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Influence of malting on selected components of soya bean, black bean, chickpea and barley

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Abstract

The influence of malting for 24 and 48 h on selected components was studied in soya bean, black bean, chick pea and barley. Proximate composition, calcium, iron zinc, α-galactosides including ciceritol, sucrose, phytic acid, myo-inositols phosphate and lectins were determined. The malting conditions were adequate to maintain the overall proximate composition and minerals. Galactosides decreased rapidly in all samples. Two days malting promoted a decrease of 91 and 84% in black bean and barley, respectively, while 44% was observed in the soya bean and only 34% in chickpea with a loss of 43% of ciceritol. The highest total levels of inositol phosphates were found in soya bean and black bean (478 and 450 mg%, respectively). IP6 and IP5 were not intensively affected by malting with the higher decrease of 25% observed in black bean. Lectin was detected in significant amounts only in soya bean and black bean and malting promoted 76% loss after 48 h in the black bean samples. The results indicated that short time malting may be useful to improve nutritional characteristics of the samples and that within the legume seeds studied black bean showed better results.

Keywords: Malting; Legume seeds; Barley; α-Galactosides; Myo-inositol phosphates; Lectin

1. Introduction

Malting is a very well stablished process used to produce cereal substrates for fermented beverages. Malted cereals may also be used to formulate nutritious products, including baby and weaning foods. However, despite the atractive cost and nutritional value of legume seeds, malted food items based on this kind of material are not common.

The germination process which occurs during malting promotes many chemical and biochemical changes in the seed which may increase the nutritional value of the final product and also decrease the amount of some undesirable compounds such as α-galactosides, trypsin inhibitors, lectins and phytates (Malleshi, Daodu, & Chandraskhar, 1989; Marero et al., 1988; Bau, Villaume, Nicolas, & Mejean, 1997).

Many works on soya bean germination have been described in the literature, demonstrating the advantages of this process for nutrition (Bau et al., 1997) but results for other regional legume seeds, including black bean and chick pea, are not so common.

Trypsin inhibitors and lectins of legume seeds impair animal growth and may be deleterious for human nutrition if cooking conditions are not sufficient to completely inactivate their activities. Some lectins are particularly resistant to heating, and besides the growth impairment, the lectin molecule may be readily endocytosed in the small intestine and transported into the circulatory system, affecting different organs (Peumans & Van Damme, 1996).

Recent interest in phytate has been directed towards the knowledge of the exact composition of the lower inositol phosphates derived from phytic acid. This interest is due to the observation that myo-inositols with different degrees of phosphorylation may have different chelation activities (Lönnerdal, Sandberg, Sandström, & Kunz, 1989).

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Since the effects of germination on the chemical composition of seeds are dependent on the plant species, seed varieties or cultivars and also on the germination conditions, it is relevant to have a better knowledge of the modifications involved during malting of different seeds with potential to be used alone or in combination for production of dietary formulas with attractive nutritive and economic characteristics.

In the present work a short period of malting was applied to three legume seeds (black bean, soya bean and chick pea) and also to one cereal (barley) and the changes in the chemical composition were followed after 24 and 48 h of malting. The background information obtained in this study will be important to compare the potential of these seeds for use in the formulation of products with higher nutritional value and a relative low cost. Barley was included in the study as a representative of the cereal group since the combination of legume with cereal may be potentially interesting to obtain formulations with better protein and energy quality.

No data are available in the literature for myo-inositol phosphates of black beans and chick peas and enphasis was directed to the speciation of myo-inositols with different degree of phosphorylation.

2. Materials and methods

2.1. Samples

Seeds of soya bean (*Glycine max* cv BR16) and black bean (*Phaseolus vulgaris* sp) were provided by EMBRAPA (Brazil) while chickpea (*Cicer arietinum* sp) and barley (*Hordeum vulgare* sp) were acquired in the local market of Chile.

2.2. Malting

The seeds were desinfected with sodium hypochlorite solution containing 25% (w/v) of chlorine and left soaking for 5 h in distilled water. Malted seeds were obtained by germination during 24 and 48 h periods in the dark at 30°C and the seeds were dried in an air oven at 60°C until reaching 7–12% of moisture. Dried samples were then milled to pass a 100 mm sieve prior to the analyses.

2.3. Proximate analysis, calcium, iron and zinc

Proximate analysis was carried out according to standardized AOAC methods (Williams, 1984). Calcium was determined in the ash solution of samples by the methyl-thymol blue reaction (Gindler & King, 1972) and iron and zinc were analyzed by flame absorption spectrophotometry according to detailed procedures described previously (Trugo, Farah, & Trugo, 1993).

2.4. α-galactosides

Galactosides were determined based on a procedure previously described (Muzquiz, Rey, Cuadrado, & Fenwick, 1992). Ground samples (0.5 g) were extracted with 80% (v/v) methanol for 1 min. The mixture was then centrifuged for 5 min at 3500 ***g*** and the supernatant decanted. This procedure was repeated twice and the combined supernatants evaporated to dryness under vacuum at 35°C. The residue was dissolved in double-deionized water (1 ml) and passed through Dowex 50WX8 and Waters QMA minicolumns by means of a Supelco vacuum system. The eluate was then used directly for HPLC. A Beckman HPLC System Gold (USA) consisting of a pump, a refractive index detector and a Rheodyne injection valve (20 μl loop) and an eletronic integrator was used. A Lichrosorb-5-NH2 column (250×4.6 mm i.d.) (Merck, Gernany) was employed with a mixture of acetonitrile/water (65:35, v/v) at 1 ml/min as the mobile phase. Individual sugars were quantified using external standardization, based on peak areas.

2.5. Phytic acid

The inositol phosphates were determined by the Lehrfeld (1994) method. The sample (0.5 g) was extracted with 5 ml of 0.5 M HCl using stirring for 3 h. The extract (2.5 ml) was diluted with 25 ml of water and placed onto a SAX column. The column was washed with 2 ml of water, and then the inositol phosphates were eluted with 2 ml of 2 M HCl. The eluate was evaporated to dryness and the residue was dissolved in a buffer solution. The solution was centrifuged at 7000 ***g*** for 6 min to remove any suspended material prior to injection into the HPLC.

The inositol tri- (IP3), tetra- (IP4), penta- (IP5) and hexaphosphate (IP6) were determined by ion-pair C18 reverse phase HPLC. The mobile phase was 51.5:48.5, methanol:water with addition of 8 ml of TBN-OH, 1 ml of 5M sulfuric acid, 0.5 ml of formic acid and 2 ml of a phytic acid solution (16.6 mg/ml) at a pH of 4.1 and a flow rate of 1 ml/min. The column consisted of a macroporous polymer PRP-1 5 mm (150×4.1mm, 5 mμ) which was used at 45°C. A Beckman System Gold HPLC equipment with a refractive index detector an electronic integration system and a fixed loop (20 μl) injection valve was used.

2.6. Lectins

Raw and processed ground samples were extracted with a solution of 0.1 M PBS (pH 7.4) at a concentration of 200 mg/ml using an Ultraturrax homogenizer (2 min). After centrifugation (4300 ***g***, 20 min, 4°C) PBS-diluted samples (4 and 100-fold, respectively) were used for the hemagglutination test and ELISA quantification.

Hemagglutination tests were carried out as described by Grant (1991). Two hemagglutination assays were used: native rat blood cells and cells treated with trypsin to increase the sensitivity of the assay. *Phaseolus vulgaris* cv. Processor (kidney bean) was included in each assay as a control. One hemagglutination unit (HU) was defined as the amount of material (g) in the last dilution at which 50% of the cells were agglutinated.

Competitive indirect ELISA assay was performed as previously described (Hajós et al., 1995) with some modifications. Antibody against phytohemmaglutinin was kindly supplied by Dr. Pusztai (Rowett Research Institute, Aberdeen, UK). Plates coated overnight at 4 C with 1mg/ml of phytohemmagglutinin (PHA) (in 0.05 M sodium carbonate–bicarbonate buffer, pH 9.8) were washed three times with PBST (0.01 M phosphate buffer–0.9% (w/v), NaCl–0.01% (v/v), Tween 20 , pH 7.4). Then 0.2 ml/well of PBSG (0.01 M phosphate-buffered saline containing 0.5% gelatin) was added and after incubation for 1h at 37°C the plates were washed three times with PBST. After this, 0.05 ml/well of standard PHA diluted in PBS or kidney bean samples with unknown content of lectin were added, followed by 0.05 ml/well of rabbit anti-PHA (E_2L_2) IgG antibody (diluted 1:2000 with PBS). Determinations were performed in triplicate for each data point. After incubation for 1 h at 37°C, the plates were washed three times with PBST and 0.1 ml of goat anti-rabbit IgG biotin conjugate (Sigma B-9642) diluted with PBS (1:10000 v/v) was added to each well and incubated for a further 1 h at 37°C. After washing (three times) with PBST, 0.1 ml of ExtrAvidin-peroxidase (Sigma E-8386) diluted 1:500 in PBS was added. After incubation for 1 h at 37°C the plates were washed five times with PBST and then 0.1 ml of a solution of OPD–H_2O_2 (0.34 mg/ml o-phenylene-diamine in 0.05 M phosphate–citrate buffer, pH 5.0–0.03% (v/v) hydrogen peroxide) was added to each well. After 5 min, the reaction was stopped by adding 0.05 ml of 3M H_2SO_4 and the optical density measured at 492 nm using a plate reader. The lectin content of the samples was estimated from the calibration curve (0.001–1000 mg/ml of standard PHA) using a half-logarithmic linear regression analysis.

3. Results and discussion

3.1. Proximate analysis and minerals

No marked variation was observed in the results of proximate analysis and minerals obtained from control and processed samples. However, there was a tendency to decrease of fat with a relative correspondent increase in protein as a function of the malting time, particularly in soya bean and black bean. Other small variations in the results, shown in Table 1, may be considered within the expected method error. The time and conditions used for malting the samples appear to be adequate to maintain their overall nutritive characteristics. As expected, the malted legume seeds represent good sources of macro- and micronutrients, including calcium, iron and zinc.

Table 1
Proximate composition, calcium iron and zinc of the samples

Sample	Moisture[a]	Ash	Fat	Protein	Calcium	Iron	Zinc
	g/100g				mg/100g		
Black bean	11.8	4.4	1.5	21.7	204	9.2	4.8
24 h malting	10.4	4.4	1.2	23.0	227	9.1	4.6
48 h	7.9	4.2	1.4	24.4	237	9.4	4.6
Soy bean	11.2	4.3	23.2	34.3	330	9.3	8.5
24 h malting	6.7	5.3	21.7	36.4	268	9.2	7.9
48 h	7.2	4.8	21.9	38.2	293	10.6	7.8
Chick pea	8.7	2.8	7.0	13.4	287	7.4	3.6
24 h malting	7.9	3.3	7.5	12.8	315	7.9	3.7
48 h	7.8	3.0	7.0	13.9	324	7.7	3.6
Barley	9.4	2.0	2.4	11.3	94.4	5.4	2.2
24 h malting	8.3	1.9	2.3	10.0	90.7	5.2	2.2
48 h	8.5	1.9	2.3	12.1	79.8	5.2	2.2

[a] After drying.
Results are averages of duplicate determinations, dry basis.

3.2. α-Galactosides

Raffinose oligosaccharides have been shown to contribute to flatulence production in man and animal; therefore their reduction/elimination in legumes is desirable (Sathe & Salunkhe, 1983). In this work the main α-galactosides found in the beans were raffinose and stachyose. Chickpea contained ciceritol but, in barley, the sugars found were only sucrose and raffinose (Table 2). Ciceritol is a less studied trisaccharide which was described by Quemener and Brillouet (1983) and should also be included in the α-galactoside group. The highest content of total α-galactosides was found in chickpea.

The effects of the malting process on the sucrose and α-galactoside contents in the different samples studied are shown in Table 2. These oligosaccharides decreased significantly during the malting in all samples analyzed. At the end of two days of malting a loss of 91 and 84% of total α-galactosides were observed in black bean and barley, respectively. In this period reduction was 44% in soya bean whereas the chickpea showed (at the end of the process) a reduction of 34% (Table 2). This behavior is in agreement with the results found in lentil by Corchete and Guerra (1987) who observed that galactosidase activity increased considerably during the first 24 h germination.

Table 2
Influence of malting process on sucrose and α-galactoside contents (g/100g, dry basis) of black bean, soybean, chickpea and barley

Sample	Sucrose	Raffinose	Ciceritol	Stachyose	Verbascose	Total galacroside
Black bean	1.85 ± 0.05	0.43 ± 0.03		2.34 ± 0.02	0.02 ± 0.005	2.79 ± 0.03[a]
1 day malting	1.31 ± 0.03	0.18 ± 0.02		0.62 ± 0.03		0.80 ± 0.04[b]
2 day malting	1.67 ± 0.08	0.01 ± 0.002		0.22 ± 0.03		0.23 ± 0.03[c]
Soybean	4.90 ± 0.14	0.88 ± 0.04		2.26 ± 0.09	0.21 ± 0.02	3.35 ± 0.13[a]
1 day malting	3.96 ± 0.09	0.42 ± 0.03		1.40 ± 0.06		1.82 ± 0.06[b]
2 day malting	4.37 ± 0.06	0.49 ± 0.03		1.39 ± 0.02		1.88 ± 0.03[b]
Chickpea	2.39 ± 0.02	0.52 ± 0.01	2.91 ± 0.02	2.20 ± 0.01	0.19 ± 0.02	5.82 ± 0.04[a]
1 day malting	1.27 ± 0.05	0.19 ± 0.01	1.59 ± 0.05	0.79 ± 0.02	0.33 ± 0.03	2.91 ± 0,09[b]
2 day malting	2.19 ± 0.09	0.37 ± 0.03	1.95 ± 0.06	1.20 ± 0.02	0.33 ± 0.03	3.85 ± 0.13[c]
Barley	1.05 ± 0.02	0.75 ± 0.02				0.75 ± 0.00[a]
1 day malting	0.55 ± 0.00	0.30 ± 0.04				0.30 ± 0.02[b]
2 day malting	0.34 ± 0.10	0.12 ± 0.04				0.12 ± 0.04[c]

Means followed by the same superscripts are not significant different at 5% level by *t*-test.

Sucrose in the legume seeds decreased only about 8 to 11% after 2 days of malting while in barley the decrease was of 67%. This is an indication that, while sucrose was readly available for seed matabolism, its turnover in the legume seeds was higher, since more galactosides were available for degradation compared to barley.

Except for chick pea, all malted products presented a considerable improvement in the digestible carbohydrate ratio (sucrose/total α-galactosides) (Trugo, Farah, & Cabral, 1995) as a function of the malting time, with black bean presenting higher improvement in the ratio after 48 h of malting (0.66 to 7.0) and chick pea showing the lowest upgrade (0.4 to 0.6).

3.3. Inositol phosphates

The nutritional importance of phytic acid lies in its ability to chelate several minerals, especially the divalent metals such as Ca, Fe, Zn and Mo, thereby reducing their availability in the intestinal tract. They may also interact with proteins to form insoluble complexes which inhibit the peptic digestion of ovoalbumin and elastin (Phillippy & Johnston, 1985; Lee & Karuranithy, 1990).

The determination of individual inositol phosphates has received much attention recently due to evidence that the chelation power is a function of the increased degree of phosporylation (Lönnerdal et al., 1989). In the present work a comprehensive evaluation of the inositol phosphates distribution was carried out and the results are presented in Table 3.

The highest total inositol phosphates found were for soya bean and black bean. Although some degree of hydrolysis was observed during processing, one and two days of malting were not sufficient to degrade much phytic acid in the samples. The soya bean phytate somehow presented more resistance to the process. The results indicate that there is a delayed activation of phytase during seed germination and, in the first stages of germination, the seeds use preferentially more readily-available inorganic phosphates. IP6 was the major inositol phosphate found in all samples and the relative proportion of IP3 to IP5 was low indicating a low degree of phytic acid hydrolysis in the seeds. IP6 and IP5 are in fact the inositol phosphates involved more intensively in the decrease of absorption of minerals in the gastrointestinal tract (Lönnerdal et al., 1989; Sandgerg, Carlsson, & Svanberg, 1989) and these were not much affected by the malting process. These results are somehow in agreement with data of Akpapunam,

Table 3
Influence of malting process on inositol phosphate content (mg/100 g, dry basis) of black bean, soybean, chickpea and barley

Sample	IP3	IP4	IP5	IP6	Total
Black bean		5 ± 1	61 ± 3	384 ± 17	450 ± 20[a]
1 day malting	26 ± 1	53 ± 4	68 ± 6	138 ± 6	285 ± 15[b]
2 day malting	15 ± 1	51 ± 3	113 ± 8	221 ± 13	400 ± 25[a]
Soybean		14 ± 1	82 ± 2	382 ± 15	478 ± 18[a]
1 day malting	1 ± 0	20 ± 4	92 ± 9	372 ± 29	485 ± 38[a]
2 day malting		19 ± 1	101 ± 4	362 ± 17	482 ± 20[a]
Chickpea		3 ± 0	33 ± 3	258 ± 19	294 ± 22[a]
1 day malting		6 ± 1	33 ± 2	210 ± 5	249 ± 7[b]
2 day malting		5 ± 1	37 ± 1	209 ± 1	251 ± 0[b]
Barley	2 ± 0	7 ± 1	53 ± 2	194 ± 7	256 ± 10[a]
1 day malting	4 ± 0	13 ± 1	37 ± 1	163 ± 7	217 ± 10[b]
2 day malting	3 ± 0	16 ± 2	48 ± 1	186 ± 4	253 ± 5[a]

Means followed by the same superscripts are not significant at 5% level by *t*-test.

Igbedioh, and Aremo (1996) in their study with soya bean. They observed a high percentage reduction of phytic acid only after 120 h of malting. Bau et al. (1997) also observed a decrease of 17% in soya bean phytic acid content only after five days of germination. In the present work only two days of malting was studied since the longer the process the more costly will be the final product. Black bean and soya bean presented similar and highest values of IP5 and IP6; however, black bean showed a decline of around 25% in the sum of the IP5 and IP6 during malting with the consequent generation of higher amounts of IP3 and IP4, indicating that it presents a better mineral availability when compared to soya bean.

3.4. Lectins

An initial screening of lectin content in unprocessed black bean, soya bean, barley and chickpea was carried out by using the hemagglutination assay (Table 4). In accordance with literature data (Grant, More, McKenzie, Steward, & Pusztai, 1983), the present results indicated that the highest hemagglutination activity (lectin content) corresponded to *Phaseolus vulgaris* (black bean), using native rat blood cells, but due to the low reactivity of soya bean with native cells, trypsin-treated rat blood cells were also used to determine its lectin content. The hemagglutination activities of barley and chickpeas were minimum against both native and trypsinated erythrocytes; consequently, the effect of malting on lectin content was studied only in black bean and soya bean. In addition to the hemagglutination test, competitive ELISA was also applied to the analysis of black bean (Table 5). The results showed that the malting process promoted a considerable reduction of hemagglutinin content in the black beans. After 1 day of malting, the initial hemagglutinin content (0.17 g%) was decreased by 46% and a reduction of 76% was achieved when the malting continued two days. This was in agreement with the decrease also detected by means of the hemagglutination assay. The influence of malting process on lectin content of soya bean showed a similar trend although not so intense (Table 5). Some authors have indicated that the germination over 4 days considerably reduces the lectin concentration of *Phaseolus vulgaris* and soya bean (Chen, Thacker, & Pan, 1977; Nielsen & Liener, 1988; Savelkoul, van der Poel, & Tamminga, 1992). In the present work a rapid decrease of hemagglutination activity was observed after two days of malting both for black bean and soya bean. From the results obtained, it is quite apparent that malting promoted a clear improvement in the nutritional characteristics of the legume seeds with a more pronounced effect on black bean.

The results obtained in this work indicate that short time malting may be a viable process to improve nutritional characteristics of legume seeds. Most of the anti-nutritional factors studied showed some degree of decrease, with black bean presenting the better results. The combination of the malted legume seeds with barley may represent useful ingredients for the elaboration of highly nutritive food formulas.

Table 4
Screening of lectin content of black beans, soybean, barley and chickpeas by determination of hemagglutination activity

Sample	Native rat blood cells		Trypsin treated rat blood cells	
	HA	HU (g/kg)	HA	HU (g/kg)
Black bean	195	5.13	4.5	250
Soybean	12500	0.08	390	2.56
Barley	12500	0.08	12500	0.08
Chickpeas	12500	0.08	1560	0.6
Standard Processor	49	20.4	0.2	5000
Pinto	12 500	0.08	49	20.4

HA: hemagglutination activity (amount of material (μg) containing 1 HU).
HU: hemagglutination unit or lectin equivalent.

Table 5
Influence of malting process on heamagglutination activity and lectin content (mean ± standard error) of black beans and soybean

Sample	HU (g/kg)	PHA (g/100 g)	ELISA PHA (g/100g)
Black bean			
0 day	5.13 ± 0.00^{a}	2.00 ± 0.00^{a}	0.17 ± 0.04^{a}
1 day malting	1.92 ± 0.64^{b}	0.75 ± 0.25^{b}	0.09 ± 0.01^{b}
2 day malting	1.28 ± 0.00^{b}	0.50 ± 0.00^{b}	0.04 ± 0.01^{c}
Standard (Processor)	20.41 ± 0.00	7.96 ± 0.00	0.59 ± 0.06
Soya bean			
0 day	2.56 ± 0.00^{a}		
1 day malting	1.60 ± 0.96^{a}		
2 day malting	0.80 ± 0.48^{a}		
Standard (Processor)	5000		

Means followed by the same superscripts are not significantly different at 5% level by *t*-test. Results expressed on dry basis.
PHA = Phaseolus hemagglutinin.

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References

Akpapunam, M. A., Igbedioh, S. O., & Aremo, I. (1996). Effect of malting time on chemical composition and functional properties of soya bean and bambara groundnut flours. *Int. J. Food Sci. Nut., 47*, 27–32.

Bau, H. M., Villaume, C., Nicolas, J. P., & Mejean, L. (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (*Glycine max*) seeds. *J. Sci. Food Agric., 74*, 1–9.

Chen, L. H., Thacker, R. R., & Pan, S. H. (1977). Effect of germination on hemagglutinating activity of pea and bean seeds. *J. Food Sci., 42*, 1666–1668.

Corchete, M. P., & Guerra, H. (1987). Alpha- and beta-galactosidase activities during germination of lentil seeds. *Plant Physiol. Biochem., 25*, 105–109.

Gindler, E. M., & King, J. D. (1972). Rapid colorimetric determination of calcium in biological fluids with methylthymol blue. *Am. J. Clin. Pathol., 58*, 627–630.

Grant, G. (1991). Lectins. In J. P. F. D'Mello, C. M. Duffus, & J. H. Duffus (Eds.), *Toxic Substances in Crop Plants* (pp. 49–67). Cambridge, UK: The Royal Society of Chemistry.

Grant, G., More, L. J., McKenzie, N. H., Steward, J. C., & Pusztai, A. (1983). A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. *British J. Nutr., 50*, 207–214.

Hajós, G., Gelencsér, E., Pusztai, A., Grant, G., Sakhri, M., & Bardocz, S. (1995). Biological effects and survival of trypsin inhibitors and the agglutinin from soybean in the small intestine of the rat. *J. Agric. Food Chem., 43*, 165–170.

Lee, Ch. K., & Karuranithy, R. (1990). Effects of germination on the chemical composition of Glycine and Phaseolus beans. *J. Sci. Food Agric., 51*, 437–445.

Lehrfeld, J. (1994). HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: problems and solutions. *J. Agric. Food Chem., 42*, 2726–2731.

Lönnerdal, B., Sandberg, A. S., Sandström, B., & Kunz, C. (1989). Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr., 119*, 211–214.

Malleshi, N. G., Daodu, M. A., & Chandraskhar, A. (1989). Development of weaning food formulations based on malting and roller drying of sorghum and cowpea. *Int. J. Food Sci. Technol., 24*, 511–517.

Marero, L. M., Payumo, E. M., Librando, E. C., Lainez, W.N., Gopez, M.D., & Homma, S. (1988). Technology of weaning food formulation prepared from germinated cereals and legumes. *J. Food Sci., 53*, 1391–1397.

Muzquiz, M., Rey, C., Cuadrado, C., & Fenwick, G. R. (1992). Effect of germination on the oligosaccharide content of lupin species. *J. Chrom., 607*, 349–352.

Nielsen, S. S., & Liener, I. E. (1988). Effect of germination on trypsin inhibitor and hemagglutinating activities in *Phaseolus vulgaris*. *J. Food Sci., 53*, 298–301.

Peumans, W. J., & Van Damme, E. J. M. (1996). Prevalence, biological activity and genetic manipulation of lectins in foods. *Trends in Food Science and Technology, 7*, 132–138.

Phillippy, B. Q., & Johnston, M. R. (1985). Determination of phytic acid in food by ion chromatography with post-column derivatization. *J. Food Sci., 50*, 541–542.

Quemener, B., & Brillouet, J. (1983). Ciceritol, a pinitol digalactoside from seeds of chickpea, lentil and white lupin. *Phytochemistry, 22*, 1745–1751.

Sandgerg, A. S., Carlsson, N. G., & Svanberg, U. (1989). Effects of inositol tri-, tetra-, penta- and hexaphosphates on *in vitro* estimation of iron availability. *J. Food Sci., 54*, 159–161.

Sathe, S. K., & Salunkhe, D. K. (1983). Technology of removal of unwanted components of dry legumes. In D. K. Salunkhe, & S. S. Kadan (Eds.), *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization* (Vol III, pp. 249–270.) Boca Raton, FL: CRC Press.

Savelkoul, F. H. M. G., van der Poel, A. F. B., & Tamminga, S. (1992). The presence and inactivation of trypsin inhibitors, tannins, lectins and amylase inhibitors in legume seeds during germination. A review. *Plant Foods Hum. Nutr., 42*, 71–85.

Trugo, L. C., Farah, A., & Trugo, N. M. F. (1993). Germination and debittering of lupin seeds reduce alpha-galactosides and intestinal carbohydrate fermentation in humans. *J. Food Sci., 58*, 627–630.

Trugo, L. C., Farah, A., & Cabral, L. (1995). Oligosaccharide distribution in Brazilian soybean cultivars. *Food Chem., 52*, 385–387.

Williams, S. (1984). *Official methods of the Association of Official Analytical Chemists* (14th ed., pp. 152–169.) Arlington, VA: AOAC.