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RESEARCH ARTICLE

THE EFFECT OF BOILING PERIODS ON NUTRIENTS COMPOSITION AND ANTI-NUTRIENT OF AFRICAN NUTMEG (*Monodora myristica*)

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ABSTRACT

The effect of different duration of boiling on nutrients composition and anti – nutrient content of African nutmeg (Monodora myristica) was investigated using proximate, Amino acid, minerals, Gross energy and anti nutritional factors. The samples were subjected to 30 minutes, 60 minutes and 90 minutes boiling. Both the raw and processed forms were later dried, milled and chemically analyzed for nutrients and anti-nutrients. There were significant ($p < 0.05$) differences among the treatment means. The raw seeds (T_1) had the highest value of crude protein 16.88%; followed by treatment 2, 14.53%; T3, 11.74% and T4, 10.57% making treatment 2 a choice treatment among the processed forms. For micro minerals in mg/kg, T_1 (Fe, 91.36; Zn, 84.36; Cu, 21.70; Mn, 306.3), T_2 (Fe, 88.50; Zn, 81.70; Cu, 19.60; Mn, 296.2), T_3 (Fe, 74.46; Zn, 69.72; Cu, 16.70; Mn, 279.4), T_4 (Fe, 65.56; Zn, 51.36; Cu, 14.56; Mn, 263.6) and macro minerals in %, T_1 (Na, 0.08; P, 0.94; Ca, 0.34; Mg, 0.34; K, 0.54;), T_2 (Na, 0.07; P, 0.81; Ca, 0.30; Mg, 0.27; K, 0.48), T_3 (Na, 0.06; P, 0.62; Ca, 0.22; Mg, 0.21; K, 0.40), T4 (Na, 0.03; P, 0.48, Ca, 0.17; Mg, 0.14; K, 0.28), it followed similar pattern like that of proximate and gross energy with treatment 2 having the highest value among the processed forms. Also the essential amino acid in g/100g, T_1 (Isoleucine, 1.78; Leucine, 1.67; Methionine, 1.49; Valine, 1.61; Threonine, 0.69; Lysine, 0.10), T_2 (Isoleucine, 1.60; leucine, 1.51, Methionine, 1.34; Valine, 1.45; Threonine, 1.15; lysine, 0.09), T_3 (Isoleusine, 1.42; leucine, 1.51; Methionine, 1.19; Valine, 1.29; Threonine, 1.02; Lysine, 0.08), T_4 (Isolusine, 1.24; leucine, 0.76; Methionine, 1.04; Valine, 1.13; Threonine, 0.97; Lysine, 0.71),and non essential in g/100g, T_1 (Aspatate, 0.00; Phenylalanine, 1.95; Glutamate, 1.11), (Aspatate, 0.00; Phenylalanine, 1.75; Glutamate, 1.00), T_3 (Aspatate, 0.00; Phenylalanine, 1.56; Glutamate, 0.89), T_4 (Aspatate, 0.00; Phenylalanine, 1.36; Glutamate, 0.78), favored treatment 2, followed by 3 and 4. There were general reduction in the values of anti nutritional factors in %, T_1 (Trypsin inhibitor, 31.59; Tannin, 0.30; HCN, 26.36), T_2 (Trypsin inhibitor, 0.76; Tannin, 0.23; HCN, 11.65), T_3 (Trypsin inhibitor, 0.00; Tannin, 0.17; HCN, 0.00), T_4 ((Trypsin inhibitor, 0.00; Tannin, 0.12; HCN, 0.00), with highest reduction in treatment 4, 3, 2 and 1. Considering the highest value of crude protein, gross energy, mineral composition and highest value of both essential and non-essential amino acid and the reduction in the anti nutritional factors, Treatment 2 is recommended among others.

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INTRODUCTION

Spices are pronutrients used orally in a relatively small amounts to improve the intrinsic value of the nutrient mix in an animal diet (Rosen, 1996). Jones (2002) reported that spices have played a significant role in the health and nutrition of animals due to their additional potentially beneficial properties such as anti-oxidative, antimicrobial, fungicidal and physiological activities. Various spices such as nutmeg and "Rosemary" have been used in animal diet (Abdel-Wahlab and Aly, 2003; Tatsuya *et al*, 2003; Krause and Ternes, 2000). One of the spices that come to mind is African nutmeg (*Monodora myristica*) with crude protein content of 14.88% (Okwu, 2001). Its yield is about 7 metric tonnes per hectare (Celnet spice guide, 2007).The effect of boiling periods on proximate composition, amino acid profile, gross energy, mineral composition and anti-nutritional factors of raw African nutmeg. (*Monodora myristica*) are yet to be reported.This forms the objective of the study.

MATERIALS AND METHODS

Processing of the test spice

The raw African nutmeg was purchased from Umuahia central market of Abia state, the seeds were randomly collected, mixed thoroughly, cleaned and weighed. The seeds were divided into four parts, one part was boiled for 30 minutes, in water at 100^0C – 105^0C, a part was boiled for 60 minutes while the other part was boiled for 90 minutes and the remaining part was used as raw. The boiled seeds were then oven-dried at 60^0C before being milled and used for chemical analysis.

Chemical analysis

Proximate and gross energy composition determination

The raw beans and processed beans were analyzed for proximate composition using the procedure of (AOAC, 1990) the gross energy was determined using Gallenkamp Ballistic Bomb Calorimeter.

Mineral determination

The Milled and raw processed seeds were subjected to wet digestion with hydrochloric and nitric acid by the Johnson and (Ulrich, 1959) method. Following the digestion, the mineral elements Na, K, Ca, were determined by Flame photometric using Jenway Digital photometre. Cu, Zn, Mn, Fe, were determined by Atomic absorption spectrophotometric using buck 600 AAS. Phosphorus, sulphur and Magnesium were determined using spectronic 21A digital spectrophotometer.

Determination of hydrocyanic acid (hydrocyanide)

Knowles *et al,* (1980) method was used in determining the hydrocyanic acid during which 5g of each sample was weighed into 250 ml conical flask. Each sample was soaked with 25 ml of distlled water for 3 hours, incubated for another 16 hours at a temperature of 38^oc and after the extraction, filtration was done using double layer of harden filter paper. The distillation was done using Markham distillation apparatus. Each sample extracted was transferred into a 2 necked 500 ml flask connected to a steam generator, and this was steam–distilled with saturated sodium bicarbonate solution contained in a 50 ml flask for 60 minutes. 1 ml of starch indicator was added to 20 ml of each distillate and was titrated with 0.02N of Iodine solution. The colour change was from colourless to blue which is the end point. The percentage (%) hydrocyanide was calculated with the formula:

$$\% \text{ Hydrocyanic acid} = \text{Titre} \times \frac{100}{10} \times \frac{0.27}{1000} \times \frac{100}{\text{weight of sample}}$$

Determination of phytic acid (phytate)

The phytic acid was determined using the procedure described by lucas and Markaka (1975). The entails the weighing of 2 2 g of each sample into 250 ml conical flask. 100 ml of 2% concentrated hydrochloric acid was used to soak each sample in the conical flask for 3 hours. This was filtered through a double layer of hardened filter paper. 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was

added in each case to give proper acidity. Ten millilitres of 0.3% Ammonium thiocyanic solution was added into each solution as indicator. This was titrated with standard iron (iii) chloride solution, which contained 0.00195 g iron per ml. the end-point was slightly brownish-yellow which persisted for 5 minutes. The percentage phytic acid was calculated using the formula.

$$\% \text{ phytic acid} = \frac{X \times 1.19 \times 100}{2}$$

Where X = Titre value x 0.00195

Determination of tannic acid (tannin)

The tannin in the test feedstuffs were determined according to the method of Maga (1982). Thus 2 g of each sample were weighed into a beaker. Each filtrate was in the water bath for 4 hours, after which the filtrates were removed. The samples were filtrate through double layer filter paper to obtain filtrate. A set of standard solution of tannic and was prepared ranging from 10 ppm to 50 ppm. The absorbance of the standard solution as well as that of the filtrates were read at 500 nm on a spectronic 20. The percentage tannin was calculated using the formulate:

$$\% \text{ Tannin} = \frac{\text{Absorbance} \times \text{Average gradient} \times \text{Dilution factor}}{100}$$

Determination of trypsin inhibitors

The determination of trypsin inhibitor was carried out according to the procedure outlined by Kakade *et al* (1969). This involves weighing of the samples into a srew cap centrifuge tube. 10ml of 0.01m phosphate buffer was added and the contents shaken at room temperature for one hour on a UDY shaker. The suspension obtained was centrifuge at 5000 rpm for 5mins and filtered through ehatman No 42 filter paper. The volume of each was adjusted to 2ml with phosphate buffer. The test tubes were placed in water bath, maintained at 37^{o}C. six millilitres of 5% TCA solution was added at one of the tubes to serve as a blank. 2 ml of casein solution was added at one of the tubes. Which were previously kept at 37^{0}C. These were incubated for 20 mins. The reaction was stopped after 20 minutes by adding 6 ml of TCA solution to the experimental tubes and the tubes were shaken. The reaction was allowed to proceed for 1 hour at room temperature. The mixture was filttered through whatman No. 42 filter paper. Absorbance of filtered from samples and trypsin standard solutions were read at 280nm. The trypsin inhibitor in mg/g was calculated using the formular:

$$\text{T.I mg/g} = \frac{\text{A standard} - \text{A sample} \times \text{Dilution factor}}{0.1\text{g} \times \text{sample weight in gram} \quad 1000\text{x sample size}}$$

Determination of amino-acid profile

The amino acid composition of both raw and boiled *African nutmeg (monodora myristica) s*eed were carried out as described by AOAC (2006). The dried and pulverized samples were made to be free of water by ensuring constant weight for a period of time in the laboratory .10.0 of the sample was weighed into the 250ml conical flask capacity. The sample was defatted by extracting the fat content of the sample with 30ml of the petroleum spirit three times with soxhlet extractor that was equipped with thimble. The sample was hydrolysed by using 30ml of deionised water three times. The amino acid content of the sample was recovered by extraction with 30ml of the methylene chloride thrice before concentrated to ImI for gas chromatography analysis. The gas chromatography conditions were as given below.

GC:	Hp 5890 Power With Hp Chem. Station Rev. A 09.01 (1206) software
Injection temperature	Split injection
Split ratio.	20.1
Carrier Gas	Nitrogen
Inlet temperature:	250^{0}C
Column type	HP5
Column Dimension	30m x 0.25 x 0.25µm
Over program:	Initial Temperature @ 50^{0}c First Ramping @ 10^{0}C/min for 20 min, maintained for 4min second ramping @ 15-0-C/min for 4min, maintained for 5min
Detector:	PFPD
Detector temperature:	320^{0}C
Hydrogen pressure:	20psi
Compressed Air:	30psi

RESULT AND DISCUSSION

The proximate and gross energy composition of both raw and boiled African nutmeg is as shown in Table1. There were general reductions in all the parameters considered, this is in line with earlier reporter Akinmutimi *et al* (2009) who reported that processing reduces nutrient composition. These reductions were attributed to solubilization of nutrients and leaching as a result of boiling. The crude protein, ether extract, crude fibre, ash and nitrogen free extract values followed similar pattern in that the highest value occurred in the raw African nutmeg and significantly decrease progressively down ward making African nutmeg subjected to 90 minutes boiling having the least value. Among the processed forms, there were significant ($P < 0.05$) differences among the processed forms with treatment 2 having the highest value and treatment 4 having the least value. This down ward decrease is in line with earlier reporter (Akinmutimi *et al*., 2009)

Since nutrients in raw African nutmeg may not be available in terms of digestibility and utilization by the animals, the need then to concentrate on the processed form arises. Among the processed form, it seems that African nutmeg subjected to 30 minutes boiling had the highest value for all the parameters considered making it a choice treatment in terms of proximate and gross energy compositions This was followed by treatment 3 and lastly treatment 4 subjected to 90 minutes boiling. With crude protein of 14.53% and gross energy content of 3.85kcal/g for treatment 2, the use of treatment of 2 as feed additives will not only add flavor but contribute to protein and energy requirements, provide aroma, colour, stimulate appetite and enhance digestion (Agbara , 2010).

There were significant differences ($p < 0.05$) among the treatment means with the raw values being significantly ($p < 0.05$) higher than the processed seeds. This was followed by treatment 2, 3and 4.

Table 1. proximate and gross energy composition of both raw and boiled Africa nutmeg

	C.P	%Rd	C.F	%Rd	E.E	%Rd	Ash	%Rd	N.F.E	%Rd	Gross Energy	%\Rd
T1	16.88[a]		4.32[a]		3.79[a]		5.68[a]		57.32[d]		3.88[a]	
T2	14.53[c]		3.93[b]		3.62[b]		5.32[b]		62.51[b]		3.87[b]	
		-13.92%		-9.03%		-4.49%		-6.34%		9.05%		-0.25%
T3	11.74[b]		3.67[c]		3.55[c]		5.05[c]		63.69[c]		3.85[c]	
		-12.67%		-15.0%		-6.33%		-11.0%		11.1%		-0.77%
T4	10.57[d]		3.56[d]		3.44[d]		4.82[d]		69.48[a]		3.83[d]	
		-37.34%		-17.5%		-9.23%		-15.1%		21.2%		-1.28%
SE	0.03		0.00		0.00		0.00		0.00		0.00	

a-d treatments means in the same column with different superscript are significantly different ($p<0.05$). % RD = percent reduction

Table 2. The macro mineral composition of both raw and processed African nut meg

	Na	%red	P	% red	Ca	% red	Mg	% red	K	% red
T1	0.08[a]		0.94[a]		0.34[a]		0.34[a]		0.54[a]	
T2	0.07[b]		0.81[b]		0.30[b]		0.27[b]		0.48[b]	
		-12.5%		-13.82%		-11.76%		-20.8%		-11.11%
T3	0.06[c]		0.62[c]		0.22[c]		0.21[c]		0.40[c]	
		-25%		-34.04%		-35.29%		-38.2%		-25.9%
T4	0.03[d]		0.48[d]		0.17[d]		0.14[d]		0.28[d]	
		-62.5%		-49.94%		-50%		-8.8%		-48.1%
SEM	0.00		0.00		0.00		0.00		0.00	

Table 3. Micro mineral composition of both raw and processed form micro mineral composition of both raw and processed

	Fe	%Rd	Zn	%Rd	Cu	%Rd	M n	%Rd
T1	91.3667[a]		84.3667[a]		21.7000[a]		306.3[a]	
T2	88.5000[b]	-3.14%	81.7000[b]	-3.16%	19.6000[b]	-9.68%	296.2[b]	-3.29%
T3	76.4667[c]	-16.3%	69.7200[c]	-17.3%	16.700[c]	-23.0%	279.4[c]	-8.78%
T4	65.5667[d]	-28.2%	51.3667[d]	-39.1%	14.5667[d]	-32.9%	263.6[d]	-13.94%
SEM	0.081		0.08		0.065		0.057	

Table 4. The Anti – nutritional factors of raw and processed African nutmeg

	TRYPSIN INHIBITOR	%Rd	TANNIN	%Rd	HCN	%Rd
T1	31.5967[a]		0.3000[a]		26.3667[a]	
T2	0.7667[b]	-97.6%	0.2367[b]	-21.1%	11.6567[b]	-55.8%
T3	0.0000[c]	-100%	0.1767[c]	-41.1%	0.0000[c]	-100%
T4	0.0000[d]	-100%	0.1200[d]	-60%	0.0000[c]	-100%
SEM	0.0000		0.0000		0.0000	

a-d treatments means in the same column with different superscript are significantly difference ($p<0.05$).

Table 5. The Essential Amino acid in raw and processed African nutmeg in g/100 g

	Isoleucine	%Rd	Leucine	%rd	Methionine	%Rd	Valine	%Rd	Threonine	%Rd	Lysine	%Rd
T1	1.780a[a]		1.678[a]		1.496[a]		1.617[a]		0.696[d]		0.102[a]	
T2	1.602[b]	-10%	1.510[c]	-10%	1.3468[b]	-9.9%	1.4554[b]	-9.9%	1.1578[a]	+66.3%	0.0918[b]	-10%
T3	1.424[c]	-20%	1.515[b]	-9.7%	1.197[c]	-20.0%	1.293[c]	-19.9%	1.029[b]	-47.8%	0.081[c]	-20%
T4	1.246[d]	-30%	0.761[d]	-54.7%	1.047[d]	-29.9%	1.132[d]	-30%	0.974c	-39.9%	0.714[d]	-60%
SEM	0.00		0.00		0.0000		0.0000		0.0000		0.0000	

a-d treatments means in the same column with different superscript are significantly difference ($p<0.05$). % RD = percent reduction.

Table 6. The Non – essential amino acid in raw and processed African nutmeg g/100g

	Aspatate	%Rd	Phenylalanine	%Rd	glutamate	%rd
T1	0.0027[a]		1.9550[a]		1.1167a	
T2	0.0024[ab]	-11.1%	1.7595[b]	-10%	1.0051b	-9.9%
T3	0.0021[b]	-22,2%	1.5640[c]	-20%	0.8934[c]	-19.9%
T4	0.0015[c]	-44.4%	1.3685[d]	-30%	0.7817[d]	-29.9%
SEM	.0.000		0.000		0.000	

a-d treatments means in the same column with different superscript are significantly difference ($p<0.05$). % RD = % reduction

that reported that processing reduces nutrient composition due to boiling. This implies that treatment 2 is a choice treatment in terms of macro minerals and that formation of ration with the seed from treatment 2 will enhance neural conduction and muscular contraction, blood coagulation and bone and teeth formation in animals due to highest values of phosphorus, calcium, magnesium, sodium and potassium (Agbara, 2010). There were significant differences ($p < 0.05$) for all the elements considered. The trend followed similar pattern like that of macro mineral with the raw having the highest value. There were significant ($P < 0.05$) differences among the processed seeds. Among the processed seeds, the seed boiled for 30 minutes had the highest value while the seeds boiled for 90 minutes had the least value, this probably may be due to solubilization and leaching. It then implies that addition of seeds boiled for 30 minutes in ration will enhance blood formation because of high value of iron among others (Robert *et al*., 2006), normal utilization of carbohydrate in the body because manganese is a co – factor of many enzymes e.g. Kinase, decarboxylase, peptidase (Eburuaja, 2010), better utilization of iron in haemoglobin formation as a result of copper (Robert *et al*., 2003) and prevent skeletal abnormalities since zinc plays important role in calcification of bones (Olomu, 1995). There were general reductions in the value of anti – nutritional factors. The highest value occurred in the raw and the least value occurred in seeds boiled for 90 minutes. This is in the line with earlier reporters that wet heat treatment reduces anti nutritional factors (Akinmutimi, 2004, Akanji *et al*., 2003). The result also shows the thermostability of tannin and phytate as oppose to thermolability of trypsin inhibitors and hydrocyanic acid (linenar 1990, Eburuaja, 2010). The use of seed boiled for 30 minutes expose the animal to problem such as formation of complexes with protein and bitter taste (Vohra, 1962) as a result of poor detoxification of tannin (Olomu, 1995; Eburuaja, 2010). This is followed by treatment 3 boiled for 60minutes, making treatment 4 a choice treatment in terms of detoxification. There was general reduction in the values of essential amino acids with the exception of threonine, making the raw to have the highest value that was significantly different ($p < 0.05$) from the processed seeds while the least value occurs in seeds boiled for 90 minutes. This general reduction could be attributed to solubilization and leaching of these amino acids as a result of boiling (Akinmutimi, 2004, Akinmutimi *et al*., 2010). Among the processed seeds treatment 2 had the highest value that was significantly different ($p< 0.05$) from others making it a desired treatment. For threonine, processed seeds had values that were significantly higher than the raw but the values decreased as the boiling periods increased, this probably may be due to the fact that at 30 minutes, there was release of these amino acid and probably threonine is a component of anti nutrient that has been broken down leading to it increased, but at 60 minutes, and 90 minutes, the degree of solubility of this amino acid increased leading to leaching and hence down ward decreased in value. This is to say that treatment 2 became a choice treatment. Comparing treatment, 2 amino acid especially lysine (1.51) and methionine (1.35) with conventional protein sources such as groundnuts cake with lysine value of (2.13) and methionine value of (0.19) and soy bean with lysine value of (3.54) and methionine value of (0.66) (Nwankwo 2010). It is higher in methionine than the convensional protein sources but lower than the convensional protein sources for lysine. The higher value of methionine than the convensional protein sources is an added advantage in using it as a spice. It follows similar trend like the essential amino acid in that the highest value occurred in raw and the least occurred in seeds boiled for 90 minutes also among the processed seed treatment 2 had the highest value, this probably may be due to solubilization of nutrient and leaching (Nwankwo, 2010)

Conclusion

In conclusion, considering the highest value of crude protein, gross energy, minerals composition (macro and micro elements) and highest value of both essential and non – essential amino acid and the reduction in anti-nutritional factors, T2 is recommended among others.

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