Original Research Article

Bioactive Compounds, Amino acid content /Score of Composite Wheat, African walnut, Moringa biscuits

ABSTRACT:

Moringa and African walnut seeds were processed into flour and evaluated for tannin, total phenol and anti-oxidant activity. Different formulations of wheat, African walnut and Moringa seed composite flour (A: WHF 100%: AWF 0;MSF 0, B= WHF 77.5%:AWF 20%: MSF 2.5%, C=WHF 75%: AWF 20%: MSF 5.0%, D= WHF 72.5%: AWF 20%: MSF 7.5%. E = WHF 70%: AWF 20%: MSF 10%, F = WHF 90%: AWF 0 : MSF 10%, G = WHF 80% : AWF 20% : MSF 0) were used in the production of biscuits. The Amino acid content of the prepared biscuits were determined and the Amino acid score using Hens egg and FAO standard to evaluate protein quality. Results reveal thatTannin and Total phenol content of African walnut flour was significantly higher with values 49.82mg/100g and54.90mg/100g respectively, with Moringa seed flour having an anti-oxidant activity of 60.55%. Amino acid content of the biscuits showed that glutamic acid was higher in all the samples ranging from 10.96 – 12.33g/100g. Amino acid score results indicate that the amino acid content of the composite biscuits were higher than the FAO standards for adults above 18years.

KEYWORDS: Anti-oxidant activity, Tannins, Amino acid score, African walnut, Biscuits, Moronga seed, Flour, Total Phenol.

INTRODUCTION:

Biscuits are ready to eat snack made from wheat dough and baked for a short period of time. Increasing urbanization and change in consumption pattern due to changing food habits and preferences has led to increased consumption of biscuits. The association of wheat consumption with problems such as celiac disease makes it pertinent to utilize composite flour in biscuit

manufacture. Consequently, the improvement in protein quality of cereal products have become a research focus in developing countries. This has led to the need of producing food products containing functional ingredients to meet the requirements of individuals with health challenges (1, 2).

Moringa is a rich source of essential nutrients such as proteins, essential amino acids, minerals and low amounts of anti-nutrients. The seeds contain bioactive compounds such as flavonoids and phenolic compounds and tannins (3). These compounds exhibit anti-oxidant properties. Anti-oxidants play a role in inhibiting and scavenging free radicals thus providing protection to human against infection and degenerative diseases. Moringa extract have been shown to offer protection against oxidative damage (4). Also reported is the use of Moringa oleifera in the treatment of rheumatism, ascities, while extracts of the leaf is capable of reducing hyperglycemia and dyslipidemia (5, 6, 7).

Polyphenols have been found to be strong antioxidants that can neutralize free radicals by donating an electron or hydrogen atom. Polyphenols suppress the generation of free radicals, thus reducing the rate of oxidation by inhibiting the formation of or deactivating the active species and precursors of free radicals. They act as direct radical scavengers of the lipid peroxidation chain reactions (chain breakers). Chain breakers donate an electron to the free radical, neutralizing the radicals and themselves becoming stable (less reactive) radicals, thus stopping the chain reaction.

Some studies have shown that flavonoids displayed higher antioxidant capacity against metal induced peroxidation. The antioxidant capacity of flavanoids is determined by the number of hydroxyl groups they contain and the position on the molecular ring structure (8).

Udedi et.al (9) reported that African walnut has the potential of combating food insecurity due to its phytochemical components such as phenols, flavonoids and ascorbic acid. Also, the African walnut have been shown to be a good source of high biological proteins, and fats containing polyunsaturated fatty acids, as well as reasonable amounts of ash and crude fiber (10, 11).

Ayo-ola et.al,(12) reported the use of African walnut of African walnut in the treatment of stomach disorders and for controlling high blood pressure. Barber and Obinna-Echem (13) assessed the nutritional composition, physical and sensory properties of wheat African walnut cookies and recommended that the African Walnut flour could be used successfully as a partial substitute for wheat at a range of 5% - 15%.

Proteins vary in their content of constituent amino acids and can be devoid of or low in one or more dietary indispensable amino acid. The quantity of protein within a given food source is primarily determined as a function of true nitrogen content and this is not considered a reliable indicator of the ability of the dietary protein to meet the metabolic needs of the consumer. The quality of a dietary protein is typically defined by the extent to which the constituent amino acids match the needs of the individual. The capacity of the protein to provide metabolically available nitrogen to organ and tissues is of great consideration in determining amino acid quality (14). An amino acid score is a score which expresses overall healthiness of a diet which are developed either for the general population or for the purposes of preventing specific dietary disease (15). Protein quality have been previously evaluated in terms of protein efficiency ratio (PER) and Net Protein Utilisation (NPU) (16). However FAO evaluates protein requirements as the quantity of amino acids that is required to meet metabolic needs for maintenance and these could depend on age group, infants, children or adults and on pregnancy and lactation (17). The objective of the study was to formulate composite biscuits from wheat, African walnut and Moringa seed flour

blends and evaluate the amino acid content and the amino acid scores of the biscuit using the FAO Standard.

MATERIALS AND METHODS:

2.1 Processing of African walnut flour.

African walnut (Tetracarpidium conorphorum) was processed int flour in accordance with the method of Barber and Obinna-Echem (13). Sorting operations were carried out on matured seeds to remove debris and undersized materials. The seeds were washed and boiled for 90mins, allowed to cool , dehull manually and the husks discarded. Size reduction operations was carried out by cutting the seeds to dimensions of 1cm in diameter and then drying in an electric air oven at a temperature of 54^0C for 6hr. The dried kernel was milled to finer particles that will pass through a 250µm sieve aperture and packaged.

2.2. Processing Moringa seed flour.

Matured and dried Moringa seeds were processed into flour using the method described by Ogunsina et.al, (18). The seeds were sorted according to various parameters viz size, color and shape and debris removed. The seeds were de-hulled manually and the husks discarded. The seeds were boiled in water 3:1 (v/w), drained and dried in an air oven at 54^0C for 4hr, allowed to cool to room temperature and milled to flour.

2.3 Baking of biscuits.

The method of Agu and Okoli (19) was used in the baking of biscuits. Flour blends, sugar, margarine and baking powder were mixed manually in a mixing bowl to achieve the desired consistency, then one whipped egg and 5 ml of vanilla flavor added and mixed thoroughly again for about 10 mins to obtain the desired dough quality. The batter was then spread out on a baking

table and rolled to uniform thickness. Biscuit cutters were used to cut the biscuits to uniform sizes and transferred to aluminum baking trays whose surfaces have been previously lubricated with margarine to prevent burning.

The biscuits were then perforated, glazed with whipped egg and transferred to a pre-set electric oven at 180^0C and baked for 25mins. The baked biscuits were then removed from the oven, allowed to cool before packaging.

2.4 Chemical Analysis.

2.4.1 Anti-Oxidant Determination

Plant Extraction

The antioxidant activity was determined using the DPPH (2, 2- Diphenyl-1- picrylhydrazyl) method as described by Akter et.al, (20).

One Hundred grams of dry powdered sample was weighed into a beaker or conical flask and 100 ml of ethanol added and shaken vigorously for 2 minutes. This was stirred with a magnetic stirrer or vortex for 15 minutes, and allowed to stand for 2 hrs for proper extraction. Thereafter, it was centrifuged at 2500 rpm for 10 minutes and the supernatant poured into another beaker. The supernant was concentrated by evaporating in a water bath at 80°C. The concentrated extract was kept for assay.

One mM DPPH (2, 2- Diphenyl-1-picrylhydrazyl) in ethanol was prepared (394.32mg DPPH dissolved in one litre of ethanol) and 10 mg of the concentrated sample extract dissolved in 10 ml of ethanol (1 mg/ml). And 1.5 ml of the sample extract was pipetted into a test tube and 1.5 ml DPPH solution added into the test tube. The spectrophotometer was calibrated with ethanol as blank and the value of the absorbance/optical density of the control (DPPH solution) was taken. Absorbance of the test sample was read at 517nm.

Calculation:

$$\text{DPPH Scavenged \%} = \frac{Abs.\ of\ control - Abs.of\ test\ sample}{Abs.\ of\ control \times wt\ of\ sample} \times 100$$

2.4.2 Determination of Total phenol.

The total phenol content of the samples was determined using Folin – Ciocalteu reagent as described by Singleton et al, (21) and modified by Stankovic (22).

Sample extraction (Using Ethanol)

One gram of powdered sample was weighed into a conical flask and 10 ml of ethanol added, and then plugged it with aluminium foil. This was vigorously shaken and allowed to stand for 30 min for proper extraction. Thereafter, Centrifugation was done and then filtered to obtain clear supernatant. The supernatant was used for total phenolic assay

One ml of the solution (extract/supernatant) was pipetted into a test tube and 0.5 ml 2N Folin-Ciocalteu reagent and 1.5 ml 7% $NaCO_3$ solution added. This was made up to 10ml with distilled water and shaken vigorously. This was allowed to stand for 90 minutes and the absorbance read at 765 nm. Also, prepared were the following concentrations of Tannic acid standard 20 mg/l, 40 mg/l, 60 mg/l, 80 mg/l, 100 mg/l, and 120 mg/l. The various absorbances of the Tannic acid concentrations were read off and calibration curve for the Tannic acid standard drawn. That is absorbance against concentration. Extrapolation was done by tracing the absorbance of the sample down the concentration axis to obtain the concentration of the sample.

Calculation:

$$\text{Phenol content mg/100g (TAE)} = \frac{\text{Conc.obtained in mg per litre x Vol of sample x DF}}{Wt\ of\ sample} \qquad (13)$$

DF: Dilution factor. If not diluted, then DF = 1

2.4.3 Determination of Amino Acid Profile

The Amino Acid profile in the known sample was determined using methods described by Benitez (23). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Defatting Sample:

The sample was defatted using chloroform/methanol mixture of ratio 2:1. Four grammes of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (21).

Nitrogen Determination:

Nitrogen determination was carried out according to A.O.A.C methods(24). A small amount (200 mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated Sulphuric acid (10 ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10 m1) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected.

The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V}{W \times C} \times 100$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100ml)

W. = Weight of dried sample (mg)

C. = Aliquot of the sample used (10ml)

14. = Nitrogen constant in mg.

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. Seven (7 ml) of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^0C \pm 5^0C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. **It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis.**

The filtrate was then evaporated to dryness using rotary evaporator (Buchi R 100). The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

The amount loaded was 60 microlitre. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Calculation Amino Acid Values

An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

Determination of Tryptophan

The tryptophan in the known sample was hydrolyzed with 4.2 M Sodium hydroxide (25). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

De-fatting of samples and Nitrogen determination were carried out as previously described.

2.4.5 Amino Acid Scores

Amino acid scores were estimated using whole egg as reference protein and the FAO/WHO (17) standard. Estimation of dietary protein quality was done by calculating the amino acid scores using 2 different parameters. The amino acid was calculated using whole hen's egg amino acid profile using the formula below:

$$\text{Amino acid score} = \frac{mg\ of\ amino\ acid\ in\ 1g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1g\ of\ reference\ protein}$$

Also, the amino acid scores was calculated using FAO/WHO reference pattern for adults above 18 years. Determination of aromatic amino acids and Sulphur amino acids were calculated from the amino acid profile table.

RESULTS AND DISCUSSION:

TABLE 1. Bioactive Compounds of African walnut flour (AWF) and Moringa seed flour (MSF).

Parameter	AWF	MSF
Tannin (mg/100g)	49.82 ± 0.03a	32.01 ± 0.02b
TotalPhenol (mg/100g)	54.90 ± 0.03a	43.18 ± 0.01b
Antioxidant Activity%	47.63^{c} ± 0.25b	60.55 ± 0.28^{a}

Values are Mean ± SEM of triplicate determination.

Means with different superscripts within a row are significantly ($p \leq 0.05$) different.

The results of bioactive compounds shown in Table 1, for the raw material (African walnut and Moringa seed flour) indicated than African walnut were significantly higher than Moringa seed flour in Tannin content and Total phenol content with values of 49.82mg/100g and 54.90mg/100g respectively. Khanbabaee and Ree (26) reported that tannins are secondary metabolites of plants which can be classified based on their structure into four major groups viz: Gallotannins, Ellagitannins, Complex tannins and Condensed tannins. Tannins are mainly found in fruits, especially berries, Cocoa, beverages like wine, beer, tea and cereals such as Sorghum and barley.

However, the antioxidant activity of Moringa seed flour (60.55%) was significantly higher than African walnut flour with a value of 47.63%. This result shows that moringa seed flour has a more potent antioxidant than African walnut. Results of amino acid content of African walnut flour shown in Table 2 revealed appreciable amounts of glutamic (13.85g/100g), aspartic 9.61g/100g leucine 5.30g/100g , and Arginine 5.50g/100g respectively.

TABLE 2: Amino acid contents of African walnut flour (AWF) samples.

Amino acid	Concentration:g/100g
	AWF
Leucine	7.29
Lysine	5.30
Isoleucine	3.27
Phenylalanine	3.81
Norleucine	-
Tryptophane	1.07
Valine	3.45
Methionine	0.96
Proline	3.96
Arginine	5.50
Tyrosine	3.09
Histidine	2.55
Cystine	3.30
Alanine	4.06
Glutamic acid	13.85
Glycine	3.85
Threonine	3.05
Serine	3.35
Aspartic	9.61

TABLE 3.Amino acid contents of biscuit samples produced from wheat flour (WHF), African walnut flour(AWF) and Moringa seed flour(MSF) blends

-Amino acid	Concentration: g/100g protein						
	A	B	C	D	E	F	G
Leucine	5.28	5.89	6.30	6.63	7.00	6.34	6.13
Lysine	4.13	4.35	4.93	5.66	5.75	5.86	4.5
Isoleucine	3.11	3.40	3.40	3.67	3.73	3.44	3.40
Phenylalanine	3.64	4.08	4.26	3.90	4.97	4.32	4.26
Norleucine					-		
Tryptophan	0.88	0.89	0.89	0.89	1.15	0.97	1.05
Valine	4.32	4.27	4.50	4.60	4.91	4.48	4.79
Methionine	1.04	1.17	1.23	1.28	1.44	1.24	1.39
Proline	3.04	3.25	3.25	3.29	3.55	3.38	3.35
Arginine	5.07	5.16	5.42	6.70	6.63	5.94	5.25
Tyrosine	3.04	3.18	3.27	3.53	3.53	3.45	3.26
Histidine	2.14	2.36	2.17	2.68	2.56	2.40	2.49
Cystine	0.95	0.97	1.69	0.97	1.21	1.25	0.97
Alanine	4.12	3.87	4.17	3.79	4.10	4.43	4.40
Glutamic acid	10.96	11.66	11.96	12.33	11.57	11.96	12.11
Glycine	3.13	3.56	3.89	3.42	3.51	3.32	3.70
Threonine	2.44	2.61	2.83	3.66	3.27	3.12	3.05
Serine	3.34	3.35	3.40	3.94	3.78	3.50	3.62
Aspartic acid	7.03	8.49	7.50	9.12	8.43	7.78	8.06

Sample A: WHF 100%: AWF 0;MSF 0, B= WHF 77.5%:AWF 20%: MSF 2.5%, C=WHF 75%: AWF 20%: MSF 5.0%, D= WHF 72.5%: AWF 20%: MSF 7.5%. E = WHF 70%: AWF 20%: MSF 10%, F = WHF 90%: AWF 0 : MSF 10%, G = WHF 80% : AWF 20% : MSF 0

The Amino acid content of biscuit samples produced from wheat flour (WHF), African walnut flour (AWF) and Moringa seed flour (MSF) are presented in Table 3.. The results indicated that all the African walnut biscuits had high amounts of glutamic acid ranging from 10.96 to 12.33 g/100g for sample D, Aspartic acid values were 7.03 to 9.12 g/100g. Leucine values showed that sample E had higher values of 7.00g/100g and Lysine content was between 4.13 to 5.86g/100g, with Arginine having a value of 5.07 g/100g to 6.70 g/100g for sample D. The values of Phenylalanine, Valine and Alanine were 4.20g, 4.55g, and 4.13g. Isoleucine, Tyrosine, Glycine, Threonine, and serine amino acids had values ranging between 3.08 – 3.92g/100g. Cystine and methionine had values of 1.30g and 1.16g, with Tryptophan being the least at 0.79g. The results also showed that the lysine and leucine content of the biscuits increases in samples D which contained 7.5% moringa seed flour and also in sample E which contained 10% moringa seed flour compared to the other formulations. Within the samples product sample E had the highest content of Leucine 7.00 g/100g, Lysine 5.75 g/100g, Isoleucine 3.75 g/100g, phenylalanine 4.97g/100g, Tryptophan 1.15 g/100g and Valine 4.91 g/100g.

Table 4. shows the Amino acid score of biscuit samples produced from wheat flour (WHF), African walnut and Moringa seed flour (MSF) compared to whole egg. The result indicated that the Histidine value of samples B, F and G of the formulated biscuit samples are comparable to hens egg content of histidine. Also, all the samples showed good amounts of glutamic acid content when compared to Hens egg except for sample A which is the control. The result of the glycine content of the biscuits showed that these amino acids were slightly higher in the biscuits than in whole egg with amino acid score values above 1.0. The arginine content of the biscuits showed that samples D and E showed higher Arginine content than Hens egg with values of 1.01 and 1.09 respectively. The most limiting of the amino acids was methionine with values ranging from 0.32g to 0.45g, and serine with a value of 0.42g to 0.49g. Similar, low values of amino

acid score for methionine have been reported by Fasuan et.al, (27) for protein isolates from Sesamum indicum with values of 0.57g

TABLE 4: Amino acid scores of biscuit samples produced from wheat flour (WHF), African walnut flour(AWF) and Moringa seeds flour(MSF) blends.

Amino acid	Hens Egg	A	B	C	D	E	F	G
Leucine	8.30	0.70	0.71	0.76	0.68	0.84	0.77	0.74
Lysine	6.20	0.66	0.70	0.79	1.06	0.93	0.94	0.73
Isoluecine	5.60	0.55	0.61	0.61	0.65	0.78	0.61	0.61
Phenylalanine	5.10	0.71	0.80	0.84	0.76	0.97	0.85	0.84
Norleucine								
Tryptophane	1.16	0.77	0.77	0.77	0.77	0.99	0.83	0.91
Valine	7.50	0.57	0.57	0.60	0.61	0.65	0.60	0.64
Methionine	3.20	0.32	0.37	0.38	0.40	0.45	0.39	0.43
Proline	3.80	0.80	0.86	0.86	0.84	0.93	0.89	0.88
Arginine	6.10	0.83	0.85	0.89	1.01	1.09	0.97	0.86
Tyrosine	4.00	0.76	0.80	0.82	0.88	0.88	0.86	0.81
Histidine	2.40	0.89	0.98	0.90	1.11	1.06	1.00	1.03
Cystine	1.80	0.52	0.54	0.63	0.54	0.67	0.69	0.53
Alanine	5.40	0.76	0.72	0.77	0.70	0.76	0.82	0.81
Glutamic acid	12.00	0.91	0.97	1.00	1.02	1.05	0.99	1.01
Glycine	3.00	1.04	1.19	1.29	1.14	1.17	1.10	1.73
Threonine	5.10	0.47	0.51	0.55	0.72	0.64	0.65	0.68
Serine	7.90	0.42	0.42	0.43	0.49	0.48	0.44	0.46
Aspartic acid	10.70	0.66	0.79	0.70	0.85	0.79	0.73	0.75

Sample A: WHF 100%: AWF 0;MSF 0, B= WHF 77.5%:AWF 20%: MSF 2.5%, C=WHF 75%: AWF 20%: MSF 5.0%, D= WHF 72.5%: AWF 20%: MSF 7.5%. E = WHF 70%:AWF20%:MSF10%, F=WHF90%:AWF0:MSF10%, G=WHF80%:AWF20%:

TABLE 5 :Amino Acid Score (FAO 2011) of biscuit samples produced from wheat flour(WHF),African walnut flour(AWF) and Moringa seed flour(MSF) blends (mg/g protein)

Amino Acid	FAO	A	B	C	D	E	F	G
His	15	24	24	22	26	26	24	25
IIe	30	31	37	34	36	37	34	34
Leu	59	58	59	63	66	70	63	61
Lys	45	41	44	49	57	57	58	45
SAA	22	20	22	29	23	26	25	24
AAA	38	75	82	84	83	85	87	86
Thre	23	24	26	28	32	33	31	31
Trp	6.0	8	9	9	11	11	9	10
Val	39	43	44	45	48	48	45	47

Sample A: WHF 100%: AWF 0;MSF 0, B= WHF 77.5%:AWF 20%: MSF 2.5%, C=WHF 75%: AWF 20%: MSF 5.0%, D= WHF 72.5%: AWF 20%: MSF 7.5%. E = WHF 70%:AWF20%:MSF10%, F=WHF90%:AWF0:MSF10%, G=WHF80%:AWF20%:

Table 5. shows the Amino acid score FAO (28) of biscuit samples produced from wheat flour (WHF), African walnut flour (AWF) and Moringa seed flour (MSF) blends for adults 18years and above. The results showed that the Histidine values of the various biscuits formulations (samples A to G) were higher than the FAO Standard, with values ranging from 22 mg/g for sample C to 26 mg/g for samples D and E. Also, Isoleucine and leucine values of the biscuits were higher than the FAO standard for all the samples of the biscuits formulated. The lysine content of samples A and B were lower than the FAO standard (45 mg/g) with values of 41.0

mg/g and 44.0 mg/g respectively. The amino acid score of the biscuits showed high amounts of aromatic amino acids which indicated that all the samples of the biscuit were higher than the FAO standard. Also, except for sample A, the value of the Sulphur amino acids (SAA) which ranged from 22 mg/g to 29 mg/g of protein are comparable to the FAO standard. Aromatic amino acids values were higher for all the samples of the biscuits produced as well as the Threonine values (24 mg/g – 33 mg/g of protein). The tryptophan and valine content of the biscuit samples showed that the formulated biscuits contained higher amounts of both amino acids. Enhanced protein values of the biscuit resulting from higher amino acid content indicates an improvement nutritional quality of the biscuits. The results showed that the protein quality of the biscuits which is an index of how well a protein meets the requirements of essential amino acids are above the FAO recommended standard.

CONCLUSION:

Substitution of the flour with Moringa seed four and African walnut resulted in significantly higher values of leucin, lysine, glutamic and Aspartic content of the biscuits.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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