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IMPLICATION OF PROCESSING ON OLIGOSACCHARIDE CONTENTS OF COWPEA VARIETIES

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Target Audience: Plant breeders, feed and feeds toxicologists, nutritionists.

ABSTRACT

Five cowpea varieties were used. The seeds were subjected to autoclaving, cooking, germination and soaking. No raffinose and little stachyose were detected in the germinated samples but autoclaving and cooking gave appreciable losses intheseoligosaccharides. Soaking was ineffective in the removal of raffinose and stachyose as losses were insignificant. The different processing methods significantly reduced the oligosaccharides at varying degrees (P <0.05). Since raffinose and stachyose concentratins were influenced by genetic variation (P <0.05), elimination of these oligosaccharides could be effected by selection.

Key words: Processing, oligosaccharides, cowpea varieties.

DESCRIPTION OF PROBLEMS

Result of surveys carried out in the various countries with relatively large legume intakes among ,the adult populations showed that mothers did not give legume food to small children andmuch lessto infants. The reasons given are that they are not well - tolerated and caused diarrhoea and vomiting (1). These digestive disorders could be caused by unavailable carbohydrates in grain legumes.

Due to the inherent structures of assoicated proteins and carbohydrates in legume seeds, other factors beside antinutritonal factors, are responsible for the poor digestibility observed in man and livestock when used as feed ingredients. Legumes contain considerable quantities of poorly digested non starch polysaccharides (NSP) in the form of Oligosaccharides, consisting of raffinose, stachyose and verbascos (2) Originally the oligosaccharides were considered to make minor contributions to the nutrition of simple stomach animals (3) Now, there is evidence that some oligosaccharides posses anti-nutritive activities that affect energy and protein utilisation in young animals especially poultry (4) are specific for α – linked galactose containing oligosaccharides found in legumes. Lack of the necessary α – galactosidase enzyme in all monogastric species, including man, means that

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oligosaccharides are fermented by gut microflora and yield volatile fatty acids and gases instead of usable monosaccharides and disaccarides produced by hydrolysis with the α – galactosidase enzyme (5) Fermentation, in lieu of enzymatic hydrolysis, yields less energy and creates digestive disturbances in many animal species (6,7,8). In order to improve the utilisation of legume carbohydrates by removing the alpha (1 --> 6) linkages in oligosaccharides, the objective of this study was directed towards subjecting cowpea seeds to different processing treatments and then identify the most effective method.

MATERIALS AND METHODS

Seeds of cowpea varieties used for this study were supplied by IITA Ibadan. The varieties included IT 84E-1-108, IT 82D889, IT 81D-1137, IT 82E - 16 and Ife Brown.

(a) **Autoclaving:** Air - dried cowpea grains were milled and the flour autoclaved at 105°C and 10546.05 kgm-2 pressure for 30 minutes. Autoclaved samples were then dried at 65°C for 48 hours, milled and stored in screw-capped bottles at 4°C.

(b) **Germination:** Seeds were immersed in 2% sodium hypochlorite solution for two minutes to kill all pathogenic organisms that they may contain and then spread thinly or a tray.

Every 24 hours, seeds were sprayed with distilled water at 25°C according to the method of some workers (9). Germinated seeds were dried at 65°C for 48 hours, milled and stored in acrew-capped bottles at 4°C.

(c) **Cooking:** Raw cowpea grains were cooked with a pressure cooker at 105°C for 30 minutes. The cooked beans were dried at 65°C for 48 hours, milled and then stored in screw-capped bottles at 4°C.

(d) **Soaking:** Raw seeds were soaked with distilled water for 24 hours, rinsed thrice with distilled water, dried at 65°C for hours, milled and stored in screw-capped bottles at 4°C.

Estimation of oligosaccharides:

Raffinose and stachyose were estimated by extracting the oligosaccharides with 70% ethanol and concentrating the extract in a rotary evaporator at 40°C. The syrup obtained was made up to 25cm^3 volume with distilled water and then subjected to paper chromatography. In the latter procedure, the extracts were spotted along the short edge of a 46 x 57mm What man No. 1 filter paper next to authentic raffinose and stachyose (10). Area corresponding to each sugar was cut, eluted with distilled water and colorimetrically quantified (11).

Statistical analysis: Results were subjected to a randomised complete block analysis of variance while treatment means were compared using Duncan's Multiple Range Test (12).

RESULTS AND DISCUSSION

Raffinose contents of cowpea varieties as influenced by different processing methods are shown in Table 1. Values in the raw samples ranged from 1.25g/100g in Ife Brown to 2.44g/100g in IT 81D-1137, with a mean value of 1.97g/100g.

Table 1: Effect of Processing on Raffinose Content of different Cowpea varieties (g/100g DM)

	Processing				
Cowpea Variety	Auto-claving	Cooking	Germi-nation	Soaking	Raw
IT 84E-1-108	0.61	0.75	0.00	1.86	2.33
IT82D-889	0.65	0.46	01/200	1.26	1.85
IT 81D-1137	0.95	0.73	0.00	1.83	2.44
IT 92E-16	0.74	0.68	0.00	1.40	2.00
Ife Brown	0.58	0.63	0.00	0.93	1.25
Mean	0.71a	0.65a	0.00d	1.46b	1.97c
SE-x	±0.04	±0.03	±0.00	±0.10	±0.12

Each value is a mean of three determinations.
a,b,c,d = Means followed by different letters are significantly different (P 0.05).

Raffinose contents in the autoclaved samples ranged from 0.58/100g in Ife Brown to 0.95g/100g in IT 81D-1137 with an average of 0.71g/100g. Cooked samples had values that ranged from 0.46g/100g in IT 82D-889 to 0.75/100g in IT 84E-1-108, having 0.65g/100g as the average. Soaked samples recorded values ranging from 0.93/100g in Ife brown to 1.86g/100g in IT 84E - 1 - 108. Interestingly however, germinatin resulted intotal elimination of raffinose in all the selected cowpea varieties. On the average, percentage loss of raffinose in other processing methods are 65.73 by cooking, 63.27 by autoclaving and 22.50 by soaking (Table 2).

As for stachose, raw samples had values ranging from 2.66g/100g in Ife brown to 4.35g/100g in IT 81D - 1137, with an average of 3.65g/100g while soaking, with low efficiency, had the lowest value of 2.43g/100g in Ife Brown and the highest value of 3.87/100g in IT 84E - 1 - 108. The average value obtained for the soaked samples was 3.34g/100g. Germination gave a drastic reduction in stachyose content with values ranging between 0.13g/100g in Ife Brown and 0.40g/100g in IT 82E-16. The mean value wa 0.26g/100g. Cooked samples had 1.05g/100g as the highest value in IT 8 1 - 108 while 1.45g/100g was

Table 2: Percent Loss in Raffinose content of process cowpea varieties

Cowpea Variety	Processing			
	Auto-Claving	Cooking	Germination	Soaking
IT 84E-1-108	73.82	67.81	100.00	20.17
IT 82D-889	64.86	51.14	100.00	31.89
IT 81D-1137	61.07	70.08	100.00	25.00
IT 82E-16	63.00	66.00	100.000	30.00
Ife Brown	53.60	49.60	100.00	25.60
Mean	63.27a	65.73a	100.00b	22.50c
SE-x	±2.49	±2.49	±0.00	±1.19

Each value is a mean of three determinations.
a,b,c,d = Means followed by different letters are significantly different (P 0.05).

recorded as the average.(Table 3). The ascending order of effectiveness in the removal of stachyose by the different processing methods is as follows: soaking < autoclaving,< cooking < germination respectively (Table 4). variety and processing significantly ($P<0.001$) affected raffinose and stachyose contents. The varietal effect may be due to genetic variation (7,13).

Table 3: Effect of Processing on Stachyose content of different cowpea varieties (g/100g DM)

Cowpea Variety	Processing				
	Auto-claving	Cooking	Germination	Soaking	Raw
IT 84E-1-168	1.55	1.42	0.22	3.41	3.88
IT 82D-889	1.27	1.10	0.18	3.21	3.33
IT 81D-1137	2.18	1.90	0.37	3.76	4.35
IT 82E-16	1.46	2.05	0.04	3.87	4.05
Ife Brown	0.80	1.05	0.13	2.43	2.66
Mean	1.45a	1.50a	0.26b	3.34c	3.65d
SE-x	±0.13	±0.12	±0.03	±0.15	±0.17

Each value is a mean of three determinations.
a, b, c, d, = Means followed by different letters are significantly different ($P<0.05$)

Table 4: **Percent loss in stachyose content during Processing**

Cowpea Variety	Processing: Autoclaving	Cooking	Germination	Soaking
IT 84E-1-108	60.05	63.40	94.33	12.11
IT 82D-889	61.86	66.97	94.59	3.09
IT 81D-1137	49.89	56.32	91.49	13.56
IT 82E-16	63.95	49.38	90.12	4.44
Ife Brown	69.92	60.53	95.11	8.65
Mean	61.13a	59.32a	93.13b	8.37c
SE- x	±1.89	±0.75	±0.57	±1.19

Each value is a mean of three determinations.
a, b, c, d, = Means followed by different letters are significantly different (P<0.05)

The result of this study has proved germination as the most effective method of eliminating raffinose and stachyose from cowpea varieties. Germinating soybean seeds have been shown to utilise oligosaccharides rapidly apparently removing raffinose and stachyose (14,15).

Similarly, rduction in oligosaccharide content of legumes during germination with concomitant conversion to glucose and fructose has been reported (16,17,18). The results of this investigation are in conformity with the reported findings.

The reduction in oligosaccharide content due to germination had been ascribed to enzyme activities (19). Alpha-galactosidase present in both dormant and germinating seeds has been reported to be responsible for the hydrolysis of oligosaccharides to their constituent units. Sequential action of enzymes has also been shown to accelerate oligosaccharide hydrolysis. Some workers (7,20) have demonstrated that a rapid and complete removal of sucrose bysucrase stimulated raffionose and stachyose hydrolysis thermodynamically.

The inability of soaking to appreciably reduce oligosaccharides in the cowpea varieties may be due to a feed-back mechanism between enzyme activity and hydrolysis products as it has been copiously proved that there is enzyme activity in dormant seeds (19,20,21). This shows that products ofthe oligosaccharide break down (glucose and fructose) are present in dormant seeds, but as soon as the seeds are soaked, hydrolytic activities of sucrase and alpha-galactosidase start resulting in the production of fructose and glucose.

Because these sugars are not utilised or removed, they would accumulate and inhibit further breakdown of galactose by the enzymes. Consequently, high level of the oligosaccharides would be found in soaked samples. hence, is ineffectiveness as a processing method.

Autoclaving and cooking methods are characterised by thermal application. Since enzymes are proteins, inactivation of alpha-galactosidase by thermal processing would be inevitable. Hence, the appreciable loss in raffinose and stachyose by autoclaving and cooking would be non-enzymatic. It is possile that glucose and fructose linkages within raffinose and stachyose molecules were susceptible to thermal hydrolysis but the linkages were more susceptible to enzyme activity.

CONCLUSION AND APPLICATIONS

Conclusion:

1. Germination was the most effective method of removing the oligosaccharide content of cowpea seeds.
2. Raffinose was completely removed during germination while a greater proportion of stachyose ws removed during germination
3. Removal of oligosaccharides during germination was enzymatic
4. Ife Brown had the least oligosaccharide content while IT 81D -1137 had the highest value.

Applications:

1. Since monogastric animals and man do not possess enzymes that could hydrolyse the oligosaccharides, the result of this study would increse utilisation of cowpea carbohydrates by simple - stomached animals including man when germinated cowpeas are incorporated into livestock feeds and human foods.
2. Because all the stachyose were not removed by germination, to enhance complete removal, plant breeding can select cowpea varieties with low oligosaccharide content.

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