



EFFECT OF PRE-PROCESSING OPERATIONS ON ANTIOXIDANT PROPERTIES AND ACTIVITIES OF *PROSOPIS AFRICANA* SEED

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ABSTRACT: *The study investigated the effect of pre-processing operations on antioxidant properties and activities of Prosopis africana seeds. The following pre-processing operations were considered: boiling, germination, soaking and roasting. The antioxidant properties and activities of the processed Prosopis africana seed were analyzed using standard methods. The data obtained were analyzed using one-way ANOVA while Turkey HSD was adopted for its post hoc test. All statistical tests were conducted at 5 % level of significance. The roasted samples were higher in Total Phenol (7.49 mgGAE/g), Anthocyanin (6.44 mg/100g) but Vitamin E was found to be the highest in the germinated seed (3.87 mg/100g). The boiled seeds were higher in Beta carotene (103.85 mg/100g) while the germinated and soaked seeds were higher in Vitamin C (8.59 mg/100g) and Flavonoid (6.50 mg/100g respectively). Roasting gave a higher OH scavenging activity (70.68 %) and Reducing Power Activity (80.30%) while soaking gave a higher DDPH activity (70.64). The Flavonoid, Total Phenol, Anthocyanin and Vitamin E are significantly correlated to OH and FRAP while Beta carotene is significantly correlated to DPPH. The study had revealed that pre-processing operations had a significant effect on the antioxidant properties and activities of Prosopis africana seed, which offer nutritional and health benefits. Processed Prosopis africana seed are recommended for consumption as a good source of antioxidants.*

KEYWORDS: Legumes, Antioxidants Properties, Pre-processing, *Prosiopis africana* and Antioxidant Activity.



INTRODUCTION

The growing world population is concerned with the availability of agricultural and highly nutritious food that can meet the nutritional requirements of the populace. These have been a major concern especially in developing and under-developed countries. The most important aspect of food and nutrition security in contemporary society is the processing of agricultural products (Smith *et al.*, 2018). Of interest in achieving food and nutrition security are these classes of food: cereals, pulses and legumes; which are grains used globally as staple foods. Legumes in particular are grains used globally as staple food that contains many bioactive and functional components, such as antioxidants, minerals etc, that affect health positively. They serve as rich source of dietary proteins in many developing countries and mostly synthesize certain biologically active substances considered to be anti-nutritional in nature since they inhibit the utilization of very important and necessary nutrients (Bouchenak & Lamri, 2013; King *et al.*, 2024). They are rich with wide variety of phytochemicals like: phytosterols, natural antioxidants and bioactive carbohydrates and as such have been useful in the prevention and treatment of several diseases in the developed countries (Amarowicz & Pegg, 2008).

To access these antioxidants present in legumes and *Prosopis africana* in particular, processing technique is of great essence. Among five common processing techniques for legumes are soaking, boiling, roasting, fermentation and germination. Most of these processing methods are generally non-complex and less expensive in achieving desirable nutritional changes in the composition of the legume seeds as well as increase in protein content, dietary fiber, vitamins and bioavailability of trace elements and minerals (Kaushik *et al.*, 2010). This study therefore seeks to examine the effect of pre-processing operations on the antioxidant properties and activities of *Prosopis africana* seed.

LITERATURE/THEORETICAL UNDERPINNING

Legume consumption has been found to be helpful in the management of coronary heart disease, type II diabetes mellitus and obesity. Reports confirms that it lowers the Low Density Lipoprotein (LDL) cholesterol but increases High Density Lipoprotein (HDL) cholesterol (Rizkalla *et al.*, 2002). Bazzano *et al.* (2001) have shown that there is a relationship between consumption of legumes and reduction in risk of several diseases such as cancer, heart related diseases, obesity and diabetes. African mesquite (*Prosopis africana*) is a perennial leguminous tree of the sub-family Mimosaidae and it grows in the savanna region of Senegal and Nigeria. It is a flowering plant species in the legume family found in Africa. Its common names include African mesquite, iron tree, geré (malinke) (traditional djembe tree), or somme tree (Nwokocha, 2021). There are numerous social, economic, cultural, medical, and agricultural benefits associated with African mesquite (*Prosopis africana*). It is consumed and utilized extensively throughout the nation and beyond. It is well-known for its seeds, expensive spice, condiment, and abundance of protein, fatty acids, and other essential minerals and nutrients (Asoiro & Ohagwu, 2017).



METHODOLOGY

Procurement of the Sample

The *Prosopis africana* seeds were purchased from Okwo Main Market Ngbo in Ohaukwu Local Government Area of Ebonyi State, Nigeria. Extraneous and foreign matters such as insect-infected seed, sand and chaff were removed manually from the sample.

Sample Preparation

Boiling

This was carried out according to the traditional way of processing *Okpehe* (condiment). The *Prosopis africana* seeds were boiled for 3 hours and allowed to cool and then dehulled manually. The dehulled seeds were wrapped in plantain leaf and kept at room temperature for four days. The fermented sample was sun-dried. The dried fermented sample was milled and sieved using a No. 30 mesh sieve. The ground seed flour was stored in zip locked polyethylene bags until analyses. The boiled-fermented sample served as control in this study.

Germination

Germination was done using the method described by Uleh and Fagbemi (2017). The *Prosopis africana* seeds were soaked in hot water of 100 °C at a ratio of 1:3 w/v for 72 hours with change of the hot water every 8 hours. The sample was kept in a lagged box to maintain a temperature of approximately 80-90 °C; after 72 hours, the water was drained, and the seeds germinated for 14 days at room temperature. During germination, the seeds were sprinkled with cold water every twelve (12) hours. Seeds with sprouts of 1 mm or more were considered germinated. The germinated seeds were wrapped in plantain leaf and kept at room temperature for four (4) days to ferment. The fermented samples were sun-dried, milled and sieved using a No. 30 mesh sieve. The ground seed flour was stored in zip locked polyethylene bags until the analyses.

Soaking

Soaking was done using the method described by Uleh and Fagbemi (2017). The *Prosopis africana* seeds were soaked in hot water of about 100 °C at a ratio of 1:3w/v for 24 hours with the change of boiled water every six (6) hours. At the end of the 24 hours, the soaked seeds were de-hulled, then wrapped in plantain leaf and kept at room temperature for four days to ferment. The fermented sample was sun-dried, milled and sieved using a No. 30 mesh sieve. The ground seed flour was stored in zip locked polyethylene bags until analyses.

Roasting

The *Prosopis africana* seeds were put in a frying pan and roasted for about 30 minutes when the hulls were seen being separated from the cotyledon. Complete dehulling was manually carried out and the roasted seeds were wrapped in plantain leaf and kept at room temperature for four days to ferment. The fermented sample was sun-dried, milled and sieved using a No. 30 mesh sieve. The ground seed flour was stored in zip locked polyethylene bags until analyses.



Antioxidant Analysis

Determination of Ascorbic Acid (Vitamin C)

The ascorbic acid contents of the samples were determined by 2, 6-dichlorophenol indophenol (DCPIP) titration method described by Rao and Deshpande (2006). Five (5) mL of the ascorbic acid working standard (500 µg/5 mL) and 10 mL of 4 % oxalic acid were pipetted out into a 100 mL conical flask. The contents in the flask were titrated against the dye solution (V1) until the appearance of a pale pink colour that persisted for a few minutes. Five (5) mL of the test sample was similarly titrated against the dye solution (V2). Ascorbic acid content present in the test samples were determined using the formula:

$$\text{Amount of Ascorbic content (mg/100g)} = \frac{500 \times V_2 \times 25 \times 100}{V_1 \times 5 \times 5} \quad (1)$$

where 500 = µg of standard ascorbic acid taken for titration, V_1 = Volume of dye consumed by 500 µg of standard ascorbic acid, V_2 = volume of dye consumed by 5 mL of test sample, 25 = corresponds to total volume of the extract, 100 = ascorbic acid content/100g of the sample, 5 = weight of sample taken for extraction, and 5 = volume of the test sample taken for titration.

Determination of Total Flavonoid Content

The aluminum chloride colorimetric method, as outlined by Chang *et al.* (2002), was used to determine the total flavonoid concentration. One and half milliliters of methanol was combined with an aliquot of 0.5 mL of seed extracts (1 mg/mL). Next, 2.8 mL of distilled water, 0.1 mL of 10 % aluminum chloride, and 0.1 mL of potassium acetate (1 M) were added. For thirty minutes, the reaction mixture was allowed to sit at room temperature. At 415 nm, the reaction mixture's absorbance was measured using an electronic spectrophotometer (Jenway Model 6000). Plotting the calibration curve (0–8 µg/mL) with rutin as a standard was done. Rutin equivalent milligrams per gram of dry weight was used to express the total flavonoids. The equation is given thus:

$$\text{Total Flavonoid Content (mg/100g)} = \frac{(A_{\text{sample}} - A_{\text{blank}}) \times C_{\text{standard}}}{(A_{\text{standard}} - A_{\text{blank}}) \times V_{\text{total}} \times 1000} \quad (2)$$

where A_{sample} = absorbance of sample at 415 nm, A_{blank} = absorbance of the blank (solvent without the sample), C_{standard} = concentration of the flavonoid standard solution used for calibration (mg/mL), A_{standard} = absorbance of the standard at the same wavelength (415 nm), V_{total} = total volume of the extract (mL) and 1000 = factor to convert the result into mg of phenolic content per g or mL of sample.



Determination of Total Phenolic Content

The Folin-Ciocalteu reagent (FC) was used to colorimetrically quantify the total phenolic content (TPC) as proposed by Chuah *et al.* (2008). A glass tube was filled with a half milliliter aliquot of the sample extract solution. After five minutes, 0.5 milliliters of reactive FC was added, and then 2 milliliters of Na₂CO₃ solution (200 mg mL⁻¹) was added and agitated. A vortex mixer was used to mix the sample, and the reaction continued for 15 minutes at room temperature. Following the addition of 10 mL of ultra-pure water, the precipitate was extracted using centrifugation for 5 minutes at 4000 × g. A Jenway model 6000 electronic spectrophotometer was used to measure the absorbance at 765 nm, and the results were compared to a calibration curve for gallic acid (GA). Results were expressed as mg GA100 g⁻¹ dry matter. The content is determined mathematically thus:

$$\text{Total Phenolic Content (TPC)} = \frac{(A_{\text{sample}} - A_{\text{blank}}) \times C_{\text{standard}}}{(A_{\text{standard}} - A_{\text{blank}}) \times V_{\text{total}} \times 1000} \quad (3)$$

where A_{sample} = absorbance of sample at a given wavelength usually 765 nm, A_{blank} = absorbance of the blank (usually solvent without the sample), C_{standard} = concentration of the phenolic standard solution used for calibration (mg/mL), A_{standard} = absorbance of the standard at the same wavelength, V_{total} = total volume of the extract (mL) and 1000 = factor to convert the result into mg of phenolic content per g or mL of sample.

Determination of Vitamin E

Zeng *et al.* (1999) described the use of a methanol-BHT (1 mg mL⁻¹) solution for sample extraction. A 150 × 4.6 mm, 5 m symmetry column was used for the separation from water, and a methanol:acetonitrile (1:1 v v⁻¹) mobile phase was used, with a flow rate of 1.2 mL min⁻¹ wavelengths, respectively. Every measurement was carried out in triplicate. The amount of vitamin E was stated in milligrams per 100 grams of dry matter. The content is determined mathematically thus:

$$\text{Vitamin E content (mg/100g)} = \frac{A_{\text{sample}}}{A_{\text{standard}} \times C_{\text{standard}}} \quad (4)$$

where A_{sample} = area under the chromatographic peak for the sample, A_{standard} = area under the chromatographic peak for the standard, and C_{standard} = the concentration of the standard.

Determination of Total Anthocyanin Content

The method described by James *et al.* (2020) was employed in the determination of total anthocyanin content. The pH differential method was used to determine the total anthocyanin content. Using a vortex, 0.5 mL of the sample extract was combined with approximately 3.5 mL of 0.025 M potassium chloride buffer pH 1.0. A Jenway model 6000 electronic spectrophotometer was used to measure the absorbance at 515 nm after the mixture had stood for 15 minutes. Following a similar treatment with 0.4M sodium acetate buffer pH 4.5, the



extract's absorbance was measured using the same wavelengths. The following formula was used to get the total anthocyanin concentration, which was then represented as mg of cyanidine-3-glucoside equivalent in 100 g of dried sample (mg c-3-gE/100 g dried sample).

$$\text{Total anthocyanin (mg/100g)} = \frac{A \times MW \times DF \times 103}{\epsilon \times 1} \quad (5)$$

where A = (A_{515 nm} – A_{700 nm}) pH 1.0 – (A_{515 nm} – A_{700 nm}) pH 4.5;

Molecular weight (MW) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor of samples; 1 = path length in cm; ϵ = 26,900 molar extinction coefficient, in L x mol⁻¹ x cm⁻¹ for cyd-3- glu; 103 = factor for conversion from g to mg.

Determination of Beta-Carotene

The beta-carotene was determined using (Pearson, 1976) method. Chromatography is typically used to separate carotenoids. The technique is based on employing an adsorbent with variable affinities for the various pigments to separate the physiologically active carotenoid pigments from the overall carotenoid pigment in an extract. A sample weighing 10 g was combined with 40 ml of acetone and then filtered. A Whatman's No. 1 filter paper was used to filter the aforementioned sample after 0.1 g of MgO₂ was added and diluted with 60 ml of hexane. Two layers of the filtrate separated when it was moved into a separatory funnel. Hexane's top transparent layer, or supernatant, was used for estimation. After transferring the top layer to a 100 ml volumetric flask, 100 ml of hexane was added to make up the volume. Aluminum and sodium sulfate were taken in a 1:1 ratio (20 g + 20 g) for the column's construction. Using a 10-milliliter beaker, the silica gel was poured into the column; then air was forced into the column and packed in using a suction pump. Four to five centimeters were left on top of the adsorbent so that the solvent (acetone and hexanes) could be added. The pre-elution procedure was finished and the column was prepared for loading when hexanes and acetone solution were added to the top of the silica gel and the bottom solvent level reached the bottom of the column. After dissolving the material to be purified in a tiny quantity of solvent, like acetone or hexanes, it was put onto the column. The sample was taken in a cuvette, and the Jenway model 6000 electronic spectrophotometer was used to measure the absorbance at 436 nm.

Calculation:

$$\begin{aligned} &\text{BetaCarotene(mcg/100g)} \\ &= \frac{\text{Conc. of carotene soln read as standard soln(mcg/100g)} \times \text{Final dilute vol.}}{\text{Weight of the sample}} \times 100 \end{aligned} \quad (6)$$

Antioxidant Activity Analysis

Determination of DPPH Radical Scavenging Activity

The 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) technique was used to assess the samples' capacity to scavenge free radicals (Turkmen *et al.*, 2005). The extracts were made in triplicates in various dilutions. A test tube containing 1 mL of the sample extract was filled with an aliquot of 2 mL of 0.15 mM DPPH radical in ethanol. After 30 seconds of vortex mixing, the reaction mixture was allowed to stay at room temperature in the dark for 20 minutes. An electronic



spectrophotometer of the Jenway model 6000 was used to test the absorbance at 517 nm. For the spectrophotometer's calibration, 80 % ethanol was utilized. Extract was not included in the preparation of the control sample. The percentage inhibition of the DPPH radical was used to express total antioxidant activity (TAA), which was calculated thus:

$$\%TAA = 1 - \left(\frac{Abs_{sample}}{Abs_{control}} \right) \times 100 \quad (7)$$

where TAA = total antioxidant activity, Abs = absorbance.

Determination of OH Scavenging Activity

The method described by Zhao *et al.* (2014), for the determination of OH scavenging activity, was adopted. Each extract (0.5 mL) was combined with 3.5 mL of distilled water, 0.5 mL of 9.1 mmol/L salicylic acid–ethanol solution, and 0.5 mL of 9 mmol/L Fe^{2+} solution. For the Fenton reaction to begin, 5 mL of 88 mmol/L H_2O_2 was then added. The reaction product's absorbance A_1 was measured at 510 nm. Instead of utilizing the 9 mmol/L Fe^{2+} solution, 0.5 mL of distilled water was used to determine the absorbance A_2 , whereas 0.5 mL of distilled water was used to test the absorbance A_3 . Using the following formula, the hydroxyl radical (.OH) scavenging activity was determined:

$$\% \text{Scavenging activity} = [1(A_1 - A_2) / A_3] \times 100 \quad (8)$$

where A_1 = Absorbance of control, A_2 = Absorbance of sample.

Reducing Power Activity Determination

The assay of reducing power was determined using the method described by Singhal *et al.* (2011). Samples in different concentrations (0.2 mg/ml to 1 mg/ml) were made. To the various sample quantities, 1 milliliter of newly made 1% potassium ferric cyanide and 0.2 milliliters of sodium phosphate buffer (pH 6.6) were added. After 20 minutes of incubation at 50°C in a water bath, 1 milliliter of 10 % trichloroacetic acid (TCA) was added to the mixture. The mixtures were centrifuged for ten minutes at 3000 rpm. Five hundred microliters of freshly made 1% ferric chloride and two milliliters of distilled water were combined with two milliliters of the supernatants. Rutin was used as standard. The amount of rutin was calculated from a standard calibration curve for lowering power activity after the absorbance values were measured at 700 nm using a Jenway model 6000 electronic spectrophotometer. Increased absorbance will be a sign of increased lowering power activity. The percentage of the reducing power was calculated using the formula given below:

$$\text{Reducing power} = \frac{Abs_{extract} - Abs_{blank}}{Abs_{blank}} \times 100 \quad (9)$$

Data Analysis/Statistical Design

The study adopts completely randomized design (CRD). Data obtained were analyzed with the Statistical Package for Social Science (SPSS) version 26. Summary statistics (mean and standard deviation) was estimated for each treatment group (pre-processing operations).



One way Analysis of Variance (ANOVA) was adopted to test the difference in mean among several treatment groups (pre-processing operations). Turkey HSD multiple test was deployed as a post-hoc test for determination of treatment groups that differ significantly from each other. Moment Pearson correlation coefficients were adopted to ascertain the relationship between antioxidant properties and activities of the processed seed. All tests were conducted at 5% level of significance, that is, $p < 0.05$ is considered significant.

RESULTS

Table 4.1: Anti-oxidant Properties of Processed *Prosopis africana* Seeds

Pre-processing operations	Anti-oxidant properties					
	Flavonoid (mg/100g)	Total Phenol Content (mgGAE/g)	Anthocyanin (mg/100g)	Beta Carotene (mg/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)
Control	6.13 ^a ±0.23	3.91 ^d ±0.14	5.52 ^b ±0.49	103.85 ^a ±2.02	5.88 ^b ±0.10	2.72 ^c ±0.03
Germination	4.15 ^c ±0.10	4.92 ^c ±0.28	5.72 ^{ab} ±0.15	88.66 ^b ±0.89	8.59 ^a ±0.34	3.87 ^a ±0.11
Soaking	6.50 ^a ±0.40	6.06 ^b ±0.25	4.85 ^b ±0.20	72.59 ^c ±0.56	6.05 ^b ±0.17	2.53 ^c ±0.13
Roasting	4.95 ^b ±0.10	7.49 ^a ±0.36	6.44 ^a ±0.41	82.23 ^c ±1.92	4.73 ^c ±0.14	3.42 ^b ±0.04

Values are means ± standard deviation of triplicate determinations. Values along the same column with different alphabetical superscripts indicate significant difference ($p < 0.05$).

Table 4.1 depicts the result of the antioxidant properties of processed *Prosopis africana* seeds using different pre-processing operations. Total Phenol (3.91–7.49 mgGAE/g) was the most abundant anti-oxidant followed by Beta Carotene (72.59–103.85 mg/100g) while the least was Vitamin E (2.72–3.87 mg/100g). A significant difference ($p < 0.05$) was observed in the anti-oxidant properties among different pre-processing operations without any particular order. Flavonoid was highest in Control (6.13 mg/100g), Total Phenol (7.49 mgGAE/g) and Anthocyanin (6.44 mg/100g) was highest in Roasting while Vitamin C (8.59 mg/100g) and Vitamin E (3.87 mg/100g) were highest in germinated sample.

Table 4.2: Antioxidant Activities of Processed *Prosopis africana* Seeds

Pre-processing operations	Anti-oxidant activities (%)		
	OH	DPPH	FRAP
Control	56.30 ^c ±0.65	49.67 ^c ±1.09	59.85 ^d ±0.25
Germination	65.67 ^b ±0.85	55.91 ^b ±0.47	72.33 ^b ±0.58
Soaking	55.53 ^c ±0.74	70.64 ^a ±0.57	62.55 ^c ±0.44
Roasting	70.68 ^a ±0.65	56.75 ^b ±0.25	80.30 ^a ±0.86

Values are means ± standard deviation of triplicate determinations. Values along the same column with different alphabetical superscripts indicate significant difference ($p < 0.05$). OH = Hydroxyl Radical, DPPH = 2,2-Diphenyl-1-Picrylhydrazyl, FRAP = Ferric Reducing Antioxidant Power.



The results in Table 4.2 are the mean and standard deviation of the anti-oxidant activities of processed *Prosopis africana* seeds. Three activities were considered and each of them was at least approximately 50 % and ranged from (50–80 %). OH was more abundant in Roasting (70.68 %) and less available in Soaking and Control (55.53 %/56.30 %). There is no significant difference ($p > 0.05$) in the OH of the Control and Soaked seed. Soaking (70.64 %) had the highest DPPH value on average while Control (49.67 %) had the lowest DPPH. The DPPH in Germinated was not significantly different from that in Roasting. Roasting (80.30 %) had the highest RP activity while Control (59.85 %) had the least RP activity.

Table 4.3: Correlation between Antioxidant Properties and Activities

Antioxidant properties	Antioxidant activities		
	OH	DPPH	FRAP
Flavonoid	-0.813**	0.366	-0.735**
Total Phenol Content	0.588*	0.455	0.719**
Anthocyanin	0.812**	-0.523	0.738**
Beta Carotene	-0.132	-0.909**	-0.291
Vitamin C	-0.029	-0.054	-0.099
Vitamin E	0.822**	-0.369	0.756**

Values are Pearson correlation coefficient (r), * = significant at 5% ($p < 0.05$), ** = significant at 1% ($p < 0.01$)

The result of the measure of relationship between the antioxidant properties and activities are as represented in Table 4.3; the values are the correlation coefficient between each of the various antioxidant properties and activities. The result revealed that OH is significantly and inversely (negatively) related to Flavonoid ($r = -0.813$), but significantly and directly (positively) related to Anthocyanin ($r = 0.812$), Total Phenol Content ($r = 0.588$) and Vitamin E ($r = 0.822$). OH had no significant ($p > 0.05$) relationship with Beta Carotene and Vitamin C. DPPH was only significantly ($p < 0.05$) but inversely related to Beta Carotene ($r = -0.909$) among all the antioxidant properties. FRAP was significantly ($p < 0.05$) and inversely related to Flavonoid ($r = 0.735$) but significantly ($p < 0.05$) and directly related to Total Phenol Content ($r = 0.719$), Anthocyanin ($r = 0.738$) and Vitamin E ($r = 0.756$). The result further revealed that OH and FRAP had the same pattern and direction of correlation with the antioxidant properties.

DISCUSSION

Anti-oxidant Properties of Processed *Prosopis Africana* Seeds

With the range of total phenol content of processed *Prosopis africana* seeds obtained in this study, it is considered an excellent source of phenols. The variations and range of the phenol is within the recommended phenol content of legume (Nwaoguikpe *et al.*, 2014). The low value seen in control might be attributed to possible oxidation of some components of lipophilic polyphenols and their leaching out in the fermentation medium (Wollgast & Anklam, 2000). Total phenolic content found in germinated seed obtained in this study was higher than what



was reported by James *et al.* (2020) for germinated cowpea (2.69 mgGAE/g), germinated red bean (3.24 mgGAE/g), germinated pigeon pea (1.42 mgGAE/g), but lower than that observed in germinated bambaranut (7.01 mgGAE/g) and germinated African breadfruit (10.80 mgGAE/g). Increases in phenolic content of germinated seeds have been explained to be as a result of migration of phenolic compounds to the outer layer of the seed during germination (Duenas *et al.*, 2009). Increase in total phenolic in soaked seed could also be as a result of possible solubilization of condensed tannins during soaking (Sokrab *et al.*, 2012). Roasting had the highest total phenolic content in this study and this concurs with the total phenolic content of maize and beans when subjected to certain kinds of thermal processing (Kwon, 2007). As cellular constituents and membranes break down, heat processing may release more bound phenolic acid. Phenolic compounds are known to accumulate in vacuoles (Dewanto *et al.*, 2002). Phenols contain hydroxyl, which can produce more phenolic hydroxyl and hydrogen atoms with a higher number of total phenols, making it a better source for scavenging impact on free radicals (Zhao *et al.*, 2014). Processed *Prosopis africana* seed is a major source of antioxidant activity in food, as evidenced by its high phenolic content.

Anthocyanin content of processed *Prosopis africana* seed, as reported in Table 4.3, was found to be low. This finding is in agreement with the finding by Morata *et al.* (2003) which could be attributed to the absorption mechanism between the fermenting flora and anthocyanin. Natural enzymes (like glucosidase, peroxidase, polyphenol oxidase) activated during fermentation might oxidize secondary metabolites like anthocyanin, thus reducing their concentration (Afoakwa *et al.*, 2012). The anthocyanin content for the various pre-processing operations obtained in this study was much higher when compared with the result obtained for red bean (2.41 mg/100g), pigeon bean (2.52 mg/100g), African breadfruit (2.35 mg/100g), African yam bean (2.30 mg/100g) and African oil bean (2.16 mg/100g) (James *et al.*, 2020). This positions *Prosopis africana* seed as a better source of anthocyanin than aforementioned legumes.

By chelating or reducing soluble iron, Vitamin C, a water-soluble antioxidant, promotes its absorption. It can rebuild other antioxidants, including tocopheroxyl, from their radical species and scavenge free radicals (Mubarak, 2015). Vitamin C is vital for the healthy operation of the thyroid and adrenal glands as well as for strong teeth, gums, and bones. The vitamin C obtained in the study was lower than what was reported by Omokpariola *et al.* (2021) for fried (38.67 mg/100g), boiled (29.73 mg/100g) and roasted (28.86 mg/100g) *Afzella africana*. The low Vitamin C content of *Prosopis africana*, as shown in this investigation, is in concordance with the assertion made by Oboh (2006) that legumes consist mainly of extremely low vitamin C compared to leafy vegetables and fruits. This could be attributed to the dry nature of most legumes as reports have shown that sun drying reduces vitamin C; this will increase the risk of scurvy and impair iron absorption, since vitamin C enhances the absorption of non-heme iron present in plant food materials (Oboh, 2006).

Different pre-processing operations adopted in this study showed a very low content of flavonoid content in *Prosopis africana* seed, as shown in Table 4.3, when compared with other legumes, such as bambaranut (14.97 mg/100g), cowpea (11.32 mg/100g), red bean (11.50 mg/100g), pigeon pea (11.51 mg/100g) and African yam (11.22 mg/100g) (James *et al.*, 2020). Germination showed the lowest content of flavonoid, which concurs with the finding that germination decreases flavonoid (James *et al.*, 2020). Low flavonoid content will lead to lower antioxidant capacity and reduced anti-inflammatory effect.



Carotenoids being lipophilic plant pigments are found in nature in large quantities. Their pro-vitamin A activity, anti-oxidant activity, capacity to control gene transcription, improvement of gap junction communication, phase II enzyme inducing activity, and capacity to enhance immune function make them useful biological pigments that are frequently used in food (Hu, 2003). The carotenoid contents for different pre-processing operations shown in this study provide evidence that processed *Prosopis africana* seed is a very good and desirable source of beta-carotene when compared to some legumes studied by James *et al.* (2020). This difference in the carotene content of the different legumes may be as a result of species, breeding, soil quality, climate and temperature and light exposure.

Vitamin E was the least antioxidant present in processed *Prosopis africana* seed, as revealed in this study. These values however are higher than what is reported for some legumes like: African yam bean (0.19 mg/100g), kidney bean (0.10 mg/100g), lima bean (0.18 mg/100g) but far below that of Mung bean (0.51 mg/100g) and Marama bean (6.27 mg/100g) (Charrondiere *et al.*, 2020). The low amount of Vitamin E found in this study is consistent with a previous finding that heat reduces the vitamin content of legumes. This may be explained by the fact that vitamins are very susceptible to oxidation and leaching into water-soluble media, which causes them to be lost during processing (Dary *et al.*, 2010). The values for Vitamin E obtained in this study are lower than what Oluwafemi *et al.* (2021) reported for unprocessed *Prosopis africana* seed (11.67 mg/100g). Further, when compared with fried (6.35 mg/100g) and roasted (7.46 mg/100g) *Afzella Africana*, the values observed in this study were higher than those obtained for boiled *Afzella africana* seed (0.19 mg/100g). Vitamin E shields bodily tissues from the damaging effects of free radicals, which can damage organs, tissues and cells, as supported by Omokpariola *et al.* (2021), making them a very desirable food content.

Antioxidant Activities of Processed *Prosopis Africana* Seeds

Roasting having the highest total phenolic content (8.43 mgGAE/g) also had the highest Ferric reducing antioxidant power activity. This agrees with the report by Oboh (2006) which provides evidence of significant correlation between total phenol content and antioxidant activity. Control, having the lowest content of phenol, as obtained in this study, also exhibited the lowest ferric reducing antioxidant power activity. This could be attributed to the plants' capacity to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which is reduced since there are fewer molecules available to give electrons. The FRAP values obtained was higher than what Marathe (2011) reported for pigeon pea (16.19 $\mu\text{mol/g}$), lablab bean (6.26 $\mu\text{mol/g}$) and fenugreek (37.85 $\mu\text{mol/g}$). *Prosopis africana* seed under various pre-processing operations adopted for this studies had a better ferric reducing power activity, as shown in Table 4.4 when compared to groundnut (41.44 %), African oil bean (7.18 %), African yam bean seed (39.48 %) and *Africana* breadfruit (9.3 %) (James *et al.*, 2020).

The ability of *Prosopis africana*'s seeds to scavenge free radicals is widely assessed using the DPPH scavenging assay due to its straightforward, sensitive and reproducible procedure (Mayachiew & Devahastin, 2008). The values obtained for DPPH in this study was very high compared to what was reported by Zhao *et al.* (2014) for lentils (11.8 %), lima bean (14.5 %) and navy bean (14.8 %), placing *Prosopis africana* seed as an excellent free-radical scavenging food. Roasting with the highest total phenolic content also showed the highest DPPH radical scavenging activity; this could be due to the fact that phenols are excellent free radical scavengers. Polyphenols protect cells against the effects of oxidative stress through the ability



to scavenge free radicals (Scalbert, 2005). The high DPPH ability of *Prosopis africana* seed is advantageous due to its protection against oxidative stress and anti-inflammatory effects.

One extremely reactive free radical produced by the Fenton reaction is the hydroxyl radical (OH). Under specific physiological conditions, the human body may produce this free radical from hydrogen peroxide and the superoxide anion. When OH reacts with aromatic compounds, double bonds are created, which results in the production of other oxygen-reactive free radicals, including the hydroxy-cyclohexadienyl radical (Lee *et al.*, 2004). Though slightly lower than that recorded for black kidney beans (85.68 %), the OH activity found in this study is comparable to that reported by Zhao *et al.* (2014) for cowpeas, particularly for the boiled and soaked samples.

Relationship between Antioxidant Properties and Activities

The result of correlation analysis between anti-oxidant properties and activities of the processed *prosopis africana* seed showed a significant relationship, as revealed in Table 4.5. The finding in this study is in concordance with the result obtained by Sarah (2020) for cowpea and groundnut, Campos-Vega *et al.* (2010) for commonly consumed dry bean in United States, Gujral *et al.* (2013) for 6 different legumes: [green gram (*Vigna radiate*), red kidney beans (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), red lentil (*Lens esculenta*), soybean (*Glycine max*), and moth beans (*V. aconitifolia*)] and Wojdyło *et al.* (2001) for 32 selected herbs. All the aforementioned literature affirmed that higher total phenolic content increases antioxidant activities, which in this study are OH and FRAP but not DPPH. However, our findings were not in agreement with the discovery of Oboh (2006), which reported that higher phenolic content leads to a higher free radical scavenging activity in cowpea, *V. unguiculata* (brown) and underutilized legumes, *C. cajan* (brown) and *S. sternocarpa*. According to Lee *et al.* (2021), the total phenolic content of adzuki bean, common bean and soybean is significantly and positively correlated to FRAP, and this is in agreement with our findings. Also, total phenolic content of adzuki bean and soybean is significantly and positively correlated with DPPH but negatively correlated for common beans.

IMPLICATION TO RESEARCH AND PRACTICE

This research shows that the type of pre-processing method adopted in the processing of *Okpehe* has an effect on the antioxidant properties and activities of the *Okephe*.

CONCLUSION

The effect of pre-processing operations on the antioxidant properties and activities of processed *Prosopis africana* seeds was examined in this study. Beta carotene was the most abundant antioxidant (72.59–103.85 %) but the least is Vitamin E. The result further revealed that all the antioxidants except Vitamin C had a significant relationship with one or two antioxidant activities. Based on the findings of this study, we recommend processed *Prosopis africana* seed for consumption as a good source of antioxidants. Boiling and germination are recommended as better pre-processing operations for good availability of beta carotene, which is the highest antioxidant present in processed *Prosopis africana* seed.



FUTURE RESEARCH

Studies can be carried out on the effect of pre-processing methods on the anti-nutrient composition of *Okpehe* and possible ways of extracting the antioxidants present in *Prosopis africana* seed.

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