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Oligosaccharide Composition and Trypsin Inhibitor Activity of *P. vulgaris* and the Effect of Germination on the α-Galactoside Composition and Fermentation in the Human Colon

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ABSTRACT

Ten varieties of P. vulgaris *were analyzed for their oligosaccharide composition and trypsin inhibitor activity. One of these varieties was studied further to verify the effect of cooking time and germination on trypsin inhibitor activity and oligosaccharide contents. This sample was also submitted to a group of volunteers to compare untreated and three days-germinated beans as to their flatulence capacity, by measuring H_2 production in the expired air. Cooking for 60 min was sufficient to inactivate over 90% of trypsin inhibitors but promoted only about 15% of α-galactoside loss. Germination promoted a progressive decrease of α-galactosides with 77% loss after the third day. Germination for 3 days proved to be efficient to reduce flatulence with all volunteers showing no H_2 responses above the cut off level. Cooking and germination may be useful procedures to inactivate trypsin inhibitors and also to decrease flatulence in legumes.*

INTRODUCTION

Legumes are good and relatively inexpensive sources of protein and energy and they represent an important food item for developing countries. Black bean is one of the most important legumes consumed in South America, particularly in Brazil (IBGE, 1978). However, it presents some undesirable characteristics which somehow may limit its wider use especially as an

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ingredient of infant diets or formulas for recovery of undernourished children. Anti-nutritional factors and α-galactosides are examples of undesirable components present in legumes (Rachie, 1973). Oligosaccharides of the raffinose family have been quoted as flatulence producers due to the absence of α-galactosidases in the human intestine, consequently, undergoing bacterial fermentation (Jood *et al.*, 1986).

Germination appears to be a potential method to reduce flatulence caused by legumes since during this process α-glactosides will be degraded producing available sugars (King & Puwastien, 1987). Besides, it increases the nutritive value of the seeds and does not require sunlight or soil, with high production yield (Chen *et al.*, 1975).

Extensive work has been carried out by Calloway and her group (1971) on the measurement of flatulence, based on gas chromatography determination of specific gases formed by fermentation in the human intestine, particularly methane, hydrogen and CO_2 by monitoring flatus and breath samples. The hydrogen determination in breath samples seems to be an easy and reliable approach for assessing gas-forming properties of legumes (Solomons, 1983, 1984).

In the present work the oligosaccharide composition of different Brazilian cultivars of *P. vulgaris* was determined and one variety of black bean was studied regarding the effect of germination on the oligosaccharide contents. Non-germinated and germinated beans were also compared in relation to the production of hydrogen in the large intestine of volunteers by the analysis of expired breath air samples using the hydrogen breath test. In addition, trypsin inhibitor activity was determined in the different cultivars studied and for monitoring the cooking time of germinated beans.

MATERIALS AND METHODS

Samples

Samples of 10 cultivars of *Phaseolus vulgaris*, Jalo, Rio Fato Tibagi, Rio Negro, Vermelho, FT 83-25, FT 83-100, RAI 103, RAI 180, FT 120 and CNF 0178, were supplied by the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA, Brazil). All samples were milled to pass a 0·75 mm sieve prior to analysis. Whole grains of sample CNF 0178 were also used for processing (cooking and germination) and for the biological assays with volunteers.

Trypsin inhibitor activity

Trypsin inhibitor activity was measured according to the method of Kakade *et al.* (1974) using bovine trypsin (Merck, FRG) and *N*-benzoyl-DL-arginine-

p-nitroanilide (Merck) as substrate. The trypsin inhibitor activity was expressed as inhibited trypsin units (ITU) per mg of dried sample.

Oligosaccharide

Extraction of oligosaccharides was carried out following the procedure described by Macrae and Zand-Moghaddam (1978) including a further clarification step by means of activated charcoal (0·2 g for 20 ml of extract). The extract was then filtered through a Millipore 0·45 μm filter and the filtrate used directly for chromatography. Chromatography was performed using a Knauer (FRG) chromatograph consisting of a pump, a refractive index detector and a Reodhyne injection valve with a 20 μl fixed loop. Packed column (250 × 4·6 mm id) and a guard column (30 × 4·6 mm id), filled with Spherisorb-NH_2-5 μm stationary phase were also supplied by Knauer. Mobile phase was acetonitrile/water (72:28 v/v) at a flow rate of 1·5 ml/min. Quantification was by comparison of peak height with external standard solutions of sucrose, raffinose (Merck) and stachyose (C. Roth, FRG).

Cooking

Batches from sample CNF 0178 were soaked in distilled water for 16 h at room temperature, with grain/water in the proportion of 1:3 (w/v). The soaking water was discharged and the seeds were cooked at 100°C (1:3 w/v) for 15, 30, 80 and 90 min. Cooked samples were used for chemical analysis after freeze-drying and milling. The 60 min-cooked sample was also used for biological assays after addition of salt.

Germination

Germination of the grains (batches from sample CNF 0178) was carried out according to the method of Khaleque *et al.* (1985) at 25°C in the dark up to 3 days. Germinated samples (3 days) used in the biological assay were kept frozen until the trial when they were cooked and submitted to the volunteers. Germinated samples for chemical analysis were freeze-dried, milled and kept frozen until analyzed.

Biological assay (hydrogen breath test)

The fermentation of flatulent sugars was monitored in human volunteers by measuring the hydrogen produced in the large intestine and eliminated through expired air at different time intervals after a challenge with cooked non-germinated and germinated black beans. General procedures and care

employed in the hydrogen breath test (HBT) were based on the work of Solomons (1984). Fourteen healthy adult volunteers participated in this assay. Subjects had no recent history of gastrointestinal disorders or antibiotics usage. They were selected from an initial larger group after measuring their breath hydrogen response to water (100 ml) and to lactulose (100 ml of a 10% w/v solution in water) following an overnight fast. Positive responders to water (increments higher than 20 ppm in breath hydrogen above basal levels) and/or negative responders to lactulose (increments lower than 20 ppm) from the initial group did not take part in the hydrogen breath test with the beans.

The selected volunteers received a challenge meal of cooked beans, equivalent to 70 g uncooked beans, after an overnight fast. Samples of expired alveolar air were then collected for 8 h at 30 min intervals, by breathing through a low-resistance one-way valve connected to an appropriate collection bag of aluminium foil (Quintron Instruments, USA). Specimens of the expired air were transferred immediately to 50 ml plastic syringes fitted with a three-way stop-cock and stored until analysis, which was performed at the end of the test day. Baseline samples of expired air were obtained before the challenge. During the test, the volunteers were only allowed to drink water and no other food was consumed except for a standard meal of polished rice cooked in non-fat reconstituted lactose-hydrolysed dry milk (LactAid Inc., USA), given 4 h after the challenge. This meal proved not to give positive results for the HBT when previously tested with the same volunteers. About 1 week after the challenge with cooked beans the same volunteers received another challenge meal of beans germinated for 3 days and cooked for 60 min, equivalent to 70 g of uncooked non-germinated beans. The same procedure for breath H_2 determination was followed as for the test with cooked, non-germinated beans.

The concentration of H_2 in the expired air was determined using a Microlyzer Model 12 (Quintron Instruments, USA) calibrated with a standard reference gas of 104 ppm of H_2 in room air (Quingas, USA). An increment in breath H_2 concentration above baseline levels of 20 ppm at any of the post-dose intervals was considered a biologically significant intestinal fermentation of the undigested oligosaccharides (Rosado & Solomons, 1983).

RESULTS

The activities of the trypsin inhibitors present in the different varieties of *P. vulgaris* studied, expressed as the capacity of each sample to inhibit added trypsin to their extracts (inhibited trypsin units per mg of sample, dry basis),

TABLE 1
Oligosaccharide Composition and Trypsin Inhibitor Activity in *P. vulgaris*

Variety	*Sucrose*[a] (g%)	*α-Galactosides (g%)*[a]			*ITU*[b]
		Raffinose	*Stachyose*	*Total*	
Jalo	3·6	1·4	3·2	4·6	98
Rio fato tibagi	3·5	1·2	3·3	4·5	103
Rio negro	3·4	1·1	3·6	4·7	132
Vermelho	3·7	1·3	3·5	4·8	71
FT 83-25	3·1	1·1	3·4	4·5	126
FT 83-100	3·1	1·3	4·7	6·0	112
RAI 103	3·1	1·0	4·3	5·3	111
FT 120	3·3	1·1	3·5	4·6	160
RAI 180	3·5	1·0	3·3	4·3	120
CNF 0178:					
uncooked	3·0	0·5	4·2	4·7	119
cooked for:					
15 min	2·3	0·6	3·7	4·3	nd
30	2·2	0·5	3·7	4·2	8·0
60	2·1	0·4	3·8	4·2	8·1
90	1·9	0·5	3·6	4·1	7·9
germinated for:					
1 day	1·9	0·6	3·4	4·0	122
2	2·1	0·5	2·7	3·2	98
3	4·2	0·2	0·9	1·1	101

[a] Figures are average of duplicate determinations, dry matter.
[b] ITU = inhibited trypsin unit per mg of dried sample; figures are average of four determinations.
nd = not determined.

varied from 71 to 160 ITU/mg (Table 1). The effect of germination on inhibited trypsin units in variety CNF 0178 was small, decreasing from 122 to 101 ITU/mg, from the 1st to the 3rd day of germination, whereas cooking caused a more pronounced effect, with a decrease from 119 to 8 ITU/mg in 30 min of cooking at 100°C. Cooking times of 60 and 90 min gave similar results to that of 30 min (Table 1).

Total oligosaccharides in the different cultivars of *P. vulgaris* showed values around 8·1% (dry basis) with small variation between the samples. Stachyose was the main α-galactoside present in all samples with values from 3·2 to 4·7% (Table 1). Cooking promoted a small loss (around 10%) but germination produced a progressive decrease in α-galactosides which was followed by an increase in sucrose (Table 1). Verbascose was present only in trace amounts in the samples and it was not considered in this study.

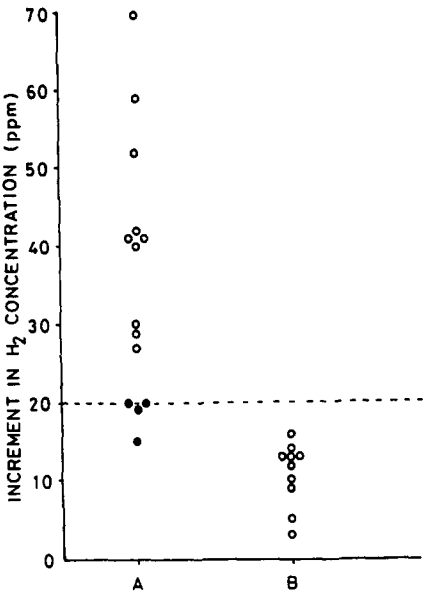

Fig. 1. Individual distribution of maximum increments of hydrogen concentration in the expired air of volunteers after challenging with black bean meals. A, Cooked black beans. B, Germinated and cooked black beans. ●, Volunteers who did not take part in test B. Increment in hydrogen concentration is calculated by the difference between H_2 concentration at a given time interval after ingestion of the test meal and the H_2 concentration at baseline, obtained just before the ingestion of the test meal.

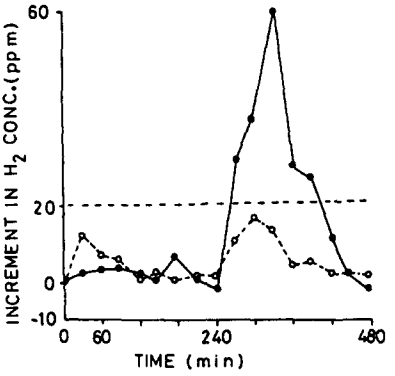

Fig. 2. Increment in hydrogen concentration in the expired air of subject No. 9. ○, Germinated and cooked beans. ●. Non-Germinated and cooked beans. Increment in H_2 concentration calculated as in Fig. 1.

The results of the hydrogen breath test with the cooked and with the germinated and cooked sample CNF 0178 are presented in Fig. 1. The maximum increment in H_2 concentration in the expired air attained by each volunteer after challenging with the test meals shows that 10 out of 14 subjects presented increased (above 20 ppm) H_2 production after ingesting the cooked beans. However, when these 10 volunteers received the germinated and cooked beans, their increment in H_2 concentration was always below 20 ppm, similar to the results found when they were challenged with water or with the rice-based meal, indicating a decrease in intestinal fermentation when beans were germinated for three days. Figure 2 shows the breath H_2 response of one subject during the extent of the test, at each time interval, following ingestion of cooked and germinated beans.

DISCUSSION

The variations in the activity of the trypsin inhibitor (71-160 inhibited trypsin units/mg sample, dry basis) between the different varieties of *P. vulgaris* presented in this work are in agreement with variations for trypsin inhibitors determined in other varieties and species of legumes (Mancini & Lajolo, 1981; Thorn *et al.*, 1983; Khokhar & Chauhan, 1986).

One variety of black beans (CNF 0178) was submitted to processing after soaking in water for 16 h. Determination of the trypsin inhibitor activity before and after soaking and in the water showed no loss of activity. We found a slight reduction (about 15%) on the trypsin inhibitor activity as the length of the period of germination increased up to 3 days. Other authors have found more marked effects of germination on trypsin inhibitors in other varieties of *P. vulgaris*, with Sathe *et al.* (1983) finding 25·4% reduction after 3 days and El-Hag *et al.* (1978) finding 50% reduction after 10 days of germination. With other species of legumes the effect of germination is more conflicting, with some authors describing high reductions (El-Hag *et al.*, 1978; Sathe *et al.*, 1983; Khokhar & Chauhan, 1986) whereas others report an increase (Kute *et al.*, 1984) or no differences (King & Puwastien, 1987) during germination.

The effect of cooking on the trypsin inhibitor activity, followed the pattern usually found for other species and varieties of beans (Philips *et al.*, 1983; Thorn *et al.*, 1983; Khokhar & Chauhan, 1986) with a decrease of about 93% at 100°C for 30 min.

The extraction method used for oligosaccharide determination was based on the procedure described by Macrae and Zand-Moghadan (1978) for lupin and soybean analyses. However, when it was applied to black beans a strong red coloured extract was obtained after addition of Carrez solutions. This was probably due to structural modifications of black bean pigments that occurred at the pH used for analysis. This problem was overcome by including a further clarification step by the use of activated charcoal which produced a clear extract for chromatography. The whole extraction procedure showed recovery of 95% for sucrose and 90% for raffinose.

Oligosaccharide composition of cooked beans was not intensively affected by cooking time and, although about 23% of loss has been observed in total oligosaccharides after 90 min of cooking, this was mainly due to some sucrose degradation. Germination produced a considerable reduction in α-galactosides (64% for raffinose and 79% for stachyose) after the third day. These results are somehow higher than the values found by Savitri & Desikachar (1985) for soybeans but are in agreement with the findings of Sathe *et al.* (1983). The reduction of α-galactosides was followed by an increase of about 40% of sucrose after the third day of germination and this

was a consequence of the seed metabolism which needs available sugars as energy sources (King & Puwastien, 1987). Based on these results, beans germinated for 3 days were used for the breath test. Sixty minutes was the time selected for cooking since it gave an adequate texture and it was sufficient to inactivate most of the trypsin inhibitor present.

The flatus-promoting capacity presented by legumes has been related to the fermentative action of intestinal bacteria on non-digested carbohydrates, which are not degraded by human digestive enzymes (Calloway *et al.*, 1971). This intestinal fermentation produces gases such as hydrogen. Although most of the H_2 produced by fermentation is expelled in the flatus, 14–21% is absorbed from the lumen into the blood and eliminated by the lungs in the expired air (Solomons, 1984). Therefore, the concentration of H_2 in the expired air has been used as an indicator of gas production in the large intestine (Solomons, 1984). An advantage of the determination of H_2 in the expired air is that it is a non-invasive technique and, although indirect, represents a very specific method of detecting malabsorption of carbohydrates, since H_2 is one of the products of bacterial fermentation of non-absorbable carbohydrates but it is not found when other nutrients are not digested and absorbed (Dowes *et al.*, 1985).

The results for the breath H_2 concentration in the present work showed that germination of black beans for 3 days was sufficient to decrease intestinal fermentation and the consequent production of gases in subjects who presented biologically significant H_2 production when previously challenged with non-germinated, cooked beans. The volunteers also reported absence of abdominal discomfort when they were fed the germinated beans. All subjects found germinated beans more palatable than the non-germinated samples indicating that structural modifications occurred improving the texture and flavour of the beans.

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REFERENCES

Calloway, D., Hickey, C. A. & Murphy, E. L. (1971). Reduction of intestinal gas-forming properties of legumes by traditional and experimental food processing methods. *J. Food Sci.*, **36**, 251–5.

Chen, C. H., Wells, C. E. & Fordhan, J. R. (1975). Germinated seeds for human consumption. *J. Food Sci.*, **40**, 1290–4.

Dowes, A. C., Schaap, C. & van der Klei-Van Moorsel, J. M. (1985). Hydrogen breath test in school children. *Arch. Dis. Child.*, **60**, 333–7.

El-Hag, N., Haard, N. F. & Morse, R. E. (1978). Influence of sprouting on the digestibility coefficient, trypsin inhibitor and globulin proteins of red kidneybeans. *J. Food Sci.*, **43**, 1874–5.

IBGE-Instituto Brasileliro de Geografia e Estatistica (1978). Estudo nacional das despesas familiares; tabelas de composiçao dos alimentos. Rio de Janeiro, V. 3.

Jood, S., Mehta, U. & Singh, R. (1986). Effect of processing on available carbohydrates in legumes. *J. Agric. Food Chem.*, **34**, 417–20.

Kakade, M. L., Rackis, J. J., McGhee, J. E. & Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Am. Assoc. Cereal Chem.*, **51**, 376–82.

Khaleque, A., Elias, L. G., Braham, J. E. & Bressani, R. (1985). Studies on the development of infant foods from protein sources. Part I: Effect of germination of chickpea (*Cicer arietinum*) on the nutritive value and digestibility of proteins. *Arch. Latinoam. Nutr.*, **35**, 315–23.

Khokhar, S. & Chauhan, B. M. (1986). Antinutritional factors in moth bean (*Vigna aconitifolia*): Varietal differences and effects of methods of domestic processing and cooking. *J. Food Sci.*, **51**, 591–4.

King, R. D. & Puwastien, P. (1987). Effects of germination on the proximate composition and nutritional quality of winged bean seeds. *J. Food Sci.*, **52**, 106–8.

Kute, L. S., Kadam, S. S. & Salunkhe, D. K. (1984). Changes in sugars, starch and trypsin inhibitor activity in winged bean during seed development. *J. Food Sci.*, **49**, 314–5.

Macrae, R. & Zand-Moghaddam, A. (1978). The determination of the component oligosaccharides of lupinseeds by high pressure liquid chromatography. *J. Sci. Food Agric.*, **29**, 1083–6.

Mancini, F. J. & Lajolo, F. M. (1981). Fatores antinutricionais em diferentes variedades de feijoes. *Ciencia e Cultura*, **33**, 94–8.

Phillips, R. D., Chhinnan, M. S. & Mendoza, I. G. (1983). Effect of temperature and moisture content on the kinetics of trypsin inhibitor activity, protein *in vitro* digestibility and nitrogen solubility of cowpea flour. *J. Food Sci.*, **48**, 1863–7.

Rachie, K. O. (1973). Improvement of food legumes in tropical Africa. In *Nutritional Improvement of Food Legumes by Breeding*. ed. M. Milner. PAG, United Nations, New York, pp. 83–92.

Rosado, J. L. & Solomons, N. W. (1983). Sensitivity and specificity of the hydrogen breath-analysis test for detecting malabsorption of physiological doses of lactose. *Clin. Chem.*, **29**, 545–8.

Sathe, S. K., Deshpande, S. S., Reddy, N. R., Goll, D. E. & Salunke, D. K. (1983). Effects of germination on proteins, raffinose oligosaccharides, and anti-nutritional factors in the great northern beans (*Phaseolus vulgaris* L.). *J. Food Sci.*, **48**, 1796–1800.

Savitri, A. & Desikachar, R. (1985). A comparative study of flatus production in relation to the oligosaccharide content of some legumes. *Nutr. Rep. Int.*, **31**, 337–44.

Solomons, N. M. (1983). The use of hydrogen breath-analysis tests in gastrointestinal diagnosis. *Curr. Conc. Gastroent. Jan./Feb.*, 30–40.

Solomons, N. M. (1984). Evaluation of carbohydrate absorption: The hydrogen breath test in clinical practice. *Clin. Nutr.*, **3**, 71–8.

Thorn, K. A., Tinsley, A. M., Weber, C. W. & Berry, J. W. (1983). Antinutritional factors in legumes of the sonoran desert. *Ecol. Food Nutr.*, **13**, 251–6.