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## Effects of Processing Treatments on the Antinutrients in Bambaranut (*Vigna Subterranea*) and Moringa Oleiferaseed Flour

**Chibuzo Mary Abiaeye**

Researcher/ Ph.D. Student, Department of Food Science and Technology,  
Ebonyi State University, Abakaliki, Nigeria

**Eric Chigozie Okoli**

Professor/Director, Ebonyi State University, Abakaliki, Nigeria

### **Abstract:**

The aim of this work was to study the effects of soaking Bambaranut and *Moringa oleifera* seeds separately in water at room temperature for 12,24 and 48hours and blanching at 100°C for 15,30, and 48 minutes respectively. The bambaranuts were subsequently dehulled and milled into flour while the *Moringa oleifera* was milled into flour of 100µm size without dehulling. Tannin, Trypsin inhibitor activity, Phytate and Oxalate content of the samples were determined and the values obtained were used in the estimation of the antinutrient factor in the variously treated samples of Bambaranut and *Moringa oleifera* seed flours. Results showed that the soaking and blanching significantly reduced ( $P<0.05$ ) the antinutrients in Bambaranut and defatted *Moringa oleifera* flours and the reduction of the antinutrients increased with increase in soaking and blanching time. Soaking bambaranuts for 24hours and blanching at 100°C for 45minutes reduced Tannin, Trypsin inhibitor activity, Phytic acid and Oxalate by 68.33%, 85.91%, 52.63% and 66.67% respectively over the control values. Similarly, *Moringa oleifera* seed soaked for 24hours and blanched at 100°C for 45minutes reduced Tannin, Trypsin inhibitor activity, Phytic acid and Oxalate by 64%, 84.47%, 78.18% and 100% over the control values.

**Keywords:** Bambaranut, *Moringa oleifera* seed, soaking, blanching, antinutrients.

### **1. Introduction**

There is wide spread of biologically active constituents throughout the plant kingdom (Igile.,1996). But their use as food could be limited by the presence of these biologically active constituents such as polyphenols, trypsin inhibitors, lecithins, phytates, oxalates and flatulence factors that depress the biological value of dietary proteins and hinder mineral adsorption from the diet (Singh,1984; Rackis *etal.*, 1986).

Bambaranut (*Vigna subterranean* (L)) is agrain legume widely cultivated in West and Central Africa (Mazahib *etal.*, 2013). However, its consumption as food appears to be limited because of several factors such as low protein and starch digestibility (Negi *etal.*, 2001), poor mineral bioavailability (Kamchan *etal.*, 2004) and high antinutritional factors.

*Moringa oleifera* plant is considered to be the most nutrient-rich plant on earth (Mbah *etal.*, 2012; Oluwole *et al.*,2013). *Moringa oleifera* seed cake are highly nutritious, however it requires to exclude the antinutritional and or toxic factors which could interfere with its digestion and absorption ion the human body (Oliveira *etal.*, 1999). However, for human consumption plant foods are processed in a variety of ways depending upon taste and cultural preferences which are known to affect the level of the antinutrients (Jood *etal.*, 1985). In this study, effort would be made to investigate the effect of processing treatments on nutritional attributes of Bambaranut and *Moringa oleifera* seed flours.

### **2. Materials and Methods**

Matured dried seeds of Bambaranut and *Moringa oleifera* were purchased from Abakpa market in Abakaliki, Nigeria.

#### **2.1. Sample Preparation**

*Moringa oleifera* seed: The flour was produced using the method described by Ogunsina *et al.*, (2010) with slight modifications. The seeds were defatted by soaking in petroleum ether for 48 hours, drained and air dried for 24hours. The defatted seeds were soaked for 0,12,24, and 48hours. Each of the soaked samples were further divided into four parts and blanched at 100°C for 0,15,30,45minutes respectively. They were dried and milled to pass through 100µm mesh sieve.

Bambaranut (*Vigna subtranea*): The flour was produced using the method described by Fadahunsi (2009) with slight modification. The Bambaranut was soaked in water at 28°C for 12, 24, 48hours. Each of these samples were further divide into four parts and

blanched at 100°C for 0,15,30 and 45minutes respectively. The seeds were subsequently dehulled dried, and milled to pass 100µm mesh sieve.

## 2.2. Analytical Methods

### 2.2.1. Tannin

This was determined by the Folin-Dennis spectrophotometric method described by Onwuka (2005). 1g of sample was dispersed in 10ml distilled water and agitated. This was left to stand for 30minutes at room temperature. After it was centrifuged and extract gotten. 2.5ml of the supernatant was dispersed into a 50ml volumetric flask. Similarly, 2.5ml of standard tannic acid solution was dispersed into a separate 50ml flask. A 1ml folin dennis reagent was measured into each flask, followed by 2.5ml of saturated Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was diluted to 50ml in the flask and incubated for 90minutes at room temperature. The absorbance of each sample was measured at 250nm with reagent blank at zero.

The tannin content was calculated as follows:

$$\%Tannin = \frac{A_n}{A_s} \times C \times 100 / w \times V_f$$

Where: A<sub>n</sub>=absorbance of test sample

A<sub>s</sub>=absorbance of standard solution

C=concentration of standard solution

W=weight of sample used

V<sub>f</sub>=total volume of extract

V<sub>a</sub>=volume of extract analysed.

### 2.2.2. Phytate

This was determined by the method of Haug and Lantzsch (1983). 2g of sample was soaked in 20ml of 0.2 N HCl solution for 3hours. 2.5ml of the extract was mixed with 5ml of Ferric ammonium sulphate solution in a test tube and stoppered. The mixture was boiled for 30minutes in a water bath. The tubes were cooled in ice water for 15minutes, allowed to adjust to room temperature and centrifuged for 15minutes at 3000rpm. 2ml of the supernatant was mixed with 3ml 2,2 Bipyridine solution, absorbance was measured at 519nm against a reagent blank in a spectrophotometer. The absorbance of the test sample is used to obtain the concentration of phytic acid from the standard curve.

$$\% \text{ Phytic acid} = \frac{Y V_f}{10 W V_a}$$

Where Y = conc. of phytic acid in the sample (mg/ml)

W = weight of sample used (g)

V<sub>f</sub> = total volume of extract (ml)

V<sub>a</sub> = aliquot volume analysed (ml)

### 2.2.3. Oxalate

This was determined using the method of Onwuka (2005). 2g of sample was soaked in 190ml distilled water in a 250ml volumetric flask. 10ml of 6M HCl solution was added and the suspension digested at 100°C for 1 hour. This was cooled and made up to 250ml mark of the flask. The sample was filtered and duplicate portion of 125ml of the filtrate was measured into beakers, and four drops of methyl red indicator was added followed by the addition of concentrated NH<sub>4</sub>OH solution changed from pink to yellow colour. Each portion was then heated to 90°C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90°C and 10ml of 5% CaCl<sub>2</sub> solution was added to it while stirring consistently. This was cooled and left overnight. This solution was centrifuged at 2500rpm for 5minutes. The supernatant was decanted while the precipitate was completely dissolved in 10ml of 20% H<sub>2</sub>SO<sub>4</sub>. The total filtrate resulting from the digestion of 2g sample was made up to 300ml. Aliquots of 125ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized KMnO<sub>4</sub> solution to a faint pink colour which persisted for 30seconds.

The Calcium oxalate content will be calculated as:

$$\frac{T \times (V_{me}) [DF]}{(ME) \times M_f} \text{ (mg/100g)}$$

Where T=titre of KMnO<sub>4</sub>(ml),

V<sub>me</sub>=volume-mass equivalent (i.e. 1cm<sup>3</sup> of 0.05M KMnO<sub>4</sub> solution is equivalent to 0.00225g anhydrous oxalic acid).

Df= dilution factor V<sub>T</sub>/A (2.4)

where V<sub>T</sub>= Total volume of titrate (300ml)

A= Aliquot used (125ML),

ME=molar equivalent of KMnO<sub>4</sub> in oxalate (KMnO<sub>4</sub> redox reaction)

M<sub>f</sub>=mass of flour used.

### 2.2.4. Trypsin Inhibitor Activity

The trypsin inhibitor activity will be tested using the spectrophotometric method described by Arntfield *et al.*, (1985). 1g of sample was dispersed in 50ml of 0.5M NaCl solution. The mixture was stirred for 30minutes at room temperature and centrifuged at 1500rpm

for 5minutes. The supernatant was filtered using whatman No41 filter paper. 2ml of standard trypsin solution was added to 10ml of the substrate. The absorbance of the mixture was taken at 410nm using 10ml of the same substrate as blank.

The trypsin inhibitor activity was expressed as the number of trypsin units inhibited (TUI) per unit weight (g) of the sample analysed:

$$\text{TUI/mg} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 0.01F$$

$$\text{TUI/mg} = \frac{b-a}{0.01} \times F$$

Where b= absorbance of test sample solution

a = absorbance of the blank (control)

F = experimental factor, given by

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

Where w =weight of sample

Vf = total volume of extract

Va = volume of extract used in the assay

D = dilution factor (if any)

### 2.5. Statistical Analysis

Data were analysed using SPSS version 17.0 by one-way analysis of variance (ANOVA), and significant means were compared using Duncan multiple range test. The results are presented in Table 1 and 2.

## 3. Results and Discussion

| Sample  | Tanninmg/100g            | R%    | Trypsin TUI/mg          | R%    | Phytic acid%             | R%    | Oxalate mg/100g          | R%    |
|---------|--------------------------|-------|-------------------------|-------|--------------------------|-------|--------------------------|-------|
| BS0B0   | 0.59 <sup>a</sup> ±0.01  | -     | 9.64 <sup>a</sup> ±0.01 | -     | 0.18 <sup>a</sup> ±0.01  | -     | 0.03 <sup>a</sup> ±0.00  | -     |
| BS12B0  | 0.44 <sup>b</sup> ±0.01  | 25.00 | 6.52 <sup>b</sup> ±0.01 | 32.33 | 0.16 <sup>b</sup> ±0.01  | 10.53 | 0.02 <sup>b</sup> ±0.00  | 33.33 |
| BS12B15 | 0.37 <sup>c</sup> ±0.01  | 36.67 | 5.74 <sup>d</sup> ±0.01 | 39.38 | 0.14 <sup>c</sup> ±0.01  | 21.05 | 0.02 <sup>b</sup> ±0.00  | 33.33 |
| BS12B30 | 0.30 <sup>e</sup> ±0.01  | 48.33 | 4.82 <sup>e</sup> ±0.01 | 49.95 | 0.13 <sup>cd</sup> ±0.01 | 26.32 | 0.01 <sup>c</sup> ±0.00  | 66.67 |
| BS12B45 | 0.24 <sup>f</sup> ±0.01  | 58.33 | 4.20 <sup>h</sup> ±0.01 | 56.37 | 0.12 <sup>de</sup> ±0.01 | 31.58 | 0.01 <sup>c</sup> ±0.00  | 66.67 |
| BS24B0  | 0.33 <sup>d</sup> ±0.01  | 43.33 | 5.89 <sup>c</sup> ±0.01 | 38.86 | 0.13 <sup>cd</sup> ±0.01 | 26.32 | 0.02 <sup>b</sup> ±0.00  | 33.33 |
| BS24B15 | 0.26 <sup>h</sup> ±0.01  | 55.00 | 4.29 <sup>g</sup> ±0.01 | 55.44 | 0.11 <sup>ef</sup> ±0.01 | 36.84 | 0.02 <sup>b</sup> ±0.00  | 33.33 |
| BS24B30 | 0.19 <sup>hi</sup> ±0.01 | 66.67 | 2.74 <sup>i</sup> ±0.01 | 71.50 | 0.09 <sup>gh</sup> ±0.01 | 47.37 | 0.01 <sup>c</sup> ±0.00  | 66.67 |
| BS24B45 | 0.18 <sup>±</sup> 0.01   | 68.33 | 1.35 <sup>±</sup> 0.01  | 85.91 | 0.08 <sup>hi</sup> ±0.01 | 52.63 | 0.01 <sup>c</sup> ±0.00  | 66.67 |
| BS48B0  | 0.27 <sup>f</sup> ±0.01  | 53.33 | 4.69 <sup>f</sup> ±0.01 | 51.29 | 0.11 <sup>ef</sup> ±0.01 | 36.84 | 0.01 <sup>c</sup> ±0.00  | 66.67 |
| BS48B15 | 0.20 <sup>h</sup> ±0.01  | 65.00 | 3.65 <sup>±</sup> 0.01  | 62.07 | 0.10 <sup>ef</sup> ±0.01 | 42.11 | 0.01 <sup>c</sup> ±0.00  | 66.67 |
| BS48B30 | 0.13 <sup>j</sup> ±0.01  | 76.67 | 1.84 <sup>k</sup> ±0.01 | 80.82 | 0.09 <sup>gh</sup> ±0.01 | 47.37 | 0.003 <sup>d</sup> ±0.00 | 96.67 |
| BS48B45 | 0.09 <sup>k</sup> ±0.01  | 83.33 | 1.06 <sup>±</sup> 0.01  | 88.91 | 0.07 <sup>±</sup> 0.01   | 57.89 | 0.002 <sup>c</sup> ±0.00 | 99.67 |

Table 1: The effect of soaking, dehulling and blanching treatments on antinutrients in Bambaranut

Where B= Bambaranut

S= Soaked for 0,12,24,48 hours respectively

B= Blanched for 0,15,30,45 minutes respectively

R=Percentage reduction.

Means in the same column with varying superscripts differ significantly (P<0.05)

Table 1 shows that Bambaranut contain tannin, trypsin inhibitor, phytic acid and oxalate which may limit the nutritional value of the legume, but the soaking, dehulling and boiling treatments at varying times adopted in this study significantly (P<0.05) reduced the levels of these anti-nutrient substances when compared to the levels in the raw sample.

Result of this study found dehulled raw Bambaranut to have 9.64 ± 0.02 TIUmg/100g which compared favourably with those of Apatha *et al.*, (1997) who found that Trypsin inhibitor activity (TIA) in two raw Bambaranut varieties to be 9.4 and 12.2TIUmg in 100g samples. On soaking for 24hours the trypsin inhibitor activity reduced to 5.89±0.01TIUmg/100g which corresponds to 38.86% reduction. Similarly, Rasha *et al.*, (2011) reported that soaking of beans overnight reduced trypsin inhibitor activities by 6.3% and cooking of the soaked beans further reduced the trypsin inhibitor activity by 66.7%.

Also blanching of the soaked seeds for 45minutes significantly (P<0.05) decreased the trypsin inhibitor to 1.36TIUmg/100g which corresponds to 85.91% when compared with the control.

This observation conforms with the report of Havalala (1995) who noted that soaking of mugbeans, chickpeas and broadbeans for 24hour removed 66%,50% and 10% of their initial trypsin inhibitor content respectively.

Trypsin inhibitor activity are inactivated by heat especially moist heat, because of even distribution of heat (Bressani and Sosa, 1990; Liener, 1995; Shimelis and Rakshit,2007). Ayagari *et al.*, (1980) stated that boiling destroys TIA in cereal and leguminous grains there by enhancing its protein digestibility. The value of 1.35±0.01TIUmg/100g obtained in this analysis is quite lower than 200mg/100g which is reported as lethal dose in man by Inuwa *et al.*, (2011).

The value of tannin content in dehulled raw Bambaranut was 0.59±0.01mg/100g, which was higher than 0.16±0.58mg/100g reported by Abiodun and Adepoju (2011). Soaking for 24hours significantly (P<0.05) decreased tannin to 0.33±0.01mg/g which corresponds to 43.33%. While soaking for 24hours and blanching for 45minutes further decreased tannin to 0.18±0.01mg/100g showing 68.33% reduction. Similar trends were observed by Mazahib *et al.*, (2013) for bambaranut, Mubarak (2005) for Mugbeans respectively.

Mazahib *et al.*, (2005) suggested that the reduction in polyphenols after soaking may be due to washing out of soluble polyphenols in water and after cooking might be due to interaction with protein during cooking, forming poorly extractable protein-phenolic complexes.

The value of  $0.18 \pm 0.01$  mg/100g obtained compares favourably to the maximum recommended limit of 0.1% condensed tannin content allowed in Tsabana baby food (Ohiokpehai, 1994). Preet and Punia (2000) observed in cowpea variety that condensed tannin content is concentrated in the seed coat with trace amount in the cotyledons. Apata and Ologbo (1997) also indicated that different processing methods such as soaking, dehulling, cooking and discarding of cooking water significantly reduced tannic acid content in Bambaranut. Reduction of tannic acid content in the dehulled flour is expected to improve its nutritional value because tannins form complex substances with proteins and thus reduce their digestibility and palatability Eka, (1985).

From the result, dehulled raw bambaranut seed contained  $0.18 \pm 0.01$  % phytic acid. Soaking for 24 hours in water significantly ( $P < 0.05$ ) reduced phytic acid to  $0.13 \pm 0.01$  % corresponding to 26.32% reduction. While soaking for 24 hours and blanching for 45 minutes further decreased phytic acid to  $0.08 \pm 0.01$  % which corresponds to 52.63% reduction. Results revealed that soaking in water lowers the level of phytic acid below the control value. Similar results on reduction of phytic acid in beans soaked in water were reported by Bishnoi *et al.*, (1994) and Mazahib *et al.*, (2013). El Maki *et al.*, (2007) also observed loss of phytic contents during cooking of beans. According to El Maki *et al.*, (2007) the decrease in phytic acid during cooking could be because insoluble complexes between phytate and other components were formed and accordingly, the amount of free phytate was reduced.

Abiodun and Adepeju (2011) reported that Bambaranut coat had higher phytate content while dehulling and boiling drastically reduces its phytate content. The value for phytic acid were found to be lower than reported lethal dose. The lethal dose of phytate is reported to be from 250-500 mg/100g (Bushway *et al.*, 1998).

Oxalate content of the raw dehulled bambaranut flour significantly ( $P < 0.05$ ) decreased from 0.03 mg /100g to 0.02 mg/100g after soaking for 24 hours. While soaking for 24 hours and boiling for 45 minutes further reduced oxalate to 0.01 mg/100g showing 66.67% reduction. Boiling causes considerable leakage of soluble oxalate into the cooking water (Albihn and Savage, 2001). Therefore, the reduction in the levels of oxalate in the dehulled flour may be due to its solubility in hot water (Bradbury and Nixon, 1998). The value of oxalate content after soaking, dehulling and boiling were found to be lower than reported lethal doses. Hui, (1992) reported that intake of 5g or more of oxalic acid could be fatal to human while Munro and Basir (1969) estimated the threshold of oxalate toxicity in man to be 200-500 mg/100g of the sample.

Several food processing methods such as soaking, dehulling, cooking and fermentation are *known* to reduce antinutritional factors effectively and upgrade the nutritional quality of legumes (Rashaet *et al.*, 2011). Processing treatments given to the raw dehulled bambaranut flour showed significant ( $P < 0.05$ ) decrease in its antinutrient contents as the soaking and boiling time increased, to a safe margin for human consumption.

| Sample  | Tannin mg/100g       | R%    | Tryps in TU/mg    | R%    | Phytic acid%      | R%    | Oxalate mg/100g      | R%  |
|---------|----------------------|-------|-------------------|-------|-------------------|-------|----------------------|-----|
| MS0B0   | $0.74^a \pm 0.01$    | -     | $1.02^a \pm 0.01$ | -     | $0.54^a \pm 0.01$ | -     | $0.02^a \pm 0.00$    | -   |
| MS12B0  | $0.49^b \pm 0.01$    | 33.33 | $0.78^b \pm 0.01$ | 23.30 | $0.45^b \pm 0.01$ | 16.36 | $0.02^b \pm 0.00$    | 0   |
| MS12B15 | $0.42^c \pm 0.01$    | 42.67 | $0.67^c \pm 0.01$ | 33.98 | $0.29^d \pm 0.01$ | 45.45 | $0.01^b \pm 0.00$    | 50  |
| MS12B30 | $0.38^{de} \pm 0.01$ | 48    | $0.50^e \pm 0.01$ | 49.51 | $0.19^e \pm 0.01$ | 63.64 | $0.01^b \pm 0.00$    | 50  |
| MS12B45 | $0.36^f \pm 0.01$    | 50.67 | $0.22^j \pm 0.01$ | 77.67 | $0.13^h \pm 0.01$ | 74.55 | $0.00^d \pm 0.00$    | 100 |
| MS24B0  | $0.39^d \pm 0.01$    | 46.67 | $0.83^b \pm 0.01$ | 18.45 | $0.33^c \pm 0.01$ | 38.18 | $0.01^b \pm 0.00$    | 50  |
| MS24B15 | $0.29^e \pm 0.01$    | 54.67 | $0.63^d \pm 0.01$ | 37.86 | $0.16^g \pm 0.01$ | 69.09 | $0.01^b \pm 0.00$    | 50  |
| MS24B30 | $0.28^e \pm 0.01$    | 61.33 | $0.48^h \pm 0.01$ | 52.43 | $0.13^h \pm 0.01$ | 74.55 | $0.01^b \pm 0.00$    | 50  |
| MS24B45 | $0.26^i \pm 0.01$    | 64.00 | $0.15^k \pm 0.01$ | 84.47 | $0.11^i \pm 0.01$ | 78.18 | $0.00^b \pm 0.00$    | 100 |
| MS48B0  | $0.37^{ef} \pm 0.01$ | 49.33 | $0.80^d \pm 0.01$ | 35.92 | $0.28^c \pm 0.01$ | 47.27 | $0.00^d \pm 0.00$    | 50  |
| MS48B15 | $0.29^{gh} \pm 0.01$ | 60    | $0.43^h \pm 0.01$ | 21.36 | $0.18^h \pm 0.01$ | 65.45 | $0.00^d \pm 0.00$    | 100 |
| MS48B30 | $0.25^h \pm 0.01$    | 65.33 | $0.25^i \pm 0.01$ | 71.84 | $0.12^i \pm 0.01$ | 76.36 | $0.00^{cd} \pm 0.00$ | 100 |
| MS48B45 | $0.22^j \pm 0.01$    | 69.33 | $0.14^k \pm 0.01$ | 85.44 | $0.08^i \pm 0.01$ | 83.64 | $0.00^c \pm 0.00$    | 100 |

Table 2: Effect of soaking and blanching on antinutrients in *Moringa oleifera*

Where M= *Moringa oleifera* seed

S= Soaked for 0,12,24,48 hours respectively

B= Blanched for 0,15,30,45 minutes respectively

R=Percentage reduction.

Means in the same column with varying superscripts differ significantly ( $P < 0.05$ ).

Table 2 showed that defatted *Moringa oleifera* seed flour contained tannin, trypsin, phytate and oxalate. The *Moringa oleifera* seeds were defatted, soaked and boiled at varying times which significantly ( $P < 0.05$ ) reduced the levels of these antinutrients when compared to the levels in the defatted raw sample used as control.

Result from this study found *Moringa oleifera* seed to contain tannin  $0.74 \pm 0.01$  mg/100g which is higher than the value of  $0.45 \pm 0.17$  mg/100g obtained by Abiodun *et al.*, (2011). On soaking for 24 hours the tannin content reduced to  $0.39 \pm 0.01$  mg/100g which corresponds to 46.67%. While soaking for 24 hours and blanching for 45 minutes further decreased tannin to  $0.26 \pm 0.01$  mg/100g showing 64% reduction. Mazahib *et al.*, (2005) suggested that the reduction in polyphenols after soaking may be due to washing out of soluble polyphenols in water and after cooking might be due to interaction with protein during cooking, forming poorly extractable protein phenolic complexes. However, the value of  $0.26 \pm 0.01$  mg/100g compares favourably to the maximum limit of 0.1% condensed tannin content allowed in Tsabana baby food (Ohiokpehai, 1994).

From the result, defatted raw *Moringa oleifera* seed contained  $0.54 \pm 0.01\%$  phytic acid, which is lower than the value of  $1.49 \pm 0.15 \text{ mg/g}$  obtained by Abiodun *et al.*, (2011). Soaking for 24 hours in water significantly ( $P < 0.05$ ) reduced phytic acid to  $0.33 \pm 0.01\%$  corresponding to 38.18% reduction. While soaking for 24 hours and blanching for 45 minutes further decreased phytic acid to  $0.11 \pm 0.01\%$  which corresponds to 78.18%. Results revealed that soaking in water lowers the level of phytic acid below the control value. The value of phytic acid were found to be lower than reported lethal dose of 250-500 mg/100g by Bushway *et al.*, (1998). Oxalate content of the defatted raw *Moringa oleifera* seed flour significantly ( $P < 0.05$ ) decreased from 0.02 mg to very negligible value. It was observed that boiling caused considerable reduction of the oxalate. Therefore, the reduction in the levels of oxalate during blanching at  $100^\circ\text{C}$  at varying times may be due to its solubility in hot water (Bradbury and Nixon, 1998). The value of oxalate content after soaking and blanching were found to be lower than reported lethal doses. Munro and Basir (1969) estimated the threshold of oxalate toxicity in man to be 200-500 mg/100g of the sample.

Defatted raw *Moringa oleifera* seed was found to have  $1.02 \pm 0.01 \text{ TUI/mg}$ . On soaking for 24 hours the trypsin inhibitor activity reduced to  $0.83 \pm 0.01 \text{ TUI/mg}$  which corresponds to 18.45%. Also blanching of the soaked seeds for 45 minutes significantly ( $P < 0.05$ ) reduced the try [psin inhibitor activity to  $0.15 \pm 0.01 \text{ TUI/mg}$  which corresponds to 84.47% reduction when compared to the control. It was also observed that blanching further facilitated reduction of the trypsin inhibitor activity. This conforms with the reports that trypsin inhibitor activity is inactivated by heat especially moist heat because of even distribution of heat (Bressani and Sosa, 1990; Liener, 1975). The value of  $0.15 \pm 0.01 \text{ mg/100g}$  obtained in this analysis is quite lower than 200 mg/100g reported as lethal dose in man by Inuwa *et al.*, (2011).

#### 4. Conclusion

The overall result revealed that soaking, dehulling and / or boiling of bambaranut and defatted *Moringa oleifera* seeds separately for varying periods significantly ( $P < 0.05$ ) reduced the antinutrients. Longer periods of soaking and boiling reasonably reduced these antinutrients to a safer level below their lethal dosage for human consumption.

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