Food Chemistry **38** (1990) 153–158

Short Communication

Changes in Alpha-Galactosidase Activity and Oligosaccharides During Germination and Incubation of Cowpeas (*Vigna unguiculata*)

ABSTRACT

Germination of cowpeas at 24 and 30°C resulted in increased alpha-galactosidase activity during the first 6 to 12 h and leveled off subsequently. Stachyose content declined by 38% during 12 h of germination at 24°C. Incubation of a cowpea flour paste for 24 h at 24°C resulted in a large reduction of the stachyose and sucrose concentrations, and in their practical elimination at 34°C. Incubation for 12 h at 24 and 34°C of a cowpea paste made from 6-h germinated seeds resulted in a large decrease in stachyose content. Incubation of flour or germination of seeds effectively reduced the stachyose concentration of cowpeas.

INTRODUCTION

Cowpeas (*Vigna unguiculata*) are the main source of protein for many people in the world. However, cowpeas, like other legumes, contain alpha-galactosides which cannot be hydrolyzed and absorbed because of lack of alpha-galactosidase (EC 3.2.1.22) activity in the small intestine (Taeufel *et al.*, 1965). Consumption of legume seeds by humans may result in gastro-intestinal distress and flatulence (Fleming, 1981; Olson *et al.*, 1981). Nausea, cramps and diarrhea may also occur in varying degrees (Rackis *et al.*, 1970*a*). Although the presence of raffinose and stachyose in the gas-producing fraction of beans has been demonstrated, positive identification of all flatulence-causing factors has not been made (Rackis *et al.*, 1970*a*; Reddy *et al.*, 1984).

Food Chemistry 0308-8146/90/$03·50

Several approaches have been taken to reduce or eliminate the flatus activity of legumes, e.g. induced seed germination (Suparmo & Markakis, 1987), ethanolic and aqueous extraction of the sugars (Rackis *et al.*, 1970*b*; Kim *et al.*, 1973), and incubation of flour suspensions of small white beans (Becker *et al.*, 1974). Breeding of lima beans has also been suggested for decreasing flatulent oligosaccharides (Meredith *et al.*, 1988). Antibiotics have been successfully employed to suppress microbial activity in the lower ileum and thereby reduce flatus (Richards & Steggerda, 1966).

There is little doubt that seed alpha-galactosidases play an important role in the early stages of germination by hydrolyzing galactose-containing oligosaccharides and thereby providing metabolites for the developing seedling (Pridham & Dey, 1974; Dey & Campillo, 1984; Dey *et al.*, 1986). McCleary and Matheson (1974) reported a decrease in the raffinose family of oligosaccharides and galactomannans in germinating seeds of lucerne, guar, carob and soybean.

The objective of this research was to study the alpha-galactosidase activity and oligosaccharide changes occurring during the germination of cowpeas and incubation of cowpea flour slurries to provide practical recommendations for reducing the flatus activity of cowpeas.

MATERIALS AND METHODS

California cowpeas or black-eye peas were used. For germination, washed cowpeas were soaked at room temperature (24°C) for 2 h, placed in Petri dishes lined with wet filter paper, and kept in the dark at 24 and 30°C. Periodically, during the 24-h germination period, samples were withdrawn and analyzed for alpha-galactosidase activity according to Dey and Pridham (1969), and for oligosaccharides using high-performance liquid chromatography (HPLC), as described by Liu and Markakis (1987).

A cowpea paste was prepared from flour made by the dry procedure of Dovlo *et al.* (1976). A Wiley mill was used for grinding the cowpea seeds to 20 mesh fineness and 100 g of flour was mixed with 140 ml distilled water. The paste was divided into two portions, one of which was incubated at 24°C and the other at 34°C. At 0, 3, 6, 12 and 24 h of incubation, samples were removed and their pH and oligosaccharide content were determined.

Cowpeas were also soaked for 2 h, germinated for 6 h at 24°C, ground into a paste using a mortar and pestle, and then incubated at 24 and 34°C for 0, 3, 6 and 12 h. After each time period, pH and oligosaccharide content of the paste were determined.

A comparative study was made of the invertase activity in dormant and 12-h germinated seeds, as well as in a cowpea flour paste incubated for 12 h at 24°C. Invertase activity was assayed as described by Al-Bakir and Whitaker (1978), except that sucrose was quantified by HPLC (Alani *et al.*, 1989).

The AOAC (1980) procedure No. 14.003 was used for moisture determination. All experiments were performed in triplicate. Microstat (1985) software was used for basic statistics, regression analysis and analysis of variance, where applicable.

RESULTS AND DISCUSSION

Germination effect on cowpea alpha-galactosidase and oligosaccharides

The alpha-galactosidase activity more than doubled (from 41·7 to 86·7 units/mg dry sample) during the first 6 h of germination at 24°C and remained unchanged subsequently. Germination of cowpeas at 30°C showed similar changes in alpha-galactosidase activity but the curve leveled off after 12 h. Stachyose content declined by 38% from 3·4 g/100 g sample during 12 h germination at 24°C, but did not change after 12 h germination. Raffinose content did not change significantly in cowpeas during 24 h of germination ($p > 0{\cdot}05$). Sucrose content declined during the first 6 h of germination from 1·7 to 1·4 g sucrose/100 g cowpeas, then increased. Cowpeas contained 2·4 g sucrose/100 g cowpeas after 24 h germination. Nnanna and Phillips (1988) reported similar changes in alpha-galactosidase activity when cowpeas were germinated for 24 h at 25 and 30°C. They also reported a decrease in sucrose concentration in cowpeas during the first 12 h of germination, followed by an increase to 2·87 g/100 g cowpea after 72 h of germination.

Incubation of cowpea flour paste

The changes in oligosaccharide content during 24 h of cowpea flour paste incubation at 24 and 34°C are shown in Table 1. The sucrose and stachyose contents decreased significantly ($p < 0{\cdot}05$) during incubation at either temperature. Stachyose and sucrose concentrations approached zero after 24 h of incubation at 34°C. The cowpea paste contained 0·28 g raffinose/100 g dry sample. The raffinose content did not change significantly ($p > 0{\cdot}05$) at 24°C, although a small but significant decrease was observed at 34°C ($p < 0{\cdot}05$). The initial pH of the cowpea flour paste was 6·4. There was a small but statistically insignificant drop in pH during incubation at both temperatures. The oligosaccharide content of the paste decreased in the following order during incubation: stachyose > sucrose > raffinose.

TABLE 1
Oligosaccharide Content of Cowpea Flour Paste during Incubation at 24 and 34°C (g/100 g flour, dry wt)

Oligosaccharide	*Incubation temperature (°C)*	*Incubation time (h)*				
		0	*3*	*6*	*12*	*24*
Sucrose	24	1·74	1·45	0·89	0·82	0·25
	34	1·74	1·29	0·93	0·46	0
Raffinose	24	0·26	0·26	0·24	0·25	0·18
	34	0·26	0·26	0·25	0·22	0·11
Stachyose	24	2·81	2·60	1·78	1·96	2·19
	34	2·81	2·11	1·67	1·07	0·

Germination plus incubation of cowpea flour paste

Table 2 illustrates changes in stachyose, raffinose and sucrose concentrations during 2 h of soaking and 6 h of germination at 24°C, followed by 12 h of incubation at either 24 or 34°C of a paste made from germinated seeds. Germination was stopped after 6 h because no increase in alpha-galactosidase activity was observed. The incubation of the paste was stopped at 12 h because considerable oligosaccharide breakdown was achieved without apparent microbial spoilage.

The stachyose content significantly ($p < 0{\cdot}01$) decreased at both 24 and 34°C. The rate of decrease was faster at 34°C than at 24°C. The raffinose content decreased slightly but significantly ($p < 0{\cdot}05$) at both temperatures. The rates of decrease (slopes) at both temperatures were much lower for raffinose than stachyose. Sucrose increased significantly ($p < 0{\cdot}01$) at 24°C, but did not change significantly ($p > 0{\cdot}05$) at 34°C. The pH increased significantly ($p < 0{\cdot}01$) from 6·36 to 6·57 at 24°C, but did not change significantly ($p > 0{\cdot}05$) at 34°C.

TABLE 2
Oligosaccharide Content of Cowpeas during 2-h Soaking, 6-h Germination and 12-h Incubation of a Paste at 24 and 34°C (g/100 g cowpea, dry wt)

Oligosaccharide	*Incubation temperature (°C)*	*Incubation time (h)*				
		0	*8*	*11*	*14*	*20*
Sucrose	24	1·74	1·76	1·84	1·93	2·04
	34	1·74	1·73	1·71	1·61	1·49
Stachyose	24	2·89	2·39	2·14	1·91	1·42
	34	2·89	2·13	1·75	1·32	0·51

These results showed that it was possible to decrease the flatus-related sugar content of cowpeas, especially stachyose, using germination of seeds, incubation of a paste or a combination of germination and incubation. Precautions should be taken to prevent the growth of pathogens in the paste during incubation. Lowering the pH below 4·6 might be helpful. Alani *et al.* (1989) reported that cowpea alpha-galactosidase was optimally active at pH 5·0 but possessed over 80% of its activity at pH 4·0.

Invertase activity

The invertase activity of dormant seeds was 96·7 units/mg dry sample; that of seeds germinated for 14 h at 24°C was 171·2 units/mg sample; and that of ungerminated seed paste after incubation for 12 h at 24°C Was 333·4 units/mg sample. Invertase activity was lower in dormant and germinated seeds than in the paste made from cowpea flour. The higher invertase activity might explain the rapid disappearance of sucrose in the paste. Sucrose is a product of the hydrolysis of stachyose and raffinose by alpha-galactosidase, while invertase converts sucrose to glucose and fructose. The changes in sucrose concentration during germination or incubation were caused by changes in alpha-galactosidase and invertase activity. The paste prepared by soaking and germination prior to incubation at 24°C did not contain sufficient invertase activity to suppress sucrose accumulation caused by hydrolysis of stachyose and raffinose.

REFERENCES

Alani, S. R., Smith, D. M. & Markakis, P. (1989). Alpha-galactosidases of cowpeas (*V. unguiculata*). *Phytochem.*, **28**, 2047–51.

Al-Bakir, A. & Whitaker, J. R. (1978). Purification and characterization of invertase from dates (*Phoenix dactylifera* L. var. *zahdi*). *J. Food Biochem.*, **2**, 132–60.

AOAC (1980). *Official Methods of Analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC.

Becker, R., Olson, A. C., Frederick, D. P., Kon, S., Grubmann, M. R. & Wagner, J. R. (1974). Conditions for the autolysis of alpha-galactosidase and phytic acid in California small white beans. *J. Food Sci.*, **39**, 766–9.

Dey, P. M. & Campillo, D. E. (1984). Biochemistry of the multiple forms of glycosidase in plants. *Adv. Enzymol.*, **56**, 141–249.

Dey, P. M. & Pridham, J. B. (1969). Purification and properties of alpha-galactosidase from *Vicia faba*. *Biochem. J.*, **113**, 49–55.

Dey, P. M., Naik, S. & Pridham, J. B. (1986). Further characterization of alpha-galactosidase I–glycoprotein lectin from *Vicia faba* seeds. *Phytochem.*, **25**, 1035–41.

Dovlo, F. E., Williams, C. E. & Zoaka, L. (1976). Cowpeas: Home preparation and use in West Africa. IDRC-055E, International Development Research Center, Ottawa, Canada.

Fleming, S. E. (1981). Influence of processing on physical forms of beans and on intestinal fermentation. *J. Food Sci.*, **53**, 777–82.
Kim, W. J., Smith, C. J. B. & Nakayama, T. O. M. (1973). The removal of oligosaccharides from soybeans. *Lebensm. Wiss. u. Technol.*, **6**, 201–4.
Liu, K. & Markakis, P. (1987). Effect of maturity and processing on the trypsin inhibitor and oligosaccharides of soybeans. *J. Food Sci.*, **52**, 222–3, 225.
McCleary, B. V. & Matheson, N. K. (1974). Alpha-galactosidase activity and galactomannan and glactosylsucrose oligosaccharide depletion in germinating legume seeds. *Phytochem.*, **13**, 1747–57.
Meredith, F. I., Thomas, C. A., Snook, M. E., Himmelsbach, O. S. & Halbeek, H. V. (1988). Soluble carbohydrates, oligosaccharides and starch in lima bean seeds. *J. Food Sci.*, **53**, 768–71.
Microstat 4.0 (1985). Ecosoft, Indianapolis, IN.
Nnanna, I. A. & Phillips, R. D. (1988). Changes in oligosaccharide content, enzyme activities and dry matter during controlled germination of cowpeas. *J. Food Sci.*, **53**, 1782–6.
Olson, A. C., Gray, G. M., Grubmann, M. R. & Wagner, J. R. (1981). Flatus causing factors in legumes. In *Antinutrients and Natural Toxicants in Food*, ed. R. L. Ory. Food and Nutrition Press, Westport, CT, pp. 275–94.
Pridham, J. B. & Dey, P. M. (1974). The nature and function of higher plant alpha-galactosidase. In *Plant Carbohydrate Biochemistry*, ed. J. B. Pridham. Academic Press, London, pp. 83–96.
Rackis, J. J., Sessa, D. J., Steggerda, F. R., Shimizu, T., Anderson, J. & Pearl, S. L. (1970*a*). Soybean factors relating to gas production by intestinal bacteria. *J. Food Sci.*, **35**, 634–9.
Rackis, J. J., Honig, D. H., Sessa, D. J. & Steggerda, F. R. (1970*b*). Flavor and flatulence factors in soybean protein products. *J. Agric. Food Chem.*, **18**, 977–82.
Reddy, N. R., Pierson, M. D., Sathe, S. K. & Salunkhe, D. K. (1984). Chemical, nutritional and physiological aspects of dry bean carbohydrates. A review. *Food Chem.*, **13**, 25–68.
Richards, E. A. & Steggerda, F. R. (1966). Production and inhibition of gas in various regions in the intestine of the dog. *Proc. Soc. Exp. Biol. Med.*, **122**, 573–6.
Suparmo & Markakis, P. (1987). Tempeh prepared from germinated soybeans. *J. Food Sci.*, **52**, 1736–7.
Taeufel, K., Ruttlof, H., Krause, W., Taeufel, A. & Vetter, K. (1965). Zum intestinalen verhalten von galakto-oligosacchariden beim menschen. *Klin. Wochenschr.*, **43**, 268–74.

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(Received 6 June 1989; revised version received 29 August 1989; accepted 31 August 1989)

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