Table I. Amino Acid Analyses (Percent Residues) of Supercritical CO_2- and Hexane-Extracted Seed Meals

amino acid	soybean		lupine		jojoba	
	hexane	CO_2	hexane	CO_2	hexane	CO_2
Lys	5.7	5.7	5.2	5.2	4.3	4.4
His	2.2	2.2	2.4	2.4	1.7	1.7
Arg	5.7	5.8	8.0	7.9	5.6	5.5
Asp	12.0	12.0	10.4	10.3	10.0	9.8
Thr	4.5	4.5	4.4	4.3	6.0	5.9
Ser	6.9	7.0	7.0	7.0	6.9	6.8
Glu	16.5	16.4	21.6	21.5	10.4	10.5
Pro	6.2	6.2	5.0	4.9	6.0	5.9
Gly	7.7	7.7	7.5	7.5	16.2	16.2
Ala	6.6	6.6	5.5	5.5	6.3	6.3
$^1/_2$-Cys	1.5	1.4	1.4	1.6	3.5	3.5
Val	4.7	4.7	3.9	4.0	5.4	5.5
Met	1.1	1.1	0.5	0.5	0.9	0.9
Ile	4.1	4.1	4.0	4.1	3.1	3.3
Leu	7.8	7.7	7.0	7.0	6.8	6.9
Tyr	2.8	2.8	3.2	3.3	3.6	3.6
Phe	4.0	4.0	3.0	3.0	3.3	3.3

changes in the initial rate of enzymic hydrolysis of seed globulins. Hence, we conclude that treatment with supercritical CO_2 at 350 bars and 40 °C for 2 h did not denature the lupineseed proteins.

Amino acid analysis (Table I) showed no significant differences between the control meals and their CO_2-treated counterparts. The jojoba meal has an unusual composition; it is relatively low in glutamic acid plus glutamine and high in cystine and glycine compared with the other seed meals. These differences are presumably related to the virtual absence of salt-soluble globulins; the major subunits of the jojoba proteins extracted with water or salt solutions are of low molecular weight (see Figure 1) compared with other oilseed proteins.

The constant amino acid composition, coupled with the evidence above that there has been a negligible influence on protein solubility and enzymic digestibility, leads us to conclude that treatment with supercritical CO_2 at 40 °C will have no deleterious effects on the nutritional value of oilseed meals.

ACKNOWLEDGMENT

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Chemical Constitution of Starch and Oligosaccharide Components of "Desi" and "Kabuli" Chickpea (*Cicer arietinum*) Seed Types

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Investigations on the chemical constitution of the chickpea seed types "desi" and "kabuli" have been performed. The breeding lines of the desi types contain higher levels of acid detergent fibers (9.4–14.7%; cf. 3.8–7.6% for kabuli) and higher average seed coat weights, but there appeared to be no difference in the total protein content. Variations have been noted in the total starch and percent amylose contents of desi and kabuli type seeds. The levels of the raffinose-series oligosaccharides were higher in kabuli than in desi types. Soluble sugar profiles revealed a substantial difference in the distribution of sucrose in the two types. Stachyose was the predominant sugar in desi types, but the majority of the kabuli types indicated sucrose as the main component. On the average, oligosaccharides did not show any differences in the two types although the sucrose content of kabuli types was 46.9% higher than the desi types. Verbascose represented only a small fraction (range 0.09–0.41 g/100 g of seed weight) of the total soluble sugars. Quantification of oligosaccharides and amylose offers a useful criteria for identification of genotypes of high nutritional quality.

Grain legumes are an important component of both human and livestock diets. In addition to complementing cereal protein, grain legumes also make a significant contribution to total energy intake. The quality as well as the quantity of grain legume carbohydrates is thus a major consideration in the development of new cultivars that have desirable nutritional properties.

Starch is often the major component of many grain legumes (Naivikul and D'Appolonia, 1979; Lineback and Ke, 1975). On ingestion, salivary α-amylase splits the starch molecules to liberate glucose to provide energy. However, the digestibility and hence the energy value of starch are often determined by the branching of the starch molecule and its ability to interact with the hydrolytic enzymes (Geervani and Theophilus, 1981).

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Oligosaccharides of the raffinose family are an integral component of many food legumes (Shallenberger and Moyer, 1961; Schweizer et al., 1978). Antinutritional activity of grain legumes, is sometimes associated with the raffinose-series oligosaccharides (Rackis, 1975). Due to the absence of α-galactosidase activity in the upper gut, these oligosaccharides remain intact and enter the lower intestine where they are metabolized by bacterial action. Methane, hydrogen, and carbon dioxide accumulations, caused by bacterial degradation of galactose, can lead to flatulence and diarrhea. Oligosaccharides are thus a factor limiting the utilization of grain legumes in monogastric diets.

Chickpea is a major component of the human diet in many temperate regions. Significant tonnages are also included in pig diets especially in Mexico (Arias, 1980). In chickpeas, the existence of distinct seed types, in particular "desi" and "kabuli", has long been recognized (Vander Maesen, 1972). However, information is still lacking on their respective chemical constitution. Therefore, the main objective of this investigation was to derive new chemical and biochemical data and to correlate variations in these data with seed type. In this paper, the chemical properties of two major chickpea seed types are compared and the nutritional implications discussed.

EXPERIMENTAL SECTION

Seed Sampling and Analysis. The lines were grown in 1978 under essentially indentical agronomic conditions at the Wagga Agricultural Research Institute. For the determination of seed coat weights, the seeds were soaked in distilled water and stored at 5 °C overnight. Coats were removed by using forceps, and the separated coats and cotyledons were oven-dried to determine the seed coat percentages. Acid detergent fiber (ADF) was determined by using the method of Goering and Van Soest (1970).

Estimation of Protein. Protein content (nitrogen × 6.25) was estimated by micro-Kjeldhal nitrogen analysis (Association of Official Analytical Chemists, 1975). Portions of the material were oven-dried to determine the moisture content and appropriate corrections made to express results on a moisture-free basis.

Extraction and Examination of Soluble Sugars. For the extraction of soluble sugars, the seeds were ground in a Udy cyclone mill (100-mesh sieve). A 5.0-g sample was macerated in 70% aqueous ethanol (250 mL) and the mixture boiled for 5 min. After cooling, the solution was filtered through scintered glass and the residue washed with ethanol and then dried. The ethanol extracts were concentrated by rotary evaporation (below 40 °C) to 50 mL and extracted twice with chloroform in a separating funnel. Final traces of chloroform and denatured protein were removed by centrifugation. The aqueous solution was used for the determination of mono-, di-, and oligosaccharide components.

The extracts were examined qualitatively by paper chromatography (solvent system, 1-butanol–pyridine–water–benzene, 5:3:3:1, upper phase) and thin-layer chromatography (solvent system, 1-propanol–ethanol–water, 7:1:3). The papers were developed with *p*-anisidine–HCl (Pridham, 1956) or with silver nitrate (Trevelyan et al., 1950) spray reagents. The TLC plates were developed by spraying with 5% H_2SO_4 in ethanol and then heating at 105 °C. A suitable aliquot (0.5–1.0 mL) of the extract was also fractionated by gel filtration on polyacrylamide (Bio-Gel P-2, –400 mesh). The column fractions collected were assayed with anthrone (Loewus, 1952) to distinguish the oligomer peaks. The peaks thus obtained were identified by comparing their elution volumes with those of the standard sugars run under the same conditions.

Determination of Total Starch. The residue obtained after ethanol extraction was used for the determination of total starch. A portion of the residue flour (50 mg) was incubated in Me_2SO (5 mL/g of flour), at 37 °C overnight, and the digest was suspended in acetate buffer (100 mM, pH 5.0, 20 mL) in a 50-mL Quickfit tube. The suspension was mixed thoroughly on a vortex mixer and heated in a boiling water bath for 15 min. On cooling, the tubes were shaken vigorously and amyloglucosidase (Sigma, type IV, 1 mL, 50 units) was added. The suspension was incubated in a water bath for 2 h at 55 °C with occasional shaking. On cooling to room temperature, salivary α-amylase (human, 0.2 mL) was added, and the tubes were incubated for 1 h at 37 °C and shaken occasionally. The incubation mixture was transferred to a 25-mL volumetric flask and the volume was adjusted to the mark. Glucose liberated was determined by using the glucose oxidase–peroxidase method of Trinder (1969) after slight modifications. To a 5.0-mL aliquot of incubation mixture, an equivalent quantity of phenol tungstate reagent (of double concentration) was added. The mixture was gently shaken and centrifuged at 5000 rpm for 10 min. A suitable aliquot (0.1–1.0 mL) of the clear solution was then taken for color development and determination of glucose content. Starch content was calculated by multiplying the amount of glucose by a factor of 0.9.

Isolation of Starch Granules. For the isolation of starch granules the seeds were soaked in water at 4 °C overnight. The cotyledone were then macerated in a 0.01 M $HgCl_2$–0.1 M NaCl solution containing toluene (5 mL) in a Waring blender. The suspension was filtered through four layers of muslin, and the residue was again macerated and filtered twice without the addition of toluene. The combined filtrates were then centrifuged at low speed (600*g*, 30 min, 25 °C). The supernatant was poured off from the centrifuge bottles by tilting gently to remove the upper toluene lipid and aqueous layers. The bottom solid layer of starch granules was resuspended in $HgCl_2$–NaCl solution in a beaker. The granules were allowed to settle and the supernatant was decanted. The process was repeated until the granules were free from cell debris. The granules were finally collected by filtration through a scintered funnel under gentle suction, washed with ethanol, acetone, and ether, and dried under vacuum.

Iodine Affinity and Percent Amylose Contents. Iodine affinity and percent amylose contents of the isolated starch granules were determined by the method of Matheson (1975). Starch granules (50 mg) were treated with dimethyl sufoxide (Me_2SO) containing 0.01 M $HgCl_2$ (5 mL/g of granule sample) and incubated at 37 °C for 18 h. The incubation mixture was dispersed in a small volume of 0.05 M KI–0.05 M KCl solution and shaken until the starch was in solution. The final volume was made to 50 mL in a volumetric flask by adding 0.05 M KI–0.05 M KCl solution. A suitable aliquot (20 mL) was taken for the determination of amylose content.

Microscopic Examination. The gelatinization temperatures of the isolated granules were determined on a Leitz heating stage fitted on an Olympus microscope.

RESULTS AND DISCUSSION

Physical and Chemical Characteristics. The 15 lines of chickpeas examined have been used as breeding lines by the New South Wales Department of Argiculture. From appearance characteristics, chickpea seeds can be arbitrarily divided into three distinct types (Figure 1), namely, desi, kabuli, and pea. Pea seeds are nearly spherical, except for the characteristic chickpea beak. Although common in the progeny from kabuli × desi

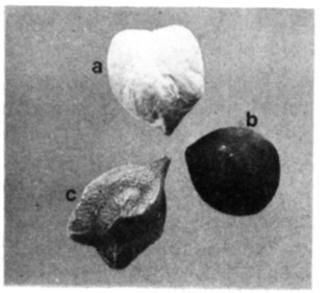

Figure 1. Illustration of kabuli-type (a), pea seed type (b), and desi-type (c) chickpeas.

Table I. Average Values of Some Constituents of Desi- and Kabuli-Type Chickpeas[a,b]

line, variety, and type	weight/100 seeds, g	seed coat, %	A/D fiber, %	protein,[c] %
1, WWC1, desi	22.2	14.4	12.6	23.3
2, CPI 61277, desi	14.2	16.7	14.2	24.4
3, CPI 53007, desi	13.2	15.8	14.7	25.4
4, CPI 7473, desi	13.9	15.3	13.2	28.0
5, CPI 56566, desi	33.9	11.8	12.3	23.9
6, CPI 71180, desi	18.6	13.4	9.4	22.4
7, CPI 56288, desi	17.3	12.3	11.8	21.3
8, CPI 56565, kabuli	16.3	6.9	6.0	25.0
9, K 583, kabuli	33.4	4.5	3.8	21.2
10, K 1480, kabuli	21.8	5.4	4.0	22.0
11, CPI 56329, kabuli	15.0	6.8	7.6	25.7
12, CPI 60557, kabuli	28.6	4.5	4.3	19.6
13, CPI 6051, kabuli	23.9	5.0	5.0	21.3
14, CPI 56296, kabuli	18.9	6.0	5.6	25.9
15, K 368, pea type	23.7	9.5	12.2	24.1

[a] Mositure-free basis. [b] Average of two determinations. [c] N × 6.25.

crosses (Hawtin and Singh, 1980), they are poorly represented germ plasm collections. This is most likely due to the vulnerability of their seed coat to cracking. Desi types have a pronounced angularity and strongly ridged surface. Kabuli types are intermediate in shape with a less wrinkled surface. The seed coat of desi types is considerably thicker than that of kabuli types, but in both types there is a good adherence of the seed coat to the cotyledons (Knights, 1980).

The analysis of some constituents of seven lines of each of the desi and kabuli type and one of the pea type are shown in Table I. Seed size varies within both types, but the average seed coat weights are higher in desi types. The seed coat constitutes 14% of the desi seeds, and this compares with the seed coat weights of kabuli types, which constitute only 6%. However, there is no evidence of a difference in the protein content of the two types examined.

Mono-, Di-, and Oligosaccharide Profiles. Several methods have been reported for the extraction and identification of soluble sugars from seeds (Black and Bagley, 1978). The most frequently employed method is the extraction of ground seeds in hot aqueous ethanol. Major proportions of the soluble sugars are extractable by this procedure, but higher oligomers such as verbascose and ajugose may escape extraction due to their relatively insoluble nature in organic solvents. Using hot aqueous ethanol (70% v/v) as the extraction medium, the total amounts of soluble sugars obtained from various chickpea lines are presented in Table II. The total amount of oligosaccharides, expressed as a percent of dry matter, varies from 10.4 to 17.0%. On an average basis, kabuli chickpeas indicated 3.2% higher levels than desi types, and these levels were consistently higher in all the kabuli lines examined. An average level of 4.7% has been reported by

Table II. Total Soluble Sugars and Component Mono-, Di-, and Oligosaccharides of Desi- and Kabuli-Type Chickpeas

line, variety, and type	total soluble sugars, g/100 g of seed	soluble sugar components,[a] g/100 g of seed				
		Ver	Stach	Raff	Suc	Glc
1, WWC1, desi	11.8	0.41	4.97	1.56	4.87	tr
2, CPI 61277, desi	10.8	tr	5.35	1.83	3.66	tr
3, CPI 53007, desi	10.4	0.14	5.21	1.94	3.09	tr
4, CPI 7473, desi	10.6	0.19	5.08	1.04	4.33	tr
5, CPI 56566, desi	10.4	0.17	4.96	1.42	3.85	tr
6, CPI 71180, desi	10.4	0.12	5.07	1.00	4.20	tr
7, CPI 56288, desi	10.6	tr	4.78	1.58	4.22	tr
average	10.7	0.15	5.06	1.48	4.03	tr
8, CPI 56565, kabuli	14.4	0.13	6.48	1.99	5.80	tr
9, K 583, kabuli	17.0	0.18	6.15	2.10	8.75	tr
10, K 1480, kabuli	11.4	0.09	5.37	1.16	4.77	tr
11, CPI 56329, kabuli	12.4	tr	4.04	1.17	7.16	tr
12, CPI 60557, kabuli	13.4	0.12	5.11	1.12	7.06	tr
13, CPI 6051, kabuli	11.4	0.17	4.68	1.56	5.00	tr
14, CPI 56296, kabuli	11.8	0.12	5.30	1.22	5.13	tr
average	13.1	0.12	5.30	1.47	6.2	tr
15, K 368, pea type	13.2	tr	5.42	2.00	5.78	tr

[a] tr = trace. Ver = verbascose, Stach = stachyose, Raff = raffinose, Suc = Sucrose, and Glc = glucose.

Rao and Belavady (1978) in some varieties of chickpeas. Äman (1979) reported a value of 13.1% in one variety, whereas values ranging from 6.7 to 8.4% have been obtained by Schweizer et al. (1978).

The identification of the extracted products is often accomplished by paper chromatography (Rao and Belavady, 1978), thin-layer chromatography (Celga and Bell, 1977), gas–liquid chromatography (Schweizer et al., 1978), and, more recently, by high–pressure liquid chromatography (Macrae and Zand-Moghaddam, 1978). Occasionally colorimetric quantification of the oligosaccharides after separation by gel filtration has been employed (Cerning-Beroard, 1976).

In the present studies, a PC and TLC examination on selected extracts showed that oligosaccharide fractions consist of verbascose, stachyose, and raffinose; the disaccharide fraction was all sucrose except for a trace of melibiose. Reducing monosaccharides were glucose and a trace of fructose detected in some samples. For a quantitative separation of the soluble sugars, portions of the extracts were chromatographed on Bio-Gel P-2, and the patterns obtained are shown in Figure 2. A distinct separation of various components as determined by the

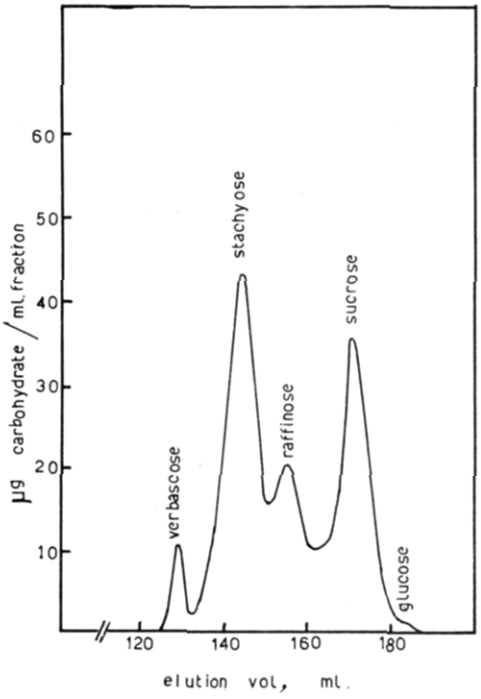

Figure 2. Gel chromatographic separation of chickpea oligosaccharides on Bio-Gel P-2 (–400 mesh).

Table III. Corrected Average Distribution of Component Soluble Sugars of Chickpea Seed Types

seed type	component sugars[a]				
	Ver	Stach	Raff	total[b]	Suc
desi	0.016	0.541	0.158	0.749	0.431
kabuli	0.015	0.694	0.192	0.901	0.812

[a] Equation used for the calculations: (average sugar component × average total soluble sugars)/100 g of seed. [b] The sum total of verb + stach + raff.

anthrone assay was achieved.

A difference in the distribution of mono-, di-, and oligosaccharide components of kabuli- and desi-type chickpeas has been observed (Table II). Stachyose ranges from 4.04 to 6.48 g/100 g of seed weight in the lines examined. Verbascose represented only a small fraction of the total oligosaccharides. Glucose was present only in traces in all the lines examined. On the average (Table III), the combined amounts of verbascose, stachyose, and raffinose were higher in desi types by 16.8% whereas kabuli types indicated 46.9% more sucrose than the desi types. These results show a very similar "culinary" properties of kabuli and desi types as the degree of flatulence has been reported to be associated with the levels of galactose-containing oligosaccharides, i.e., raffinose, stachyose, and higher oligomers present in the seeds (Rackis, 1975).

Amounts and Properties of Isolated Starches. Total starch contents of various lines were determined. Often starch values are determined by subtracting all other values from 100 and assuming the difference, expressed as a percentage, represents the total starch content. Earlier workers either have used this difference method (Meiners et al., 1976) or have determined the starch content alone without analyzing for other constituents (Srinivasa Rao, 1976). In the present investigations, starch was chemically determined after enzymic hydrolysis and the released glucose was estimated. The levels of starch thus determined varied from 35.4 to 57.2% of seed weights in the lines examined (Table IV). Kabuli types had, on the average, 8% more starch than the desi types. A similar difference between the two types has been reported by

Table IV. Some Chemical Characteristics of Desi- and Kabuli-Type Chickpeas

line, variety, and type	total starch, % of dry matter	gel. temp	I_2 affinity	% amylose
1, WWC1, desi	39.2	68–72	4.9	25.4
2, CPI 61277, desi	43.6	64–71	7.7	40.4
3, CPI 53007, desi	46.6	68–72	7.9	41.3
4, CPI 7473, desi	35.4	65–68	7.3	38.1
5, CPI 56566, desi	45.8	60–65	6.6	34.6
6, CPI 71180, desi	51.0	66–72	6.2	32.3
7, CPI 56288, desi	48.4	72–75	8.0	42.2
8, CPI 56565, kabuli	46.2	65–70	5.7	29.8
9, K 583, kabuli	55.0	65–70	5.6	28.9
10, K 1480, kabuli	53.6	68–72	7.1	37.1
11, CPI 56329, kabuli	48.4	62–67	6.6	35.0
12, CPI 60557, kabuli	57.2	ND[a]	ND	ND
13, CPI 6051, kabuli	52.4	ND	ND	ND
14, CPI 56296, kabuli	51.0	68–73	7.2	36.8
15, K 368, pea type	44.0	68–75	7.6	39.5

[a] ND = not determined.

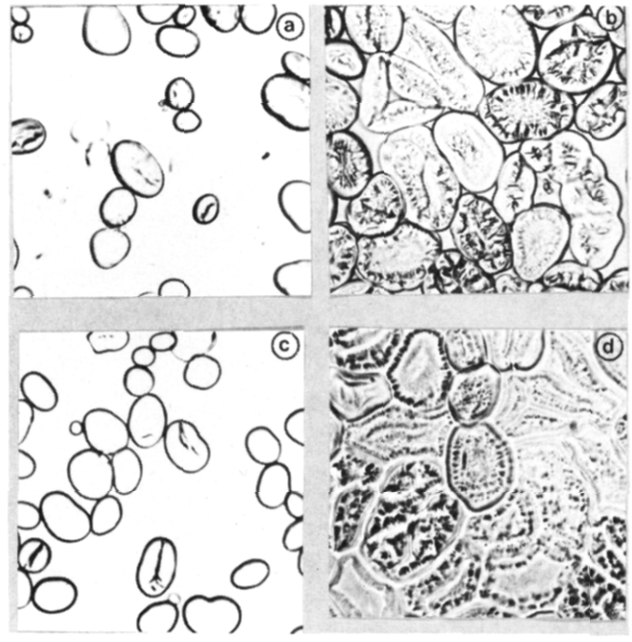

Figure 3. Photomicrographs of chickpea starch granules at ambient and gelatinization temperatures (×200). (a) WWC1 (desi), 30 °C; (b) WWC1 (desi), 72 °C; (c) K 583 (kabuli), 30 °C; (d) K 583 (kabuli), 70 °C.

Jambunathan and Singh (1980).

Starch granules were isolated from various varieties of desi and kabuli types and their properties examined. The granule size of various starches ranged from large oval-shaped (18–27 μm) to small spherical (5–9 μm) granules (Figure 3). When the granules were examined under the light microscope, a fissuring phenomenon was observed. A similar effect has been reported by Lineback and Ke (1975). Srivastava et al. (1970) also reported that chickpea starch has round granules (3–4 μm with a central hilum) that were sometimes fissured. Gelatinization temperature ranges of the isolated starch granules, determined by using a microscope equipped with a Kofer hot stage, were between 60 and 75 °C (Table IV). These values agree with those previously reported for chickpea starches (Schoch and Maynald, 1968). Srivastava et al. (1970) reported a higher birefringence end point temperature range (71–74 °C) for chickpea starch.

Iodine affinity values for starch granules and percent amylose contents were determined by potentiometric titration, assuming that pure amylose absorbs 200 mg of

iodine/g of amylose (Table IV). The I_2-affinity values thus obtained ranged from 4.9 to 8.0 g of I_2/100 g of starch sample and compares with previously reported values ranging from 5.65 to 7.70 (Srivastava et al., 1970; Schoch and Maynald, 1968; Rosenthal et al., 1971; Singh et al., 1956). The percent amylose content of the isolated starches varied from 25.4 to 42.2% (Table IV). Singh et al. (1956) reported an amylose content of 26.8% to 29.0% for starches from two varieties of chickpeas. Tolmasquim et al. (1971) reported that iodine affinity values for legume starches generally range from 6.0 to 7.5%, indicating an amylose content of 30.0–37.5% based on the assumption of an iodine affinity of 20.0% for pure amylose. In this study we observed that the starch of desi seeds contained, on an average, 2.8% more amylose than that of kabuli seeds. Granules with high amylose content had higher gelatinization temperatures.

Desi and kabuli seeds are broadly representative of two gene pools within chickpeas. These populations have evolved in separate regions under different environmental conditions and have been subjected to different artificial selection pressures. As a result, there has been a tendency for small colored desi genotypes to evolve in the Middle East and Indian subcontinent and for the large white or cream kabuli genotypes to evolve in the Mediterranean region. Chemical differences between desi and kabuli seeds might thus be attributable to seed type and/or the combined effects of natural and artificial selection pressures.

The quantification of amylose and oligosaccharide content of chickpea provides two useful selection criteria. With respect to human consumption, certain genotypes could be identified as causing only low levels of flatulence on the basis of their oligosaccharide contents. Likewise, costly and time-consuming animal feeding trails could be obviated by analyzing for oligosaccharide and amylose contents. Low oligosaccharide levels would permit a higher percentage of grain to be included in the diets of monogastrics, and amylose content would provide an index of the available energy levels.

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Registry No. Amylose, 9005-82-7; starch, 9005-25-8; sucrose, 57-50-1; stachyose, 470-55-3; verbascose, 546-62-3; raffinose, 512-69-6.

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