Antinutrient and Micronutrient contents of processed "Nturukpa" (Pterocarpus santalinoides) seed powder

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Abstract:
The antinutrient and micronutrient (minerals and vitamins) contents of processed and raw seeds of "Nturukpa" (Pterocarpus santalinoides), a wild plant in Nigeria were analyzed using accepted methods. The various quantities (in mg/100g sample) of the antinutrients ranging from hydrogen cyanide (11.25±1.46), through alkaloids, saponins, flavonoids, oxalates, tannins, phytic acid to phynols (0.18±0.02) contained in raw "Nturukpa" (Pterocarpus santalinoides) seed powder were reduced by 4.98% (in saponins) to 63.64% (in oxalates) when processed. In mg/100g sample, the micronutrients: P (86.21±16.48), Na (16.07±1.76), Fe (0.68±0.02) and K (0.48±0.12) and, Ascobate (5.87±0.84), B_3 (1.32±0.41), and B_2 (0.31±0.05) were respectively the most abundant minerals and vitamins in the raw sample. Except for potassium and vitamin D that were not affected by processing, the concentrations of other micronutrients were diversely increased. The processed plant food, relative to the established physiologically tolerable limits for the antinutrients is safe for human consumption and an excellent source of the minerals: Fe, Zn, P, I, Mn, Cu and Se; the B vitamins and Ascobate (vitamin C) as 1kg could supply substantial proportion of the RDA for the nutrients.

Keywords: Antinutrients, micronutrients, minerals, Pterocarpus santalinoides, vitamins.

1. Introduction

Minerals and vitamins constitute the class of nutrients called micronutrients because they are required in minuscle (milligram or microgram) amount by the body, and yet crucial in the production of enzymes, hormones and other substances essential for proper growth and development (Anyalogbu, et al., 2014; Monanu et al., 2014). Minerals; microminerals when the amount needed by the human body per day is less than 100mg and macrominerals when greater than 100mg; are required as vital components of body fluids and tissues, constituents

of enzyme, hormonal and nervous systems, and for the development of bones and teeth. On the other hand, vitamins: either fat- or water- soluble, are involved in cell production, tissue overhaul, and other essential processes that make up the body's metabolic system (Anyalogbu et al., 2014). Essential minerals and vitamins needed for development and maintenance of optimal growth and health, as noted by Misra and Misra (2014), could be provided by leafy vegetables (edible leaves, stems, roots, fruits or seeds) cherished by Africans as part of their dietary regimen. In 2016, Agiang et al. reiterated that plant foods are the cheapest and most accessible sources of minerals and vitamins in developing countries. According to Ndukwe and Ikpeama (2013), and Offor et al. (2015), the leaves of Pterocarpus santalinoides is used for soup making in the South Eastern part of Nigeria. Pterocarpus santalinoides, of the Leguminosae: papilionoideae family is an evergreen tree with a dense crown of drooping branches. It is essentially bi-continental in distribution being native to tropical Western Africa and South America (Prado, 1998) and usually called red sandal wood in English (Offor, et al., 2015), . The plant grows wild in Nigeria and is known in various Nigerian vernaculars as nturukpa (Igbo); okumeze (Edo); nja (Efik); gbengbe (Yoruba); gunduru or gyadar kurmi (Hausa); maganchi (Nupe); ikyarakya or kereke (Tiv) (Odeh and Tor-Anyiin, 2013). A fully grown tree: 9 -15 m tall, trunk up to 1 m in diameter and flaky bark, pinnate leaves (10–20 cm long), flowers orange-yellow and produced in panicles; bears fruit in pods 3.5 - 6 cm long, with a wing extending three-quarters around the margin (Keay,1989).

Antinutrients (anti-nutritional factors) are present at varying amounts in almost all plant foods (Soetan and Oyewole, 2009). These are phytochemicals which themselves may not be toxic but could reduce nutrient intake, digestion, absorption and utilization, or deter most animals from consuming foods containing them (Sango et al. 2016; Anyalogbu and Ezejiofor, 2017). According to Omenna et al. (2016), many traditional methods employed in processing plant food into food reduce their antinutrient load. According to Carvalho and Barata (2017) many people all over the world depend on many wild plant species for food and medicines. As a component of food, micronutrients are essential in the maintenance of human health (Anyalogbu et al., 2014). This work is therefore intended to appraise the seed powder of 'nturukpa', a wild plant in Nigeria, for its content of micronutrients (minerals and vitamins) and antinutrients and, possible effect of heat treatment on these.

2. Materials and Methods

2.1. Samples Pterocarpus santalinoides pods were picked from among those fallen from 'Nturukpa' tree at Umuigwe Eziama community in Ikeduru L. G. A. of Imo State, Nigeria and authenticated by a botanist in the Dept. of Biological Sciences, Federal Uni. of Tech. Owerri, Imo State, Nigeria.

2.2. Sample processing

The pods were opened with a hammer and wholesome seeds collected, washed in several changes of deionized water, air-dried for 24 hr. and divided into two portions. It is expected that the plant food, if found finally safe for human consumption would be employed as

condiment/thickener in soup. In-lieu of the anticipated mode of preparation, one portion of the air-dried sample was wrapped tightly in cellophane, heated in boiling water for 40 min and labelled 'Processed'. The other portion was labelled 'Raw'. The raw and processed samples were dried at 60ºC for 48 hr. in an air-circulatory oven (Model OVE.100.130M.Gallenkamp, UK), ground in a hand mill (Model BL357. Kenwood, Birmingham UK), passed through a 60-mess size screen and used as stock samples in the analyses.

2.3. Quantitative analyses of antinutrients

The phytochemicals: Alkaloids, flavonoids, saponins, tannins, oxalate, phytic acid, phynols and cyanogenic glycoside were quantitatively determined in triplicates in the stock samples using established methods. Gravimetric methods of Edeoga et al. (2005), and Boham and Kocipai-Abyazan (1994) were used to quantify alkaloids and flavonoid respectively. The double solvent extraction gravimetric method described by Aluko et al. (2012) was used for saponins content determination. Tannin and total phenol contents were evaluated spectrophoto- metrically as enunciated by Van-Burden and Robinson (1981) and, Santhi and Sengottuvel (2016) respectively. Oxalate (oxalic acid) contents were determined using the Precipitation-Titrimetric method of Oke (1966) while Cyanogenic glycosides contents were evaluated by the AOAC (2006) quantitative alkaline titrimetric method No. 915.03. Phytate concentrations in the stock samples were quantified using colorimetric assay of Vaintraub and Lapteva (1988) as modified by Lorenz et al. (2007).

2.4. Analyses of mineral content

The concentration of minerals (Ca, K, Mg, Na, P, Pb, Cu, Fe, I, Mn, Zn, and Se) in the stock samples were quantified using Association of Official Analytical Chemists' Atomic absorption Spectrophotometric method (2000) described by Anyalogbu et al. (2014). Each of the stock samples (3.0 g) was incinerated in a muffle furnace set at 550ºC until ash of constant weight was obtained. The minerals as non-combustible inorganic contents of the ash were extracted with 20ml of 2.5% HCl and then reduced to 8.0 ml by heating in a water-bath (98±0.03ºC). The concentrated extracts were diluted to 50ml with deionized water and the mineral contents determined using atomic absorption spectrophotometer (Model 2380, Perkin-Elmer, USA) calibrated with standard solution of the minerals being estimated. The results were given in mg per 100g sample.

2.5. Fat-soluble (fsv) and Water-soluble (wsv) vitamin contents analyses.

Fsv: A, D_1, D_3, E and K contents of the stock sample were quantified by subjecting the extract to gas chromatographic analyses (AOAC, 2006) using HP 5890 Powered with HP Chem Station Rev. A09.01 (1206) software (Hewlett-Packard, California, USA) as described by Monanu et al. 2014. One hundred miligramme of the stock sample and 50 mg of vitamin C as antioxidant, were weighed into 16x126mm test tube and 5ml of reagent alcohol (90.2% ethanol, 4.9% methanol, and 4.9% isopropanol) and, 0.5ml of 80% KOH (w/v) added. The tube was vortexed for 30 seconds, flushed with N_2, capped, and then incubated for 30min in a water bath (set at 70ºC) with intermittent vortexing. The tube was placed in an ice bath for 5

min., deionized water (3ml) and 5ml of hexane added and then vortexed again for 30 sec, centrifuged at 1000xg for 10 min. The supernatant (hexane extract) was decanted into another test tube and the residue re-extracted two times with 5ml hexane. The extracts were pooled, concentrated to 1ml by evapouration (in a water bath) under N_2 flow and the fsv contents analyzed under standard conditions in a HP Gas chromatograph calibrated with selected standards. The results were given in mg/100g sample by an enhanced integrator.
Wsv: B_1, B_2, B_3, B_5, B_6, B_9, and C, on the other hand were extracted and quantified as described by Santos et al. (2012) using HPLC-MS/MS system; an Accela liquid chromatograph equipped with a diode array detector and coupled to a MS analyzer via an electrospray interface. To extract the wsv, 0.25g of the stock sample and 0.05g of 0.1% butylated hydroxytoluene were weighed into a 16 x 126mm test tube, 16ml of 10mM ammonium acetate/methanol 50:50 (v/v) added and vortexed for 15min. Then the test tube was flushed with N_2, capped, incubated for 15min in an ultrasound bath (set at 25ºC) and centrifuged at 14000g for 15min. The methanol layer (supernatant) was filtered through a 0.45µm nylon filter and 1.0 ml concentrated by evaporation under N_2 stream.
Wsv contents of the concentrated extract (10µL) were evaluated in a HPLC-MS/MS system optimized by direct injection of wsv standards and the result given in mg/100g sample.

2.6. Statistical Analysis
All the analyses were done in triplicate determinations. Means and standard deviations of the data generated were obtained using Descriptive Statistics in statistical package for social sciences (SPSS) version-20. Presented values are therefore Means ± SDs.

3. Results and Discussion

3.1. Antinutrient contents of the sample seed powder.

The antinutrient contents of the plant sample powders, raw and processed Pterocarpus santalinoides seeds are presented in Table 1. Effect of processing on the Antinutrient contents expressed as percentage reduction in the antinutrient concentration upon processing is as depicted in Figure 1 below.

Analysis of the antinutrient contents of the samples shows that hydrogen cyanide (11.25±1.46mg/100g sample) had the highest concentration followed by alkaloids (6.80±0.88mg/100g sample) and then saponins (6.02±0.78mg/100g sample) while phynols (0.18±0.02mg/100g sample) had the least concentration. Ndukwe and Ikpeama (2013) also noted that the concentration of HCN and phynols were also the highest and least respectively in the leaves of P. santalinoide. The antinutrient contents of the sample were all reduced by the processing method (Fig. 1). The reduction was highest in oxalates (63.64%) followed by flavonoids (61.11%) and least in saponins (4.98%). The observed reduction may be as a result of inactivation or degradation of the antinutrient initiated by the heat of processing (Akinyele and Oloruntoba, 2013; Palermo et al., 2014). Earlier researches showed that thermal processing reduced most antinutrient contents of plant foods; African elemi and African walnut (Anyalogbu and Ezejiofor, 2017), Cowpea (Omenna et al., 2016), Telfairia occidentalis leaves (Imaobong and Bassey 2012), Hibiscus Sabdariffa (Musa and Ogbadoyi, 2012), Legumes (Khokhar and Owusu-Apenten, 2003).

Table 1: Antinutrient contents of raw and processed Pterocarpus santalinoides seed powder

Antinutrient	Concentration (mg/100g sample)[a]		Dietary (Physiologically) Tolerable limit	References
	Raw	Processed		
Alkaloids	6.80±0.88	5.20±0.68	20mg/100g	Fowomola, 2010
Flavonoids	3.60±0.47	1.40±0.18	None yet	Anyalogbu and Ezejiofor, 2017
Saponins	6.02±0.78	5.72±0.74	None yet	Cressey and Thomson, (2007)
Tannins	0.63±0.08	0.43±0.06	3mg/100g	Inuwa et al., 2011
Oxalates	0.66±0.09	0.24±0.03	200-500mg/100g	Udousoro and Akpan (2014).
Phytic acid	0.39±0.05	0.26±0.03	22.10 - 25mg/100g	Oly-Alawuba and Obiakor-Okeke, 2014; Udousoro and Akpan, 2014
Phynols	0.18±0.02	0.13±0.02	0.06 – 0.20 mg[b]	IPCS, 1994
Hydrogen cyanide	11.25±1.46	9.40±1.22	50-60g/kg	Inuwa et al., 2011

[a]Values are means and standard deviations of triplicate determinations. [b]Recommended as the upper limit of total human daily phenol intake per kilogramme body weight.

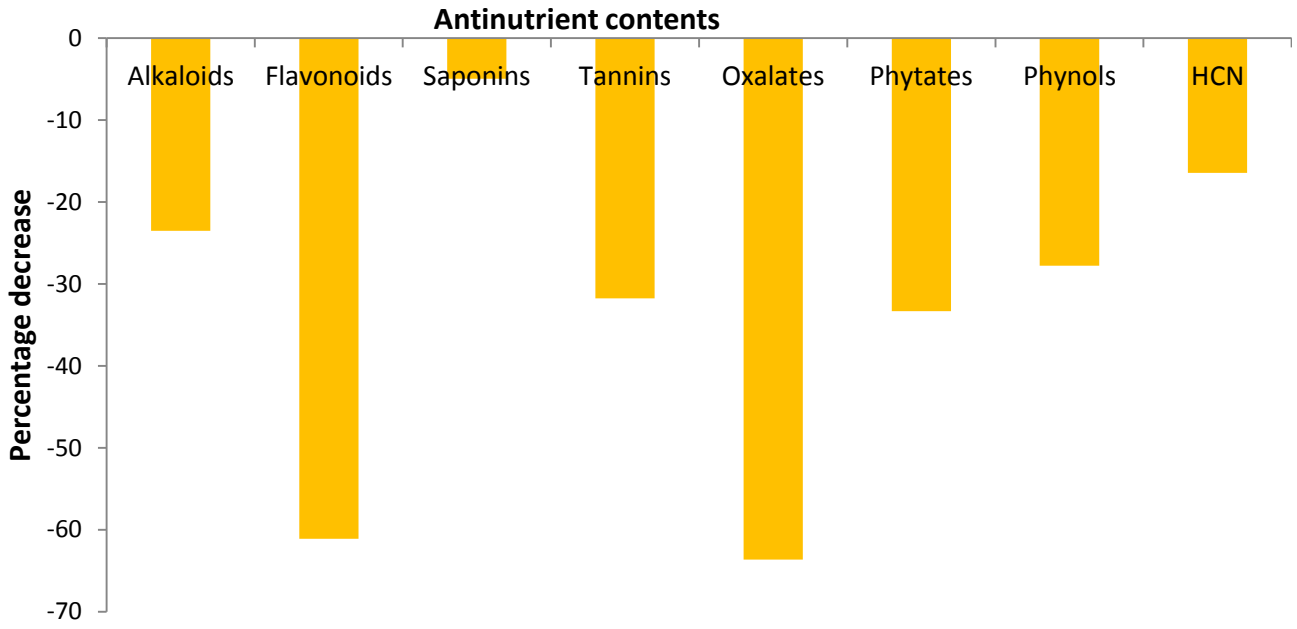

Fig.1: Effect of processing on Antinutrient contents of Pterocarpus santalinoides seed powder

The presence of antinutrients or anti-nutritional factors (phytochemicals) through various and variant ways interfere with bioavailability of nutrients to animals (Gemede et al., 2015; Udousoro and Akpan, 2014). High doses of alkaloids such as glycoalkaloids: solanine, according to Fowomola (2010), cause gastrointestinal upsets and neurological disorders: mainly through disruption of membrane integrity (Cressey and Thomson, 2007). Saponins, a

varied and chemically complex collection of compounds, possess throat-irritating activity (Bureau et al., 1998), inhibit digestive enzymes such as trypsin and chymotrypsin (Makkar et al., 2007), and reduce feed conversion efficiency (Christopher and Dosunmu 2011) while HCN, produced on hydrolysis of cyanoglycosides by β-glycosidase (Fennema, 1985), binds and inhibits a number of proteins and enzyme systems e.g. mitochondrial cytochrome oxidase system -resulting in anoxic cell death and other characteristic symptoms of cyanide poisoning (Cressey and Thomson, 2007: Anyalogbu and Ezejiofor, 2017).

High concentrations of oxalate cause absorptive poisoning (Sasanka and Gurumoorthi, 2011), reduce minerals bioavailability by chelation (Sango et al. 2016, Udousoro and Akpan, 2014), form non-digestible complexes with nutrients such as proteins (Akande et al., 2010; Adesuyi et al., 2012), and could precipitate within the kidney causing kidney stones (Ibrahim and Fagbohun. 2013). Tannin on the other hand reduces feed digestibility by forming series of complexes with macromolecules: enzyme proteins (Kraus et al., 2003; Gemede et al., 2015), nutrient proteins and some cell wall carbohydrates (Udousoro and Akpan 2014), protein and cell tissues in rumen bacteria (Anyalogbu and Ezejiofor, 2017), glycoproteins of saliva and mouth mucosal membrane proteins (Reed, 2001). Flavonoids act as antinutrient at very high concentrations: chelate metals (Egbuna and Ifemeje, 2015), precipitate proteins and inhibit digestive enzymes (Enechi et al., 2013), interact with specific molecular targets like nucleic acids (Kanakis, 2005), polysaccharides (Zheng et al., 2004), proteins (Hollosy and Keri, 2004) and vitamin C (Song et al., 2002). High levels of dietary phytic acid limit absorption (by chelation) of some physiologically important minerals, proteins and starches (Admassu, 2009; Sango et al. 2016). This is predicated on the fact that monogastric animals including humans lack phytase enzyme required for phytate hydrolysis in their digestive tract (Gupta et al., 2015). Although polyphenols are antioxidant compounds, phenol itself is inactive as an antioxidant (Landete, 2013). Phenols may form complexes with essential amino acids, enzymes and other proteins and nutrients. The antinutrient forms insoluble iron-phenol complex in the gastrointestinal tract and thus makes the iron unavailable for absorption (Shahidi and Naczk, 1992). The residual concentrations of the assayed antinutrients were all below the established physiologically tolerable limits (Table 1). Consequently, the processed plant sample relative to tolerable limits of its antinutrients contents could be considered safe for human consumption.

3.2 Micronutrient contents of the raw and processed samples.

Table 2 and Figure 2 contain respectively, the mineral concentrations and the corresponding effect of processing. From Table 2, the plant sample contains various levels of Na, Ca, Mg, K, Fe, Zn, P, I, Mn, Cu, Se and Pb assayed. The highest value in mg/100g sample (86.21±16.48) was obtained for phosphorous followed by Na (16.07±1.76) and then iron (0.68±0.02) and potassium (0.48±0.12) while the least value (in part per million) was obtained for Pb (0.002±0.001), and then selenium (0.05±0.03). The observed values for the minerals in the plant sample, except for K that was not affected, were all increased by processing (Fig. 2). The highest increase was observed in Pb (50%) followed by Mg (21.21%) and then P (20.35%) and least in Cu (4.76%).

Table 2: Mineral concentrations[a] of raw and processed Pterocarpus santalinoides seed powder

Mineral	Concentration (mg/100g sample)*		RDA for Adult (Anyalogbu, et al., 2014; IOM, 2006)	% of RDA in 1kg processed sample
	Raw	Processed		
Na	16.07±1.76	17.93±2.73	1200 - 1500mg	11.95 - 14.94
Ca	0.19±0.02	0.21±0.04	1000 - 1300mg	0.16 - 0.21
Mg	0.33±0.04	0.37±0.08	310- 400mg	0.93 - 1.19
K	0.48±0.12	0.48±0.12	4700mg	0.10
Fe	0.68±0.02	0.75±0.06	8mg	93.75
Zn	0.31±0.06	0.33±0.04	8-11mg	30.0 – 41.25
P	86.21±16.48	103.72±4.85	700mg	148.17
I	0.29±0.05	0.32±0.04	150µg	213.33
Mn	0.39±0.09	0.41±0.11	1.8-2.3mg	178.26 - 227.78
Cu	0.21±0.04	0.22±0.04	900µg	244.44
Se (ppm)*	0.05±0.03	0.06±0.03	55µg	109.09
Pb (ppm)*	0.002±0.001	0.003±0.001	232.14µg[b]	3.0µg/1kg[c]

[a]Values are means and standard deviations of triplicate determinations, [b]The daily permissible amount of lead for adult, [c]Lead content of 1kg processed sample.

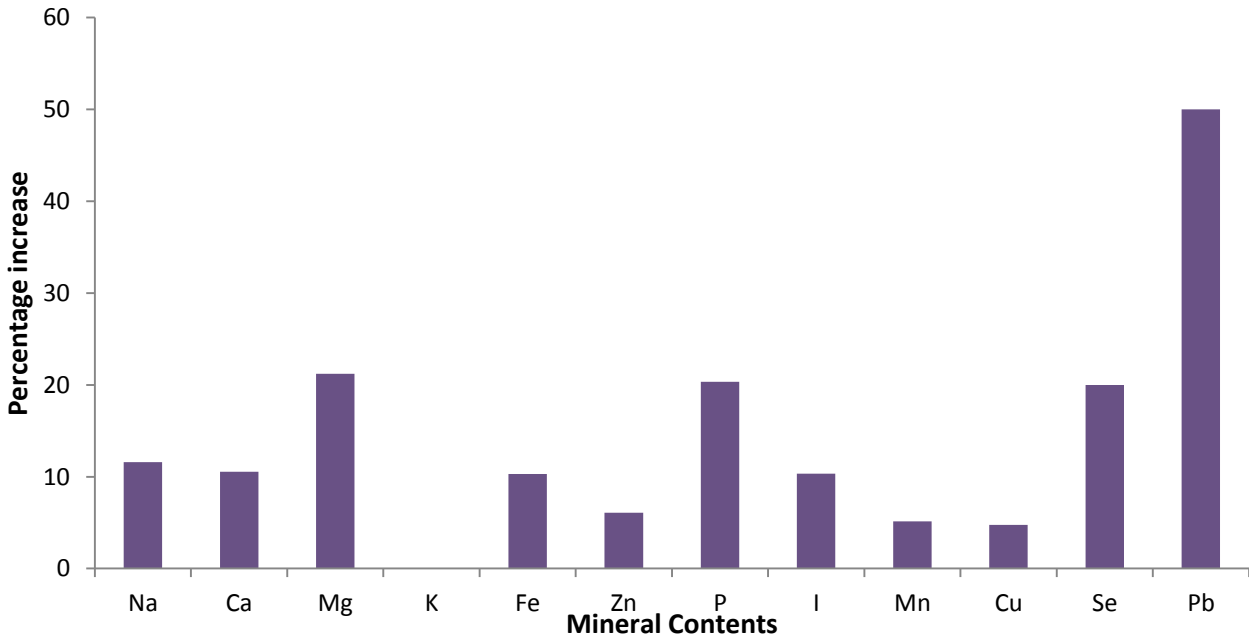

Fig.2: Effect (%) of processing on mineral content of Pterocarpus santalinoides seed powder

Relative to the recommended dietary allowance (RDA) of minerals for adult, the processed plant food is an excellent source of the minerals: Fe, Zn, P, I, Mn, Cu and Se, as 1kg of the sample could supply more than or substantial proportion of the RDA (Anyalogbu et al., 2014; IOM, 2006) of them (Table 2). RDA, according to Institute of Medicine (IOM, 2006), is the

average daily dietary intake level that is sufficient to meet the nutrient requirements of 97-98 percent of healthy individuals in a group.

Minerals serve a variety of functions in the body. For instance, iodine is an essential nutrient that is required by both the developing foetus due to its effect on brain development and, the body for the synthesis of thyroid hormones (Rienecke, 2017; Delange, 1994). Phosphorus is a component of nucleotide molecules, involved in the control of the acid-alkaline state of the blood (Anyalogbu et al., 2014); bone, teeth and muscle growth and maintenance and, blood formation (Umar et al, 2007). Fe is required for normal function of central nervous system, energy metabolism and together with Cu participates in haemoglobin formation and function (Anyalogbu et al., 2014). On the other hand, Mn is a cofactor in many enzymes actions (McDonald, 1995), involved in reproduction and actions of the CNS and a component of the bone (Norman and Joseph, 1995). Zn plays important roles in male sexual health: promote testosterone and sperm production, retard prostate growth by inhibiting the production of dihydrotestosterone (DHT) from testosterone by the enzyme 5-alpha reductase (Anyalogbu et al., 2014). It is also involved in the actions of B-complex vitamins and many othermetabolic processes such as cholesterol, protein, carbohydrate and nucleic acid metabolism (Camera and Amaro, 2003; Oderinde, et al., 2009). While selenium functions in the body's defense against infection and as an antioxidant protects vitamin E from degradation (Brown. and Arthur, 2001). Being part of selenoenzymes (Endogenous antioxidants) – e.g. glutathione peroxidases and thioredoxin reductases, Se protect the tissues against extremely reactive oxygen-containing metabolites like hydrogen peroxide and lipid hydroperoxide (Papp et al., 2007). With the understanding of some of the functions of these minerals, considering the proportion of their RDA derivable from 1kg of the plant food, it becomes evident that the sample could be invaluable in animal nutrition.

On the other hand the vitamin concentrations of the raw and processed Pterocarpus santalinoides seed powder and the corresponding effect of processing on these are shown on Table 3 and Figure 3 respectively. The analysis of the vitamin contents shows that the plant food contains both the fat-soluble (A, D, E and K) and water-soluble (B_1, B_2, B_3, B_5, B_6, B_9 and C) vitamins with the later quantitatively predominating. The highest value (in mg/100g sample) for the vitamins was obtained for vitamin C (5.87±0.84), followed by B_3 (1.32±0.41) and then B_2. The least (in µg/100g sample) was vitamin D (0.02±0.02) followed by K (1.57±0.44) and then E (1.74±0.52). While vitamin A (3.75±0.71 µg/100g sample) was the highest fat-soluble vitamin. The concentrations of the vitamins in the raw sample, except for vitamin D, were variously increased by the heat treatment (Fig. 3). The highest increment was observed in B_2 (48.39%) and least in B_9 (4.0%). Vitamin E (37.36%) was the most affected water-soluble vitamin.

Weighed on the scale of the recommended dietary allowance (RDA) of vitamins for adult (IOM, 2006) the plant food is an excellent source of the B vitamins (Thiamine B_1, Riboflavin B_2, Niacin B_3, Pantothenate B_5, Pyridoxine B_6, Folate B_9) and Ascobate (vitamin C). One kg of the processed sample could provide between 45.78 and 418.18% of the RDA of the vitamins for adult. B-vitamins identified in this sample largely function as cofactors (coenzymes) in the metabolism of macronutrient - carbohydrate, fat and protein- yielding energy and metabolites for the body functions (Albers et al., 2002: Gernah et al., 2012). In addition, they participate in red blood cell formation, support healthy immune and nervous

systems and are tangentially involved via DNA replication, in cell growth and multiplication.

Table 3: Vitamin concentrations[a] of raw and processed Pterocarpus santalinoides seed powder

Vitamin	Concentration		RDA for Adult (IOM, 2006)	% of RDA in 1kg processed sample
	Raw	Processed		
A (µg/100g sample)	3.75±0.71	4.27±0.48	600-900µg	4.74 - 7.12
D (µg/100g sample)	0.02±0.02	0.02±0.01	15.0µg	1.33
E (µg/100g sample)	1.74±0.52	2.39±0.28	15.0mg	0.16
K (µg/100g sample)	1.57±0.44	1.76±0.17	90 - 120µg	14.67 - 19.56
Thiamine, B_1 (µg/100g sample)	86.15±3.53	122.41±12.52	1.1 -1.2mg	102.01 - 111.28
Riboflavin, B_2 (mg/100g sample)	0.31±0.05	0.46±0.05	1.1 -1.3mg	353.85 - 418.18
Niacin, B_3 (mg/100g sample)	1.32±0.41	1.58±0.31	14 – 16mg	98.75 – 112.86
Pantothenate, B_5 (mg/100g sample)	0.30±0.08	0.32±0.07	5 – 6.0mg	53.33 – 64.0
Pyridoxine, B_6 (mg/100g sample)	0.24±0.05	0.29±0.08	1.3 -1.7mg	170.59 – 223.08
Folate, B_9 (µg/100g sample)	17.49±1.39	18.19±0.94	400µg	45.78
Ascobate, C (mg/100g sample)	5.87±0.84	6.77±1.33	75 – 90mg	75.22 – 90.27

[a]Values are means and standard deviations of triplicate determinations

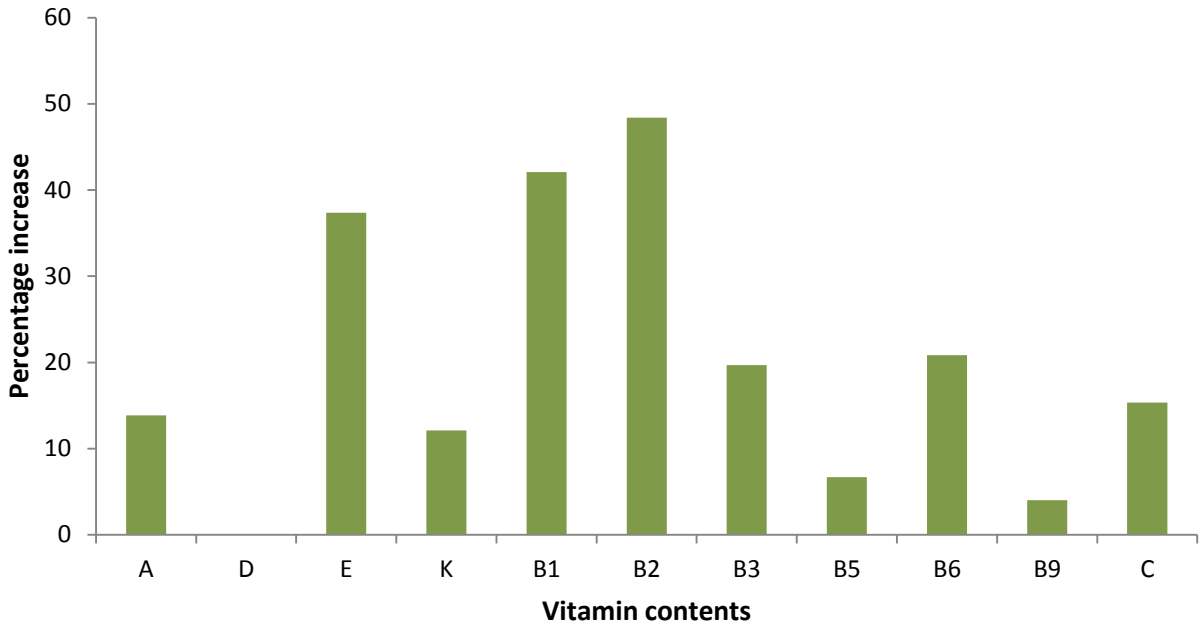

Fig.3: Effect of processing on Vitamin contents of Pterocarpus santalinoides seed powder

Each vitamin (co-enzyme) is unique in function and specialized on specific metabolic reactions. For instance, B_1 is involved in carbohydrate metabolism; B_2 and B_3 energy metabolism, B_5 fat metabolism and energy conversion, B_6 amino acid metabolism and, B_9

Amino- and nucleic acid metabolism (Albers et al., 2002). Ascorbic acid on its part, according to Albers et al. (2002), exhibits antioxidant activities in co-operation with other antioxidant vitamins. It is also involved in collagen synthesis, regulation of calcium metabolism, function of macrophages, granulocytes and lymphocytes in the immune system (Anyalogbu et al., 2014). A deficiency/absence of one or more of these vitamins may lead to several errors of metabolism resulting in reduced performance, growth impedance, fertility problems or diseases.

Conclusion.

The observation that processing reduced the antinutrients below the established physiologically tolerable limits while increasing most of the micronutrients to a level that are impactful on the recommended dietary allowances accentuates the immense potentials of the plant food to contribute to nutrition and wellbeing of consumer.

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