Research Center, U.S. Department of Agriculture, Berkeley, Calif., for help in interpretation of certain mass spectra.

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# Nature of Carbohydrates in Pulses

P. Srinivasa Rao

Four commonly consumed pulses, Bengalgram, greengram, redgram, and blackgram, were studied to ascertain the chemical nature of the carbohydrates contained in them. Total carbohydrates, starch, and soluble sugars were determined. The nature of starch with respect to amylose content and its degree of polymerization was found to be different in the four pulses tested. Carbohydrate tolerances in children fed Bengalgram and greengram were also found to be different. The significance of these in vivo studies on the utilization of carbohydrates is discussed in relation to the chemical nature of carbohydrates in these pulses.

In an attempt to explain the flatulence associated with ingestion of pulses, investigations were carried out to test the in vitro digestibility of carbohydrates ($\alpha$-amylolysis) of commonly consumed pulses. The results of these studies revealed marked differences in the rate of in vitro $\alpha$-amylolysis of pulses. Of the legumes tested, maximal differences were seen between Bengalgram (*Cicer arietinum*) and greengram (*Phaseolus areus*) (Srinivasa Rao, 1969). These studies were, therefore, extended to ascertain (1) whether the in vitro observations have any relevance to the in vivo situation and (2) whether the nature of the carbohydrates contained in these pulses is different. The results of these investigations are reported here.

## EXPERIMENTAL SECTION

**In Vivo Studies in Children.** These studies were carried out on a group of eight children (six males and two females) between the ages of 3 and 4 years, using a crossover design. A test meal of 20 g of cooked Bengalgram or greengram dhal, mashed to give homogenous slurry, was given to children in the morning under fasting conditions. Four children received greengram while four others received Bengalgram. Capillary blood was collected by finger prick before the meal and at intervals of 30, 60, 120, and 180 min after the meal. After 1 week, the children received a meal of the second pulse. Greengram was fed to those children who had received Bengalgram earlier and vice versa. Fasting blood samples were collected before and after the meal as described earlier. Blood glucose was estimated by the ferricyanide method of Park and Johnson (1949).

**Chemical Analysis.** Chemical analyses were carried out on pooled decorticated samples of pulses collected from different sources. Bengalgram (*Cicer arietinum*), greengram (*Phaseolus areus*), redgram (*Cajanus cajan*), and blackgram (*Phaseolus mungo*) were finely ground and passed through a 60 mesh sieve prior to analysis.

The starch content was determined by the method of McCready et al. (1950) while total carbohydrate and soluble sugars were estimated according to methods described by Friedmann and coworkers (1967). Isolation of starch from pulses was carried out following the procedure of Badenhuizen (1964).

**Determination of Amylose Content of Starch.** Amylose content of isolated starches was determined by measuring iodine affinity potentiometrically according to the method of Schoch (1964). A preliminary calibration curve was obtained to relate the emf reading (in millivolts) to the amount of free iodine in solution under conditions identical with those employed in starch titration.

**Titration of Isolated Pulse Starches.** An accurately weighed amount of defatted starch (about 40 mg) from the pulse was transferred to a clean dry 250-ml beaker and 1 ml of water was added to suspend the sample. Five milliliters of 1 N KOH was added and the sample kept at 4 °C for 30 min with occasional stirring until clear. After neutralizing with HCl (Methyl Orange as indicator), 10 ml of 0.5 N KI solution was added. The contents were titrated against iodine solution and the emf noted. Values for free iodine were derived from the earlier calibration curve. The bound iodine was obtained by deducting free iodine values from the total iodine. The free iodine values for each sample were then plotted against bound iodine. The upper linear portion of the curve was extrapolated back to intersect the zero axis, and from this the amount of bound iodine was calculated. The percentage iodine affinity (which corresponds to the percentage amylose content) was derived as follows: % iodine affinity = (mg of bound iodine for the sample at zero intersect/sample weight in mg) × 100. One hundred milligrams of pure potato amylose in our experiments gave 18.9 mg of bound iodine. Hence the amylose content of the pulse starch sample was calculated from the relation: % amylose = (mg of bound

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Table I. Carbohydrate Tolerance in Children Fed Bengalgram and Greengram Meal

| Subject no. | Bengalgram | | | | | Greengram | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Blood glucose, mg %, at time (min) | | | | | Blood glucose, mg %, at time (min) | | | | |
| | Fasting | 30 | 60 | 120 | 180 | Fasting | 30 | 60 | 120 | 180 |
| 1 | 83.0 | 95.0 | 74.0 | 79.0 | 72.0 | 81.0 | 94.5 | 85.5 | 90.0 | 81.0 |
| 2 | 81.0 | 95.0 | 86.0 | 80.0 | 81.0 | 96.8 | 96.8 | 87.8 | 148.5 | 76.5 |
| 3 | 58.5 | 67.5 | 65.3 | 60.8 | 58.5 | 58.5 | 76.5 | 67.5 | 64.1 | 59.6 |
| 4 | 72.0 | 85.5 | 90.0 | 78.8 | 73.1 | 74.3 | 103.5 | 99.0 | 98.3 | 81.0 |
| 5 | 78.8 | 81.0 | 96.8 | 85.0 | 81.0 | 85.5 | 112.5 | 86.4 | 85.5 | 85.5 |
| 6 | 99.0 | 103.5 | 99.0 | 96.8 | 92.3 | 96.8 | 114.8 | 105.8 | 108.0 | 99.0 |
| 7 | 87.8 | 85.5 | 87.8 | 103.3 | 96.8 | 87.8 | 96.8 | 110.3 | 96.8 | 101.3 |
| 8 | 85.5 | 92.3 | 92.3 | 96.8 | 87.8 | 85.5 | 96.8 | 90.0 | 81.0 | 87.8 |
| Mean ± SE | 80.70 ± 4.197 | 88.16 ± 3.868 | 86.40 ± 4.044 | 86.06 ± 4.167 | 80.31 ± 4.37 | 83.28 ± 4.212 | 99.03 ± 4.212 | 91.54 ± 4.759 | 86.53 ± 8.756 | 83.96 ± 4.649 |

Table II. Blood Glucose Levels in Children Fed Bengalgram and Greengram (mg of Glucose/100 ml of Blood)

| Subject no. | Bengalgram | | | | Greengram | | | |
|---|---|---|---|---|---|---|---|---|
| | Fasting value | Peak value | Increase over fasting value | % increase over fasting value | Fasting value | Peak value | Increase over fasting value | % increase over fasting value |
| 1 | 83.0 | 95 | 12 | 14.46 | 81.0 | 94.5 | 13.5 | 16.67 |
| 2 | 81.0 | 95.0 | 14 | 17.28 | 96.8 | 148.5[a] | 51.7 | 53.41 |
| 3 | 58.5 | 67.5 | 9 | 15.38 | 58.5 | 76.5 | 18.0 | 30.77 |
| 4 | 72.0 | 90.0[a] | 18 | 25.00 | 74.3 | 103.5 | 29.2 | 39.30 |
| 5 | 78.8 | 96.8[a] | 18 | 22.84 | 85.5 | 112.5 | 27.0 | 31.58 |
| 6 | 99.0 | 103.5 | 4.5 | 4.55 | 96.8 | 114.8 | 18.0 | 18.60 |
| 7 | 87.8 | 101.3[a] | 13.5 | 15.38 | 87.8 | 110.3[a] | 22.5 | 25.63 |
| 8 | 85.5 | 96.8[a] | 11.3 | 13.22 | 85.5 | 96.8 | 11.3 | 13.22 |
| | | | Mean ± SE | 16.01 ± 2.201[b] | | | Mean ± SE | 28.65 ± 4.689[b] |

[a] Indicates that peak level in these cases was reached later than 30 min after the pulse meal. [b] The difference between the means is significant ($P < 0.05$).

Table III. Total Carbohydrate, Starch, Soluble Sugars, and Amylose Content of Starch in Pulses

| Source | Total carbohydrates[a] by acid hydrolysis (1 N HCl), g % | Starch[a] | Soluble[a] sugars, g % | Starch soluble sugars, g % | Amylose,[b] % linear fraction of starch |
|---|---|---|---|---|---|
| Bengalgram | 61.2 | 46.8 | 3.5 | 50.3 | 45.81 |
| Greengram | 61.2 | 53.6 | 3.9 | 57.5 | 35.01 |
| Redgram | 58.7 | 48.2 | 3.5 | 51.7 | 38.56 |
| Blackgram | 63.7 | 47.9 | 3.0 | 50.9 | 43.86 |

[a] Average of duplicate determinations. [b] Single determination (see text).

iodine at zero intersect × 100 × 100/sample weight in mg × 18.9).

**Determination of Degree of Polymerization of Isolated Amylose Samples.** Amylose was isolated from starch samples by the method of Lilo et al. (1964). The degree of polymerization of amylose molecules was determined by the procedure of Whelan (1964). The principle involved is an initial acid hydrolysis of isolated amylose at 70 °C followed by oxidation with periodate. Small aliquots of acid hydrolysate after neutralization were oxidized with periodate at different intervals. The mean rate of liberation of formaldehyde was measured using maltotetraose (Sigma Chemicals) of a known degree of polymerization (dp), i.e. 4 glucose units, as a reference compound. The average degree of polymerization of each sample at different intervals of hydrolysis was thus determined. The linear relationship between 1/dp and time of hydrolysis was thus determined. Thus, the possible degree of polymerization at zero time of unhydrolyzed amylose could be determined for all four pulse amyloses.

## RESULTS AND DICSUSSION

Blood glucose patterns, after consuming Bengalgram and greengram, of the eight subjects are shown in Table I. In four of the eight subjects fed the Bengalgram meal, peak values were reached at 60 min or later. In the case of greengram in six of the eight subjects peak values for blood glucose were reached at 30 min.

The percentage increases in blood sugar (peak values) over the fasting level in each case are presented in Table II. The mean increase after greengram meal was 28.7% vs. 16.0% with Bengalgram. The difference between the two values is statistically significant ($P < 0.05$). These data may be considered as supporting the earlier in vitro observation that carbohydrates in greengram are more rapidly digested and more easily available than those in Bengalgram (loc cit).

Values for total carbohydrates, starch, and soluble reducing sugars are presented in Table III. The total carbohydrate content ranged from 58 to 63%. Starch and soluble reducing sugars which represent available carbohydrates varied between 50 and 57% in the different pulses. The available carbohydrate content of greengram was found to be higher than that of the other three pulses studied. The difference between total carbohydrate (determined by acid hydrolysis) and available carbohydrates comprising starch and soluble sugars may be explained on the basis of the observations of Nigam and Giri (1961) who have shown that these legumes contained unusual nonreducing oligosaccharides of higher molecular weight such as raffinose, stachyose, and verbascose.

**Amylose Content of Starches.** Data on the amylose contents of the four legumes are also summarized in Table III. Greengram was found to have the lowest and Ben-

Table IV. Oxidation of Acid Degraded Amyloses from Pulses with Sodium Metaperoxide

| Source | Time of acid hydrolysis, h | Rate of form.[a] of formaldehyde on oxidtn of hydrolyzed amylose, mol/h per D-glucose residue | Av dp of hydrolyzed amylose | $10^3$/dp |
|---|---|---|---|---|
| Bengalgram | 1 | 4.46 | 95.94 | 10.42 |
| | 2 | 10.80 | 39.69 | 25.23 |
| | 3 | 14.20 | 30.14 | 33.18 |
| | 4 | 18.92 | 22.62 | 44.20 |
| | 5 | 23.33 | 18.35 | 54.49 |
| Greengram | 1 | 2.52 | 169.8 | 5.88 |
| | 2 | 4.08 | 104.9 | 9.53 |
| | 3 | 6.04 | 66.88 | 14.95 |
| | 4 | 8.15 | 52.51 | 19.04 |
| | 5 | 9.84 | 43.49 | 22.99 |
| Redgram | 1 | 2.5 | 171.20 | 5.84 |
| | 2 | 4.0 | 107.00 | 9.35 |
| | 3 | 5.3 | 80.75 | 12.38 |
| | 4 | 7.7 | 55.58 | 17.99 |
| | 5 | 9.7 | 44.12 | 22.67 |
| Blackgram | 1 | 2.5 | 171.20 | 5.84 |
| | 2 | 4.1 | 104.39 | 9.58 |
| | 3 | 7.5 | 57.14 | 17.5 |
| | 4 | 9.9 | 43.23 | 23.13 |
| | 5 | 12.4 | 34.52 | 28.97 |

[a] The rate of formation of formaldehyde from maltotetraose (dp = 4) under the same conditions was 197 mmol of HCHO/h per D-glucose residue.

Table V. Chain Length (Degree of Polymerization) of Amylose from Pulses

| Source | $10^3$[a]/dp | dp |
|---|---|---|
| Bengalgram | 0.6 | 1667 |
| Greengram | 1.5 | 667 |
| Redgram | 1.85 | 540 |
| Blackgram | 0.25 | 4000 |

[a] Values correspond to $10^3$/dp at 0 h of hydrolysis calculated from Figure 1A-D.

galgram the highest content of amylose. This corresponds to the faster rate of $\alpha$-amylolysis observed in greengram as compared to that of Bengalgram in earlier in vitro studies, and the earlier peaking of blood sugar levels, and better utilization of carbohydrates in children with greengram in the present study. The amylose content of several varieties of rice determined by the same method (Srinivasa Rao, 1971) was found to be about 20%, a value much lower than that seen in pulses—a finding in line with the common experience that cereals are more easily digested than are pulses.

**Degree of Polymerization of Amylose Isolated from Legume Starches.** The degree of polymerization (dp) which is an indicator of chain length of the amylose molecule was determined using an authentic sample of maltotetraose with a known number of glucose units (i.e., 4 units) as the reference compound. The results of these analyses are presented in Table IV and Figure 1, which consists of plots for individual pulses, arriving at the degree of polymerization in each case. In Table V, the possible number of glucose units per amylose unit of these pulses are presented. Amylose units of the four different pulses vary widely in their degree of polymerization. Bengalgram and blackgram amylose had a higher number of glucose units than did amylose from greengram and redgram. The rates of in vitro $\alpha$-amylolysis, i.e. digestion with $\alpha$-amylase, seemed to be influenced by the degree of polymerization of their amylose unit. Amyloses with a greater degree of polymerization appeared to be more difficult to digest. It is pertinent to point out here that all analyses were carried out on freshly isolated samples to exclude the possibility of retrogradation.

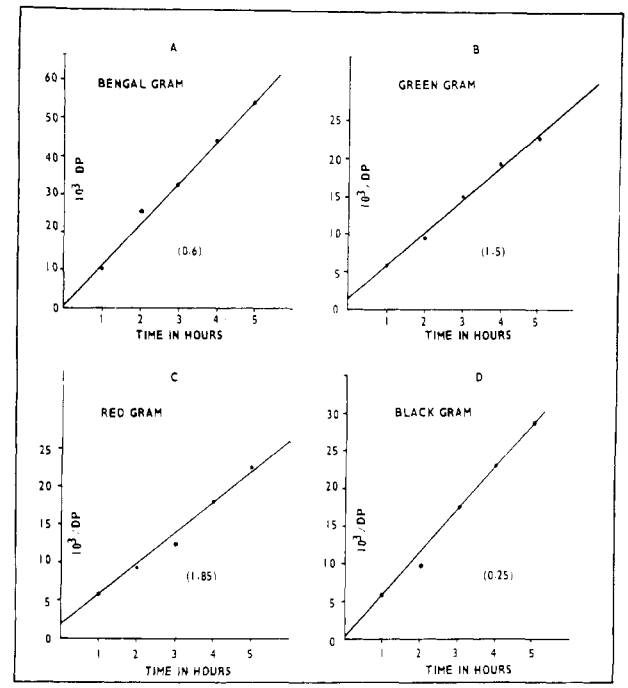


Figure 1. Relation between 1/dp and time of hydrolysis (in hours) of amylose samples. Values in the individual graph correspond to $10^3$/dp at 0 h of hydrolysis.

Langworthy and Deuel (1920) examined the digestibility of raw starches derived from corn, wheat, and potato in human subjects and found that potato starch was the least digestible of the three. Beazell et al. (1939) repeated these studies and found that a major part of the undigested starch was not excreted in feces of human subjects. They have presumed that this had undergone bacterial fermentation leading to flatulence. These workers, however, did not explain the reasons for the differences in digestibility. It later became evident from the work of Potter and Hassid (1948) that the chain length of potato amylose (980 glucose residues) was far different from that of either wheat or corn (540 and 490 glucose residues, respectively). These data thus show that the makeup of starch is an

important factor in the utilization of carbohydrates.

Data presented on the nature of starch with respect to amylose moiety and results of earlier studies on the in vitro digestibility of carbohydrates in these pulses and in vivo experiments in children clearly indicate that both the carbohydrate makeup and their utilization in different pulses are different and that legumes whose amylose has a longer chain length are difficult to digest.

Murphy (1963) implicated low molecular weight oligosaccharides such as raffinose and stachyose contained in beans as being responsible for flatulence. The pulses investigated here also have these oligosaccharides, but their concentrations are essentially similar in Bengalgram and greengram (Nigam and Giri, 1961) and these oligosaccharides by themselves cannot account for the higher amounts of flatus produced following the ingestion of Bengalgram (Narayana Rao et al., 1973).

These observations may be interpreted as suggesting that among the factors that can cause flatulence is the extent of digestibility of carbohydrates in pulses. It is, however, premature to conclude that poor digestibility may cause flatulence, although present observations on pulses and earlier work in the field of carbohydrate utilization point to such a possibility.

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# Fluorescence Behavior of Sterigmatocystin

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The fluorescence and absorbance spectra of sterigmatocystin and its *O*-methyl derivative were determined in buffered aqueous solutions and concentrated sulfuric acid solutions. The excited state species of sterigmatocystin undergoes phototautomerism prior to emission resulting in a zwitterionic structure. This mechanism was supported by the acidity dependent spectral shifts of sterigmatocystin and *O*-methylsterigmatocystin. Fluorescence intensity in sulfuric acid solutions was enhanced due to carbonyl protonation. Analysis in 60% sulfuric acid solution was demonstrated to have a sensitivity limit of 0.01 μg/ml.

Sterigmatocystin (I), a mycotoxin structurally and biosynthetically (Hsieh et al., 1973) related to the aflatoxins, has been demonstrated under laboratory conditions to be a causative agent in production of hepatocellular carcinoma in rats (Purchase and Van der Watt, 1970). Additionally, hepatotoxic effects have been observed with vervet monkeys (Van der Watt and Purchase, 1970). The fungus *Aspergillus versicolor* will produce sterigmatocystin under optimum conditions (Holzapfel et al., 1966), and the mycotoxin has occasionally been detected in agricultural commodities (Scott et al., 1972; Purchase and Pretorius, 1973).

Analytical methodology available for quantitation of sterigmatocystin levels includes a semiquantitative thin-layer chromatographic (TLC) method (Reiss, 1975) and a gas chromatographic assay (Manabe et al., 1971). The mycotoxin is reported to exhibit weak fluorescence on silica gel which may be magnified by an aluminum chloride treatment (Stack and Rodricks, 1971). This report details the fluorescence behavior of sterigmatocystin in various aqueous media and describes a fluorescence assay capable of detecting levels expected in contamination of agricultural commodities.

EXPERIMENTAL SECTION

**Apparatus.** Fluorescence measurements were obtained on a Turner, Model 430, recording spectrofluorometer equipped with a xenon light source. Absorption spectra were recorded using a Coleman 124 spectrophotometer.

**Materials.** Sterigmatocystin was obtained from cultures of *Aspergillus versicolor* (Vuill.) Tiraboschi, American Type Culture Collection no. 18643. The cultures were grown on Czapek Dox Agar for a period of 15 days. The mycelial mats along with the agar were extracted with

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