Effect Of Processing On Antinutrients Contents Of African Elemi (Canarium Schweinfurthii) And African Walnut (Plukenetia Conophora) Consumed As Traditional Snacks In Nigeria.

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Abstract: It has been reported that seeds and fruits, but for their content of antinutrients, could offer a cheap means of providing adequate nutrients to their consumer. The effect of processing on antinutrients contents of the pulp of African elemi, AE (Canarium schweinfurthii) and kernel of African walnut, AW (Plukenetia conophora) consumed as traditional snacks (masticatories) in Nigeria were investigated. The levels of the antinutrients in raw samples of the traditional snacks as well as their corresponding residual levels in samples subjected to graded doses of heat treatment were evaluated using standard methods. Saponins, alkaloids, cyanogenic glycosides and oxalate were obtained in both samples while AE contained tannins and flavonoids in addition. The concentrations of saponins (1.00±0.19) and flavonoids (14.98±2.91) in raw AE and saponins (1.05±0.13) in AW were increased by the processing methods. The rest of the antinutrients were decreased in both samples. The effects on saponins and oxalate became significant ($p<0.05$) as the processing time was increased. The plant foods, with respect to the tolerable limits of the antinutrients, could therefore be considered safe for human consumption at all levels of processing.

Keywords: heat processing, maceration, masticatories, plant foods.

Introduction

Processed pulps of African elemi and seed kernels of African walnut are among the masticatories widely consumed as traditional snacks in West and Central Africa [1]. African elemi also known as bush candle (Canarium schweinfurthii) is a big tree with a long and straight bole of more than 50m. It is of the family Burseraceae and native to West and Central Africa. The tree is known locally in Nigeria as; 'Ube mgba' (Igbo), 'Atili' (Hausa) or 'Origbo'(Yoruba). African elemi fruit (a drupe) contains a single triangular shaped seed that is surrounded by purplish green pulp [2] (Plate 1). The pulp is consumed raw or softened in warm water as traditional snacks in Nigeria African walnut (Plukenetia conophora) on the other hand is a Liane (climbing and twining woody plant) of the family Euphorbiaceae and could be more than 30m long. The plant is variously known in Nigeria as 'Ukpa' (Igbo), 'Asala' or 'Awusa' (Yoruba), 'Okhue'or 'Okwe' (Edo) [3]. The plant bears capsular fruit that is 6-10cm long by 3-11cm wide and contains sub globular seeds of 2-2.5cm long wrapped in a thin brown shell. (Plate 2). The kernel is eaten roasted or cooked as traditional snacks (masticatory) in Nigeria. Antinutrients or anti-nutritional factors (ANFS) are phytochemicals that interfere with bioavailability of nutrients [4], [5]. Though not necessarily toxic per se, through their action, they reduce nutrient intake, digestion, absorption and utilization and may produce other adverse effects [6] or deter most animals from consuming foods containing them [7]. Some levels of antinutrients are found in almost all plant foods [8], but are reduced by many traditional methods of food preparation [4], [9]. Morton earlier [10] stated that masticatories are veritable sources of antinutrients. Often, these masticatories are not properly processed that some of the raw repugnant acid taste persists. Thus, toxicologically significant dietary intake of these phytochemicals results mainly from inadequate processing [11], [8]. This study was therefore carried out to evaluate the levels of some antinutrients in raw samples of the traditional snacks as well as their corresponding residual levels in samples subjected to graded doses of heat processing. The percentage differences are reported as the effects of processing on the antinutrients contents of the samples.

Plate 1: *African elemi (Canarium schweinfurthii) fruits.*

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Plate 2: *African walnut (Plukenetia conophora) seeds.*

Materials and Methods

Samples: Wholesome African elemi fruits were bought from Ngwa Road market in Aba, Aba South Local Government Area of Abia State, Nigeria while seeds of African walnut were obtained from fresh capsules bought from a farmer at Ojoto, Idemili South Local Government Area of Anambra State, Nigeria.

Sample processing: The samples were processed following the methods enunciated by Anyalogbu et al. [12], [13]. The plant samples were severally washed in deionized water and divided into four (4). The first portions were labelled AE_{raw} and AW_{raw} for African elemi and African walnut samples respectively and used raw. Accepted eating tenderness was obtained for African elemi by macerating in hot water (55°C) for 30 min [12] and, for African walnut by boiling in water (99±1°C) for 90 min [13]. The remaining portions of African elemi were labelled AE_{15}, AE_{30}, and AE_{45} and macerated in hot water (55°C) for 15, 30 and 45 min respectively. On the other hand, the remaining portions of African walnut were labelled AW_{45}, AW_{90}, and AW_{135} and boiled in water (99±1°C) for 45, 90, and 135 min respectively. The processing water was thrown away. The pulps and kernels of African elemi and African walnut samples respectively were dried for 48 hr in an air-circulatory oven (50°C) and ground in a mill. The powdered samples were then passed through a screen of 60 mesh size (i.e. 0.25mm openings) and used in the analyses.

Phytochemical Analyses

Qualitative tests (Screening): To prepare the water extract used in the qualitative tests, 10g of each of the sample was weighed into a 250ml conical flask and 100ml of distilled water added. The mixture was left to stand for 24hr at room temperature with occasional stirring and then filtered through Whatman Number 1 filter paper and the filtrate/extract collected. Screening tests for phytochemical/antinutrient constituents were carried out on the samples (AW_{raw}, AW_{45}, AW_{90}, $AW_{135,}$ AE_{raw}, AE_{15}, AE_{30}, and $AE_{45,}$) using standard procedures: Saponins and Flavonoids [14], Alkaloids and Tannins [15], Oxalate and Cyanogenic glycoside [16].

Quantitative analyses: Determination of the levels of the antinutrients indicated by the screening tests was carried out in triplicates using the recommended methods. The gravimetric method of Edeoga et al. [17] was used for alkaloids and, for saponins the double solvent extraction gravimetric method of Obadoni and Ochuko [18] as described by Aluko et al. [19] was used. Cyanogenic glycosides content was evaluated by the AOAC [20] quantitative alkaline titrimetric method No. 915.03 while oxalate (oxalic acid) was determined using the Precipitation-Titrimetric method of Oke [21]. The Spectrophotometric method of Van-Burden and Robinson [22] was used for tannin contents and the Gravimetric method of Boham and Kocipai-Abyazan [23] for flavonoid levels.

Statistical Analysis Test for statistical significance was done using one-way analysis of variance (ANOVA) and treatment means compared by the Duncan's [24] multiple range test utilizing statistical package for social sciences (SPSS) version-20. All values were expressed as Mean ± SD. Differences in the means were considered significant at $p < 0.05$ in all cases.

Results:

Phytochemical contents of raw and macerated African elemi seed pulp. The results of the qualitative analyses of the phytochemical contents of raw and macerated African elemi seed pulp are shown in Table 1. The plant food at all levels of maceration contained saponins, alkaloids, cyanogenic glycoside, oxalate, tannins and flavonoids. The contents of alkaloids and flavonoids were highest followed by oxalate with lower contents of saponins, cyanogenic glycosides and tannins. Table 2 shows the quantitative phytochemical contents of the plant food. This showed that alkaloid had the highest concentration which ranged from 16.35mg/100g in AE_{45} to 19.50mg/100g in AE_{raw} followed by flavonoids with values ranging from 14.98mg/100g in AE_{raw} to 17.27mg/100g in AE_{45}. The lowest value was in tannins (0.03mg/100g) Effect of processing expressed as the variation of the phytochemical contents of the sample with maceration time, is shown in Fig. 1. The concentrations of saponins and flavonoids increased ($p > 0.05$) and while those of alkaloids, cyanogenic glycoside, oxalate decreased significantly ($p < 0.05$), tannins decreased non-significantly as maceration time was increased.

Phytochemical contents of raw and cooked African walnut seed kernel. The result of the qualitative analyses of African walnut is shown in Table 3. The presence of saponins, alkaloids, cyanogenic glycoside and oxalate in the sample at all levels of processing was shown. Highest phytochemical content was in oxalate while tannins and flavonoids were absent. The quantitative phytochemical contents of African walnut are presented in Table 4. The data show the concentration of oxalate as highest and saponins lowest at every level of processing of the sample. The effect of heat treatment represented as the percentage difference between the phytochemical contents of raw and cooked samples is shown in Fig.2. Saponin content increased while alkaloids, cyanogenic glycoside and oxalate contents decreased with increase in cooking time. The effect of the processing method on the oxalate content of the sample was significant ($p < 0.05$).

Table 1: *Phytochemical screening of raw and heat processed African elemi seed pulp flour.*

Phytochemical	Samples			
	AE_{raw}	AE_{15}	AE_{30}	AE_{45}
Saponins	+	+	+	+
Alkaloids	+++	+++	+++	+++
Cyanogenic glycoside	+	+	+	+
Oxalate	++	++	++	++
Tannins	+	+	+	+
Flavonoids	+++	+++	+++	+++

KEY: + = present in a minute amount, ++ = present in a moderate amount,
+++ = present in an appreciable amount.

Table 2: *Phytochemical contents (mg/100g sample) of raw and macerated African elemi seed pulp flour.*

Phytochemical	Samples			
	AE_{raw}	AE_{15}	AE_{30}	AE_{45}
Saponins	$1.00±0.19^{a}$	$1.00±0.13^{a}$	$1.10±0.03^{a}$	$1.90±0.50^{b}$
Alkaloids	$19.50±1.86^{a}$	$19.50±1.55^{a}$	$17.64±1.17^{ab}$	$16.35±0.62^{b}$
Cyanogenic glycoside	$1.98±0.05^{a}$	$1.75±0.10^{b}$	$1.43±0.05^{b}$	$1.35±0.06^{b}$
Oxalate	$10.59±0.50^{a}$	$9.46±0.13^{b}$	$9.26±0.08^{b}$	$9.09±0.01^{b}$
Tannins	$0.03±0.07^{a}$	$0.02±0.01^{a}$	$0.02±0.01^{a}$	$0.02±0.12^{a}$
Flavonoids	$14.98±2.91^{a}$	$15.25±0.50^{a}$	$15.25±0.48^{a}$	$17.27±1.54^{a}$

Values are means ± standard deviations of triplicate determinations. Values in the same row bearing the same superscript letters are not significantly different at the 5% level (p>0.05).

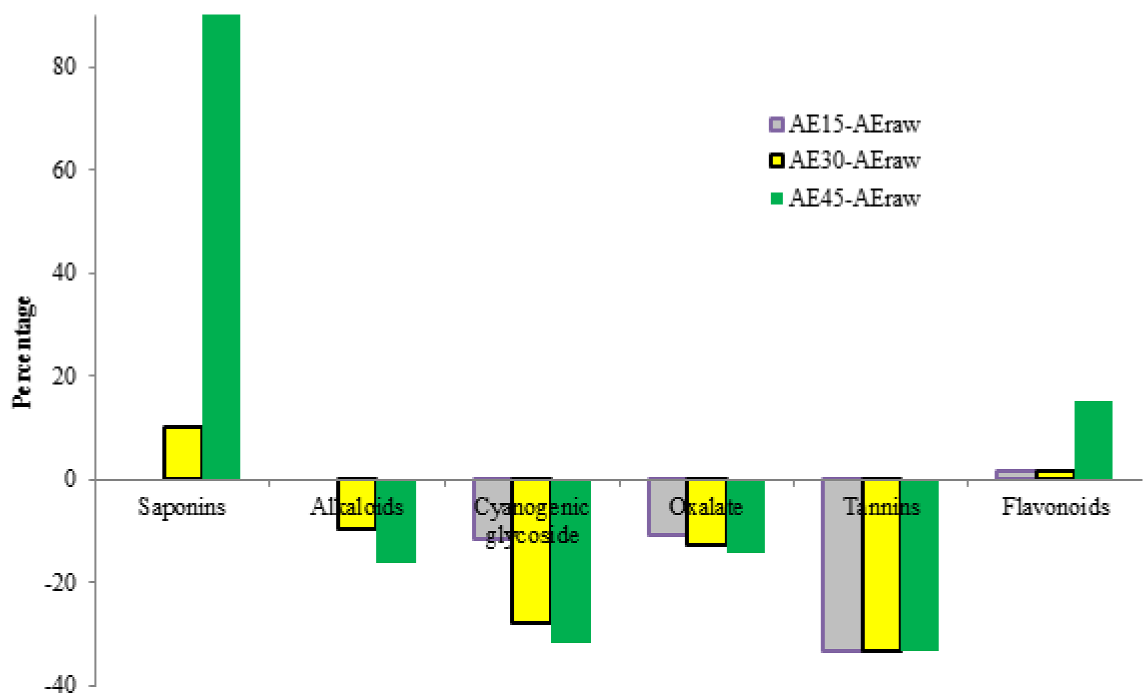

Fig. 1: Percentage difference in phytochemical contents of of raw and macerated African elemi seed pulp flour

Table 3: *Phytochemical screening of raw and heat processed African walnut seed kernel flour.*

Phytochemical	Samples			
	AW_{raw}	AW_{45}	AW_{90}	AW_{135}
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Cyanogenic glycoside	+	+	+	+
Oxalate	++	++	++	++
Tannins	ND	ND	ND	ND
Flavonoids	ND	ND	ND	ND

KEY: + = present in a minute amount, ++ = present in a moderate amount, ND = not detected

Table 4: *Phytochemical contents (mg/100g sample) of raw and cooked African walnut seed kernel flour.*

Phytochemical	Samples			
	AW_{raw}	AW_{45}	AW_{90}	AW_{135}
Saponins	1.05 ± 0.13^{a}	1.05 ± 0.06^{a}	1.65 ± 0.47^{ab}	1.80 ± 0.14^{b}
Alkaloids	3.40 ± 0.57^{a}	3.30 ± 0.57^{a}	3.10 ± 0.12^{a}	3.00 ± 0.69^{a}
Cyanogenic glycoside	1.79 ± 0.05^{a}	1.79 ± 0.05^{a}	1.73 ± 0.08^{a}	1.70 ± 0.05^{a}
Oxalate	6.81 ± 0.25^{a}	5.80 ± 0.25^{b}	5.30 ± 0.26^{bc}	5.05 ± 0.51^{c}

Values are means ± standard deviations of triplicate determinations. Values in the same row bearing the same superscript letters are not significantly different at the 5% level (p> 0.05).

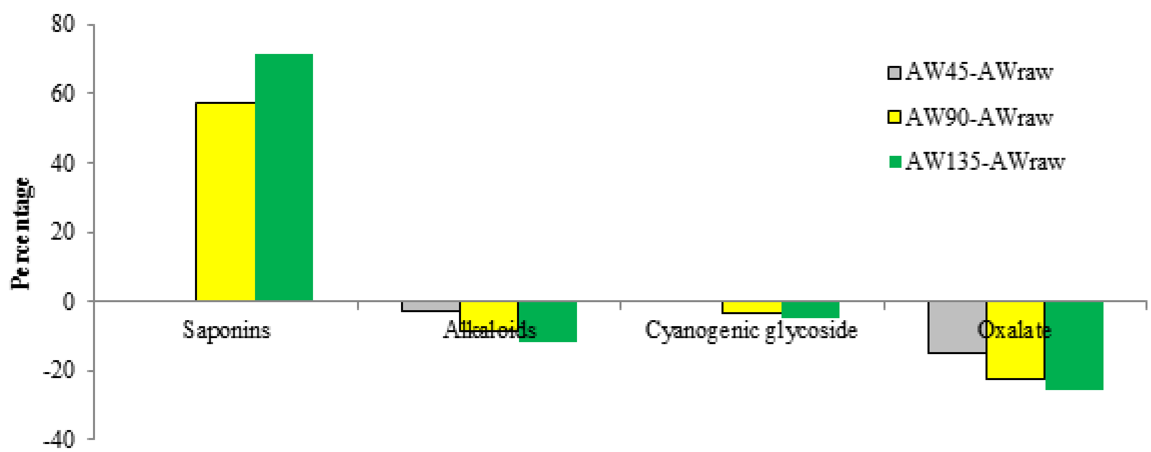

Fig. 2: Percentage difference in phytochemical contents of raw and cooked African walnut seed kernel flour

Discussion

The results show that the antinutrient concentrations in the plant foods were variously affected by processing. The observed changes may be attributable to two opposite phenomena: thermal inactivation/degradation which reduces their concentration [25], [26], and matrix softening effect resulting from the disruption of cell structure and membrane partitions of plant foods by the wet-heat/hydro-thermal processing leading to their increased extractability and consequent higher concentrations relative to the raw sample [27]. The saponin contents of the samples were low (1.00±0.19 - 1.90±0.50) compared to the values of 3.37±0.31, 2.85±0.17, 3.66±0.02, 3.08±0.05, 5.46, 7.17±0.58 and 9.83±0.17 mg/100gsample obtained for Brown African yam bean, Marble-coloured African yam bean, African stare apple (Chrysophyllum africanum) fruit, dried water melon pulp, Cnidoscolus aconitifolius leaves, Cowpea (Vigna unguiculata) local variety 'Kannanado', and raw Moringa oleifera seed flour respectively [28], [29], [30], [31], [32], [33] and comparable to the value of 1.58±0.60 obtained for African nutmeg (Monodora myristica) [34]. The antinutrient was made slightly more available in both samples as processing time increased (Figs.1 and 2). This increase became statistically significant (p<0.05) beyond AE_{30} and AW_{90}. Saponins are phytocompounds found as non-cardiac steroid glycosides that produce foams, have bitter taste and characteristically hemolyze erythrocytes [35], [36]. Ilelaboye and Pikuda [37] stated that saponins in a forage crop affect palatability and intake of nutrients. Saponins were reported to reduce nutrient utilization and conversion efficiency [29]. They act as growth inhibitor by reducing feed intake due to their bitterness and throatirritating activity [38] and also by affecting protein digestibility through inhibition of various digestive enzymes such as trypsin and chymotrypsin [39]. Alkaloid was high in African elemi (Table 2), but was decreased by maceration beyond 15min (Fig. 1). The decrease became significant ($p<0.05$) after 30min. Though the concentration of the antinutritional factor was relatively low in African walnut, it was also decreased by processing (Fig.2). The observed values were lower than the value (17.33±0.17mg/100g) for raw Moringa oleifera seed flour [33] and 12.07mg/100g for raw Cnidoscolus aconitifolius leaves (Iyana Ipaja) which was also decreased to 5.33mg/100g by cooking [31]. High level of alkaloids exerts toxicity and adverse effects to humans, especially in physiological and neurological activities. According to Fowomola [35], some alkaloids such as solanine cause gastrointestinal upsets and neurological disorders, especially when taken in excess of 20mg/100g sample. However, doses differentiate between toxicity and pharmaceutical effects of alkaloids [40]. For instance, lower dose of alkaloids mediate important physiological activities, such as analgesic, reducing blood pressure, destroying tumour cells, stimulating circulation and respiration [39]. The plant foods are safe especially African walnut relative to alkaloid toxicity especially when properly processed. Low cyanogenic glycoside content was observed in both raw samples (1.98±0.05 and 1.79±0.05mg/100g for African elemi and African walnut respectively) relative to the value of 21.6mg/100g for Terminalia catappa (almond), 16.1±0.14 mg/100g for Chanca piedra (Stone breaker), and 2.16-2.46mg/100g for solvent extract of Buchhholzia coriacea [41], [42], [43]. This was further decreased by processing. The decrease in cyanogenic glycoside content of African elemi was statistically significant ($p<0.05$) at all levels of processing. The decrease was consistent with the finding of Ogbadoyi et al. [44] and Ilelaboye et al. [46] that various food processing methods decrease cyanide content in plants. The observed decrease with processing may be due to vaporization of free cyanide by heat. Besides, both free and bound cyanide are water soluble and may be leached out during processing of samples in water [44], [45]. Cyanogenic glycosides per se are nontoxic [46], [47], but on hydrolysis by β-glycosidase, releases a sugar and a cyanohydrin which quickly decomposes further to toxic hydrocyanic acid (HCN) and an aldehyde or a ketone [48]. The toxicity of cyanogenic glycosides depends on this HCN released which readily and reversibly binds and inhibits a number of proteins and enzyme systems in the body. It significantly binds mitochondrial cytochrome oxidase system, the terminal enzyme of the mitochondrial electron transport chain [49]. This inhibits oxidative phosphorylation, paralyzing the vital cellular process of aerobic respiration resulting in anoxic cell death [50]. The concentration of cyanogenic glycosides in all the samples (Tables 2 and 4) was far below the lethal dose of 50-60g/kg [51]. Thus, the plant foods are free of HCN toxicity risk. The oxalate content of African elemi was higher than that of African walnut but the anti-nutrient was significantly ($p<0.05$) decreased in both samples by heat processing. Aye [31] also reported decrease in the level of the anti-nutrient by cooking in Cnidoscolus aconitifolius leaves (Iyana Ipaja) giving the value 0.81-2.03mg/100g. The observed decrease is in agreement with the observation of Adeboye and Babajide [52] and Ogbadoyi et al. [46] who reported that various processing methods decrease oxalate content in plant foods. The values obtained for the two samples (9.09±0.01-10.59±0.50mg/100g and 5.05±0.05-6.81±0.25mg/100g respectively) were higher than 4.995 ±0.01 reported for African star apple (Chrysophyllum africanum) fruit [29] but lower than 26.40mg/100g, 24.30mg/100g and 40.50mg/100g obtained for Terminalia catappa (almond) seeds, white yam (Discorea alta) and cocoyam (Colocasia esculenta L.) respectively [41], [53]. The oxalate content of African walnut was comparable to the value 6.21±0.11mg/100g obtained for cowpea (Vigna unguiculata) local variety 'Kannanado' [32]. Oxalates impart acrid taste to food, exert irritations to the intestinal tract and cause absorptive poisoning [54]. At high levels in the body oxalate binds (chelates) minerals like calcium, iron and magnesium converting them to less soluble salts thus interfering with their metabolism and bioavailability [53], [4]. Oxalates also bind other nutrients making them unavailable. For instance, it reacts with proteins to form complexes which have an inhibitory effect in peptic digestion [55], [56]. Oxalate may also precipitate around soft tissue such as the kidney, causing kidney stones [42]. The two plant foods are safe from any risk of oxalate toxicity as the observed oxalates levels are lower than 2-5g/kg recorded as the physiological tolerance level for the anti-nutrient [42], [4]. The tannin content of raw African elemi pulp was very low (0.03mg/100g). This was however still decreased though non-significantly ($p>0.05$) to 0.02mg/100g at all levels of macerations. Omenna, et al. [9] and Aye [31] also reported decreased in the tannin content of cowpea (0.34 to 0.31mg/100g) and Cnidoscolus aconitifolius leaves (3.72 to 2.97mg/100g) respectively by cooking. The observed tannin decrease may be due to leaching or to the formation of insoluble tannin–protein complexes [35], [45]. The anti-nutrient was not detected in the African walnut seed kernel sample. The observed tannin level was lower than 39.40, 15.2± 0.13 and 6.01±0.01mg/100g and, 1.23, 2.33±0.03 and 0.24±0.04mg/100g obtained for almond seed, Chanca piedra (Stone Breaker) and Cowpea (Vigna unguiculata) local variety 'Kannanado' and, three common spices: Gongronema latifolium, Piper guineense and Xylopia aethiopica respectively [57], [41], [42], [32], [58]. The phytochemical negatively affect an animal's feed intake through its astringency which is the consequence of its complex formation with glycoproteins of saliva and proteins of mucosal membrane of the mouth during the mastication of food [59]. Tannin affects feed digestibility through its complex formation with metabolic proteins (enzymes) [60], [5], with dietary proteins and cell wall carbohydrates like cellulose and hemicellulose[4], [61], and also with protein and cell tissues in rumen bacteria. These precipitate reduction in the animal's efficiency of production. Considering tannin toxicity, African elemi is safe as the observed level of tannins was far below the lethal dose of 30mg/kg [51] for the phytochemical. The flavonoid content of the raw African elemi (14.98±0.19mg/100g) was made more available (Fig.1), though non-significantly ($p>0.05$) by maceration giving the range 14.98±2.91 – 17.27±1.54mg/100g. The phytochemical was not detected in African walnut at all levels of processing. Although flavonoids have protective effects including anti-inflammatory, anti-oxidant, anti-viral, and anti-carcinogenic properties, at very high concentrations they manifest a myriad of antinutrient activities. They chelate metals such as iron, copper and zinc, and decrease their absorption [40]. They also inhibit digestive enzymes and may precipitate proteins [62]. At the cellular level, flavonoids exert a variety of biological effects mediated by specific interaction with molecular targets. For instance, they interact with nucleic acids [63], polysaccharides [64], proteins [65] and vitamin C [66]. Neither Dietary Reference Intake (DRI) nor Tolerable Upper Intake Level (UL) has been established for Flavonoids by the National Academy of Sciences (NAS) although the USDA estimates that in the U.S., daily total flavonoid consumption by the average adult is approximately 250-275mg [67]. Consequently, the flavonoids content of African elemi (14.98±2.91 – 17.27±1.54mg/100g) could not be considered of any toxicological risk to the consumer.

Conclusions:

It could be concluded that the effect of heat processing on the antinutrient contents of the masticatories: African elemi (Canarium schwenfurthii) pulp and African walnut (Plukenetia conophora) seed kernel used as traditional snacks in most parts of Nigeria varied with processing method as well as the heat contact time. However, with respect to the tolerable limits of the antinutrients, the plant foods may be considered safe for human consumption at all levels of processing.

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