IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 12 Ver. IV (Dec. 2016), PP 08-12
www.iosrjournals.org

Variations in Selected Anti-Nutrient Contents of Infested (*Callosobruchus Maculatus Coleoptera: Bruchidae* and *Acanthoscelides Obtectus*) and Non-Infested Legumes Commonly Consumed in Nigeria.

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Abstract: *Investigations were carried out to determine variations in selected anti-nutrients of commonly consumed legumes in Nigeria. Results of Callosobruchus maculatus and Acanthoscelides obtectus infestation on the anti-nutrients of Vigna unguiculata, V. aconitifolia, Phaseolus vulgaris, P. lunatus and P. autifolius revealed significant (p<0.05) variations in infested seeds. Infestation engendered significant (p< 0.05) variations in hydrocyanic acid with mean values of 2.28 ± 0.02 for (P. autifolius), 1.85 ± 0.01 (P. lunatus), 1.51 ± 0.01 (V. unguiculata), 1.19 ± 0.01 (P. vulgaris) and 1.13 ± 0.01 mg/100 g (V. aconitifolia). Hydrocyanic acid had variations in mean values of 1.93 ± 0.01, 1.78 ± 0.02, 1.30 ± 0.01, 1.25 ± 0.01 and 1.08 ± 0.01 mg/100 g in non infested P. autifolius, V. unguiculata, V. aconitifolia, P. lunatus and P. vulgaris respectively. Vigna aconitifolia had highest total oxalate of 28.06 ± 0.01 while V. unguiculata had the lowest value of 21.46 ± 0.01 mg/100 g. Phytate was highest in infested V. unguiculata and lowest in P. lunatus. Results have shown variations in anti-nutrients arising from infestation and non-infestation of different legumes consumed in Nigeria. Findings of this research will guide quantity consumption of these legumes.*
Keywords: *Infestation, Anti-nutrients variation, Legumes.*

I. Introduction

Antinutrients are known plant's metabolites which reduce the utilization of nutrient in food [1].They are natural or synthetic compounds which interfere with the absorption of nutrient. Required nutrients can also possess antinutrient action when taken in excess. Excessive intake of fiber reduces the transient time through the intestine to such an extent that other nutrients cannot be absorbed. This is so because calcium, iron, zinc and magnesium share the same transport system within the intestine, thus, excessive consumption of one of these minerals will result in the saturation of the transport system thereby reducing the absorption of other minerals [2]. Some proteins such as trypsin inhibitors and lectins found in legumes can also be anti-nutrients. These enzymes inhibitors interfere with digestion. Other forms of antinutrients are flavonoids, which are a group of polyphenolic compounds that include tannins. These compounds chelate metals such as iron and zinc and reduce the absorption of these nutrients, but they also inhibit digestive enzymes and may also precipitate proteins [3].

Phaseolus vulgaris L. (Common beans), *P. lunatus* L. (Commonly called Akara beans), *P.* autifolius L. (Aloka or Pinto beans), V. *unguiculata* L. (Blackeyed pea) and *V. aconitifolia* L. (Ife Brown or Brown beans) are commonly consumed pulses in Nigeria. Legumes are plants in the family Fabaceae or Leguminoceae characterized by edible seed pods though sometimes poisonous. Members of this family are important food sources; and food legumes are those species of the legume family that are commonly eaten by human beings and animals [4]. Examples include; peas, beans, lentils, peanut and other podded plants used as food [5].

Callosobruchus maculatus (Coleoptera: Bruchidae) and *Acanthoscelides obtectus* Say infestation constitutes a serious threat to the growing of legumes in most regions of the world. Pest infestation may start from the field and continue during the period of storage [6]. In Nigeria losses incurred during storage by these storage pests are estimated at 3.6 billion naira per year [7]. Previous reports have shown that storage pest affected the quality of plant food and altered their chemical content negatively and the quality of plant-based antioxidant [8]. The severity of infestation depends on the developmental stages and the intensity of attack by the pest. *Callosobruchus maculatus* infestation of selected cowpea varieties brought about increase in phytate [9]. Infestation of *V. unguiculalta* by *C. maculatus* brought about significant increase in phytate, and oxalate. Wheat, maize and sorghum infested by *Trogoderma granarium* showed increase in phytic acid with progressive levels of infestation (25%, 50% and 75%). Substantial reduction in the level of nutrients during storage has been reported [10]. Cowpea seeds contain more phytate than oxalate and tannins [11]. Anti-nutrients are present in plant-based food. Phytate and oxalate are reported by [12] in two local cowpea species.

Anti-nutrients are present at some levels in almost all food for a number of reasons. Levels of antinutrients vary within and across species [13]. Food crop vary in their levels of nutrients and anti-nutrients contents. Knowledge of which is important in determining the amount consumed. The present research is therefore intended to investigate variations in selected ant-nutrients of some commonly consumed legumes in Nigeria as affected by *Callosobruchus maculatus* and *Acanthoscelides obtectus*.

II. Materials And Methods

2.1 Seed collection

Seeds of the five legumes were procured at the Goldie Market in Calabar, Nigeria. On procurement, the infested seeds were sorted from the non-infested ones and stored separately for three months. After which, the dust particles were removed from infested seeds and the seeds sun-dried for five days and milled into powdered form used for analysis of the anti-nutrients; hydrocyanic acid, phytic acid, soluble oxalate and total oxalate.

2.2 Determination of anti-nutrients

Antinutrient contents of infested and non-infested seed samples of *Vigna unguiculata, V. aconitifolia, Phaseolus vulgaris, P. lunatus and P. autifolius* were determined using the following methods; hydrocyanic acid content of samples was determined by the method of [14]. Phytic acid and oxalate were determined according to methods as described by [15] and [16].

2.3 Data analysis

Results obtained in this were subjected to Student T- test at 95% limit of confidence. Values presented are means of three replicates. Results were also expressed as percentage difference between the means for infested and non-infested.

III. Results

Table 1: Variation in antinutrient contents of infested (*Callosobruchus maculatus*) and non-infested of *Vigna unguiculata* L.

	mg/100 g				
Antinutrients	Infested	Non-Infested	% difference	Tcal	Ttab p< 0.05
Hydrocyanic acid	1.51 ± 0.01	1.76 ± 0.02 *	14.2	3.9	2.78
Total oxalate	21.46 ± 0.01	17.43 ± 0.2**	23.1	42.9	2.78
Soluble oxalate	12.77 ± 0.01	10.55 ± 0.01**	21.0	185	2.78
Phytic acid	0.64 ± 0.01	0.61 ± 0.01*	4.9	5.2	2.78

Values are mean ± SD, N=3 replicates, p<0.05, * = significant

Variation in antinutrients of infested *(Callosobruchus maculatus*) and non infested seeds of *V. unguiculata* are shown in (Table 1). The non-infested seeds had higher amount of hydrocyanic acid compared to the infested with a percentage reduction of (14.2%). Infested seeds had higher amount of total oxalate, soluble oxalate and phytic acid with percentage increase of 23.1%, 21.0% and 4.9% respectively.

Table 2: Variation in antinutrient contents of infested (*Callosobruchus maculatus*) and non-infested of *Vigna aconitifolia L.*

	mg/100 g				
Antinutrients	Infested	Non-Infested	% difference	Tcal	Ttab 0.05
Hydrocyanic acid	1.13 ± 0.01	1.30 ± 0.01	13.1	3.09	2.78
Total oxalate	28.06 ± 0.01	25.95 ± 0.01	8.1	162.3	2.78
Soluble oxalate	13.09 ± 0.01	10.68 ± 0.02	22.6	170.2	2.78
Phytic acid	0.53 ± 0.02	0.48 ± 0.02	10.4	1.96	2.78

Values are mean ± SD, N=3 replicates, p<0.05, * = significant

Hydrocyanic acid also showed a reduction in content due to *C. maculatus* infestation of *V. aconitifolia* with percentage reduction of 13.1%. Soluble oxalate, phytic acid and total oxalate had increase in infested seeds of 22.6%, 10.4% and 8.1% respectively. Corresponding T-test analysis of results also revealed significant (p<0.05) reduction and increase in anti-nutrients of infested seeds (Table 2).

Table 3: Variation in antinutrient contents of infested (*Acanthoscelides obtectus Say*) and non-infested of *Phaseolus vulgaris* L.

	mg/100 g				
Antinutrients	Infested	Non-Infested	% difference	Tcal	Ttab 0.05
Hydrocyanic acid	1.19 ± 0.01	1.08 ± 0.01*	9.2	40.5	2.78
Total oxalate	24.12 ± 0.02	25.85 ± 0.01*	6.7	422.0	2.78
Soluble oxalate	11.24 ± 0.02	10.43 ± 0.02*	7.8	45.0	2.78
Phytic acid	0.49 ± 0.01	0.61 ± 0.02**	19.7	18.6	2.78

Values are mean ± SD, N=3 replicates, p<0.05, * = significant

Phaseolus vulgaris showed variation in anti-nutrients Results revealed that non-infested seeds had lower amount of hydrocyanic acid and soluble oxalate while *Acanthoscelides obtectus* infested seeds had higher amount with percentage increase of 9.2% and 7.8%. Non-infested seeds had higher amount of phytic acid and total oxalate while infested had lower amount with percentage reduction of 19.7% and 6.7% (Table 3).

Table 4: Variation in antinutrient contents of infested (*Acanthoscelides obtectus Say*) and non-infested of *Phaseolus lunatus L.*

	mg/100 g				
Antinutrients	Infested	Non-Infested	% difference	Tcal	Ttab 0.05
Hydrocyanic acid	1.85 ± 0.01	1.25 ± 0.01**	32.4	28.1	2.78
Total oxalate	25.94 ± 0.01	26.87 ± 0.01*	3.5	11.2	2.78
Soluble oxalate	10.54 ± 0.02	11.80 ± 0.02*	10.8	79.7	2.78
Phytic acid	0.42 ± 0.02	0.46 ± 0.01*	8.7	98.3	2.78

Values are mean ± SD, N=3 replicates, p<0.05, * = significant

Results in Table 4 highlighted variations in antinutrients due to *Acanthoscelides obtectus* infestation and non-infestation of *P. lunatus*. Hydrocyanic acid had 32.4% increase with decrease of 10.8% (soluble oxalate), 8.7% (phytic acid) and 3.5% (total oxalate) in infested seed samples.

Table 5: Variation in antinutrient contents of infested (*Acanthoscelides obtectus Say*) and non-infested of *Phaseolus autifolius L.*

	mg/100 g				
Antinutrients	Infested	Non-Infested	% difference	Tcal	Ttab 0.05
Hydrocyanic acid	2.28 ± 0.02	1.93 ± 0.01*	18.1	28.9	2.78
Total oxalate	26.51±0.01	28.50±0.02*	7.0	284.7	2.78
Soluble oxalate	9.38±0.03	10.65±0.02*	11.9	55.5	2.78
Phytic acid	0.50±0.03	0.48±0.02*	4.2	1.9	2.78

Values are mean ± SD, N=3 replicates, p<0.05, * = significant

Hydrocyanic acid (18.1%) content of infested seeds was higher than non-infested. Soluble oxalate, total oxalate and phytic acid were significantly lower in infested compared to the non-infested with percentages of 11.9%, 7.0% and 4.2% respectively.

Results revealed significant (p<0.05) variations in anti-nutrients in infested and non-infested legumes among the commonly consumed legumes. Hydrocyanic acid content was highest (2.28 ± 0.02) in infested seeds of *P. autifolius* and lowest (1.13 ± 0.01 mg/100 g) in *V. aconitifolia* while non-infested also had highest amount (1.93 ± 0.01) in *P. autifolius* but lowest amount (1.08 ± 0.01 mg/100 g) in *P.* vulgaris.

IV. Discussion

The study revealed significant (p<0.05) variations in anti-nutrient contents of commonly consumed legumes in Nigeria. Also revealed are the high and low amounts of antinutrients in the various beans species arising from *Callosobruchus maculatus* and *Acanthoscelides obtectus* infestation and non-infestation. Results of this research depicts significant (p<0.05) variations in oxalate contents with highest amount in infested *V. aconitifolia,* followed by *P. autifolius, P. lunatus, P.* vulgaris and lowest in *V.* unguiculata. This increase agrees with earlier reports by [17] that oxalate content of three beans species showed increase as a result of infestation. Hydrocyanic acid and phytic acid contents also showed a similar trend of high and low amounts in all species studied. Antinutrients contents investigated were either high or low in infested compared to the non-infested. The increase in all the anti-nutrients after storage due to infestation may be due to the selective consumption of the beans seeds endosperm by the storage pest thus, leaving behind portions rich in anti-nutrients. The low levels of anitnutrients caused by infestation could be due to insect feeding activities [18].

Increased amounts of hydrocyanic acid caused by infestation in this research should not be treated with laxity because hydrocyanic acid (HCN) sometimes called prussic acid is an organic compound [19], extremely poisonous and flammable. In the air, hydrogen cyanide concentration in the range of 100-200 ppm will kill human within 10 and 60 minutes [20], while concentration of 2000 ppm (about 2380 mg/m^3) will a human in about 1 minute. Cyanide ion (CN^-) toxicity halts cellular respiration by acting as a non-competitive inhibitor for mitochondria enzyme (cytochrome c oxidase). This ion binds to iron in the heme subunit in the cytochrome interrupting electron transfer [20]. Hydrogen cyanide is one of the chemical warfare agents known as blood agent [21]. It has been used in a number of poisoning murders and suicides [22]. Some beneficial effects of HCN in mammals includes; vasodilation, antihypertensive effect, neurotransmission and neuromodulation. It has been reported by [23] that leukocytes generates HCN production during phagocytosis, and can kill bacteria,fungi and other pathogens by generating several different toxic chemicals, one of which is hydrogen cyanide. Vasodilation caused by sodium nitroprusside has been shown to be mediated not only by NO generation but also by endogenous cyanide generation, which adds not only toxicity, but some additional antihypertensive efficacy compared to nitroglycerine and other non-cyanogenic nitrates which do not cause a rise in blood cyanide levels [24]. It has been showed by some authors that [23] neurons can produce hydrogen cyanide upon activation of their opioid receptors by endogenous or exogenous opioids. Also shown is that neuronal production of HCN activates NMDA receptors and play a role in neurotransmission (signal transduction between neuronal cells). Moreover, under opioids increase in endogenous neuronal HCN production was seemingly needed for adequate opioid analgesia, as analgesic action of opioids was attenuated by HCN scavengers. They considered endogenous HCN to be a neuromodulator.

Phytate is a complex group of naturally occurring compounds that can significantly influence the functionality of food. Phytate has a strong mineral binding affinity and when it bind to minerals such as calcium, zinc, magnesium and iron, it becomes insoluble, precipitates and non absorbable making the minerals unavailable for absorption in the intestine resulting in mineral deficiencies [25]. It can also act as an acid, chelating the vitamin niacin, the deficiency known as pellagra. Phytic acid mineral binding potential is believed to prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract [26]. [27], reported that chelating effect may serve to prevent, inhibit and even cure some cancers by depriving those cells of minerals (iron) they need to reproduce. [28] documented that several studies though a bit scare in humans have demonstrated the positive effects of phytic acid in fighting cancerous tumour cells. The antioxidant effect of phytic acids particularly in regards to iron is of great importance since iron is known to behave as a free radical contributing to oxidative stress in the body. Phytic acid's ability to sequester and trap iron is beneficial in this context. It binds to iron and effectively neutralize any free radical. It also has the ability to bind essential minerals examples, iron, calcium, zinc and magnesium. [29], reported the capacity of phytic acid in reducing cholesterol and triglycerides, and impact positively the glycemic response of certain foods. Some phytic acid seems to have the ability to slow down a potential blood sugar spike following the consumption of certain high-carbohydrate food. This may explain why food high-fiber have been associated with improved blood sugar control [30]. Phytic and oxalic acids are among the major anti-nutrients present in plant protein sources [31].

The negative effect of anti-nutrient lies in their ability to affect absorption and nutrients utilization. According to Rao, legumes contain enzyme inhibitor called trypsin inhibitor which prevents the digestion of dietary proteins and make them unavailable, leading to protein deficiencies and other health disorders. Antinutrients are found in wide range of plant food. They are present in significant quantities in cereals (wheat) and legumes (beans). Rao reported that phytate can cause unavailability of calcium and zinc resulting in the classical dwarf syndrome known in Egypt. In Egypt, people sometimes eat high levels of phytates in unleavened bread which can lead to severe zinc deficiency and growth impairment in children [32]. Phytic acid and oxalic acid reduce mineral availability which lead to various mineral deficiency diseases such as anaemia [33] or form deleterious complexes with metal ions example calcium-oxalate which leads to renal damage [34]. Scientist discovered that some antinutrients bind to nutrients, thereby preventing their digestion and utilization in the body, while others appear to be toxic at high levels [32]. Apart from cyanogenic glycosides, food poisoning arising from antinutritional factors has not been properly addressed in developing countries like Nigeria. People have died out of ignorance, poverty and inadequate nutrition information and education especially in African countries. Reports of death from time to time after people consumed cooked beans abound. Also cases of renal and liver diseases are increasing. Legumes contain high concentrations of antinutritional factors [35]. These anti-nutrients for examples, phytate, oxalate and tannins [36] are of great importance both to the grains as well as human. They confer resistance of grains to insect pest. Thus, their reduction affects the grains susceptibility to insect attack. Anti-nutrients action depends on the metabolic processes of the ingesting animal, they are not inherent [31].

V. Conclusion

Antinutrient variations and their high levels observed in infested and non-infested seeds samples of the various legumes demands urgent attention. Legume constitutes a part of the diet of nearly all humans. The consumption of these infested and non-infested legumes tends to be limited by the presence of anti-nutrients which have been reported by many researchers to affect the digestibility and the bioavailability of plant nutrients. Though most processing methods such as soaking, fermentation, cooking, dehuling and genetics however, have been shown to significantly reduce or totally eliminate some antinutrients [37].

Variations found in this study calls for caution in the consumption of these legumes since recommended amounts have not yet been given by Dietary Reference Intakes (DFI), as the consumption of high amounts is dangerous, moderate amount is however, recommended to meet humans health needs. This recommendation is in line with a new theory which suggests that low levels of antinutrients may contain beneficial properties for the body [33]. This is not a surprise since a body of evidence links the consumption of whole plant food with a range of health benefits. The findings of this research also revealed the need for routine check of legumes seeds during storage in warehouses and market packing stores to avoid damage and increase amount arising from infestation in order to maximize the benefits of antinutrients.

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