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## Physicochemical quality and consumer acceptability of condiment from pumpkin (*Cucurbita pepo*) seeds

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### Abstract

Effect of fermentation on the quality attributes of pumpkin seeds was investigated. Pumpkin seeds were fermented in a digital incubator at 35 °C using fresh gmelina leaves. Fermentation was carried out at different intervals of 24, 36, 48 and 72 hours to obtain samples F1, F2 and F3 respectively. The non-fermented sample (F0) was used as control. Samples were subjected to sensory evaluation and the most preferred sample was compared with the control based on the proximate composition, microbiological analysis and sensory attributes using standard analytical methods. The best sample (C) was finally compared with the control in terms of pH, mineral and vitamin contents. Result of the proximate composition indicated that protein, crude fat, crude fibre, ash, moisture and carbohydrates were in the range of 38.36-40.6, 40.13-44.63, 0.76-0.92, 4.95-5.43, 5.54-6.61 and 0.83-1.91% respectively. Sample F3 had highest values except in carbohydrates. The total bacteria count ranged from  $1.3 \times 10^2$  -  $9.3 \times 10^5$  Cfu/g, while the mould count ranged from  $1.0 \times 10^3$  -  $7.4 \times 10^3$  cfu/g. Sample F3 had highest values of bacteria count and the least value of mould count. Mean sensory scores for appearance were in the range of 4.83-6.47, texture was 5.73-7.13, flavour 6.13-6.57 and overall acceptability 6.66-6.93, respectively. Sample F3 was rated best in all the attributes except for appearance. The comparison of sample F3 with the control (F0) showed that, F3 increased with fermentation period in pH while F0 was constant. Sample F3 had 13.29, 135.67, 742.65, 996.76 and 327.95mg/100g for Fe, Ca, K, P and Mg respectively while sample F0 had 13.30, 134.36, 742.66, 996.74 and 327.96 respectively. The vitamin content of F3 and F0 were almost constant for  $\beta$ -carotene,  $\alpha$ -carotene, thiamine and vitamin C with values of 214.66  $\mu$ g, 48.84  $\mu$ g, 0.07mg/100g and 1.69mg/100g respectively for F3 and 214.66  $\mu$ g, 48.96  $\mu$ g, 0.07mg/100g and 1.70mg/100g respectively for F0. It was therefore possible to convert pumpkin seeds into a nutritious and flavourable condiment for human consumption.

**Keywords:** pumpkin, fermentation, quality, acceptability and condiment

### Introduction

Traditional diets in West Africa often lack variety and consist of large quantities of the staple food (cassava, yam, maize,) with supplements of plantain, cocoyam, rice, and beans depending on availability and season <sup>[1]</sup> (Steinkraus, 1996a). The staple foods provide the calories but are poor in other nutrients. Soups and sauces eaten with the staples are an essential component of the diet. They are the main sources of proteins and minerals and one of the ways to improve the diet has been to improve thnutrient content of soups by adding nutritious fermented condiments from legumes and oil seeds <sup>[2]</sup> (Ademiluyi *et al.*, 2015). Traditionally, legumes and oil seeds are usually fermented into local condiments which serve as soup thickeners and flavour enhancers in food preparation <sup>[3]</sup> (Chukwu *et al.* 2018). However, these condiments may account for up to 80% of dietary protein and may be the only source of protein for some groups in Nigeria. <sup>[4]</sup> (Kolapo *et al.* 2007). The condiments therefore act as substitutes for fish or meat in populations that may find it difficult to afford the protein from these sources <sup>[5]</sup> (Ibeabuchi *et al.* 2014). The supply of locust bean is dwindling and in recent years it's been in short supply as a result of deforestation, long gestation period and charcoal production. In addition, the preparation of locust bean daddawa is tedious and time consuming <sup>[6]</sup> (Appiah *et al.* 2012). Soybean daddawa has been used as an accepted alternative to locust bean daddawa for some time. However, the popularity of soybean daddawa is marred by the perception that, it is prone to faster deterioration than locust bean daddawa; at the end of the fermentation period <sup>[7]</sup> (Adedayo *et al.*, 2018). This has led to the search for more alternative raw material for preparing the valued condiment <sup>[8]</sup> (Obatolu *et al.*, 1998).

Pumpkin seeds, commonly known as 'pepitas', are flat, encased in yellow-white husk <sup>[9]</sup> (Abdel-Rahman, 2006). In many cultures, pumpkin seeds are consumed directly as snack

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food. Pumpkin seeds are rich in protein content (25-52%) and also contain phyto-compounds. It has been reported that oil content is also high, ranging from 40- 60%. Out of this, up to 60.8% is contributed from fatty acids oleic acid (up to 46.9%), linolenic acid (up to 40.5%), palmitic and stearic acid up to 17.4%, the ratio of monounsaturated to polyunsaturated acids from 0.60 to 0.75 g. Approximately 1% each of phytosterols, squalene and chlorophyll pigments is present. Phytosterols are present in free and bound form. Minerals including selenium, zinc, calcium, copper, iron, manganese, phosphorus and potassium are present at 4-5%. Pectin content is 30% <sup>[10]</sup> (Bombardelli and Morazzoni, 1997).

Fermentation is defined as the anaerobic decomposition or breakdown of complex food materials to simple ones with the evolution of gas; example the conversion of sugar to alcohol and carbon dioxide. The changes are brought about by enzymes that are produced by microorganisms (e.g. Bacteria, mould and yeast). Basically fermented foods are agricultural products which have been converted by enzyme activities of microorganisms into desirable products whose properties are considered more attractive than those of the original raw materials <sup>[11]</sup> (Achi, 2005). It is about the only method of preservation that encourages the growth and activities of desirable microorganisms that bring about desirable changes in food system. Therefore optimal conditions of hydrogen ion concentration (pH), temperature, moisture and nutrient are provided for growth of these microorganisms. The fermentation process may be complete oxidation where sugar is converted to carbon dioxide and water or its partial oxidation in which the resulting products are acetic acid, lactic acid, citric acid, alcohol, aldehyde, and some flavoured compounds. It is one of the unit operations widely used in Africa and contributes to development of acceptable textures, flavour and improves the safety of food. Examples of fermented foods are tempeh, dawadawa, yoghurt, beer, palm-wine, sauerkraut, bread etc. Fermentation plays different roles in food processing.

Since the widely used local condiment (dawadawa) obtained from the African locust bean (*Parkia biglobosa*) tree is subject to unavailability, as the tree is not being cultivated presently, efforts have been made to overcome this challenge and ensure the availability of a cheap source of protein by using gbaaye (*Prosopis africana*) as the starting material. However, this project is conceived out of synthetic imagination to produce related local condiment from pumpkin seed considering its relatively wide availability and ease of cultivation than *Parkia biglobosa* and *Prosopis Africana*. Furthermore, in many rural areas of Nigeria and other developing/underdeveloped countries, the supply of animal protein is inadequate to meet the protein needs of the rapidly growing population. This has necessitated contemporary research efforts geared towards the study of the food properties and potential utilisation of protein from locally available food crops, especially from underutilised or relatively neglected high protein oilseeds such as pumpkin <sup>[12]</sup> (Giami, 1993). This underutilization is attributable to ignorance of the benefits embedded in the seed. Hence no literature has been found on the fermentation of pumpkin seed so far.

Pumpkin contributes to food sector, nutrition, dietary and culinary diversification, health and income generation <sup>[13]</sup> (Tawheed 2013). This research explores the nutritional as well as health benefits of its seeds so that the awareness for

nutritive and quality food by health conscious population (especially the rural populace) can be met. The objective of this research is to determine effect of fermentation on the quality attributes of fermented pumpkin seeds.

## Materials and Methods

### Raw Material

Matured, dry pumpkin fruits were obtained from Railway market Makurdi, Benue State of Nigeria. The pumpkin fruits were broken by hand and the seeds removed. The dry seeds were moistened to get soft and dehulled manually with hands.

### Sample Preparation

About 100 g seeds were cooked for 45 minutes to tenderise detoxify and create a favourable surface for faster fermentation. Cooked seeds were drained and allowed to cool using a plastic basket. The seeds were then spread on the stainless steel tray lined with gmelina leaves after which there were covered with another layer of gmelina leaves. The pumpkin seeds were allowed to ferment for three days at the room temperature. The fermented seeds were dried in a vacuum and packaged appropriately into a plastic container and covered tightly.

### Measurement of fermentation rate

The rate of fermentation was measured by monitoring the pH of the samples from the fermenting seeds. The pH was measured at 0 hour and every 24 hours and the results recorded.

### pH Determination

The pH was determined on the raw and fermented pumpkin seeds. Five grams of each sample were milled using a mortar and pestle and mixed well in 100ml distilled water. The pH was then measured using a digital pH meter model 162R

### Proximate Composition of fermented pumpkin seed

Determination of moisture, ash, crude fiber, fat and protein were carried out as described methods by <sup>[14]</sup> AOAC (2010). Carbohydrate was calculated by difference using the method described by <sup>[15]</sup> Ihekoroye and Ngoddy (1985).  

$$\% \text{ carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ fat} + \% \text{ protein} + \% \text{ ash} + \% \text{ crude fiber})$$

### Determination of Mineral Composition

Using the method described by <sup>[14]</sup> AOAC (2010). The ash of each sample as digested with 5mL of 2M HNO<sub>3</sub> and heated to dryness on a heating mantle. About 5mL of 2M HNO<sub>3</sub> was added again, heated to boil and filtered through a Whatman No 1 filter paper into 100ml volumetric flask. The filtrate was made up with distilled water. Calcium and Potassium were determined using Jenway Digital Flame Photometer (PFP7 model) while other minerals apart from phosphorus were determined using Buck Scientific Atomic Absorption Spectrophotometer (BUCK 210GP model). The phosphorus in the sample filtrate was determined by using Vanadomolybdate reagent at 400nm using colorimetric method (Colorimeter Sp 20, Bausch and Lomb).

### Determination of beta carotene content

Beta-carotene was determined using the method described by Murkovic *et al.* <sup>[16]</sup> with slight modifications. Five grams

(5.0g) of the sample was poured into a separating funnel and a solution containing 140 mL ethanol: hexane (4:3) instead of petroleum ether and acetone was added. About 2mL of 2% sodium chloride (NaCl) solution was also added to avoid formation of an emulsion. The mixture was manually shaken vigorously for about 3min., allowed to settle for 30min. and the lower layer was run off. The absorbance of the top layer was determined at the wavelength of 452nm using a spectrophotometer (Spectro Sc 20, Labomed, Inc. USA) and the concentration of  $\beta$ -carotene was calculated using Beer-Lambert law as follows:

$$\text{Beta-carotene (m g/100g)} = \frac{A \times V \times 10^6}{A_{1cm}^{\%} \times W(g)}$$

Where, A = Absorbance, V = Total volume of extract, W = Weight of sample,  $A_{1cm}^{\%} = 2590$  (Absorption coefficient of  $\beta$  and  $\alpha$ -Carotene in hexane)

### Determination of vitamin C

Vitamin C was determined using a titrimetric method as described by [17] Suntomsuk *et al.* (2002). In this method, 10 g of ground sample was mixed with 100 ml of distilled water in a 50 ml beaker and was allowed to stand for 30 min. A clear solution (extract) of 40 ml was transferred to a 250 ml volumetric flask. Two drops of 0.05% starch solution was added as an indicator. The mixture was titrated against 0.0057 M solution of iodine which has been standardized with ascorbic acid solution. Vitamin C was calculated as:

$$\text{Vita min C(mg / 100g)} = \frac{\text{Vita min Cconc}}{\text{Volume of extrat}} \times \frac{100}{1}$$

### Sensory Evaluation

The products were evaluated organoleptically by a group of semi-trained panel of fifteen judges made up of male and female students of the department of Food Science and technology, University of Agriculture, Makurdi and who are familiar with dawadawa product. These panelists were selected based on interest, availability and ability to express opinion. Panelists were provided with product information and requested to evaluate the products for appearance, texture, flavor and general acceptability using the 9 – point hedonic scale with 9: representing – like extremely and 1: representing – dislike extremely and the data obtained were statistically analysed using Analysis of Variance (ANOVA).

### Microbiological Analysis

#### Determination of the total viable count

The pour plate method was used enabled the number of living (viable) organisms and clumps of organisms (i.e. colony forming unit) in a sample to be counted.

#### Pour Plate Method

##### Mould Count

Medium – potato dextrose agar

### Discussion

The protein content increased with increase in fermentation time as shown in Table 1. In the unfermented seeds the pumpkin seeds have protein content of 38.36% and after

fermentation (72 hrs at 35 °C) the protein content increased to 40.67%. In the report obtained by [2] Campbell-Platt (1980) for locust bean fermentation, there was an increase from 30.00% to 38.50% when fermented. [18] Gernah *et al.* (2002) reported a protein content of 24.80% and 33.50% for the unfermented and fermented locust beans respectively after 72 hrs of fermentation at 35 °C. These values are somehow low compared to the value obtained from fermented and unfermented gbaaye seeds at the same temperature and fermentation time. The increase in protein content was due to the microbial activity of *Bacillus subtilis* in the fermentation. [1] Steinkraus (1995, 1996a) illustrated that during fermentation, food substrates are invaded or overgrow by edible microorganisms whose enzymes hydrolyze the proteins and lipids to non-toxic products with flavour, aroma and texture that are pleasant and attractive to human consumers. During fermentation, fat content of foods are increased. This can be seen from the result obtained from this work. After 72 hrs of fermentation at 35 °C, the fat content of the pumpkin seeds were increased. However, [18] Gernah *et al.* (2002) in his work reported a fat level of 25.30 after 72hrs at 35 °C. This value is substantially lower compared to 44.61% of fat obtained in this work. There was a marked decrease in percentage crude fibre content when processes and fermented after 72hrs of fermentation at 35°C. This is relatively low as much of the fibre content was removed during dehulling of the seeds in its preparation for fermentation.

After 72 hrs of fermentation at 35 °C, the ash content of the sample was found to be 5.42% while the unfermented sample was 4.94% as show in table 3 above which means that there was no significant different in the ash content. However, these values were higher compared to the ash content of locust bean when fermented at the same temperature, time of fermentation and incubation materials as reported by [18] Gernah *et al.* (2002). The moisture content also increased with fermentation time. At 35°C and fermentation time of 72hrs the moisture content increased from 5.53% to 6.60%. There was a marked decrease in carbohydrate level or content after the fermentation period. This could be due to the breakdown of carbohydrate by the microorganisms involved, [2] (Campbell-Platt, 1980). The fermentation of pumpkin seeds yield a decrease in carbohydrate content with time of fermentation as shown in table 3 above. The value obtained after 72hrs of fermentation (0.83%) is somewhat lower compared to that of fermented locust beans (7.83%) obtained by [18] Gernah 2002 and 11.70% obtained by [3] Chukwu *et al.* (2018) after 72hrs at 35°C using the same incubation materials.

From the Table 2 above, the microbial count was much more in the fermented than the unfermented seeds. During fermentation, microorganisms multiply and help in the breakdown of carbohydrate and other complex food materials to simple ones. The microorganisms grew very rapidly and produced a high percentage yield of protein. Ibrahim and [19] Antai (1986) reinforced this observation when they work on the production of dawadawa from African locust bean seeds.

Few counts of mould were also found to be present in the fermented sample of pumpkin seeds which increased from  $5.0 \times 10^3$  for 24hrs to  $7.0 \times 10^3$  for 72hrs fermentation.

When the fermented pumpkin seeds were passed through identification tests, the predominant microorganisms found to be associated with the fermentation of pumpkin seeds are

gram positive rods bacteria (e.g. *Bacillus species*, *Lactobacillus species*).

Figure 1 shows the different level of pH from 0hour to 72 hours for the fermentation of Pumpkin seed using Gmelina leaves as incubation materials at a temperature of 35°C. The pH increased from 5.73 to 8.10 resulting from the biochemical changes in the seeds. The main biochemical changes in the seeds are the hydrolysis of protein and the metabolism of the resultant amino acids caused by the strong proteolytic bacteria in the seeds which leads to the production of strong amoniacal odour. The liberation of ammonia during fermentation is a common phenomenon of protein on food as observed by [3] Chukwu *et al.* (2018). The level of increase follows the same trend as that obtained by [18] Gernah *et al.* (2002), [18] Antai and Ibrahim (1986) for locust bean fermentation. It was also observed that the total titratable acidity decreased with an increase in pH. This is because pumpkin fermentation is an alkaline fermentation since the fermentation process caused a rapid rise or increase in pH that reduces the acidity in the fermented

seeds. According to [6] Apiah (2012), fermentation process show a consistent drop in pH of medium with concomitant increase in the TTA and an increase in pH with a decrease in TTA expressed in percentage lactic acid.

Table 4: contains results of mineral composition of fermented and unfermented pumpkin seeds. The mineral composition of pumpkin seed was affected by fermentation as shown in table 4 above. Its iron, potassium and magnesium content decreased in the fermented sample as the values changed from 13.30, 742.66 and 327 to 13.29, 742.65 and 327.95mg/100g respectively; While calcium and phosphorus content were reduced on fermentation.

Table 5 shows the comparison of vitamin content of fermented and unfermented samples of pumpkin seeds. Beta carotene content increased insignificantly as its value changed from 214.65 to 214.66 while the alpha carotene content decreased significantly when fermented but the vitamin B1 (thiamine) was observed to be insignificant in its increase. There was significant increase in vitamin C content as shown in the fifth column.

**Table 1:** Proximate Composition of Fermented and Unfermented Pumpkin seeds

Sample	Protein	Fat	Fibre	Ash	Moisture	CHO
F0	38.36±0.01 <sup>d</sup>	41.56±0.01 <sup>c</sup>	0.92±0.01 <sup>a</sup>	4.95±0.01 <sup>c</sup>	5.54±0.01 <sup>d</sup>	1.91±0.01 <sup>a</sup>
F1	39.05±0.02 <sup>c</sup>	40.13±0.02 <sup>d</sup>	0.76±0.01 <sup>d</sup>	5.31±0.01 <sup>b</sup>	5.91±0.01 <sup>c</sup>	1.62±0.01 <sup>b</sup>
F2	39.55±0.04 <sup>b</sup>	43.11±0.02 <sup>b</sup>	0.77±0.01 <sup>c</sup>	5.42±0.01 <sup>a</sup>	6.23±0.01 <sup>b</sup>	1.45±0.02 <sup>c</sup>
F3	40.67±0.04 <sup>a</sup>	44.63±0.01 <sup>a</sup>	0.78±0.01 <sup>b</sup>	5.43±0.02 <sup>a</sup>	6.61±0.01 <sup>a</sup>	0.83±0.01 <sup>d</sup>
LSD	0.05	0.02	0.01	0.02	0.02	0.02

Mean in a column with different superscripts differ significantly ( $p < 0.05$ ). F0 = control sample (unfermented), F1 =24hours fermented seeds, F2 = 48hours fermented seeds, F3 = 72hour fermented seeds, LSD = least significant difference

**Table 2:** Microbiological Analysis of Fermented and Unfermented Pumpkin Seeds

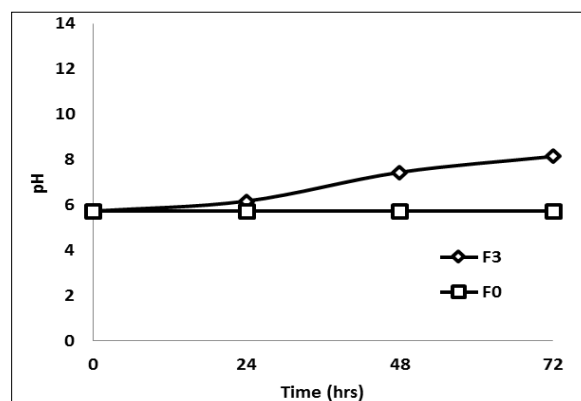
Samples	Total Bacterial Count (CFU/g)	Mould Count (CFU/g)
F0	1.3X10 <sup>2</sup>	7.4X10 <sup>3</sup>
F1	4.0X10 <sup>4</sup>	7.0X10 <sup>3</sup>
F2	8.2X10 <sup>5</sup>	5.0X10 <sup>3</sup>
F3	9.3X10 <sup>5</sup>	1.0X10 <sup>3</sup>

F0=0 hours (unfermented pumpkin seeds), F1=24 hours fermented pumpkin seeds, F2=48 hours fermented pumpkin seeds, F3= 72 hours fermented pumpkin seeds, Cfu= coliform forming units

**Table 3:** Mean Sensory Scores of Fermented and Unfermented Pumpkin Seeds

Samples	Appearance	Texture	Flavour	Overall Acceptability
F0	6.47 <sup>a</sup>	5.73 <sup>a</sup>	6.13 <sup>a</sup>	6.60 <sup>a</sup>
F1	5.53 <sup>a</sup>	6.47 <sup>a</sup>	6.50 <sup>a</sup>	6.80 <sup>a</sup>
F2	4.93 <sup>b</sup>	6.47 <sup>a</sup>	6.40 <sup>a</sup>	6.87 <sup>a</sup>
F3	4.83 <sup>b</sup>	7.13 <sup>b</sup>	6.57 <sup>a</sup>	6.93 <sup>a</sup>
LSD	1.049	0.996	1.257	0.834

F0=0 hours (unfermented pumpkin seeds), F1=24 hours fermented pumpkin seeds, F2=48 hours fermented pumpkin seeds, F3= 72 hours fermented pumpkin seeds, LSD=least significant difference



**Fig 1:** pH of Fermented (F3) and Unfermented (F0) Pumpkin Seeds with Respect to Time



**Table 4:** Mineral Composition of Fermented and Unfermented Pumpkin Seeds (mg/100g)

Samples	Fe	Ca	K	P	Mg
F3	13.29±0.01	135.67±0.03	742.65±0.04	996.76±0.04	327.95±0.02
F0	13.30±0.01	134.36±0.03	742.66±0.03	996.74±0.03	327.96±0.03

F0=unfermented pumpkin seeds, F3=72 hours fermented pumpkin seeds

**Table 5:** Vitamin Composition of Fermented and Unfermented Pumpkin Seeds

Samples	Beta carotene(µg/g)	Alpha carotene(µg/g)	Thiamine(mg/100)	Vitamin C (mg/100)
F3	214.66±0.04	48.84±0.03	0.07±0.001	1.69±0.01
F0	214.66±0.03	48.96±0.03	0.07±0.002	1.70±0.02

F0=unfermented pumpkin seeds, F3=72 hours fermented pumpkin seeds

## Conclusion

It was observed that fermented pumpkin seeds possessed improved physical and chemical characteristics with increased period of fermentation (72 hrs) which gives it a substantial preference above the unfermented and other samples. Fermented pumpkin seeds were observed to be significantly improved in its sensory attributes such as general acceptability, flavour and texture except in appearance where it suffered a mild rejection when fermented for 72 hrs but accepted better for 24 hrs fermentation. The predominant microorganisms found to be associated with the fermentation of pumpkin seeds are gram positive rods bacteria (*Bacillus* species, *Lactobacillus* species) the microbial population proliferates with increase in fermentation time from 24 hr to 72hrs. But the pace of increase was observed to decline as a result of the formation of certain metabolites from the microbial activities

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