Nature of Some Indian Legume Lipids

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Total lipids, constituting 1.7–2.2%, from seven breeding varieties of five legumes, viz. the cow pea (*Vigna catiang*), field bean (*Dolichos lablab*), red gram (*Cajanus cajan*), horse gram (*Dolichos biflorus*, two varieties), and black gram (*Phaseolus mungo*, two varieties), were extracted with chloroform–methanol, purified (Sephadex G-25), resolved by silicic acid column chromatography, and analyzed for component fatty acids by gas chromatography. Neutral lipids, which were mostly triglycerides carrying 17–32% of total sterols (15–20% free and 2–11% as esters), constitute 46–52% of the total lipids, phospholipids 35–40%, and glycolipids 10–12%. All seven legume varieties showed similar proportions of lipid classes. In five varieties, the neutral lipids resembled phospholipids in fatty acid composition, but this was not so in the two black gram varieties. The latter, moreover, carried high proportions (33–60%) of linolenic acid in all lipid classes whereas the other legume lipids were all rich in linoleic acid (30–60%). Cultivars within a species exhibited identical lipid profiles.

Legumes constitute some 10% of the total food grains consumed in India, daily intake per head averaging 34 g (Gopalan et al., 1971). They represent an important source of dietary protein which being rich in lysine and threonine complements cereal diets (Patwardhan, 1962). For a major foodstuff like legumes, the nature of the lipids present is therefore also of importance. The cholesterol-lowering effects shown by the black gram (Devi and Kurup, 1972) and the chick pea (Devi and Kurup, 1970; Mathur et al., 1961, 1963, 1968) have indeed been attributed in part to the high content of linoleic and linolenic acids present in the constituent lipids.

The common pea (*Pisum sativum*) is the only legume whose lipid classes and corresponding fatty acid compositions appear to have been studied (Miyazawa et al., 1974 a,b; 1975). Fatty acid components in the total lipids of several common legumes have been determined (Baker et al., 1961; Korytnyk and Metzler, 1963; Shah, 1975). The proportions of various lipid classes in well-identified high-yielding strains of five Indian legumes and the fatty acid components of these lipid classes are reported in this paper.

EXPERIMENTAL SECTION

Materials. Seven strains of high-yielding varieties of five legumes were kindly supplied by the University of Agricultural Sciences, Hebbal, Bangalore, India: cow pea, *Vigna catiang* C152; field bean, *Dolichos lablab* Hebbal Avare; red gram, *Cajanus cajan* SA I; horse gram, *Dolichos biflorus* Hebbal 1 (termed A) and PLKU 32 (termed B); black gram, *Phaseolus mungo* T9 (termed A) and Khargan 3 (termed B). All had been grown in Bangalore during the winter in adjacent fields.

Lipid Extraction and Purificiation. Seed material finely ground in a mill was dried to constant weight at 50 °C, and 25-g portions were Soxhlet-extracted with chloroform–methanol (2:1 v/v, 8–10 h, tocopherol acetate in chloroform as antioxidant). Following solvent removal in a rotary flash evaporator under nitrogen, the lipids were stored in chloroform–methanol at −20 °C. Nonlipid contaminants were removed by passing through Sephadex G-25 (100–300 μ) and lipids recovered from the eluates were weighed (Wuthier, 1966; AOAC, 1960).

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Lipid Classes. Following the method of Rouser et al. (1967), the total lipid in chloroform was resolved on a 100–200 mesh silicic acid column (V.P. Chest Institute, New Delhi) using chloroform (neutral lipids), acetone (glycolipids), and methanol (phospholipids), and the fractions were isolated in a rotary flash evaporator under nitrogen and weighed. Total recoveries amounted to 80–95%. An aliquot of the neutral lipid fraction was used to estimate total sterols (Courchaine et al., 1959; Zlatkis et al., 1963); free sterols were determined by digitonin precipitation, followed by estimation as for total sterols; and esterified sterols were estimated by difference.

Fatty Acid Composition. Methyl esters were derived from total lipids and neutral lipids by transmethylation with 14% boron trifluoride in methanol (Sigma), followed by extraction with *n*-heptane and concentration under nitrogen (Van Wijngaarden, 1967). Glycolipids and phospholipids were transmethylated using 3% methanolic HCl for 2 h (Kates, 1964a). For gas chromatography a Varian Aerograph 1400 series with a flame ionization detector was used (column 160 cm × 4 mm, 15% DEGS on Chromosorb Q, 185 °C), and the peak areas were determined by triangulation, no correction factors being used.

RESULTS AND DISCUSSION

Table I shows the proportions (average of three determinations) of lipid classes present in the seven legumes examined. Table II shows the fatty acid compositions (average of three determinations) of the lipid classes and of the total lipids.

Lipid Content and Classes. The total lipids present in these Indian legumes constitute 1.7–2.2%, a range of the same order as in other studies (Baker et al., 1961; Korytnyk and Metzler, 1963; Choudhury and Rahman, 1973; Miyazawa et al., 1974 a, b). The main exceptions so far to this low lipid level are the Bengal gram, *Cicer arietinum*, and two Phaseolus species, *P. trilobus* or mugani bean and *P. aconitifolius* or moth bean, which have 4–7% of total lipid (Baker et al., 1961; Suresh et al., 1971; Shah, 1975).

Neutral lipids in the present studies constitute 46–52% of the total, phospholipids 35–40%, and glycolipids 10–12%. These neutral lipids consist mostly of triglycerides accompanied by very small proportions of hydrocarbons and free fatty acids. Total sterols constituted 17–32% of the neutral lipids, these being mostly free sterols accompanied by 2–11% of sterol esters.

Fatty Acid Composition. The fatty acids present in neutral lipids and phospholipids resemble each other in

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Table I. Classes of Legume Lipids and Nature of Neutral Lipids

	cow pea	field bean	red gram	horse gram A	horse gram B	black gram A	black gram B
lipid classes, % dry wt weight							
total lipids	2.048	1.680	2.194	2.246	2.153	1.694	1.578
neutral lipids	0.960	0.800	1.120	1.030	1.066	0.810	0.818
glycolipids	0.184	0.165	0.194	0.243	0.258	0.152	0.145
phospholipids	0.754	0.606	0.754	0.524	0.520	0.580	0.568
neutral lipid composit., % wt							
glycerides, free fatty acids and hydrocarbons	83.3	72.2	82.1	75.5	73.3	69.9	68.9
sterols	13.9	20.1	15.8	16.5	17.6	20.6	20.2
esterified sterols	2.8	7.7	2.1	8.0	9.1	9.5	10.9

Table II. Fatty Acid Composition of Legume Lipid Classes

		cow pea	field bean	red gram	horse gram A	horse gram B	black gram A	black gram B
neutral lipids, % weight	16:0	19.5	14.0	24.9	24.0	24.7	8.0	8.7
	18:0	3.4	2.6	6.5	3.5	2.8	2.9	2.8
	20:0	1.6		1.9				
	22:0	4.4						
	18:1	8.9	10.5	9.0	18.8	18.9	15.6	15.8
	18:2	36.3	60.4	51.5	44.5	44.3	12.4	12.2
	18:3	25.9	12.5	6.2	9.2	9.3	61.1	60.5
glycolipids, % weight	16:0	28.6	19.7	30.0	42.0	40.0	20.5	21.5
	18:0	3.4	2.6	5.1	trace	trace	2.8	2.9
	20:0							
	22:0							
	18:1	8.0	9.3	9.5	16.3	16.5	14.9	14.8
	18:2	39.5	54.6	50.9	38.6	40.6	10.2	9.9
	18:3	20.5	13.8	4.5	3.1	2.9	51.6	50.9
phospholipids, % weight	16:0	30.0	29.0	29.9	27.5	27.5	28.0	29.9
	18:0	2.8	2.9	3.6	1.2	1.5	6.9	7.0
	20:0							
	22:0							
	18:1	7.8	6.5	6.6	15.5	15.5	21.2	21.0
	18:2	44.3	53.5	57.5	46.0	45.6	9.9	9.7
	18:3	15.1	8.1	2.4	9.8	9.9	34.0	32.4
total lipids, % weight	16:0	23.5	20.2	20.5	27.3	26.9	18.7	16.9
	18:0	5.6	4.6	6.9	1.6	1.8	5.6	6.2
	20:0	0.6		0.8				
	22:0	2.2						
	18:1	8.4	6.5	10.5	13.0	13.0	15.6	18.9
	18:2	34.0	56.0	56.3	44.4	44.7	11.7	11.5
	18:3	25.7	12.7	5.0	13.7	13.6	48.4	46.5

five of the legumes studied. The exceptions are the two black gram varieties, which will be considered separately. In the five varieties, palmitic acid is the major saturated fatty acid in all three lipid classes, constituting 15–25% in the neutral lipids, 20–40% in the glycolipids, and 26–30% in the phospholipids. Linoleic acid is prominent (30–60%) in all lipid classes, while oleic and linolenic acids each fall in the 5–20% range.

The two black gram (*P. mungo*) varieties stand apart in several respects. While the proportions of the lipid groups are similar to those in the other legumes (Table I), the fatty acid profile is characterized by exceptionlly high levels of linolenic acid in all lipid classes: 60% in the neutral lipids, 50% in the glycolipids, and 33% in the phospholipids, accompanied in all categories by about 10% of linoleic acid and 15–20% of oleic acid. Furthermore, unlike in the other five legumes studied, neutral lipids do not resemble phospholipids in the content either of saturated acids (11–12% against 35–37%) or of linoleic acid (60–61% against 32–34% in phospholipids). A high linolenic acid (70%) and low linoleic acid (5%) level for *P. mungo* neutral lipids (ether-extracted) was likewise earlier reported by Choudhury and Rahman (1973), and this was in contrast to 54–67% contents of linoleic acid in four other legumes studied alongside. High levels of the two unsaturated essential fatty acids could have nutritional implications.

Varietal Differences between Species. Extraordinary similarity, both in gross lipid composition and in the fatty acid profiles of the lipid classes, marks the two cultivars of horse gram and of black gram now studied. Since the varieties were grown in the same area, this suggests a very strong effect of climatic factors on lipid composition. It is also possible, however, that the strains, though differently named, may have been bred from common ancestors.

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Extraction and Separation of Guar Seed Proteins

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The solubility of guar meal protein was determined in various aqueous solvents using different acids and bases. The total proteins were characterized by the techniques of gel filtration, polyacrylamide gel electrophoresis, and ultracentrifugation. They were found to consist of three fractions, two of high molecular weight and one of low molecular weight. Neither the total proteins nor the fractions showed any hemagglutinin activity. Maximum trypsin inhibitor and proteolytic activity was observed with the lowest molecular weight fraction.

Guar bean, *Cyamopsis tetragonoloba*, is a commercially important crop of India and serves as the raw material for the production of the galactomannan gum (guar gum). Guar meal left after the extraction of the gum contains 38–55% protein and some antinutritional factors and objectionable flavors (Subramanian and Parpia, 1975; Ambegaokar et al., 1969; Couch et al., 1966). This investigation was undertaken to characterize the nature of guar proteins and explore the possibilities of obtaining a protein isolate free from deleterious constituents.

Since there were no reports on the solubility and the fractionation profiles of guar seed protein, studies were undertaken on these aspects. In addition, guar meal and protein fractions were assayed for trypsin inhibitor (Couch et al., 1966; Sumathi and Pattabiraman, 1976), hemagglutinin, and proteolytic activities (D'Souza, 1972) which have been reported to be present.

MATERIALS AND METHODS

Preparation of Defatted Guar Meal. Guar seeds of the variety FS-277 obtained from the Haryana Agricultural University, Hissar, India were used. The seeds were broken into splits and grits in an electric blender and the gum splits separated from the protein-rich germ fraction (grits) by passing through a 12-mesh sieve (British Standard Sieve). The grits were ground, solvent extracted thrice with *n*-hexane, and once again ground to 60-mesh size. The moisture content of the flour was 8% and the protein content 50%.

Nitrogen Solubility Experiments. To 2 g of the meal 20 mL of the aqueous solvent was added and the pH of the suspension adjusted to the desired value by the addition of 1 N HCl or 1 N NaOH. The suspension was then shaken for 1 h at room temperature (about 28 °C) and centrifuged at 4000 rpm for 20 min, and the pH of the supernatant was noted. Aliquots of 10 mL were taken for nitrogen estimation by the microKjeldahl method. The percentage of the total meal nitrogen extracted was calculated. The solvents used were water, 0.5 and 1.0 M NaCl, 0.1 and 0.2 M $CaCl_2$, and 2% sodium hexametaphosphate (SHMP) solutions in distilled water. A comparative study was also made to find out the efficiency of extraction using some acids such as HCl, H_2SO_4, and H_3PO_4 and alkalies such as NaOH, $Ba(OH)_2$, and $Ca(OH)_2$. In the case of the mineral acids, 2 g of the meal was suspended in 20 mL of water and the pH adjusted in the acidic range with the respective acids. In the case of the bases, solid calcium and barium hydroxides and 40% sodium hydroxide solution were added to obtain the desired pH.

Ammonium Sulfate Fractionation. Guar meal was extracted with 1 M NaCl solution (1:10), the clear extract was dialyzed extensively against 1 M NaCl solution, and the dialyzate was diluted to about 1% protein concentration. Solid $(NH_4)_2SO_4$ was added to the protein solution and in each case the volume made up to 10 mL so as to obtain 10, 20, ...60% $(NH_4)_2SO_4$ concentration. After thorough mixing and dissolution of $(NH_4)_2SO_4$, the samples were kept in a water bath at 30 °C for 30 min and centrifuged to remove the precipitates, and the absorbance values of the supernatant were read at 280 nm in a Carl-Zeiss Spectrophotometer.

Gel Filtration. Sephadex G-200 (Pharmacia, Sweden), which had been equilibrated with 1 M NaCl, was packed into a 2.0 × 85.0 cm column. Four milliliters of the total 1 M NaCl extract of guar meal containing about 100 mg of protein was loaded onto the column and allowed to be absorbed. The protein was eluted with 1 M NaCl solution, 3.0-mL fractions collected in an automatic fraction col-

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