Effect of Several Treatments on the Oligosaccharide Content of a Brazilian Soybean Variety [*Glycine max*. (L.) Merril]

A study was carried out on the effect of different treatments such as soaking in water, cooking in an autoclave, soaking plus cooking, and soaking plus seed coat removal plus cooking for different periods of time and at different soybean to water ratios on the oligosaccharide content of soybeans [*Glycine max*. (L.) Merril, var. Santa Rosa]. Residual sucrose, raffinose, and stachyose levels were determined by thin-layer chromatography in soybeans submitted to these treatments as well as in dry beans. Statistical analysis of the results showed that soaking in water and seed coat removal had no significant effect on oligosaccharide content, whereas cooking decreased the oligosaccharide amounts originally present in dry beans. The most effective cooking time was 90 min and the most effective soybean to water ratio was 1:10. Under these conditions, significant reduction was obtained in the sucrose (85.3%), raffinose (86.5%), and stachyose (86.7%) content of soybeans.

The main oligosaccharides present in soybeans are sucrose, raffinose, and stachyose (Smith and Circle, 1972). Considerable evidence that raffinose and stachyose are factors responsible for the flatulence and abdominal discomfort often experienced after ingestion of soybeans and their products has been reported in the literature (Rackis et al., 1970; Cristofaro et al., 1974). Fleming (1981), in a study of seven different types of legume seeds, showed significant positive correlation between hydrogen production and the following chemical components: stachyose, raffinose plus stachyose, glucans, and pentosanes hydrolyzable in diluted acids. The same investigator observed significant negative correlation between hydrogen production and amide and lignine content.

Oligosaccharides of soybeans have been generally considered undesirable, and many attempts have been made to remove them from seeds and their products or to degrade them by methods such as fermentation, treatment with commercial enzyme preparations, germination, soaking, combined soaking and germination, and others.

Ku et al. (1976) showed that soybean cooking for different periods of time and at different soy to water ratios permitted the removal of oligosaccharides. Silva and Braga (1982) also observed that cooking decreased the oligosaccharide content of a variety of beans (*Phaseolus vulgaris* L.) consumed in Brazil. Garg et al. (1980) reported that cooking of chickpeas and peas caused a decrease in gas production in relation to that of uncooked seeds. In contrast, Rao and Belavady (1978) reported that cooking increased the oligosaccharide content of four different legume seeds consumed in India, and more recently, Reddy et al. (1980) detected a slight increase in the stachyose and sucrose content of black gram (*Phaseolus mungo* L.) cotyledons and seeds after cooking.

Since the preparation of soy for consumption requires soaking of the seeds in water prior to cooking with or without seed coats, the objective of the present study was to evaluate the effect of these procedures on the final oligosaccharide content of soybean seeds also in view of the controversy existing on this subject and the lack of studies of this type on legume seeds in Brazil.

MATERIALS AND METHODS

Soybean Seeds. Yellow soybean seeds [*Glycine max*. (L.) Merril], Santa Rosa variety, were supplied by Faculdade de Ciencias Agrarias e Veterinaria de Jaboticabal, UNESP, Jaboticabal, SP, Brazil. The moisture content was 7.04%. On a dry weight basis, the seed composition was 4.5% ash, 20.7% fat, 38.2% protein, 5.10% crude fiber, and 24.5% total carbohydrates.

Soaking. Soybean samples were soaked in water at 1:3 and 1:10 bean to water ratios for 3, 6, 12, 18, and 24 h at 25 °C (room temperature).

Cooking. Other soybean samples were cooked in an autoclave at 120 °C and at a pressure of 1 kg/cm^2 at 1:3 and 1:10 soybean to water ratios for 30, 60, and 90 min.

Soaking plus Cooking with and without Seed Coats. Other soybean samples were soaked for 12 h as described above, the soaking water was discarded, and the samples, with and without coats, were cooked in fresh water for 90 min as described above.

Oligosaccharide Extraction, Identification, and Quantitation. Whole soybean seeds were pulverized with a manual disk mill. Soaked and/or cooked seeds as well as cotyledons were transferred to a blender and homogenized. Appropriate samples of these materials were brought to a boil under reflow with 100 mL of an aqueous solution of 80% ethanol for 1 h. The residue was separated and heated with a fresh 100-mL amount of 80% ethanol for an additional 30 min, filtered, and washed with 200 mL of 80% ethanol. The extracts were combined and treated as described in a previous paper (Silva and Braga, 1982) for oligosaccharide identification and quantification by thin-layer chromatography.

Moisture. For moisture determination, the cooked and/or soaked seeds were quickly crushed in a mortar after drying with absorbent paper and transferred in known weights to an air oven where they were heated as recommended by AOAC (1975).

Statistical Analysis. The data were analyzed statistically by the Student's t test and by the Tukey test.

RESULTS AND DISCUSSION

Soaking promoted no reduction in oligosaccharide content regardless of the time or soybean to water ratio used since during soaking times of 3, 6, 12, 18, and 24 h, at both soybean to water ratios, the amounts that remained in the soaked beans were statistically equal ($P = 0.05$) to those originally present in the dry beans, i.e., 4.35% sucrose, 0.74% raffinose, and 3.53% stachyose (g/100 g of seeds, on a dry weight basis). The effect of soaking on soy oligosaccharides has been studied by other investigators (Kim et al., 1973; Wang et al., 1979; Hand, 1966), but their results cannot be compared with ours because of the different conditions used in each study. In contrast, when the seeds were cooked, time and soybean to water ratio had a significant effect at the 5% level on oligosaccharide content (Table I). A cooking time of 90 min and the 1:10 soybean to water ratio were the most effective in reducing oligosaccharide levels. Ku et al. (1976) showed that cooking

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Table I. Oligosaccharide Content of Soybean Seeds Cooked in Water for Different Periods of Time and at Different Soybean to Water Ratios

cooking time, min	residual oligosaccharides, g/100 g of seeds, on a dry weight basis					
	sucrose		raffinose		stachyose	
	1:3 ratio	1:10 ratio	1:3 ratio	1:10 ratio	1:3 ratio	1:10 ratio
0	4.35 a[a]	4.35 a	0.74 a	0.74 a	3.53 a	3.53 a
30	2.34 b	1.27 b	0.37 b	0.24 c	2.03 b	1.05 d
60	2.09 b	1.09 d	0.31 b	0.22 c	1.82 b	1.04 d
90	1.89 c	0.64 e	0.30 b	0.10 d	1.68 c	0.47 e

[a] For each oligosaccharide, means followed by different letters in the column or in the row were statistically different at the 5% level of significance.

of whole soybean seeds in a 1:10 soybean to water ratio removed variable percentages of raffinose and stachyose depending on cooking time. According to these investigators, the solubility, molecular weight, location, and natural binding form of the sugars within the cell all play an important part in the extent to which saccharides are extracted from soybean seeds. The alternative treatments of soaking in association with cooking of coated and uncoated seeds showed no significant difference at the 5% level. On a dry weight basis, 1.46% sucrose, 0.32% raffinose, and 1.28% stachyose were detected in seeds soaked for 12 h and then cooked for 90 min with or without coats in fresh water at the 1:3 soy to water ratio, whereas 0.76% sucrose, 0.11% raffinose, and 0.67% stachyose were detected when the 1:10 soy to water ratio was used.

The reduction in oligosaccharide content, on a percentage and dry weight basis, produced by cooking the seeds at the most effective soy to water ratio was 83.0% for sucrose, 86.5% for raffinose, and 86.7% for stachyose, which are statistically identical values ($P = 0.05$).

In view of these data, we may conclude that soybean soaking before cooking is superfluous for the reduction of saccharides as long as cooking is carried out for 90 min using a 1:10 soybean to water ratio. Coat removal from soaked seeds before cooking is also ineffective.

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Registry No. Sucrose, 57-50-1; raffinose, 512-69-6; stachyose, 470-55-3.

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Chemical and Biochemical Nature of Fodder Tree Leaf Tannins

The chemical nature and biochemical nature of tannins such as the relative degree of polymerization (estimated from the ratio of total flavon-3-ol residues to terminal flavon-3-ol residues), protein precipitating capacity as tannic acid equivalents, and relative tannin specific activity (estimated from the ratio of absorbance in the protein precipitation assay to absorbance in assay of total phenols), in relation to crude protein digestibility of fodder tree leaves, are determined. The results suggest that the tannin content and their protein precipitating capacity, not the polyphenolic with a high relative degree of polymerization, reflect on the crude protein digestibility in a negative relationship.

Fodder obtained from tree/shrub leaves (top feed) has been classified as emergency fodder for livestock, but it forms an integral part of feed for ruminants in arid and semiarid regions. In general, top feeds are a good source of protein for grazing animals (Jain and Beniwal, 1982). However, in some cases not only their crude protein digestibility has been observed to be low (Nath et al., 1969; Lohan et al., 1980; Diagayete, 1981) but also several episodes of loss of livestocks have been ascribed to the presence of a high quantity of tannins in them (Wolter,

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