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Functional, Proximate and Mineral Composition of a Lesser Known Fermented Legume (Fermented Seeds of Cathormion Altissimum, Oso)

Jolaoso A.A, Ajayi J.O,Ogunmuyiwa S.I.O And Albert O.M

Moshood Abiola Polytechnic,
Ojere.Abeokuta.Ogun State.

Corresponding Author: Jolaoso A.A,

Abstract
The seeds of *cathormion altissimum i*s one of the lesser-known legumes in the world. *Cathormion altissimum* is a leguminous plant with seeds that can be fermented into local fermented food, *Oso*. Seeds of legume may account for 80% of dietary protein and may be the only affordable protein for some people. The local fermented food could be eaten as main food, delicacies or used as condiments to flavour soups and stews. The functional properties, proximate analysis and mineral contents of this seed was carried out. The foaming capacity of 7.84,water absorption of 160.5%,fat absorption of 126.4,emulsifying properties of 52.0%,gelation properties of 13.4% and bulk density of 0.7240g/cm3 were recorded. The percentage proximate analysis showed a high protein value of 25.3% and a fibre value of 6.40 after fermentation. The mineral content recorded high calcium of 129.5mg/100g and phosphorus of 720mg/100g.The research was carried out to harness the nutritional value of lesser –known fermented legumes in order to encourage food security and also to create awareness on the availability of an affordable source of protein for people

Keywords: legume, lesser-known, fermented, functional properties, nutritional

INTRODUCTION

Seeds of legumes may account for up to 80% of dietary protein in Nigeria and may be the only source of protein for some people in Nigeria (Achi, 2005). Among these legumes include melon seeds (*Citrullus vulgaris*) fermented to ogiri, oilbean (*Pentachlera macrophylla*) fermented to ugba, sesame seed (*Sesanum indicum*), African locust bean (*Parkia biglobosa*), soybean (*Glycine max*) fermented to dawadawa, *Cathormion altissimum* seeds fermented to 'Oso'. These food flavouring condiments are prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of protein and carbohydrate components (Eka, 1980). Apart from increasing the shelf life and reduction in anti-nutritional factors (Odunfa 1985b; Reddy and Pierson, 1999; Barimalaa, Achinewhu, Yibatima, Amadi, 1989; Achi and Okereka, 1999), fermentation markedly improves the digestibility, nutritive value and flavours of the raw seeds.

Seed proteins should possess the requisite functionality for their successful utilization in various food products. These functional properties are intrinsic physio-chemical characteristics that affect the behaviour of properties in food systems during processing, manufacturing, storage and preparation (Aremu, Olaofe and Akintayo, 2007).Hence,the objective of this study is to determine the functional properties and the proximate analysis of this lesser known fermented seeds of Cathormion altissimum (Oso).

LIMITATION OF THE STUDY

Oso is a seasonal plant and therefore must be preserved till the next planting season.

MATERIALS AND METHODS

Collection of Samples

Cathormion altissimum ('oso') samples used for this research were bought from Ipobe market in Yewa region of Ogun state in Nigeria.

DETERMINATION OF SOME FUNCTIONAL PROPERTIES

Bulk Density

This was determined by the method of Narayana and Narasingo Rao (1984). An empty calibrated centrifuge was weighed .The tube was then filled with a sample to 5ml by constant tapping until there was no further change in volume. The weight of the sample alone was determined by difference. Bulk density was calculated from the values obtained as follows:

$$\text{Bulk density (g/ml)} = \frac{\text{Weight of sample}}{\text{Volume occupied}}$$

Dispersibility Of 'oso' Flour

The dispersibility of meal in water indicated its ability to reconstitute. The higher the dispersibility, the better. The method of Kulkarni,Kulkarni and

Ingle (1991) was adopted. 10g of sample was weighed into a 100ml measuring cylinder. Distilled water was added up to 100ml volume. The sample was vigorously stirred and allowed to settle for 3hours.volume of settled particles was recorded and subtracted from 100 to give a difference that is taken as percentage dispersibility.

Protein Solubility

Ph dependent protein solubility was studied using the method of Akanda (1989). 1g of flour was dissolved in $10cm^3$ 1mHcl. The solution was then centrifuged for 15 minutes at 3500rpm before the protein content of the supernatant was determined by microkjedahl method (Pearson, 1976). %N was converted to crude protein multiplying the % by 6.25

Foaming Properties

The method of Coffman and Garcia, 1977 was used for the determination of the foaming capacity and stability of legume flours .1g of legume flour was dispersed in 50ml distilled water .The resulting solution was vigorously whipped for ten minutes in a Kenwood blender and then poured into a 100ml graduated cylinder .Volume was recorded before and after whipping and the % volume increase was calculated according to the following equation.

$$\%\text{volume increase} = \frac{\text{Volume after-volume before} \times 100}{\text{Volume before}}$$

Foaming stability was determined as the volume of foam that remained after 8 hours expressed as a percentage of the initial foam volume .Effect of flour concentration on foaming property was determined by whipping 2,4,6,8 and 10%w/v slurries as described above.

Water and Absorption Capacity

The method of Beuchat (1977) was used for water and oil absorption capacity determination.1g of flour was added, mixed with 10ml distilled water (density gcm^3) or oil (Executive chef vegetable oil with sp. gravity of $0.989ml^{-1}$) in a mixer and kept at room temperature for 30min . It was later centrifuged for 30minutes and the supernatant was noted in a 10ml graduated cylinder. The excess absorbed by the flour was expressed as the % of water or oil bound by 100g sample. Studies were conducted to investigate the effect of legume flour concentration using 2, 4, 6, 8, 10%w/v slurries as described above.

Emulsifying Properties

Emulsion capacity and stability was prepared by using Beuchat's (1977) procedure. One gram of sample was blended in a Kenwood major blender with 50ml distilled water for 30seconds.At maximum speed, exec chef oil was added in 5ml portions with continued blending. A drop in consistency was considered to be the point at which oil addition was discontinued. The emulsion so prepared was then allowed to stand in a graduated cylinder and the volume of water separated after 24hours was recorded as emulsion stability .studies was conducted in triplicates.

Gelation Properties

Gelation properties of the sample flour was be determined by employing the method of Coffman and Garcia (1977). Flour suspensions of 2 – 12%w/v was prepared in distilled water. The test tubes containing these suspensions was heated for 1hour in boiling followed by rapid cooling under running tap. The test tubes were then cooled for 2hours at 40^oC.The least gelation concentration was taken as the concentration when the sample from inverted test tube did not fall or slip. Studies on the effect of flour concentration on gelation property was conducted by preparing 2,4,6,8 and 10%w/v slurry.

PROXIMATE ANALYSIS OF FERMENTED 'OSO'

The proximate compositions of samples at different times during fermentation process was carried out. parameters like moisture, ash, fat, crude protein, crude fibre, lipid were evaluated as follows.

Determination of Crude Fat Content

The samples were first dried in an oven at 80^oC for 48hours, ground into powder and then sieved using a 250um wire mesh. 2grams each of the samples was extracted in soxhlet extraction thimble for 6hours using petroleum ether (Boiling point60^0c – 80^0c). The residues were then dried in the oven at 105^oC for 24 hours and weighed. The loss in weight between the initial sample and the residue was taken as the lipid content expressed as the percentage of the original sample (A.O.A.C, 2000).

Determination of Moisture Content

5grams of samples were weighed into pre-weighed foil drying dish and dried to constant weight in an oven at 105^oC for 3hours.The dishes were then removed and placed in a dessicator to cool before weighing. The dishes were then returned to the oven for 2 hours, cooled and weighed again. This process was continued until a constant weight was obtained for each sample. From the final weight, the moisture content of samples was determined from the mean values of triplicate determinations.

$$\%\text{ moisture} = \frac{\text{initial weight (before drying)} - \text{final weight (after drying)} \times 100}{\text{Initial weight (before drying)}}$$

Determination of Total Crude Protein Content
1gram of each sample was transferred into 50ml kjedahl flask. Two millimeters (2mls) of distilled water was added and the flask was allowed to stand for 30 minutes. 0.02g pumice, 1.33g K_2SO_4 catalyst mixture and 1.5 mls concentrated H2SO4 were then added. Heating was done on the digestion rack until frothing stopped. Heating was again increased to gentle boiling so that the H_2SO_4 condensed to about one third way up the neck of the flask. The isolated particles were washed with 30% H_2O_2 solution before boiling again for 1 hour. On cooling, 10ml deionized water were added slowly with swirling. 2mls aliquots of each diluted solution were measured and total nitrogen determined spectrophotometrically .The crude protein contents were determined from total nitrogen values (A.O. A.C, 2000).

Determination of Ash Content
The basis of the ashing is to determine the amount of residual inorganic substances after ignition of samples. An amount (2g) each of dried samples was quantitatively transferred into pre-weighed porcelain crucibles, the weights of the samples with the crucibles were recorded .The crucible containing the samples were placed in a pre-heated muffle-furnace at 550 – 570^oC for 1 hour, they were then removed ,cooled to room temperature in a dessicator and then weighed. The differences between the final weights and the porcelain dish gave the ash contents of the samples that were expressed as percentages of the initial weights as follows;

$$\%ash = \frac{\text{weight of ash} \times 100}{\text{Initial weight of sample}}$$

Determination of Crude Fibre
Two grams each of samples was transferred into clean fitter crucibles. One hundred and fifty millimetres of 0.128M H_2SO_4 previously pre-heated in the reagent system was added to prevent foaming. The contents of the beakers were boiled for 30 minutes and filtered through a Buchner funnel with the aid of a suction pump. The residues were washed with hot deionized water until acid free. The residues left after acid digestion were carefully transferred into a 400ml beaker.150ml of 0.22M KOH solution and a few drops of octanol were added to each sample. The contents of the beaker was filtered through a Buchner funnel and 15cm diameter whatman no 4 filter paper on cooling. The residue was washed several times with hot water and once with methylated spirit until free of KOH. The residues was finally washed three times with acetone, carefully transferred into porcelain crucibles and dried at 130^oc for 2 hours. They were allowed to cool in desiccators before weighing. (A.O.A.C, 2000).

Determination of Carbohydrate
Carbohydrate was determined by the difference method (A.O.A.C, 2000)

Determination of Mineral Elements
The minerals were analysed from solutions obtained by first dry-ashing the fermented and unfermented samples of Cathormion altissimum,oso at 550oc.The residues of both samples were dissolved in 10ml of 50% of nitric acid solution and made up to final volume of 25ml of distilled waterThe mineral elements that were determined were calcium,copper,iron ,zinc and phosphorus.All the elements were determined by using the atomic absorption spectrophotometer(Perkin –Elmer 380) except the phosphorus which used the spectronic 20 spectrophotometer.

STATISTICAL ANALYSIS
All datas were statistically analysed by analysis of variance (ANOVA)

RESULTS AND DISCUSSION
The foaming capacity of *Cathomion altissimum* is 7.84(TABLE 1), the value of the foaming capacity is higher than that of prosposis africana, 3.9 (Aremu et al, 2006). The value is however in line with other varieties of legumes ranging from 7.9 – 155% (Olaofe and Akintayo, 2006). The value was however higher than that of soybean 66% (Lin, 1974) and varieties of African yambean, 54 – 55% (Oshodi et al, 1997).

Water absorption capacity of 'oso' recorded is 160.5%. This was higher than that reported for soybean (130%) (Linn et al, 1974) and sunflower (107%). The result was also higher than that of varieties Liman bean 130 – 142% (Oshodi, 1989). The WAC was however lower than that of prosposis africana; 340.00 (Aremu et al, 2006).

The fat absorption capacity of *Cathormion altissimum* is 126.4. The value is close to that recorded for proposes africana of 120.0 (Aremu et al, 2006). This is also found to be closer to the value obtained, for varieties of legume seeds ranging from 127.8 to 172.0% (Olaofe and Akintayo, 2006). The value is however, lower than that recorded for cowpeas with the range 281 – 310% (Olaofe et al, 1993).

The value obtained for emulsifying properties of *Cathomion altimssimum* is 52.0%. This was however higher than that of prosposis africana 30.0% (Aremu et al, 2006). The value can compare favourably with benniseed, peer millet and quinoa, 63.0, 89.0 and 104.0% respectively. The value is however, higher than that reported for soybean, 18% (Lin, 1994) and pigeon pea, 7 – 11% (Oshodi and Ekpesigun, 1989).

For the least Gelation properties, the values obtained for *Cathormion altissimum* is 13.4%.

The value compares favourably with that obtained for bambara groundnut flour of 12.0%. However the value is lower than that for cowpea flour with 16% least gelation properties. (Aremu et al, 2007).

The bulk density value of *Carthormion altissimum* was 0.7240glcm^3. The bulk density value of *Cathormion altissium* is higher than that of prosposis aficana, which recorded a bulk density of 0.5268glcm3 (Aremu et al, 2007).

The value of 0.724glcm^3 of *Cathormion altissimum* is higher than the values reported for various samples of extrusion texturized soya products with varied protein and soluble sugar contents of 0.2382 – 446.0 gmL^{-1}(Cherry, 1981).

The sample is even higher than that of fluted pumpkin seed flour of (180 – 380L-1) (Fagbemi et al, 2006). The bulk density of *Cathormion altissimum* is comparable to cowpea and pen protein isolates recording 0.71 $\pm$ 0.05 and 0.68 to 04gem^{-3} respectively.

TABLE 1: Functional Properties Of *Cathormion Altissimum* Seeds

Parameters	Functional Values
FOAMING CAPACITY	7.84%
EMULSIFYING PROPERTIES	52.0%
BULK DENSITY	0.7240g/cm3
WATER ABSORPTION	160.5%
OIL ABSORPTION	126.4%
GELATION PROPERTIES	13.4%

Values are mean analysis of samples

Functional properties explicated the potential of legume for preparation of protein isolates (Butt and Batool, 2010).

Foaming capacity reported in this study could make *Cathormion altissimum* flour utilizable to form a stable form by unfolding the polypeptide chains and exposing substantial region of hydrophobic residue into air in lipid phase where they form good foaming capacity (Sathe and Salinkhe, 1981).

The high water absorption capacity reported in this study indicated that *Cathormion altissimum* may be used in formulation of some foods such as soups and baked products (Olaofe, 1998). WAC is an indication of a product to associate with water in conditions where water is limiting (Giami and Bekebain, 1992).

The observed high water absorption capacity may imply that in wet form of Cathormion altissimum, less water is available for microbial activities fat absorption capacity could be attributed to the physical entrapment of oils which is related to some numbers of non-polar side chains on the proteins that bind hydrocarbon chains of the fatty acids (Giami and Bekebain, 1992).

The fat absorption capcity is important as oil acts as a flavour retainer and improves the mouth feel of food (Kinsella, 1976).

The higher emulsifying value in *Cathormion altissimum* indicated that *Cathormion altissimum* might be useful in the production of sausages, soups and cakes (Kinsella, 1976).

The least gelation concentration (LGC) is defined as the lowest protein concentration at which gel remained in the inverted tube. This is used as an index of gelation capacity. The lower the gelation concentration, the better the gelating ability of the protein ingredient (Akintayo et al, 1999). The least gelation value of Cathormion altissimum 13.4% is an asset in the use of these legume for formulation of curd or as an additive to other gel-forming materials in food products (Oshodi et al, 1997).

The ability of protein to form gels and provide a structural matrix for holding water, flavours, sugars and food ingredients is useful in food applications and in new product development, thereby providing an added dimension to protein functionality (Oshodi et al, 1997).

The bulk density of *Cathormion altissimum* was low and this could be an advantage in complementary foods (Akpata and Akubor, 1999). The result obtained from this research work showed that *Cathormion altissimum* flour could be a good substitute for flour from other legumes such as soybean, cowpea, in some food formulation.

Table 2: Percentage Proximate Analysis At 12hour Interval Of Fermentation(G/100g)

Analysis/hour	Unfermented	12hrs	24hrs	36hrs	48hrs	60hrs	72hrs	96hrs
Crude protein	29.50^{ab}	29.50^{ab}	28.20^{bc}	29.60^{a}	28.40^{b}	26.30^{c}	25.30^{d}	25.30^{d}
Lipid	16.50^{bc}	16.50^{bc}	15.50^{cd}	15.90^{d}	16.00^{c}	16.80^{b}	16.90^{a}	16.90^{a}
Moisture content	24.30^{c}	24.30^{c}	26.60^{d}	30.65^{c}	34.40^{bc}	37.93^{b}	40.30^{a}	40.20^{a}
Crude fibre	10.30^{a}	10.30^{a}	9.40^{b}	8.40^{c}	7.30^{d}	7.00^{de}	6.45^{e}	6.40^{ef}
Ash	3.82^{a}	3.82^{a}	2.91^{b}	2.00^{bc}	1.99^{c}	1.20^{d}	1.03^{e}	1.03^{e}
Carbohydrate	15.58^{a}	15.58^{a}	17.39^{b}	13.45^{c}	12.21^{d}	10.77^{e}	10.02^{ef}	10.17^{f}

Mean values followed by different superscripts within rows are significantly different ($p<0.05$)

In table 2, Significant increase ($P < 0.05$) in moisture content was recorded in the samples. The spontaneous fermented sample of *Cathormion altissimum* have a 39% increase in moisture content. The increase in moisture content recorded was similar to fermented sample of soy-bean and an increase was also recorded in moisture content of African locust bean and melon seeds of 51.9 and 56.7% and 43.0 and 44.1% (Omafuvbe et al, 2004).

The increase in moisture content of most of the samples of *Cathormion altissimum* was as a result of boiling in water followed by further washing in water (Omafuvbe et al, 2004). Decrease in ash content was recorded for all the *Cathormion altissimum* fermented samples. This was in accordance with the values recorded for African locust bean and melon seeds (Eka, 1980) and (Omafuvbe, 2004). Boiling, washing and further boiling of *Cathormion altissimum* seeds led to a loss in ash. This means that a reasonable amount of mineral content might have been leached during processing.

The condiment fibre values in all fermented samples were also reduced by fermentation. The decrease in fibre content was reported for African locust bean and melon seed after fermentation (Omafuvbe, 2006), (Enuyieba, 1992).At the end of the boiling process, the water used for boiling the *Cathormion altissimum* seeds was more viscous than it was at the beginning of fermentation process. This is an indication of the presence of mucilaginous materials in the boiling water. The reduction in crude fibre may also be as a result of production of extracellular enzymes.

Decrease in fibre value was also recorded in fermentation of soybean (Popoola and Akueshi, 1986b). The fibre values of 6.40 and 6.30 agree with the values recorded for most fermented legumes (Akpata and Ologhobo, 1994).

The increase in ether extract of *Cathormion altissimum* was in agreement with earlier findings in which melon seed increased in ether during fermentation (Eka, 1980).

The slight increase in lipid values is also in agreement with that of Wokoma and Aziagba (2001) who recorded increase in lipid values of fermented African yam bean to dawadawa.

The decrease in crude protein values from 29.5% to 25.3% was probably due to the reduction in the content of ash, crude fibre and carbohydrate (Omafuvbe, Abiose and Shonukan, 2002).There was a significant decrease in protein value 29.5% to 25.3%, this was in conformity with the decrease in protein contents of soybeans fermented to dawadawa by Popoola and Akueshi (1986) in the fermentations of soybean to dawadawa. Significant decrease($p>0.05$) in carbohydrate values,15.58% to 10.17% is likely due to bioconversion of the substrate which is usually accompanied by the release of heat that also accounted for the steady increase in temperature during fermentation(Jolaoso,2005) .

TABLE 3: Mineral Elements Determined In Fermented And Unfermented Seeds.(Mg/100g)

Mg/100g	Zinc(zn)	Iron(Fe)	Calcium(Ca)	Phosphorus(P)	Copper(Cu)
Unfermented	5.81^a	2.05^a	127.4^a	560^a	2.6^a
fermented	6.38^b	1.08^b	129.5^b	720^b	1.4^b

Mean values followed by different superscripts are significantly different

A significant increase $p<0.05$ in the values of zinc, calcium and phosphorus was observed. zinc increased from 5.81mg in unfermented sample to 6.38mg in fermented sample. Calcium increased from 127.4mg in unfermented sample to 129. 5mg in fermented sample. phosphorus also recorded an increase from 560mg to 720mg.This increase compares well with the result of Eka (1980) who reported an increase in the level of zinc, calcium and phosphorus during the fermentation of locust bean to iru. Obizoba and Atu (1993) also observed that fermentation increased the zinc, sodium and phosphorus in castor oil seeds and oil bean seeds after 4 days of fermentation. The fermented seeds of Cathormion altissimum contains phosphorus combined with calcium is needed in the formation of strong bones and teeth .Phosphorus also plays role in energy metabolism of the cells (Trimmer, 1994)

The results from this study has shown that fermented seeds of Cathormion altissimum is nutritionally rich in some essential nutrients needed in the body. it could therefore be recommended as an amino acid supplements in young children.

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