Effect of dehulling and fermentation on availability of vitamins, anti-nutritional constituents and microbial load of Mesquite (Prosopis africana Guill. and Perr.) seed flours

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ABSTRACT

Dehulled and fermented flours were produced from mesquite (Prosopis africana Guill. and Perr.) seeds and each of the samples was subjected to chemical and microbiological analysis using standard analytical methods. The results of vitamin composition of the dehulled and fermented flour samples showed that beta carotene ranged from (64.83 to 91.40) iu/100 g. Thiamine (B1) ranged from (0.12 to 0.35) mg/100 g. The results of B vitamins, Riboflavin (B2) ranged from (0.11 to 0.32) mg/100 g and for Niacin (B3) the results ranged from (0.07 to 0.17) mg/100 g. The results for anti-nutrient composition of the flour samples showed total phenol in mg/100 g ranging from (1.57 to 3.66) mg/100 g; phytate ranged from (1.38 to 3.24) mg/100 g. For oxalate, the results ranged from (1.03 to 1.68) mg/100 g while tannin ranged from (0.64 to 2.21) mg/100 g. The results for total viable counts of the flour sample in cfu/g ranged from (3.5 × 10^5 to 2.5 × 10^2) cfu/g while for mould and yeasts counts ranged from (<10^1 to 2.4 × 10^4) cfu/g. All the flours produced were within safe limits as stipulated by International Standards. The results showed that flours from dehulled and fermented mesquite seeds can be incorporated to produce condiments of high nutritional and functional properties.

Keywords: Mesquite, Prosopis africana, fermented flours, seed flours, dehulling, microbiological analysis.
with plantain, cocoyam and cowpea depending on availability and season (Achi, 1999). Soups eaten with staples are an essential component of the diets derived from a variety of seeds, nuts, pulses and leaves (Campbell-Platt, 1980). The staple foods provide the calories but are poor in other nutrients and minerals. One of the ways to improve the diets has been to improve the nutrient content of the soups. Due to increased population and corresponding increased need for animal food, there is high demand on alternative sources that are close substitutes to high quality animal protein (Odunfa, 1986).

Fermentation markedly improves the digestibility, nutritive value and flavours of the raw seeds. Traditional method of production of Gbaaye made from mesquite seeds involve subjecting the seeds to 24 h boiling above 100°C followed by dehulling, fermentation, mashing and rolling into balls (Achi, 1992). The fermentation method is uncontrolled solid substrate fermentation which result to extensive hydrolysis of the protein and carbohydrate components (Fetuga et al., 1973; Eka, 1980). This processing method increases the shelf-life and reduction of the anti-nutrition factors (Odunfa, 1985a; Reddy and Pierson 1999; Barimalaa et al., 1989; Achi and Okereke, 1999).

There are different types of fermentations and examples include: alcoholic fermentation for the production of beer using yeast, lactic acid for yoghurt production using lactic acid bacteria, acetic acid for vinegar production using Acetic acid bacteria, propionic acid in blue-eyed cheese using propionic bacteria and alkaline fermentation for Gbaaye, dawadawa and other fermented condiments using Bacillus, Staphylococcus and Leuconostoc species. Moreover, Odunfa (1981) intimated that some microbes are known to hydrolyze proteins into amino acids and peptides releasing ammonia as a by-product in the process, this process is said to increase the protein content of fermented food products. Fermented foods contribute about one-third of the world’s diet (Campbell-Platt, 1994). This is because fermentation causes changes in food quality indices including texture, flavour, appearance and nutrition (Odunfa, 1985b; Reddy and Pierson, 1999). Fermentation markedly improves the digestibility, nutritive value and flavours of the raw seeds. Although, fermented food condiments have constituted a significant proportion of the diet of many people, Nigerians have exhibited an ambivalent attitude in terms of consumer tastes and preferences for such foods (Achi, 2005). The introduction of foreign high technology products especially processed ones due to globalization and liberalization of the economy has radically changed the Nigerian food culture into a mixed grill of both foreign and local dishes. Many developing countries are still preparing traditional fermented products for human consumption (Campbell-Platt, 1980). Fermented products remain of interest since they do not require refrigeration during distribution and storage. The traditional condiments have not attained commercial status due to the very short-shelf life, objectionable packaging materials, stickiness and characteristic putrid odour (Arogba et al., 1995).

The production of fermented vegetable proteins for use as food condiments is craft-based. In many areas of Nigeria, they are still made in traditional ways, with success depending upon observance of good manufacturing practices and control of environmental conditions during the manufacturing process. Starter cultures are not normally used and therefore there are variations in the quality and stability of the product (Odunfa, 1981). There is usually rise in pH due to hydrolysis of protein into amino acids and ammonia by predominant micro-organisms Bacillus species. As with any other fermentation process the understanding of the microbial ecology of vegetable fermentations requires the knowledge of the fermentation substrates, that is, the seeds of the various plants as well as, the products expected. During fermentation of condiments, amino acids is produced due to protein metabolism which are responsible for the gradual pH increase from 7.5 to 8.0 (Barimalaa et al., 1989; Achi, 1992; Barber and Achinewhu, 1992; Sarkar et al., 1997). Increase in pH into alkaline range may be physiologically important for tolerance and adaptation of fermenting micro-organisms in the environment.

Odunfa (1985b) reported low levels of lipase activity in P. biglobosa during dawadawa production. Low lipase activity in some fermented foods has been considered desirable as a result of problems of objectionable taste and development of rancidity (Odunfa, 1983, 1985a). Eka (1980) studied the effect of fermentation on the nutrient content of locust beans and reported that protein and fat increase whereas the quantity of carbohydrate decreased. He also reported increased levels of the amino-acids except for arginine, leucine and phenylalanine. Similar results were reported for other seed legumes (Odunfa, 1985b; Achinewhu, 1982; Sarkar et al., 1998).

Food condiments made from vegetable protein may be a good source of certain B vitamins but are deficient in ascorbate and some fat soluble vitamins which are lost during fermentation. Ajeigbe et al. (2012) reported decreases in the levels of phosphorus, calcium and magnesium but indicated that soaking and fermentation did not influence the concentration of sodium in Canavalia ensiformis seeds. Achinewhu (1986) also reported decrease in calcium, copper and phosphorus but increase in iron and zinc. Macro-nutrients in fermented legumes therefore, contribute to enhance food quality.

The use of Prosopis leaves and its nutritive value has been reported by Lyon et al. (1988). However, information on the nutritional quality of the flours produced from mesquite seeds (P. africana) is very scanty and such information will enhance increased utilization of this inexpensive plant protein to minimize the over dependence on African locust bean (P. biglobosa) and soya bean (Glycine max) seeds and their allied as condiment. The objectives of this study therefore, were to determine the effect of dehulling and
fermentation on the availability of vitamins, anti-nutritional constituents as well as, microbiological quality of mesquite seed flours.

MATERIALS AND METHODS

Procurement of African mesquite seeds

The African mesquite seeds (P. africana) used for this study was obtained from North Bank Market in Makurdi, Benue State. So also, the Gmelina leaf used for the fermentation process was plucked from the trees within the vicinity of Federal University of Agriculture, Makurdi. Makurdi is situated on Latitude 7.73 and Longitude 8.52 at elevation of 104 m above sea level (WorldAtlas, 2015).

Procurement of Gbaaye a local condiment

Gbaaye, a local condiment was purchase from Kaduna Central Market in Kaduna State (10°20′N 7°45′E) (Wikipedia, 2017) Nigeria. The purchased Gbaaye condiment was dried at room temperature (25 to 27°C) for 30 to 60 min and milled into flour using Model RPM 8 Laboratory Mill and packaged in a polythene wrapper and stored at ambient temperature at 25 to 27°C in a plastic jar until ready for use.

Preparations of African mesquite seeds for treatments

Preparation of dehulled and fermented African mesquite seed flour

100 g of cleaned and sorted African mesquite seeds were boiled and dehulled according to the modified method of Achi (2005). The boiling of the seeds was done in an aluminum pot for 12 h using firewood. Loss of water occurring through steaming was topped up occasionally to compensate for water lost through evaporation after which the seed coats were manually dehulled. The dehulled seeds were washed thoroughly with water, boiled for another 8 h and drained in a plastic sieve ready for fermentation. The dehulled cotyledon seeds were packed into a cleaned wooden basket padded with cleaned Gmelina leaves. Additional Gmelina leaves were used to cover the cotyledon seeds to enhance anaerobic condition. The wrapped cotyledon sees were then allowed to ferment at room temperature (25 to 27°C) for 72 h. The fermented dehulled cotyledon seeds were then removed from the leaves, mashed with pestle in a ceramic mortar and rolled into balls. The rolled ball condiments were dried using the hot air oven set at 45°C for 30 to 60 min and then cooled, milled into flour using Model RPM 8 Lab Mill and packaged in a polythene wrapper, stored in a closed plastic jar at ambient temperature of about (25 to 27°C).

Preparation of non-dehulled and fermented African mesquite seed flour

In this method, the same amount of cleaned and sorted African mesquite seeds were treated as earlier described, but the seeds were not dehulled but instead cooked over firewood for 72 h and treated with occasional adding of water as earlier described to compensate for loss of water during cooking. The process of obtaining the non-dehulled and fermented African mesquite seed flour followed the processes as earlier described.

Preparation of dehulled and non-fermented, and non-dehulled and non-fermented African mesquite seed flour

The same amount of African mesquite seeds was treated as earlier described but cooked for 12 h separately. 100 g of the seeds were dehulled and prepared as non-fermented while another 100 g was prepared as non-dehulled and non-fermented, respectively. The preparations were spread on a metal tray and dried using the hot air oven at 45°C for 30 to 60 min individually. The dried seeds were milled and stored individually as earlier described.

Preparation of uncooked, non-dehulled and non-fermented African mesquite seed flour

100 g of the African mesquite seeds were cleaned, sorted and milled with the same machine as earlier described. The product was sieved using a 2 mm diameter sieve in order to obtain its flour. The flour obtained was packaged in a polythene wrapper and stored in a plastic jar.

Chemical and microbiological analyses

Dehulled, non-dehulled, fermented and non-fermented African mesquite seed flours were analyzed for ß-carotene, thiamine, riboflavin and niacin and anti-nutritional contents (phenol, phylates, oxalates and tannin) determined using Isocratic High Performance Liquid Chromatography method as described by AOAC (1990). Microbiological analysis was determined by the method described by AOAC (1990).

Statistical analysis

The data obtained was analyzed in one way analysis of variance (ANOVA) using Genstat statistical package (2005). Confidence intervals were set at (p=0.05) using the
RESULTS

The beta carotene determination in the samples showed that samples F and A contained 91.40 and 87.97 iu/100 g, respectively and these values were not significantly different (P≥0.05) from each other but statistically and significantly different (P<0.05) from samples B (80.1 iu/100 g), E (71.27 iu/100 g), C (65.37 iu/100 g) and D (64.83 iu/100 g) which were all significantly different (P<0.05) from each other (Table 1). The Table 1 also showed that the presence of Thiamine was more pronounced in samples E and F followed by D, C, B and A with values of 0.35, 0.32, 0.23, 0.21 0.17 and 0.12 ml/100 g, respectively. Samples E and F were statistically the same but were significantly different from D and C which were significantly different from B and A.

On account of the amount of Riboflavin (B₂) content in the samples, sample F had the highest amount of 0.32 ml/100 g. This was found to be significantly different (P≤0.05) from the amount contained in samples B (0.16 ml/100 g) and E (0.15 ml/100 g) which were not statistically different (P=0.05) from each other but were found to be significantly different (P≤0.05) from the amount contained in samples A (0.13 ml/100 g), C (0.12ml/100 g) and D (0.11 ml/100 g). The amount of Niacin found in the samples as noticed in Table 1 showed that samples F and E with values of 0.17 and 0.13 ml/100 g, respectively were not statistically different (P=0.05) from each other but were significantly different from samples A and D with values of 0.11 ml/100 g, respectively, while samples B and C contained 0.8 and 0.7 ml/100 g.

Table 2 showed the effects of dehulling and fermentation on anti-nutritional composition of mesquite seed flour samples. The results indicated that sample E had the highest total phenol of 3.66 ml/100 g followed by sample D with 2.84 ml/100 g. Samples C (2.23 ml/100 g), B (1.67 ml/100 g), F (1.62 ml/100 g) and sample A showed the least value of 1.57 ml/100 g. All the values were significantly different (P≤0.05) from each other. The phytate content of the samples showed that samples E and D were not statistically different (P=0.05) from each other with values of 3.24 and 3.16 ml/100 g, respectively but were significantly different (P≤0.05) from the values of

### Table 1: Effect of dehulling and fermentation on beta carotene (iu/100 g) and b vitamins of Mesquite (Prosopis africana) seed flours (mg/100 g).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Beta carotene (iu/100 g)</th>
<th>Thiamine B₁</th>
<th>Riboflavin B₂</th>
<th>Niacin B₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>*A</td>
<td>87.97 ± 0.01ᵃ</td>
<td>0.12 ± 0.05ᶜ</td>
<td>0.13 ± 0.05ᶜ</td>
<td>0.11 ± 0.01ᵇ</td>
</tr>
<tr>
<td>B</td>
<td>80.1 ± 0.05ᵇ</td>
<td>0.17 ± 0.05ᶜ</td>
<td>0.16 ± 0.06ᵇ</td>
<td>0.08 ± 0.00ᶜ</td>
</tr>
<tr>
<td>C</td>
<td>65.37 ± 0.05ᶜ</td>
<td>0.21 ± 0.05ᵇ</td>
<td>0.12 ± 0.05ᶜ</td>
<td>0.07 ± 0.05ᶜ</td>
</tr>
<tr>
<td>D</td>
<td>64.83 ± 0.05ᶜ</td>
<td>0.23 ± 0.05ᵇ</td>
<td>0.11 ± 0.05ᶜ</td>
<td>0.11 ± 0.05ᵇ</td>
</tr>
<tr>
<td>E</td>
<td>71.27 ± 0.05ᵇ</td>
<td>0.35 ± 0.06ᵃ</td>
<td>0.15 ± 0.00ᵇ</td>
<td>0.13 ± 0.06ᵃ</td>
</tr>
<tr>
<td>F</td>
<td>91.40 ± 0.17ᵃ</td>
<td>0.32 ± 0.05ᵃ</td>
<td>0.32 ± 0.05ᵃ</td>
<td>0.17 ± 0.06ᵃ</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of triplicate determination. Means with the same superscript within the same column are not significantly different (P < 0.05) from each other. *A = Dehulled fermented flour; B = Non-dehulled fermented flour; C = Dehulled-non fermented flour; D = Non-dehulled-non-fermented flour; E = Non-dehulled non-fermented non-boiled flour; F = Commercial purchased flour condiment (control).

### Table 2: Effect of dehulling and fermentation on anti-nutritional content of Mesquite (Prosopis africana) seed flours (mg/100 g).

<table>
<thead>
<tr>
<th>Anti-nutrient composition (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>*A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>F</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of triplicate determination. Means with the same superscript within the same column are not significantly different (P > 0.05) from each other. *A = Dehulled fermented flour; B = Non-dehulled fermented flour; C = Dehulled-non fermented flour; D = Non-dehulled-non-fermented flour; E = Non-dehulled non-fermented non-boiled flour; F = Commercial purchased Flour Condiment (control).
Table 3: Effect of dehulling and fermentation on total viable count and yeasts and moulds counts of Mesquite (Prosopis africana) seed flours (cfu/g).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total viable count</th>
<th>Yeast and mold counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>*A</td>
<td>2.0×10^5c</td>
<td>&lt;10^4c</td>
</tr>
<tr>
<td>B</td>
<td>2.5×10^5a</td>
<td>2.2×10^4a</td>
</tr>
<tr>
<td>C</td>
<td>1.4×10^3c</td>
<td>2.4×10^4a</td>
</tr>
<tr>
<td>D</td>
<td>3.5×10^2d</td>
<td>2.4×10^4a</td>
</tr>
<tr>
<td>E</td>
<td>4.5×10^2d</td>
<td>5.0×10^2b</td>
</tr>
<tr>
<td>F</td>
<td>3.0×10^4b</td>
<td>&lt;10^2b</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations of triplicate determination. Means with the same superscript within the same column are not significantly different (P > 0.05) from each other. *A = Dehulled fermented flour; B = Non-dehulled fermented flour; C = Dehulled-non fermented flour; D = Non-dehulled-non-fermented flour; E = Non-dehulled non-fermented non boiled flour; F = Commercial purchased flour condiment (control).

Effect of dehulling and fermentation on beta carotene and B vitamins of mesquite seeds flour sample showed that all the values were very low when compared to the values obtained by Gernah and Sengen (2011) (52.32 iu/100 g). The very low values obtained may be attributed to the processing procedure for the manufacturing of these flours which involved excessive boiling, dehulling and milling etc all of which can reduce these essential vitamins. Vitamin A is a very important vitamin for the development of good eye sight, healthy skin, strong immunity and resistance to infection, strong bones, good growth and prevention of anaemia (Villamor and Fawzi, 2002). Deficiency of vitamin A can cause intestinal and respiratory infection, poor hair quality growth, eyeball pain, poor eye sight, night blindness and exothalmia (a dry, thickened, lusterless eye condition) which can damage the cornea and lead to blindness. The results of thiamine (B1), riboflavin (B2) and niacin(B3) obtained were all low; the low values could be attributed to leaching in boiling water, heating at very high temperatures for longer time and milling.

Vitamins are organic micro nutrient and are essential dietary constituents being required for growth, health and reproduction. In practice, evaluation of the vitamins in different food is much more difficult than that of most other food constituents. This can be attributed to the exceedingly small amounts in food and the diverse and complex nature of vitamins in general.

The effect of dehulling and fermentation on antinutritional composition shown in Table 2 showed that the results of total phenol, phytate, oxalate and tannin were very low when compared with the report of Udensi (2005) who reported a phytate content value of 4.58 ml/100 g in a study. The low value obtained could be as a result of leaching into water during boiling, dehulling and fermentation as these processes reduce anti-nutrients in food stuff. Total phenol inhibits the activity of digestive and hydrolytic enzymes such as beta-amylase, trypsin and lipases, thus, decreasing the availability of reducing sugars, vitamins and mineral (Onwuka, 2005). Phytate forms insoluble salts with metals such as calcium, iron, zinc and magnesium, thus, rendering them unavailable for absorption in the body. It also adversely affects protein and starch...
digestibility (Gernah and Sengev, 2011). Though, the smallest toxic dose of phytate in humans is not known, it appears that high doses are required for appreciable effect. Apart from causing irritation, oxalate forms insoluble complexes with some metals, thus, leading to reduction in calcium availability for metabolism. Gernah and Sengev (2011) however, reported that the risk of calcium deficiency diseases due to consumption of oxalate rich foods is very rare. This is because humans are able to efficiently utilize very low amounts of calcium in their food. The low values obtained in this study for oxalate may be as a result of boiling in water for about 12 h as boiling has reduction effect on all anti-nutritional factors with percentage level of reduction depending on the duration of boiling, dehulling, drying and milling. Toxicants can be reduced by boiling and dehulling. Health implications of anti-nutrients are well known. Reduction of the anti-nutrients during processing of some legumes such as African locust beans, soya beans, P. africana (mesquite seeds) etc, are therefore, of great importance for the safety of the product. Mesquite pods and seeds have been reported to be almost totally devoid of trypsin inhibitor activity.

Cyanoglycosides which occurs in some legumes have not been found in mesquite seeds (Ohenhen et al., 2008). These low values of anti-nutritional factors agreed with earlier observation of Bairigli et al. (2002). Fermentation, boiling and dehulling reduces anti-nutritional factors and enhances the nutritive value of legumes thereby breaking down the nutrient for easy absorption.

Effect of dehulling and fermentation on mesquite seeds flour on total viable count, moulds and yeasts counts indicated that there was reduction in the microbial composition of the flour samples. Earlier reports showed that processing operations like boiling and dehulling of the legumes meant to be fermented into condiment may drastically reduce the quantity of micro-organisms present in them (Tope, 2013). Achi (2005) attributed the composition of microbes in traditionally fermented condiments to the composition of the substrate and the hygiene of the environment during the preparation of the product. All the results obtained for the total plate, yeast and mould counts fall within the safe limits by the International Commission on Microbiology Specification of Food (ICMSF, 1978) who specified a limit of 10^6 cfu/g for aerobic, mould and yeasts counts of food.

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