Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS) 3 (4): 608-613

jeteas.scholarlinkresearch.org

Changes in Functional Properties as a Measure of Biochemical Deterioration of Oso (Fermented Seeds of Cathormion Altissimum)

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Abstract
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 behavior of properties in food systems during processing, manufacturing, storage and preparation . One of the major components of oso(fermented seeds of *Cathormion altissimum*) is fat which makes it susceptible to oxidative rancidity .The fear of the side effects of most synthetic anti-oxidants has brought about the use of ginger, ascorbic acid and salt as alternative preservatives. The water and fat absorption capacity of fermented legume was also observed. Oso (fermented seeds of *Cathormion altissimum*) was treated with various concentration of ginger, ascorbic acid and salt. The free fatty acid and peroxide value were used to quantify anti-oxidant activity of oso stored at ambient temperature of 30^{o}c.The aerobic mesophilic count were also determined. At 1600ppm concentration of ginger, 44.4% reduction in free fatty acid was observed and a significant reduction in peroxide value was also noted. A decrease of 68.9% was also observed in free fatty acid at 2400ppm concentration of ginger. The samples treated with ascorbic acid and salt also exhibited significant decrease in free fatty acid and peroxide values at various concentrations.The significance and purpose of conducting this research is the need to explore the use of non-synthetic preservatives in extending the shelf-life of the proteinous product.

Keywords: oso, *Cathormion Altissimum*, ginger, ascorbic acid, salt, synthetic, natural.

INTRODUCTION

Traditional diets in West Africa often lack variety and consists of large quantities of the staple food (cassava, yam, maize) with supplements of plantain, cocoyam, rice and beans depending on availability and season (Achi,1999).Seeds of legumes may account for up to 80% of dietary protein and may be the only source of protein for some groups (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'. The process involved in the production of 'Oso' (fermented seeds of Cathormion altissimum), the microorganisms involved during fermentation, the amino acid profile and some important mineral elements present in the fermented seeds had been documented (Popoola et al, 2006).The functional properties of the fermented products was carried out The product cannot be kept longer than one week after fermentation due to its fast deterioration, hence the need for a preservative method. Lipid oxidation which is an important factor influencing quality and acceptability of food product has been identified as one of the major factors in the spoilage of fermented legumes (Hunt et al,1999). Anti-oxidant have proved useful in reducing peroxidation in fat containing food. Natural anti-oxidants have been shown to have advantage over synthetic anti-oxidants(Ito et al,1986)(Zia-ur – Rehman et al(2003),(Kolapo et al,2010).Ginger have been used for taste enhancement and as a preservative in various foods and cuisines because of its ability to inhibit microbial growth that often contribute to lipid peroxidation in oil rich foods(Yano et al,2006).Hence this study was carried out to investigate the effect of ginger extract on peroxidation,free fatty acid and microbial load in stored oso in comparison to ascorbic acid which has been reported to be the most anti-oxidant in extracellular fluids(Tsao and Akthar,2005).This was done to improve the shelf – life of oso.

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.

Research Laboratory

The research was carried out at the Microbiology Research laboratory of Sacred Heart Hospital,Lantoro,Abeokuta,Ogun State 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.

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

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 oil 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.

Preparation of Extract and Application to 'oso'

The ginger extract, ascorbic acid and salt were prepared according to the method of Zia-ur-Rahman, et al, 2003. The concentrations of ginger used were 1600ppm and2400ppm. To prepare 1600ppm, 800mg of freshly prepared ginger extract was dissolved in 50ml sterile distilled water while in order to prepare 2400ppm,1200mg of ginger extract was dissolved in 50ml sterile distilled water.0.5ml was added to 5g of 'oso'.

For ascorbic acid preparation, 250mg ascorbic acid was dissolved in 50ml sterile distilled water to obtain a standard solution. Half a millimeter of standard solution was added to 5g 'oso' to obtain 500ppm concentration. To obtain 250ppm, the standard solution was diluted twice before adding 0.5ml of the resultant solution to 5g of 'oso' (Zia-ur-Rahman, et al, 2003). 1% salt was used which is equivalent to 10000ppm (Ikenebomoh, 2002). Control samples were prepared by adding 0.5ml of sterile distilled water. All samples of each treatment were prepared in triplicates and stored at ambient temperature (30 +

2^{o}C) for 14 days and the various analysis were done at two days interval. The effectiveness of the anti-oxidant properties of ascorbic acid compared with the ginger and salt were carried out by the determination of free fatty acid (ffa) content and peroxide value (pov) of the stored 'oso' stored at ambient temperature. The Free fatty acid was estimated by alkali titration method (A.O.A.C, 2000) while the peroxide content was measured by titration with 0.1Msodium thiosulphate solution using starch as indicator. (A.O.A.C, 2000).

Determination of Free Fatty Acid

25ml of diethyl ether was added to 25ml of alcohol and 1ml of phenolphthalein was added. 5g of oil was dissolved in the mixed neutral solvent and titrated with aqueous 0.1M NaOH shaking constantly until a pink colour which persisted for 15 seconds was obtained.

$$\text{Acid value} = \frac{\text{Titration (ml)} \times 5.61}{\text{Weight of sample}}$$

Determination of Peroxide Values

This was determined by titration with 0.1M sodium thiosulphate solution using starch as indicator (A.O.A.C, 2000) .All determinations were carried out in triplicate and mean values were calculated.

Microbial Counts

The method of Omafuvbe et al (2000) was used for the enumeration of microbial population in the stored oso samples. Counts were noted at zero time and two days interval. Counted bacteria colonies were expressed as colony forming units per gram (cfu/g) of samples. Mean values of triplicates plates were recorded.

Statistical Analysis of Data

The data obtained were subjected to 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 yam bean, 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).

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

The fat absorption capacity of *Cathormion altissimum* is 126.4. The value is close to that recorded for proposis 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 *Cathormion altissimum* is 52.0%. This was however higher than that of prosposis africana 30.0% (Aremu et al, 2006). The value can compare favorably 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 $0.7240 glcm^{3}$. 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 $0.724 glcm^{3}$ 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 ± 0.05 and 0.68 to $04 gcm^{-3}$ respectively

The free fatty acid, decreased with days of storage for all the treated samples. The decrease is a significant decrease ($P < 0.05$) to the control.

The free fatty acid value of the ginger (1600ppm) treated sample decreased significantly ($P < 0.05$) from 0.045 to 0.025 while the ginger (2400ppm) treated sample decreased from 0.045 to 0.014 .The decrease in free-fatty acid of ginger (1600ppm) treated sample was by 44.4% while the decrease in ginger (2400ppm) was by 68.9%. The FFA of 'oso' treated with ascorbic acid 250ppm concentration decreased from 0.045 to 0.022. This was a significant ($P < 0.05$) decrease in fatty acid. The 500ppm ascorbic acid treated 'oso' significantly decreased ($P < 0.05$) from 0. 045 to 0.026 .The sample treated with 250ppm ascorbic acid showed a 51.1% decrease and the 500ppm ascorbic acid treated sample of 'oso' showed a 42.2% decrease in fatty acid. The sample after 14 days of storage treated with salt showed a significant reduction ($P < 0.05$) from 0.045 to 0.030. This showed a 33.3% decrease in free fatty acid after 14 days of storage.

In Table 3 (See Appendix), the peroxide value increased from 3.500 to 21.312 after 14 days of storage.Significant decrease ($P < 0.05$) was observed in the treated 'oso 'with ascorbic acid (250ppm) concentration from 3.5 to 2.5.

Table 2: Changes in Ffa of Stored 'Oso'

Storage Time (days)	Control (oso)	('oso' + ginger) 1600ppm	('oso'+ ginger) 2400ppm	('oso' + a.a) 250ppm	('oso+a.a') 500ppm	1%salt
0	0.045^{a}	0.045^{h}	0.045^{h}	0.045^{h}	0.045^{f}	0.045^{h}
2	0.048^{b}	0.041^{g}	0.040^{g}	0.037^{g}	0.038^{e}	0.042^{g}
4	0.050^{c}	0.040^{f}	0.032^{f}	0.034	0.036^{d}	0.040^{f}
6	0.056^{d}	0.038^{e}	0.030^{e}	0.031^{e}	0.035^{d}	0.038^{e}
8	0.059^{e}	0.036^{d}	0.029^{d}	0.028^{d}	0.033^{c}	0.034^{d}
10	0.062^{f}	0.033^{c}	0.025^{c}	0.027^{c}	0.031^{b}	0.033^{c}
12	0.071^{g}	0.029^{b}	0.021^{b}	0.025^{b}	0.030^{b}	0.031^{b}
14	0.076^{h}	0.025^{a}	0.014^{a}	0.022^{a}	0.026^{a}	0.030^{a}

Values are mean of results.
values with the same superscripts are not significantly different.
a.a=ascorbic acid

Table 3: Changes In Pov Of Stored 'Oso'

Storage time	Control oso	'oso + ginger 1600ppm	'oso' + ginger 2400ppm	'oso' + a.a 250ppm	'oso' + a.a 500ppm	'oso+' 1% salt
0	3.500^{a}	3.500^{a}	3.500^{a}	3.500^{d}	3.500^{c}	3.500^{a}
2	6.322^{b}	6.211^{h}	6.301^{h}	3.000^{b}	3.500^{c}	5.052^{b}
4	10.000^{c}	5.000^{g}	6.000^{g}	3.120^{b}	3.721^{d}	6.120^{c}
6	13.200^{d}	4.901^{f}	5.320^{f}	3.330^{c}	3.941^{f}	10.000^{d}
8	14.310^{e}	4.321^{e}	4.671^{e}	3.510^{d}	3.821^{e}	12.200^{e}
10	16.240^{f}	3.810^{d}	3.921^{d}	3.211^{c}	3.510^{c}	14.000^{f}
12	19.500^{g}	3.720^{c}	3.814^{c}	3.100^{b}	3.000^{b}	16.100^{g}
14	21.312^{h}	3.700^{b}	3.800^{b}	2.500^{a}	2.700^{a}	17.213^{h}

Values are mean of results.
values with the same superscripts are not significantly different.
a.a=amino acid

A decrease of 28.57% was observed in the peroxide value after 14 days of storage (table 3). In 'oso' treated with 500ppm concentration, a decrease from 3.5 Meq kg^{-1} to 2.700 was observed and a 22.86% decrease in POV value was observed. The ginger (1600ppm) treated sample increased slightly from 3.5 to 3.7 , an increase by just 5.7% and the 2400ppm treated sample from 3.5 to 3.8 , an 8.57% increase was recorded. The salt treated sample has its POV increased from 3.5 to 17.213. This recorded 39% increase from the initial peroxide value.

There had been an observed decrease in the microbial load of samples of 'oso' stored with ginger and ascorbic acid at the end of the fermentation. In ginger of 1600ppm concentration, there was no significant difference ($P > 0.05$) between the samples treated with 2400ppm (Table 4).

The microbial count in the sample with concentration of ginger 1600ppm ranges from 7.36 to 8.27 log cfu/g while the microbial count in the ginger sample treated with 2400ppm concentration ranges from 7.36 to 8.24 log cfu/g. The ascorbic acid treated sample (250ppm) has a microbial load ranging from 7.36 to 8.28 while the sample treated with 500ppm concentration of ginger ranges from 7.36 to 8.31. The samples treated with 1% salt concentration ranges from 7.36 to 8.56. Treatments with ginger and ascorbic acid significantly ($P < 0.05$) reduced the bacteria population in stored samples. There was a significant difference ($P < 0.05$) between the

microbial load of untreated samples and those treated with anti-oxidants (ginger and ascorbic acid).
However, significant differences ($P < 0.05$) were not observed between the two anti-oxidants (ginger and ascorbic acid) at different concentration.
It is evident that ginger extract and ascorbic acid lowered the bacteria load of the preserved 'oso'. This is in agreement with earlier workers (Kolapo and Sanni, 2005 and Zia-ur-Rahman et al, 2003). Decrease in microbial load of soy-bean and soy-iru preserved with ginger and ascorbic acid were observed by the above authors. The representative bacteria species isolated from fermented 'oso' included *Bacillus substilis, B.licheniformis, Staphylococcus aureus* and *Leuconostoc mesenteroides subsp. cremoris*. These organisms have been reported to be involved in the fermentation of 'oso' (Popoola et al 2006). Their presence is an indication that even after the normal period of fermentation, these bacteria species are still associated with the product in storage.

The free fatty acid in monitoring degradation cannot be ignored as the formation of organic acids and free fatty acids is an initial step in development of rancidity and off-flavours in fatty foods. Rancidity becomes noticeable when Free fatty acid content (calculated as percentage oleic acid) is about 0.5 – 1.5% (Pearson, 1976).

The values of FFA observed on the 14th day of the research on ''oso'' were lower than these values; it was assumed that with prolonged storage, the FFA would increase and reach the specified value. The peroxide values of all the samples were less than that of standard peroxide values of $10mEqkg^{-1}$ for vegetable oil deterioration. Fresh oils have value less than $10mEqkg^{-1}$ (Akubugwo and Ugbogu, 2007). The low peroxide value indicated slow oxidation of these oils. The low peroxide values also indicated that the oil can resist lipolytic hydrolysis and oxidative deterioration (Ayoola and Adeyeye, 2010).

The decrease in fatty acid with the day of storage for ginger and ascorbic acid is in agreement with some scientists (Kolapo and Sanni, 2005), (Zia-ur-Rahman, et al, 2003). At higher concentration, ginger (2400ppm) gave a better protection against rancidity than ascorbic acid and salt. It is evident that as concentration of ginger extract increases, the inhibitory effect on free fatty acid increases too. The increase peroxide value of untreated ''oso'' after 14 days of fermentation compares favourably with that obtained from other stored fermented foods (Iwe, 1991) and this indicated that peroxidation is associated with the spoilage of ''oso''.

Increase in peroxidation has been found to be a good predictor of fat deterioration (Zia-ur-Rahman et al, 2003). A decrease in fat content of soy-iru was observed with storage (Kolapo and Sanni, 2005) and this was attributed to decrease in free-fatty-acid content as an initial step in fat deterioration (Sattar and Demen, 1973).

The difference in the pattern of changes observed for free fatty acid and peroxide value of ''oso'' suggested that other components of the substrate in addition to the lipids are being peroxidized. The reduction in peroxide value of ascorbic acid of concentrations, 250ppm and 500ppm are in agreement with earlier findings that ascorbic acid plays its antioxidative role in high concentration by reacting very rapidly with reactive oxygen species, producing semidehydroascorbic radical which breaks down rapidly in a very complex way eventually producing oxalic and L-threonic acids (Halliwell and Gutteridge, 1985). Ascorbic acid has also been found to retard the development of rancidity in fried banana chips during storage (Noor and Augustin, 1984).

The concentration of salt was able to increase peroxidation to about 391%. The adverse effect of synthetic antioxidants on human and animal health has been well documented (Ito et al, 1986). On the contrary naturally occurring materials provide advantages over synthetic anti-oxidants because they may be safer to humans (Zia-ur-Rahman et al, 2003).

Results from this study suggested that ginger and ascorbic acid are practical alternative to synthetic antioxidants thus allaying the fear of side effects of most synthetic antioxidants. Also the result is in line with the earlier submission that chemical preservatives could exercise either bactericidal or bacteriostatic effect.

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