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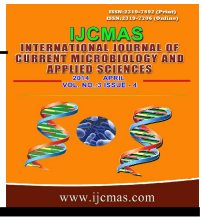

Original Research Article

Evaluation of the antinutritional properties of the seed of chinese fan palm (*Livistona chinensis)*

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Keywords

anti-nutrients, heat processing, heat resistance, whole, pulp

A B S T R A C T

The anti-nutrients present in the pulp, raw seed, blanched (4, 6, and 8min), cooked (20, 40 and 60min) and the roasted (110^0C for 5, 10 and 15min) seeds were determined. Nine (9) anti-nutrients (Tannin: 0.49%, Phytates: 1.20%, Oxalates: 0.18%, Saponins: 5.50%, Hydrogen cyanide: 1.46mg/kg, Alkaloids: 6.00%, total phenols: 0.61%, Flavonoids: 4.00% and oligosaccharides: 27.50%) were found to be present in the raw seed. During processing there was a general reduction trend of the anti-nutrients at different rates but total elimination of saponins, alkaloids and flavonoids was achieved at 40min cooking while oxalates were totally eliminated at 60min cooking. Generally, the levels of the anti-nutrients all decreased with increased heat processing (Table 3) except for tannins which remained stable with all the different heat treatments but were just slightly reduced by 40 and 60min cooking from 0.49% to 0.48% and from 0.48% to 0.47% respectively (Table 3). Tannins have been noted for their relative characteristic heat resistance. The longer persistence of tannins during the heat processing in this study may likely be as a result of the nature of the seed which is very hard and tough, and was in a whole unbroken form during the heat processing.

Introduction

Livistona chinensis commonly known as Chinese Fan palm or Chinese Fountain palm is of the family, Arecaceae (palm family) and belongs to the genus *Livistona* (Naoto *et al*., 2000). It is native to southern Japan, Taiwan and several Islands in the southern China Sea. It is a medium-sized, slow-growing, single-trunked palm tree that reaches about 15.2m tall in its natural habitat but often seen at much shorter heights of 3 – 8m (Forest, 2003). The leaf sheaths are fibrous, fluffy and brown-coloured somewhat like nests of birds. It has oval to round olive-like fruits that change from green to blue-black when ripe (Wagner *et al.,* 1999).

In most developing tropical countries, the food situation is worsening owing to

increasing population, shortage of fertile land, high prices of available staples and restrictions on the importation of food (Nwosu, 2011). This has resulted in a high incidence of hunger and malnutrition, a situation in which children and women, especially pregnant and lactating women are most vulnerable (Potter and Hotchkiss, 1995; Nwosu, 2011). Prediction of future rates of population increase and food production emphasizes the seriousness of this problem (FAO, 1990). Okaka *et al.* (1992) and Nwosu (2011) noted that there is no single solution to the problem of food shortages and crisis. In essence, all information on new sources of food will be of value in the food security struggle.

As recommended by Okaka *et al.* (1992) and noted by Nwosu (2011) that although measures are being taken to boost food production by conventional agriculture, a lot of interest is currently being focused on the possibilities of exploiting the vast number of less familiar food plant resources. Many of such plants have been identified but lack of data on their chemical composition has limited the prospects for their broad utilization (Viano *et al.,* 1995). Most reports on some lesser-known and unconventional crops indicate that they could be good sources of nutrients and many have the potentials of broadening the present narrow food base for humans (Nwosu, 2011).

Livistona chinensis (Chinese Fan Palm) tree resembles that of *Cocos nucifera* (coconut), the cross-sectional profile of the seed also resembles that of coconut and both belong to the same family, Arecaceae (Genini *et al.,* 2009).

It is mainly planted for ornamental reasons (Juliana *et al.,* 2003). The seeds have been noted to be astringent, contain phenolic compounds and used traditionally by the Chinese as an anti-cancer agent (Juliana *et al.,* 2003; Gurpreet and Roman, 2008; Singh and Kaur, 2008; Tao *et al.,* 2009). But in areas where this plant is found in Nigeria, their seeds are left to waste after maturity. Also its sparing distribution, astringent nature, high phenolic compounds composition, lack of knowledge and documentation of its chemical composition has restricted its use to traditional medication rather than food.

Application of different processing methods to *Livistona chinensis* (Chinese Fan palm) seed, its seed oil properties' determination, proximate composition determination, and its toxicological evaluation will give some useful information, which may increase the utilization of Chinese Fan palm seeds and enhance its potential in food formulations. It is envisaged that a more suitable process for the reduction or elimination of any detected anti-nutritional factors may be found for the production of safer Chinese Fan Palm seed products.

Despite the importance of the palm family, *Arecaceae,* and particularly *Livistona chinensis* which has been used traditionally by the Chinese as an anti-cancer agent (Singh and Kaur, 2008), little has been systematically documented about its utilization as food; the proximate composition, oil extract and effect of processing on the anti-nutrients it contains. Furthermore, they are mainly planted for ornamental reasons (Corlett, 2005) and the fruits are not utilized as food but rather left to waste after maturity in many places they are found in Nigeria.

The fruit *of Livistona chinensis* has also been noted by many researchers (Gurpreet and Raman, 2008; Juliana *et al.,* 2003; Singh and Kaur, 2008; Fabiana *et al.,* 2006) to be astringent and contain

phenolic compounds which could be part of the reason why it is not utilized as food. This research will seek to find answers to some of the problems of its utilization through appropriate processing.

The objectives of this research are;

To determine the anti-nutrients present in *Livistona chinensis* seed.

To evaluate the effect of blanching, cooking and roasting on the anti-nutrients present in *Livistona chinensis* seed.

This study will give an insight on the effect of blanching, cooking and roasting on the anti-nutrients in the seed of *Livistona chinensis*.

Through this way, some possible level of utilization of *Livistona chinensis* seeds will be achieved. This in essence will also be a step forward in the food security struggle in Nigeria through non-conventional food sources.

Materials and Methods

Source of Raw Material.

The fresh fruits of *Livistonia chinensis* was obtained from Amaigbo in Nwangele L.G.A of Imo State, Nigeria.

Equipment and Chemicals Used.

All equipments and chemicals used are available at National Root Crops Research Institute (NRCRI), Umudike and Federal University of Technology (FUTO), Owerri, Imo State.

Sample Preparation

The pulp of the fruit was removed manually with a knife. The separated seed was dried in an oven (Gallenkamp hot box oven) at 60^0C for 3h. The dried sample was milled and kept in airtight containers. From there, the sample for anti-nutritional properties analysis was taken. The pulp was also analysed for anti-nutritional properties. A completely randomized design and one-way analysis of variance (ANOVA) was used for the experiment.

Plate.1 *Livistona chinensis* (chinese fan palm) fruit

Plate.2 *Livistona chinensis* (chinese fan palm) seeds

Blanching, Cooking and Roasting

Blanching and cooking were done by the procedures described by Nwosu (2011). The fruit was manually removed of its pulp and the seed was taken in its whole

form (without milling) for blanching, cooking and roasting treatments. The seed was divided into Nine (9) batches (1, 2, 3, 4, 5, 6, 7, 8 and 9) of 300g each. Batches 1, 2 and 3 were given a hot water (100^0C) blanching treatment for 4, 6 and 8min respectively. Batches 4, 5 and 6 were cooked for 20, 40 and 60min respectively. While batches 7, 8 and 9 were roasted at 110^0C for 5, 10 and 15min respectively. The samples were left to cool after the treatments. The blanched and cooked samples were dried (Gallenkamp hot box oven) at 60^0C for 3h to a moisture content of 35%. The Nine (9) processed batches were all milled, allowed to cool and stored in airtight containers. Samples were taken from the airtight containers for anti-nutritional factors determination.

Anti-nutritional factors determination

The anti-nutritional factors in 'Chinese Fan Palm' seed and pulp were determined as follows:

Determination of tannins

Tannin content of the sample was determined by Folin Denis Colorimetric method (Kirk and Sawyer, 1998). A measured weight (1g) of the processed sample was mixed with distilled water in the ratio of 1:10 (W/V). The mixture was agitated for 30min at room temperature and filtered to obtain the extract.

A standard tannic acid solution was prepared 2ml of the standard solution and equal volume of distilled water were dispersed into a separate 50ml volumetric flasks to serve as standard and reagent blank respectively. Then 2ml of each of the sample extracts were put in their respective labeled flasks.

The content of each flask was mixed with 35ml distilled water and 1ml of the Folin Denis reagent was added to each. This was followed by 2.5ml of saturated Na_2CO_3 solution. Thereafter each flask was diluted to the 50ml mark with distilled water and incubated for 90min at room temperature. Their absorbance was measured at 710nm in a colorimeter (Jenway 6051) with the reagent blank at zero. The tannin content was calculated as shown below:

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va} \times D$$

Where W = weight of sample
au = absorbance of test sample
as = absorbance of standard tannin solution
C = Concentration of standard tannin solution
Vt = Total volume of extract
Va = Volume of extract analyzed
D = Dilution factor (if any)

Determination of saponins

This was done by the double solvent extraction gravimetric method (A.O.A.C., 1990). Two grams (2g) of the processed sample was mixed with 100ml of 20% aqueous ethanol solution and incubated for 12hours at a temperature of 55^0C with constant agitation. After that, the mixture was filtered through whatman No 42 grades of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30min and the extracts weighed together.

The combined extract was reduced to about 40ml by evaporation and then transferred to a separating funnel and equal volume (40ml) of diethyl ether was added to it. After mixing well, there was partition and the upper layer was discarded while the lower aqueous layer was re-extracted with the ether after which its pH

was reduced to 4.5 with dropwise addition of NaOH solution.

Saponin in the extract was taken up in successive extraction with 60ml and 30ml portion of n-butanol. The combined extract (ppt) was washed with 5% of NaCl solution and evaporated with a water bath in a previously weighed evaporation dish. The saponin was then dried in an oven (Gallenkamp Hot box Oven) at 60^0C (to remove any residual solvent), cooled in a desiccator and re-weighed. The saponin content was calculated as shown below:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W}$$

Where W = Weight of sample used
W_1 = Weight of empty evaporation dish
W_2 = Weight of dish + saponin extract

Determination of alkaloid

The alkaline precipitation gravimetric method (Inuwa *et al.,* 2011) was used.

A measured weight (1g) of the processed sample was dispersed in 30ml of 10% acetic acid in ethanol solution. The mixture was shaked well and allowed to stand for 4h at room temperature. The mixture was shaken periodically at 30min interval. At the end of this period, the mixture was filtered through whatman No.42 grade of filter paper.

The filterate (extract) was concentrated by evaporation, to a quarter of its original volume. The extract was treated with dropwise addition of concentration NH_3 solution to precipitate the alkaloid. The dilution was done until the NH3 was in excess.

The alkaloid precipitate was removed by filteration using weighed whatman No.42 filter paper. After washing with 1% NH_4OH solution, the precipitate in the filter paper was dried at 60^0C in an oven (Gallenkamp hot box oven) and weighed after cooling in a desiccator. The alkaloid content was calculated as shown below:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where W_1 = Weight of empty filter paper
W_2 = Weight of filter paper + alkaloid precipitate

Determination of total phenols

This was determined by the folin-ciocateau spectrophotometeric method (AOAC, 1990). The total phenols was extracted in 0.2g of the sample with 10ml concentrated methanol. The mixture was shaken for 30min at room temperature. The mixture was centrifuged at 500rpm for 15min and the supernatant (extract) was used for the analysis.1ml portion of the extract from each sample was treated with equal volume of folin-ciocalteau reagent followed by the addition of 2ml of 2% Na_2CO_3 solution. Standard phenol solution was prepared and diluted to a desired concentration.

Iml of the standard solution also treated with the Folin-ciocateau reagent and Na_2CO_3 solution. The intensity of the resulting blue colouration was measured (absorbance) in a colorimeter (Jenway 6051) at 540nm wavelength.

Measurement was made with a reagent blank at zero. The phenol content as calculated using the formula below:

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va} \times D$$

Where

W = weight of sample
au = absorbance of test sample
as = absorbance of standard phenol solution
C = Concentration of standard phenol solution

Vt = Total volume of extract
Va = Volume of extract analyzed
D = Dilution factor (if any)

Determination of hydrogen cyanide (HCN)

This was determined by the method used by Balagopalan *et al*. (1998).

1g of the sample was dispersed in 50ml of distilled water in a 25ml conical flask. An alkaline pikrate paper was hung over the sample mixture and the blank in their respective flasks.

The set up were incubated overnight at room temperature and each pikrate paper was eluted (or dipped) into 60ml distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solution and of the standard were measured colourimetrically at 540nm wavelength with the reagent blank at zero. The cyanide content was determined by the formula shown below:

$$\text{HCN (mg/kg)} = \frac{1000}{W} \times \frac{au}{as} \times C \times D$$

Where

W = weight of sample analyzed
au = absorbance of test sample
as = absorbance of standard HCN solution
C = Concentration of standard in mg/dl
D = Dilution factor where applicable

Determination of phytate

The method described by Nwosu (2011) was used. The phytic acid in the samples was precipitated with excess $FeCl_3$ after extraction of 1g of each sample with 100ml 0.5N HCl. The precipitate was converted to sodium phytate using 2ml of 2% NaOH before digestion with an acid mixture containing equal portions (1ml) of conc. H_2SO_4 and 65% $HClO_4$. The liberated phosphorus was measured colorimetrically (Jenway 6051 Colorimeter) at 520nm after colour development with molybdate solution.

The percentage phytate was thus calculated:

$$\% \text{ Phytate} = \frac{100}{Wt} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where

W = weight of sample used
au = absorbance of test sample
as = absorbance of standard phytate solution
C = Concentration of standard phytate solution
Vt = Total volume of extract
Va = Volume of extract analyzed

Trypsin inhibitor determination

This was done using the spectrophotometric method, described by Nwosu (2011).

A measured weight (10g) of the test sample was dispersed in 50ml of 0.5M NaCl solution and stirred for 30min at room temperature. It was centrifuged and the supernatant filtered through whatman No.42 filter paper. The filtrate was used for the assay.

Standard trypsin was prepared and used to treat the substrate solution (N – benzoyl-DI-arginine-P-anilide; BAPA). The extent of inhibition was used as a standard for measuring the trypsin inhibitory activity of the test sample extract. Into a test tube containing 2ml of extract and 10ml of the substrate (BAPA) 2ml of the standard trypsin solution was added. Also 2ml of the standard trypsin solution was added in another test tube containing only 10ml of the substrate. The latter served as the blank.

The content of the tubes were allowed to stand for 30min and then absorbance of the solution measured at 430nm wavelength with a colorimeter (Jenway 6051). One trypsin activity unit inhibited is given by an increase of 0.01 absorbance unit at 430nm. Trypsin unit inhibited was thus calculated:

$$\text{Trypsin unit inhibited} = \frac{Au}{As} \times 0.01 \times F$$

Where

Au = Absorbance of test sample
As = Absorbance of standard (uninhibited) sample
F = Experimental factor given as:

$$\frac{Vf}{Va} \times \frac{I}{W}$$

Where Vf = Total volume of extract
Va = Volume of extract analyzed
W = weight of sample analyzed

Determination of total steroids

The total steroids was determined colorimetrically with reference to the saponin content (Nwosu, 2011). The saponin crystals were dissolved in 50ml formaldehyde – Conc. H_2SO_4 mixture (1:1 v/v) and the absorbance was measured at 500nm with a colorimeter (Jenway 6051). The steroid content was calculated as follows:

$$\% \text{ Steroids} = \frac{\text{Absorbance} \times 100}{\text{Weight of sample 1}}$$

Oxalate determination

This was carried out by the procedures described by Nwosu (2011).

one gram (1g) of the sample was weighed into a 100ml beaker, 20ml of 0.30N HCl was added and warmed to (40 – 50^0C) using magnetic hot plate and stirred for one hour. It was extracted three times with 20ml flask. The combined extract was diluted to 100ml mark of the volumetric flask.

The oxalate was estimated by pipetting 5ml of the extract into a conical flask and made alkaline with 1.0ml of 5N ammonium hydroxide. A little indicator paper was placed in the conical flask to enable know the alkaline regions. It was also made acid to phenolphthalein (3 drops of this indicator added, excess acid decolorizes solution) by dropwise addition of glacial acetic acid. 1.0ml of 5% $CaCl_2$ was then added and the mixture allowed to stand for 3h after which it was then centrifuged at 300 rpm for 15min. The supernatants were discarded. 2ml of 3N H_2SO_4 was added to each tube and the precipitate dissolved by warming in a water bath (70 – 80^0C). The content of all the tubes was carefully poured into a clean conical flask and titrated with freshly prepared 0.01N $KMnO_4$ at room temperature until the fist pink colour appeared throughout the solution. It was allowed to stand until the solution became colourless. The solution was then warmed to 70 – 80^0C and titrated until a permanent pink colour that persisted for at least 30sec

was attained. The percentage (%) oxalate content was thus calculated:

$$\% \text{ Oxalate} = \frac{100}{W} \times 0.00225 \times \text{Total titre volume}$$

Where W = Weight of sample used

Oligosaccharides determination

The method of Balagopalan *et al.* (1998) was used. One gram of sample was boiled in 100ml of 2M HCl solution until it was negative to iodine starch test. It was centrifuged and the hydrolysate (supernatant) used for the analysis. 1ml of the hydrlysate was missed with 4mls of anthrone reagent in a test tube and boiled for 10min in a water bath while covering the test tubes. After boiling, the mixture was filtered and diluted with distilled water. Similarly, a standard sugar solution (glucose) was prepared and treated as described above and the absorbances of both the samples and sugar solutions were read with Jenway 6051 Colorimeter against a blank reagent at zero.

The oligosaccharides content was calculated as:

$$\text{Percentage sugar} = \frac{Au}{As} \times C \times F$$

Where

Au = Absorbance of test sample
As = Absorbance of standard sugar solution
C = Concentration of sugar solution
F = Experimental factor given by:

$$\frac{Vf}{Va} \times D \times \frac{100}{W}$$

Where Vf = Total filtrate volume
Va = Volume of aliquot analyzed
D = Dilution factor (where applicable)
W = weight of sample used

Determination of flavonoids

Flavonoid was determined using the method described by Harborne (1973).

A measured weight of the processed sample (1g) was boiled in 100ml of 2M HCl solution under reflux for 40min. It was allowed to cool before being filtered. The filtrate was treated with equal volume of ethyl acetate and the mixture was transferred to a separation funnel. The flavonoid extract (contained in the ethyl acetate portion) was received by filtration using weighed filter paper. The weight was obtained after drying in the oven and cooling in a desiccator. The weight was expressed as a percentage of the weight analysed. It was calculated as shown below:

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample 1}} \times 100$$

W_2 = Weight of filter paper + flavonoid precipitate

W_1 = Weight of filter paper alone.

Hemagglutinin determination

Hemagglutinin was determined using the method described by Onwuka (2005).

One (1g) of the sample was weighed out and dispersed in a 10ml normal saline solution buffered at pH 6.4 with a 0.01M phosphate buffer solution. The mixture was allowed to stand at room temperature for 30min and was centrifuged afterwards to obtain the extract. 1ml of trypsinized rabbit blood was added to 0.1ml of the extract diluent in a test tube.

A control sample was also prepared containing only the blood cells.

The test tubes containing the sample mixture and the ones containing the control sample were allowed to stand for 4 hours at room temperature. 1ml of normal saline was added to all the test tubes and they were allowed to stand for 10 minutes, after which their absorbances were read in a colorimeter (Jenway 6051) at 620nm. The hemagglutinin units per milligram of the sample was thus calculated:

Hamagglutinin unit/mg = (b – a) x F

Where b = Absorbance of test sample solution
a = Absorbance of the blank control
F = Experimental factor given by:

$$F = \frac{1}{W} \times \frac{Vf}{Va} D$$

Where Vf = Total volume of extract
Va = Volume of extract used in the essay
W = weight of sample used
D= Dilution factor

Data analysis

The results obtained from the data were subjected to Analysis of Variance (ANOVA) according to Onuh and Igwemma (2000) and SAS (1999).Significant means at $p<0.05$ were separated using Fisher's Least Significant Difference (LSD) test (Onuh and Igwemma, 2000).

Results and Discussion

Effect of processing on the anti-nutritional factors in *Livistona chinensis* seed

The effect of processing on the anti-nutritional factors in Livistona chinensis seed are presented in Table 3 below.
Nine anti-nutritional factors at different levels were found in raw *L. chinensis* seed. There was no trypsin inhibitory activity, hemagglutinin and steroid present in raw *L. chinensis* seed. The anti-nutrients found in the raw seed of *L. chinensis* are tannins, phytates, oxalates, saponins, hydrogen cyanide, alkaloids, total phenols, flavonoids, and oligosaccharides.

The different levels of the anti-nutrients found in the raw seed of *L. chinensis* are as follows: oligosaccharides (27.50%), alkaloids (6.00%), saponins (5.50%), flavonoids (4.00%), Hydrogen cyanide (1.46mg/kg), phytate (1.20%), while total phenols (0.61%), tannins (0.49%) and oxalates (0.18%) were below 1%. The pulp had the same level of tannin (0.49%) with the raw seed. It recorded 0.68%, 0.49mg/kg, 4.00%, 0.50%, 2.00% and 25.00% for phytate, hydrogen cyanide, alkaloids, total phenols, flavonoids and oligosaccharides respectively which were all lower than that found in the raw seed. On the other hand, the levels of oxalate (0.54%) and saponins (6.50%) were higher than that of the raw seed.

Generally, the levels of the anti-nutrients all decreased with increased heat processing (Table 3) except for tannins which remained stable with all the different heat treatments but was just slightly reduced by 40 and 60min cooking from 0.49% to 0.48% and from 0.48% to 0.47% respectively (Table 3). Tannins have been noted for their relative characteristic heat resistance (Jimoh *et al.*, 2011; Olajide *et al*., 2011; Nwosu, 2011). The longer persistence of tannins during the heat processing in this study may likely be as a result of the nature of the seed which is very hard and tough, and was in a whole unbroken form during the heat processing.

Phytates, hydrogen cyanide, total phenols and oligosaccharides, all showed some varying levels of stability during the different heat processing (Table 3). It was observed that phytates were only reduced at 60min cooking from the raw seed value of 1.20% to 0.85%, hydrogen cyanide from 1.46mg/kg to 0.49mg/kg, total phenols from 0.61% to 0.33% while oligosaccharides reduced from 27.50% to 7.5% (Table3). At 15min roasting, the phytates, hydrogen cyanide total phenols and oligosaccharides levels reduced to 1.03%, 0.98mg/kg, 0.47% and 20.00% respectively. In essence, there was no total elimination of these five anti-nutrients (tannins, phytates, hydrogen, cyanide, total phenols and oligosaccharides) with all the different heat treatments (blanching, cooking and roasting) at the different levels tested. This as noted earlier, may likely be as a result of the nature and the state of the raw seed which is hard, tough and was processed in its whole seed form.
Total elimination was achieved for oxalates (at 60min cooking), saponins (at 8min blanching), alkaloids (at 40min cooking) and flavonoids (at 40min cooking). This anti-nutrients' reduction/elimination during heat processing are also in agreement with their reduction/elimination in some other plants during heat processing as established by Ugwu and Oranye (2006), Nwosu (2011), Jimoh *et al*.(2011) and Olajide *et al*. (2011).

Oxalates bind with calcium and magnesium, and interfere with their metabolism, cause muscular weakness and paralysis (Soetan and Oyewole, 2009), Saponins haemolyse red blood cells (Nwosu, 2011), while alkaloids and flavonoids cause gastro- intestinal and neurological disorders (Soetan and Oyewole, 2009). Their total elimination at the different levels of heat treatment (Table 3) would rather make them not to be considered as risk at those levels.

For the anti-nutritional factors which weren't totally eliminated at the highest wet heat treatment of 60 minutes cooking, like the hydrogen cyanides had a value of 0.49mg/kg which is lower than the fatal dose level of 50mg/100g which is equivalent to 500mg/kg (Nwosu, 2011). Cyanide inhibits the cytochrome oxidase through combination with their copper and iron ions respectively (Onwuka, 2005) and also causes gasping, staggering and convulsion (Nwosu, 2011).

At this level of treatment/dose, hydrogen cyanide will likely not be implicated in any toxic activity of whole raw *L. chinensis* seed.

At 60min cooking, the tannins (0.47% which is equivalent to 4,700mg/kg) and total phenols (0.33% which is equivalent to 3,300mg/kg) (Table 3) are very much higher than the lethal dose level of 30mg/kg (Inuwa *et al*., 2011). Tannins are complex phenolic polymers which are capable of inhibiting the activities of trypsin, chymotrypsin, amylase and lipase (Inuwa *et al*., 2011). Tannins can provoke astringent reaction in the mouth, interfere with dietary iron absorption and also cause growth depression (Soetan and Oyewole, 2009). This very high level of tannins and phenolic compounds in *L. chinensis* seed is also in agreement with previous studies on *L. chinensis* seed which established a high level of phenolic compounds in *L. chinensis* seed (Tao *et al*., 2009; Yanna *et al*., 2001; Singh and Kaur, 2008; Huang *et al*., 2007). Tannins and phenols will likely be implicated in any toxic activities of this seed at this level of treatment/dose.

Table.1 Effect of blanching, cooking and roasting on the anti-nutritional factors in *Livistona chinensis* seed

Sample	Tannin (%)	Phytate (%)	Oxalate (%)	Saponins (%)	Hydrogen Cyanide (mg/kg)	Alkaloid (%)	Total Phenols (%)	Flavonoids (%)	Oligosacharides (%)	Trypsin inhibitor (T./u/g)	Haemagglutin (Hu/g)	Steroid (%)
Raw Seed	0.49a + 0.00	1.20a + 0.02	0.18b + 0.01	5.50b + 0.50	1.46a + 0.49	6.00a + 1.00	0.61a + 0.01	4.00a + 1.00	27.50a + 2.50	-	-	-
4min blanched	0.49a + 0.00	1.08d + 0.00	0.11d + 0.00	4.00d + 0.50	0.98ab + 0.49	6.00a + 1.00	0.61a + 0.00	3.00ab + 0.00	25.00b + 0.00	-	-	-
6min blanched	0.49a + 0.00	1.30e + 0.01	0.09e + 0.01	3.00e + 0.00	0.98ab + 0.49	6.00a + 0.00	0.53c + 0.01	3.00ab + 0.00	25.00b + 2.50	-	-	-
8min blanched	0.49a + 0.00	1.00c + 0.01	0.05f + 0.00	0.00f + 0.00	0.98ab + 0.00	4.00c + 0.00	0.47e + 0.01	2.00bc + 0.00	22.50c + 0.00	-	-	-
20min cooked	0.49a + 0.00	0.91g + 0.00	0.02g + 0.01	0.00f + 0.00	0.49b + 0.00	3.00d + 0.00	0.42f + 0.00	1.00cd + 1.00	17.50e + 0.00	-	-	-
40min cooked	0.48b + 0.01	0.88h + 0.01	0.02g + 0.00	0.00f + 0.00	0.49b + 0.00	0.00f + 0.00	0.37g + 0.00	0.00d + 0.00	15.00f + 0.00	-	-	-
60min cooked	0.47c + 0.00	0.85i + 0.02	0.00h + 0.00	0.00f + 0.00	0.49b + 0.00	0.00f + 0.00	0.33h + 0.00	0.00d + 0.00	7.50g + 0.00	-	-	-
5min Roasted	0.49a + 0.00	1.17b + 0.01	0.16c + 0.01	5.00c + 0.50	1.46a + 0.49	5.00b + 1.00	0.59b + 0.02	3.00ab + 1.00	25.00b + 2.50	-	-	-
10min roasted	0.49a + 0.00	1.11c + 0.01	0.11d + 0.00	3.00e + 0.00	1.46a + 0.49	3.00d + 0.00	0.52 + 0.01	2.00bc + 0.00	22.50a + 0.00	-	-	-
15min roasted	0.49a + 0.00	1.03e + 0.00	0.09e + 0.01	0.00f + 0.00	0.98ab + 0.00	2.00e + 0.00	0.47e + 0.02	1.00cd + 1.00	20.0d + 0.00	-	-	-
Pulp	0.49a + 0.00	0.68j + 0.02	0.54a + 0.02	6.50a + 0.00	0.49b + 0.00	4.00c + 0.00	0.50d + 0.01	2.00bc + 0.00	25.00b + 0.00	-	-	-
LSD	0.01	0.02	0.02	0.44	0.55	0.88	0.02	1.02	2.21			

Note: means with different superscripts in the same column are significantly different at $p<0.05$, LSD = least significant difference.

The phytate content was also not totally eliminated and had a value of 0.85% which is equivalent to 8,500mg/kg after 60min cooking. This value is also very much higher than the 50 – 60mg/kg lethal dose level (Inuwa *et al*., 2011). Phytates form insoluble salts with essential minerals like calcium, iron, magnesium and zinc in food, rendering them unavailable for absorption into the blood stream (Onwuka, 2005). It also causes reduction in protein availability (Nwosu, 2011). In essence, phytates also will likely be implicated in any toxic activities of whole *L. chinensis* seed at this level of processing/dose.

At 60min cooking, the oligosaccharides content (7.50% which is equivalent to 75,000mg/kg) is also very high when compared to the 5-8% oligosaccharides content range of raw legumes(beans, peas and lentils). Oligosaccharides are the major contributory factors in flatulence (Okaka *et al.*, 1992). These four anti-nutrients (tannins, phytates, total phenols and oligosaccharides) will most likely also be implicated in any toxic activity of whole *L. chinensis* seed at the 110^0C for 15min roasting done since they even had higher values of 0.49%, 1.03%, 0.47% and 20.00% for tannins, phytates, total phenols and oligosaccharides respectively.

Tannins, phytates, total phenols and oligosaccharides were present beyond lethal dose levels after 60min cooking and 15min of roasting of the whole *L. chinensis* seed thus suggesting that the seed is unsafe for human consumption at these levels of treatment.

Recommendation

Other methods of reduction of anti-nutrients in foods should be carried out on *L. chinensis* seed to ascertain their own rate and suitability in the reduction of the anti-nutrients in *L.chinensis* seed. The anti-nutrients' reduction/elimination methods should also be carried out on the cracked seed and not as a whole (uncracked) seed to also ascertain if there is any variation in the anti-nutrients' reduction/elimination rate between the whole (uncracked) and cracked *L. chinensis* seed.

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