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The Cyanogenic Glycoside Contents of Raw and Processed Limabean Varieties

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ABSTRACT

Eighteen varieties of limabean (Phaseolus lunatus), *were subjected to the processes of cooking, autoclaving, soaking in water and germination for 6 days. The effects of these processes on the free and bound HCN contents of the raw limabean varieties were investigated. Total HCN in the raw varieties ranged from 265 mg kg*$^{-1}$ *in TPL 071-33 and 553 mg kg*$^{-1}$ *in TPL 13. Considerable variability was encountered in the different varieties and processing effects tended to make these varietal differences even more pronounced. Autoclaving gave a mean total loss of 53·9 % in total HCN content while cooking effected a 64·8 %–81·9 % loss in total HCN content. Drastic reductions in both free and bound HCN contents were obtained in all cooked varieties. Soaking for 2 days effected the highest HCN loss in TPL 2 (40·1 %), closely followed by TPL 13 (39·7 %) and then TPL 3 (35·4 %). All varieties, by the sixth day of soaking, lost between 61·3 and 86·4 % of their total HCN contents. The effect of germination on HCN contents increased progressively from a mean total loss of 24·5 % in day 2 to 55·6 % in day 4 and 76·1 % in day 6. Cooking and germination for 6 days appeared to be equally effective in reducing free and bound contents. Autoclaving was the least effective of all the processes studied.*

INTRODUCTION

The importance of grain legumes in ameliorating the protein deficit in the diets of several population groups has been well established (Tandon

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et al., 1957; Aykroyd & Doughty, 1964; Khan *et al.*, 1979). The utilization of their protein has, however, been shown to be adversely affected by the presence of several natural constituents such as protease inhibitors (Phadke & Sohonie, 1962; Chernikov *et al.*, 1966; Frost & Mann, 1966), hemagglutinin (Honavar *et al.*, 1962; Salgarkar & Sohonie, 1965), tannins (Elias *et al.*, 1979) and phytic acid (Oberleas, 1973). Limabean is one such legume which, in addition to these constituents, has been shown to contain cyanogenic glucosides (Conn, 1973). Such cyanogenic glycosides yield, on hydrolysis, hydrocyanic acid (HCN) which has been shown to depress growth through interference with the absorption of certain essential amino acids by growing rats (Flux *et al.*, 1956). Boey (1972) also observed that cyanide ions inhibited the cytochrome oxidase and hydrophenol oxidase enzymes through combination with their copper and iron ions, respectively. The presence of such glycosides in limabean could therefore be expected to further affect the utilization of associated nutrients.

While the presence of cyanogenic glycosides in cassava (*Manihot esculenta*) has been extensively studied (Bolhuis, 1954; Nartey, 1968; Osuntokun, 1970), the extent of their presence in varieties and cultivars of limabeans has been little studied. The present study was therefore designed to quantify the yield of free and bound hydrogen cyanide in several limabean varieties. The effects of different processing methods on these levels were also studied.

EXPERIMENTAL

Eighteen varieties of limabean were employed in this study. The samples, which were quite heterogenous in growth habit, colour, size and texture, had been described in a previous publication (Ologhobo & Fetuga, 1983). These were accessions obtained from the National Cereals Research Institute and the International Institute of Tropical Agriculture, both in Ibadan, Nigeria. Apart from the analysis of raw bean sample, each variety was subjected to the processes described below prior to freeze-drying and analyses.

Cooking

Fifty grams of each of the eighteen varieties were cooked with an adequate amount of water, using a pressure cooker set at 15 lb per square inch pressure for 15 min.

Autoclaving

The samples to be autoclaved were first milled in the raw form and then autoclaved at 105 °C at 15 lb per square inch pressure for 30 min.

Germination

Germinations of seeds were carried out in sterile Petri dishes lined with wet cotton wool for a period of 6 days. Seed samples had to be treated with 10 % v/v bleaching solution for 6 min and washed several times with running tap water in order to remove the seed coat colour which tended to prevent germination. Samples of sprouting beans were withdrawn from the germinating batches every 48 h, rinsed in water and freeze-dried.

Soaking

Soaking was carried out for 6 days with a continuous change of water after initial washing with mercuric iodide and rinsing with distilled water to remove surface contaminants. Samples were withdrawn at 48-h intervals, rinsed in water and freeze-dried.

Analytical procedure

The method of Wood (1965) was used for the quantification of hydrogen cyanide in limabeans. This involved the liberation of HCN from the limabeans by autolysis followed by treatment with acid. Free HCN was determined by incubating 2–3 g of the sample in a flask placed in a water bath maintained at 37 °C for 24 h after a prior addition of 0·025M cold borate buffer at pH 8·5. Total (Free + bound) HCN determination involved further treatment of substrates with 0·1M citrate buffer (pH 5·5) plus 1·0 ml of crude linamarase. The crude linamarase was prepared from fresh cassava peels as described by Tewe *et al.* (1980). These samples were also incubated at 37 °C for 24 h. Released HCN was distilled into 5 % (w/v) sodium carbonate and later reacted with picric acid to yield orange coloured iso-purpric acid. The absorbances were compared at 530 nm in a Spectronic 20, using 1 cm glass cuvettes.

RESULTS

Raw samples

The data for the HCN contents of the raw limabean varieties are shown in Table 1. Free HCN was highest in TPL 13 and lowest in TPL 071-33. The range obtained was from 249 mg kg^{-1} to 499 mg kg^{-1}. Bound HCN was comparatively lower than free HCN, ranging from 13·2 mg kg^{-1}in TPL 304 to 53·4 mg kg^{-1} in TPL 13. Particularly low values were also obtained in TPL 5, TPL 7, TPL 8 and TPL 071-33, all of which contained lower bound HCN values than the mean total value of 31·5 mg kg^{-1}.

Cooked and autoclaved samples

The effects of autoclaving and cooking are summarised in Table 2. Percentage total losses obtained in autoclaved samples ranged from 39·4 in TPL 187 and 63·8 in TPL 4. Residual total HCN was, however, highest in TPL 10 and lowest in TPL 071-33. The coefficients of variation for the free, bound and total HCN in all the autoclaved samples were 21·7 %, 40·9 % and 20·5 %, respectively.

Cooking resulted in an average total loss of 77·8 %. The varieties most affected included TPL 2, TPL 4, TPL 6, TPL 7, TPL 8, TPL 9 and TPL 304, which contained 103, 65·2, 106, 75·5, 89·6, 82·0 and 106 mg kg^{-1}, in residual total HCN, respectively. These values correspond to total losses of 77 %, 81·7 %, 79·0 %, 81·9 %, 78·0 %, 73·5 % and 77·4 %, respectively in total HCN contents. Effects of cooking were also remarkable in TPL 5, TPL 11 and TPL 071-33, as indicated by their low residual total HCN contents.

Soaked samples

The effects of soaking on limabean HCN contents are presented in Table 3. Two days of soaking resulted in a maximum loss of 40·1 % and a minimum of 20·9 % in total HCN. Free HCN ranged between 172 mg kg^{-1} in TPL 5 and 346 mg kg^{-1} in TPL 17 while bound HCN ranged from 10·5 mg kg^{-1} in TPL 304 to 45·4 mg kg^{-1} in TPL 13. Soaking for 4 days increased total losses to an average of 55·6 %, ranging between 33·7 % in TPL 187 and 69·2 % in TPL 2. With the exception of TPL 1, TPL 6, TPL 7, TPL 10, TPL 13 and TPL 304, whose residual

TABLE 1
Hydrocyanic Acid Content of Raw Unprocessed Limabean
(Dry Matter)

Limabean varieties	*Free HCN ($mg\,kg^{-1}$)*	*Bound HCN ($mg\,kg^{-1}$)*	*Total HCN ($mg\,kg^{-1}$)*
TPL 1	485	45·7	530
TPL 2	414	34·0	448
TPL 3	491	50·0	541
TPL 4	312	44·0	356
TPL 5	253	28·5	281
TPL 6	481	46·0	507
TPL 7	374	23·1	397
TPL 8	382	25·9	407
TPL 9	283	27·0	310
TPL 10	422	25·6	447
TPL 11	309	31·6	340
TPL 13	499	53·4	553
TPL 14	461	33·0	494
TPL 17	451	20·6	472
TPL 183	323	24·0	347
TPL 304	457	13·2	470
TPL 187	271	25·4	299
TPL 071-33	249	15·4	265
Mean	383	31·5	415
Standard deviation	88·1	11·8	94·2
(%) Coefficient of variation	23·0	37·5	22·7

total HCN exceeded 200 $mg\,kg^{-1}$, all the other varieties ranged between 112 $mg\,kg^{-1}$ in TPL 071-33 and 197 $mg\,kg^{-1}$ in TPL 187. After 6 days of soaking, TPL 8 and TPL 304 recorded 61·3 % and 64·8 % losses in total HCN, respectively, these being the least affected limabean varieties. The lowest residual total HCN contents were obtained in TPL 5 (71·8 $mg\,kg^{-1}$), TPL 8 (96·2 $mg\,kg^{-1}$), TPL 11 (60·7 $mg\,kg^{-1}$), TPL 187 (80·8 $mg\,kg^{-1}$) and TPL 071-33 (59·8 $mg\,kg^{-1}$).

Germinated samples

The effects of germination are summarized in Table 4. After 2 days of growth, the lowest loss in HCN content was recorded in TPL 4 (10·6 %), while the highest loss was obtained in TPL 14 (30·6 %). After 4 days, a maximum of 70·8 % loss was obtained in TPL 6. Variety TPL 1 contained

TABLE 2

Hydrocyanic Acid Content of Autoclaved and Cooked Limabean Varieties (mg kg^{-1} Dry Matter)

Limabean varieties	*Autoclaved varieties*				*Cooked varieties*			
	Free HCN	*Bound HCN*	*Residual total HCN*	*% Total loss*	*Free HCN*	*Bound HCN*	*Residual total HCN*	*% Total loss*
TPL 1	181	31·3	212	60·0	106	26·7	133	75·0
TPL 2	144	26·2	170	62·0	84·9	18·2	103	77·0
TPL 3	183	38·5	222	59·0	117	29·4	146	73·0
TPL 4	119	10·0	129	63·8	57·1	8·2	65·2	81·7
TPL 5	144	22·7	167	40·8	85·9	13·2	99·1	64·8
TPL 6	175	38·0	213	58·0	76·3	30·1	106	79·0
TPL 7	143	15·7	159	60·0	65·2	10·3	75·5	81·9
TPL 8	150	21·4	171	58·0	78·4	11·2	89·6	78·0
TPL 9	119	24·0	143	53·7	64·0	18·0	82·0	73·5
TPL 10	226	20·1	246	45·0	126	13·2	139	69·0
TPL 11	151	19·4	170	50·0	79·2	16·1	95·3	72·0
TPL 13	176	39·8	216	61·0	91·4	30·1	122	78·0
TPL 14e	215	21·7	237	52·0	113	15·7	128	74·0
TPL 17	216	14·9	231	51·0	145	10·4	156	67·0
TPL 183	179	16·9	196	43·4	106	11·6	118	66·1
TPL 304	205	10·0	215	54·4	97·4	9·0	106	77·4
TPL 187	158	21·4	180	39·4	82·9	19·5	102	65·5
TPL 071-33	97·4	13·8	111	58·0	54·2	12·0	66·2	75·0
Mean	166	22·5	188	53·9	90·5	16·8	107	77·8
Standard deviation	36·0	9·2	38·5	7·53	24·6	7·5	26·5	5·4
% Coefficient of variation	21·7	40·5	20·5	14·0	27·2	44·6	24·7	6·9

TABLE 3

Hydrocyanic Acid Contents of Soaked Limabean Varieties (mg kg^{-1} Dry Matter)

Limabean varieties	*Soaked for 2 days*				*Soaked for 4 days*				*Soaked for 6 days*			
	Free HCN	*Bound HCN*	*Residual total HCN*	*% Total loss*	*Free HCN*	*Bound HCN*	*Residual total HCN*	*% Total loss*	*Free HCN*	*Bound HCN*	*Residual total HCN*	*% Total loss*
TPL 1	336	40·3	376	29·1	178	27·4	205	61·3	138	15·7	154	71·0
TPL 2	211	30·6	242	40·1	118	20·1	138	69·2	97·3	12·0	109	75·6
TPL 3	319	31·3	350	35·4	175	19·6	194	64·1	132	10·3	142	73·8
TPL 4	221	42·6	263	26·0	145	28·4	174	51·2	90·5	15·1	106	70·4
TPL 5	172	24·9	197	30·1	108	17·5	126	58·8	61·1	10·7	71·8	74·5
TPL 6	312	41·1	353	30·4	192	29·2	222	56·3	130	18·7	149	70·7
TPL 7	294	20·6	314	20·9	204	11·6	215	45·8	146	7·5	154	86·4
TPL 8	281	22·7	303	25·6	112	9·4	121	47·2	90·7	5·5	96·2	61·3
TPL 9	273	22·6	296	27·9	168	15·3	183	55·3	111	8·6	120	70·8
TPL 10	306	19·9	326	27·3	196	8·7	205	54·2	117	4·1	121	72·9
TPL 11	204	25·4	230	32·6	125	17·0	142	58·4	53·1	7·6	60·7	82·2
TPL 13	288	45·4	333	39·7	185	30·7	216	60·9	132	22·1	154	72·1
TPL 14	331	30·6	362	26·7	155	20·8	176	64·2	100	14·0	114	76·8
TPL 17	346	16·2	362	23·4	150	9·7	160	66·2	100	5·6	106	77·5
TPL 183	258	21·0	279	21·7	178	10·6	189	47·1	95·6	6·4	102	71·4
TPL 304	337	10·5	348	26·0	234	7·2	241	48·8	161	4·5	166	64·8
TPL 187	244	23·1	267	29·4	183	13·2	197	33·7	73·2	7·6	80·8	72·8
TPL 071-33	190	11·0	201	24·2	104	7·7	112	57·8	53·6	6·2	59·8	78·2
Mean	273	26·7	300	29·0	162	16·9	179	55·6	105	10·1	115	73·5
Standard deviation	55·1	10·4	56·7	6·3	37·0	7·9	38·0	8·8	31·7	5·2	33·4	4·3
(%) Coefficient of variation	20·2	38·9	18·9	21·7	22·9	46·7	21·3	15·8	30·3	51·5	29·1	5·9

TABLE 4
Hydrocyanic Acid Content of Germinated Limabean Varities ($mg\,kg^{-1}$ Dry Matter)

Limabean varieties	*Germinated for 2 days*				*Germinated for 4 days*				*Germinated for 6 days*			
	Free HCN	*Bound HCN*	*Residual total HCN*	*% Total loss*	*Free HCN*	*Bound HCN*	*Residual total HCN*	*% Total loss*	*Free HCN*	*Bound HCN*	*Residual total HCN*	*% Total loss*
TPL 1	353	39·6	393	26·0	265	27·7	293	44·7	109	12·2	121	77·2
TPL 2	395	28·2	323	27·9	193	16·7	209	53·3	92·0	9·0	99·0	77·9
TPL 3	362	30·0	392	27·5	263	19·8	283	47·8	114	7·7	121	77·6
TPL 4	286	32·0	318	10·6	178	16·0	194	45·5	73·9	7·1	81·0	77·3
TPL 5	184	23·9	208	26·2	100	11·4	112	60·3	58·4	4·5	62·9	77·6
TPL 6	320	38·9	359	29·1	126	21·9	148	70·8	52·3	9·5	61·8	87·8
TPL 7	297	19·9	317	20·3	218	10·6	229	42·5	112	2·6	114	71·2
TPL 8	294	21·1	315	22·8	200	13·8	214	47·5	41·4	5·1	46·6	88·6
TPL 9	318	21·0	339	17·2	221	9·2	230	43·8	131	3·2	134	56·8
TPL 10	309	17·3	326	27·1	184	11·6	195	56·4	77·0	3·8	80·8	81·9
TPL 11	226	24·6	251	26·2	131	14·1	145	57·5	42·6	5·6	48·2	85·9
TPL 13	386	45·0	431	21·9	261	33·1	294	46·7	127	10·8	138	75·0
TPL 14	313	29·5	342	30·6	140	18·3	159	65·6	79·8	9·9	89·7	81·8
TPL 17	335	14·8	350	25·8	190	8·4	198	58·0	90·0	2·8	92·8	80·3
TPL 183	260	19·1	279	21·7	105	10·6	115	65·3	62·2	4·2	66·4	81·4
TPL 304	321	10·0	331	29·7	170	5·2	175	61·7	80·8	2·0	82·8	82·4
TPL 187	205	21·8	227	23·5	87·6	13·7	101	65·8	43·7	5·1	48·8	83·5
TPL 071-33	173	20·8	194	26·8	76·9	6·7	83·6	68·4	39·3	2·3	41·6	84·3
Mean	297	25·4	316	24·5	173	14·9	188	55·6	79·2	6·0	85·1	76·1
Standard deviation	64·9	9·1	64·1	4·9	60·3	7·2	64·2	9·4	30·2	3·2	31·1	17·8
(%) Coefficient of variation	21·8	35·8	20·3	20·0	34·9	48·3	34·2	16·89	38·1	53·3	36·5	24·5

265 mg kg^{-1} free HCN as the highest free HCN value, closely followed by TPL 3 with 263 mg kg^{-1} and TPL 13 with 261 mg kg^{-1}. Bound HCN was generally low in all varieties with the exception of TPL 13 and TPL 1 with bound HCN values of 33·1 and 27·7 mg kg^{-1}, respectively. In beans germinated for 6 days, residual total HCN contents ranged between 41·6 mg kg^{-1} in TPL 071-33 and 138 mg kg^{-1} in TPL 13. HCN losses were remarkable in all varieties with the exception of TPL 9, which contained 134 mg kg^{-1} in residual total HCN, corresponding to 56·8% total loss in HCN content.

DISCUSSION

The results presented in the preceding section suggest a fairly high content of cyanogenic glycosides in the varieties assayed which, on hydrolysis, yielded fairly high concentrations of hydrocyanic acid. Varietal differences were quite evident in all cases as judged by the fairly high coefficient of variation, which, in the case of bound HCN, was as high as 37·5% in the unprocessed beans. It is therefore quite possible that variable levels of cyanogenic glycosides occur in different varieties of limabean. This would be consistent with the observation with respect to cassava where high and low HCN varieties have been identified (Collens, 1945; Sinha & Nair, 1968).

The different accessions analysed were obtained from two sources and these, in turn, were collected from various locations. Quite apart from possible genetic variation, it has been shown (Bruijn, 1971) that soil nitrogen and soil mineral status affect the levels of the cyanogenic glycosides which, in the case of limabean, have been shown to be only linamarin (Viehoever, 1940). The variations obtained with regard to the yield of hydrocyanic acid may therefore have been due partly to genetic factors and partly to cultural practices and nutrient composition of the soil on which they were grown.

Among the processing methods studied, cooking, soaking and germination for 6 days were, on average, equally effective in eliminating HCN. However, germination for 6 days would result in a complete alteration in the nutrient status of the bean because of the mobilization of nutrients from the cotyledons to support vegetative growth which, by this time, was quite marked. The effectiveness of cooking in the removal of HCN would suggest that the traditional cooking methods would eliminate a good amount of the toxic HCN. One study (Charavanapavan, 1944) has indicated that the consumption of cooked limabean is safe. Some studies, however, exist on the consumption of cassava products by humans and rats (Osuntokun, 1968; Osuntokun *et al.*, 1969) which suggest that long-term consumption of low levels of residual HCN produces pathological effects. The residual HCN, even in the cooked beans, could therefore have some significance in areas where limabeans are regularly consumed. Several legumes such as *Vicia faba* and *Phaseolus vulgaris* have been successfully used as components of compound livestock feeds for chicken and pigs (Vestal & Shrewbury, 1952; Wagh *et al.*, 1965) after adequate autoclaving. In these reports, these legumes contained no cyanogenic glycosides and all the other anti-nutritional factors were eliminated by autoclaving. The fact that, on average, only about 53·9% of the HCN in autoclaved limabean is eliminated suggests that cyanide toxicity could be a problem in limabean based diets. There is also the possibility of increased requirement for the sulphur amino acids in such situations because of the increased need for detoxification.

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