

to low trypsin inhibitor activity in the raw seeds and also to the inactivation of trypsin inhibitor on autoclaving.

A significant positive correlation (0.82) was observed between tyrosine released on autoclaving and average values of PER for different varieties. The grain-type variety Pb-1 has given the lowest PER and NPR values and this is due to high trypsin inhibitor activity in the raw seeds. Further, better nutritive values of vegetable-type varieties appear to be due to better amino acid patterns as a whole rather than any specific amino acid (Table III).

ACKNOWLEDGMENT

The authors are grateful to M. S. Naik of the Indian Agricultural Research Institute, New Delhi, for keen interest and encouragement and for providing facilities.

LITERATURE CITED

- Association of Official Agricultural Chemists, "Official Methods of Analysis", 8th ed, Washington, D.C., 1955.
- Bender, A. E., Doell, B. M., *Brit. J. Nutr.* **11**, 140 (1957).
- Bookwalter, G. N., Warner, K., Anderson, R. A., Mustakas, G. C., Griffin, E. L., Jr., *J. Food Sci.* **40**, 266 (1975).
- Borcheres, R., Ackerson, C. W., *Arch. Biochem.* **13**, 291 (1947).
- Bressani, R., Murillo, B., Elias, L. G., *J. Food Sci.* **39**, 577 (1974).
- Coppock, J., *J. Am. Oil Chem. Soc.* **51**, 59A (1974).
- Duggal, S. K., Eggum, B. O., Fifth International Symposium on Amino Acids, Budapest, 1977, p 1.
- Eggum, B. O., International Atomic Energy Agency, IAEA-SM-132/40, 1970, p 289.
- FAO/WHO, "Energy and Protein Requirements", Series No. 52, FAO, Rome, 1973.
- Fiske, C. H., Subbarow, Y., *J. Biol. Chem.* **66**, 375 (1925).
- Gupta, A. K., Kapoor, M., Deodhar, A. D., *J. Food Sci. Technol.* **13**(3), 133 (1976a).
- Gupta, A. K., Wahie, N., Deodhar, A. D., *Indian J. Nutr. Diet.* **13**, 244 (1976b).
- Henry, K. M., *Brit. J. Nutr.* **19**, 125 (1965).
- Kanthamani, S., "Tasty Recipes from Soybean", Directorate of Extension and USAID, J.N.K.V.V., Jabalpur, India, 1970.
- Kakade, M. L., Simons, N. R., Liener, I. E., Lambert, J. W., *J. Agric. Food Chem.* **20**, 87 (1972).
- Kapoor, A. C., Gupta, Y. P., *J. Food Sci.* **40**, 1162 (1975).
- King, E. J., *Biochem. J.* **26**, 292 (1932).
- Maneepum, S., Luh, B. S., Rucker, R. B., *J. Food Sci.* **39**, 171 (1974).
- Manna, L., Hauge, S. M., *J. Biol. Chem.* **202**, 91 (1953).
- McLaughlan, J. M., Rao, S. V., Noel, F. J., Morrison, A. B., *Can. J. Biochem.* **45**, 31 (1967).
- Mitchel, H. H., Block, R. J., *J. Biol. Chem.* **163**, 599 (1946).
- Moore, S., Stein, W. H., *J. Biol. Chem.* **211**, 892 (1954).
- Morse, W. J., "Soybean and Soybean Products", Markely, I. K. S., Ed., Wiley, New York, N.Y., 1950.
- Mustakas, G. C., Albrecht, W. J., McGhee, J. E., Black, L. T., Bookwalter, G. N., Griffin, E. L., Jr., Abstract Paper 101, 41st Fall Meeting of the American Oil Chemists' Society, Chicago, Ill., 1967, Mustakas, G. C., Albrecht, W. J., Bookwalter, G. N., McGhee, J. E., Kwolek, W. F., Griffin, E. L., Jr., *Food Technol.* **24**, 102 (1970).
- Osborne, T. B., Mendel, L. B., Ferry, E. L., *J. Biol. Chem.* **37**, 223 (1919).
- Oser, B. L., *J. Am. Diet. Assoc.* **27**, 396 (1951).
- Panemangalore, M., Parthasarathy, H. N., Joseph, K., Sankaran, A. N., Rao, M. N., Swaminathan, M., *Can. J. Biochem.* **42**, 641 (1964).
- Rathod, K. L., Williams, S. W., *Indian J. Nutr. Diet.* **10**, 18 (1973).
- Sidwell, V. D., Stillings, B. R., Knohl, G. M., Jr., *Food Technol.* **24**, 40 (1970).
- Sikka, K. C., Johri, R. P., Duggal, S. K., Ahuja, V. P., Austin, A., *J. Agric. Food Chem.* **23**, 24 (1975).
- Singh, R., *Sci. Rep.* **12**, 137 (1975).
- Spies, J. R., Chambers, D. C., *Anal. Chem.* **21**, 1240 (1949).
- Villegas, A. M., Gonzalez, A., Galderon, R., *Cereal Chem.* **45**, 379 (1968).

Received for review May 20, 1977. Accepted August 19, 1977.

Oligosaccharides in Pulses: Varietal Differences and Effects of Cooking and Germination

P. Udayasekhara Rao* and Bhavani Belavady

Oligosaccharide content of four commonly used Indian pulses [red gram or pigeon pea (*Cajanus cajan*), Bengal gram or chick pea (*Cicer arietinum*), black gram (*Phaseolus mungo*), and green gram (*Phaseolus aureus*)] was estimated. Red gram and green gram had significantly higher amounts of verbascose and stachyose than did Bengal gram and black gram. Bengal gram had higher amounts of raffinose. There were significant variations within varieties of the same pulse. Cooking brought about a significant increase in the oligosaccharide content of all the pulses. However, the increase was highest in Bengal gram and least in green gram. Germination decreased the oligosaccharide content in all the pulses. A systematic study needs to be undertaken to understand the precise role of oligosaccharides in flatulence production.

Habitual Indian diets contain pulses, and the average per capita consumption is around 40 g daily (Gopalan et al., 1971). While they are good sources of protein, many common varieties of pulses are known to contain antinutritional factors such as trypsin inhibitors (Borcheres et al., 1947), hemagglutinins (Huprikar and Sohoni, 1961), cyanogenic agents (Sharpless et al., 1939), and saponins

(Birk et al., 1963) which may affect the utilization of pulse protein. Many of these factors are heat labile and may therefore have little relevance in human nutrition. Oligosaccharides of the raffinose family, in which galactose is present in α -linkage, are present in mature legumes (Shallenberger and Moyer, 1961) and have been shown to be responsible for flatulence following the consumption of these beans (Steggerda, 1968). Unlike trypsin inhibitor and other antinutritional factors, oligosaccharides are heat stable. Isolated oligosaccharides have been shown to induce flatulence in experimental animals and in man (Calloway, 1973).

*National Institute of Nutrition, Indian Council of Medical Research, Jamai-Osmania, Hyderabad-500007, India.

Table I. Varietal Differences in the Free Sugar Content of Pulses

Pulses and variety	Verbascose + stachyose	Raffinose	Sucrose	Fructose	Glucose
	g/100 g of pulse				
Red gram (Pigeon pea)					
1. Agathi	3.6	0.8	1.3	0.4	0.1
2. ST-1	2.3	1.2	1.6	0.2	0.1
3. T-21	2.3	1.4	1.3	0.1	0.01
4. No. 148	2.3	0.7	0.6	0.2	0.1
5. Upas	2.2	1.3	1.6	0.4	0.1
6. Hy-4	1.9	1.8	1.2	0.3	0.01
7. Prabhat	1.6	1.2	1.1	0.3	0.02
8. Hy-2	1.5	0.7	0.8	0.3	0.1
9. Hy-3-A	1.5	0.6	1.4	0.3	0.1
10. Hy-5	1.3	0.4	1.2	0.3	0.1
11. PM-1	1.3	0.3	0.9	0.2	0.1
12. Hy-1	1.2	0.6	1.5	0.1	0.1
Mean ± S.E.	1.9 ± 0.19	0.9 ± 0.13	1.2 ± 0.09	0.3 ± 0.03	0.1 ± 0.01
Bengal gram (Chick pea)					
1. JG-62	1.8	3.0	1.4	0.3	0.1
2. Beg-482	1.4	2.7	1.7	0.3	0.04
3. Pant-104	1.3	2.1	1.3	0.2	0.1
4. Chaffa	1.2	1.9	2.0	0.2	0.03
5. CPS-2	1.0	1.7	1.7	0.1	0.03
6. Annegiri	0.8	1.9	1.3	0.1	0.1
7. 9-3	0.8	1.3	1.1	0.2	0.1
8. J-62	0.8	0.8	0.8	0.2	0.03
Mean ± S.E.	1.1 ± 0.13	1.9 ± 0.26	1.4 ± 0.13	0.2 ± 0.03	0.1 ± 0.01
Black gram					
1. T-9	1.8	0.4	0.5	Tr	Tr
2. K-10	1.7	0.5	1.5	Tr	Tr
3. US-132	1.6	0.7	0.7	Tr	Tr
4. G-31	1.6	0.5	0.4	Tr	Tr
5. H-70-3	1.5	0.7	0.4	Tr	Tr
6. Pant-30	1.2	0.7	1.0	Tr	Tr
7. G-104	1.0	0.2	0.1	Tr	Tr
8. G-1	1.0	0.8	0.5	Tr	Tr
9. US-228	0.6	0.3	0.2	Tr	Tr
Mean ± S.E.	1.3 ± 0.13	0.5 ± 0.07	0.6 ± 0.14	Tr	Tr
Green gram					
1. S-8	2.6	0.5	1.0	0.4	0.4
2. B-1	2.5	0.8	1.0	0.2	0.2
3. T-44	2.5	0.7	2.1	0.3	0.2
4. G-65	2.4	0.2	0.9	0.2	0.2
5. PS-16	2.1	0.5	1.2	0.3	0.2
6. L-84	1.8	0.7	1.0	0.3	0.2
7. HB-45	1.8	0.7	0.5	0.3	0.2
Mean ± S.E.	2.2 ± 0.13	0.6 ± 0.07	1.1 ± 0.18	0.3 ± 0.02	0.2 ± 0.03

Extensive studies have been carried out on the oligosaccharide content and the flatulence-producing capacity of soybeans (Murphy, 1973). However, information on the oligosaccharide content of pulses commonly consumed in India is scanty and data on the effects of some of the traditional methods of processing on the oligosaccharide profile are even more scanty. In the present investigation high-yielding varieties of four commonly consumed legumes—red gram (*Cajanus cajan*), Bengal gram (*Cicer arietinum*), green gram (*Phaseolus aureus*), and black gram (*Phaseolus mungo*)—were analyzed for their oligosaccharide content. Since pulses are consumed mainly after cooking and sometimes after germination, the effect of these two processes on their oligosaccharide content was investigated.

MATERIALS AND METHODS

Twelve varieties of red gram, eight varieties of Bengal gram, nine varieties of black gram, and seven varieties of green gram were analyzed for their oligosaccharide content. They were obtained from the Andhra Pradesh Agricultural University, Hyderabad. Oligosaccharides were extracted from powdered whole pulse according to the method of Nigam and Giri (1961). The oligosaccharides were separated on Whatman No. 1 chromatographic paper by descending chromatography using propanol-ethanol-water

(70:10:20) (Tharanathan et al., 1975). A standard sugar mixture containing glucose, fructose, sucrose, raffinose, and stachyose was simultaneously run. The sugars were located by the end strip method and their concentrations estimated colorimetrically by the phenol-sulfuric acid method (Dubois et al., 1956).

Cooking. Five grams of the whole pulse were cooked with adequate amount of water (10 mL) at 15 lb of pressure for 15 min. Oligosaccharides were extracted and estimated as described earlier; during the process of cooking most of the water had been absorbed and the oligosaccharides were extracted from the pulse along with the remaining water.

Germination. The pulse samples were washed with mercuric chloride to remove surface contamination. They were soaked overnight in water and were allowed to germinate in sterile petri dishes lined with wet filter paper. Oligosaccharides were estimated in overnight soaked samples and after 24, 48, and 72 h of germination.

RESULTS

The separation of verbascose and stachyose was not complete in all samples. These two sugars were therefore eluted and estimated together as a single constituent.

The free sugar content of the various pulses are given in Table I. Red gram and green gram contained higher

Table II. Effect of Cooking on Oligosaccharide Content of Pulses

	No. of samples	Verbascose + stachyose		Raffinose		Sucrose	
		Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking
g/p 100 g of pulse							
Red gram (Pigeon pea)	4	2.0 ± 0.15 ^a	3.3 ± 0.56	1.3 ± 0.22	2.4 ± 0.44	1.0 ± 0.16	4.4 ± 0.37
Bengal gram (Chick pea)	4	1.3 ± 0.19	2.7 ± 0.27	2.4 ± 0.30	4.4 ± 0.48	1.6 ± 0.14	3.1 ± 0.92
Black gram	4	1.6 ± 0.07	2.7 ± 0.25	0.5 ± 0.07	0.7 ± 0.03	0.7 ± 0.26	2.6 ± 0.63
Green gram	4	2.4 ± 0.10	3.1 ± 0.26	0.5 ± 0.09	0.8 ± 0.05	1.3 ± 0.05	2.7 ± 0.59

^a Values given are mean ± S.E.

Table III. Effect of Germination on Oligosaccharide Content of Pulses

	Germination period			
	0 h	24 h	48 h	72 h
g/100 g of pulse				
Red gram (4) ^a				
Verbascose + stachyose	2.0 ^b (1.6-2.3)	1.0 (0.6-1.6)	0.5 (0.3-0.7)	0.2 (0-0.4)
Raffinose	1.3 (0.7-1.7)	0.8 (0.6-0.9)	0.4 (0.3-0.7)	0.2 (0-0.4)
Bengal gram (4)				
Verbascose + stachyose	1.1 (0.8-1.4)	0.6 (0.4-0.7)	0.3 (0.1-0.5)	0.1 (0-0.2)
Raffinose	2.4 (1.9-3.0)	1.1 (0.8-1.3)	0.6 (0.2-0.9)	0.3 (0.1-0.5)
Black gram (3)				
Verbascose + stachyose	1.7 (1.6-1.8)	0.9 (0.7-1.0)	0.3 (0.1-0.5)	0.1 (0-0.3)
Raffinose	0.5 (0.4-0.5)	0.3 (0.2-0.3)	0.1 (0.1-0.2)	0.1 (0-0.2)
Green gram (3)				
Verbascose + stachyose	2.4 (2.1-2.6)	0.6 (0.4-0.7)	0.3 (0.2-0.4)	0.3 (0.2-0.4)
Raffinose	0.6 (0.5-0.7)	0.4 (0.2-0.5)	0.3 (0.1-0.4)	0.2 (0.1-0.3)

^a Numbers in parentheses indicate number of samples analyzed. ^b Values given are mean with range in parentheses.

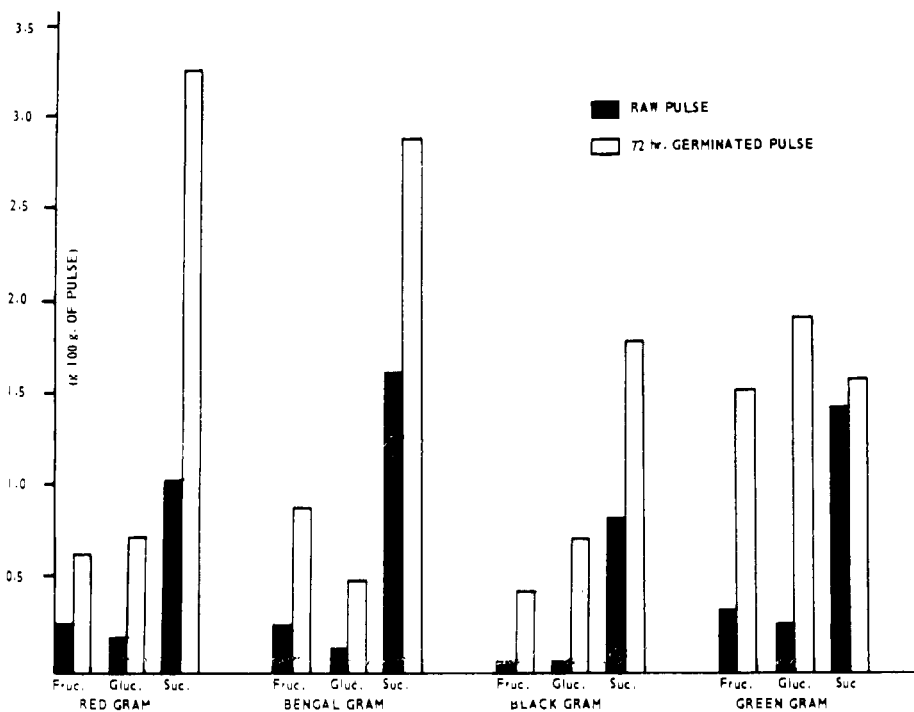


Figure 1. Effect of germination of fructose (Fruc.), glucose (Gluc.), and Sucrose (Suc.) content of red gram or pigeon pea (*Cajanus cajan*), Bengal gram or chick pea (*Cicer arietinum*), green gram (*Phaseolus aureus*), and black gram (*Phaseolus mungo*).

amounts of verbascose and stachyose (V + S) than did Bengal gram and black gram. The raffinose content of Bengal gram was significantly higher than that of the other three pulses. Black gram had significantly lower amounts of sucrose than did the other three pulses, and both glucose and fructose were present only in trace amounts. The oligosaccharide and the sucrose contents showed wide varietal differences in the same pulse.

Cooking brought about an increase in the concentrations of all the oligosaccharides and sucrose and a reduction in glucose and fructose concentrations, in all the four pulses studied (Table II). The mean increase in the oligosaccharide content after cooking was greatest in Bengal gram and the least in green gram.

Following germination, the verbascose, stachyose, and raffinose content of pulses showed a fall (Table III), while sucrose, glucose, and fructose contents showed an increase (Figure 1). Germination for 24 h brought about a greater decrease (around 75%) in verbascose and stachyose content of green gram than in the other three pulses, which was around 50% on the average. Such differences were not observed in the raffinose content.

DISCUSSION

Considerable varietal differences were observed in the oligosaccharide content of the four pulses studied here, an observation similar to that reported for soybeans (Leng, 1973). Narayana Rao et al. (1973) studied flatus production in children after feeding them diets containing large amounts of one of four pulses (red gram, Bengal gram, black gram, and green gram) and observed that the capacity to induce flatus could be graded in the following order: Bengal gram > black gram > red gram > green gram. The oligosaccharide content of the pulses used by these authors were not reported. The mean value of the oligosaccharide content of the four pulses obtained in the present study did not show much significant differences. It is possible, however, that in the particular varieties used in their study the oligosaccharide content of the pulses may have varied considerably. Factors other than oligosaccharides present in the pulses may also influence flatulence. Wagner et al. (1976) reported that the oligosaccharide free residue of California small white beans could produce flatus and that the flatus production was more when the extracted oligosaccharides were fed along with the residue than when each of them were fed separately. Helendoorn (1969) also reported that the oligosaccharide-free residue of "brown" beans was more effective in flatus production than was extracted oligosaccharides.

Cooking brought about an increase in oligosaccharide content. The source of the increased oligosaccharide needs explanation. The oligosaccharides are estimated in the alcoholic extract of the pulses. It is known that such a procedure can only extract simple sugars and oligosaccharides (Steggerda, 1968). In the raw pulse it is possible that a part of the oligosaccharides are present in bound form, bound either to proteins or other macromolecules, or be present as constituents of high molecular

weight polysaccharides. Cooking might have affected these bonds and led to the release of the oligosaccharides. Recently high molecular weight α -galactosides have been reported to be present in some legumes (Cerning-Beroard and Filiatre, 1976). Nigam and Giri (1961) have reported increased α -galactosidase activity in germinated pulses which is responsible for the decreased oligosaccharide content of germinated pulses. Venkataraman and Jaya (1975) had observed a decrease in flatus production when rats were fed cooked pulses and an increase in flatus production when germinated cooked Bengal gram was fed. These results are in variance with the effects observed on the oligosaccharide content of pulses in the present study.

It would thus appear that a systematic study on the flatus-producing factors in pulses and the effects of processing on them is essential to understand the precise relationship that exists between flatus production and the oligosaccharide content of pulses.

ACKNOWLEDGMENT

The authors acknowledge with thanks the cooperation received from M. Sriramulu, Pulse Geneticist, A.P.A.U., Hyderabad, in getting the pulse samples. They thank S. G. Srikantia, Director, National Institute of Nutrition, for his interest in this work.

LITERATURE CITED

- Birk, Y., Bondi, A., Gestetner, B., Ishaaya, I., *Nature (London)* **197**, 1089 (1963).
- Borchers, R., Ackerson, C. W., Sandstedt, R. M., *Arch. Biochem.* **12**, 367 (1947).
- Calloway, D. H., Proceedings of the Symposium on Nutritional Improvement of Food Legumes by Breeding, sponsored by PAG at FAO, Rome, Italy, July 3-5, 1972, p 263 (1973).
- Cerning-Beroard, J., Filiatre, A., *Cereal Chem.* **53**, 968 (1976).
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F., *Anal. Chem.* **28**, 350 (1956).
- Gopalan, C., Balasubramanian, S. C., Ramasastry, B. V., Visweswara Rao, K., Diet Atlas, National Institute of Nutrition, India, (1971).
- Hellendoorn, E. W., *Food Technol.* **23**, 795 (1969).
- Huprikar, S. V., Sohoni, K., *J. Sci. Ind. Res., Sect. C* **20**, 82 (1961).
- Leng, E. R., Proceedings of the Symposium on Nutritional Improvement of Food Legumes by Breeding; sponsored by PAG at FAO, Rome, Italy, July 3-5, 1972, p 277 (1973).
- Murphy, E. L., Proceedings of the Symposium on Nutritional Improvement of Food Legumes by Breeding, sponsored by PAG at FAO, Rome, Italy, July 3-5, 1972, p 273 (1973).
- Narayana Rao, M., Shurpalekar, K. S., Sundaravalli, E. E., Doraiswamy, T. R., *PAG Bull.* **3**, 53 (1973).
- Nigam, V. N., Giri, K. V., *Can. J. Biochem. Physiol.* **39**, 1847 (1961).
- Sharpless, G. R., Pearsons, J., Prato, G. S., *J. Nutr.* **17**, 545 (1939).
- Shallenberger, R. S., Moyer, J. C., *J. Agric. Food Chem.* **9**, 137 (1961).
- Steggerda, F. R., *Ann. N.Y. Acad. Sci.* **150**, 57 (1968).
- Tharanathan, R. N., Wankhede, D. B., Raghavendra Rao, M. R., *J. Sci. Food Agric.* **26**, 749 (1975).
- Venkataraman, L. V., Jaya, T. V., *Nutr. Rept. Int.* **12**, 387 (1975).
- Wagner, J. R., Becker, R., Gumbmann, M. R., Olson, A., *J. Nutr.* **106**, 466 (1976).

Received for review May 13, 1977. Accepted September 23, 1977.