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Effect of domestic processing on oligosaccharide content of some dry legume seeds

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Raffinose family oligosaccharides (raffinose, stachyose and verbascose) present in legume seeds have been reported to cause flatulence. The object of this work was to study the effect of methods of domestic processing, including soaking in tap water and 0·5% sodium bicarbonate solution, cooking and autoclaving of unsoaked and soaked seeds, on oligosaccharide contents of four commonly consumed legumes in Egypt, namely: faba bean (*Vicia faba* L.), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*) and cowpea (*Vigna sinensis*).

The identification and quantitative determination of oligosaccharides using gas-liquid chromatography (GLC) indicated that the four raw legume seeds contained sucrose, raffinose, stachyose and verbascose. The raffinose family oligosaccharides account for 67·3, 63·2, 53·0 and 51·0% of the total soluble sugars in cowpea, faba bean, lentil and common bean, respectively. The major oligosaccharide was verbascose in faba bean and stachyose in the other three legumes. The soaking of legume seeds in tap water or sodium bicarbonate solution resulted in a decreased concentration of sucrose and the raffinose family of sugars in each case. The extent of losses was enhanced as the time of soaking was increased. The removal of oligosaccharides from legume seeds was higher in alkaline medium than in water. In addition, the loss of an individual sugar was not related to its solubility or molecular weight.

Cooking of unsoaked seeds led to a large decrease in oligosaccharide content of all studied legumes compared with soaking for 12 h. The loss in the raffinose family oligosaccharides after cooking of soaked seeds was highest in common bean and lowest in faba bean. Autoclaving had a greater effect on removal of oligosaccharides than did cooking. In all cases, autoclaving of soaked seeds resulted in complete disappearance of raffinose.

INTRODUCTION

Food legumes are an important dietary component for the majority of the population in Egypt and several other developing countries. The great need to bridge the protein-calorie gap in the developing countries is well documented (Nnanna & Phillips, 1988). Despite the nutritional potential of legumes as an economic source of significant amount of proteins, calories and some B-vitamins, the ability of legume seeds to stimulate intestinal gas formation is one of the main reasons why people limit their consumption of pulses (Iyer *et al.*, 1980; Askar, 1986). A number of investigators have demonstrated that the oligosaccharides raffinose, stachyose and verbascose are the principal causes of flatulence in human and animal studies (Calloway *et al.*, 1971; Fleming, 1981; Jood *et al.*, 1985). Owing to the absence of an enzyme (α-galactosidase) capable of hydrolyzing the α-1,6-galactosidic linkage, these oligosaccharides accumulate in the lower intestine and undergo anaerobic fermentation by bacteria (Reddy *et al.*, 1980). Therefore, to utilize legumes as a more acceptable source of inexpensive proteins, it is desirable to reduce their flatulence production (Jood *et al.*, 1985).

Various processing methods have been tried to reduce the effect of these undesirable carbohydrates as well as to improve the digestibility of available carbohydrates in a variety of legumes (Rao & Belavady, 1978; Jood *et al.*, 1986). The traditional method for home preparation for most legume seeds consists of a water soaking period (usually overnight) followed by cooking of the rehydrated seeds after discarding the soaking water. Legume seeds become edible after prolonged boiling in water or after a shorter time when a pressure cooker is used.

Information on the oligosaccharide contents in the pulses usually prepared for consumption in Egypt is rarely in the literature. In addition, very limited data are available about the effects of traditional methods of processing on the oligosaccharide profiles of legumes (Silva & Braga, 1982; Jood *et al.*, 1985). The present

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study was, therefore, undertaken to investigate the effect of domestic processing, viz., soaking in tap water and in sodium bicarbonate solution for different times, cooking and autoclaving of unsoaked and soaked seeds, on oligosaccharide content of some dry legumes, and to evolve a suitable method of processing to decrease the flatus-producing factors in legumes.

MATERIALS AND METHODS

Materials

Samples of four common legumes; namely: faba bean (*Vicia faba* L.), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*) and cowpea (*Vigna sinensis*) were used in this study. The faba beans (Giza 402 variety) and lentil seeds (Giza 9 variety) were obtained from the Department of Agronomy, whereas the common bean (Giza 3 variety) and cowpea (Azmerly variety) were obtained from the Department of Horticulture, Faculty of Agriculture, Assiut University. All legume samples were obtained during the 1990 season.

Processing

Soaking
Samples of dry legume seeds were soaked in tap water and in 0·5% sodium bicarbonate solution for periods of 4, 8 and 12 h at room temperature (25 ± 2°C). The seeds to water or to sodium bicarbonate solution ratio was 1 : 4 (w/v). After soaking, the water was drained; then the soaked samples were dried at 50°C in a hot air oven for 48 h and ground in an electric grinder to pass through a 100-mesh (0·15 mm) sieve. Finally, the ground samples were kept in closed bottles and stored in a refrigerator at 5°C until analysis.

Ordinary and pressure cooking
Unsoaked seeds and those soaked for 12 h in tap water and in sodium bicarbonate solution were boiled (1 : 5 w/v) in water for 60 min. Autoclaving of samples was carried out at 10 psi (68·94 K Pa) in double amount of water for 20 min. The cooked and autoclaved legume seeds were drained, homogenized in a blender, then dried and stored as described before.

Analytical methods

Moisture content
The moisture content of ground samples was determined according to AOAC methods (1980).

Total sugars and oligosaccharides
Total sugars and oligosaccharides of raw, soaked and cooked legume seeds were extracted with hot 70% ethanol according to the method of Tanaka *et al.* (1975). Ground legume seeds (5 g) were exhaustively extracted in 70% aqueous ethanol (65°C, 2 h, 3 × 50 ml). The extracts were combined and taken to a constant volume. Aliquots were analyzed for total ethanol-soluble sugars and oligosaccharides with a degree of polymerization (DP) of less than 6.

Total ethanol soluble sugars
These were obtained by extraction with hot 70% ethanol, were determined by the phenol—sulfuric acid method (Dubois *et al.*, 1956) and expressed in terms of glucose.

Oligosaccharides
Sucrose, raffinose, stachyose and verbascose were identified and quantified using gas-liquid chromatography according to the method of Davison & Young (1969). A Hewlett-Packard (5730 A) gas chromatograph was equipped with dual flame ionization detector. A 3 ft (0·91 m) glass column, 0·16 in (4·1 mm) i.d., was packed with 3% SE-54 on 80–100 mesh chromosorb W (AW-DMCS) and pre-conditioned for 48 h at 250°C. The injector and detector temperature were 260°C and 280°C, respectively. The oven temperature was programmed at 200°C for 6 min then with 24°C per min increase to 290°C and, thereafter, held at 290°C until the last peak. The nitrogen carrier gas flow was 60 ml per min. Hydrogen and air flow rates (ml/min) were 45 and 400, respectively. Aliquots containing 1–10 mg oligosaccharides were taken to dryness under vacuum and their trimethylsilyl derivatives were prepared by heating at 60°C for 90 min in the presence of pyridine (2 ml), hexamethyldisilazane (0·4 ml) and chlorotrimethylsilane (0·2 ml). Components were identified by comparing retention times with the pure reference compounds including sucrose, raffinose, stachyose and verbascose. The components were quantified by including an internal standard, myo-inositol, and by determining comparative response factors with each reference compound.

RESULTS AND DISCUSSION

Total ethanol-soluble sugars

Sugars soluble in hot 70% ethanol included monosaccharides and their polymers to a DP of 5. Although the monosaccharide compositions will not be reported in detail in this study, they included mainly glucose and fructose. The total quantity of all sugars which could be dissolved in hot ethanol ranged from 4·90% for common bean to 6·05% for cowpea (Table 1). The total ethanol-soluble sugars content, expressed as glucose, in faba beans (5·98%) was in the range observed by Cerning *et al.* (1975) and Cerning and Filiatre (1976). The content of ethanol-soluble sugars in lentil (5·13%) was less than the 6·31% reported by Schweizer *et al.* (1978) and that in common bean (4·90%) was greater than the values 3·2 to 4·4% indicated by Kon *et al.* (1973) for ten varieties of white beans (*Phaseolus vulgaris*). The

Table 1. Oligosaccharides and total sugar contents of some dry legume seeds[a]

Legumes	Total sugars (%)	Sucrose (%)	Raffinose family oligosaccharides			Oligosaccharides[b] as percent of total sugars
			Raffinose (%)	Stachyose (%)	Verbascose (%)	
Faba bean	5·98	1·37	0·52	1·41	1·85	63·2
Lentil	5·13	1·14	0·45	1·65	0·62	53·0
Common bean	4·90	1·25	0·45	1·80	0·25	51·0
Cowpea	6·05	1·51	0·77	3·00	0·30	67·3

[a] Each value is the mean of double determinations (on dry weight basis).
[b] Includes oligosaccharides of the raffinose family of sugars.

total sugars value of cowpea (6·05%) was also greater than the 5·8% reported by Nnanna and Phillips (1988). These differences are due to the fact that different methods have been used and that the varieties were not the same.

Oligosaccharides of legumes

GLC revealed the presence of sucrose, raffinose stachyose and verbascose in the ethanol extracts of the four legume seeds. The sucrose content of the legume seeds, ranged from 1·14% for lentil to 1·51% for cowpea (Table 1). Since sucrose is critically involved in the biosynthesis of legume seed carbohydrates, the size of the sucrose pool would be expected to vary with maturity and perhaps growing conditions (Shallenberger & Moyer, 1961).

The α-galactosides are known to constitute the major portion of sugars in legume seeds and were reported to be involved in flatus formation (Fleming, 1981). The results in Table 1 showed that the major oligosaccharide was verbascose in faba bean and stachyose in the other three legumes. Raffinose constituted 0·45–0·77% of the seed weight for all legumes. The stachyose contents varied from 1·41% for faba bean to 3·0% for cowpea. Values of less than 0·7% verbascose were determined for most legume seeds, but faba bean gave a value of 1·85%. These results are in agreement with previously reported values for faba beans (Cerning *et al.*, 1975), lentil (Naivikul & D'Appolonia, 1978; Schweizer *et al.*, 1978), beans (*Phaseolus vulgaris* L.) (Silva & Braga, 1982) and cowpea (Nnanna & Phillips, 1988).

Effect of soaking

The content of oligosaccharides (sucrose, raffinose, stachyose and verbascose) remaining in the legume seeds after soaking in tap water is shown in Table 2. Table 2 indicates that soaking of seeds resulted in a decreased concentration of oligosaccharides. The extent of losses was enhanced as the time of soaking was increased to 12 h. After soaking for 12 h in tap water, the loss in sucrose content ranged from 20·2% for

Table 2. Effect of soaking on tap water in oligosaccharide contents of legume seeds (on dry weight basis)[a]

Legumes	Soaking time (h)	Sucrose (%)	Raffinose family oligosaccharides			Percent oligosaccharides reduced[b]
			Raffinose (%)	Stachyose (%)	Verbascose (%)	
Faba bean	0	1·37	0·52	1·41	1·85	—
	4	1·28	0·49	1·29	1·71	7·7
	8	1·15	0·37	1·12	1·50	20·9
	12	0·99	0·32	1·10	1·32	27·5
Lentil	0	1·14	0·45	1·65	0·62	—
	4	1·12	0·50	1·43	0·59	7·4
	8	0·96	0·37	1·32	0·60	15·8
	12	0·91	0·28	1·04	0·51	32·7
Common bean	0	1·25	0·45	1·80	0·25	—
	4	1·19	0·40	1·59	0·22	11·6
	8	1·10	0·32	1·42	0·20	22·4
	12	0·96	0·24	1·25	0·18	35·6
Cowpea	0	1·51	0·77	3·00	0·30	—
	4	1·42	0·59	2·84	0·27	9·1
	8	1·30	0·52	2·61	0·23	17·4
	12	1·14	0·45	2·28	0·22	27·5

[a] Each value is the mean of double determinations.
[b] Includes oligosaccharides of the raffinose family of sugars.

Table 3. Effect of soaking in sodium bicarbonate solution on oligosaccharide contents of legume seeds (on dry weight basis)[a]

Legumes	Soaking time (h)	Sucrose (%)	Raffinose family oligosaccharides			Percent oligosaccharides reduced[b]
			Raffinose (%)	Stachyose (%)	Verbascose (%)	
Faba bean	0	1·37	0·52	1·41	1·85	—
	4	1·22	0·41	1·23	1·67	12·4
	8	1·07	0·36	1·02	1·45	25·1
	12	0·90	0·28	0·87	1·25	36·5
Lentil	0	1·14	0·45	1·65	0·62	—
	4	1·00	0·38	1·38	0·60	13·2
	8	0·92	0·30	1·26	0·44	26·5
	12	0·85	0·20	1·01	0·49	37·5
Common bean	0	1·25	0·45	1·80	0·25	—
	4	1·11	0·35	1·55	0·21	15·6
	8	0·96	0·29	1·23	0·19	31·6
	12	0·87	0·18	1·08	0·17	42·8
Cowpea	0	1·51	0·77	3·00	0·30	—
	4	1·35	0·52	2·72	0·24	14·5
	8	1·18	0·47	2·39	0·21	24·6
	12	1·00	0·42	2·15	0·19	32·2

[a] Each value is the mean of double determinations.
[b] Includes oligosaccharides of the raffinose family of sugars.

lentil to 27·7% for faba bean. In addition, removal of the raffinose family oligosaccharides from faba bean (27·5%), lentil (32·7%), common bean (35·6%) and cowpea (27·5%) was assessed. The highest loss in raffinose (46·7%), stachyose (37·0%) and verbascose (28·6%) was observed in common bean, lentil and faba bean, respectively. The loss of an individual sugar cannot be explained solely on the basis of its solubility or molecular weight. Thus, the loss of sucrose (19·6–27·8%) was less than that of raffinose (38.5–46·7%) although sucrose is a more soluble sugar and has the smallest molecular weight among these four sugars. This observation suggests that, in addition to such simple factors as solubility and molecular size, the location and the natural bonding form of the sugar within the cell also plays an important role in affecting the rate as well as the extent of extraction. The present findings are in agreement with those reported by Silva and Braga (1982), who found that the loss of raffinose (32·4%) was greater than that of sucrose (19·5%) when the dry beans were soaked in water for 24 h. In contrast, Jood *et al.* (1985) reported a greater decrease in sucrose than in verbascose, stachyose or raffinose after soaking of broad beans in plain water for 12 h.

The effects on the content of oligosaccharides of soaking in sodium bicarbonate solution are given in Table 3. Likewise, there was a gradual decrease in the oligosaccharide content as the soaking period was increased. The reduction was higher in sodium bicarbonate solution-soaked seeds than in tap water-soaked seeds. Sodium bicarbonate solution, at 0·2–0·5% concentration, has been reported as effective for tenderization of beans (Low, 1955) and of lentil seeds (Moharram *et al.*, 1986) during soaking and cooking. It was considered that soaking in sodium bicarbonate solution might tenderize the seed coat as well as the cotyledon and thus enhance the extraction of oligosaccharides. The study of Kim *et al.* (1973) indicates that the soaking of soybeans in buffer solution of pH 8 caused a rapid decrease in the content of oligosaccharides. Similar observations have been reported in red bean, chick pea, red gram, black gram and broad bean by Jood *et al.* (1985).

Effect of ordinary cooking

Changes in the contents of oligosaccharides after cooking of unsoaked, 12 h tap water-soaked and 12 h sodium bicarbonate solution-soaked seeds are presented in Table 4. The cooking of unsoaked seeds decreased the sucrose content of faba bean, lentil, common bean and cowpea by 39·4, 34·2, 44·0 and 41·7% of the original content, respectively. In addition, the removal of the other three oligosaccharides (raffinose, stachyose and verbascose) ranged from 41·5% for faba bean to 47·2% for common beans. Among the four oligosaccharides, the highest loss was found in raffinose after cooking of the four legume seeds. About 56–71% of raffinose was removed during the cooking of unsoaked legume seeds. On the other hand, greater decreases in oligosaccharide contents were found when the soaked seeds were cooked. The soaking of seeds in tap water or sodium bicarbonate solution for 12 h, followed by cooking, caused losses of sucrose, raffinose, stachyose and verbascose to the extent of 45·6–64·2, 78·8–100, 43·3–62·8, and 41·9–60%; respectively. Raffinose was detected in trace amounts after cooking of 12 h tap water-soaked lentil and common bean seeds, but not in either legume

Table 4. Effect of ordinary cooking of unsoaked and soaked legume seeds on oligosaccharide contents[a]

Samples	Sucrose		Raffinose family oligosaccharides			Percent of loss in raffinose family oligosaccharides[b]
	Content (%)	Loss (%)[b]	Raffinose (%)	Stachyose (%)	Verbascose (%)	
Faba bean						
Unsoaked	0·83	39·4	0·20	0·88	1·13	41·5
12 h soaking in water	0·72	47·4	0·11	0·80	0·99	49·7
12 h soaking in sodium bicarbonate solution	0·62	56·2	Trace	0·63	0·82	61·6
Lentil						
Unsoaked	0·75	34·2	0·15	0·91	0·40	46·3
12 h soaking in water	0·62	45·6	Trace	0·83	0·36	56·3
12 h soaking in sodium bicarbonate solution	0·50	56·1	ND	0·66	0·30	64·7
Common bean						
Unsoaked	0·70	44·0	0·20	0·95	0·17	47·2
12 h soaking in water	0·65	48·0	Trace	0·76	0·14	64·0
12 h soaking in sodium bicarbonate solution	0·54	56·8	ND	0·67	0·11	68·8
Cowpea						
Unsoaked	0·88	41·7	0·22	1·75	0·21	46·4
12 h soaking in water	0·70	53·6	0·15	1·40	0·17	57·7
12 h soaking in sodium bicarbonate solution	0·54	64·2	0·10	1·33	0·12	61·9

[a] Oligosaccharides content of cooked seeds (%, on dry weight basis).
[b] The loss expressed as percent of corresponding value for raw seeds.
ND - Not detectable.

when 12 h sodium bicarbonate solution-soaked seeds were cooked. The raffinose was found in trace amounts also after cooking of sodium bicarbonate solution-soaked faba bean seeds. The loss in the content of raffinose family oligosaccharides after cooking was greatest in common bean and least in faba bean. In addition, the cooking of sodium bicarbonate solution-soaked seeds in water led to a greater decrease in all oligosaccharides than that observed after cooking of tap-water soaked seeds. The above results agree with those obtained by Ku *et al.* (1976) for whole soybean seeds, who reported that boiling of soybean in a 1 : 10 bean/water ratio removed 33–59% of oligosaccharides, depending on the time in the soaking water. A loss of 82·5% sucrose, 75·6% raffinose, 60·0% verbascose and 52·2% stachyose in black gram seeds over a period of soaking in water for 12 h was obtained by Iyengar and Kulkarni (1977). Silva and Braga (1982) reported that cooking of the whole dry beans leads to a greater decrease in oligosaccharide content, especially when large amounts of water are used. On the other hand, the results of the present study differ from those obtained by Rao and Belavady (1978), who in a study of four Indian pulses, found a significant increase in oligosaccharide content after cooking of the whole seeds.

Effect of autoclaving (pressure cooking)

The effect of autoclaving on the removal of oligosaccharides is illustrated in Table 5. This Table indicates that the autoclaving had more effect on the elimination of oligosaccharides from legumes than ordinary cooking. Raffinose completely disappeared after autoclaving of soaked legume seeds. The autoclaving of 12 h water-soaked seeds and 12 h sodium bicarbonate solution-soaked seeds caused further removal of the raffinose family oligosaccharides from faba bean (40·5 and 45·8%), lentil (48·6 and 53·4%), common bean (53·4 and 58·0%) and cowpea (50·5 and 56·2% of the corresponding value for soaked seeds), respectively.

An attempt was made to investigate the final effect of soaking of legumes in tap water or sodium bicarbonate solution for 12 h, followed by autoclaving, on extraction of oligosaccharides from legume seeds. The combination of these treatments leads to elimination of the raffinose family oligosaccharides from faba bean, lentil, common bean and cowpea to the extents of 56·8–65·6, 65·4–72·4, 70·0–76·0 and 64·1–70·3%, respectively.

It may be concluded that soaking of legumes for 12 h in tap water, or sodium bicarbonate solution, followed by autoclaving, causes high losses of raffinose, stachyose and verbascose. Although the soaking in alkaline medium leads to higher removal of oligosaccharides from legumes than in water, the soaking in tap water may be preferable to that in sodium bicarbonate solution because of susceptibility of some vitamins of the B-complex group to alkali, particularly thiamine and riboflavin, which are known to be destroyed slowly in alkaline medium at room temperature (Kon, 1979).

Table 5. Effect of autoclaving (pressure cooking) of unsoaked and soaked legume seeds on oligosaccharide contents[a]

Samples	Sucrose		Raffinose family oligosaccharides			Percent of loss in raffinose family oligosaccharides[b]
	Content (%)	Loss (%)[b]	Raffinose (%)	Stachyose (%)	Verbascose (%)	
Faba bean						
Unsoaked	0·77	43·8	0·10	0·75	1·07	49·2
12 h soaking in water	0·67	51·1	ND	0·67	0·96	56·8
12 h soaking in sodium bicarbonate solution	0·52	62·0	ND	0·53	0·77	65·6
Lentil						
Unsoaked	0·70	38.6	0·12	0·80	0·34	53·7
12 h soaking in water	0·55	51·8	ND	0·63	0·31	65·4
12 h soaking in sodium bicarbonate solution	0·42	63·2	ND	0·49	0·26	72·4
Common bean						
Unsoaked	0·63	49·6	0·08	0·87	0·15	56·0
12 h soaking in water	0·52	58·4	ND	0·65	0·10	70·0
12 h soaking in sodium bicarbonate solution	0·40	68·0	ND	0·51	0·09	76·0
Cowpea						
Unsoaked	0·75	50·3	0·10	1·54	0·16	55·8
12 h soaking in water	0·60	60·2	ND	1·31	0·15	64·1
12 h soaking in sodium bicarbonate solution	0·53	64·9	ND	1·11	0·10	70·3

[a] Oligosaccharide content of autoclaved seeds (%, on dry weight basis).
[b] The loss expressed as percent of corresponding value for raw seeds.
ND = Not detectable.

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