

## Full Length Research Paper

# Comparative evaluation of anti-oxidative potentials of fermented locust bean condiment and its moringa fortified variant

Gibson L. Arueya<sup>1\*</sup>, Maimuna Sani<sup>2</sup> and Akeem Olayemi Raji<sup>2</sup>

<sup>1</sup>Department of Food Technology, Faculty of Technology, University of Ibadan, Ibadan, Oyo State, Nigeria.

<sup>2</sup>Department of Food Agriculture and Biological Engineering, College of Engineering and Technology, Kwara State University, Malete, Ilorin, Kwara State, Nigeria.

Received 21 June, 2017; Accepted 28 September, 2017

The need to boost daily intake of natural antioxidants to combat free radicals causing degenerative diseases has become more imperative. Fermented locust bean condiment was fortified with moringa leaf powder (rich in antioxidants) at 20, 30 and 50% substitution levels. Proximate composition, phenolic content, total flavonoid, reducing power, and the sensory properties of the condiments were determined using standard methods. Data obtained were analyzed using analysis of variance (ANOVA) at  $p \leq 0.05$  and compared with catechin as standard. Possible correlations between trends in the proximate components and antioxidative properties were examined. Proximate components of the fortified condiments: Moisture (6.92 to 7.17%), crude protein (39.23 to 42.91%), crude fibre (5.21 to 5.63%), total ash (4.24 to 6.27%), and carbohydrate (12.21 to 16.31%) compared favorably in most parameters with those of the unfortified. The phenolic content, total flavonoid, and reducing power of the fortified condiments (with rising substitution level ( $p \leq 0.05$ )) increased significantly ranging from 13.5 to 20.20, 5.35 to 18.3, and 10.6 to 22.1  $\mu\text{g/g}$ , respectively at concentration of 1000  $\mu\text{g/g}$ . Apparently, crude fibre and ash contents correlated positively with reducing power potential across several concentration gradients. Mean sensory scores [taste (5.00 to 5.29) and mouth feel (4.86 to 5.43)] for the fortified variant was not significantly different from those of the control. Condiment fortified with 20% moringa leaf exhibited the highest antioxidant capacity among the samples and may be a sustainable means of meeting the recommended daily requirement of antioxidants among the populace.

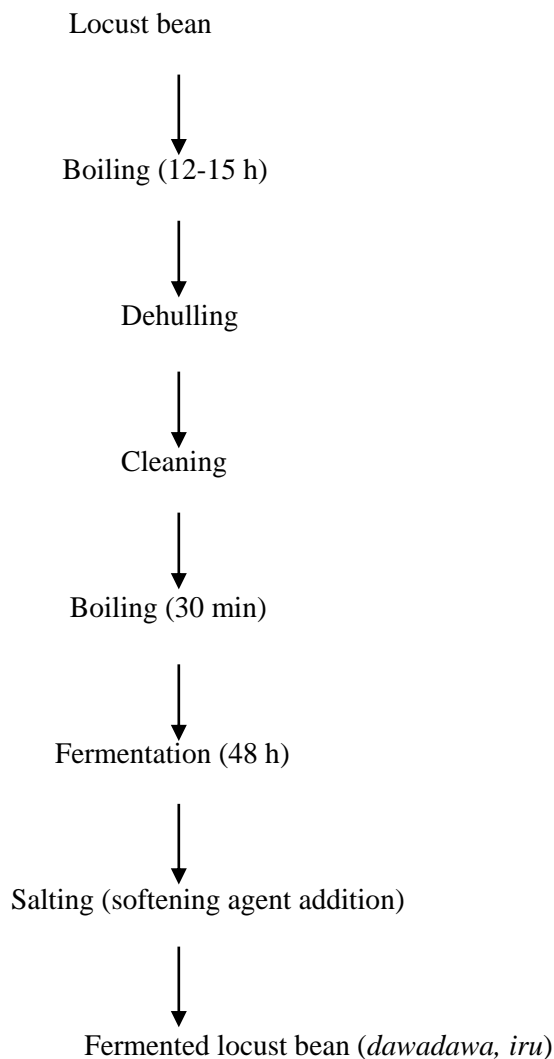
**Key words:** Antioxidants, condiment, locust beans, *Moringa oleifera* leaf, fortification.

## INTRODUCTION

Many humans are exposed to free radicals virtually every day. This emanates mostly from ultraviolet (UV) light, cigarette smoke, ionizing radiation, certain organic

solvents, pollutants and industrial waste (Boonchum et al., 2011). Free radicals have generally been recognized as harmful to health, negatively impacting several cellular

\*Corresponding author. E-mail: arueyagibson@yahoo.com.



**Figure 1.** Production of fermented African locust bean (*dawadawa, iru*) (Aworh, 2008).

metabolic pathways. Reactive oxygen and nitrogen species, such as hydroxyl radicals and hydrogen peroxides, make up some of these free radicals (Pezzuto and Park, 2002). Their presence in body fluids may trigger degenerative diseases such as coronary heart disease, cancer and Alzheimer's disease (Pezzuto and Park, 2002). However, consumption of foods dense in antioxidants is being increasingly known as an effective neutralizer of these free radicals, thus fortifying the body against such harmful tendencies (Moon and Shibamoto, 2009). Presently, aside from those retained naturally in foods, a number of antioxidants are now being synthesized to meet commercial demands, but have been linked sadly to health complications (Chen et al., 1992). Strict governmental regulations and enforcement have not stemmed this 'tide', thus raising safety concerns among consumers. The search for alternatives has become necessary (Yingming et al., 2004).

Phytochemicals such as phenols and flavonoids have for long been known to protect body cells by mopping up reactive oxygen species, scavenging free radicals, and chelating metal catalysts, thereby preventing diseases linked to oxidative stress (Oboh and Akindahunsi, 2004; Alia et al., 2003; Zhang et al., 2001).

A regimen of robust antioxidant supplementation has been advocated as an important strategy in improving protection against free radicals (Percival, 1998). *Moringa oleifera* plant had since been identified as a natural source of antioxidants (Iqbal and Bhangar, 2006; Siddhuraju and Becker, 2003), but largely underexploited. The leaves are rich in essential pro-vitamins including ascorbic acids, carotenoids (Lako et al., 2007) and tocopherols (Sanchez-Machado et al., 2006; Gomez-Conrado et al., 2004). The use of condiments as a 'carrier' or 'vehicle' to enhance the intake of these antioxidants inherent in moringa leaves evidently appears intriguing but unexplored. For instance, a traditional fermented condiment (Dawadawa, Iru, Ogiri and Ugba) are consumed by different ethnic groups in the West African sub-region, and has long been the pride of culinary tradition for centuries. In Nigeria, Togo and Ghana, the estimated average consumption of the locust bean condiment are 10, 4 and 2 g/body weight/day, respectively (Simonyan, 2012). They are generously added to soups sometimes as low-cost meat substitute by low-income families in some parts of Nigeria (Eka, 1980; Odunfa, 1981). Fortification of such a food condiment with moringa leaves in appropriate proportion might enhance the intake of these natural antioxidants. The aim of this work therefore, is to evaluate comparatively the anti-oxidative and sensory properties of fermented locust beans in relation to its moringa leaf fortified variant.

## MATERIALS AND MEHODS

### Source

African locust bean seeds (*Parkia biglobasa*) were purchased from Oja Oba market in Ilorin, Kwara State, while moringa leaves (*M. oleifera*) was purchased from the Botanical gardens, University of Ibadan, Nigeria. All chemical reagents used were of analytical grade (Sigma-Aldrich, United States).

### Preparation of samples

#### Fermented locust beans

Production of fermented locust beans was carried out according to the method described by Aworh (2008). African locust bean seeds were weighed, sorted and winnowed to remove extraneous matter. The cleaned seeds were boiled in water for 12 h until soft and were next dehulled by gentle pounding in a mortar creating abrasion for proper removal of the hull. A softening agent called 'kuru' containing sunflower seed and 'kaun' (sodium sesqui-carbonate) were added before the second boiling to enhance further softening of the cotyledons. The de-hulled seeds were thereafter boiled for 30 min, molded into small balls, wrapped in paw-paw leaves, placed in covered calabash and allowed to ferment for 2 days (Figure 1).

### **Dehydration of fermented locust bean**

Oven drying of fermented locust bean samples were carried out according to the method described by Ijarotimi and Keshinro (2012). Fermented locust beans were dried in hot air oven dryer (Carbolite PF 200, England) at 60°C for 20 h. The dried fermented locust beans were ground into fine powder and packaged in polyethylene bags for subsequent processing.

### **Drying of moringa leaves**

The fresh leaves were cleaned dust free using distilled water and then dried in hot air dryer (Carbolite PF 200, England) at a temperature of 53°C for 6 h. These were milled using Marlex blender (Model no: 7962804) to fine particle size of about 125 to 212 µm.

### **Formulation of the fortified locust bean condiment**

The fermented locust bean powder and the moringa leaf powder were blended in the ratio of 50:50, 70:30, and 80:20, respectively. The aforementioned mixing ratios were adopted in order to achieve the recommended daily requirement for phenols (>500 mg/day) and flavonoids (150 to 300 mg/day) (Williamson and Holst, 2008) and to increase intake of antioxidant to 1/10 of the daily requirement, since the total phenol in fermented locust bean is 1.02 mg/g (Obboh et al., 2008). The formulations were weighed in required quantities of 20 g and thoroughly blended in a mini planetary mixer (N-50, Hobart, Sweden) for 2 min.

### **Proximate composition of the samples**

The proximate compositions (done in three replicates) of the samples were determined using the method of AOAC (2000).

### **Determination of antioxidant activities**

#### **Preparation of the samples extract**

The sample extracts were prepared following the method of Kumar et al. (2006). Ten grams of each formulated condiment were poured into 100 ml of boiling distilled water and vortexed for 10 min. The samples were cooled and centrifuged at 3000 rpm for 15 min at room temperature (27±3°C) and the supernatants (aqueous extracts) of the condiments were stored in the refrigerator at 4°C for further analysis.

#### **Total phenol content**

The total phenol contents of the samples were carried out according to the modified method described by Singleton and Rossi (1965). Folin ciocalteau reagent (1 ml) was added into a test tube containing 1 ml of sample extract of appropriate dilutions (10 to 1000 µg), some 3 min thereafter, the reaction was neutralized by adding 1 ml of saturated solution of 15% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). The overall mix was made up to 5 ml using 2 ml distilled water and then incubated for 20 min at 40°C. The absorbance was read at a wavelength of 760 nm in UV-visible spectrophotometer (Spectrum lab 752s). This procedure was repeated for catechin as standard and results were in triplicate.

#### **Determination of total flavonoid content**

The total flavonoid content of the samples was determined by the

Aluminium chloride colorimetric method according to Jia et al. (1999). Aliquot amounts (10 to 1000 µg) of the extract in 1 ml of distilled water were added to 75 µl of 5% sodium nitrite. Some 5 min later, 150 µl of 10% Aluminium chloride was added, followed by 500 µl of 1 M sodium hydroxide (NaOH) and 275 µl of distilled water after 6 min. The absorbance of the mixture was read at 510 nm and catechin was used as standard.

### **Reducing power assay**

The reducing powers of the condiments were determined according to the modified method of Yen and Chen (1995). Different concentrations of the condiments extracts (10 to 1000 µg) in 1 ml distilled were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (K<sub>3</sub>Fe(CN)<sub>6</sub>; 2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10% Concentration) was added to the mixture to terminate the reaction and thereafter centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with equal portion of distilled water and aliquot (0.5 ml) of FeCl<sub>3</sub> (0.1%). The resulting reaction mixture was next allowed to stand for 10 min at room temperature. The absorbance was measured at 700 nm and compared with the catechin standard.

### **DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity**

The DPPH free radical scavenging activity assay was performed using the modified method of Brand-Williams et al. (1995). Various concentrations of the extracts (40 to 2000 µg/ml) in 4 ml of distilled water were added to the methanol solution of DPPH (1 mM). One millilitre (1 ml) of the resulting mix was shaken and allowed to stand for 30 min at room temperature. Catechin standards were also prepared at similar concentrations along with the blank using methanol and DPPH. They were next incubated in a water bath at 37°C for 30 min after which absorbance of samples and standards were read against the blank at 517 nm. The percent inhibition was calculated as:

$$\% \text{ Inhibition} = [(C - S)/C] \times 100$$

Where S is the sample absorbance and C is the absorbance of the blank. The 50% inhibitory concentration (IC<sub>50</sub>) was expressed as the quantity of the extract necessary to react with one half of DPPH radicals.

### **Sensory evaluation**

Sensory evaluation of soups prepared with the condiments was performed by trained twenty panelists, consisting of postgraduate students and technologists of Department of Food Technology, University of Ibadan. A 7-point hedonic scale (1-dislike extremely, 7-like extremely) was used in rating the sensory attributes (colour, taste, aroma, appearance and overall acceptability) of the soups (Iwe, 2002). Each panelist ranked 5 coded samples, which were presented randomly along with clean water for mouth rinsing in-between evaluations (5 min interval) (Iwe, 2002).

### **Statistical analysis**

Means of readings obtained were subjected to analysis of variance (ANOVA) and separated using Duncan's multiple range test. These statistical analyses were amply conducted with SPSS version 14.0, Chicago.

**Table 1.** Proximate composition of the condiments.

Sample	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Total ash (%)	CHO (%)
100% Locust beans	6.75±0.08 <sup>b</sup>	44.91±0.89 <sup>a</sup>	36.21±0.17 <sup>a</sup>	4.66±0.08 <sup>e</sup>	2.76±0.12 <sup>e</sup>	4.68±1.14 <sup>d</sup>
100% moringa leaves	7.50±0.48 <sup>a</sup>	31.09±0.89 <sup>e</sup>	7.65±0.08 <sup>e</sup>	6.21±0.18 <sup>a</sup>	9.51±0.03 <sup>a</sup>	38.04±0.37 <sup>a</sup>
50% Locust beans + 50% moringa leaves	7.17±0.13 <sup>ab</sup>	39.23±0.02 <sup>d</sup>	21.78±0.07 <sup>d</sup>	5.63±0.12 <sup>b</sup>	6.27±0.01 <sup>b</sup>	16.31±2.61 <sup>b</sup>
70% Locust beans + 30% moringa leaves	7.01±0.01 <sup>b</sup>	41.38±0.01 <sup>c</sup>	26.04±0.06 <sup>c</sup>	5.48±0.02 <sup>c</sup>	4.80±0.01 <sup>c</sup>	13.33±0.03 <sup>c</sup>
80% Locust beans + 20% moringa leaves	6.92±0.02 <sup>b</sup>	42.41±0.01 <sup>b</sup>	27.01±0.01 <sup>b</sup>	5.21±0.02 <sup>e</sup>	4.24±0.07 <sup>d</sup>	12.21±0.06 <sup>c</sup>

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ( $p < 0.05$ ). CHO denotes carbohydrate content.

## RESULTS AND DISCUSSION

### Proximate composition of the condiments

The results of the findings are shown in Table 1. Increasing fortification with moringa leaves appears to increase the moisture content of the condiments. This parameter for the condiments (6.75 to 7.17%) did not quite follow any specific trend with the reducing power potential (2.22 to 22.1  $\mu\text{g/g}$ ) and DPPH radical scavenging capacity (16.96 to 83.20%). A similar conclusion was reached when possible relationship between moisture content and antioxidative properties in Thai plants was investigated (Maisuthisakuls et al., 2008).

Inclusion of moringa leaves in the fermented locust bean (at 20 to 50% substitution levels) evidently reduced the overall protein content by an average of 9%. There was no positive correlation between protein content per se and the antioxidative properties in this study. However, numerous investigations have indicated that when plant/animal proteins are hydrolyzed, they can act as direct scavengers of diverse free radicals or behave as antioxidants in model systems (Ajibola et al., 2011).

The fat content of the moringa fortified variants (21.78 to 27.01%) was relatively lower than the unfortified ones (36.21%). This parameter like its protein counterpart did not follow a definite order in relation to antioxidant activity. Also, it contrasts, sharply with foods such as full fat milk associated with fat soluble compounds ( $\beta$ -carotene and fat soluble vitamins) considered to have antioxidant activity (Full-fat milk generally exhibits higher antioxidant activity than skimmed milk) (Rashidinejad et al., 2016).

Fortified condiment with the highest inclusion (50%) of moringa leaf had the highest crude fibre content (5.63%). In most of the samples evaluated, this parameter positively correlated with reducing power potential and DPPH free radical scavenging capacity across several concentration gradients (100 to 1000  $\mu\text{g/g}$ ). This may be related to the reported free radical scavenging activity due to polyphenols associated with dietary fibre (Ubando-Rivera et al., 2005).

Ash content of the fortified samples (5.21 to 5.63%) generally rose as the level of addition of moringa leaf increased. This translated to higher reducing power potential and DPPH free radical scavenging capacity at most concentration gradient. However, the aforementioned observation is at variance with those of Maisuthisakul et al. (2008) who reported negative correlation between ash content and antioxidant activity among 28 Thai plants studied. A number of minerals depending on the prevailing micro-environment have been recognized to enhance anti-oxidant activities (Khairy and El-Shiekh, 2015). This may well explain the differences.

The fortified condiment carbohydrate content (12.21 to 16.31%) increased with level of inclusion of moringa leaf characterized as high in carbohydrate (38.04%). This though did not positively correlate with the antioxidative properties for most of the variants studied. The reason for this is unclear but Biliaderis and Izydorczyk (2006) reported that some carbohydrate types do contribute to antioxidant activities.

### Antioxidant activity of the condiments

#### Total phenol

Phenolic concentration of test samples and catechin (reference standard) varied with the concentration gradients [0  $\mu\text{g/g}$  (blank) to 1000  $\mu\text{g/g}$ ] rising from 0.01  $\mu\text{g/g}$  to a peak of 20.29  $\mu\text{g/g}$  (Table 2). The total phenol of 100% fermented locust bean and moringa leaf powder at 1000  $\mu\text{g/g}$  concentrations were 13.46 and 19.41  $\mu\text{g/g}$ , respectively. Evidently at higher gradient concentration (500-1000  $\mu\text{g/g}$ ) phenolic content of the moringa leaf powder is some 20 to 45% higher than those of fermented locust bean. This makes the former a good complementary source of phenolics (Leone et al., 2015), boosting the overall phenolic content of the condiments. Interestingly, the fortified condiment phenolic contents (19.67 to 20.21  $\mu\text{g/g}$ ) peaked in the 20% moringa fortified variant at which point it was not statistically different from standard ( $p \leq 0.05$ ) (Table 2). Further increases in substitution did not significantly influence the phenolic

**Table 2.** Phenolic content of the condiments ( $\mu\text{g/g}$ ).

Sample	Concentration ( $\mu\text{g/g}$ )							
	0	10.0	50.0	100.0	250.0	500.0	750.0	1000.0
Catechin	0.1 $\pm$ 0.02 <sup>b</sup>	1.55 $\pm$ 0.4 <sup>c</sup>	6.02 $\pm$ 0.2 <sup>b</sup>	10.84 $\pm$ 0.2 <sup>c</sup>	16.49 $\pm$ 0.1 <sup>c</sup>	17.08 $\pm$ 0.1 <sup>b</sup>	20.02 $\pm$ 0.3 <sup>a</sup>	20.29 $\pm$ 0.2 <sup>a</sup>
100% Locust bean	0.1 $\pm$ 0.02 <sup>b</sup>	2.07 $\pm$ 0.2 <sup>b</sup>	5.14 $\pm$ 0.1 <sup>c</sup>	7.88 $\pm$ 0.8 <sup>e</sup>	10.93 $\pm$ 0.1 <sup>e</sup>	11.00 $\pm$ 1.6 <sup>d</sup>	11.69 $\pm$ 1.3 <sup>c</sup>	13.46 $\pm$ 0.5 <sup>c</sup>
100% moringa leaves	0.1 $\pm$ 0.02 <sup>b</sup>	2.43 $\pm$ 0.3 <sup>ab</sup>	0.82 $\pm$ 0.1 <sup>d</sup>	9.30 $\pm$ 0.6 <sup>d</sup>	12.04 $\pm$ 0.3 <sup>d</sup>	13.49 $\pm$ 0.30 <sup>c</sup>	15.61 $\pm$ 1.5 <sup>b</sup>	19.41 $\pm$ 0.7 <sup>b</sup>
50% Locust beans + 50% moringa leaves	0.5 $\pm$ 0.01 <sup>a</sup>	2.40 $\pm$ 0.2 <sup>ab</sup>	7.57 $\pm$ 0.1 <sup>a</sup>	11.54 $\pm$ 0.1 <sup>b</sup>	18.11 $\pm$ 0.0 <sup>b</sup>	19.06 $\pm$ 0.3 <sup>a</sup>	19.43 $\pm$ 0.1 <sup>a</sup>	20.02 $\pm$ 0.4 <sup>ab</sup>
70% Locust beans + 30% moringa leaves	0.52 $\pm$ 0.01 <sup>a</sup>	2.28 $\pm$ 0.1 <sup>ab</sup>	7.44 $\pm$ 0.0 <sup>a</sup>	12.77 $\pm$ 0.1 <sup>a</sup>	17.85 $\pm$ 0.0 <sup>b</sup>	18.61 $\pm$ 0.0 <sup>a</sup>	19.61 $\pm$ 0.1 <sup>a</sup>	19.78 $\pm$ 0.1 <sup>ab</sup>
80% Locust beans + 20% moringa leaves	0.51 $\pm$ 0.03 <sup>a</sup>	2.62 $\pm$ 0.1 <sup>a</sup>	7.70 $\pm$ 0.1 <sup>a</sup>	12.80 $\pm$ 0.5 <sup>a</sup>	19.00 $\pm$ 0.2 <sup>a</sup>	19.67 $\pm$ 0.0 <sup>a</sup>	19.88 $\pm$ 0.0 <sup>a</sup>	20.21 $\pm$ 0.1 <sup>a</sup>

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ( $p < 0.05$ ).

**Table 3.** Total flavonoids content of the condiment ( $\mu\text{g/g}$ ).

Sample	Concentration ( $\mu\text{g/g}$ )							
	0	10.0	50.0	100.0	250.0	500.0	750.0	1000.0
Catechin	0.02 $\pm$ 0.0 <sup>a</sup>	0.80 $\pm$ 0.0 <sup>a</sup>	3.45 $\pm$ 0.02 <sup>a</sup>	9.98 $\pm$ 0.01 <sup>a</sup>	17.63 $\pm$ 0.0 <sup>a</sup>	19.3.0 $\pm$ 0.0 <sup>a</sup>	19.54 $\pm$ 0.1 <sup>a</sup>	19.65 $\pm$ 0.1 <sup>a</sup>
100% locust bean	0.02 $\pm$ 0.0 <sup>a</sup>	0.12 $\pm$ 0.1 <sup>c</sup>	0.17 $\pm$ 0.01 <sup>d</sup>	0.52 $\pm$ 0.01 <sup>d</sup>	1.15 $\pm$ 0.8 <sup>d</sup>	1.79 $\pm$ 1.4 <sup>d</sup>	3.86 $\pm$ 0.1 <sup>d</sup>	5.35 $\pm$ 0.2 <sup>d</sup>
100% moringa leaves	0.02 $\pm$ 0.0 <sup>a</sup>	0.35 $\pm$ 0.0 <sup>b</sup>	1.66 $\pm$ 0.2 <sup>b</sup>	2.65 $\pm$ 0.3 <sup>b</sup>	9.54 $\pm$ 0.3 <sup>b</sup>	12.12 $\pm$ 0.7 <sup>b</sup>	15.88 $\pm$ 3.1 <sup>b</sup>	18.27 $\pm$ 0.4 <sup>b</sup>
50% locust beans + 50% moringa leaves	0.02 $\pm$ 0.0 <sup>a</sup>	0.07 $\pm$ 0.0 <sup>cd</sup>	0.19 $\pm$ 0.0 <sup>d</sup>	0.31 $\pm$ 0.4 <sup>d</sup>	1.18 $\pm$ 0.0 <sup>d</sup>	2.28 $\pm$ 0.0 <sup>d</sup>	3.21 $\pm$ 0.1 <sup>d</sup>	3.76 $\pm$ 0.2 <sup>e</sup>
70% locust beans + 30% moringa leaves	0.01 $\pm$ 0.0 <sup>a</sup>	0.11 $\pm$ 0.0 <sup>c</sup>	0.32 $\pm$ 0.0 <sup>cd</sup>	0.51 $\pm$ 0.0 <sup>d</sup>	1.21 $\pm$ 0.0 <sup>d</sup>	2.32 $\pm$ 0.1 <sup>d</sup>	3.19 $\pm$ 0.0 <sup>d</sup>	3.73 $\pm$ 0.2 <sup>e</sup>
80% locust beans + 20% moringa leaves	0.01 $\pm$ 0.0 <sup>a</sup>	0.01 $\pm$ 0.0 <sup>d</sup>	0.43 $\pm$ 0.1 <sup>c</sup>	1.00 $\pm$ 0.1 <sup>c</sup>	2.78 $\pm$ 0.1 <sup>c</sup>	5.10 $\pm$ 0.1 <sup>c</sup>	6.43 $\pm$ 0.4 <sup>c</sup>	7.63 $\pm$ 0.5 <sup>c</sup>

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ( $p < 0.05$ ).

content, a chemical property usually associated with mopping up of free radicals through hydrogen or electron-donating as well as metal ion chelating agents (Rice-Evans and Bourdon, 1993).

### Total flavonoid content

The superiority of the flavonoid content of the moringa leaf powder over those of fermented locust bean is clearly obvious throughout the entire spectrum of concentration gradients

(10-1000  $\mu\text{g/g}$ ) (Table 3). For instance at 1000  $\mu\text{g/g}$ , the flavonoid content of 100% *M. oleifera* leaf powder (18.27  $\mu\text{g/g}$ ) is three times that of 100% fermented locust bean powder (5.35  $\mu\text{g/g}$ ). Paradoxically (with the exception of 10  $\mu\text{g/g}$  concentration gradient) Increased inclusion of the moringa leaf in the condiment (at 20 to 50%) led to about 50% overall decrease in the flavonoid content of the condiment. This may be as a result of enhanced covalent interaction between the flavonoids and the proximate components (proteins, carbohydrates and fats) directly

associated with physico-chemical properties (Gonzales et al., 2015). Although generally known for their antioxidative property (Arueya et al., 2015), the flavonoids in this study represent a fairly low fraction (2-38%) of the total phenolic content of the condiments depending on the concentration gradient.

### Reducing power

Like the preceding parameter the reducing power

**Table 4.** Reducing power potential of condiments.

Sample	Concentration ( $\mu\text{g/g}$ )							
	0	10.0	50.0	100.0	250.0	500.0	750.0	1000.0
Catechin	0.4±0.0 <sup>a</sup>	0.4±0.2 <sup>e</sup>	2.3±3.2 <sup>d</sup>	5.7±0.1 <sup>c</sup>	19.5±1.1 <sup>b</sup>	23.1±0.3 <sup>a</sup>	23.2±0.6 <sup>a</sup>	23.5±2.7 <sup>a</sup>
100% Locust bean	0.2±0.1 <sup>b</sup>	2.2±0.1 <sup>d</sup>	3.3±0.1 <sup>c</sup>	4.6±0.1 <sup>d</sup>	5.8±2.1 <sup>e</sup>	8.9±1.2 <sup>c</sup>	9.9±0.0 <sup>c</sup>	10.6±0.5 <sup>c</sup>
100% moringa leaves	0.2±0.1 <sup>b</sup>	6.6±0.9 <sup>a</sup>	11.3±0.8 <sup>a</sup>	14.5±0.8 <sup>a</sup>	21.1±5.8 <sup>a</sup>	22.0±0.1 <sup>b</sup>	22.0±0.0 <sup>b</sup>	22.1±0.0 <sup>ab</sup>
50% Locust beans + 50% moringa leaves	0.2±0.1 <sup>b</sup>	4.2±0.4 <sup>bc</sup>	7.8±0.6 <sup>b</sup>	10.6±0.0 <sup>b</sup>	15.5±0.2 <sup>c</sup>	21.7±0.2 <sup>b</sup>	21.0±1.8 <sup>b</sup>	22.1±0.1 <sup>ab</sup>
70% Locust beans + 30% moringa leaves	0.2±0.1 <sup>b</sup>	4.6±0.5 <sup>b</sup>	7.6±0.6 <sup>b</sup>	10.0±0.4 <sup>b</sup>	16.9±1.1 <sup>c</sup>	21.7±0.3 <sup>b</sup>	22.1±0.1 <sup>b</sup>	21.9±0.3 <sup>ab</sup>
80% Locust beans + 20% moringa leaves	0.2±0.1 <sup>a</sup>	3.4±0.2 <sup>c</sup>	5.7±0.2 <sup>bc</sup>	8.0±0.6 <sup>c</sup>	16.4±0.7 <sup>cd</sup>	21.7±0.1 <sup>b</sup>	22.0±0.0 <sup>b</sup>	2.7±0.7 <sup>ab</sup>

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ( $p < 0.05$ ).

**Table 5.** DPPH radical scavenging activity (%) of the condiments.

Sample	Concentration ( $\mu\text{g/ml}$ )						
	40.0	100.0	250.0	500.0	750.0	1000.0	2000.0
Catechin	36.92±7.9	42.20±4.40	40.84±1.50	47.58±1.54	51.11±6.64	59.86±1.08	63.29±1.54
100% Locust bean	23.74±2.05	36.21±1.88	43.45±3.20	70.62±0.86	77.68±1.00	61.16±10.17	51.99±1.40
100% moringa leaves	29.38±2.17	36.04±1.98	79.79±1.86	83.20±0.60	77.20±1.69	65.80±1.88	20.20±4.22
50% Locust beans + 50% moringa leaves	75.59±0.69	57.16±1.40	40.10±1.65	43.93±1.99	39.43±2.10	39.53±2.80	23.63±6.70
70% Locust beans + 30% moringa leaves	26.58±2.05	26.93±2.25	33.84±1.59	79.20±1.08	65.26±4.03	65.90±1.06	46.22±1.88
80% Locust beans + 20% moringa leaves	70.19±1.03	24.66±2.21	82.36±0.69	34.00±1.10	36.95±1.72	38.80±1.82	16.96±2.79

Values are mean ± standard deviation of three replicate of percentage inhibition (%).

potential is concentration dependent with all the condiments exhibiting high reducing power comparing favorably with the catechin reference standard (Table 4) The fortified condiments (50, 30 and 20% substitution levels) had reducing powers of 22.06, 21.85 and 20.71  $\mu\text{g/g}$  respectively at 1000  $\mu\text{g/g}$  concentration gradient. These values are significantly higher than those obtained for 100% fermented locust bean (10.6  $\mu\text{g/g}$ ). Reducing power of the extracts is a reflection of their ability to donate electrons to facilitate reduction (ferric ions  $\text{Fe}^{3+}$  to ferrous ions

$\text{Fe}^{2+}$ ). While not being statistically significant ( $p < 0.05$ ), the 30% moringa fortified variant showed higher reducing power potential than those of the other two in these concentrations (10, 250, 750  $\mu\text{g/g}$ ). This may be related to possible marginal differences in concentrations of reducing substances from fermentation such as free amino acids, peptides, and oxidized lipid – amino acid reaction products reputed for antioxidative activity (Daramola, 2014). Indeed, reducing power remains a significant indicator of potential antioxidant activity (Meir et al., 1995).

#### **DPPH scavenging activity**

At concentration gradients (40-2000  $\mu\text{g/ml}$ ) evaluated, the DPPH scavenging activities (% inhibition) for the condiments varied across all substitution levels [50%(75.59-23.63), 30%(79.20-26.58), 20%(82.36-16.96)]. Majority of the samples exhibited the highest percentage inhibition at a concentration of 500  $\mu\text{g/ml}$  (Table 5). The 100% locust bean (70.6% inhibition), 100% moringa leaf (83.20% inhibition), condiments fortified with moringa leaf at 50%

**Table 6.** Sensory evaluation of the condiments.

Condiment	Colour	Aroma	Taste	Mouth feel	Appearance	Overall acceptability
100% locust bean	6.29 <sup>a</sup>	5.00 <sup>a</sup>	5.43 <sup>a</sup>	5.43 <sup>a</sup>	6.57 <sup>a</sup>	6.43 <sup>a</sup>
100% moringa leaves	3.29 <sup>d</sup>	4.29 <sup>b</sup>	4.86 <sup>a</sup>	4.71 <sup>a</sup>	4.0 <sup>d</sup>	4.86 <sup>b</sup>
50% locust beans + 50% moringa leaves	4.57 <sup>c</sup>	4.86 <sup>a</sup>	5.14 <sup>a</sup>	5.29 <sup>a</sup>	4.86 <sup>bc</sup>	5.14 <sup>b</sup>
70% locust beans + 30% moringa leaves	4.86 <sup>bc</sup>	5.14 <sup>a</sup>	5.00 <sup>a</sup>	4.86 <sup>a</sup>	5.43 <sup>ab</sup>	5.43 <sup>b</sup>
80% locust beans + 20% moringa leaves	5.86 <sup>ab</sup>	5.29 <sup>a</sup>	5.29 <sup>a</sup>	5.43 <sup>a</sup>	5.43 <sup>ab</sup>	5.71 <sup>ab</sup>

Values are means of three replicates. Mean values having different superscripts within a column are significantly different ( $p < 0.05$ ).

(43.9% inhibition), 30% (79.2% inhibition) and 20% (34% inhibition), respectively all showed strong scavenging potentials in that order. The pattern of predominantly high values for the 30% moringa fortified condiment (sometimes greater than the reference) may be due to synergistic effects exerted by the phenolic content (Songsungkan and Chantai, 2014). The rather reduced effect at 20 and 50% substitution levels could be that these were outside optimum combination levels for best of the phenolic interactions therein (Palafox-Carlos et al., 2012). Undoubtedly, DPPH effectively neutralizes compounds that have proton donating ability and so the condiment could serve as free radical inhibitor or scavenger acting possibly as primary antioxidant.

### Sensory evaluation

The results of sensory evaluation of the condiments are shown in Table 6. The mean sensory scores of the fortified condiments for attributes such as taste, aroma and mouth feel were not significantly different from those of 100% locust bean ( $P \leq 0.053$ ). In terms of overall acceptability, the latter was most preferred (6.47), followed by the 20% (5.71), 30% (5.43) and 50% (5.14) in that order. Traditional taste, habits and cultural preferences may have accounted for this trend.

### Conclusion

The antioxidant activity of condiments fortified with *M. oleifera* leaf increased significantly and could be of possible health benefit. It is apparent from the results that the addition of moringa leaf powder at all levels did not significantly alter the organoleptic properties of the condiments. Evidently, condiment fortified with 20% moringa leaf is the most promising in terms of anti-oxidative potential and sensory attributes.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

### REFERENCES

Ajibola CF, Fashakin JB, Fagbemi TN, Aluko RE (2011). Effect of

- peptide size on antioxidant properties of African yam bean seed (*Sphenostylis stenocarpa*) protein hydrolysate fractions. *Int. J. Mol. Sci.* 12(10):6685-6702.
- Alia M, Horcajo C, Bravo L, Goya L (2003). Effect of grapefruit dietary fibre on the total antioxidant capacity and the activity of Liver antioxidant enzyme in rats. *Nutr. Res.* 23:1251-1267.
- Arueya GL, Irewolede O, Adaramoye O (2015). Physicochemical Characterization and Enhancement of the Antioxidant Potential of *Ocimum gratissimum* Enriched Pepper Soup Mix Advance. *J. Food Sci. Technol.* 7(6):408-415.
- Association of Official Analytical Chemists (AOAC) (2000). *Official Methods of Analysis 17<sup>th</sup> edition* Washington DC, USA.
- Aworh OC (2008). The Role of Traditional Food Processing Technologies in National Development: The West African Experience. In: *Using Food Science and Technology to improve Nutrition and promote National Development*, Chapter 3, (Robertson, GL and Lupien JR Eds.), International Union of Food Science and Technology (IUFoST).
- Biliaderis CG, Izydorczyk MS (Eds.) (2006). *Functional food carbohydrates*. CRC Press. 570p.
- Boonchum W, Peerapornpisal, Y, Kanjanapothi D, Pekkoh J, Pumas C, Jamjai U, Vacharapiyasophon P (2011). Antioxidant activity of some seaweed from the Gulf of Thailand. *Int. J. Agric. Biol.* 13(1):95-99.
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol.* 28(1):25-30.
- Chen C, Pearson AM, Gray JI (1992). Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. *Food Chem.* 43(3):177-183.
- Daramola B (2014). Comparative Analysis of anti-oxidative potentials of Extracts of defatted unfermented and fermented locust beans. *Bangl. J. Sci. Ind. Res.* 49(4):275-280.
- Eka OU (1980). Effect of fermentation on the nutrient status of locust beans. *Food Chem.* 5:305-308.
- Gomez-Conrado DJM, Ibanez E, Barbas C (2004). Tocopherol measurement in edible products of vegetable origin. *J. Chrom. A* 1054:227-223.
- Gonzales GB., Smaghe G, Grootaert C, Zotti M, Raes K, Camp JV (2015). Flavonoid interactions during digestion, absorption, distribution and metabolism: a sequential structure-activity/property relationship-based approach in the study of bioavailability and bioactivity. *Drug Metab. Rev.* 47(2):175-190.
- Ijarotimi OS, Keshinro OO (2012). Comparison between the amino acid, fatty acid, mineral and nutritional quality of raw germinated and fermented African locust bean (*Parkia biglobosa*) flour. *Acta Sci. Pol. Technol. Aliment.* 11(2):151-165.
- Iqbal S, Bhangar MI (2006). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J. Food Compost. Anal.* 19:544-441.
- Iwe MO (2002). *Handbook of sensory methods and analysis*. Rejoint Communications Services Ltd., Enugu, pp. 64-75.
- Jia ZS, Tan MC, Wu JM (1999). The determination of flavonoid contents in mulberry and then scavenging effects on superoxide radicals. *Food Chem.* 64:555-559.
- Khairy HM, El-Sheikh MA (2015). Antioxidant activity and mineral composition of three Mediterranean common seaweeds from Abu-Qir Bay, Egypt. *Saudi J. Biol. Sci.* 22(5):623-630.
- Kumar S, Stohlgrevn TJ, Chway GW (2006). Spatial heterogeneity

- influences native and non-native plant species richness. *Ecology* 89:3186-3199.
- Lako J, Trenery VC, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem.* 101(4):1727-1741.
- Leone A, Fiorillo O, Criscuoli F, Ravasenghi S, Santagustini L, Fico G, Spadafranca A, Battezzati A, Schiraldi A, Pozzi F, Lello S, Fillipini S, Bertoli S (2015). Nutritional characterization and Phenolic profiling of *Moringa oleifera* leaves grown in Chad, Saharawi Refugee Camps and Haiti. *Int. J. Mol. Sci.* 16:18923-18937
- Maisuthisakul P, Pasuk S, Ritthiruangdej P (2008). Relationship between antioxidant properties and chemical composition of some Thai plants. *J. Food Compos. Anal.* 21(3):229-240.
- Meir SJ, Kanner BA, Hadas SP (1995). Determination and involvement of aqueous reducing compounds in oxidative defence systems of various senescing leaves. *J. Agric. Food Chem.* 43:1813-1817.
- Moon JK, Shibamoto T (2009). Antioxidant assays for plant and food components. *J. Agric. Food Chem.* 57(5):1655-1666.
- Oboh G, Akindahunsi AA (2004). Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. *Nutr. Health* 18(1):29-36.
- Oboh G, Alabi KB, Akindahunsi AA (2008). Fermentation changes the nutritive value, polyphenol distribution and antioxidant properties of *Parkia biglobosa* seeds (African locust beans). *Food Biotechnol.* 22:1-14.
- Odufa SA (1981). A note on the micro-organism associated with the fermentation of African locust bean (*Parkia filicoidiea*) during *Iru* production. *J. Plant Foods* 3:245-250
- Palafox-Carlos H, Gil-chavez J, Sotelo-Mundo RR, Namiesnik J, Gorinstein S, González- Aguilar GA (2012). Antioxidant Interactions between Major Phenolic Compounds found in 'Ataulfo mango: Chlorogenic, Gallic, Protocatechnic and Vanillic Acids. *Molecules* 17:12657-12664.
- Percival M (1998). Antioxidants. *Clin. Nutri. Insights* 31:1-4.
- Pezzuto JM, Park EJ (2002) Auto oxidation and antioxidants. *Encyclopedia of pharmaceuticals technology*. Vol. 1. 2nd ed. New York: Marcel Dekker Inc. pp. 97-113.
- Rashidinejad A, Birch EJ, Everett, DW (2016). Interactions between milk fat globules and green tea catechins. *Food Chem.* 199:347-355.
- Sanchez-Machado DI, Lopez-Cervantes J, Vazquez NJR (2006). High performance liquid chromatography method to measure alpha and gamma tocopherol in leaves, flowers and fresh beans from *Moringa oleifera*. *J. Chrom. A* 1105:111-114.
- Siddhuraju P, Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro climatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Food Chem.* 51(8):2144-2155.
- Simonyan KJ (2012). Africa Locust Bean seed processing. Prospects, status and challenges (Naphtali prints). P 14. ISBN 978-978-923-050-5.
- Singleton V, Rossi J (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Viticult.* 16:144-158.
- Songsungkan J, Chantai S (2014). Determination of synergic antioxidant activity of methanol/ethanol extract of allacin in the presence of total phenolics obtained from the garlic capsule compared with fresh and baked garlic clove. *Int. Food Res. J.* 21(6):2377-2385
- Ubando-Rivera J, Navarro-Ocaña A, Valdivia-López MA (2005). Mexican lime peel: Comparative study on contents of dietary fibre and associated antioxidant activity. *Food Chem.* 89(1):57-61.
- Williamson G, Holst B (2008). Dietary reference intake (DRI) value for dietary polyphenols: Are we heading in the right direction? *Br. J. Nutr.* 99(3):S55-S58.
- Yen GC, Chen HY (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* 43:27-32.
- Yingming P, Ying L, Hengshan W, Min L (2004). Antioxidant activities of several Chinese medicine herbs. *Food Chem.* 88(3):347-350.
- Zhang Z, Chang Q, Zhu M, Huang Y, Ho WK, Chen ZY (2001). Characterization of antioxidants present in hawthorn fruits. *J. Nutr. Biochem.* 12(3):144-152.