Variations in Lipid Composition Among Chickpea Cultivars[1]

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Seeds of 60 cultivars of chickpea, *Cicer arietinum*, from three countries were dehulled and the ground cotyledons evaluated for composition of lipids and lipoxygenase. The average lipid contents of Iraqi-, Indian- and Canadian-grown samples were 5.3, 6.6 and 7.3%, respectively. While palmitic and stearic acids were uniformly about 9.3 and 1.5% of total fatty acids in the lipids, the proportions of oleic and linoleic acids varied from 36 and 50% for Iraqi samples to 17 and 68% for Canadian-grown samples, respectively. Linolenic acid values ranged from 2.1 to 4.5% of total fatty acids and, with lipoxygenase activities averaging one-half that of soybean, the potential for oxidative rancidity in chickpea flours was substantial, especially in northern-grown samples.

Chickpea (*Cicer arietinum* L.) is a valuable legume for human and animal nutrition in certain parts of the world because of its high protein and lysine content as well as energy components such as starch and lipid (1,2). A few investigators have noted that the lipids in chickpea, which constitute about 4–6% of the dry matter, contain a high proportion of oleic and linoleic acids, about 28 and 56%, respectively (3). This high level of unsaturated lipid would have a marked influence on functional properties and storage stability of processed flours from this legume (4).

Recently, chickpeas have been introduced as a new crop in Western Canada, and it was of interest to determine the effects of the northern environment on lipid composition and the genetic variability among adapted cultivars. Because the early maturing cultivars being evaluated in Western Canada are not commonly grown in traditional chickpea production areas, a large sampling of cultivars was taken from India and Iraq for comparison with the locally-adapted strains. The objective was to compare the lipid content and fatty acid composition of cultivars which would represent the commercial crop in each country, rather than to compare the same cultivars grown at each location. In addition to analyses for lipoxygenase activity, a few of the 60 cultivars were extracted with chloroform/methanol (2:1) and the total lipids purified for silicic acid fractionation and quantitation of the neutral and polar lipids.

EXPERIMENTAL METHODS

Sixty chickpea cultivars were evaluated; each sample was a bulk lot from four to six replicates grown in experimental field trials. Nine samples were obtained from the Sulaimaniya Agricultural Station, Iraq, 25 from the International Crop Research Institute for Semi-Arid Crops, Begumpet, India, and 26 from the University of Saskatchewan, Saskatoon. The cultivars were categorized into the large-seeded Kabuli and small-seeded Desi types and stored at 4 C except for subsampling and analysis. An early maturing soybean cultivar, X930-1, was harvested when fully ripened to serve as a reference standard for the lipoxygenase assay.

Chemical analysis. Proximate analyses were conducted on ground (40-mesh) whole seed and ground dehulled seeds by official methods. Crude lipids were extracted from ground dehulled seeds (chickpea flour) with anhydrous diethyl ether in a Goldfisch extractor for five hr. Lipid extractions were done in duplicate for GLC analysis. Methyl esters of the fatty acids were prepared by the sodium methoxide procedure of Stringham and McGregor (5), using heptadecanoic acid as the internal standard. Exactly 2.3 μl of the hexane layer were injected onto a glass column (4 mm I.D. × 1.83 m) packed with GP 3% SP-2310/2% SP-2300 on 100/120 Chromosorb W AW (Supelco, Inc., Bellefonte, Pennsylvania) in a Hewlett Packard Model 402 gas chromatograph equipped with a dual flame ionization detector. The injection port and flame detector were operated at 250 C, while the column temperature was maintained at 175 C. The flow rate of the helium carrier gas was 40 ml/min. Relative peak areas were determined by multiplying the peak height by width of the peak at half-height. Weight percentage compositions were calculated using response factors obtained with known mixtures. Peaks were tentatively identified by co-chromatography with known standard fatty acids.

Lipid fractionation. Purified lipids for silicic acid chromatography were obtained by the procedure of Folch et al. (6). The lipids were extracted with chloroform/methanol (2:1) at a sample:solvent ratio of 1:4 with 150, 100 and 75 ml of the solvent mixture for 5, 3 and 2 min, respectively. The combined supernatants were sampled, and the remainder was washed twice with 0.04% $CaCl_2$. The organic phase was evaporated to dryness, taken up in ether and applied to a silicic acid column (Sigma Chemicals, St. Louis, Missouri, 60-200 mesh). The neutral lipids, glycolipids and phospholipids were eluted with four column volumes each of ether, acetone and methanol, respectively. Completion of elution was confirmed by thin layer chromatography (TLC) on glass plates precoated with 0.25 mm Silica Gel G using chloroform/methanol/28% ammonia/water (79:25:3.5:1.5, v/v/v/v) as solvent.

Lipoxygenase assay. Crude lipoxygenase was extracted from all chickpea cultivars and soybean by stirring 0.5-g samples with 100 ml 0.2 M sodium phosphate buffer (pH 6.6) at 4 C for 60 min. Insoluble material was separated by centrifugation for 30 min at 12,000 × g and the supernatant made up to 100 ml with phosphate buffer. One hundred μl of the substrate, linoleic acid (99.5%), was mixed with an equal volume of Tween 20 (polyoxyethylene sorbitan monolaurate), and 5 ml of 0.1 N potassium hydroxide was added slowly until the mixture became clear. The reaction mixture was diluted to 1.55 mM linoleate, which was determined to be optimal for reaction with five-μl sam-

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TABLE 1

Hull Content and Proximate Analysis of Four Cultivars Each of Kabuli and Desi Chickpeas Grown in Three Countries[a]

Source of seed	Biotype	Hull %	Whole seed, %				Dehulled seed, %			
			Protein[b]	Fat	Fiber	Ash	Protein[b]	Fat	Fiber	Ash
Iraq	Kabuli	4.4	23.6	4.9	4.4	3.1	24.5	5.3	1.9	2.8
India	Kabuli	4.3	20.6	6.6	3.0	3.5	20.4	6.7	1.8	3.0
	Desi	11.5	18.4	5.8	6.2	3.4	20.9	6.4	1.8	2.5
Canada	Kabuli	11.5	22.1	6.5	7.8	2.6	24.6	7.2	1.7	1.9
	Desi	14.5	25.1	6.1	8.4	2.8	28.7	7.1	1.8	1.8

[a]Average of two determinations, moisture-free basis.
[b]N × 6.25.

TABLE 2

Ether-Soluble Lipids and Their Fatty Acid Compositions in Kabuli and Desi Chickpeas, % Dry Basis

Source of seed	Biotype	No. of cultivars	Statistic	Total lipids	Proportion of fatty acids				
					16:0	18:0	18:1	18:2	18:3
Iraq	Kabuli	9	Range	4.7-5.9	8.9-9.7	1.3-1.7	32.5-39.3	47.2-53.6	2.1-3.3
			Mean	5.3	9.3	1.4	36.2	50.4	2.5
			SD	0.4	0.3	0.1	1.6	2.0	0.3
India	Kabuli	12	Range	5.6-7.6	8.6-10.3	1.2-3.7	21.2-31.7	54.9-65.1	2.4-3.2
			Mean	6.6	9.3	2.0	26.3	59.7	2.8
			SD	0.5	0.6	0.7	3.6	3.1	0.2
	Desi	13	Range	5.3-7.2	8.1-10.9	1.2-2.6	14.1-23.4	61.3-67.6	2.7-4.1
			Mean	6.5	9.6	1.7	20.9	64.2	3.4
			SD	0.5	0.8	0.5	2.4	2.3	0.5
Canada	Kabuli	6	Range	6.6-7.8	8.5-10.6	1.0-1.5	17.0-17.4	66.7-69.0	3.7-4.4
			Mean	7.2	9.6	1.3	17.2	67.9	4.1
			SD	0.5	0.8	0.1	0.1	0.7	0.3
	Desi	20	Range	6.7-7.9	7.8-10.4	1.0-1.3	14.0-19.0	66.9-71.2	3.7-4.5
			Mean	7.3	8.9	1.2	17.2	68.4	4.1
			SD	0.4	0.7	0.1	1.0	1.4	0.2
Average of all cultivars		60		6.6	9.3	1.5	23.6	62.1	3.5

ples of crude lipoxygenase extract from chickpea. The reaction mixture of 2.7 ml of phosphate buffer (0.2M, pH 6.6), 0.3 ml substrate and five μl crude enzyme extract were added to a one-cm diameter cell for measurement of the increase in absorbance at 234 nm over a seven-min period on a Perkin-Elmer Model 124 double beam spectrophotometer. One unit of lipoxygenase activity was defined as an increase of 0.1 A_{234}/min. For soybean, the pH optimum for the reaction was 6.4 rather than 6.6.

RESULTS AND DISCUSSION

Chemical composition. The Kabuli cultivars from Iraq and India contained only 4.3-4.4% hulls, compared to 11.5% hulls in the Indian Desi cultivars (Table 1). Cultivars grown in Canada were selected for early maturity and, due to their relatively smaller seeds, the hull contents were higher in both Kabuli and Desi biotypes. Cultivars from Iraq tended to contain more protein and less lipid than those from India. Canadian cultivars were comparatively higher in protein and lipid contents, especially after dehulling. Differences in lipid contents between Kabuli and Desi types from India and Canada largely disappeared after dehulling.

The range in lipid contents among the 60 cultivars was 4.7-7.9%, with average values being 5.3% for Iraqi, 6.6% for Indian and 7.3% for Canadian samples (Table 2). It appeared that differences among locations were as great as cultivar differences within any one location.

Fatty acid composition. Eight fatty acids were identified in the GLC chromatograms. Lauric, myristic and arachidic acids were present in concentration of less than 0.2% in chickpea lipids, so the values are not presented in Table 2. The proportions of palmitic and

stearic acids averaged 9.3 and 1.5%, and variations among cultivars were quite small.

Quantitative differences between cultivars in composition of both oleic and linoleic acids were quite large, especially among the Indian cultivars (Table 2). Differences in the oleic/linoleic acid ratios were also apparent between biotypes at one location and between locations. For example, the mean concentrations for these fatty acids in Kabuli cultivars in Iraq were 36.2 and 50.4%, respectively. This ratio differed substantially from the Kabuli means in India (26.3:59.7%) and Canada (17.2:67.9%). The Desi cultivars exhibited very high linoleic:oleic acid ratios in both India and Canada. The trend for vegetable oils from temperate regions to contain more linoleic acid, at the expense of oleic acid, than oils from warmer climates has been observed previously (7).

The proportion of linolenic acid in the chickpea cultivars varied from an average of 2.5% in Iraq to 4.1% in Canada (Table 2). Generally, linolenic acid levels in excess of 1% will have an adverse effect on flavor stability (8). Therefore, the linolenic acid level in chickpea lipids may limit the uses for the flour in food products where flavor is noticeable. The present results suggest that cultivars grown under warmer climates will have better storage properties.

Lipoxygenase activity. Essentially all chickpea cultivars contained over 50% polyunsaturated fatty acids (Table 2), and it appeared desirable to determine the levels of lipoxygenase activity in these same samples so the potential for oxidative rancidity could be assessed. The lipoxygenase assay gave a range of 21,570 to 86,960 units for all cultivars, which represented 25.6 to 102.9% of soybean lipoxygenase activity (Table 3). It appeared that the storage properties of chickpea flours would be very dependent on the levels of this enzyme present in the flour, in addition to other factors which influence storage stability. Also, the stability of food products prepared from chickpea flours might be associated with the extent of enzyme inactivation by heat processing during food preparation.

Unlike fatty acid composition, lipoxygenase activities of the chickpea cultivars varied widely at each location (Table 3). Mean values for all cultivars grown in Iraq and India were about 54,000 units, whereas the Canadian cultivars averaged 32,800 units. Lipoxygenase activity was not correlated with linoleic acid contents of the lipid at any location except for the six Kabuli cultivars grown in Canada.

Lipid components. Approximately 8.5% of the dry matter in chickpea was extracted with chloroform/ethanol (2:1) and, after purification with 0.04% $CaCl_2$ and ether, the yields of purified lipid were 5.5 and 6.5%, respectively, for the Kabuli and Desi types (Table 4). The Desi lipids appeared to contain slightly more neutral lipid than Kabuli, the average distribution being 80:19:1 for the neutral, phospho- and glycolipids.

Polar lipids contained about 16% palmitic acid, which was substantially greater than the 10% level in neutral lipids (Table 5). Otherwise, the polar lipids of the Kabuli chickpea samples had about the same proportion of oleic and linoleic acids, 33 and 46%, respectively, as neutral lipids. The Desi phospho- and glycolipids were also less polyunsaturated than neutral lipids, but the composition of oleic, linoleic and linolenic acids of 15,

TABLE 3

Lipoxygenase Activity in Kabuli and Desi Chickpeas in Relation to Soybean Lipoxygenase Activity

Source of seed	Biotype	No. of cultivars	Statistic	Lipoxygenase activity units/g, dry basis[a]	As % of soybean activity[b]
Iraq	Kabuli	9	Range	29,490-62,060	34.9-73.5
			Mean	52,420	61.2
			SD	9,120	10.6
India	Kabuli	12	Range	38,040-86,960	45.1-102.9
			Mean	54,090	64.1
			SD	12,450	14.7
	Desi	13	Range	38,190-81,980	45.2-97.1
			Mean	55,730	66.0
			SD	10,850	12.9
Canada	Kabuli	6	Range	25,520-28,970	30.2-34.3
			Mean	27,180	32.2
			SD	1,160	1.4
	Desi	20	Range	21,570-53,140	25.6-62.9
			Mean	34,490	40.8
			SD	9,040	11.0
Average of all cultivars		60		44,970	53.2

[a]One unit activity is defined as an increase of 0.1 A_{234}/min.
[b]Soybean cultivar X 930-1.

TABLE 4

Yields of Neutral and Polar Lipids from Silicic Acid Fractionation of Purified Lipids in Kabuli and Desi Chickpeas, %[a]

Source of seed	Biotype	No. of cultivars	Crude lipid	Purified lipid	Neutral lipid	Phospholipid	Glycolipid	Recovery
Iraq	Kabuli	3	8.4	5.5	78.0±0.8	21.0±0.9	1.1±0.3	96.0±0.8
Canada	Desi	3	8.5	6.5	82.0±0.7	17.1±1.0	0.9±0.1	94.3±2.1

[a]Average of duplicate determination of three cultivars of each type.

TABLE 5

Proportions of Fatty Acids in Neutral and Polar Lipids of Three Cultivars Each of Kabuli and Desi Chickpeas in %[a]

Source of seed	Biotype	Proportion of fatty acids				
		16:0	18:0	18:1	18:2	18:3
		Neutral lipids				
Iraq	Kabuli	9.6±0.4	1.2±0.3	37.8±1.5	48.6±0.7	2.7±0.4
Canada	Desi	9.1±0.3	1.1±0.2	17.2±2.0	67.8±1.4	4.9±0.3
		Phospholipids				
Iraq	Kabuli	15.7±0.6	1.1±0.5	33.5±1.3	47.6±1.5	2.2±0.2
Canada	Desi	15.7±0.4	1.9±0.2	14.5±1.3	64.4±2.4	3.5±0.4
		Glycolipids				
Iraq	Kabuli	16.9±0.8	1.9±0.3	33.0±1.5	45.2±2.3	3.0±0.2
Canada	Desi	14.6±0.4	2.5±0.2	15.2±2.1	61.1±2.1	6.7±0.4

[a]Average of duplicate determinations.

63 and 5%, respectively, was more polyunsaturated than the Kabuli polar lipids.

It appeared that Desi cultivars would present a greater storage problem than Kabuli, although both oils were quite polyunsaturated. In addition, the northern-grown seeds contained more total lipid which was substantially greater in degree of polyunsaturation than southern-grown chickpeas. On the other hand, the lipoxygenase activities in the Canadian cultivars were only 60% the levels found in cultivars grown in Iraq and India, which should reduce the rate of lipid oxidation to a significant degree.

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