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Effect of germination on the cyanide and oligosaccharide content of lima beans (*Phaseolus lunatus*)

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Lima bean seeds (*Phaseolus lunatus*) were germinated at room temperature for 5 days. Germination resulted in a decrease in the raffinose content; this was significant by the 5th day. In contrast, sucrose, glucose and galactose increased significantly by the 5th day of germination. There was a highly significant decrease in the cyanide content of the cotyledons when the seeds were germinated for 5 days, The shoot which appeared on the 4th day contained 11·7 mg kg^{-1} of cyanide and this increased to 50·9 mg kg^{-1} on the 5th day. In contrast, the amount of cyanide present in the root on the 3rd day decreased during germination. While germination resulted in an increase in protein content, the lipid content significantly decreased.

INTRODUCTION

The dried edible seeds of leguminous plants belonging to the Leguminosae family, known as pulses, are important sources of protein in the diets of millions of people in Asia, Africa and South American countries. Legumes were among the earliest food crops to be cultivated throughout the world by man and have a protein content ranging between 17% and 25% which is nearly twice that of cereals (Verma & Mehta, 1988).

Legumes however, contain anti-nutritional factors which reduce their protein digestibility and nutritive value, and they also contain natural toxicants (Onigbinde & Akinyele, 1983; Okolie & Ugochukwu, 1988; Abudu & Akinyele, 1989; Egbe & Ahinyele, 1990).

Egbe & Akinyele (1990) reported a cyanide value of 420 mg kg^{-1} in lima beans. Okolie & Ugochukwu (1988) reported a range of 381–1093 mg of cyanide kg^{-1} dry weight of intact seeds for variety of legumes. Ingestion of foods containing these toxic cyanogens could lead to either chronic or acute poisoning (Osuntokun, 1973). There is now increasing evidence that thiocyanate, the main metabolite of cyanide in animals, may lead to the production of nitrosamine *in vivo* which may enhance carcinogenesis (Okoh, 1992).

The occurrence of oligosaccharides in legumes has been found to reduce consumer acceptance of legumes. Ingestion of foods containing the oligosaccharides raffinose, stachyose and verbascose often results in production of intestinal gas or flatulence (French, 1954; Duperson, 1955). Legumes are especially well known to produce flatulence in humans (Onigbinde & Akinyele, 1983). Flatulence is a result of the activity of microflora present in the lower intestine which are capable of synthesizing the enzyme α-galactosidase (not present in the human digestive system), which breaks down the oligosaccharides, thereby producing intestinal gas (Calloway *et al.*, 1971; Fleming, 1981).

Several processing techniques, such as cooking (Onighinde & Akinyele, 1933), soaking (Okolie & Ugochukwu, 1988) and germination have been utilized to reduce or eliminate anti-nutritional factors and toxicants in legumes. However, no information is available on changes in oligosaccharide content with germination of lima beans.

Lima beans, known as *Ikpakpa* in the Esan community in Edo State of Nigeria, are prepared with care because of their high toxicity. Traditionally, they are prepared by boiling and changing the cooking water three or four times to reduce toxicity. Cooking usually lasts for 2 days.

The objective of this study was to investigate the effect of germination on the oligosaccharide and cyanide content of lima beans and to propose a cheap processing procedure for detoxifying lima beans. This paper also reports on the possible use of germination to improve the nutritional quality of lima beans.

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MATERIALS AND METHODS

Reagents

All reagents used were of analytical grade.

Source of sample

The samples of lima beans were all bought from the same source in a local market in the Esan community of Edo state, Nigeria.

Sample preparation

Good and healthy-looking beans were separated from immature and insect-infested ones by hand-picking. Germination was done according to the method of Wu & Wall (1980). After the germination, cotyledons, radicle/root and shoot were dried in a hot air oven a 60°C for 24 h, ground into a flour and stored in air-tight containers in the freezer. Ungerminated beans were skinned and the cotyledons milled into a flour and stored in the freezer as described above.

DETERMINATION OF SOME MAJOR NUTRIENTS

Crude protein was determined by the microKjeldahl estimation of nitrogen using a conversion factor of % N × 6·25 (Egan *et al.*, 1988). Lipid was determined according to the method of Gunstone (1969) using a solvent mixture of methanol:chloroform for lipid extraction. Ash, moisture and dry matter loss were determined according to the AOAC (1970) methods.

Determination of hydrogen cyanide

The cyanide determination combined the methods of Warburg (1931) and Lundquist *et al.* (1985). Standardization of stock cyanide solution was done according to the method of Brinkler & Seigle (1989).

Extraction of cyanide

A dried bean sample (0·59 g) was hydrolyzed using concentrated H_2SO_4 in a Warburg apparatus. The released cyanide ions were trapped in 0·3 ml of 0·1 M NaOH contained in the centre well of the apparatus by microdiffusion.

Quantification of liberated cyanide

Trapped cyanide was quantified by reacting with pyridine/barbituric acid reagent to produce a chromagen whose absorbance was read at 580 nm in a colorimeter within 8–15 min. Corresponding cyanide levels were extrapolated from a standard NaCN curve and expressed in milligrams per kilogram on a dry weight basis.

Determination of oligosaccharides

Extraction and identification of oligosaccharides (raffinose and sucrose) and other soluble sugars (glucose and galactose) were done according to Onigbinde & Akinyele's method (1983). The carbohydrates were extracted with 80% ethanol at 60°C, separated using paper chromatography and identified by means of authentic standards. Further confirmation was established by the use of R_f values of the sugars with respect to glucose.

Quantification of the carbohydrates was done according to the method of Dubois *et al.* (1956). Two identically spotted papers (Whatman No. 1 (5 cm × 45 cm)) with sample extracts were eluted for 20 h in a chromatographic tank containing a solvent mixture of *n*-butanol, ethanol, ammonia and water in the ratio 8:1:1:2. Separation was by downward elution. The papers were air-dried after elution and one of the two papers was developed using 10% $AgNO_3$ in acetone and 0·5 M ethanolic NaOH. It wad dried in the oven at 80°C for 5 min and the spots identified.

Spots on the developed paper strip were marked out correspondingly on the undeveloped paper strip, cut out carefully and each of the carbohydrates recovered by soaking the spots in 2 ml of distilled water. Each respective carbohydrate was reacted with 0·1 ml 5% phenol followed by rapid addition of 0·5 ml concentrated H_2SO_4 to produce a coloured solution whose absorbance was read at 490 nm after cooling in a water bath for 20 min. Corresponding carbohydrate levels were extrapolated from freshly prepared standard curves for the respective carbohydrates and their concentration expressed in milligram per 100 g on a dry weight basis.

RESULTS AND DISCUSSION

The results for some major nutrients in the germinated and ungerminated beans are shown in Table 1. Crude protein of seeds increased significantly ($P < 0·01$) from 18·8% to 22·9% on a dry matter basis. This increase in protein due to germination is in agreement with results obtained by other workers: Idouraine *et al.* (1989) reported a 12% increase in the crude protein content of tepary beans germinated for 14 h, King & Puwastien (1987) and Akpapunam & Achinewhu (1985) gave similar reports for winged beans and Nigerian cowpea, respectively. This increase might be due to rapid synthesis of hydrolytic enzymes as well as loss of initial dry matter. About 16% dry matter was lost by the 5th day of germination in this study.

Percentage lipid dropped from 3·10% in the ungerminated beans to 2·05% after 5 days of germination. This reduction (34%) was probably caused by the breakdown of fat by β-oxidation for energy purposes in embryo development. This finding is in agreement with that reported by Idouraine *et al.* (1989) who observed a 20% decrease in fat of tepary beans germinated for 14 h.

Moisture and ash increased from 12·7% and 3·12% in the ungerminated beans to 67·9 and 5·12% respectively by the 5th day of germination. During germination there is rapid synthesis of enzymes and co-enzymes

Table 1. Some major nutrients in germinated and ungerminated lima beans (cotyledon) (n = 4)

Days of germination	% Moisture	% Dry matter loss	% Protein	% Lipid	% Ash
0	12·69 ± 0·09	0·00 ± 0·00	18·8 ± 0·08	3·10 ± 0·14	3·12 ± 0·35
1	28·04 ± 0·49	0·49 ± 0·08	20·7 ± 0·14	2·93 ± 0·05	3·99 ± 0·46
2	43·93 ± 0·86	1·15 ± 0·08	21·2 ± 0·16	2·55 ± 0·10	4·06 ± 0·70
3	57·16 ± 0·36	6·78 ± 0·08	21·8 ± 0·16	2·43 ± 0·09	4·87 ± 0·01
4	65·26 ± 0·03	12·78 ± 0·08	22·6 ± 0·09	2·18 ± 0·05	5·00 ± 0·04
5	67·94 ± 0·11	16·09 ± 0·32	22·9 ± 0·09	2·05 ± 0·05	5·12 ± 0·01

as well as mobilization of organic and inorganic substances to meet the increased metabolic rate of the growing embryo. This could explain the increase in ash with germination, in agreement with the work of Yousef *et al.* (1987) who reported a similar increase in percentage ash in faba beans germinated for 3 days.

The results for cyanide contents of the ungerminated (cotyledon) and germinated beans (cotyledon, radicle/root and shoot) are shown in Table 2. It can be seen from the Table that there is a drastic decrease in the cyanide content of the cotyledon following germination. This change was found to be highly significant ($P < 0·01$). The amount of cyanide in the radicle/root was found to be 39·1 mg kg^{-1} by the 3rd day of germination. On the 4th day, the cyanide level dropped to 7·82 mg kg^{-1} and increased slightly to 9·28 mg kg^{-1} by the 5th day of germination. Similarly, the 4-day-old shoot was found to contain 11·7 mg kg^{-1}, which increased by the 5th day to 50·9 mg kg^{-1}.

The decrease in hydrogen cyanide content of the beans with increasing length of germination could be attributed to the presence of the hydrolytic enzyme, β-glucosidase, which hydrolyzed the parent cyanogenic glucoside (linamarin) to cyanohydrin and finally to free cyanide in the presence of the appropriate enzyme (hydroxynitrile lyase). The free cyanide formed is lost either through leaching or translocation to other developing parts such as the root, shoot or leaves of the growing plant for defensive purposes. This could explain why there was an appearance of cyanide in the radicle on the 3rd day of germination which dropped from 39·1 mg kg^{-1} to 7·82 mg kg^{-1} by the 4th day of germination, with a subsequent increase in cyanide level in the shoot between day 4 and day 5. From the result obtained, it can be seen that the ratio of cyanide distribution is higher in the shoot than the root. This finding is in agreement with the reported results for other growing plants (Com, 1969; Taylorson & Hendrick, 1973).

The appropriate lethal dose of orally ingested cyanide is between 200 and 300 mg cyanide per kg wet weight. The figure of 371 mg kg^{-1} dry weight detected in this study in the ungerminated beans is therefore a lethal concentration. But by the 5th day of germination, the cyanide level in the cotyledon was drastically reduced from 371 mg kg^{-1} to 51·8 mg kg^{-1}. With a cyanide of 50·9 mg kg^{-1} in the shoot and 9·7 mg kg^{-1} in the root, acute toxicity is ruled out if the whole seedling (cotyledon enclosing the young leaves, root and shoot) is consumed. Besides, the amount of cyanide in the seedling may be reduced to an innocuous level during the normal cooking procedure.

The results for the oligosaccharides (raffinose and sucrose) and other soluble sugar contents are shown in Table 3. Glucose, galactose and sucrose increased very significantly ($P < 0·01$) from 39·5 mg/100 g, 20·0 mg/100 g and 190 mg/100 g to 299 g/100 g, 190 mg/100 g and 790 mg/100 g, respectively by the 5th day of germination. Raffinose, on the other hand, decreased very significantly ($P < 0·01$) from 620 mg/100 g to 131 mg/100 g by the 5th day of germination.

The decrease in raffinose as days of germination increased can be attributed to the presence, of the enzyme β-galactosidase which hydrolyzed raffinose to sucrose and galactose, accounting for the observed increase in these sugars. Increase in glucose could have arisen from the hydrolysis of sucrose, starch and other polysaccharides by the appropriate enzymes.

A 5-g portion of raffinose (Calloway & Murphy, 1968) causes an elevation in flatus volume in humans, equivalent to that produced by 100 g of cowpeas, on

Table 2. Mean cyanide content of germinated and ungerminated beans (n = 4) (mg kg^{-1} dry matter basis)

Day of germination	Cotyledon	Radicle/root	Shoot
0	372 ± 0·00	NA	NA
1	280 ± 0·91	NA	NA
2	176 ± 0·90	NA	NA
3	127 ± 0·91	39·1 ± 0·00	NA
4	86·1 ± 0·00	7·82 ± 0·00	11·7 ± 0·00
5	51·8 ± 0·95	9·78 ± 0·25	50·9 ± 0·00

NA, not available.

Table 3. Oligosaccharides (raffinose and sucrose) and some soluble sugars in germinated and ungerminated beans (n = 4) (mg/100 g)

Days of germination	Glucose	Galactose	Sucrose	Raffinose
0	39·5 ± 1·00	0·00 ± 0·00	190 ± 0·00	620 ± 0·50
1	70·0 ± 0·00	20·0 ± 0·00	290 ± 0·00	560 ± 0·00
2	159 ± 2·00	65·0 ± 0·00	450 ± 0·00	467 ± 1·50
3	210 ± 0·00	120 ± 0·00	599 ± 0·00	280 ± 0·00
4	250 ± 1·00	160 ± 0·00	710 ± 0·00	220 ± 1·00
5	299 ± 1·00	190 ± 0·00	790 ± 0·00	131 ± 2·00

the average, which represents an elevation of about 300 ml in flatus (Hellendoorn, 1969). In this study, a mean value of 620 mg raffinose/100 g dry seed cotyledon was found in the raw beans. An average consumption of 500 g of lima beans would produce 186 ml flatus volume, which is enough to cause the discomfort in humans. By the 2nd day of germination, the equivalent flatus volume would be 47·9 ml. However, the effect of raffinose in the beans cannot be taken as absolute because the beans could also contain stachyose, melibiose, verbascose and other galactosides, which may be potentially flatulent, though these might have been hydrolyzed too during germination.

The prolonged cooking procedure with subsequent changing of cooking water, though reducing cyanide to safe levels (Egbe & Akinyele, 1990), consumes a lot of the energy used for the cooking process. It is therefore suggested from this study that the germination of lima beans and removal of testa prior to cooking could reduce cyanide and reduce cooking time in addition to flatus factors, as well as improving the amounts of some major nutrients in the beans.

Two days of germination is sufficient to reduce raffinose to a safe level where only 47·9 ml flatus volume would be obtained. But, at this stage, the cyanide level is high enough to cause chronic toxicity (176 mg kg^{-1}). On the 5th day of germination, the cyanide level reached 51·8 mg kg^{-1} which is a safe level. A 5-day germination is therefore recommended to obtain a desirable product with only about 16·1% loss in initial dry matter.

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