

Effect of fermentation period on the chemical composition and functional properties of Pigeon pea (*Cajanus cajan*) seed flour

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Abstract: Changes in the chemical composition and functional properties of fermented seed flour of Pigeon pea, *Cajanus cajan* L. were studied for 0,1,2,3,4 and 5 days. The differently fermented seeds were analyzed for proximate composition, calorific values and functional properties. Results showed that fermentation significantly ($p \leq 0.05$) increased the moisture, protein and ash contents of the seed while the crude fat, crude fibre and carbohydrate contents were noted to be decreased. However, water/oil absorption, bulk density, swelling capacity, foam capacity/stability, viscosity and gelation power were significantly decreased ($p \leq 0.05$).

Keywords: Fermentation, Pigeon pea, proximate composition, functional properties

Introduction

Legumes are nutritious foods and a substitute for animal protein arises from the knowledge of the functional properties of the seed flour and other products. In Africa, malnutrition is prevalent due to lack of sufficient animal protein, hence the search for alternative sources of protein from lesser-known legumes in lieu of expensive and scarce animal protein (Adebowale and Lawal, 2004). Oshodi and Ekperigin (1989) reported good indications of the possible use of food of plant origin to overcome the problem of shortage of foods of animal origin. Therefore, the use of legume seeds may be the beginning of a series of formulations which will lead to a substantial drop in dependency of animal sources for nutritious foods. Unfortunately, legume seeds contain antinutritional factors like enzyme inhibitors, phytates, oxalates, saponins and polyphenolic compounds, all of which limit their utilization (Liener, 1980; Salunkhe, 1982; Vijayakumari *et al.*, 1997). Although, remarkable improvement in the nutritive value and quality of legume seeds have been achieved through dehulling, heat treatment, germination, fermentation, soaking and partial hydrolysis of proteolytic enzyme (Sharma and Sehgal, 1992; Khalil and Mansour, 1995; Vijayakumari *et al.*, 1997; Oloyo, 2004).

As part of the efforts made to solve the problem of low protein intake in Nigeria, nutritionists have advocated increase consumption of food legumes, such as *Vigna unguiculata* and *Glycine max* in campaign (Oloyo, 2004). Yet unexploited is the utility of Pigeon pea, *Cajanus cajan* L., whose cultivation is well supported by the soil and prevailing climatic conditions of the western region of Nigeria (Oloyo, 2004).

Pigeon pea, is a legume commonly referred to as 'otili' in the south-west, Nigeria and the seeds are boiled and eaten by the natives. Earlier studies showed that its protein is rich in lysine and it is easy to grow (Oyenuga, 1978). Attempts have been made to fortify protein-deficient foods with protein concentrates or to improve the limiting essential amino acids by the use of protein or protein concentrate of vegetable origin (Oshodi and Ekperigin, 1989; Uzor-Peter *et al.*, 2008).

In order to successfully introduce a new supplementation into any food item, it is imperative to find out if the supplementation possesses suitable functional properties for food applications and consumer acceptability. These functional properties are the intrinsic physico-chemical characteristics which may affect the behaviour of food systems during processing, storage and consumption, such as solubility, foamability, gelation and emulsification properties (Oshodi and Ekperigin, 1989).

Therefore, the present study was aimed to report the changes in the chemical composition and functional properties of the seeds of Pigeon pea, *Cajanus cajan* L., as affected by fermentation.

Materials and Methods

Sample collection and fermentation

A bulk of healthy seeds of Pigeon pea used for this study was purchased from a farm at Oja-odan, Ilaro. These seeds were sorted manually, to remove stones, damaged and immature seeds; salt (Dangote salt, Nigeria) was purchased from the market in Ilaro and plantain leaves were obtained from within the polytechnic premises. The seeds were boiled with tap water (1/10 v/v) for 1 h to soften the seed coat

and then dehulled manually using hand pressure. Cotyledons were grounded to paste with NaCl (1g/kg seed) and divided into two parts. The first part was kept as control (unfermented seeds). Whereas, the second part of the paste was wrapped, 50 g/pack in flame-blanching plantain leave, to provide a warm humid environment and allowed them to undergo natural fermentation at ambient for seven days (Braber *et al.*, 1989).

Sample preparation

Fermented samples were collected at a-day interval until the end of fermentation period of 5 days. Fermented and unfermented (control) pastes were oven dried in a hot air oven, grounded using laboratory mill into flour and screened through a standard sieve (40 mm mesh) and kept for analyses.

Analytical procedures

Proximate composition

Flour samples of fermented and unfermented (control) seed flour were analysed for their total nitrogen, ether extract, crude fibre and ash (A.O.A.C., 1990). Crude protein was calculated by multiplying the percent kjeldahl nitrogen by the factor 6.25. Nitrogen free extract (NFE) was estimated by difference. The energy content was determined by using the Atwater factor (Energy value= % carbohydrate×4 + % fat×9 + %protein×4)

Determination of the functional properties

Bulk density

Bulk density was determined by using the method described by Narayana and Narasinga (1984). Ten grams of each sample was weighed (W_1) into a 25 ml graduated measuring cylinder. The sample was gently tapped to eliminate spaces between the flour samples and reweighed (W_2). The study was conducted in duplicate.

$$\text{Bulk density (g/ml)} = \frac{W_1 - W_2}{\text{Volume of sample before tapping}}$$

W_1 = weight of sample before tapping

W_2 = weight of sample after tapping

Water absorption capacity

Water absorption capacity was determined using the method described by Sefa-Dedeh *et al.* (2004). Five grams of sample was weighed into a centrifuge tube and 30 ml of distilled water, at temperature of 25 and 70°C, was added. The mixture was stirred and allowed to stand for 30 min and centrifuged

using centrifuge (Model H-103N series, Kokusan Inc., Tokyo, Japan); at 3000 rpm for 15 min. The supernatant was decanted and the increased in weight noted by weighing with digital balance (Model ARC120, Ohaus corporation, China). The water absorption capacity was expressed as a percentage of the initial sample weight. The determination was done in duplicate.

Swelling power

One gram of dried sample was weighed into 100 ml conical flask and 15 ml distilled water was added. The mixture was shaken for 15 min at low speed on a stirrer and transferred into a hot water bath (Stuart, model SWB3, Bibby Scientific Ltd., Staffordshire, U.K.) and heated for 40 min between 80-85°C with constant stirring. The heated mixture was transferred to a pre-weighed centrifuge tube and 7.5 ml distilled water added; centrifuged at 2200 rpm for 20 min. The supernatant was carefully decanted and cooled in a desiccator. The precipitate with the centrifuge tube was weighed.

$$\text{Swelling power} = \frac{\text{weight of precipitate/paste}}{\text{Weight of dry flour}}$$

Foaming properties

Foaming capacity and stability were determined according to the method reported by Coffman and Garcia (1977). Two grams seed flour was whipped (homogenised) with 100 ml distilled water for 15 min in a Kenwood blender (Model KM400, Kenwood Ltd., Britain) at speed setting 'max' and then poured into a 250 ml graduated measuring cylinder. The total volume at time intervals of 0.0, 0.25, 0.50, 1.00, 1.50, 2.00, 3.00 and 4 h was noted. Percent volume increase was calculated according to:

$$\text{Foam capacity (\%)} = \frac{\text{volume after whipping (ml)} \times \text{volume before whipping (ml)}}{\text{volume before whipping/homogenization (ml)}} \times 100$$

$$\text{Foam stability (\%)} = \frac{\text{foam volume after time (t)} \times 100}{\text{Initial foam volume}}$$

Viscosity

One gram of flour sample was weighed into a centrifuge tube. 20 ml distilled water was added and shaken them thoroughly to obtain homogenous slurry. 20 ml of the slurry was pipette into 50 ml vitreosil viscopipette. Time taken in seconds by the slurry to pass through the oval bulb was noted with digital stop watch capable of reading to decimal place. Determination was done twice.

Gelation

Gelation was determined according to Coffman

and Garcia (1977) as modified by Sathe and Salunkhe (1981). Appropriate sample suspension of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) were prepared in 5 ml distilled water. The test tubes containing these suspensions were heated for 1 h in a boiling water bath, followed by rapid cooling under running cold tap water. The test tubes then further cooled for 2 hr at 4°C. The least gelation concentration was determined at that concentration when the sample from the inverted test tube did not fall down or slip out.

Statistical analysis

Mean values of duplicate determinations were reported with their standard deviations. Analyses of Variance (ANOVA) were achieved to calculate significant differences in the treatment means, and the mean separations were achieved by Duncan's Multiple Range Test ($p \leq 0.05$).

Results and Discussion

The effect of fermentation on the proximate composition of flour of pigeon pea seed is presented in Table 1. There were significant differences ($P \leq 0.05$) in protein and ash contents among the flour samples. As fermentation day increased, crude protein and ash increased progressively from 21.8 to 23.9%. Flour sample that was fermented for 5 days had the highest protein value (23.9%) while the lowest protein value occurred in day-1 of fermentation (22.6%). This increase in protein value during fermentation compare favourably with the values reported for beniseed flour (Oshodi *et al.*, 1999). The increase in protein may confer nutritional advantage on the fermented pigeon pea flour. This increase in protein value with fermentation time could be attributed to net synthesis of protein by fermenting seeds which might have resulted in the production of some amino acids during protein synthesis (Marero *et al.*, 1989; Uwagbute *et al.*, 2000).

Ash content ranged from 4.61 to 5.52%, with non-fermented seed flour had a value of 4.61% while highest value was recorded (5.52%) at day-5 of fermentation. There was gradual increase in the ash contents with the fermentation days. However, there was no significant difference ($P \leq 0.05$) occurred in the values after day-2 of fermentation.

Moisture contents varied from 10.2 to 13.24% with day-5 fermented flour having the highest value (13.24%) and non-fermented pigeon pea flour had a value of 10.2%. moisture content increased with increase in fermentation day. The moisture content of non-fermented flour sample was lower than that of fermented flours, which might be due to its low dry

matter content.

Fat contents varied from 2.74 to 1.69%. There were significant differences ($P \leq 0.05$) (see comments as above) in fat content among samples. Fat contents were found to be significantly lower in the fermented seed flour than in non-fermented seed flour (control). This decrease in fat contents might be attributed to the increased activities of the lipolytic enzymes during fermentation which hydrolyses fat components into fatty acid and glycerol (Chinma *et al.*, 2009).

Energy values varied between 325.46 to 315.33 kcal/100 g with the non-fermented (control) pigeon pea seed flour having the highest energy value (325.46 kcal/100 g) and fermented seed flour at day-5 of fermentation, with the lowest value 9315.33 kcal/100 g). This decrease in energy value could be attributed to the decrease in both fat and NFE values of the samples. Fat on its own contains about twice the food energy values of protein and carbohydrate (Osborne and Voogt, 1978).

Functional Properties

The effect of fermentation on the functional properties of flour from pigeon pea seed flour is presented in Table 2. Bulk density ranged from 0.63 to 0.80 g/ml, although, there were significant differences ($P \leq 0.05$) among the flour samples. Bulk density values decreased gradually with fermentation periods. The bulk density is a reflection of the load the flour samples can carry, if allowed to rest directly on one another. The density of processed products dictate the characteristics of its container or package product density influences the amount and strength of packaging material, texture or mouth feel (Wilhelm *et al.*, 2004). The bulk density values obtained were generally higher (0.63 to 0.80 g/ml) than that obtained by Edema *et al.* (2005) for flour from commercially sold soybean (0.38 g/ml). However, values obtained from this study were comparable with the values reported by Okaka and Potter (1979) for cowpea (0.60 g/ml) and fall in the range for Bambara groundnut (0.6 to 0.75 g/ml) reported by Onimawo *et al.* (1998).

Water absorption capacity ranged between 113.0 to 142.0 g/100 g. The values increased with the fermentation periods. The non-fermented pigeon pea flour had a value of 142.0 g/100 g while lowest value was recorded at day-5 of fermentation (113.0 g/100 g). Values obtained from this study are greater than obtained for flours from soybean (1.12 g/100 g) Alfaro *et al.* (2004); mucuna (1.2-2.0 g/100 g) Adebawale *et al.* (2005); lupin seed flour (1.20 g/100 g) Sathe *et al.* (1982), whereas by comparing the values obtained in the present study with the water absorption capacity for pigeon pea reported by Oshodi and Ekperigin

Table 1. Proximate composition and energy value of pigeon pea as affected by fermentation

Fermentation (Day)	moisture (%)	crude protein (%)	crude fat (%)	ash (%)	crude fibre (%)	NFE (%)	Energy (kcal/100g)
0	10.20	21.8	2.74	4.61	7.25	53.40	325.46
1	11.40	22.6	2.30	4.92	5.62	53.16	323.74
2	12.00	22.8	2.18	5.25	5.30	52.47	320.82
3	12.50	23.5	1.89	5.39	5.11	51.61	315.65
4	13.08	23.7	1.74	5.48	4.70	51.30	315.66
5	13.24	23.9	1.69	5.52	4.52	51.13	315.33
± S.E.M.	0.19	0.25	0.24	0.16	0.15	0.22	4.13

a Mean of duplicate determinations (wet basis)

b Mean values are significant at $P \leq 0.05$

c S.E.M: standard error of the mean.

Table 2. Effect of fermentation on the functional properties of flour from pigeon pea seed flour

Fermentation (Day)	WAC (g/ml)	BD (g/ml)	SC (%)	FC (%)	FS (%)	V (%)	GP (%)
0	142.0	0.80	6.5	8.16	2.45	1.64	56.2
1	137.0	0.74	6.1	6.52	2.51	1.59	53.8
2	136.0	0.71	5.9	6.00	2.38	1.52	51.2
3	131.0	0.67	5.8	5.05	2.32	1.39	47.9
4	121.5	0.65	5.7	5.00	2.24	1.38	45.8
5	113.0	0.63	5.5	4.17	1.97	1.19	43.8
±S.E.M	0.01	0.01	0.20	0.02	0.01	0.01	0.02

WAC= water absorption capacity; BD=bulk density; SC=swelling capacity; FC=foam capacity; FS=foam stability; V=viscosity; GP=gelation power

a Mean of duplicate determinations (wet basis)

b Mean values are significant at $P \leq 0.05$

c S.E.M: standard error of the mean.

(1989). This result suggests that fermented pigeon pea flour may find application in the production of some baked products.

Swelling capacity decreased with increasing fermentation period, with the value of non-fermented seed flour at 6.5% and the least value obtained as 5.5% on the day-5 of fermentation. Foaming capacity varied from 4.39 to 8.16% among the pigeon pea flour samples and decreased with increasing period of fermentation. Similarly, the foam stability decreased with fermentation periods, which ranged between 1.97 and 2.45%. The non-fermented seed flour had the highest foaming properties in this study. Foam formation and stability are functions of the type of protein, pH, processing methods, viscosity and surface tension (Yasumatsu *et al.*, 1972). The foaming properties recorded in this study are lower than those reported for pumpkin (13.2%) by Oshodi and Fagbemi (1992) and germinated tiger nut varieties (4.00 to 11.33%) by Chinma *et al.* (2009).

Gelation power, an index of gelling tendency (ability to form gel) of sample is very important in food preparations. Gelation power for the seed flour of pigeon pea ranged from 43.8 to 56.2%, with highest value recorded with the non-fermented seed flour. Thus the gelation power decreased with fermentation periods, these values are still higher than that reported for great northern bean flour (10%), cowpea (16%) by Sathe and Salunkhe (1981);

soybean flour (10%) by Alfaro *et al.* (2004); pigeon pea flour (4%) by Onimawo *et al.* (1998); lupin seed flour (14%) by Sathe *et al.* (1982) and African bread fruit flour (6 to 12%) by Fasasi *et al.* (2007). Such variation in the gelling properties of different legume flours may be ascribed to the relative ratios of different constituents, proteins, carbohydrates and lipids that make up the flours; suggesting that interactions between such components may also have a significant role in the functional properties (Sathe *et al.*, 1982). Sathe and Salunkhe (1981) indicated that, gelation is not a function of protein quality but could also be related to the type of protein as well as to non-protein components.

Conclusion

Fermentation generally improved the protein value of the pigeon pea; *Cajanus cajan* flour and this suggest possible use of the fermented flour of this legume as a potential source to improve the nutritional qualities of local staples like cereal, roots and tuber flours. Most of these staples have lower values of protein and when incorporated may likely be a remedy to solving the menace of protein-energy mal-nutrition in the developing countries. The functional parameters were observed to decrease as the fermentation days increased.

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