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Preliminary Studies on Antioxidative Potentials of Extracts of Defatted Locust Bean Condiment

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ABSTRACT: Preliminary investigation on antioxidative potentials of defatted fermented locust bean extract in comparison to defatted non-fermented locust bean was conducted. Qualitative antioxidative assessment on the extract was accomplished by screening the phytochemical endowment, and similar bioactive components. Also some antioxidative indices were quantitatively evaluated. Results showed that the defatted fermented samples were characterized with high amount of phytochemical and bioactive compounds notably phenolic compounds, saponins, peptides, amino acid and reductones. However, alkaloids and flavonoids were not detected. UV-Spectral characteristics of the extracts corroborated same. Similarly, quantitative antioxidative markers evaluated showed that the antioxidative capacity in terms of relative reducing power, total phenolic content and DPPH of fermented locust bean of defatted fermented locust bean extract were higher than the antioxidative capacity of the corresponding defatted non-fermented locust bean extract. Also, the antioxidant activity rate content, and EC_{50} of the extract were evaluated. This study demonstrated the antioxidant endowment of fermented locust bean condiment in comparison to non-fermented locust bean condiment.

Keywords: *Antioxidative Potentials, Defatted Extract, Locust Bean Condiment, Rate Constants.*

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Introduction

Nutritional enhancement is one of the values added to a food by a desirable food fermentation process. Fermented foods can be more nutritious than unfermented pair as a result of catabolic or breakdown of complex components or synthesis of complex vitamins such as Vit B_{12} and other growth factor. Also lock nutrients in indigestible cells could be released. Another means is by the enzymatic splitting of structural carbohydrate into simpler substances such as sugar (Potter and Hotkiss, 1996). More importantly, fermentation process facilitates detoxification of inherent food inhibitors. This is by conversion of inedible, raw beans to digestible edible commodities due to degradation of non-digestible oligosaccharides such as stachyose and raffinose which result to decreased flatulence potentials. In addition, fermentation of some in- edible foods lend reduction, or elimination of phytic and oxalic acids, proteins are hydrolysed to peptides, and amino acids, that could function directly in taste or serve as precursors for aroma active molecules (Beaumont, 2002)

Condiments are one of the principal food fermentation products and are desirable for culinary application with sole purpose of enhancing the taste and aroma of foods. Fermented locust bean is the most important condiment of natural origin in West Africa and central savannah region (Odunfa, 1986; Daramola *et al*., 2009). Previous studies (Omafuvbe *et al*., 2004) showed that fermentation enhanced the nutritional status of locust beans condiment. This claimed was expressed by increase in nutrients factors such as reducing sugars and free amino acids

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and peptides. Increase in the enzymic activities due to the fermentation processes were also reported. The presence of amino acids, sulphur containing amino acid, enzymes and tocopherols are reported in locust bean condiment by fermentation that suggest the condiment could possess antioxidant activity. This is partly the reasons for this study.In addition, recently Daramola and Osanyinlusi (2013) reported the possibility of enhancing the shelf-life of locust bean condiment by dehydration and removal of fat.

In furtherance of our studies, on locust bean condiment, this paper to my knowledge for the first time reports on the antioxidative potentials of defatted locust bean condiment extracts in comparison to the extracts of the defatted non fermented locust bean.

Materials and Methods

- *Materials*

Seeds of locust bean (*Parkia biglobosa)* used in this study were obtained from commercial centre at Ado-Ekiti, Nigeria. The seeds were decorticated and subsequently divided into four portions prior to the fermentation process.

- *Preparation of locust bean condiment*

One of the decorated locust beans was processed into condiment following the methods elicited by Omafuvbe *et al*. (2004) and the samples obtained were tagged unfermented locust bean, while the second part (half) was fermented and the sample obtained was tagged fermented locust bean. Both samples were milled afterward.

- *Solvent extraction of locust:*

Solvent extraction of both the fermented and non-fermented locust bean was accomplished in two phases. Phase one was to defat the samples and phase two was to extract the bioactive components. This was accomplished in accordance with the method described by Adegoke and Gopalakrishna (1998). Briefly, an amount of 10g of finely ground locust bean (fermented and non-fermented) was extracted with 100ml of n-hexane in soxhlet extractor for 2h. The residue obtained from the two samples (fermented and non-fermented) were then subjected to a repeat of extraction protocol but the solvent was replaced with ethanol and diethylether-chloroform mixture. The crude extracts were evaporated to remove the solvent and extract recovered.

- *Analytical methods*
- *Phytochemicals evaluation of the defatted fermented and non-fermented locust beans extracts*

Phytochemical investigations on extracts were performed as described by Trease and Evans (2002) and Harborne (1984) as follows:

- *Test for reducing sugars*

To 0.5ml of the extracts solution, 2ml of a mixture (1:1) of Fehling's solution 1 (A) and Fehling's solution II (B) were added and the mixture were boiled in a water bath for five minutes. A brick-red precipitate indicated the presence of free reducing sugars.

- *Test for flavonoids*

To 0.5ml of the extracts solution, a few drops of 10% ferric chloride solution were added. A green or blue colour indicated the presence of phenolic nucleus.

- *Test for free amino acids*

0.5ml extracts solution were treated with few drops of ninhydrin reagent, heated in water bath, a purple colour indicated the presence of amino acids.

- *Test for alkaloids*

0.5ml of extract solution was added with 0.2ml of 36.5% hydrochloric acid and 0.2ml Dragendroff's reagent. Production of orange precipitate denoted the presence of alkaloids.

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- *Test for glycosides*

0.5ml extract solution was added to 2ml of 50% hydrochloric acid. The mixtures were hydrolized for 2hrs on a water bath. After that 1ml pyridine, few drops of 1% sodium nitroprusside solution, and 5% sodium hydroxide solution were added. Pink to red colour designated the presence of glycosides.

- *Test for reducing compounds*

To 1ml of ethanolic extracts of samples, a few drops of 10% ferric chloride and potassium hexaferrate (III) solution were added. A green or blue colour indicated reducing activity.

- *Test for saponins*

2ml of double distilled water was added with 1ml of each extract solution, few drops of olive oil were added and agitated. Formation of soluble emulsion indicated the presence of saponin.

- *UV – visible absorption spectra assessment*

UV – visible absorption spectra of defatted fermented and non-fermented locust bean extract at 0.006% in ethanol were recorded on an uv-visible double recording spectrophotometer (Unicam Helios & uv/Vis/Spectrometer V.2.05 Serial No UVA 072519 year 2000 complant).

- *Quantitative assessment of antioxidative potentials*
- *Evaluation of total phenolic content.*

Total phenolic content was evaluated according to the method described by Taga *et al*. (1984). A 100µl of Folin-Ciocalteau reagent (2N wrt acid Fluka Chemic AG-Ch-9470 BUCHS) was added to each sample (20µl) and well mixed after the addition of 1.58ml of water. After 30 seconds, 300µl of 2% sodium carbonate solution was added and the sample tubes were left at room temperature for 2h. The absorbance (A) of the developed blue colour was measured at 750nm using Unicam Helios & UV/VIS/Spectrophotmeter. A plot of A750nm against conrresponding concentration was used to calculate phenolic content (g/g ascorbic acid equivalent).

- *Determination of relative reducing power*

Reducing power of each sample was determined in accordance with the method of Oyaizu (1986). Simply, each sample (1mg/ml) in ethanol (2.5ml) was mixed with sodium phosphate buffer (pH 6.6). the buffered sample was mixed with conditioning reagents (1%K_3-Fe-CN_6, 10% TCA, 0.1% $FeCl_3$), centrifuged, diluted using distilled water and absorbance was measured at 700nm. Higher absorbance indicates a higher reducing power. A plot of A_{750nm} against corresponding concentration was used to calculate the phenolic content (g/g ascorbic acid equivalent).

- *Total flavonoids contents determination*

The total flavonoids contents were determined by colorimetric method and expressed as mg ascorbic acid equivalent per g of dry weight, using the method described by Chang *et al*. (2002) with little modification. 0.5ml of each extracts (prepared from 1mg of crude extract dissolved in 1ml of methanol) were mixed with 1.5ml of methanol, 0.1ml of 10% aluminium chloride ($AlCl_3$) solution, 0.1ml of (IM) sodium hydroxide solution (NaOH) and 2.8ml of ddH_2O. The resulting mixtures were well mixed and incubated for 30 minutes at obscurity. The absorbance of the reaction mixture was measured at 430nm with a UV/visible spectrophotometer. A plot of A_{430nm} against corresponding concentration was used to calculate the phenolic content (g/g ascorbic acid activity equivalent).

- *Measurement of radical-scavenging activity*

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Radical scavenging activity of the samples on DPPH was estimated according to the method of Yamaguchi *et al*. (1998). An aliquot of samples (200uL, 0.31-2.5mg.mL), ascorbic acid (0.04-1.25mg/mL) was mixed with the 100mM Tris-HCl buffer (800uL, pH 7.4) and then added to 1mL of 500um DPPH in ethanol (final concentration of 250um). The mixture was shaken vigorously and left to stand for 20min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517nm. The capability to scavenge the DPPH radical was calculated using the following equation.

Scavenging effect (%) = [1- (absorbance of sample at 517nm/absorbance of control at 517nm)] x100

- *Determination of free amino acids*

Free amino acids in the partially defatted locust bean condiment and non-defatted samples were extracted with 80 % ethanol (v/v) in accordance with the method of Odibo *et al.* (1990). The free amino acids in the ethanolic extract were estimated using the ninhydrin colorimetric method (Rosen, 1957) using glycine as standard.

Results and Discussion

- *Phytochemical characteristics of defatted fermented and unfermented locust bean extract*

The results of the phytochemical assessment of the extracts of the defatted fermented locust bean condiment in comparison to unfermented are shown in Table 1. The samples showed reducing activity. However, the degree of reducing property was much exhibited by the extract of the defatted fermented sample in comparison to the extract of defatted unfermented sample. Consequently, the fermentation process enhanced the reducing activity of locust beans. It is important to note that the reducing power of a substance defines the primary antioxidant activity (Giese, 1996).

The screen test also showed that the reducing sugars were present in all the samples with higher intensity mark in fermented extract sample. Increase in the presence of reducing sugar is most probably due to the breaking down of both structural and non-structural polysaccharide in agreement with elicitation of Potter and Hotkiss (1996).

More importantly, the presence of free amino acid was screened. The intensity of the presence of free amino acids in the fermented sample was much higher in comparison to the unfermented sample. This was expected because, fermentation processing of locust bean for formation of condiment is based on hydrolysis of protein into peptides, amino acids, nucleotides, that are responsible for the desired sensory property of the condiment. The assessment also showed little presence of saponins, with no test positive for the presence of flavonoids and alkaloids. This does not suggest that there are no secondary of metabolites in locust beans but fermentation contributes to detoxification of inherent phenolic anti-nutritious factors present in locust beans.it is speculated that some of the phenolic components could be alkaloids or saponins. As expected glycosides are present in all the samples

- *UV- Spectral characteristics of defatted fermented and unfermented locust bean extracts*

The uv-spectral characteristics of the unfermented and fermented extracts are shown in Table 2. UV- reading ranges from 180nm to 420nm (Silverstein *et al*., 1981). A comparison of the spectral characteristics showed that there was poor primary absorption of the samples of the unfermented in comparison to the uv-absorption of the fermented extract.

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Table 1. Phytochemical Screen of fermented and unfermented defatted locust bean extracts

Sample I.D	Reducing compounds	Reducing sugars	Free amino acids	Saponins	Flavonoids	Alkaloids	Glycosides
1.	+++	-	++	+	-	-	++
2.	+++++	++	+	+	-	-	++
3.	++	+++	++++++	+	-	-	+++
4.	+++++	++++	++++++	+	-	-	+++

+ strength of presence, Means Not detected; Sample ID = Sample Identification Number; 1 = Defatted unfermented locust bean cake ethanol extract; 2 = Defatted unfermented locust bean cake diethylether-chloroform extract; 3 = Defatted fermented locust bean cake ethanol extract
4 = Defatted fermented locust bean cake diethylether-chloroform extract

Table 2. UV – spectral characteristics of fermented and unfermented defatted locust bean extracts

Sample I.D No	A	λ	A	λ	A	λ
1	0.48	396	0.21	400	-	-
2.	0.54	396	0.28	400	-	-
3.	8.65	396	8.83	400	-	-
4.	8.80	396	1.37	450	0.27	500

A = absorbance, λ = wavelength, Sample Identification Number = sample ID No,
1 = Defatted. Unfermented locust bean cake ethanol extract, 2 = Defatted unfermented locust bean cake ethanol-diethylether extract, 3 = Defatted fermented locust bean cake ethanol extract 4 = Defatted fermented locust bean cake ethanol-diethylether extract

Table 3. Some Antioxidative and Biochemical [y] parameters

Sample ID	Total Phenolic Content (TPC)	Free Amino Acid (FAA)	DPPH %	Relative Reducing Power (RPP)
1	8.80	0.3	57	13.30
2	17.00	0.215	53	10.00
3	10.00	3.3	89	29.50
4	8.48	1.66	82	23.40

[y]TPC = mg ascorbic acid activity equivalent per mg sample; FAA = mg glycine activity equivalent per mg sample; RPP = mg ascorbic acid reducing activity equivalent per mg sample
1 = Defatted unfermented locust bean cake ethanol extract, 2 = Defatted unfermented locust bean cake ethanol-diethylether extract, 3= Defatted fermented locust bean cake ethanol extract 4 = Defatted fermented locust bean cake ethanol-diethylether extract

Absorption (primary and secondary) in the ultraviolet region by samples are diagnostic feature of unsaturation or unbound election in the absorbing molecules (Shriner *et al*., 1979). Free election is a pre-requisite for antioxidative activity (Giese, 1996). However, there was no secondary absorption at the uv region both for the fermented and unfermented samples. This result signalled the absence of complex

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phenolics and other aliphatic hydroxyl compounds. This result corroborates the detection of no or low principal phenolic compounds such as flavonoids, alkaloids, and anthraquinone.

- *Selected biochemical and antioxidative indices*

Four biochemical and antioxidative indices were assessed with view to gain insight into the quantitative antioxidative endowment of defatted unfermented and fermented extracts of locust bean. The result is presented in Table 3. The Total Phenolic Contents (mg ascorbic acid activity equivalent/mg sample as determined in this study) of the unfermented extract were high, ranging from 8.80 to 17.00 in comparison to a low values (8.48-10.00) obtained for the fermented samples. This result suggests that the enzymatic activity during fermentation has resulted in hydrolysis of complex phenolic compounds like flavonoids, alkaloids. This was attested in the result of the phytochemical screen test (Table 1).

Free amino acids: The free amino acids, FAA (mg glycine equivalent per mg sample) of the defatted unfermented extract of locust bean ranged from 0.215 to 0.300. This was extremely low as compared to the high FAA of the extract of the defatted fermented locust bean with values that ranged from 1.66 – 3.30. This result is similar to the earlier work by Odunfa (1986), as reported by Beaumont (2002). The protein is hydrolysed into amino acids, peptides during fermentation and is the foremost characteristics of bio-transformation of constituents of locust beans (Odunfa, 1986; Beaumont, 2002; Omafuvbe *et al*., 2004; Daramola and Osanyinlusi, 2013).

Free radical scavenging activity of the extracts: The result of the free radical scavenging activity (FRSA) of fermented and unfermented locust bean extracts evaluated using DPPH is shown in Table 3. The FRSA (%) of defatted extract of unfermented locust bean is low (53%) in comparison to the high (82-89%) FRSA of the defatted extract of fermented locust beans. There has not been a report on FRSA of locust bean condiment or its extract and therefore one is unable to compare the findings.

Relative reducing power: The results of the relative reducing power of the defatted unfermented locust bean extract is low (10.00 -13.00) when compared to high (23.40-29.50) relative reducing power of the defatted fermented locust bean extract. Considering the result of the total phenolic content, free amino acids in relation to DPPH and relative reducing power it is important to infer that DPPH and RRP is not largely due to phenolic compounds but due to the activity of reducing substances usually free amino acids, peptides and most probably oxidized lipid-amino acid reaction products that are known for antioxidative activity (Hidalgo *et al*., 1998) and other hydrolysate products in the fermented extract of the locust beans.

Antioxidant activity constant, EC_{50}, and ARP: Table (4) shows the summary of antioxidant activity constant, EC_{50} and ARP of the unfermented and fermented defatted extracts of locust bean. It can be seen in the table that the antioxidant activity rate constant of the extract of the fermented locust bean is 2 to 8 times more potent than the antioxidant activity rate constant of the extract of the unfermented locust bean. Similarly, the result of the EC_{50} showed that lesser amount of the extract of fermented locust bean is required to accomplish EC_{50} activity in comparison with extract of the unfermented locust bean. The obtained ARP is also showed in the Table (4).

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Table 4. Antioxidative activities of the defatted fermented and non-fermented locust bean extracts as expressed by antioxidant activity constant (K), half inhibition concentration (EC_{50}) and antiradical power (ARP)

Antioxidative Reaction	Sample ID	K (mL/mg)	EC_{50} (mg/mL	ARP
DPPH Radical	1	1.3807	0.5020	1.992
	2	1.452	0.4774	2.095
	3	3.667	0.18902	5.291
	4	2.4366	0.2845	3.515
[@]RRP	1	2.6775	0.2588	NA
	2	2.500	0.2773	NA
	3	6.120	0.1133	NA
	4	18.12	0.03825	NA

[@] relative to be absorbance , NA = Not applicable; Note: ARP = $(EC_{50})^{-1}$
1 = Defatted. Unfermented locust bean cake ethanol extract, 2 = Defatted unfermented locust bean cake ethanol-diethylether extract, 3 = Defatted fermented locust bean cake ethanol extract 4 = Defatted fermented locust bean cake ethanol-diethylether extract

Conclusion

Antioxidative potential of defatted fermented locust bean extract is superior to the antioxidative potential of unfermented defatted locust bean extract as shown by both qualitative assessment using phytochemical screen test and quantitative assessment using TPC, RRP and DPPH.

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