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The Effect of Germination on the Oligosaccharide and Nutrient Content of Cowpeas (*Vigna unguiculata*)

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ABSTRACT

*Two cultivars of cowpea (*Vigna unguiculata*) seeds were germinated for 96 h and sampled at 24, 48, 72 and 96 h to determine changes in oligosaccharides as well as energy, protein, ascorbic acid, niacin, thiamin, calcium, magnesium, iron, zinc, potassium and phosphorus.*

The results showed that there was a gradual decrease in oligosaccharide content with germination while the level of monosaccharides increased. Protein and energy content increased slightly while calcium and iron decreased with germination. Both ascorbic acid and niacin increased significantly while thiamine decreased significantly. Thus, germination may be a useful process for improving the nutritive value of cowpeas.

INTRODUCTION

Cowpeas have the potential to improve local diets due to their protein content and their essential role in weaning. While they are comparatively good and cheap sources of protein, they contain some anti-nutritional factors which will reduce their use in the diet. The anti-nutritional factors of importance to children are the oligosaccharides which have flatulence-producing properties due to the human lack of the α-galactosidase enzyme and the consequent fermentation of the oligosaccharides in the colon to produce gas (Steggada, 1968; Calloway, 1973; Christofaro *et al.*, 1974; Rackis, 1975; Carpenter, 1981).

Hussain *et al.* (1984) have shown that consumption of cowpeas by young children is responsible for the development of diarrhoea, abdominal distension and vomiting. Consequently the presence of substantial amounts of the oligosaccharides impedes full nutritional utilization of cowpeas.

Many studies have used the enzyme α-galactosidase from different sources to reduce the oligosaccharides with varying degrees of success (Sugimoto & Van Buren, 1970; Thamanakul *et al.*, 1976). The economic feasibility of such treatment is yet to be evaluated. There is, however, little information on the effect of natural processes on the oligosaccharides of cowpeas and this study was carried out to determine the effect of germination on oligosaccharides and nutrient content of cowpeas.

MATERIALS AND METHODS

Two cultivars of cowpeas, the local white and local brown, commonly consumed in Nigeria were studied. Clean cowpea seeds of both cultivars were germinated using the method of Reddy and Salunkhe (1980). The seeds were washed with distilled water and soaked for 10 min before being germinated in Petri dishes lined with wet cotton wool at room temperature.

Germination was for 96 h and triplicate Petri dishes were collected for chemical analysis every 24 h for each cultivar. The germinated seeds as collected were dried separately at a low temperature (50°C) in an air oven (Gallenkamp). The dried seeds were milled into a fine powder and stored in a refrigerator prior to analysis.

Compositional analysis

Oligosaccharides were determined by paper chromatography in duplicates as previously described (Onigbinde & Akinyele, 1983). Ten grams of the cowpea powder for each cultivar was extracted using hot ethanol and filtered through a Whatman No. 1 filter paper. A mixture of *n*-butanol, ethanol, ammonia solution and water in the radio of 8:1:2:1 formed the eluent. The elution was for 40 h. The sugars were identified on the basis of their RG values.

Monosaccharides were separated also by paper chromatography and the sugar extracts eluted using a mixture of N butanol, glacial acetic acid and water in the ratio 4:1:1.

The concentration of the identified sugars was determined using the phenol sulphuric acid method of Dubois *et al.* (1956). Moisture was determined using the AOAC (1980) procedure with an air oven (Gallenkamp). Moisture content was calculated by difference and expressed as a percentage.

Protein was determined using the micro-Kjeldahl method (AOAC, 1980). Digestion was carried out on a Techne Dri-block DB 4 system and distillation was with the Markham apparatus. A factor of 6·25 was used to obtain the protein content.

The caloric values of the samples were determined using the ballistic bomb calorimeter (Gallenkamp model CB-370) with benzoic acid as standard (AOAC, 1980).

Ascorbic acid was determined using the 2, 6-dichlorophenol indophenol visual titration method (AOVC, 1966). This method is based on the reduction of the dye 2,6-dichlorophenol indophenol by an acid solution of ascorbic acid. The capacity of an extract of the sample to reduce a standard solution of the dye as determined by titration is directly proportional to the ascorbic acid content.

Niacin was determined using the AOAC (1980) procedure while thiamine was determined using the AOVC (1966) procedure. Mineral analysis was by the wet digestion method using 10 ml of combined concentrated nitric and concentrated sulphuric acid in the ratio of 3:1. The digest was made up with deionized water and 2 ml of a 5% solution of lanthanum chloride added to prevent cation–anion interference. Analysis was for calcium, magnesium, potassium, iron and zinc using the atomic absorption spectrophotometer Perkin Elmer model 4000 with the appropriate hollow cathode lamps. Phosphorus was determined using the vanado–molybdate method (Kitson & Mellan, 1944).

RESULTS

The results of the study are presented in Tables 1 to 4. Table 1 shows changes in both the monosaccharide and oligosaccharide contents as a result of germination for 96 h. The raw beans contained $4{\cdot}03 \pm 1{\cdot}5\%$ verbascose, $3{\cdot}56 \pm 0{\cdot}2\%$, stachyose, $1{\cdot}95 \pm 0{\cdot}6\%$ raffinose, $1{\cdot}48 \pm 1{\cdot}0\%$ sucrose. Both glucose and galactose were found to be $0{\cdot}55 \pm 0{\cdot}8\%$ while no detectable level of fructose was present. After 48 h germination there were no detectable levels of verbascose and raffinose while stachyose disappeared completely after 72 h. The disappearance of oligosaccharides was accompanied by increases in the levels of sucrose and other monosaccharides (Table 1).

The protein content of the cowpeas increased slightly from $25{\cdot}8 \pm 0{\cdot}3\%$ to 27·6% after 96 h germination though the increase was not statistically significant. The energy content also increased after 24 h germination which was significantly different from the raw cowpeas ($P < 0{\cdot}05$) (Table 2).

TABLE 1
Sugar Percentages in Cowpea during Germination (Mean ± SD)

Germination *(h)[a]	*Vebascose*	*Stachyose*	*Raffinose*	*Sucrose*	*Fructose*	*Glucose and galactose*	*Total oligosaccharides*	*Total sucrose, fructose, glucose and galactose*
0	4·03 ± 1·5	3·56 ± 0·2	1·95 ± 0·0	1·48 ± 1·0	0·0 ± 0·0	0·55 ± 0·8	9·54	2·03
24	2·07 ± 2·2	2·48 ± 1·6	2·65 ± 2·8	3·05 ± 0·0	0·0 ± 0·0	0·9 ± 0·35	7·2	3·95
48	0·0 ± 0·0	0·72 ± 0·0	0·0 ± 0·0	3·90 ± 0·0	0·7 ± 0·3	0·82 ± 0·06	0·72	5·42
72	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0	4·52 ± 0·0	1·27 ± 0·6	2·2 ± 0·7	0·00	7·99
96	0·0 ± 0·0	0·0 ± 0·0	0·0 ± 0·0	4·33 ± 0·6	1·47 ± 0·09	0·81 ± 0·4	0·00	6·61

* Analysis of variance showed a difference between the sugars with germination ($P < 0·05$).

TABLE 2
Changes in the Protein and Energy Content of Cowpeas (Local White and Local Brown Varieties) with Germination

	Protein (*g/100 g*)	*Energy* (*kcal/100 g*)
Raw beans	25·80 ± 0·3	428·5 ± 6·4
Germination		
24 h	26·60 ± 0·42 (+3·1%)	458·0 ± 56·5[a] (+6·9%)
48 h	27·15 ± 0·9 (+5·2%)	426·0 ± 25·4[a] (−0·6%)
72 h	29·00 ± 1·1 (+12·4%)	438·0 ± 16·3[a] (+2·1%)
96 h	27·55 ± 0·9 (+6·8%)	450·5 ± 2·12[a] (+5·1%)

Values in parentheses indicate the per cent change.
[a] Statistically significant from the raw beans ($P < 0.05$).

There was a decrease in the levels of calcium and iron with germination. Calcium decreased from 55·2 mg% in the raw beans to 52·4 mg% while iron decreased from 21·8 mg% to 13·3 mg% (Table 3). There were slight increases in both magnesium and zinc while the changes in potassium and phosphorus were statistically significant ($p < 0.05$).

The changes in the vitamin concentration of cowpeas with germination are listed in Table 4. Vitamin C increased progressively and significantly ($P < 0.05$) during the 96 h germination. Similarly, niacin increased

TABLE 3
Mean Mineral Contents of Cowpeas (Local White and Local Brown Varieties) as a Result of Germination (Mean ± SD)

	Calcium (*mg/100 g*)	*Magnesium* (*mg/100 g*)	*Iron* (*mg/100 g*)	*Zinc* (*mg/100 g*)	*Potassium* (*mg/100 g*)	*Phosphorus* (*mg/100 g*)
Raw beans	52·2	90·8	21·8	4·1	1 246	406·3
Germination						
24 h	51·3	93·6	17·3	4·2	126·9[a]	331·3[a]
48 h	49·6	90·5	18·5	4·1	1 268	470·0[a]
72h	50·3	92·5	13·2	4·3	1 298·5[a]	468·8[a]
96 h	52·4	94·0	13·3	4·5	1 239	487·5[a]

[a] Difference statistically significant from the values for the raw beans.

TABLE 4
Changes in the Concentrations of some Vitamins in Cowpea (Local White and Local Brown Varieties as a Result of Germination (Mean ± SD)

	Vitamins		
	Vitamin C (mg/100 g)	*Niacin (mg/100 g)*	*Thiamin (mg/100 g)*
Raw beans	0–2·5 ± 0·0	3·75 ± 0·8	0·87 ± 0·2
Germination			
24 h	14·8 ± 0·0[a] (+492%)	3·85 ± 0·9[a] (+2·7%)	0·81 ± 0·2[a] (−6·9%)
48 h	19·8 ± 0·0[a] (+690%)	9·47 ± 1·3[a] (+152·5%)	0·43 ± 0·2[a] (−59·6%)
72 h	20·98 ± 1·7[a] (+739%)	13·7 ± 1·7[a] (+265·9%)	0·43 ± 0·2[a] (−50·6%)
96 h	23·35 ± 1·6[a] (+834%)	12·7 ± 2·1[a] (+237·3%)	0·52 ± 0·03[a] (−40·2%)

Values in parentheses indicate the per cent change.
[a] Values are statistically significant from the values of the raw beans ($P < 0{\cdot}05$).

significantly ($P < 0{\cdot}05$) while thiamine also decreased significantly ($P < 0{\cdot}5$) (Table 4).

DISCUSSION

Cowpeas continue to be one of the local food resources which, if properly prepared and used with the staples of the region, will provide nutritious weaning foods for young children. One factor related to indigestibility, which is a deterrent to maternal acceptance of legumes for feeding young children, is flatulence.

Germination of cowpeas in this study led to a disappearance of the oligosaccharides which have been implicated in flatulence. After 72 h of germination the total oligosaccharide content had been reduced to zero. This observation is similar to the findings of Hattori and Shiroya (1951), Reddy and Salunke (1980), Rao and Belavady (1978), Silva and Luh (1979) and Mehta *et al.* (1985). The disappearance of the oligosaccharides was accompanied by an increase in the level of monosaccharides which can be utilized for energy by the growing child. The monosaccharides will also increase the palatability of cowpeas for children.

The disappearance of oligosaccharides during germination has been attributed to the increase in the level of α-galactosidase enzyme activity (Reddy & Salunkhe, 1980). Germination in this study led to significant increases of 20% in phosphorus, 4·5% increase in zinc and 3·5% increase in magnesium levels. A decrease was observed for calcium (5·1%) and iron (3·5%). These decreases were not statistically significant. This finding agrees with the report of Chen (1970) and Fordham *et al.* (1975).

The increase in the phosphorus content due to germination can be explained by the increase in phytase activity during germination (Singh & Banerjee, 1953; Mayer, 1958; Walker, 1974; Tabekhia & Luh, 1980). Phytase breaks down phytic acid phosphorus and thus increases the phosphorus level. The decreases observed in calcium, and iron, as well as potassium, may be due to utilization by the growing shoot or leaching into water during germination. The decrease in phytate will increase the bioavailability of dietary copper, magnesium, iron, manganese and zinc which form complexes with phytate.

Germination also led to statistically significant increases in vitamin C of 834% due to its production by the growing shoot. The vitamin C increase will only be useful to the human diet if the seeds are eaten raw since prolonged cooking of the beans will destroy the vitamin C. A significant increase of 237% was observed for niacin after 96 h germination. The decrease in thiamine of 40·2% was statistically significant and may be due to its utilization by the growing shoot.

The study shows that the nutritive value of cowpeas can be improved with germination to reduce the flatulence properties of the seeds, while increasing the levels of some nutrients. Cowpeas can thus be germinated for use in infant feeding. It would, however, involve drying and milling of the germinated seeds before mixing with local cereals to make pap. The germinated seeds can also be fed raw or cooked, with or without the addition of spices, as developed in China and Japan (Jelliffe, 1967). Such a preparation will help in the reduction of malnutrition among preschool children who consume them without the side effects of abdominal distension, vomiting or diarrhoea normally associated with cowpea feeding.

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