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Effect of Using Different Containers During Fermentation on Microbial and Nutritional Quality of Sesame Seed

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ABSTRACT

An investigation was carried out to study the microbial evaluation and proximate composition and nutritional constituents of fermented sesame seed (*Sesamum inducum*) under different condition. Sesame seeds were fermented in fig leaves and also in a plastic container. The microbial investigation revealed the presence of *Bacillus anthracis, B. pumilus, Pediococcus acidilactici, Lactobacillus delbrueckii* and *Lactobacillus plantarum*. The proximate composition of the samples revealed that samples fermented in fig laeves were able to retained the nutritional value of the samples than those stored in plastic containers. Fermentation also increase some of the nutritional constituent: Protein having 54.70% increase and fat 50% increase after the 9th day of fermentation for sample fermented in fig leaf while that of plastic bowl had 39.29 and 30% increase for protein and fat content, respectively. Investigation into the 12th and 15th day of fermentation also revealed reduction in the nutritional constituent of the samples as seen in protein having 19.23 and 31.27% decrease for fig fermented samples and plastic bowl fermented sample respectively. The mineral content of the sample also revealed the presence of Calcium, Phosphorus, Potassium, Magnessium, Iron, Zinc, Manganessa and Selenium with fernentation having effect on the concentration. Investigation on Physio-chemical properties of the fermented sample revealed that the pH decrease from 6.3% on the 3rd day to 5.3% on the 9th day of fermentation for fig fermented sample and 4.6% for plastic fermented sample. The 11.48% increase was also observed for total titrable acidity after the 9th day of fermentation.

Key words: Fermentation, proximate, mineral, physio-chemical

INTRODUCTION

Sesamum indicum is a flowering plant in the genus *Sesamum*. Numerous wild relatives occur in Africa and a smaller number in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds which grow in pods. It is one of the oldest oilseed crops known domesticated well over 3000 years ago. It was a major summer crop in the Middle East for thousands of years, as attested by the discovery of many ancient presses for sesame oil in the region. Sesame (*Sesamum indicum*) of the Pedaliaceae family is one of the first oil leguminous seed known to mankind (Abou-Gharbia *et al*., 2000). The oil of the seeds are rich sources of protein, carbohydrate, phyto-nutrients, omega-6 fatty acids, flavonoid, phenolic anti-oxidants, vitamins and dietary fiber, ash and major minerals of nutritional importance (Kahyaoglu and Kaya, 2006).

Ficus carica Linn. belongs to family Moraceae. It is known as fig tree and in Yoruba language is known as "Igi opoto". The fig tree (*Ficus carica* L.) is one of the unique Ficus species widely spread in tropical and subtropical countries which has edible fruits with high commercial value.

Commercial fig production is either located around the Mediterranean Sea or is realized in countries possessing Mediterranean climate as in the case of California, Australia or South America (Aksoy *et al*., 2001).

Nigeria produces *Sesamum indicum* about 90,000 mt annually mainly in the northern region where it is commercialized for exportation (FAO, 2008). The lack and improper information to the nutritional constituent of sesame had limited its use even among the local producers. In addition to this, the utilized portions of the seeds were even discovered to be under-utilized. The study is therefore intended to compare the proximate, microbiological and some physicochemical properties of this seed kept under different conditions during fermentation.

MATERIALS AND METHODS

Collection of samples: White variety of sesame seeds were purchased from the central market in Kwara state, Nigeria and transported to the laboratory in an air tight polythene bag. Sesame grains were manually sorted and winnowed to remove stones, debris and defective seeds.

Processing: Dehulled samples, were prepared Dehulling was done by soaking in water (1:5 w/v) ratio for 4 h at 29±2°C to allow sufficient loosening of the seed to allow stripping off of the coat. Ruptured seeds were removed by rubbing with palms, washing with cold water and drained using the method of Elleuch *et al*. (2007). The ruptured seed coats were then removed by rubbing between palms and washing with cold water and drained. Dehulled seeds were divided into two parts, with one part subjected to fermentation while the other without further treatment served as control (raw sesame seed).

Fermentation of the seed: The dehulled seed were boiled and put in a glass container. Distilled water was added to the cake and the mixtures were then well kneaded to prepare a firm paste. The paste was pressed by hand to minimize the amount of air. Some were kept in fig leaves while others in plastic bowl and left to ferment at room temperature (28±2°C) for 15 days. Fermented samples were withdrawn after 3 days. Part of the fermented samples was reserved for microbiological tests. The rest of the ferments was then dried at 70°C, milled (0.4 mm sieve) and kept in containers at 4°C until chemical analysis (Yagoub and Ahmed, 2012).

pH and TTA determination: The pH and TTA were determined using the method of Achinewhu (1986). The mixture of 10 g flour and 100 mL water was allowed to stand for 15 min shaken at 5mins intervals and centrifuged at 3000 rpm for 15 min using a Denley Centrifuge (ModelBS 4402/D Denley England. Supernatant was decanted and pH was measured with a pH meter (Model 3505, England). Ten milliliter aliquot in triplicates were titrated against 0.1 M NaOH using 1% phenolphthalein as indicator. Titrable acidity was expressed as the gram lactic acid/100 g of sample and calculated using the equation:

$$TA = M\ NaOH \times mL\ NaOH \times 0.09 \times 100\ mL\ of\ sample$$

where, TA is the titratable acidity, M NaOH is the molarity of NaOH used, mL NaOH is the amount (in mL) of NaOH used, 0.09 is the equivalent weight of lactic acid.

Microbiological evaluations: The total viable counts were made on Plate Count Agar (PCA). Yeasts and mould counts were determined on Malt Extract Agar (MEA) containing 100 mL streptomycin and MacConkey agar for enterobacteriacae. Plates were incubated at 30°C for 24 h.

For the PCA 30°C, for 48 h for MRS, 37°C for 24 h for MacConkey and 4-5 days for the MEA medium. After the incubation period the plates were observed for growth and the colonies were counted and randomly selected for sub-culturing to obtain pure culture before the biochemical identification.

Proximate composition: The proximate chemical composition of each sample was determined using the standard procedure of AOAC (2005).

Minerals content: The mineral constituents (Ca, P, K, Mg, Fe, Zn, Mn and Se) were analyzed using atomic absorption spectrophotometer (Hitachi Z 6100, Tokyo, Japan) (AOAC, 2005).

Antinutritional content: Antinutritional factors the phytate content was determined by the chemical method described by Maga (1982). The titration method was used to determine the oxalate content according to Day and Underwood (1986).

Statistical analysis: Determinations were carried out in triplicates and error reported as standard deviation from the mean. One-way analysis of variance (ANOVA) was performed and the least significant differences were calculated with the SPSS version of 16.00 Software. Significance was accepted at $p<0.05$ levels.

RESULTS

The nutritional constituent of the seed upon fermentation revealed that fermentation improved the quality of the food up to the 9th day. For instance in the fig fermented sample, the seed contain 17% protein before fermentation and after the third, sixth and ninth day of fermentation the yield increased to 19, 24 and 26%, respectively. In plastic fermented sample, there was reduction of the protein content from 17-14% before the gradual increase upon the elongation of the fermentation period till the 9th day (Fig. 1-3). The same trend was also observed for the fat content as it

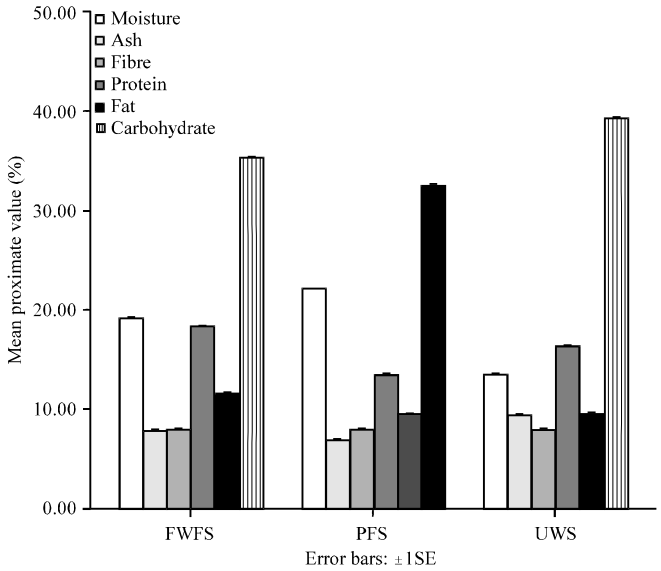

Fig. 1: Results of 3rd day of fermentation period, FWFS: Fig wrapped fermented sample, UWS: Unfermenyed sample, PFS: Plastic bowl fermented sample

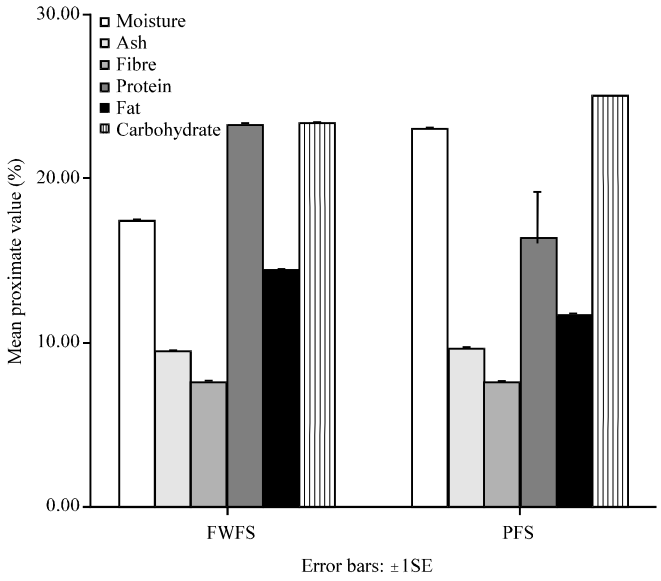

Fig. 2: Results of 6th day of fermentation period, FWFS: Wrapped fermented sample, PFS: Plastic fermented sample

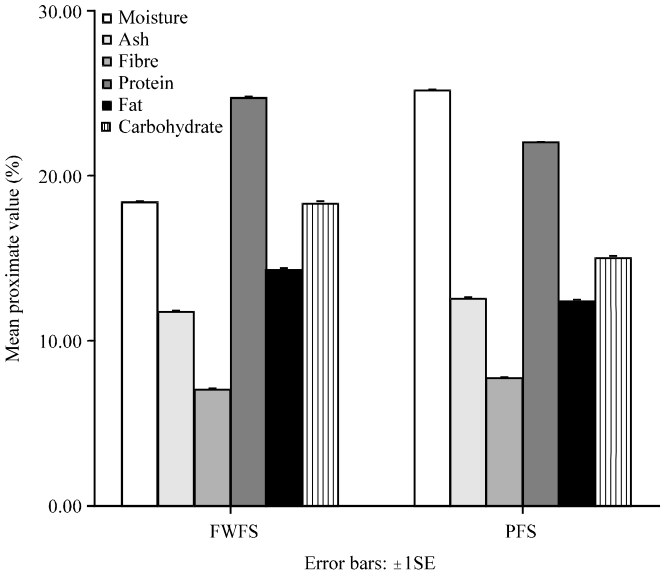

Fig. 3: Results of 9th day of fermentation period, FWFS: Fig wrapped fermented sample, PFS: Plastic fermented sample

increases from 10-15 and 13% in fig fermented sample and plastic fermented sample respectively on the ninth day of fermentation. The fibre content of the both fermented samples was stabilized till the ninth day of fermentation. Fig fermented sample showed a higher yield in the nutritional constituent than the plastic fermented sample. For instance on the sixth day of fermentation fig fermented sample gave a protein content of 24% while the plastic fermented sample gave 20%

(Fig. 2). There was reduction in the carbohydrate content of the samples as shown in Fig. 1-3. Investigation into the nutritional quality after the 9th day of fermentation showed that nearly all the components were tending to diminishing except the carbohydrate and moisture content (Fig. 4 and 5).

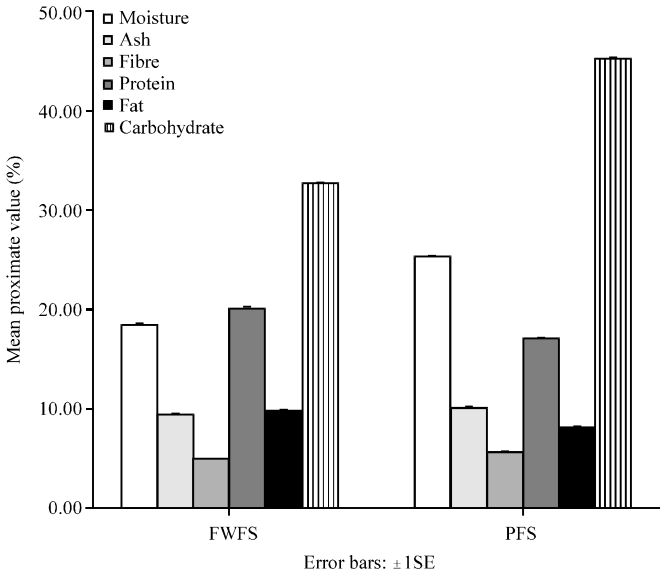

Fig. 4: Results of 12th day of fermentation period, FWFS: FWFS: Fig wrapped fermented sample, PFS: Plastic fermented sample

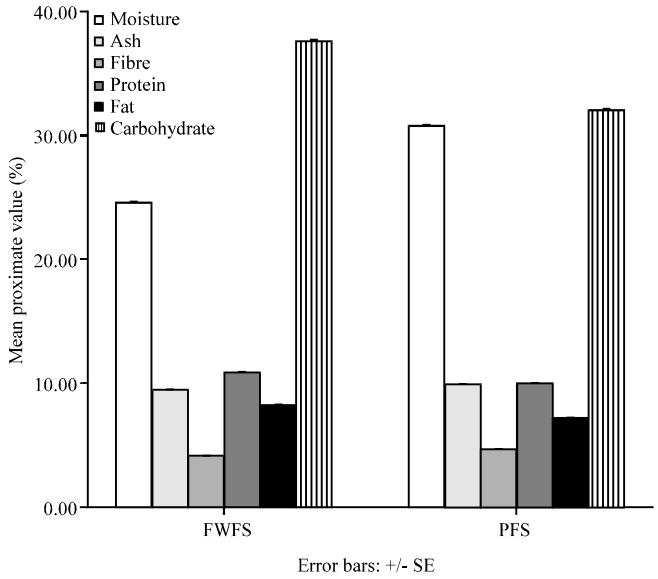

Fig. 5: Results of 15th day of fermentation period, FWFS: FWFS: Fig wrapped fermented sample, PFS: Plastic fermented sample

The microbial investigation of the sample also revealed the presence of *Bacillus subtilis, B. pumilus, Pediococcus acidilactici, Lactobacillus delbrueckii* and *Lactobacillus plantarum*. The occurrence of these microorganisms varied with the fermentation for instance *B. subtilis* was present in the sample till the third day of fermentation while *B. pumilus* occurred till the sixth day. *Lactobacillus* delbruekii appear on the third day of fermentation till the 15th day of fermentation (Table 1).

Fermentation was also found to improve the mineral constituent of the micro nutrients (Ca, P, K,m and Mg) in the fig fermented sample and the plastic samples till the ninth day of fermentation and reduction upon the elongation of the fermentation period (15th day) (Table 2 and 3). The pH and TTA of the samples as shown in Table 4 and 5 also revealed a reduction in pH and increase in the acidity. For instance the pH on the third day of fermentation for fig fermented sample was 6.3 with the corresponding 0.56 TTA on the third day while a pH value of 5.6 and a corresponding 0.61 TTA was observed on the twelfth day. Table 6 also revealed the presence of tannin and oxalate with variations upon the fermentation period.

Table 1: Micro flora of the seed

	Fermentation period (days)					
Microorganisms	0	3	6	9	12	15
Bacillus subtilis	✓	✓	×	×	×	×
B. pumilus	✓	✓	✓	×	×	×
Pediococcus acidilactici	×	×	×	✓	✓	✓
Lactobacillus delbrueckii	×	✓	✓	✓	✓	✓
Lactobacillus plantarum	×	×	✓	✓	✓	✓

×: Absent and ✓: Present

Table 2: Mineral content for fig fermented seed

Fermentation period (days)	Ca	P	K	Mg	Fe	Zn	Mn	Se
3	443.66±0.17^{b}	422.67±0.056^{b}	301.13±0.057^{a}	341.33±0.057^{a}	6.12±0.057^{a}	7.66±0.015^{a}	1.43±0.015^{a}	0.02±0.004^{b}
9	448.18±0.58^{c}	439.13±0.260^{c}	341.63±0.057^{c}	348.69±0.081^{c}	6.13±0.057^{ab}	7.66±0.055^{a}	1.51±0.000^{a}	0.02±0.000^{a}
15	444.82±2.57^{a}	418.14±0.057^{a}	321.03±0.050^{a}	339.17±0.050^{a}	6.13±0.050^{b}	7.66±0.050^{a}	1.43±0.000^{a}	0.02±0.000^{a}

Table 3: Mineral content for plastic fermented seed

Fermentation period (days)	Ca	P	K	Mg	Fe	Zn	Mn	Se
3	515.86±0.17^{b}	439.34±0.25^{b}	449.27±0.05^{b}	347.37±0.25^{b}	6.36±0.05^{b}	9.42±0.11^{c}	3.26±0.05^{b}	0.053±0.00^{a}
9	518.43±0.36^{c}	496.27±0.58^{c}	451.30±0.10^{c}	349.20±0.10^{c}	6.37±0.15^{b}	9.34±0.28^{b}	3.51±0.10^{c}	0.05±0.000^{a}
15	514.22±0.01^{a}	487.30±0.15^{a}	442.13±0.05^{a}	338.13±0.57^{a}	6.34±0.05^{a}	9.12±0.05^{a}	2.94±0.11^{a}	0.05±0.000^{a}

Table 4: pH value of the fermented sample

	Days				
Sample	3	6	9	12	15
FWFS	6.3	5.8	5.3	5.6	5.9
PFS	6.1	5.5	4.9	5.1	5.3

Table 5: TTA value of the fermented sample

	Days				
Sample	3	6	9	12	15
FWFS	0.56	0.58	0.63	0.61	0.57
PFS	0.46	0.55	0.58	0.51	0.48

Table 6: TTA antinutritional constituent of fig fermented sample

Fermentation period (days)	Oxalate	Phytate
3	7.54±0.005^{a}	2.65±0.010^{a}
6	5.24±0.075^{b}	2.65±0.010^{a}
9	3.21±0.010^{c}	1.73±0.005^{c}
12	2.93±0.005^{d}	1.50±0.005^{d}
15	2.90±0.005^{e}	1.00±0.000^{e}

DISCUSSION

Investigation into the changes in the proximate content of the fermented samples revealed that samples wrapped with fig leaves was able to improve the nutritional constituent of the fermented sample more than the plastic fermented sample. However, the general view of fermentation of sesame seed showed that the nutritional contents of the samples were improved upon fermentation period. Figure 1-3 revealed that the protein content of the fig leaves wrapped fermented sample (FWFS) increases from 19.0-26.3% upon the extension of fermentation period from 3-9th day, respectively. This observation was also observed for samples fermented in a plastic bowl (PFS) having 14.0-23.5%. For example, FWFS showed a 9% increase in the protein content while PFS gave a 4% increase after the 9th day of fermentation. The high percentage of protein content observed in the FWFS may be attributed to the inhibitory activity of fig leaf thereby preventing the proteolytic enzyme from degrading the protein content. In addition to this, some phytoconstituent of the leaves may also act on the sample thereby yielding more protein than the sample kept in the plastic bowl. This observation is in accordance with (Olufemi and Olusegun, 2013) who reported that fig leaves has preservative effect on *Capsicum frutescens* while working on the assessment of the preservative efficacy of ethanolic extract *Ficus carica* on *C. frutescens* linn. The observed increased in protein content may also be connected to the activity of the available microorganisms secreting proteinous material into the sample. This observation is in accordance with Simon and Azam (1989) who worked on the protein content and protein synthesis rates of planktonic marine bacteria and reported that bacteria protein synthesis is as an attractive approach because it can directly estimate bacteria carbon production. In addition to this, on the 12th day of fermentation there was reduction in the protein content of about 5% for both PFS and FWFS (Fig. 4-5). The same trend was also observed on the 15th day of fermentation. The observed reduction in the protein content during this period of fermentation may be due to the effect of the proteolytic enzyme which are been secreted by microorganism acting on the available protein thereby causing the reduction in the protein content. This is in accordance with the findings of (Macfarlane *et al.*, 1986) who reported that gut microflora could potentially play a major role in proteolysis in human colon while working on protein degradation by human intestinal bacteria. The observed reduction in the protein content of the samples may also be connected

to the increase moisture content by rendering the protein more available for degradative attack through the creation of more surface area for the activity of microorganism.

Investigation into the carbohydrate content of the samples also revealed drastic reduction on the 9th day of fermentation. The FWFS gave a reduction of 21.61% while a higher percentage was noticed in the sample kept in the plastic bowl after the same period of fermentation. These findings could be traced to the ability of the lactic acid fermenter acting on the carbohydrate content of the sample and utilizing it as a substrate for their metabolic activity (Kimaryo *et al*., 2000). For crude fat sesame seeds are good sources of oil which might make it a good source of vegetable oil for nutritional and industrial process. Increase in the fat content during fermentation agrees with the finding of Achinewhu (1986) for linseed with fat content of 40%, cotton seed 24% and groundnut 46%. The observed increase in the fat content agrees with the findings of (Gernah *et al*., 2012) while working on the effect of malting and lactic fermentation on some chemical and functional properties of *Zea mays*. The mineral constituent of the fermented samples revealed that fermentation increases the mineral constituent till the ninth day (Table 2-3). The lesser value observed in PFS compared to the FWFS could be attributed to leaching (Geankoplis, 2004). The observed increase in mineral composition may be due to the contribution from fermentation microorganisms. And the subsequent decrease may be linked to the acidic medium produced as a result of elongation of the fermentation period. The mineral composition showed that calcium was the predominant macro-mineral followed potassium phosphorus and magnesium. Micro-element such as iron, selenium, Manganese and selenium. Which were present in low concentration. The observed increase in the mineral content of the samples till the 9th day may also be due to the effect of fermentation. The high phosphorus and calcium in the fermented samples indicate that fermented sesame seeds are good and may enrich the food in development of bone. The increment could also be linked to the conversion of the insoluble reserve foods by enzymes during fermentation (Mukhopadhyay, 2001). Titratable acidity measures the total organic acid in a sample. The observed increase in the TTA (Table 5) during the fermentation period may be due to the effect of the microorganisms in utilizing the nutrient most especially the carbohydrate. Acid production has been reported to be responsible for product stability and Flavor development (Maga, 1982). The reduction in the crude fibre content upon fermentation period further established the process as a means of enriching and improving food nutrient as fibre control cholesterol and blood sugar and promotes bowel health. Excess dietary fibre can also bind with other nutrient thereby limiting their availability inside the body. Fermentation had effect on the antinutritional constituent (Table 6) of the seed and the reduction was more significant on FWFS than the PFS. Phytate are known to chelate some divalent minerals such as Ca, Mg, Zn and Mg making them metabolically unavailable for body use (Oshodi *et al*., 1999). The reduction in phytate level upon the elongation of the fermentation period could be interpreted as the main reason behind the observed increase in the concentration of the mineral till the 9th day of fermentation. The effect of fermentation on phytate and oxalate may be also be due to the activity of enzymes like phytase and oxidase produced by the fermenting micro flora (Table 1) (Hachmeister and Fung, 1993).

CONCLUSSION

From the study, there is all indication that the fig leaves (*Ficus carica*) had a great influence in enhancing the nutritional value of sesame seed and was also able to preserve the seed by

complimenting fermentation. The approach can therefore be recommended to processors in the developing countries as it is desirable in nutritional enhancement, preservative properties, low cost and the wide availability of the leaf.

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