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Effect of processing on the proximate composition and functional properties of cowpea (*Vigna unguiculata*) flour

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The proximate composition and functional properties of raw, germinated, fermented and heat-treated cowpea flour were studied. The functional properties investigated were protein solubility, water and fat absorption, bulk density, foam capacity and stability.

Germination increased the crude protein, iron and total phosphorus but decreased the carbohydrate, fat and total polyphenols content. Protein solubility was pH-dependent with a minimum at pH 4·0. Maximum protein solubility (0·39 mg/ml) was recorded for germinated flour which also showed excellent fat absorption properties. The water absorption capacity of the heat-treated cowpea flour was significantly higher ($P < 0·05$) than those of raw, germinated or fermented samples. Bulk densities of the germinated and fermented flours were reduced by 70·6% and 35·3% respectively. The foam of the raw flour was more stable than those of the processed samples. Incorporation of NaCl up to 0·2 M improved the foam capacity of the raw and processed flours.

Calculated on a crude protein basis, raw cowpea flour showed comparatively better water and fat absorption properties than raw winged bean or soyflour and hence it may find useful applications in fabricated foods such as bakery products and ground meat formulations.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.). is one of the most important grain legumes in diets in developing countries, especially in Nigeria. The seed has about 25% protein, 67% carbohydrate (Oyenuga, 1978) and has been reported to contain polyphenolic compounds which can reduce its nutritional value (Ekpenyong, 1985). Cowpea can be incorporated into soups or stews or can be processed into paste or flour and used as a food ingredient or starting material for a variety of local food (Dovlo *et al.*, 1976; McWatters, 1983). Cowpea is also used extensively as a weaning food in Nigeria (Uwaegbute, 1991). Few studies have been made on the functional properties and functionality of cowpea flour in foods formulations (Abbey & Ibeh, 1988; McWatters, 1985). However, information on the functional properties of cowpea, as influenced by simple domestic processing methods, is needed in view of its increased utilization.

Essential in determining potential uses for cowpea flour is the identification and improvement of its functional properties. A process of germination and fermentation has often been proposed as a means by which the nutrient composition and functional properties of legume seeds might be improved. Zamara & Fields (1979) reported that the nutritive quality of cowpea was markedly improved as a result of fermentation.

Germination has been reported to increase the water and fat absorption capacities of mung bean (del Rosario and Flores, 1981), and the protein solubility of winged bean (King & Puwastien, 1987).

This study was aimed at examining the effect of germination, fermentation and heat treatment on the proximate composition and functional properties of cowpea flour. The potential food uses for the flour were evaluated by laboratory tests for protein solubility, water and fat absorption, bulk density, foam capacity and stability and the results obtained compared with those of raw soyflour and raw winged bean flour.

MATERIALS AND METHODS

Materials

Cowpea (*Vigna unguiculata* L. Walp) seeds were bought from three batches at a local market in Port Harcourt,

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Nigeria, and stored at 5°C throughout the experimental period. From each batch, triplicate samples were selected and subjected to the various processing methods.

Processing methods

Germination

Germination was carried out as described by Akpapunam & Achinewhu (1985). Cowpea seeds were placed in sterile Petri dishes containing wet cotton wool and germinated at room temperature (29–30°C) for 72 h. The cotton wool was moistened with distilled water at regular intervals of 12 h. Germinated beans were oven-dried (60°C, 24 h) and milled to pass through a 0·5 mm sieve.

Fermentation

Fermentation was carried out at room temperature for 72 h according to the procedure described by Zamora & Fields (1979). The fermented samples were oven-dried (60°C, 24 h) and milled to pass through a 0·5 mm sieve.

Heat treatment

The samples to be heat treated were first ground in the raw form, then autoclaved (121°C, 1·05 kg cm^{-2}, 15 min). The autoclaved samples were oven-dried (60°C, 24 h) and then milled to pass through a 0·5 mm sieve.

Raw flours

Raw cowpea flour was made from ground seeds which were dried (60°C, 24 h) and then milled to pass through a 0·5 mm sieve. Raw and processed samples to be used for studies on functional properties were defatted by solvent extraction using *n*-hexane. Soybean (*Glycine max.* L.) and winged bean (*Psophocarpus tetragonolobus*) obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were processed in a similar manner to obtain defatted raw flour samples. All samples were stored in capped bottles at 4°C until required for use.

Chemical analysis

Crude protein, ether extract, crude fibre, ash and moisture contents of the raw and processed samples were determined according to standard methods of the Association of Official Analytical Chemists (AOAC, 1984). The factor 6·25 was used throughout for conversion of nitrogen to crude protein. Carbohydrate content was calculated by difference.

Iron and total phosphorus were determined colorimetrically according to standard methods (AOAC, 1984). Total polyphenols was determined using the vanillin–H_2SO_4 assay as described by Wilson & Blunden (1983). The results are expressed as grams phloroglucinol equivalents per 100 g dry weight flour. The calorific value was obtained by standard calculations (Osborne & Voogt, 1978). All reagents used were of analytical grade.

Functional properties

Protein solubility

Protein solubility was determined in the pH range 2–12 for the raw and processed cowpea flours at room temperature (29–30°C) using the method of Sathe & Salunkhe (1981) as modified by Abbey & Ibeh (1988). The flour sample (10 mg) was dissolved in 10 ml N NaOH and the pH adjusted to the desired value with N HCl. It was then centrifuged at 5000*g* for 15 min and the soluble protein in the supernatant determined by Lowry's method (Lowry *et al.*, 1951) using bovine serum albumin as a standard.

Water and fat absorption

Water absorption capacity was determined by the method of Sosulski *et al.* (1976) while fat absorption capacity was determined by the method of Lin *et al.* (1974) using a 4 g flour sample and refined palm oil (King's Brand, Devon Ind., Singapore; density 0·88 g/ml). Values were expressed as grams of water or oil absorbed by 1 g of flour or protein.

Bulk density

The method described by Narayana & Narasinga Rao (1984) was used to determine bulk density. A calibrated centrifuge tube was weighed and samples were filled to 5 ml by constant tapping until there was no further change in volume. The contents were weighed and from difference in weight the bulk density of the sample was calculated.

Foam capacity and stability

This was determined as described by Narayana & Narasinga Rao (1982) at pH 7·0. The volume of foam at 30 s after whipping was expressed as the foam capacity and the volume of the foam over a time period 10–120 min, as foam stability, for the respective time periods. The volume increase (%) was calculated according to the following equation:

$$\text{Volume increase} = \frac{\text{Volume after whipping (ml)} - \text{Volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100$$

Foam capacity measurements were also made using NaCl solution of 0·2–1·0 M concentration.

Statistical analysis

All the experiments were conducted in triplicate and the mean ± standard deviation of three values reported. The data were subjected to analysis of variance and Duncan's multiple range test (Steel & Torrie, 1960) to determine the significance of differences between treatment means.

RESULTS AND DISCUSSION

Proximate composition

The results of the proximate composition of raw, germinated, fermented and heat-treated cowpea flour are presented in Table 1. On a dry weight basis, germination resulted in a decrease in fat (17·65%) and carbohydrate (4·34%) while the crude protein, total ash and crude fibre increased by 3·75%, 3·23% and 4·76% respectively. Iron and total phosphorus contents of the germinated flour were also increased by 7·69% and 15·6% respectively. Calorific value and total polyphenolic content of the germinated flour were, however, observed to be decreased by 2·71% and 40·0% respectively. Akpapunam & Achinewhu (1985) reported an increase of 2·76% in crude protein content and a decrease of 4·63% in carbohydrate content of cowpea germinated for 72 h. However, an increase in fat content reported by these workers as a result of germination was not evident in this study. The depletion of fat in germinating legumes has been reported by Kylen & McCready (1975) for lentils, mung beans and soybeans and by King & Puwastien (1987) for winged beans. Germination and heat treatment reduced the total polyphenols of cowpea by 44·4% and 55·6% respectively. Similar findings have been reported by other workers. Ekpenyong (1985) reported losses in polyphenols varying between 36·7 and 56·7% during heat processing of cowpea while losses obtained by Ologhobo & Fetuga (1984) ranged between 23·8 and 40·4% in ten germinated cowpea varieties.

The crude protein content of the fermented flour increased slightly by 2·50% which was not significant ($P < 0.05$) while the carbohydrate content decreased by 3·84% during a 72 h fermentation period. Several other researchers have reported similar or greater increases in crude protein, as well as decreases in carbohydrate of various fermented Nigerian legumes and oilseeds. Akpapunam & Achinewhu (1985) observed that there was not much difference in the crude protein content of fermented and unfermented cowpea but noted that fermentation decreased its carbohydrate content by 2·87% during a 72 h period. Studies by Isichei & Achinewhu (1988) showed that the crude protein content of African oil bean increased slightly (by 2·0%) while the carbohydrate content decreased by 26·5% during a 6-day fermentation period. In a previous study, fermentation has been reported to increase the crude protein content of fluted pumpkin seed by 2·50% while the carbohydrate content decreased by 16·3% during a 5-day fermentation period (Giami & Bekebian, 1992).

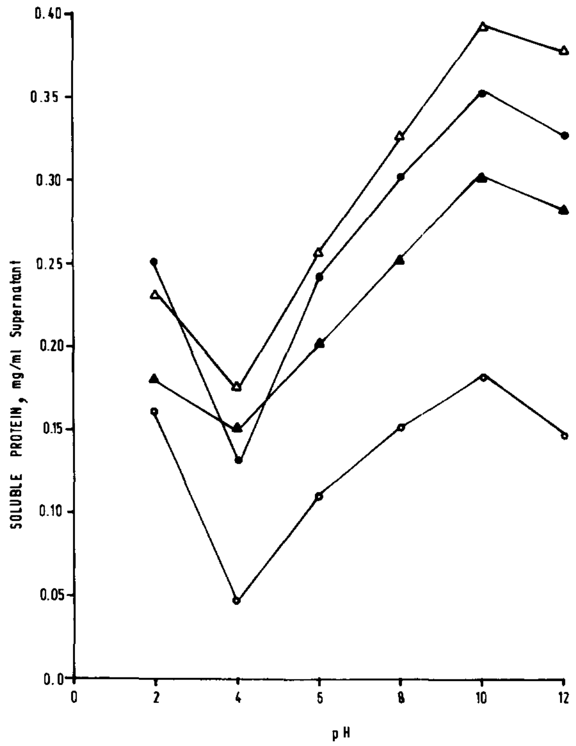

Fig. 1. Effect of pH on protein solubility profile of raw and processed cowpea flour (●, raw; ○, heat treated; ▲, fermented; Δ, germinated).

Table 1. Proximate composition of raw and processed cowpea flour[a]

Component	Sample			
	Raw	Germinated	Fermented	Heat treated
Moisture (%)	9·2 ± 0·1	11·0 ± 0·4	10·7 ± 0·2	10·3 ± 0·5
Crude protein (%)	24·0 ± 0·4	24·9 ± 0·5	24·6 ± 0·3	22·7 ± 0·3
Ether extract (%)	1·7 ± 0·1	1·4 ± 0·1	1·9 ± 0·1	1·7 ± 0·1
Total ash (%)	3·1 ± 0·2	3·2 ± 0·1	3·2 ± 0·2	3·2 ± 0·2
Crude fibre (%)	2·1 ± 0·2	2·2 ± 0·2	2·0 ± 0·1	2·3 ± 0·3
Carbohydrate (%) (by difference)	59·9 ± 0·7	57·3 ± 0·6	57·6 ± 0·4	59·8 ± 0·4
Iron (mg/100 g)	6·5 ± 0·3	7·0 ± 0·3	6·8 ± 0·2	6·2 ± 0·3
Total phosphorus (mg/100 g)	385·0 ± 2·4	445·0 ± 2·1	415·0 ± 2·2	360·0 ± 1·4
Total polyphenols (g/100 g)	0·45 ± 0·03	0·27 ± 0·01	0·25 ± 0·02	0·21 ± 0·01
Calorific value (kcal/100 g)	350·9	341·4	345·9	345·3

[a] Mean ± standard deviation of the mean for three determinations from each of the three purchased batches; values except moisture expressed on dry weight basis.

Table 2. Water and fat absorption of raw and processed cowpea flour[a]

Sample	Crude protein[b]	Water absorption capacity (g/g)		Fat absorption capacity (g/g)	
		Flour	Protein[c]	Flour	Protein[c]
Raw cowpea flour	25·2 ± 0·3	2·6 ± 0·1[b]	10·3 ± 0·6[a]	2·8 ± 0·4[b]	11·1 ± 0·6[b]
Germinated cowpea flour	26·1 ± 0·4	1·5 ± 0·4[c]	5·7 ± 0·3[b]	4·1 ± 0·5[a]	15·7 ± 0·8[a]
Fermented cowpea flour	25·6 ± 0·2	1·0 ± 0·2[c]	3·9 ± 0·2[c]	3·6 ± 0·1[a]	14·1 ± 0·7[a]
Heat-treated cowpea flour	24·7 ± 0·6	3·4 ± 0·3[a]	13·8 ± 0·7[a]	3·1 ± 0·3[a]	12·6 ± 0·5[b]
Raw winged bean flour	43·5 ± 0·8	2·1 ± 0·1[b]	4·8 ± 0·4[c]	1·5 ± 0·2[c]	3·4 ± 0·2[c]
Raw soyflour	45·4 ± 0·9	3·0 ± 0·5[a]	6·6 ± 0·3[b]	1·2 ± 0·1[c]	2·7 ± 0·3[c]

[a] Mean ± standard deviation of the mean for three determinations from each of the three purchased batches; flour samples were defatted.
[b] Expressed on dry weight basis.
[c] Expressed on crude protein basis.
a,b,c: means with the same superscripts within the same column do not differ ($P < 0·05$).

Protein solubility

The protein solubility profiles of the four types of flour studied are shown in Fig. 1. Protein solubility was observed to be pH dependent. The solubility profile of raw, germinated and fermented flour did not vary widely, but heat-treated flour exhibited the least solubility values in the pH range 2–12 investigated compared to soyflour or winged bean flour which have been shown to have minimum protein solubility at pH 4·5 (Smith & Circle, 1972; Narayana & Narasinga Rao, 1982), cowpea flour had minimum protein solubility at pH 4·0.

Maximum protein solubilities of 0·39 mg/ml, 0·35 mg/ml, 0·30 mg/ml and 0·18 mg/ml were recorded for germinated, raw, fermented and heat-treated flour samples respectively. Heat-treatment resulted in a 48·6% reduction in protein solubility of cowpea at pH 10·0, compared to the raw flour. Reduction in protein solubility due to heat processing has been reported in the case of winged bean flour (Narayana & Narasinga Rao, 1982) and cowpea flour (Abbey & Ibeh, 1988).

Germination was observed to improve protein solubility. A similar observation was made by del Rosario & Flores (1981) for mung bean and Giama & Bekebian (1992) for fluted pumpkin. Since high proteolytic activity within germinating seeds might be expected, an increase in the protein solubility resulting from hydrolysis of the storage proteins would also be expected (King & Puwastien, 1987). The applicability of germinated flour in food preparations where maximum solubility of proteins is desired, looks very promising.

Water and fat absorption

The water absorption capacity of raw cowpea flour (2·6 g/g flour) was similar to that of raw winged bean flour (2·1 g/g flour) and both were significantly lower ($P < 0·05$) than the value of 3·0 g/g flour obtained for raw soyflour (Table 2). Since cowpea, winged bean and soyflour had different protein contents, water and fat absorption capacities were also calculated on a crude protein basis. The values obtained showed that raw cowpea flavour has a higher water absorption capacity (10·3 g/g protein) than either raw winged bean flour (4·8 g/g protein) or raw soyflour (6·6 g/g protein).

While heat-treatment improved the water absorption capacity of cowpea flour, germination and fermentation did not. Heat-treatment has been reported to increase the water absorption capacity of mung bean flour (del Rosario & Flores, 1981), winged bean flour (Narayana & Narasinga Rao, 1982) and sunflower proteins (Lin *et al.*, 1974). Water absorption characteristics represent the ability of a product to associate with water under conditions where water is limiting, such as doughs and pastes. The results obtained suggest that the raw and heat-treated cowpea flour would be useful in food systems such as bakery prod-

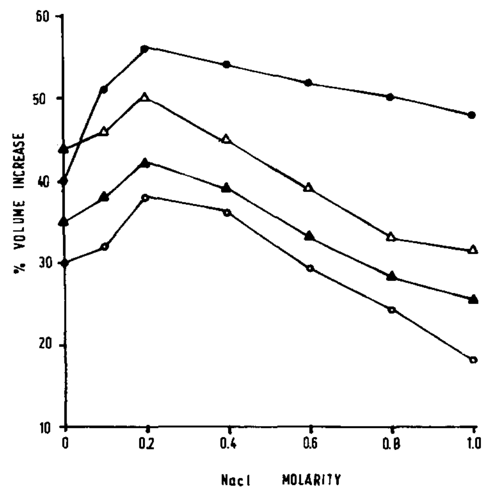

Fig. 2. Effect of NaCl concentration on the foam capacity of raw and processed cowpea flour (●, raw; ○, heat treated; ▲, fermented; Δ, germinated).

Table 3. Bulk density, foam capacity and stability of raw and processing cowpea flour[a]

Cowpea flour sample	Bulk density (g/ml)	Foam capacity[b] (ml)	Volume Increase (%)	Foam vol. (ml) after time (min)			Decrease over 2 h (%)
				30	60	120	
Raw	0·34 ± 0·02	141·0 ± 1·3	40	126·5 ± 2·5	115·0 ± 1·4	100·0 ± 2·0	29
Germinated	0·10 ± 0·03	145·0 ± 2·0	44	100·0 ± 1·2	90·0 ± 1·0	80·0 ± 0·5	45
Fermented	0·22 ± 0·01	110·5 ± 3·0	35	75·5 ± 2·0	70·5 ± 1·5	66·0 ± 0·2	40
Heat treated	0·30 ± 0·04	125·0 ± 1·5	30	115·0 ± 3·0	95·4 ± 2·0	80·0 ± 0·5	36
LSD[c] (P = 0·05)	0·09	15·0	5·9	17·0	11·5	10·0	6·5

[a] Mean ± standard deviation of the mean for three determinations from each of the three purchased batches.
[b] Determined at room temperature (29–30°C), pH=7.
[c] LSD: Difference of two means between treatments exceeding this value are significant.

ucts which require hydration to improve handling characteristics. The uses of heated and unheated cowpea flour in the making of biscuits, sugar cookies and doughnuts have been reported (McWatters, 1985). This worker encountered no problems in preparing and handling the doughs that contained up to 30% cowpea flour.

The fat absorption capacity of raw cowpea flour (2·8 g/g flour) was lower than those of the germinated flour (4·1 g/g flour), fermented flour (3·6 g/g flour) and heat-treated flour (3·1 g/g flour) but was, however, higher than the values obtained for raw soyflour (1·2 g/g flour) and winged bean flour (1·5 g/g flour) (Table 2). The fat binding capacity of cowpea protein would find useful application in ground meat formulations, meat replacers and extenders. Studies by McWatters (1985) showed that, during frying, the percentage of fat retained by ground beef patties extended with steamed cowpea meal increased as the level of cowpea meal increased.

Foam capacity and stability

Germination increased the foam capacity of cowpea flour, but, like fermentation and heat treatment, decreased the foam stability compared to the raw sample (Table 3). A similar effect of germination on foam capacity and stability of protein isolates from yellow pea, faba bean and lentil has already been reported (Hsu *et al.*, 1982).

The formability of cowpea flour is a desirable characteristic for the production of several traditional cowpea-based food products in Nigeria (McWatters, 1983, 1985). Foam stability has been suggested to be related to the amount of native protein (Lin *et al.*, 1974). Native protein has been shown to give higher foam stability than denatured protein (Yasumatsu *et al.*, 1972).

Addition of salt (NaCl), up to 0·2 M concentration, increased the foam capacity of the raw and processed cowpea flour; greater concentrations of NaCl decreased it considerably (Fig. 2). With raw cowpea flour, the foam capacity at 1·0 M NaCl was higher than in water but, with the processed samples, it was lower. The beneficial effect of low concentrations of NaCl on the foam capacity of raw winged bean flour and soyflour has been previously reported (Narayana & Narasinga Rao, 1982).

Bulk density

Germinated flour had a lower bulk density (0·10 g/ml), compared to either the raw flour (0·34 g/ml), the fermented flour (0·22 g/ml) or the heat-treated flour (0·30 g/ml) (Table 3). It may be expected that decreased bulk density would be an advantage in the preparation of weaning food formulations. Among the various traditional technologies which could be followed for the preparation of low bulk weaning food, germination has been reported to be very useful (Desikachar, 1980). Studies by Malleshi *et al.* (1989) have shown that the bulk density of a weaning food formulation prepared from a blend of germinated sorghum and cowpea flours was reduced by 12·3% compared to the raw food prepared from ungerminated raw materials.

This study has shown that cowpea flour has a great potential as a functional agent in fabricated foods and supplements in diets.

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