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Effect of Thermal Processing on the *In Vitro* Bioavailability of Minerals and Anti-Nutritional Factors in Indian Almond *(Terminalia catappa)* Nut

G.M. Saibu[1*], O.B. Adu[1], O.O. Ogunrinola[1], S.O. Ogun[1], G.A. Adeyemo[1] and O.O. Oguntibeju[2]

[1]*Department of Biochemistry, Faculty of Science, Lagos State University, Lagos, Nigeria*

[2]*Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health & Wellness Sciences, Cape Peninsula University of Technology, Bellville Campus, South Africa*

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Saibu, G.M.
https://orcid.org/0000-0002-5068-9630

ABSTRACT

Terminalia catappa L. (Indian almond) is an underutilized crop rich in minerals, but investigation and information on the bioavailability of minerals and the presence of anti-nutritional factors in the nut are lacking. Several traditional food-processing methods are used domestically to enhance the bioavailability of micronutrients and decrease the effects of anti-nutrients in plant-based diets. This study was carried out to determine the effect of thermal processing on anti-nutritional factors and *in vitro* digestibility of minerals of *Terminalia catappa* nuts. The mineral concentration (Na, Ca, P, Mg, Cu, Fe, and Zn) of raw and processed *T. catappa* seed were determined. The mineral concentration of the raw *T. catappa* nut was significantly higher ($p<0.05$) than those of processed nuts. The pressure cooking led to the highest loss in mineral content, followed by boiling, drying, and roasting in descending order. A similar pattern was observed in the digested sample except for Ca and P, which occurred the least in boiled nuts. Mineral digestibility was generally low in the raw *T. catappa* nut. The various processing methods employed had different effects on the digestibility of the different minerals. There was a reduction in the concentration of the anti-nutrients in the processed samples. The highest reduction in tannin and phytic acid occurred in boiled samples, followed by pressure-cooked and roasted ones. The thermal processing methods which enhanced the digestibility of minerals of *T. catappa* nut were those that showed the greatest significant reduction of the anti-nutrients in the nuts of *Terminalia catappa.*

[*]Corresponding author -gbemisola.saibu@lasu.edu.ng

INTRODUCTION

Terminalia catappa, also known as Tropical almond, Indian almond, is a tree with a height that ranges from 3-8 m. It bears fruit that consists of edible flesh and a hard shell enclosing the edible seed (Akpakpan & Akpabio, 2012; Thomson & Evans, 2006). The hard shell and often small size of the seeds make it difficult to extract the nut. These factors may have led to its lack of use in many areas (Thomson & Evans, 2006). *T. catappa* has various uses ranging from food, shade, ornamental purposes, aphrodisiac activity, antioxidant activity, and anticarcinogenic and hepatoprotective properties (Thomson & Evans, 2006; Akpakpan & Akpabio, 2012; Adu *et al.,* 2013; Ladele *et al.,* 2016).

Minerals are essential nutrients necessary for various biological functions like the development of bones, homeostasis, proper muscle, heart, and nerve functioning (Soetan *et al.,* 2010). They also serve as cofactors for enzymes responsible for various biochemical reactions in the body. *T. catappa seed* is rich in minerals like sodium, potassium, phosphorus, iron, magnesium, and calcium (Ladele *et al.*, 2016). However, they have also been reported to contain antinutritional factors such as tannin, oxalate, and Hydrogen cyanide (Akpakpan & Akpabio, 2012). Antinutritional factors directly or indirectly reduce the nutritional value of foods leading to decreased bioavailability and absorption of nutrients (Sandberg, 2002).

On a household basis, several processing and preparation methods are used to increase the bioavailability of micronutrients and macronutrients in foods. These methods include thermal processing, fermentation, and mechanical processing (Hotz & Gibson, 2007). Thermal processing improves the bioavailability of micronutrients and macronutrients by destroying certain antinutritional factors (Hotz & Gibson, 2007; Wangui, 2015).

Although, mineral bioavailability in foods is majorly determined through precise feeding trials, in vitro methods of evaluating mineral bioavailability are important as quick, sensitive, and a good alternative to animal trials, which are expensive, time-consuming, and incapable of detecting small differences (Adu *et al.*, 2015; Kamchan *et al.*, 2004).

Adu et al (2014) reported the effect of thermal processing on the protein quality and free amino acid profile of T. *catappa*. However, information on the effect of thermal processing on the bioavailability of minerals and the presence of anti-nutritional factors in the nut is lacking (Adu *et al.*, 2015). This study was carried out to determine the effect of thermal processing- boiling, drying, roasting, and pressure cooking- on the *in vitro* bioavailability of minerals (Na, Ca, P, Mg, Cu, Fe, and Zn) and anti-nutritional factors (Tannin and Phytic acid) of *T. catappa* nuts.

METHODOLOGY

Collection of samples

Fruits of *T. catappa* were collected from three different locations- Surulere, Oshodi, and Ojo, all within Lagos metropolis in Nigeria. The fibrous outer flesh of the fruits was removed with a cutter knife, and the thick shell was sun-dried for two weeks. After drying, the hard shells were split by smashing with a hammer and the kernels were separated from the nut. Porcine intestinal fluid was collected from the intestine of a freshly slaughtered pig at a slaughterhouse of the Tejuosho main market, Yaba, Lagos. The intestinal fluid was placed in an ice box and transferred to the laboratory.

Processing of samples

Five hundred grams of *T. catappa* nut was divided into five batches, and each batch was respectively treated as follows.

Preparation of boiled sample

One hundred grams of the nut was put into a clean beaker, and 200 cm^3 of deionized water was added. It was then placed on a Griffin hot plate preset at 100℃ for 35min. The seeds were drained after boiling, allowed to cool, placed in an airtight plastic container, and stored in a refrigerator at 4℃.

Preparation of roasted sample

The Indian almond nuts were roasted for about 10min in a frying pan over a smokeless flame (hot plate) until the nut was crispy. The sample was then allowed to cool and stored, as previously mentioned.

Preparation of dried sample

Fresh almond nuts were split into four batches in a foil and placed in a Gallenkamp hot air oven (model 300 plus) at 60℃ for 5hrs. The sample was then allowed to cool and stored.

Preparation of pressure-cooked sample

One hundred grams of seed was put in a beaker with 200cm^3 of deionized water. It was placed in an autoclave to cook under pressure for 30min. The sample was thereafter allowed to cool and then transferred into an airtight container and stored.

Digestion of samples

For the digestion of the samples, the in vitro method of Miller and Nolan (1984) was used. A 0.5 g portion of the milled samples was placed in different Erlenmeyer flasks, and 20 mg of pepsin in 10 cm^3 of 0.075 N HCl was added and mixed. The mixture was incubated in a water bath at 37℃ for 4hrs. After the peptic digestion, the mixture was neutralized with 0.2M NaOH, and 10cm^3 of porcine intestinal fluid was added and the mixture was further incubated for 4hrs at 37℃. The content after the second round of incubation was then centrifuged for 10 min at 125 x *g,* and the residue was transferred to a pre-weighed filter paper and dried. The dried residue was then subjected to quantitative minerals analyses for Na, Ca, P, Mg, Zn, and Fe.

The utilizable portion of minerals in *T. catappa* was taken as:

Utilizable portion
= Concentration (Undigested sample)
− Concentration (Digested sample)

The % Bioavailability of each mineral was calculated as:

$$\%\ Bioavailability = \frac{Concentration\ (utilizable)\ \ x\ 100}{Concentration\ (undigested)}$$

Quantitative mineral analyses

Quantitative analyses of sodium (Na), calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn), magnesium (Mg), and iron (Fe) in the digested and undigested *T. catappa* samples were carried out in samples extracts prepared by dry ash method (Pearson, 1981). The concentrations of Cu, Fe, and Zn were determined according to the analytical method of atomic absorption spectroscopy (Philips PU9100X). Calcium and magnesium were determined using the Chapman and Pratt (1961) titration method (Chapman & Pratt, 1961). Sodium and phosphorus were determined according to the AOAC (1990) method using a flame photometer (Corning EEL) (AOAC, 1990).

Determination of Tannin Concentration

The concentration of Tannin was determined by the modified method of Mailoa *et al.* (2013). The sample was prepared by boiling 1 g of the processed nuts for 30 minutes in 50 mL of water, which was cooled and transferred into a 100 mL volumetric flask and diluted to mark, the solution was filtered through Whatman No 01 filter paper to get a clean filtrate. 1 mL of the filtrate was pipetted into a test tube with the addition of 0.5 mL Folin Ciocalteu reagent (1:5 dilution), 2.5 mL of Na_2CO_3, and 1.0 mL of distilled water. The standard solution was prepared by dissolving 10 g of the tannic acid in 10 mL of distilled water; the stock solution was pipetted as 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL respectively in a test tube instead of the samples. The solution was then incubated at room temperature for 40 minutes after which the absorbance read at 725 nm. The absorbance of the sample was then compared with that of the standard on a tannin standard curve to determine the concentration of tannin in the processed sample.

Determination of Phytate Concentration

Phytate was determined by the modified method as described by Adeyemo & Onilude (2013). Four gram of the sample was soaked in 100 mL of 2% HCl for 3 hours and then filtered through Whatman filter paper, 25 mL of the filtrate was poured into a 100 mL conical flask, and 5 mL of 0.3% ammonium thiocyanate solution was added as indicator. Then, 53.5 mL of distilled water was added to the mixture; this was titrated with a standard Iron (iii) chloride solution (which contains about 0.00195 g of Iron per milliliter) until a brownish-yellow colour appeared, which persisted for five minutes (Adeyemo & Onilude, 2013).

CALCULATION;
Phytate content (mol/kg) = T × 564.11 / M
Where; T = Titre value, M = Molar mass of phytate = 660.04g/mol

Statistical analysis

All analyses were carried out in triplicates and data were expressed as mean ± standard deviation. Means were compared using a Univariate analysis of variance, and Fisher's least significant difference (LSD) test was used for post hoc analyses using the SPSS (version 11) software.

RESULTS AND DISCUSSION

In vitro methods for evaluating the bioavailability of minerals have become increasingly common in recent years due to their precision, speed, and relatively low cost. It is assumed that the in vitro approach for estimating the bioavailability of minerals is more descriptive of in vivo conditions (Wolters *et al.*, 1993). Humans and animals need a mineral balance because deficiency, overdose, or any other imbalance has an adverse impact on health. However, it is not the amount of a mineral that is important to maintain balance but rather the bioavailable quantity (Mertz, 1981; Wolters *et al.*, 1993).

Diverse factors may affect nutrient bioavailability. For example, in the small intestine, several constituents of our food form complexes with minerals which may affect the bioavailability of these minerals by influencing their availability for absorption (Barde *et al.*, 2012). Also, several food components have been shown to have either positive (Citric acid, ascorbic acid, lactose, and certain amino acids) or negative effects (phytic acid, tannin, dietary fiber, and polyphenolic compounds) on the bioavailability of minerals (Brune *et al.*, 1989; Hallberg *et al.*, 1986; Torre *et al.*, 1991; Wolters *et al.*, 1993).

The mineral concentration of undigested *Terminalia catappa* nuts (Table 1) for all minerals analyzed was significantly higher ($p < 0.05$) in raw samples compared to processed samples. The macro elements determined included Na (17.54-23.25mg/100g); Ca (33.90-42.80mg/100g); P (30.99-50.70mg/100g); and Mg (29.26-42.95mg/100g) while the micro elements were Cu (7.96-19.59g/100g); Fe (3.75-6.25mg/100g) and Zn (0.44-21.76mg/100g). Pressure cooking of nuts led to the greatest loss in mineral content, followed by boiling, drying, and roasting in descending order. A similar pattern was observed in the digested sample (Table 2) except for Ca and P occurring the least in boiled nuts.

Boiling, pressure cooking, drying, and roasting brought a significant loss of minerals (Na, Ca, P, Mg, Cu, Fe, Zn) in the processed undigested sample compared to the raw samples seen in Table 1. This result is similar to that of Yagoub *et al*. (2008) where there was an obvious reduction in the mineral content of soaked, sprouted, and cooked seeds. As observed, moist heat techniques (boiling and pressure cooking) resulted in a more significant loss in mineral content than the dry heat method (drying and roasting). This may be attributed to heat and the leaching of minerals out into the water (Saikia *et al.*, 1999; ElMaki *et al.*, 2007; Yagoub *et al.*, 2008). The losses observed due to drying and roasting of seeds could be due to the destruction of minerals by heat (Ijeh *et al.*, 2010).

Table 1. Mineral concentration in undigested *Terminalia catappa* nuts (mg/100g)*

Element	Raw	Boiled	Pressure cooked	Dried	Roasted
Na	23.24±0.11^{c}	17.99±0.11^{a}	17.54±0.11^{a}	19.39±0.05^{b}	19.61±0.05^{b}
Ca	42.80±0.29^{c}	34.18±0.15^{a}	33.90±0.14^{a}	37.44±0.14^{b}	37.45±0.28^{b}
P	50.70±0.28^{e}	31.83±0.28^{b}	30.99±0.00^{a}	44.36±0.14^{d}	35.35±0.14^{c}
Mg	42.95±0.29^{e}	30.12±0.31^{b}	29.26±0.12^{a}	39.88±0.13^{d}	35.19±0.13^{c}
Cu	19.59±0.28^{d}	9.18±0.21^{ab}	7.96±0.20^{a}	15.60±0.11^{c}	10.41±0.20^{b}
Fe	6.25±0.00^{c}	5.00±0.00^{b}	3.75±0.00^{a}	5.00±0.00^{b}	3.75±0.00^{a}
Zn	21.76±0.22^{c}	0.55±0.10^{a}	0.44±0.00^{a}	15.93±0.11^{b}	16.26±0.00^{b}

*Values with different superscripts are significantly different at p<0.05 across rows

Table 2. Mineral concentration in digested *Terminalia catappa* nuts (mg/100g)

Element	Raw	Boiled	Pressure cooked	Dried	Roasted
Na	22.13±1.01^{c}	16.87±0.11^{ab}	15.74±0.10^{a}	17.39±0.05^{ab}	18.55±0.11^{b}
Ca	40.79±1.06^{c}	31.30±0.19^{a}	31.62±0.12^{a}	34.75±0.14^{b}	34.75±0.14^{b}
P	48.17±0.28^{d}	28.00±0.28^{a}	28.87±0.14^{a}	40.14±0.70^{c}	32.25±0.14^{b}
Mg	42.22±0.24^{d}	27.65±0.10^{a}	27.04±0.12^{a}	37.16±0.12^{c}	32.71±0.12^{b}
Cu	16.94±0.20^{c}	5.10±0.21^{a}	5.51±0.20^{a}	10.61±0.41^{b}	6.12±0.41^{a}
Fe	5.00±0.00^{c}	3.75±0.00^{b}	2.50±0.00^{a}	3.75±0.00^{b}	2.50±0.00^{a}
Zn	20.88±0.66^{c}	0.44±0.00^{a}	0.22±0.00^{a}	13.66±0.25^{b}	14.18±0.11^{b}

*Values with different superscripts are significantly different at p<0.05 across rows

The mineral concentration of digested *T. catappa* nuts (Table 2) for all minerals analyzed was significantly higher (p< 0.05) in raw samples compared to processed samples. The macro elements determined included Na (15.74-22.13) mg/100g; Ca (31.30-40.79) mg/100g; P (28.00-48.17) mg/100g; and Mg (27.04-42.22) mg/100g while the micro elements were Cu (5.10-16.94) mg/100g; Fe (2.50-5.00) mg/100g and Zn (0.22-20.88) mg/100g.

The mineral content of digested raw and processed *T. catappa* nuts varied with the different processing methods as was observed for the undigested samples(Table 2). There were losses in the mineral concentrations as indicated above but with much more severe loss compared to the undigested sample. The drastic loss of minerals observed in the processed digested *T.catappa* as compared to the undigested sample might be due to the combination of minerals with co-nutrients or non-food components. They may become unavailable for digestion due to these interactions Watzke,(1998).

Table 3. Mineral digestibility (D) and Relative digestibility (RD) of *Terminalia catappa* nuts

Element	Raw		Boiled		Pressure cooked		Dried		Roasted	
	D (%)	RD (%)	D (%)	RD (%)	D (%)	RD (%)	D (%)	RD (%)	D (%)	RD (%)
Na	9.09	**100.0**	6.23	**68.5**	10.14	**111.6**	7.53	**82.8**	5.41	**59.5**
Ca	4.76	**100.0**	8.43	**177.1**	6.73	**141.4**	7.18	**150.8**	7.2	**151.3**
P	4.99	**100.0**	8.86	**177.6**	8.84	**177.2**	11.09	**222.2**	8.77	**175.8**
Mg	1.69	**100.0**	8.93	**528.4**	7.59	**449.1**	6.82	**403.6**	7.05	**417.2**
Cu	13.53	**100.0**	44.44	**328.5**	30.78	**227.5**	31.99	**236.4**	41.21	**304.6**
Fe	20.00	**100.0**	25.00	**125.0**	33.33	**166.7**	32.5	**162.5**	32.5	**162.5**
Zn	4.04	**100.0**	20.00	**495.0**	50.00	**1237.6**	14.25	**352.7**	12.79	**316.6**

*D- Mineral digestibility, RD- Relative digestibility

Mineral digestibility was generally low in the raw *T. catappa* nut (Table 3); it ranged from 1.69% (Mg) to 20.00% (Fe). The various processing methods employed had different effects on the digestibility of the different minerals. Relative digestibility of Na was reduced by boiling (68.5%), drying (82.8%), and roasting (59.5%) but was increased by pressure cooking (111.6%). On the other hand, Relative digestibility of Ca (141.4% - 177.1%), P (175.8% - 222.2%), Mg (403.6% - 528.4%) , Cu (227.5% - 328.5%), Fe (125.0% - 166.7%) and Zn (316.6% - 1237.6%) were all increased by all the processing methods.

The relative digestibility of all the minerals except Na, in the processed samples, increased due to better availability of the minerals following heat treatment as seen in Table 3. Reduced digestibility of Sodium was seen in the boiled, dried, and roasted samples which may be due to possible interactions of sodium with co-nutrients or non-nutrient components like phytic acid Watzke, (1998).

Raw *T. catappa* naturally contains high levels of antinutrients e.g phytic acid and tannin (Udotong *et al.*, 2015). The deleterious effect of anti-nutritional factors has been shown to be reduced during processing (Xu *et al.*, 2016). Tannin is known to bind irreversibly to proteins, rendering it indigestible by intestinal enzymes

Tannin concentration was significantly reduced ($p< 0.05$) in the boiled and pressure-cooked sample compared to the raw (Figure 1).

All processing methods were shown to reduce tannin concentration, and boiling was the most effective method for reducing tannin. Heat degradation, leaching out effects, and formation of insoluble complexes might be the factors that resulted in the significant reduction of these nutrients by boiling (Saikia *et al.,* 1999; Alonso *et al.,* 2000; Yagoub *et al.,* 2008).

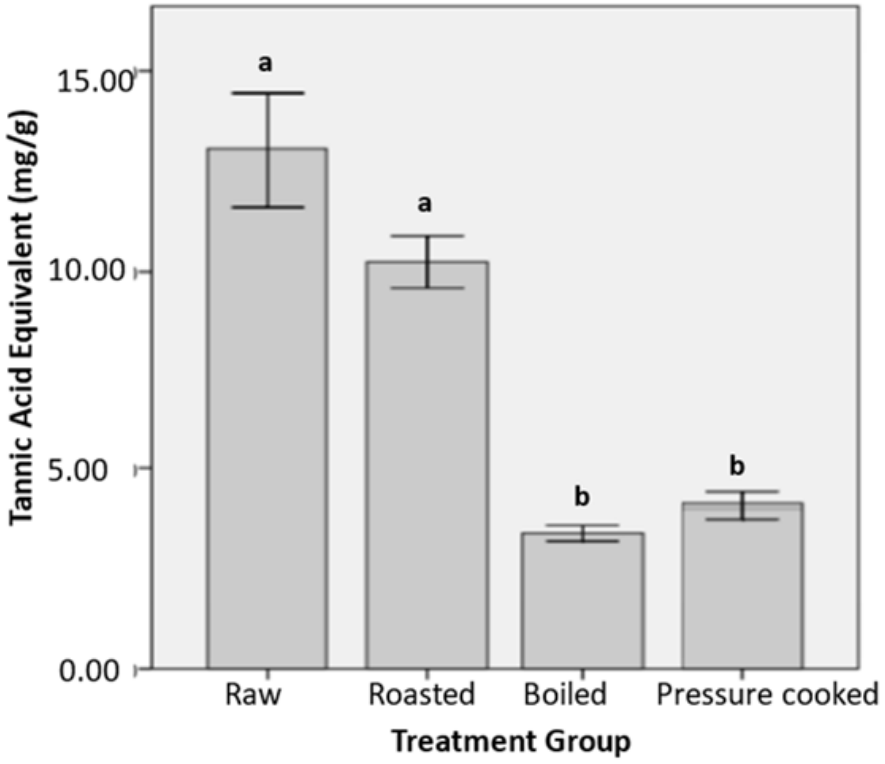

Figure 1: Effect of Processing Methods on Tannin Concentration. [ab] Bars with different superscripts are significantly different at $p<0.05$. Values are represented as mean ± standard error of 4 samples determination

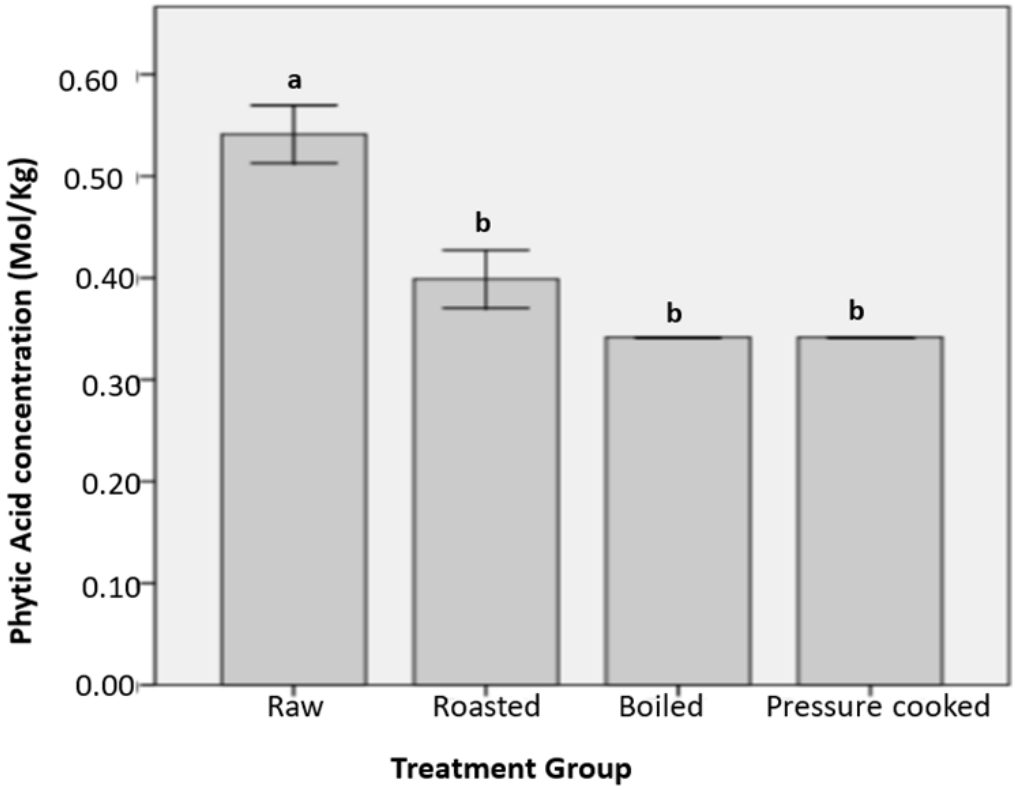

Figure 2: Effect of Processing Methods on Phytic acid Concentration.
[ab] Bars with different superscripts are significantly different at $p<0.05$. Values are represented as mean ± standard error of 4 samples determination

Phytic acid concentration showed that there was a significant reduction in phytate concentration for all samples subjected to the processing methods (Figure 2), however, the greatest reduction in phytic acid concentration was evident in the boiled and pressure-cooked samples.

The phytic acid concentration was also reduced by the different processing methods used, with boiling and pressure cooking being the most efficient in its reduction (Figure 2). This is important, because of bioavailability of divalent minerals, especially Ca, Mg, and Fe are adversely affected by the presence of phytic acid, a metal-binding food constituent. By breaking down phytate, the heat treatment methods minimized the concerns posed by metal chelation brought about by the phytate naturally present in the nut (Chitra *et al.*, 1996; Ghavidel & Prakash, 2007).

Furthermore, the study showed that the processing methods, that had the greatest effect on antinutritional factors (boiling and pressure cooking) also had the most positive effect on mineral digestibility. This suggests that the presence of antinutrients could be a key factor militating against the availability of minerals in *T. catappa* nut. As such, processing methods that effectively reduce or inactivate these antinutrients will improve mineral availability.

CONCLUSIONS

Different heat methods, whether moist-heat method (boiling), roasting or drying, improved mineral digestibility and reduced anti-nutritional agents in *T. catappa*. It can be deduced from this study that the various processing methods used reduced the mineral content – both micro and macro by varying degrees but increased their digestibility. The increase in digestibility might be due to the reduction in the concentration of tannin and phytic acid by the various heat methods employed.

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