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Effect of Soaking, Dehulling and Fermentation on the Oligosaccharides and Nutrient Content of Cowpeas (*Vigna Unguiculata*)

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ABSTRACT

*Two cultivars of cowpea (*Vigna unguiculata*) seeds were fermented for 24 h or soaked for 4 h or dehulled and samples obtained 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 fermentation caused a 79·7% decrease in the level of verbascose and a 5·9% decrease in stachyose but raffinose increased by 12·8%. Increases in the levels of sucrose (8·1%), fructose (105%) glucose and galactose (56·4%) were observed. Four hour soaking of cowpea seeds in water led to a 49·4% decrease in verbascose, 29·8% in stachyose and 1·0% in raffinose; sucrose increased by 41·9% and fructose by 43% but glucose and galactose levels decreased by 55%.

Dehulling of the beans led to a decrease of 76·4% in verbascose, 16·9% in stachyose, 56% in raffinose, 63·6% in glucose and galactose and a 45·9% increase in sucrose. Fermentation led to a significant increase in the levels of thiamin, niacin, phosphorus, energy and protein. Thus, fermentation soaking and dehulling are acceptable methods for decreasing the flatulence properties of cowpeas and increasing acceptability among consumers.

INTRODUCTION

Fermented foods are important in the diet of Nigerians since several foods are fermented at various stages of their preparation. These products may be

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in the form of beverages, spices, flavouring agents or staple foods. In the fermentation process, micro-organisms utilise the biochemical constituents of the food material, changing them from one form to another with the aid of microbial enzyme systems.

Several attempts have been made to ferment cowpeas and other legumes (Oke, 1967; Zamora & Fields, 1979). These attempts led to the identification of the organisms responsible for the fermentation (Zamora & Fields, 1979) as well as the characteristics of the fermenting system (Oke, 1967). Fermentation may enhance the nutritive value of foods by an increase in the level of essential nutrients or reduction in the level of toxicants present in the food.

Cowpeas are especially important in the diet of Nigerians, being made into several food preparations for consumption. However, the presence of oligosaccharides has restricted their use by children whose mothers have associated discomfort with the consumption of cowpeas (Hussain *et al.*, 1984). This factor alone causes a decrease in the full nutritional use of cowpeas.

This study was carried out to determine the effects of soaking, dehulling and fermentation on the oligosaccharide and nutrient contents of cowpeas.

MATERIALS AND METHODS

Two varieties of cowpeas (*Vigna unguiculata*), the local white and the local brown, were purchased from the market and used in the study.

Fermentation of the beans was carried out whole according to the method of Reddy & Salunkhe (1980). The seeds were washed in distilled water to remove surface micro-organisms followed by soaking in distilled water for 30 min. At the end of the period, the cowpeas were ground into a fine paste in an Osterizer blender for 3 min. The slurry was then placed in covered plastic containers and allowed to ferment in an incubator at 30°C. Samples were obtained in triplicate at 16 h and 24 h. These were dried in the air oven (Gallenkamp) at 60°C overnight before being milled to powder for chemical analyses.

Soaking of the seeds was carried out by placing distilled water-washed seeds into covered beakers with water for 4 h, after which the water was discarded, the seeds were washed again and dried in the oven at 60°C overnight after which they were milled to powder for chemical analyses.

Dehulling of the seed was carried out by soaking the seeds in water for 10 min after which the seeds were rubbed between the palms to loosen the testa from the cotyledons. The testa were removed by flotation in water. The cotyledons were then washed in distilled water and dried in the air oven

(Gallenkamp) at 60°C overnight before being milled into powder for chemical analyses. All experiments were repeated in triplicate on different days.

Compositional analysis

Oligosaccharides were determined by paper chromatography in duplicate as previously described (Onigbinde & Akinyele, 1983). Ten grams of the cowpea powder for each cultivar were 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 ratio 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 (RG = distance travelled by the sample spot from origin/distance travelled by glucose from origin).

Monosaccharides were also separated by paper chromatography and the sugar extracts eluted using a mixture on *n*-butanol, glacial acetic acid and water in the ratio 4:1:1 (AOAC, 1980).

The concentration of the identified sugars was determined using the phenolsulphuric 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 (AOAC, 1980).

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; 0·01 N HCl was used to titrate the distillate and the percentage nitrogen calculated. This was multiplied by 6·25 to obtain the protein content.

The caloric value of the samples was determined using the ballistic bomb calorimeter (Gallenkamp model CB-370) with benzoic acid as standard (AOAC, 1980). The basic principle was to measure, by a galvanometer and thermocouple system, the temperature rise due to the combustion of a known weight of sample in excess oxygen. The maximum deflection on the galvanometer was read and used to calculate the energy content per gram of sample.

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 deionised 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 AND DISCUSSION

The results of the study are presented in Tables 1–5. Tables 1 and 2 show changes in both the monosaccharide and oligosaccharide contents due to fermentation, soaking and dehulling.

Fermentation at 16 h led to a decrease of 49·9% in verbascose, 35·4% in stachyose, 38·9% in raffinose and 45·9% of sucrose (Table 1) and an increase of 105% was observed for fructose and 9·1% for glucose and galactose. Total oligosaccharides decreased by 42·2% and total sucrose plus monosaccharides increased by 20·7%.

At 24 h of fermentation, there was a decrease in verbascose by 79·7% and a decrease of 5·9% in stachyose. Raffinose increased by 12·8% and there was an increase of 8·1% in sucrose, 105% in fructose and 56·4% in glucose and galactose. At 24 h fermentation, total oligosaccharides decreased by 33·2% and total sucrose plus monosaccharides increased by 72·9%.

Fermentation of cowpeas led to a 3·9% increase in protein at 16 h and an 8·3% increase at 24 h. These increases were not, however, statistically significant (Table 4). The energy content of fermented cowpeas was also higher than the raw seeds. The 13·8% increase in energy at 16 h of fermentation was statistically significant ($P < 0{\cdot}05$). These increases in protein and energy are due to the activities of the micro-organisms involved in fermentation.

Fermentation led to a decrease in all the minerals calcium, iron, magnesium, zinc and potassium both at 16 h and 24 h of fermentation. This could be due to their utilisation by the micro-organisms involved in fermentation. However, a 96·2% increase in phosphorus was observed at 24 h fermentation.

There was a statistically significant increase ($P < 0{\cdot}05$) in niacin and thiamin as a result of fermentation both at 16 h and 24 h (Table 5). This is in contrast to the findings of Zamora & Fields (1979) who observed a decrease in thiamin as a result of fermentation of cowpeas for 45 h. A decrease was, however, observed at 24 h when compared to the level of the vitamin at 16 h of fermentation.

TABLE 1
Sugar Concentrations (%) in Cowpea During Fermentation (mean + SD)[a]

Fermentation (h)	*Verbascose*	*Stachyose*	*Raffinose*	*Sucrose*	*Fructose*	*Glucose plus galactose*	*Total oligosaccharides (verbascose, stachyose, raffinose)*	*Total sucrose, fructose, glucose plus 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
16	2·02 ± 0·4 (49·9%)	2·3 ± 0·4 (−35·4%)	1·19 ± 0·8 (−38·9%)	0·80 ± 0·9 (−45·9%)	1·05 ± 1·5 (+105%)	0·60 ± 0·8 (+9·1%)	5·51 (−42·2%)	2·45 (+20·7%)
24	0·82 ± 0·3 (−79·7%)	3·35 ± 3·0 (−5·9%)	2·2 ± 0·9 (+12·8%)	1·6 ± 0·7 (+8·1%)	1·05 ± 1·5 (+105%)	0·86 ± 0·7 (+56·4%)	6·37 (−33·2%)	3·51 (+72·9%)

[a] Values in parentheses indicate the percentage change.
Values of sugars are not statistically significant from each other as a result of fermentation ($P > 0{\cdot}05$).

TABLE 2

Sugar Concentrations (%) in Cowpea During 4 Hours of Soaking in Water and Dehulling (mean + SD)[a]

Type of treatment	*Verbascose*	*Stachyose*	*Raffinose*	*Sucrose*	*Fructose*	*Glucose plus galactose*	*Total oligosaccharides (verbascose, stachyose, raffinose)*	*Total sucrose, fructose, glucose plus galactose*
Raw beans	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
4 h soaking in water	2·04 ± 0·3 (−49·4%)	2·5 ± 0·6 (−29·8%)	1·93 ± 0·2 (−1·0%)	2·1 ± 0·9 (+41·9%)	0·43 ± 0·6 (+43%)	0·0 ± 0·00 (−55%)	6·47 (−32·2%)	2·53 (+24·6%)
Dehulling	0·95 ± 0·5 (−76·4%)	2·96 ± 0·6 (−16·9%)	0·85 ± 0·06 (−56%)	0·85 ± 0·4 (45·9%)	0·0 ± 0·0 (0%)	0·20 ± 0·22 (−63·6%)	4·76 (−50·6%)	1·05 (−50·7%)

[a] Values in parentheses indicate the percentage change.

Changes in sugar pattern were statistically significant with soaking and dehulling ($P < 0{\cdot}05$).

TABLE 3

Mineral Contents of Cowpeas (Local White and Local Brown Varieties) as a Result of Fermentation, Soaking and Dehulling (mean ± SD)

Type of treatment	*Minerals (mg/100 g)*					
	Calcium	*Magnesium*	*Iron*	*Zinc*	*Potassium*	*Phosphorus*
Fermentation						
16 h	48·3 ± 10·5 (−12·5%)	90·7 ± 3·9 (−0·1%)	15·4 ± 0·2 (−29·4%)	3·8 ± 0·1 (−3·8%)	1 230·5* ± 44·5 (−1·3%)	362·5* ± 53·0 (−10·8%)
24 h	52·2 ± 10·0 (−5·4%)	88·3 ± 5·1 (−2·8%)	12·6 ± 0·5 (−42·2%)	4·0 ± 0·3 (−2·4%)	1 150·0 ± 33·2 (−7·7%)	431·3 ± 167·9 (+6·2%)
Soaking in water for 4 h	52·8 ± 8·34 (−4·3%)	15·0 ± 1·1 (−0·8%)	15·0 ± 1·1 (−31·2%)	4·95 ± 0·9 (+20·7%)	1 149·0* ± 35·4 (−7·8%)	475* ± 106·1 (+16·9%)
Dehulling	42·8 ± 12·4 (−4·3%)	85·5 ± 1·3 (−5·8%)	11·5 ± 0·6 (−47·2%)	4·3 ± 0·3 (+4·9%)	1 130·5* ± 123·7 (−9·3%)	(0%)

* Significantly different from the raw bean values ($P < 0{\cdot}05$).

TABLE 4
Protein and Energy Contents of Cowpeas (Local White and Local Brown Varieties) as a Result of Fermentation, Soaking and Dehulling (mean ± SD)

Type of treatment	*Protein (g/100 g)*	*Energy (kcal/100 g)*
Fermentation		
16 h	26·80 ± 0·1 (+3·9%)	487·50* ± 61·5 (+13·8%)
24 h	27·95 ± 26·7 (+8·3%)	437·50 ± 16·3 (+2·1%)
Soaking in water for 4 h	27·26 ± 1·1 (+5·7%)	436·00 ± 2·8 (+1·8%)
Dehulling	26·50 ± 0·0 (+2·7%)	444·00 ± 2·8 (+3·7%)

Values in parentheses indicate the percentage change.
* Statistically significant from the raw beans ($P < 0{\cdot}05$).

The raffinose, stachyose and verbascose contents of beans have been reported to decrease proportionately with the time of soaking (Mehta *et al.*, 1985). This was confirmed by this study which showed a significant decrease ($P < 0{\cdot}05$) of 49·4% in verbascose, 29·8% in stachyose, 1% in raffinose an increase of 41·9% sucrose, 43% fructose and a decrease of 55% in glucose

TABLE 5
Concentrations of Some Vitamins in Cowpea (Local White and Local Brown Varieties) as a Result of Fermentation, Soaking and Dehulling (mean ± SD)

Type of treatment	*Vitamin (mg/100 g)*		
	Vitamin C	*Niacin*	*Thiamin*
Fermentation			
16 h	0–2·5 ± 0·0 (0%)	7·47* ± 1·9 (+99·2%)	1·80* ± 0·3 (±106·9%)
24 h	0–2·5 ± 0·0 (0%)	10·4* ± 1·5 (+177·3%)	1·16 ± 0·3 (+33·3%)
Soaking in water for 4 h	0–2·5 ± 0·0 (0%)	12·7* ± 4·0 (+238·7%)	0·39* ± 0·2 (−55·2%)
Dehulling	0–2·5 ± 0·0 (0%)	12·60* ± 3·7 (+236%)	0·26* ± 0·2 (−70·1%)

Values in parentheses indicate the percentage change.
* Values are statistically significant from the values of the raw beans ($P < 0{\cdot}05$).

and galactose in cowpea (Table 2). Soaking led to a decrease of 32·2% in total oligosaccharides which could make cowpea more digestible and utilisable by man.

Soaking of seeds in water expectedly led to a decrease in all the water-soluble nutrients because the soak-water was discarded. Soaking led to a decrease in all the minerals except phosphorus and zinc although this was not statistically significant ($P > 0.05$) (Table 3).

Soaking increased the niacin content of cowpea by 238·7% and a decrease of 55·2% in thiamin was observed. A 5·7% increase in protein and a 1·8% increase in energy were also observed (Tables 4 and 5). As explained earlier, the increases in some of the nutrients must have been as a result of the fermentation which was likely to take place during soaking.

Dehulling of cowpeas led to a decrease in all the minerals except zinc although these decreases were not statistically significant. There was a decrease of 70·1% in thiamin as a result of dehulling while there was a 236% increase in the niacin level. Protein increased by 2·7% and energy increased by 3·7% (Tables 4 and 5). Decreases in the minerals and thiamin can be attributed to the testa of the cowpeas being discarded.

Developments of new varieties of cowpeas emphasise the importance attached to the crop as a source of food for people in developing countries. However, the presence of oligosaccharides continues to pose problems to full utilisation of the nutrients by young children. The problem is due to the human lack of the α-galactosidase enzyme needed to digest the oligosaccharide of cowpeas. These oligosaccharides are only fermented anaerobically in the large intestine to produce gas which causes flatus and/or diarrhoea.

Fermentation thus represents a mechanism for the removal of these oligosaccharides. Fermentation enhances the nutritive value of foods by increasing the levels of essential nutrients or reduction in the level of toxicants in the food. In this study, fermentation was effective in reducing the higher oligosaccharides after 24 h. The decrease in the concentration of the oligosaccharides during fermentation may be due to some lactic acid micro-organisms that possess α-galactosidase activity to utilise oligosaccharides such as raffinose, stachyose and verbascose (Mital *et al.*, 1973).

This has been supported by the observation that there was an increase in the population of lactic acid micro-organisms up to 16–18 h and a decline thereafter (Mital & Steinkraus, 1975). Two heterofermentative bacteria were identified to be responsible for these activities. These were *Lactobacillus Celobiosis* and *Lactobacillus Fermenti.*

Soaking of cowpeas in water is a usual practice prior to the preparation of some cowpea dishes in Nigeria. The period of soaking depends on the purpose and the variety. It has been observed that, during the soaking of beans in water, fermentation also takes place as shown by the foaming which occurs. Oligosaccharides have been found to decrease proportionally with the time of soaking (Mehta *et al.*, 1985) which is similar to the findings in this study. Soaking in water may be preferable to that in sodium bicarbonate solution because of susceptibility of some vitamins of the B-complex group to alkali, particularly thiamin and riboflavin which are destroyed slowly in alkaline medium at room temperature. Similarly, soaking in water is expected to have a negative effect on water-soluble nutrients in the seeds which complicates the simultaneous fermentation.

The findings of this study show that the processes of fermentation, soaking and dehulling all have a beneficial effect in the reduction of oligosaccharides of cowpeas. Such practices should therefore be encouraged at home level during preparation of cowpea foods to facilitate their utilisation by young children who would benefit from the additional intake of protein from this source.

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