

Antinutritional Factors of Five Selected Underutilized Legumes

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Abstract
Antinutritional factors (ANFs) are a major factor reducing the wider use of underutilized legumes as human food and animal feeds, as a result of the deleterious effects produced by ANFs in man and animals. This study evaluated the levels of some common ANFs in selected in five selected underutilized legumes (ULs) from the Genetic Resources Unit of International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The selected ULs were Winged bean (WB) (*Psophocarpus tetragonolobus*) (Tpt-48), Lima bean (LB) (*Phaseolus lunatus*) (2006-009), Bambara groundnut (BG) (*Vigna subterranea*) (TVSU- 1482), Jack bean (JB) (*Canavalia ensiformis*) (Tce-4) and Sword bean (SB) (*Canavalia gladiata*) (Tcg-4). All the ANFs were analyzed using standard methods. Statistical analysis was done using descriptive statistics. The results showed that trypsin inhibitors ranged from 13.76±0.02 mg/g (LB) to 39.71±0.04 mg/g (SB), hydrogen cyanides ranged from 9.30±0.02 mg/kg (LB) to 41.41±0.04 mg/kg (SB), oxalates ranged from 0.11±0.00% (BG) to 0.38±0.001% (SB), phytates ranged from 0.24±0.00% (BG) to 0.69±0.002% (SB), saponins ranged from 0.22±0.00% (WB) to 0.35±0.00% (BG), tannins ranged from 0.04±0.00% (WB) to 0.08±0.003% (SB), alkaloids ranged from 0.14±0.00% (WB) to 0.15±0.00% (LB) and haemagglutinins ranged from 25.27±0.01% (LB) to 66.92±0.04% (BG). The study concluded that all the selected underutilized legumes in this study contained antinutritional factors at varying levels and there is a need to investigate the suitable processing methods to either reduce or eliminate the inherent ANFs in these ULs.
Keywords: Antinutritional factors, underutilized, legumes, human food, animal feeds

Introduction
Legumes are important components of a balanced human and animal diet in many parts of the world as a result of their high starch and protein contents (Adebowale *et al*. 2009). They are usually consumed traditionally as whole seeds or as ground flour post-dehulling. The rapid growth in the food industry demanding that new ingredients, has led food scientists to discover and exploit new legume varieties (Adebowale *et al*. 2009).
The ever increasing demand for plant proteins *in lieu* of expensive animal proteins have been reported by many researchers (Adebowale *et al*. 2009). This calls for the need to intensify research efforts aimed at identifying new legume varieties.

Winged bean (*Psophocarpus tetragonolobus*) is an under-exploited legume which is a potential source of food for humans and animals in the tropics (Amoo *et al.,* 2006). Several parts of the plant like seeds, leaves, pods and tubers are rich in protein and edible, which it unique among leguminous crops (Amoo *et al.,* 2006).
Lima bean (*Phaseolus lunatus*) is mostly cultivated in South America (Yellavila *et al.*, 2015). And is sometimes referred to as butter beans, sugar beans, haba beans, civet beans, Guffin beans, Burma beans, Pallar beans, Hibbert beans, Sieva beans and Madagascar beans (Yellavila *et al.*, 2015).

Bambara groundnut (*Vigna subterranea*) originated from Africa and is applied locally as a vegetable (Ayo *et al*., 2014). It is known by different names in Nigeria; 'Epakuta' (Yoruba tribe), 'Okpa' (Ibo tribe), 'Gurjiya or Kwaruru' (Hausa tribe) and 'Kwam' (Geom, Plateau State). Bambara groundnut is consumed in different forms (either at immature green or mature stages) as a vegetable and it is capable of boosting food availability and reducing malnutrition (Ayo *et al*., 2014).

Jack bean (*Canavalia ensiformis*) is an under-exploited tropical dry beans widely distributed in Africa, Asia, Latin America and the West Indies (Marimuthu and Gurumoorthi, 2013). Raw jack bean seed contains approximately 300 g/kg protein and 600 g/kg carbohydrates (Marimuthu and Gurumoorthi, 2013).

Sword bean (*Canavalia gladiata*) originated either from southern Asia or Africa (Adebowale *et al.*, 2006). Sword bean possesses good agronomic characteristics which make it thrive in adverse climates and is thus desirable for improving food and nutrition security in the tropics (Adebowale *et al.*, 2006).

Despite the desirable nutritive features of underutilized legumes, they are not optimally utilized as human food and animal feeds for monogastrics and ruminants mainly due to the presence of potent inherent anti-nutritional factors (Udedibie *et al*., 2000; Esonu, 2009; Emiola, 2011). Emphasis has been placed on the various effective techniques for inactivating the anti-nutritional factors in legume seeds for the improvement of their nutritive value (Emiola, 2011). Anti-nutritional factors either by themselves or through their metabolic products, obstruct feed utilization and adversely affect animal health and production. ANFs act by reducing nutrient intake, digestion, absorption and utilization of human food and animal feeds and may produce other adverse effects

(Akande *et al*., 2010). Many plants and legumes seeds contain in their raw forms wide varieties of potentially toxic antinutrients (D'Mello, 2000). Some of these anti-nutrients are known as ''secondary metabolites'' and they have been widely documented as biologically active compounds. Most of these secondary metabolites can cause very harmful biological responses (Soetan, 2008). The major ANFs in plants are saponins, tannins, oxalates, phytates, cyanogenic glycosides, gossypol, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors (Akande *et al*., 2010).

Several justifications for the continued surveillance and research on antinutritional factors naturally present in plants used as human foods and animal feeds have been reported (Osagie, 1998). As efforts are ongoing to discover the numerous nutritional and functional properties of new and underutilized legumes, there is the need to have knowledge of the levels of their inherent antinutritional factors. The aim of this study is to evaluate the concentrations of the common antinutritional factors is some selected underutilized legumes.

Materials and Methods

Source of the Selected Underutilized legumes

The Winged bean (WB) (*Psophocarpus tetragonolobus*) (Tpt-48), Lima bean (LB) (*Phaseolus lunatus*) (2006-009), Bambara groundnut (BG) (*Vigna subterranea*) (TVSU- 1482), Jack bean (JB) (*Canavalia ensiformis*) (Tce-4) and Sword bean (SB) (*Canavalia gladiata*) (Tcg-4) used for this study were supplied by the Genetic Resources Center (GRC) of the International Institute for Tropical Agriculture (I.I.T.A), Ibadan, Oyo State, Nigeria.

Quantification of the Antinutritional factors

Determination of the Trypsin inhibitor concentration

Trypsin inhibitor activities were analysed according to (Liener, 1979).

Determination of Hydrocyanide levels

Hydrogen cyanide levels were quantified according to (Bradbury *et al*., 1999).

Determination of the Oxalate level

Oxalate level was determined by the method of (Fasset, 1996).

Determination of the Phytate level

Phytate level was analysed according to (Maga, 1983).

Determination of the Saponin content

Saponin level was determined according to (Brunner, 1984).

Determination of the Tannic acid (Tannin) level

Tannin level was quantified according to (Dawra *et al*., 1988).

Determination of the Total Alkaloids concentration

Alkaloid concentration was determined by the method of (Henry, 1973).

Determination of the Heamagglutinin Unit

Haemagglutinin unit was determined according to (Jaffe, 1979).

Statistical analysis

Statistical analysis was done using descriptive statistics, mean$\pm$standard deviation.

Results and Discussion

The results of the antinutritional factors (ANFs) in the selected underutilized legumes are presented in Tables 1 to 8.

Table 1: Trypsin Inhibitors content of some selected Underutilized legumes

Legumes	Winged bean Tpt-48	Lima bean 2006-009	Bambara Groundnut TVSU-1482	Jack bean Tce-4	Sword bean Tcg-4
Trypsin Inhibitors (mg/g)	24.08± 0.02	13.76± 0.02	29.75± 0.04	35.79± 0.03	39.71± 0.04

Mean±standard deviation

Trypsin inhibitors ranged from 13.76±0.02 mg/g (LB) to 39.71±0.04 mg/g (SB). These values were lower than the trypsin inhibitors values reported for the different varieties of lablab beans (LB) (*Lablab purpureus*), an underutilized legume (UL) which ranged from 39.5±0.29 u/mg protein to 44.8±0.57 u/mg protein (Soetan, 2012).

The values of trypsin inhibitors reported for the ULs in this study, were however, higher than the reported values for the different accessions of African yam bean (AYB) (*Sphenostylis stenocarpa*), another underutilized legume, with AYB trypsin inhibitors ranging from 9.8360±0.118 mg/g to 15.9210±0.267 mg/g (Soetan, 2017).
Trypsin inhibitors occur in a wide range of foods. They have been studied extensively in plants belonging to the Leguminoseae (Savage and Morrison, 2003). Trypsin inhibitors that inhibit the activity of the enzymes trypsin and chymotrypsin in the gut, thus preventing protein digestion, are found in many plant species (Hill, 2003).
Protein digestibility may be reduced, and dietary protein is excreted in the feces. There is also reduced nitrogen and sulfur absorption. One of the effects of the inactivation of digestive enzymes in the intestine is the stimulation of trypsin and chymotrypsin secretion from the pancreas, which can create an increased demand for the sulfur amino acids methionine and cystine (Hill, 2003). In turn, this leads to increased endogenous loss of both nitrogen and sulfur. The trypsin inhibitors can stimulate the release of cholecystokinin into the blood stream, which further increases pancreatic secretion (Hill, 2003).

Table 2: Hydrogen Cyanides content of some selected Underutilized legumes

Legumes	Winged bean Tpt-48	Lima bean 2006-009	Bambara Groundnut TVSU-1482	Jack bean Tce-4	Sword bean Tcg-4
Hydrogen Cyanides (mg/kg)	21.15± 0.03	9.30± 0.02	24.35± 0.03	37.27± 0.04	41.41± 0.04

Mean±standard deviation

Hydrogen cyanides ranged from 9.30±0.02 mg/kg (LB) to 41.41±0.04 mg/kg (SB). These values were lower than the cyanogenic glycosides values recorded for the different varieties of *Lablab purpureus*, which ranged from 175±0.00 mg/kg to 195±0.57 mg/kg (Soetan, 2012).

The toxic effects of hydrogen cyanide are well documented and affect a wide spectrum of organisms since their mode of action is inhibition of the cytochromes of the electron transport system (Laurena *et al*., 1994).
Cyanogenic glucosides are hydrolysed to yield toxic hydrocyanic acid (HCN). The cyanide ions depress several enzyme systems, inhibit growth of animals through interference with several essential amino acids and suppress the optimum utilization of nutrients resulting in acute toxicity, neuropathy and death (Osuntokun, 1972; Fernando, 1987).

Hydrogen cyanide inactivates cytochrome oxidase in the mitochondria of cells through binding to Fe^{3+}/Fe^{2+} contained in the enzyme, which causes a decrease in the utilization of oxygen in the tissues (Jones *et al*., 2000). Cyanide also causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism. Cyanide also decreases the rate of glycolysis and inhibits the tricarboxylic acid (TCA) cycle. Hydrogen cyanide will reduce the energy availability in all cells, but its effect will be most immediate on the respiratory system and heart (Jones *et al*., 2000).

Table 3: Oxalates content of some selected Underutilized legumes

Legumes	Winged bean Tpt-48	Lima bean 2006-009	Bambara Groundnut TVSU-1482	Jack bean Tce-4	Sword bean Tcg-4
Oxalates (%)	0.34± 0.00	0.21± 0.00	0.11± 0.00	0.35± 0.001	0.38± 0.001

Mean±standard deviation

Oxalates ranged from 0.11±0.00% (BG) to 0.38±0.001% (SB). These values were higher than the oxalates values reported for AYB, which ranged from 0.0785±0.00% to 0.1160±0.00% (Soetan, 2017). The values obtained for the ULs in this study were however, lower than those reported for LB, which ranged from 8.2±0.12 mg/g to 9.8±0.17 mg/g (Soetan, 2012).

Oxalates (oxalic acid) forms strong bonds with various other minerals like calcium, potassium, sodium and magnesium. This chemical combination results in the formation of oxalate salts (Habtamu and Negussie, 2014). Oxalate is an anti-nutrient which under normal conditions is confined to separate compartments. However, when it is processed and/or digested, it comes into contact with the nutrients in the gastrointestinal tract. When released, oxalic acid binds with nutrients, rendering them inaccessible to the body.

If feed with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut (Habtamu and Negussie, 2014).

Oxalates react with calcium to produce insoluble calcium oxalate, reducing calcium absorption. This leads to a disturbance in the absorbed calcium: phosphorus ratio, resulting in mobilization of bone mineral to alleviate the hypocalcemia. Prolonged mobilization of bone mineral results in nutritional secondary hyperparathyroidism or osteodystrophy fibrosa (Rahman and Kawamura, 2011). Excessive body concentration of soluble oxalate prevents the body's absorption of calcium ions because the oxalate binds the calcium ions to form a calcium-oxalate complex which is insoluble. People with the tendency to form kidney stones are mostly advised to avoid oxalate-rich foods (Adeniyi *et al*., 2009).

Table 4: Phytates content of some selected Underutilized legumes

Legumes	Winged bean Tpt-48	Lima bean 2006-009	Bambara Groundnut TVSU-1482	Jack bean Tce-4	Sword bean Tcg-4
Phytates (%)	0.57± 0.00	0.42± 0.00	0.24± 0.00	0.63± 0.001	0.69± 0.002

Mean ± standard deviation

Phytates ranged from 0.24±0.00% (BG) to 0.69±0.002% (SB). These values were higher than the phytates values in AYB, which ranged from 0.1385±0.00% to 0.2000±0.00% (Soetan, 2017). The phytates in the UL in this study were however, lower than those in LB, which ranged from 13.6±0.27 mg/g to 14.4±0.06 mg/g (Soetan, 2012).

Phytates are known to bind many minerals like calcium, iron, magnesium and zinc, making them unavailable for normal body processes (Walter *et al*., 2002).

Phytate, which is also known as inositol hexakisphosphate, is a phosphorus containing compound that binds with minerals and inhibits mineral absorption. The cause of mineral deficiency is commonly due to its low bioavailability in the diet. The presence of phytate in feeds has been associated with reduced mineral absorption due to the structure of phyate which has high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing non-availability for intestinal absorption (Walter *et al*., 2002).

Table 5: Saponins content of some selected Underutilized legumes

Legumes	Winged bean Tpt-48	Lima bean 2006-009	Bambara Groundnut TVSU-1482	Jack bean Tce-4	Sword bean Tcg-4
Saponins (%)	0.22± 0.00	0.27± 0.00	0.35± 0.00	0.24± 0.002	0.29± 0.002

Mean ± standard deviation

Saponins ranged from 0.22±0.00% (WB) to 0.35±0.00% (BG). These values were higher than the saponins values recorded for AYB, which ranged from 0.1190±0.00% to 0.1390±0.00% (Soetan, 2017). The saponins in the ULs in this study were however, lower than those in LB, which ranged from 11.3±0.17 mg/g to 12.1±0.06 mg/g (Soetan, 2012).

Saponins are reported to reduce the uptake of some nutrients, including cholesterol and glucose in the gut through intra-lumenal physicochemical interaction (Umaru *et al.,* 2007). The structural complexity of saponins results in many of biological and chemical properties like bitterness, foaming and emulsifying and haemolytic properties (Habtamu and Ngusse, 2014). Saponins caused haemolysis of red blood cells of rats (Johnson *et al*. 1986).

Table 6: Tannins content of some selected Underutilized legumes

Legumes	Winged bean Tpt-48	Lima bean 2006-009	Bambara Groundnut TVSU-1482	Jack bean Tce-4	Sword bean Tcg-4
Tannins (%)	0.04± 0.00	0.07± 0.00	0.06± 0.00	0.07± 0.003	0.08± 0.003

Mean ± standard deviation

Tannins ranged from 0.04±0.00% (WB) to 0.08±0.003% (SB). These values were higher than the tannins values recorded for AYB, which ranged from 0.0061±0.00% to 0.0096±0.00% (Soetan, 2017). The tannins in the UL in this study were however, lower than those in LB, which ranged from 3.5±0.84 mg/g to 4.7±0.06 mg/g (Soetan, 2012).

The consumption of tannins by animals resulted in diminished consumption of feeds by the animals. It also binds dietary protein and some digestive enzymes forming undigestible complexes (Aletor, 1993). Tannins also decreased palatability of animal feed and their growth rate (Roeder, 1995).

Tannins exhibit their antinutritional ability largely by precipitating dietary proteins, digestive proteins and digestive enzymes to form indigestible complexes (Omoyeni and Adeyeye, 2009). Tannin sours the mucus secretions and contracts the membranes so that the secretions from the cells are restricted. Excessive use of herbs containing high quantity of tannins is not recommended (Reed, 1995).

Table 7: Alkaloids content of some selected Underutilized legumes

Legumes	Winged bean Tpt-48	Lima bean 2006-009	Bambara Groundnut TVSU-1482	Jack bean Tce-4	Sword bean Tcg-4
Alkaloids (%)	0.14± 0.00	0.15± 0.00	0.18± 0.00	0.16± 0.003	0.17± 0.003

Mean ± standard deviation

Alkaloids ranged from 0.14±0.00% (WB) to 0.15±0.00% (LB). These values were lower than the alkaloids values recorded for AYB, which ranged from 12.0595±0.01% to 15.015±0.00% (Soetan, 2017) and also alkaloids values for LB, which ranged from 3.7±0.15 mg/g to 6.8±0.15 mg/g (Soetan, 2012).
Alkaloids are reported to cause gastrointestinal and neuron disorders (Aletor, 1993) and they also reduce appetite (Yadav *et al*., 2014). The glycoalkaloids present in some root crops like potatoes caused toxicicity to fungi and animals (Saito *et al*., 1990; Aletor, 1991).

Table 8: Haemagglutinins units of some selected Underutilized legumes

Legumes	Winged bean Tpt-48	Lima bean 2006-009	Bambara Groundnut TVSU-1482	Jack bean Tce-4	Sword bean Tcg-4
Haemagluttinins (HU/mg)	46.16± 0.02	25.27± 0.01	66.92± 0.04	62.83± 0.03	66.36± 0.02

Mean ± standard deviation

Haemagglutinins ranged from 25.27±0.01% (LB) to 66.92±0.04% (BG). These values were higher than the haemagglutinins values reported for LB, which ranged from 18.7±0.17 U/mg to 28.6±0.06 U/mg (Soetan, 2012).

Haemagglutinins are capable of damaging the intestinal mucosa, thus reducing the permeability of the mucosa, subsequently affecting the reabsorption of nutrients but also damages the immune system (Aletor and Fetuga, 1987). It also resists digestive breakdown and substantial quantities of ingested lectins may be recovered intact from the faeces of animals fed diets (Aletor and Fetuga, 1987).

Legumes have been underutilized because of the presence of anti-nutritional factors such as enzymes (trypsin, chymotrypsin, α-amylase) inhibitors, phytic acid, flatulence factors, saponins and other toxic factors, also due to the need for prolonged cooking. These factors adversely affect the nutritive value of legumes. ANFs inhibit carbohydrate and protein digestibilities, inhibit some enzymes and bind nutrients making them unavailable and they also induce pathological changes, (Bressani, 1993).

To increase biological benefits, ease of digestion and decrease antinutrient compounds in legumes, traditional procedures such as heating or blanching, soaking and fermentation, roasting are generally used. Most of the methods employed are based on the use of water and thermal treatments (Bressani, 2002; Gilbert, 2002; Diallo and Berhe, 2003).

Conclusion

All the selected underutilized legumes in this study contained antinutritional factors at varying levels. There is a need to investigate the most suitable processing methods to reduce or eliminate the antinutritional factors in these underutilized legumes. This could assist in enhancing the various nutritional and functional properties of these legumes for the benefit of human and animals.

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