Evaluation of the Anti-nutrients, Amino Acid Profile and Physicochemical Properties of*HuraCrepitan* Seed Flour used in Food Production.

Abstract:

The study examined the antinutritional factors, amino acid profiles, and chemical characteristics of sandbox seed.~~The antinutritional, amino acid profiles, and chemical characteristics of sandbox seed were examined in this study.~~Four treatments of the seed flour samples—raw, heated, roasted, and fermentedwere created ~~Using~~ using conventional techniques, ~~four treatments of the seed flour samples—raw, heated, roasted, and fermented—were created,~~ and their physicochemical composition, amino acid content, and antinutritional components were examined. Standard methods were used to extract and characterize the seed oil from the flour samples ~~and characterize it~~. The flavonoids (17.50% in raw seed), alkaloids (6.20% in raw), and tannins (5.24% in raw) were the anti-nutrients in the seed flour that were of concern. The anti-nutrients in the sand box seeds were shown to be more effectively reduced by fermentation and moist cooking, also known as moist heat treatment. Twenty amino acids were found in the seed flour after the amino acid profiles were assessed. Arginine (3.25g/100g cooked and 8.05g/100g fermented), glutamic acid (6.05g/100g cooked and 10.2g/100g fermented), and valine (8.03g/100g raw and 8.58g/100g fermented) were the three main ones implicated. With a chemical score of 44.52%, methionine is the limiting amino acid. The free fatty acid values of the sandbox seeds under evaluation ranged from 3.60% to 6.03%, with no significant variations (P>0.05) seen in their physicochemical attributes. The samples' peroxide values vary from 2.96% to 44.81%, and their iodine values range from 104.94% to 126.90%. Thus, the study proposed that the anti-nutrients in the sandbox seed might be significantly reduced by using fermentation and/or moist cooking. *Huracrepitan*~~has also been shown to~~ ha~~ve~~d strong physicochemical qualities and significant levels of necessary amino acids; as a result, it can be used in several food industries where protein (amino acids) is crucial.

Keywords: *sandbox seed, anti-nutrients, amino acids, physicochemical properties, cooking, fermentation, iodine value, Peroxide value.*

1.0 Introduction

Huracrepitan, commonly referred to as "possum wood" and "*jabillo*" in Spanish, is an evergreen tree belonging to the spurge *Euphorbiaceae* family. It is indigenous to the Amazon Rainforest in tropical parts of North and South America (PIER, 2005). Its brown bark bears numerous black, pointy spines that help identify it. According to reports, the *Huracrepitan* tree (plate 1.1) can reach heights of up to 30 m~~etres(100 feet)~~ in the South American Amazon region. Its big, oval leaves are extremely thin, like paper (Adewale et al., 2014). The trees can reach a height of 10 m and are planted as shade trees along the walkways in church premises, parks, cities, and schools. ~~They can reach a height of 10 metres.~~ The fruits are tyre-shaped capsules (plate 1.3), measuring from 3 to 4 cm thick and from 5 to 6 cm in diameter. Around the central axis of the capsule are

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sixteen carpels distributed radially (Adewale et al., 2014). Capsules burst when ripe and dry, releasing some seeds up to 14 yards away. The ripe, dried fruit makes an explosion sound when it splits into segments, hence the name "Dynamite" tree. The seeds have a smooth brown hue and a round, flattened diameter of approximately 2 cm. There are several applications for this tree. For example, the plant secretes a yellowish-milky latex that the Native Americans used to make poisoned darts (Jones, 2007; Charles et al., 2007).

In Nigeria, presently, the *Huracrepitan* tree is seen as an ornamental tree used to provide shade along walkways and within large ~~premises~~premises, such is its present relevance within the campus of the Federal University of Technology, Owerri.

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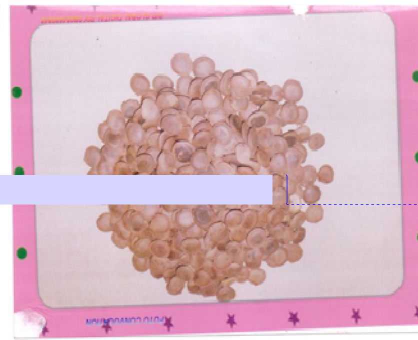

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Plate 1.1: ~~Friuts~~Fruits of *Huracrepitan*Tree

Plate 1.2: Seed of *Huracrepitan*

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Plate 1.3: Matured Dried Fruit of *Huracrepitan*

The best source of nutrients can be found in many wild plants and seeds, particularly during periods of food scarcity (Hamza, 2002). However, little is known about the nutritional and anti-nutritional characteristics of some of these wild plants and seeds. Research on these kinds of plants and seeds could be useful in determining the qualities and possibilities of the plant or seed. If the anti-nutritional profiles of the plant or seed are not investigated, using them in food or pharmaceutical preparations may also result in long-lasting adverse effects, some of which may be lethal. One such seed that needs research is the ~~*hura*~~ *Huracrepitan* seed. The potential of the seed for use in human and animal diets is thought to be revealed by an excellent nutrient balance in the seed flour, particularly with regard to protein and amino acids. As a result, the study will look at the Huracrepitan seed flour's anti-nutritional content, amino acid profile, and phytochemical analysis.

2.0. MATERIALS AND METHODS

2.1. Collection of Raw Materials

The mature dry intact (indehiscent) fruits of the sand box (*Huracrepitan*) seeds were harvested from some plants on Federal University of Technology Owerri campus.

2.2. Processing of the *HuraCrepitan* Seeds

To get rid of dirt and other foreign objects, the seeds were separated. Four sections of the cleaned seeds were placed in separate glass containers with unfastened lids for additional processing. The seeds in the first container (E1) were ground into flour using a Monilex blender after being dried for ~~six hours~~6 h at 60 ~~degrees Celsius~~°C in a Gallen Kamp moisture extraction oven. For ~~two hours~~2 h, the seeds in the second container (E2) were cooked. The cooked seeds were placed on a dish and let to dry for 24 h~~ours~~ at 65 ~~degrees Celsius~~°C in a Gallen Kamp oven. Additionally, the dry seeds were ground into a fine flour using a Monilex blender. After 50 min~~utes~~ at 70 ~~degrees Celsius~~°C, the seeds in container E3 were roasted. Additionally, the roasted seeds were ground into a fine flour using a Monilex blender. After ~~two hours~~2 h of boiling, the cotyledons of the seeds in the fourth container (E4) became extremely tender. After discarding the water, the roasted seeds were placed in a basket and covered with plantain leaves. For three days, they were allowed to ferment. After three days, the fermented seeds were crushed into a flour using a Monilex mixer and dried in a Gallen Kamp moisture extraction oven set at 60°C for ~~six hours~~6 h. All of the samples were held as stock in the freezer (OC) and preserved in clearly labelled, airtight glass containers.

2.3. Determination of Anti-nutrient in *Huracrepitan* Seed Flour

2.3.1. Alkaloid Content

A 250 ~~ml~~ mLbeaker containing ~~five grammes~~5 g of the sample was filled with 200 ~~ml~~ mLof 20% ethanolic acetic acid, covered, and left to stand for ~~four hours~~4 h. The extract (filtrate) was concentrated to one-fourth of its original volume using a hot-water bath after the 4-h~~our~~ period had ended. The sample had been filtered. Once the extraction had reached the point of precipitation, a concentrated ammonium hydroxide solution was added drop by drop to the extract. Filtration was used to gather the precipitate after the entire solution was given time to

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settle. There was a filter paper that was pre-weighed. An oven set to 70°C was used for six hours to dry the filter paper and its contents. Then, in accordance with Odimegwu et al. (2019)'s instructions, it was weighed after cooling in a desiccator.

2.3.2. Determination of Tannin Content

Tannin was measured spectrophotometrically using a Unico UV-2102 Spectrophotometer using the Price and Butler (1977) technique. A 100 mL plastic bottle was filled with 500 mg of the sample. After adding 50mL of distilled water, the sample was agitated on a mechanical shaker for 1hour. After filtering, the sample was filled to the mark with distilled water in a 50 mL volumetric flask. Three millilitres of 0.1 M FeCl in 0.1N HCl and 0.008M potassium ferocyanide solution were combined with 5mL of the filtrate that had been pipetted out into a tube. In less than 10min, the absorbance of the sample at 120 nm was determined using a spectrophotometer. A blank sample was prepared and the colour was developed and read at the same wavelength. A standard was prepared using tannin acid to get 100ppm and measured.

$$mg/cm^3\ Tannin = \frac{Absorbance\ of\ sample}{Absorbance\ of\ Standard}\ x\ \frac{Conc.of\ Standard}{1} \qquad (1)$$

2.3.3. Determination of Saponins

The saponin content was determined by the method described by Okwu (2005). Twenty grams of the sample was dispersed in 200mL of 20% ethanol. The suspension was heated in water bath at 55°C for 4 h while stirring. The mixture was filtered and the residue re-extracted with another 200mL of 20% ethanol. The combined extract was concentrated to 10mL, in a water bath at 90°C. The concentrate was transferred into a 250mL separating funnel and 20mL of diethylether was added. The sample was agitated and allowed to settle. The aqueous layer was recovered while the other layer was discarded. The purification process was conducted by adding 60mL of n-butanol into the aqueous layer. The mixture was shaken and allowed to separate. The extract was washed twice with 10mL of 5% aqueous sodium chloride in a separating funnel. The twice washed extract was poured into a beaker and evaporated to dryness on a boiling water bath. The extract was cooled reweighed and the value was recorded.

2.3.4. Flavonoid Content

The flavonoid content was determined by the method described by Ofoedum et al (2023). A 100 g of the sample was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a hot water bath and weighed.

2.3.5. The Phenol Content of *Huracrepitan*

Phenols were ascertained using the AOAC (2010) description. A 100 mL Erlenmeyer flask was filled to the brim with 200 mL of *Huracrepitan* seed flour. Methanol (10 mL) was added. For 1hour, the contents of the flask were continually and slowly swirled. Whatman filter paper was utilized to filter the mixture. A test tube was measured to hold 300 µL of filtrate. The Folin-Ciocalteau solution was diluted ten times and added in 1.5 mL. To the filtrate, 1.2 mL of a 7.5% sodium carbonate solution were

also added. The mixture was left to stand for half ~~an hour~~1 h. A UV spectrophotometer was used to measure the absorbance of the mixture~~'sabsorbance~~ at 765 nm. Additionally, a blank was made with 300~~µl~~ µLof distilled water.

Serial dilutions of a standard solution ranging from 0 to 60µg/~~ml~~ mLwere made. The procedure used on the sample was carried out once more. A calibration curve was produced by measuring the absorbance of the working standard~~'sabsorbance~~ at the same wavelength. The calibration curve helped determine the amount of phenol present in the sample.

2.3.6. The Phytate Content of the *Huracrepitan* Seed Flour

The method of A.O.A.C (2010) was adopted for this determination. The phytic acid in the sample was precipitated with excess $FeCl_2$ after extraction of 1g of each sample with 100~~ml~~ mLof 0.5N HCL. The precipitate was converted to sodium phytate using 2ml of 2% NaOH before digestion with an acid mixture that contain equal portion of concentrated H_2SO_4 and 65% $HClO_4$. The liberated phosphorous was measured calorimetrically at 520nm after color development with molybdate solution. The percentage phytate was calculated as;

$$\%\ \mathbf{Phytate} = \frac{100}{Wt}\ X\ \frac{au}{as}\ X\ CX\ \frac{Vt}{Va} \qquad (2)$$

Where;

~~Au~~au= Absorbance of test samples

C= Concentration of Standard Phytate solutions

Vt= Total Volume of extract

Wt= Weight of sample used

~~As~~as= Absorbance of standard phytate solution

Va= volume of extract analyzed

2.4. Determination of Amino Acids of *HuraCrepitan* Seed Flour

The spectrophotometric approach reported by Schroeder et al. (1990) was ~~utilised~~utilized. Ninhydrin interacts with amino acids to generate coloured complexes, the intensity of which is determined by the amount of amino acid present. One gram~~me~~ of well-ground *Huracrepitan* seed flour was placed in a stoppered 250~~ml~~ mLconical flask, and 100~~ml~~ mLof 6MHCl was added to the sample. To hydrolyze the sample, it was cooked in an oven at 50 degrees Celsius for 16 h~~ours~~. The resultant mixture was filtered into another 25~~ml~~ mLconical flask using a double layer of Whatman No. 42 filter paper. A pipette was used to transfer 2 ~~ml~~ mLof the hydrolysate into a 30~~ml~~ mLtest tube. Ten millilitres of buffered ninhydrin reagent were added. The mixture was cooked in a boiling water bath for 15 min~~utes~~. After cooling to room temperature, 3~~ml~~ mLof 50% ethanol was added immediately. Each standard amino acid solution yielded approximately 5µg/~~ml~~ mLworking standard amino acids. These dilutions (working standards) were heated with the buffered ninhydrin reagent, as described above for the sample hydrolysate. The absorbance of sample hydrolyzate and working standards was measured at the appropriate wavelength for each amino acid (Table 1). The calibration curves were created by plotting the absorbance of

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each working standard solution against its corresponding concentration. The slope of each amino acid's calibration curve was determined and used to calculate the % amino acid.

$$\%\ Amino\ acid = \frac{Absorbance\ of\ sample\ x\ gradient\ factorx\ Dilution\ factor}{Weight\ of\ sample} \quad (3)$$

Table 1: Wavelength of colour developed by each amino acid

AMINO ACID	WAVELENGTH	COLOUR
Asparagine	505nm	Light blue
Alanine	620nm	Blue
Aspartic acid	465nm	Purple
Cysteine	600nm	Blue
Glutamic Acid	560nm	Purple
Glycine	525nm	Purple
Histidine	460nm	Purple
Isoleucine	580nm	Light Purple
Leucine	590nm	Purple
Lysine	450nm	Orange yellow
Methionine	525nm	Greenish yellow
Omithine	570nm	Purple
Phenylanine	545nm	Yellow
Proline	470nm	Yellow
Pyrrolysine	455nm	Yellowish
Serine	485nm	Blue
Threonine	615nm	Bluish green
Thyrosine	530nm	Greenish blue
Typtophan	565nm	Yellowish Blue
Valine	490nm	Greenish Blue

2.5. The Chemical Properties of the *Huracrepitan* Seed Oil

2.5.1. Determination of the Acid Value

The determination was done according to the method described by AOAC (1990). One gram of each treatment oil sample was weighed into a 100~~ml~~ mLbeaker; 50 ~~ml~~ mLof neutral alcohol was poured into the beaker and 3 drops of phenolphthalein indicator was added. The solution was heated on a steam bath until boiling. The solution was then titrated with 0.2N ~~Potassium~~ potassium hydroxide solution until permanent slightly pink solution was obtained. The burette reading was noted and the acid value was calculated.

2.5.2. Determination of the Saponification Value

Two ~~grammes~~ grams of each treatment oil sample were weighed into a conical flask (AOAC, 1990). Twenty-five millilitres of 0.5N alcoholic potassium hydroxide solution were then added. On a steam bath, the flask was heated to boiling while the contents were shaken occasionally. It was ~~let~~ allowed to boil slowly for 60 min~~utes~~. The solution was then left to cool slightly. A few drops of phenolphthalein indicator were added, and the sample was titrated with 0.5NHCl until the pink hue of the indicator faded away. The burette reading was recorded. The same technique was used for the blank. The saponification value was then computed and recorded.

2.5.3. Determination of Iodine Value

The iodine value was calculated using the method provided by the AOAC (1990). The oil sample (0.4g) was weighed into a conical flask. To dissolve the oil, add ~~ten millilitres~~ 10 mL of carbon tetrachloride. A well-fitting glass stopper was used to close the flask, and 20~~ml~~ mL of Wij's solution was added to it. The flask was more effectively sealed by moistening the stopper with a minimum of a 10% potassium iodide solution. The flask's contents were briskly stirred before being placed in the dark at 20°C for 21/2 h~~ours~~. At the conclusion of this time, 15 mL of 10% potassium iodide solution and 100 mL of distilled water were added. The content was titrated with 0.1M sodium-thiosulphate solution until the yellow tint faded almost completely. A few drops of 1% starch indicator were added, and the titration was carried out by adding thiosulphate drop by drop until the blue tint faded after vigorous shaking.The iodine value was estimated using the same approach as with the blank.

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2.5.4. Determination of the Peroxide Value

The determination was carried out in accordance with AOAC methodology (1990). One gram~~me~~ of the oil sample was weighed into a clean dry boiling tube, and while it was still liquid, one gramme of powdered potassium iodide and 20 ~~ml~~ mL of a glacial acetic acid-chloroform mixture were added. The tube was then immersed in boiling water until the liquid boiled within 30 sec~~onds~~. The contents of the tube were transferred to a flask containing 20ml of 5% potassium iodide solution. The tube was rinsed twice with 25 mL of distilled water. A few drops of starch solution were added to the mixture, which was then titrated with 0.002 M sodium thiosulphate. The identical method was used for the blank at the same time. The peroxide value was calculated at the conclusion of the experiment.

2.5.5. Determination of Fatty Acid Constituents of *Huracrepitan* Seed Oil

The spectrophotometric method as described by AOAC (2010) was applied. Two grams of oil extracted from the samples of Huracrepitan seeds by Sohxlet extraction, was put into a 100~~ml~~ mL conical flask containing 20~~ml~~ mL of Benzene and shaken thoroughly to extract the fatty acids. The mixture was transferred into a separatory funnel and 2~~ml~~ mL of 10% Copper acetate solution was added to separate the benzene extract. About 10ppm standard solutions of each fatty acids were prepared from each specific standard fatty acid. The ~~absorbances~~ absorbance of the different standard solutions and the sample fatty acids (benzene extracts) were read on a spectrophotometer at a wavelength specified for each fatty acids as follows: Lauric Acid (671nm); Stearic Acid (650nm); Palmitic Acid (630nm); Oleic Acid (670nm); Linoleic Acid (660nm); Linolenic Acid (680nm); Arachidonic Acid (690nm); Behenic Acid (615nm);

Palmitoleic Acid (625nm); Myristic Acid (635nm); Capric Acid (640nm); Caprylic Acid (645nm) Lignoceric Acid (695nm). Using the absorbance of each specific standard fatty acid solutions and their corresponding concentration, the calibration curves were plotted. From the calibration curve of each fatty acid, its gradient was calculated and used in determining the percentage fatty acid as follows;

$$\%\ Fatty\ Acid = \frac{Absorbance\ of\ oil\ extract\ x\ Gradient\ Factor\ x\ Dilution\ Factor}{Weight\ of\ Sample\ taken\ x\ 10{,}000} \quad (4)$$

3.0 RESULTS AND DISCUSSION

Table 2: Mean values of Anti-nutrients (mg/100g) in *H. crepitan*seed flour sample

	Raw seed flour	**Cooked seed flour**	**Roasted seed flour**	**Fermented seed flour**	**LSD**
Alkaloids	6.20 [a]	5.67 [a]	6.00 [a]	5.47 [a]	**3.82**
Tannin	5.24 [b]	2.4 [a]	1.98 [a]	1.50 [a]	**1.98**
Saponin	0.09 [a]	0.08 [a]	0.11 [a]	0.07 [a]	**1.01**
Flavonoids	17.50 [a]	14.50 [a]	16.50 [a]	12.16 [a]	**6.54**
Phenols	0.37 [a]	0.12 [b]	0.19 [a,b]	0.09 [b]	**0.21**
Phytate	0.56 [a]	0.25 [b]	0.32 [a,b]	0.12 [b]	**0.25**

Means with similar superscripts in the same row are not significantly (P>0.05) different.

Table 3: Mean values for the amino acid of *H. Crepitan*seed flour sample (g/100g protein)

	Raw seed flour	**Cooked seed flour**	**Roasted seed flour**	**Fermented seed flour**	**FAO/WHO UNU reference value**	**% chemical score (fermented)**
Alanine	2.61 [a]	1.84 [a]	2.69 [a]	2.78 [a]		
Arginine	7.22 [a]	3.25 [a]	7.28 [a]	8.05 [a]		
Aspartic acid	2.87 [a]	1.79 [a]	3.04 [a]	3.26 [a]		
Cysteine	2.03 [a]	1.54 [a]	2.07 [a]	2.18 [a]		
Glutamic acid	9.87 [a]	6.05 [a]	10.05 [a]	10.21 [a]		
Glycine	2.95 [a]	1.38 [a]	3.21 [a]	3.65 [a]		
Histidine	2.57 [a]	1.27 [a]	2.69 [a]	2.87 [a]	3.4	84.41

Isoleucine	1.69 [a]	1.15 [a]	1.81 [a]	1.95 [a]	4.2	46.43
Leucine	3.21 [a]	2.75 [a]	4.08 [a]	4.14 [a]	4.2	98.57
Lysine	1.93 [a]	1.69 [a]	2.01 [a]	2.08 [a]	4.2	49.52
Methionine	0.78 [a]	0.45 [a]	0.93 [a]	0.98 [a]	2.2	44.55
Phenylalanine	3.66 [a]	1.33 [a]	3.72 [a]	3.77 [a]	2.8	134.64
Proline	2.59 [a]	1.12 [a]	2.71 [a]	2.74 [a]		
Threonine	1.47 [a]	1.04 [a]	1.62 [a]	1.68 [a]	2.8	60.00
Trytophan	1.16 [a]	1.19 [a]	1.23 [a]	1.29 [a]		
Tyrosine	3.14 [a]	1.63 [a]	3.21 [a]	3.26 [a]		
Ornithine	0.25 [a]	0.14 [a]	0.32 [a]	0.35 [a]		
Cystine	1.95 [a]	1.39 [a]	2.09 [a]	2.12 [a]		
Serine	0.72 [a]	0.43 [a]	0.88 [a]	0.97 [a]		
Valine	8.03 [a]	4.66 [a]	8.15 [a]	8.58 [a]	4.2	204.29

Means with similar superscripts in the same row are not significantly ($P>0.05$)~~{p>0.05}~~ different.

Table 4: Mean values of the chemical properties of *H. Crepitan*seed oil

	Oil from Raw seed	Oil from Cooked seed	Oil from Roasted seed	Oil from Fermented seed	LSD
Acid value %	7.63 [a]	5.05 [a]	6.31 [a]	9.12 [a]	6.02
Saponification value (mg KOH/g)	107.99 [a]	99.58 [a]	116.4 [a]	105.19 [a]	20.51
Iodine Value (I_2g/100g)	126.9 [a]	120.5 [a]	171.32 [a]	152.28 [a]	55.98
Peroxide Value (meqO_2/kg of oil)	2.96 [a]	2.54 [a,b]	0.72 [a]	2.94 [b]	2.01
Free fatty acid (%)	3.6 [a]	2.54 [a]	3.15 [a]	4.56 [a]	3.92

Means with similar superscripts in the same row are not significantly ($P>0.05$)~~{p>0.05}~~ different.

3.1. Antinutrients in *Huracrepitan* Seed Flour Samples.

Flavonoids and alkaloids were the most common antinutrients in Huracrepitan seed flour, with values dropping from 17.5% (raw flour) to 12.16% (fermented flour) for flavonoids and 6.2% (raw flour) to 5.47% (fermented flour) for alkaloids (Table 2). There were no significant differences ($P>0.05$) between the alkaloid and flavonoid values of the flour samples. However, there was a significant ($P<0.05$) difference between the oxalate levels in raw flour and cooked and fermented flours. Phenols, phytates, and saponins were detected in the seed flour samples, however their concentrations were less than 1.0 in all treatment samples. The anti-nutrient levels in the *H. crepitan* seed sample were lower than those found by Sani et al. (2013) in their study on L. siceraracia seeds. They found phytate levels (10.70 and 20.84 mg/g), oxalate (5.85 and 6.45 mg/g), and tannin (5.41 and 22.92 mg/g) in raw and fermented seed. Also, the reported phytate and tannin concentrations of Jatropha curcas were high, at 3688.42mg/100g and 590.00mg/100g, respectively (Oladele&Oshodi, 2008).

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Though the levels of all anti-nutrients investigated were lower in roasted seed flours than in raw seed flours, the roasting process had a smaller effect on degrading (lowering) the anti-nutrients studied. Alkaloids and flavonoids appeared to be more resistant to all of the treatments among the nutrients investigated.

Anti-nutrients can be extremely detrimental if present in significant levels in our diets. Phytic acid can bind phosphorus, calcium, iron, magnesium, and zinc in the food, limiting their bioavailability and increasing the risk of certain health problems, such as anaemia caused by iron deficiency (Inuwa et al., 2011). Tannin is an enzyme inhibitor that hinders proper digestion, resulting in protein shortage and gastrointestinal issues. Oxalates limit the absorption of plant amino acids, resulting in proteins of "poor quality". Saponins have an effect on the gastrointestinal lining, which can lead to 'leaky gut syndrome' and auto immunological problems. They are highly resistant to human digestion and can penetrate the bloodstream, eliciting immunological responses (Inuwa et al., 2011). Cyanogenic glycoside is an efficient cytochrome oxidase inhibitor that disrupts aerobic respiratory systems (Bolhuis, 2003).Thus, fermenting the seed would help to solve the problems of nutrient malabsorption and the seeds' toxicological effects.

3.2. Amino Acid Profile of the *Huracrepitan*

Twenty amino acids were found in Huracrepitan seed flour samples: alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, ornithine, cystine, serine, and valine (Table 3). Eleven of these acids are important for human nutrition, including arginine, which babies require. In terms of the amount of amino acids in the raw seed sample (before processing), only three of them, arginine (7.22g/100g), glutamic acid (9.87g/100g), and valine (8.03g/100g), could be considered major in the seed flour. These three also had values that varied from 3.25g/100g (arginine) to 6.05g/100 g (glutamic acid) in cooked seed flour, 7.28g/100g (arginine) to 10.05g/100g (glutamic acid) in roasted seed flour, and 8.05g/100g (arginine) to 10.21g/100g

(glutamic acid) in fermented seed flour. Roasted and fermented seed flours showed valine values of 8.15g/100g and 8.58g/100g, respectively, whereas raw seed flour had a value of 8.03g/100g. Aside from arginine (mostly for newborns) and valine, only leucine, phenylalanine, and tyrosine showed amounts of up to 3.0g/100g in the treatment samples (not cooked). Cooked samples exhibited the lowest amounts for all amino acids tested. This observation implied that the amino acids were soluble in water and hence leached into the cooked water. Roasted samples had slightly higher values (differences not significant at $P>0.05$) ~~$p>0.05$)~~ for all amino acids examined than raw samples. This could be attributable to the increased dry matter resulting from moisture loss (8.0% moisture) in roasted samples versus 10% moisture in raw seed samples. Fermented seed samples likewise exhibited greater values for all amino acids found in the seed than the other treatment samples, although there were no significant ($P>0.05$~~$p>0.05$~~) differences in the values of each amino acid in the samples. Other seeds, such as African locust bean, have shown a similar increase in amino acid levels during fermentation (Omafuvbe et al., 2000). In general, when it comes to nutrition for all ages, the most prevalent essential amino acid in *Huracrepitan* is valine, which has a value of at least 8.09 g/100g and can withstand roasting and fermentation. Glutamic acid is the most abundant amino acid (about 10.0g/100g).

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The nutritive value of plant protein is typically assessed by comparing its essential amino acid content to the World Health Organization's (FAO/WHO/UNU, 1991) reference standards for ideal protein quality, which are based on the amino acid requirements for children aged 2 to 5 years (Nweke et al., 2011). As a result, our findings revealed that Huracrepitan seed contains all of the essential amino acids required, with phenyalamine and valine having relative chemistry scores greater than 100% at 134.64% and 204.29%, respectively. Methionine is the limiting amino acid, with a chemical score of 44.55%. The seed contains a high concentration of essential and non-essential amino acids, making it a nutritious diet for humans and animals alike. Amino acids are regarded as critical for human health since they contribute significantly to the health of the neurological system, hormone production, and muscular construction. Furthermore, they are required for critical organs and cellular structure. Essential amino acid deficiencies can induce hormonal imbalances, lack of attention, irritability, and even depression (Papes et al., 2001). The amino acids valine, leucine, and isoleucine contribute to muscle strength, endurance, and stamina. During periods of valine deprivation, the gastrointestinal system absorbs all other amino acids less adequately (Papes et al., 2001).

3.3. Chemical Properties of *Huracrepitan* Seed Oil

The acidity levels of the materials tested ranged from 5.05% (cooked) to 9.12% (fermented flour). The raw seed flour exhibited an acidity level of 7.63% (Table 4). This observation implied that heating caused the evaporation of certain volatile acids in the samples, but fermentation created additional acids as expected. Huracrepitan seed oil has higher acid values than groundnut oil (5.99%) as reported by Atasie et al. (2009) or soya bean oil (4.28%) as reported by Akanni et al. (2005). A high acid level suggests that the oil is likely to oxidise. The acid value, which is

twice the free fatty acid (FFA) value, indicates how many fatty acids (a component of oil) are separated from their parent molecules (triglycerides or phospholipids). Cleavage of a free fatty acid from a parent molecule indicates hydrolytic breakdown and is commonly employed as an indicator of stress in biological systems (Nas et al., 2004).

The saponification values of the oil samples varied from 99.58mgKOH/g (cooked flour sample) to 116.4mgKOH/g (roasted flour sample). The readings for the oil samples were lower than the range of 188mgKOH/g to 196mgKOH/g for most plant-based oils (Pearson et al., 1986). Furthermore, the saponification value reported for palm oil is 200mg KOH/g, whereas the value for groundnut is 193mg KOH/g and the value for coconut oil is 257mg KOH/g. Ejikeme et al. (2010) found that *Huracrepitan* seed oil has a saponification value of 116.00mgKOH/g, comparable to *Afzeliaafricana* seed oil and *Perseagratesima*(106.60mgKOH/g). The saponification values observed for *Huracrepitan* seed oil indicated that it might be combined with oils having greater saponification values, such as coconut oil, in soap manufacturing. This theory is supported by the fact that Huracrepitan seed oil contains a reasonably high concentration of lauric acid (14.67%/100g), which is also found in coconut oil.

The iodine levels of seed flour oil samples ranged from 120.5 g I2~~g~~/100g (cooked sample) to 171.32g I2~~g~~/100g (roasted sample), however there were no statistically significant ($P>0.05$~~p>0.05~~) variations among the samples. Because the higher the iodine value of an oil, the more unsaturated it is. This observation revealed that frying may have aided in the leaching of unsaturated fatty acids, whereas roasting aids in their retention in oil. The *Huracrepitan* oil's iodine value range of 120.5 - 171.32 places it in the semi-drying region, making it suitable for usage in the surface coatings industry to modify alkyl resins (Ejikeme et al., 2010). *Huracrepitan* seed oil has an iodine value comparable to soybean (117-143) and rapeseed oil (94-120), but it is greater than coconut oil (10.0) (Obasi et al., 2012). The iodine value represents the number of double bonds in the oil. It is not a quality metric, but rather an indicator of oil composition.

The peroxide levels in the seed flour oil samples varied from 0.72 meq O_2/kg (roasted seed flour oil) to 2.96 meqO_2/kg (raw seed flour oil). The roasted flour oil had a significant ($P<0.05$~~p<0.05~~) difference in peroxide value (0.72 meq O_2/kg of oil) compared to other samples. Roasted seed flour oil had the lowest peroxide value among the oil samples. This indicated that oil oxidation was lower in roasted seeds. The peroxide values found for *Huracrepitan* seed oil ranged from 0.72 meqO_2/kg to 2.96 meqO2/kg, which are comparable to the 1.50 meqO2/kg reported for groundnut oil and 2.5 meqO2/kg recorded for cotton seed oil by Kyari (2008). The low number suggests that the oil can withstand lopolytic hydrolysis and oxidative degradation. The ~~perioxide~~peroxide value assesses hydroperoxide compounds. It is an excellent indication of the oil's major oxidation products (Nas et al., 2004).

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4.0. Conclusion and Recommendation.

The findings of this study revealed that *Huracrepitan* seeds contain a high concentration of proteins in the form of amino acids. The high amino acid profile of *Huracrepitan* seed showed that it could be effective as a food supplement for human nutrition, particularly for newborns and children whose protein-energy and malnutrition levels have continued to impede growth and development. Similarly, it could be employed in the composition of animal diets to prevent nutrient deficiencies. The study also found that *Huracrepitan* seed includes several anti-nutritional components, with flavonoids, alkaloids, and tannins being the most prominent. The fermentation technique was shown to be more successful at reducing or degrading each of the antinutrients under consideration.

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As a result, the oil or flour should be refined to improve their edibility because they have good physical and chemical properties.

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