterol glycoside	38.0	0.5	17.2	1.0	21.6	55.3	4.4
monogalactosyldiacylglycerol	9.3	2.5	35.3	8.4	21.3	30.1	2.4
unidentified	3.6	0.7	49.7	3.2	18.0	26.1	2.3
sterol glycoside[c]	10.2						
cerebrosides	9.0	tr	32.5	2.3	20.3	42.4	2.4
monogalactosylmonoacylglycerol[b]	3.9	2.4	41.2	5.7	11.2	28.3	11.2
digalactosyldiacylglycerol	20.9	3.7	57.8	4.8	14.7	15.2	3.8
digalactosylmonoacylglycerol[b]	5.1	tr	10.6	10.3	13.6	38.9	26.6

[a] Total lipids extracted by hot WSB were fractionated on a column of silicic acid, and the glycolipid fraction was separated by preparative TLC using chloroform–methanol–acetone–diethylamine–water (120:35:37.5:6:4.5, v/v/v/v/v). [b] Identified by quantitation of deacylation products (Kates, 1972). [c] Bound sterols analyzed by GLC and quantified by colorimetric reaction (Kates, 1972).

Table VII. Composition of Sorghum Seed Phospholipids[a] (Variety SSH_3)

phospholipid	wt, %	fatty acid compn (area, %)					
		14:0	16:0	18:0	18:1	18:2	18:3
lysophosphatidylcholine	41.8	tr	50.7	1.9	15.1	31.0	1.1
lysophosphatidylethanolamine[b]	13.6	0.6	52.2	1.2	9.6	33.9	2.5
phosphatidylcholine[c]	36.3	tr	20.0	0.8	23.8	51.6	3.7
phosphatidylethanolamine	5.5	0.9	37.7	1.7	10.4	47.0	2.3
phosphatidylglycerol	1.2	4.9	38.3	3.2	20.2	29.9	3.5
phosphatidic acid	1.6	3.9	57.0	5.8	15.6	16.6	1.1

[a] The phospholipid fraction was separated by preparative TLC using chloroform–acetone–methanol–acetic acid–H_2O (10:4:2:2:1, v/v/v/v/v). [b] Includes phosphatidylserine. [c] Includes phosphatidylinositol.

comprising almost 80% of this lipid class (Table VII). Smaller amounts of lysophosphatidylethanolamine (LPE) and phosphatidylethanolamine (PE) were present, along with small quantities of phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidic acid (PA). The overlapping of PC with PI and LPE with PS in one-dimensional TLC using chloroform–acetone–methanol–acetic acid–H_2O (10:4:2:2:1, v/v/v/v/v) was confirmed by the use of authentic standards and two-dimensional TLC (Osagie and Kates, 1984).

The PL composition has been reported for corn (Weber, 1979), barley (Price and Parsons, 1979), wheat (Morrison et al., 1975) and other cereals (Price and Parsons, 1975; Morrison, 1978). Comparisons between results in this study and some of those cited are difficult because of differences in extraction procedures and solvents. The weight distribution of the individual phospholipids could be influenced by the extraction solvents because lysophospholipids are quantitatively extracted only by hot-water-saturated butanol. As shown in Table VII, lysophospholipids constitute 55.4% of sorghum seed phospholipids. The fatty acid composition of the phospholipids showed significant amounts of myristic (14:0) acid in PG and PA, and high proportions of palmitic (16:0) acid in the lysophospholipids. Higher proportion of linoleic (18:2) acid were found in PC and PE. The different patterns of fatty acid spectra exhibited by LPC/LPE on one hand and PC/PE on the other hand indicate that the lysophospholipids are not formed as breakdown products of PC and PE during the extraction process.

Phospholipids are essential components of the cytoplasmic membranes of vegetative and reproductive tissues even though they only represent less than 0.6% of the dry weight of cereal grains. The identification of large quantities of lysophospholipids as normal components of cereal grains is interesting. In particular, these lysophospholipids exist as inclusion complexes with amylose within the starch granules and regulate the amylose to amylopectin ratio in cereal starch (Acker, 1977; Baisted, 1981; Downton and Hawker, 1975). Carbohydrate metabolism and mobilization during seed germination is partially controlled by the associated lysophospholipids. These relationships are currently under study in our laboratories.

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Digestibility and Protein Quality of Raw and Heat-Processed Defatted and Nondefatted Flours Prepared with Three Amaranth Species

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The study was carried out to determine whether removing hexane extractables from three amaranth species improved protein quality of the grain as compared to heat processing. It also examined whether or not feeding the grain induced pathological damage to rats organs. Hexane-extracted samples were cooked at atmospheric pressure or drum dried. These preparations were fed in 10% protein diets to weanling rats for standard PER digestibility studies. Macroscopic examination of the organs was done on rats fed *Amaranthus cruentus*. Atmospheric cooking of the whole and of the defatted materials gave higher food intake, weight gain, and PER than non-heat-treated samples. Drum drying of the fat-free flours always resulted in lower quality than atmospheric cooking. Organ weight and appearance for *A. cruentus* fed rats were normal. Results showed growth-reducing factors of amaranth grain to be still present in the raw fat-free flour.

Amaranth grain is considered an excellent source of nutrients for man. Its composition has been fully described by various authors (Becker et al., 1981; Carlsson, 1980; Sánchez-Marroquín et al., 1980). These studies report protein and lipid contents of 12–17% and 6–9%, respectively, with its protein being rich in lysine and sulfur amino acids (Carlsson, 1980; Sánchez-Marroquín et al., 1980) and its fat containing high amounts of squalene (Becker et al., 1981), an intermediate in the biosynthesis of cholesterol.

The nutritional value of amaranth seeds has been reported as comparable to that of casein (Sánchez-Marroquín et al., 1980). Moreover, other biological evaluations (Betschart et al., 1985; Bressani, 1983) have pointed out that wet heat-processed amaranth seeds give a higher PER value than nonprocessed seeds, suggesting the existence of unknown thermolabile factors or of chemical structures not readily available to the animal. Even though it has been reported that amaranth grain contains tannins, trypsin inhibitors, and hemagglutinins (Imeri, 1985), the amounts are too small to explain the improvement in protein quality obtained with controlled heat processing. There is need, therefore, to study the significance of other organic components in the grain. In a previous study (García et al., 1986) the nutritive value of the oil from three species was evaluated. The results indicated that oil induced normal animal performance although its true digestibility was lower than that of cottonseed oil.

The purpose of this study was to evaluate the effect of wet-heat treatment on the protein value of defatted and nondefatted amaranth seed flours and on the possible damage caused to organs of rats fed these flours.

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MATERIAL AND METHODS

One selection from each of three species of amaranth was evaluated: *Amaranthus caudatus*, *Amaranthus cruentus*, and *Amaranthus hypochondriacus*. The seeds were obtained from INCAP's experimental farm in Guatemala and were milled to prepare the flours.

Protein (AOAC, 1975) and ether extract content (AOAC, 1975) were determined in the individual flours prior to defatting. The results have been reported previously (García et al., 1986). Portions from each flour were extracted in a Soxhlet apparatus with hexane during 28–32 h. Percentage oil extractions varied from 75 to 85% of the original content in each species (García et al., 1986).

Defatted and nondefatted flours of each of the three cultivars were divided into three portions. The first had no further processing, while the second was cooked for 10 min at atmospheric pressure with constant manual stirring in three parts of boiling water, dehydrated in an air oven at 60 °C, and ground. The third portion was mixed with three parts of cold water and drum dried. The drums were heated with vapor pressure at 70 psi, which gave a temperature of approximately 134 °C. The drums were set to rotate at 3 rpm for a residence time of 10–12 s. The product was ground into a flour.

The biological assay was done using 21-day-old rats of the Wistar strain, from INCAP's animal colony. A total of 144 animals was divided by weight in groups of eight, four males and four females, and placed in individual all-wire screen cages with raised screen bottoms. They were fed diets containing the amaranth flours at a protein level of 10% for 28 days. These diets were supplemented with a vitamin solution (Manna and Hauge, 1953), mineral mixture (Hegsted et al., 1941), 5% cottonseed oil, 1% cod

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