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ANTINUTRIENTFACTORS IN SOME EDIBLE COWPEA LEGUMES

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ABSTRACT

Seven varieties of cowpea (*Vigna unguiculata*), Ife Brown, IT98K-128-3 , IT98K-506-1, IT93K-452-1 and IT95K-1072-57, IT06K-149-1, and IT06K-136 obtained from the International Institute of Tropical Agriculture, Ibadan and cultivated at the Biological Garden, College of Education, Agbor, were evaluated for levels of anti nutrient factors. Tannins were determined following the vanillin-HCl method while phenols and polyphenols were determined using the method of Folin-Denis. Similarities in anti nutrient factors among the cowpea varieties were described using cluster analysis. The results obtained were shown as follows: Ife Brown (Tn=0.67g; Ph=0.05g; Po=12mg), IT98K-128-3 (Tn= 0.47g; Ph=0.04g;Po= 16mg), IT98K-506-1(Tn=.54g; Ph=0.04g; Po=11mg), IT93K-452-1 (Tn=0.73g; Ph=0.03g; Po=18mg), IT95K-1072-57(Tn=0.31g; Ph=0.06g; Po=20mg), IT06K-149-1 (Tn=0.29g; Ph=0.05g; Po=24mg) and IT06K-136 (Tn=0.33g; Ph=0.06g; Po=15mg). It was observed that the values of tannins were generally low, being less than one gram in all the cowpea varieties studied. The cowpea variety IT93K-452-1 had the highest value of tannin which was 0.73 g/100g^{-1}. Phenols (0.03-0.06 g/100g^{-1}) and polyphenols (11-24mg/100g^{-1}) were comparatively low in all the samples. The study recommends that IT06K-136 which recorded low values of anti nutrient factors as well as Ife Brown, IT93K-452-1 and IT98K-128-3 which recorded low tannins and moderate levels of phenols and polyphenols should be grown by farmers in Agbor in Ika land.

Key words: Tannins (Tn), Phenols (Ph) and Polyphenols (Po).

INTRODUCTION

Cowpea, (*Vigna unguiculata* [L.] Walp.) is an important legume crop found in the tropical as well as subtropical countries covering about 65 countries in Asia and Oceania, the Middle East, Southern Europe, Africa, Southern USA, Central and South America (Singh *et al.*,1997; Chinma *et al.*, 2008). Mahalakshmi *et al.* (2007) reported that cowpea belongs to the family Fabaceae sub-family Papilionoideae, tribe Phaseoleae and genus, *Vigna*. Okpara and Oshilim (2001) further noted that this important tropical and subtropical legume is grown for forage, green pods, and grains. Generally it is an excellent source of protein. White seeded varieties and black-eyed types are commonly grown for grain and table use, while viny varieties that mature late are preferred for forage.

According to Chang *et al.* (1994), cowpea grain legumes are increasingly important for its cheap source of protein. The writers further observed that although the crop is loaded with high protein content, the quality of protein in cowpea is low because of the relatively low concentration of sulfur-containing amino acid and the presence of anti nutritional factors. Consequently, cowpea is underutilized in various countries because of anti nutrient factors, such as enzyme (trypsin, chymotrypsin and α-amylase) inhibitors, phytic acid, flatulence factors, polyphenols, lectins, cyanogenic compounds, oestrogens, saponins, antivitamins and allergens etc (Urbano *et al.*, 2003; Ramakrishna *et al.*, 2006; Amata and Lebari, 2012). According to Suarez *et al.* (1999), and Wang *et al.* (2003), indigestible substances include the flatulence producing oligosaccharides, namely rafinose, starchyose and verbascose which due to the absence of α-galactosidase in humans, are fermented anaerobically by microorganisms to produce carbon dioxide, hydrogen and methane. As the name suggests, anti-nutrient factors are constituents found within foods that inhibit the absorption of some nutrients and in some instances interfere with digestion. Despite the inaccurate perception that anti-nutrients are bad things that need to be avoided, anti-nutrients are found in almost all fruits and vegetables where they function to protect from invaders such as insects. Examples of anti-nutrients that can be found in legumes are lectins (khokhar and Apenten, 2003). While they may function as an anti-nutrient if not prepared well, they are also therapeutically beneficial with clinical studies showing that lectins found within legumes have the ability to induce death of cancerous cells. Heat

treatments destroy most of the anti-nutrient compounds, but it is necessary to process at optimum temperatures for an optimum period of time in order to maintain nutritional quality. Some anti-nutrient components of the cowpea, V. *unguiculata*, include the following:

Phenols and Polyphenols

According to Ramakrishna *et al.* (2006), phenols and polyphenols are a structural class of natural, synthetic and semi-synthetic organic chemicals, found in cowpea grain legumes and other foods. They help the body fight premature aging and disease by absorbing free-radicals the cells produce due to poor diet, lack of exercise, environmental toxins and stress. Phenols and polyphenols are anti-oxidants found in some foods which include grain legumes (Pandey and Rizvi, 2009). It was believed that the antioxidant polyphenols help protect humans from environmental carcinogens such as passive tobacco smoke. On the other hand some phenols are anti-nutritional factors responsible for the bitterness and astringency of many foods and beverages (Ramakrishna *et al.*, 2006). These writers further noted that the nutritional value of grain legumes depends primarily on their nutrient contents and presence or absence of anti-nutritional toxic factors. Nutritive value is the ability of food to provide a usable form of nutrients: proteins, carbohydrates, vitamins and minerals. The food processing methods including soaking, germination, decortications, fermentation and cooking greatly influence their nutritive values. Of these, cooking and germination plays an important role as it influences the bioavailability and utilization of nutrients and also improves palatability which incidentally may result in enhancing the digestibility and nutritive value (Bakr, 1996; Obohet *al.*, 2000). The presence of anti-nutritional factors in legumes was reported to be reduced at varying degrees based upon the food preparation involved and the properties exhibited by various types of legumes themselves.

However, some anti-nutritional factors are essential for their health-promoting and disease preventive effects. According to Djocgoue *et al.* (2007), low molecular weight phenolics are involved in plant resistance mechanisms to insect attacks and pathogen infections. Nana *et al.* (2002), reported that following pathogen infection, the concentration of constitutive phenols is enhanced as a result of the production of compounds toxic to pathogens. Furthermore, Lattanzio *et al.* (1997), demonstrated that cowpea which interacted with arbuscular mycorrhizal fungi (AMF) produced enhanced concentration of two isoflavonoids, quercetin and kaempferol. However, Nana *et al.* (2002), cited in Fokom *et al.* (2010) concluded that plants colonized by AMF produced high phenol concentration necessary to resist against destructive insect attacks.

Tannins

Tannins are high- molecular weight, phenol-rich polymers that exist in foods, including legumes, beverages, cereals, fruits, coffee and tea (Chang *et al.*, 1994). Of the varieties of beans, red-colored beans appear to contain the most tannin. Tan-and-red speckled cranberry beans contain quite a lot of tannin. Black beans contain a moderate-to-high amount of tannins. Chick peas, yellowish in color, contain small amounts of tannins. White-colored beans, however, contain few if any tannins (Navia, 1998). Tannins are divided into two major types, hydrolysable and condensed (Chang *et al.*, 1994).

Hydrolysable tannins are basically derived from simple phenolic acids like gallic acid or ellagic acid and when heated they give away pyrogallol. Pyrogallol is also known as hepatotoxic and has antiseptic as well as caustic properties. Owing to the hepatotoxic property, plants that have a concentration of tannins are not appropriate for application on open wounds (Herbs, 2000). When out in the open air hydrolysable tannins normally change to a brownish color and are accountable for the brown color of many plant dyes.

Condensed tannins are polymers of flavin-3-ols linked through acid-labile carbon-carbon bonds (Chang *et al.*, 1994). Condensed tannins, also known as non-hydrolysable tannins, do not split easily and hence they are difficult to analyze. Condensed tannins are basically flavonoid dyes formed through bio-synthesis of flavins and catechins. When these non-hydrolysable tannins are heated up in acids they synthesize to yield a red insoluble substance known as tannin reds or phlobaphenes. Phlobaphenes are flushed precipitates found in some plants that have reddish tints and this is an indication that these plants have rich concentration of condensed tannins. Furthermore, the writers noted that when condensed tannins are heated, catechol emerges as the final product. Unlike hydrolysable tannins, condensed tannins do not possess any trace of hepatotoxicity or any adverse side effects and hence are favourable for use. Condensed tannins are not as nutritionally important because of their limited solubility (Chang *et al.*, 1994).

According to Patte (1985), tannin-protein interactions, was long believed to be responsible for the formation of astringency in unripe fruits and various beverages. According to Salunkhe *et al.* (1982), and Oh and Hoff (1986), tannins may decrease nutritional quality of some cereals and legumes and inactivate certain digestive enzymes. They are anti-nutritional factors which can form complexes with proteins, proteases and amylases of the intestinal tract, thereby inhibiting the absorption and utilization of protein and minerals. Chang *et al.* (1994) reported that tannins would continue to be prominent components of human and livestock diets because of their presence in high yielding crops and in crops that can be grown on marginal areas. All tannins have several

common properties amongst them. While the tannins are soluble in water and alcohol, they do not dissolve in organic solutions. Furthermore, Chang *et al.* (1994), documented that all tannins form precipitates when reacted with nitrogenous bases, polysaccharides, some alkaloids, few glycosides and proteins. Medically, tannins are used as antidotes to poisoning by alkaloids depending on their capacity to form insoluble tannates. However, only dilute solutions of tannins are applied for this work. Finally, almost all tannins consumed remain exuded during the digestive process, and different quantities of it enter the body fluids and are emitted by the kidney.

According to Kalidass and Mohan (2012), other remedial values of tannins include application on burns to heal the injury and on cuts to stop bleeding. Tannin's ability to form a strong 'leather' resistance on the exposed tissues helps in protecting the wounds from being affected further. While it stops infection from above, internally tannin continues to heal the wound. In case of third degree burns using strong tannin sources will not only prevent septicemia, but also help to save life. This traditional method has been practiced by most medical practioners in all countries (Herbs, 2000). On the other hand, when a tannin-rich solution is poured on the flesh, it generates a sealing 'eschar' that often helps in growing new skin albeit temporarily. These writers posited that this technique requires repeated washing of the wound with tannins and this helps to eliminate the bacteria too. Hence, tannins are also said to have antiseptic properties. They reiterated that phenols and tannins are known to inhibit the activities of digestive enzymes and noted that the presence of ever low levels of tannins and phenols is not desirable from nutritional point of view. However, they inferred that in legumes, the soaking and cooking process is known to reduce phenols and tannins significantly (Vijayakumari, *et al.*, 1996).

This paper seeks to determine through biochemical analysis, the level of anti nutrient factors among some selected cowpea varieties that would adapt to the ecological zone of Agbor, Ika land of DeltaState.

METHODOLOGY

The research comprised of three field trials that were carried out at the Biological Garden of the College of Education Agbor, Delta State. The seeds for the experiments were obtained from the plant breeding unit of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The experiments comprised of seven cowpea varieties of *V. unguiculata (L.) Walp.* namely: Ife Brown, IT98K-128-3 , IT98K-506-1, IT93K-452-1 and IT95K-1072-57, IT06K-149-1, and IT06K-136 that were laid out in a complete randomized block design (CRBD) with seven replications. The cowpea samples were dried at 80°C to remove moisture for about 8hrs.The sa were put in a desiccator and allowed to cool. cowpeas were then broken into smaller bits w porcelain mortar and then ground to powder usi blender and thereafter analysed for the follo biochemical compositions.

Phenols and polyphenols

Phenols and polyphenols were determined by F Denis method (A.O.A.C. 2005). About 200 mg defa seed material was taken in a 250 ml round bottom f and 100 ml of 1 % HCl in methanol was added. contents were refluxed for 2 hours, cooled, filtered the volume made up to 100 ml with acid-methanol a few washing. 0.2 ml of extract was taken and 7.5 m water and 0.5 ml of Folin-Denis reagent were added mixed. To it, 1 ml of saturated sodium bicarbon solution was added and volume made up to 10 ml w water, mixed and the absorbance was measured at 7 nm after 30 minutes. The results were calculated as tannic acid equivalents/ g sample and expressed as m 100 g dry weight.

Tannins

Tannins were determined by Vanillin-HCl method of Pr *et al.* (1978). Five gram of defatted seed material w used for the extraction of tannins by using acid methanol. One ml of suitably diluted extract was taken a test tube and 5 ml of freshly prepared Vanillin-H reagent was added slowly with mixing and colou developed was read at 500 nm. A standard curve tannic acid was prepared according to A.O.A.C. (200 method of measurement of the concentration of tannin in sample.

RESULTS

Phenol

The level of phenols among the cowpea varieties was relatively low, ranging from 0.03g/100g^{-1} to 0.06g/100g^{-1}. Both IT95K-1072-57 and IT06K-136 recorded the highest level of 0.06g/100g^{-1}, followed by Ife Brown and IT06K-149-1 (0.05g/100g^{-1}) respectively. Moderately lower levels of phenols were recorded in IT98K-506-1 (0.04g/100g^{-1}), IT06K-128-3 0.04g/100g^{-1}) and IT93K-452^{-1} (0.03g/100g^{-1}) (Table 1).

Figure 1 showed that three clusters showing absolute similarities in each case were formed between IT95K-1072-57 (0.06 g/100g^{-1}) and IT06K-136 (0.06 g/100g^{-1}) with the highest value of phenols, Ife Brown (0.05 g/100g^{-1})

Table 1. Anti nutritional compositions of seven cowpea varieties studied.

S/no.	Cowpea Varieties	Tannins (g/100 g^{-1})	Phenols (g/100 g^{-1})	Polyphenol (myricetin mg/100 g^{-1})
1	Ife Brown	0.67	0.05	12
2	IT93K–452–1	0.73	0.03	18
3	IT98K–506–1	0.54	0.04	11
4	IT95K–1072–57	0.31	0.06	20
5	IT06K–128–3	0.47	0.04	16
6	IT06K–136	0.33	0.06	15
7	IT06K–149–1	0.28	0.05	24

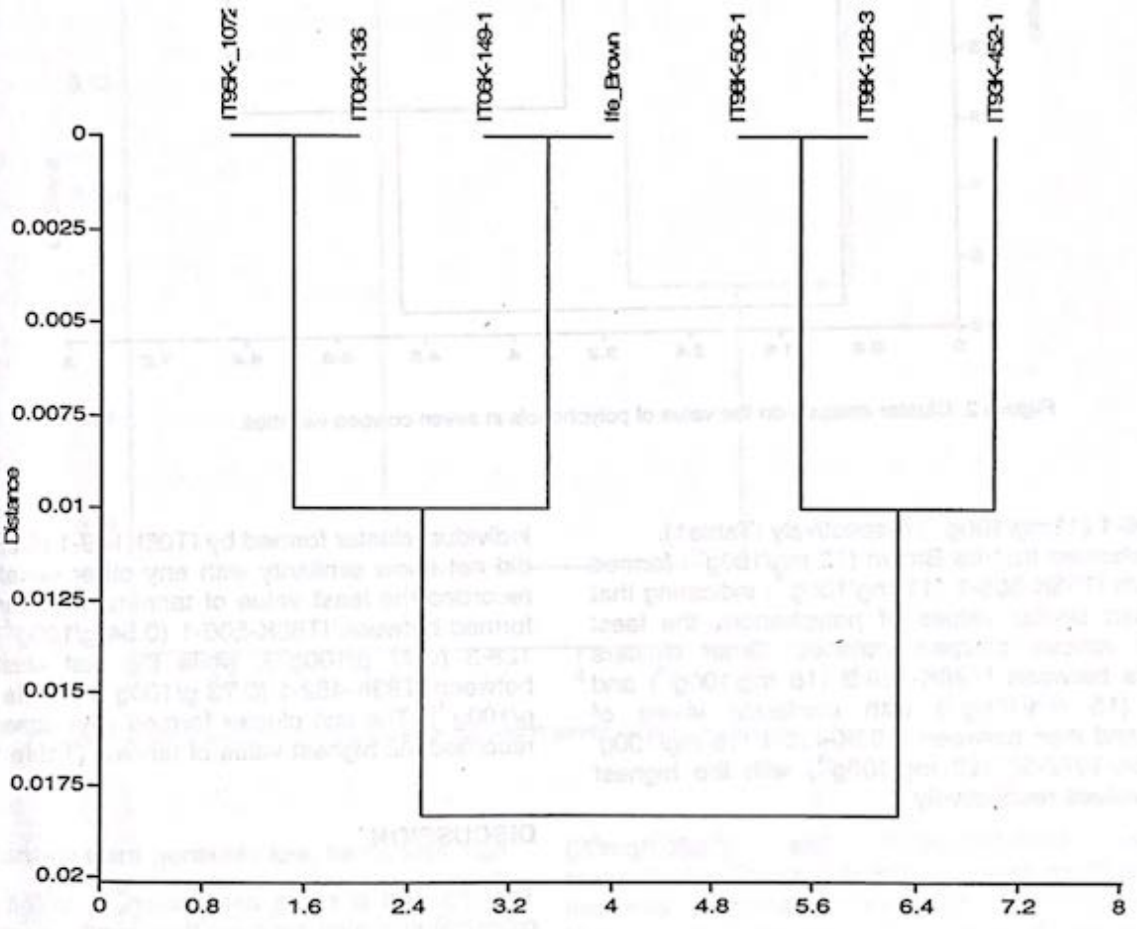

Figure 1. Cluster analysis on the value of phenols in seven cowpea varieties.

and IT06k-149-1 (0.05 g/100g^{-1}) with moderate value of phenol, as well as IT98K-506-1 (0.04 g/100g^{-1}) and IT98K-128-3 (0.04 g/100g^{-1}) with lower phenol values. Distantly apart from these clusters was an individual cluster formed by IT06K-149-1 (0.28 g/100g^{-1}) in which the least value of phenols was recorded. Cowpea varieties with low to moderate levels of phenols were preferred from the nutritional point of view.

Polyphenols

The principal polyphenol compound investigated in the study was myricetin. Its highest value of 24mg/100g^{-1} was recorded in IT06K-149-1, followed by IT95K-1072-57 (20mg/100^{g-1}) and IT93K-452-1 (18mg/100g^{-1}) respectively. It was progressively lower in IT06K-128-3 (16mg/100g^{-1}), IT06K-136 (15mg/100g^{-1}), Ife Brown (12 mg/100g^{-1}) and

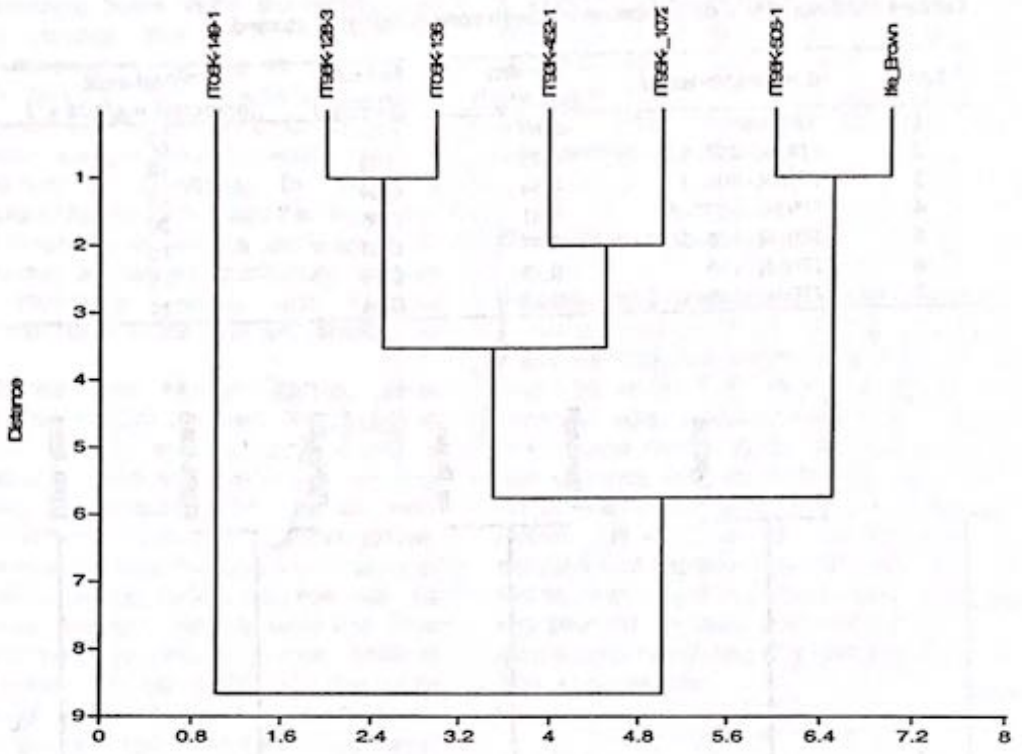

Figure 2. Cluster analysis on the value of polyphenols in seven cowpea varieties.

in IT98K-506-1 (11mg/100g^{-1}) respectively (Table1).

Figure 2 showed that Ife Brown (12 mg/100g^{-1}) formed a cluster with IT98K-506-1 (11 mg/100g^{-1}) indicating that they recorded similar values of polyphenols, the least among the various cowpea varieties. Other clusters formed were between IT98K-128-3 (16 mg/100g^{-1}) and IT06K-136 (15 mg/100g^{-1}) with moderate levels of polyphenol and then between IT93K-452-1 (18 mg/100g^{-1}) and IT95K-1072-57 (20 mg/100g^{-1}) with the highest polyphenol values respectively.

Tannins

The highest level of tannins was recorded in IT93K-452-1 (0.73g/100g^{-1}), followed by Ife Brown (0.67g/100g^{-1}). Lower tannin values were recorded for other cowpea varieties namely: - IT98K-506-1 (0.54g/100g^{-1}), IT06K-128-3 (0.47g/100^{-1}), IT06K-136 (0.33g/100^{-1}), IT95K-1072-57 (0.31g/100g^{-1}) and IT06K-149-1 (0.28g/100g^{-1}) respectively (Table 1).

From Figure 3, it was shown that IT95K-1072-57 (0.31g/100g^{-1}) and IT06K-136 (0.33 g/100g^{-1}) formed a cluster which indicated that they had similar values of tannins which was comparatively low among the various cowpea varieties studied. Distantly joined to this was an individual cluster formed by IT06K149-1 (0.28 g/100g^{-1}). It did not show similarity with any other variety because it recorded the least value of tannins. Another cluster was formed between IT98K-506-1 (0.54 g/100g^{-1}) and IT98K-128-3 (0.47 g/100g^{-1}), while the last cluster occurred between IT93K-452-1 (0.73 g/100g^{-1}) and Ife Brown (0.67 g/100g^{-1}). The last cluster formed was observed to have recorded the highest value of tannins (Table 1).

DISCUSSION

Tannins and phenols are known to inhibit activities of digestive enzymes because they combine with proteins to make the cowpea grains less digestible and unavailable to the body (Enwere, 1998). Consequently, the presence of even low levels of tannins and phenolics is not desirable from the nutritional point of view (Soris and Mohan, 2011). However, the soaking, dehulling and decanting of water is known to reduce tannins and phenolics significantly (Vadivel and Pugalenthi, 2008; Preet and Punia, 2000). Recently, phenolics have been suggested to exhibit health related functional properties such as anticarcinogenic, anti-inflammatory, antimicrobial, antioxidant, antiviral and hypotensive properties (Shetty, 1977, in Kalidass and Mohan, 2012).

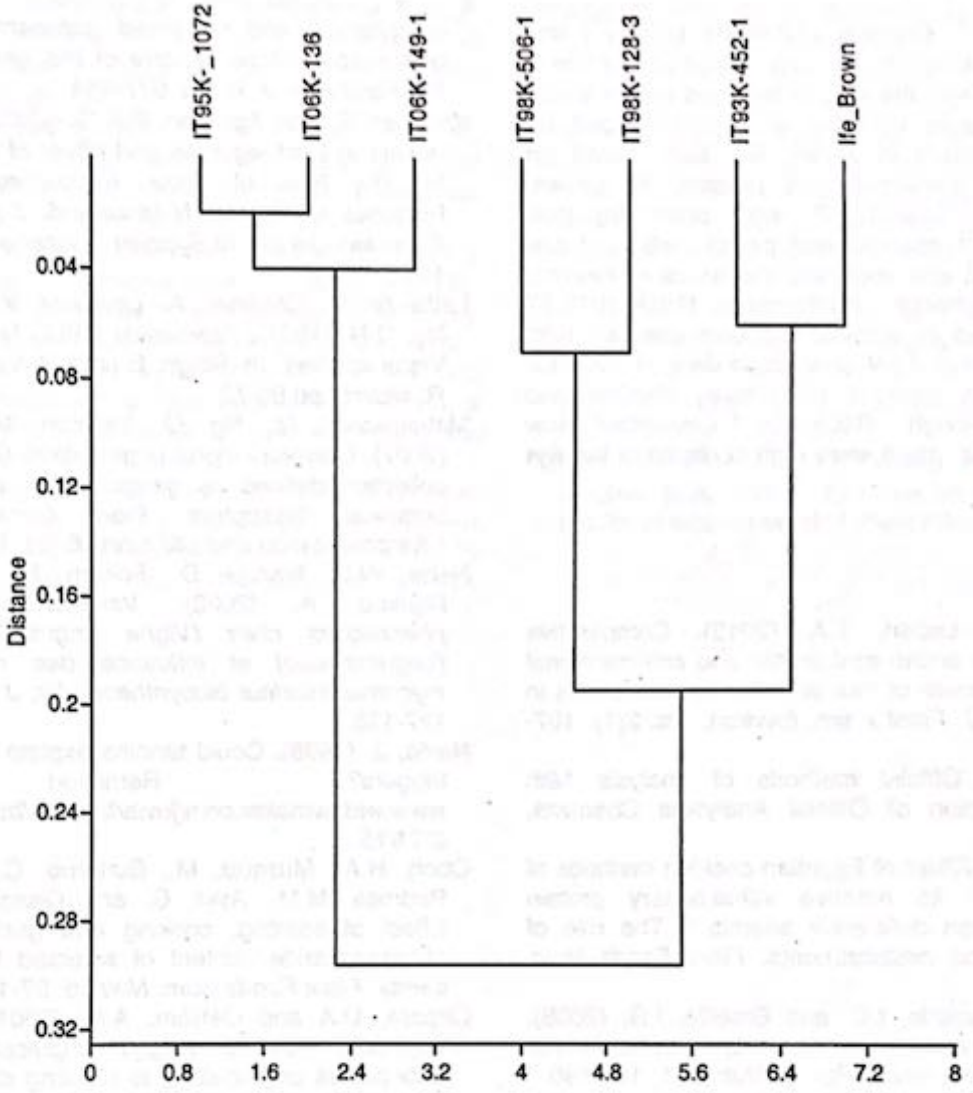

Figure 3. Cluster analysis on the value of tannins in seven cowpea varieties.

The values of tannins were generally low, being less than one gram in all the cowpea varieties studied. Comparatively among the cowpeas, very low tannin levels were recorded in IT95K-1072-57 (0.31g/100g^{-1}), IT06K-136 (0.33g/100g^{-1}) and IT06K149-1 (0.28g/100g^{-1}). It was also noted that phenols .were low in IT93K-452-1(0.03 g/100g^{-1}), IT98K-506-1(0.04 g/100g^{-1}) and IT98K-128-3 (0.47 g/100g^{-1}), but comparatively higher in Ife Brown (0.05), IT06K-149-1(0.05 g/100g^{-1}), IT95K-1072-57 (0.06 g/100g^{-1}) as well as IT06K-136 (0.06 g/100g^{-1}). Similar levels phenols have been recorded in cowpeas by Okwu and Orji (2007). Polyphenols were low in Ife Brown (12mg/100g^{-1}), IT98K-506-1(11mg/100g^{-1}), and moderate in both IT06K-136 (15mg/100g^{-1}) and IT98K-128-3(16mg/100g^{-1}). The results reported higher polyphenol values for IT93K-452-1 (18mg/100g^{-1}), IT95K-1072-57 (20mg/100g^{-1}), and IT06K-149-1(24 mg/100g^{-1}) respectively. These cowpea varieties could still be of immense nutritional benefits if the culinary procedures of Vadivel and Pugalenthi (2008), Preet and Punia (2000), who had suggested that the soaking, dehulling and decanting of water is known .to reduce tannins and phenolics significantly are followed. It is therefore recommended that farmers should cultivate these varieties with low anti nutrient content.

CONCLUSION AND RECOMMENDATIONS

The values of tannins were generally low, being less than one gram in all the cowpea varieties studied. The cowpea variety IT93K-452-1 had the highest value of tannin which

was 0.73 g/100g^{-1}. Phenols (0.03-0.06 g/100g^{-1}) and polyphenols (11-24mg/100g^{-1}) were comparatively low in all the samples. From the results reported in this study, the following cowpea varieties are recommended for cultivation by farmers in Agbor, Ika land based on contents of anti nutrient factors present. Ife Brown, IT98K-506-1 and IT98K-128-3, were noted for their moderate levels of phenols and polyphenols, but low tannins. IT06K-136 also recorded low levels of tannins, phenols and polyphenols. Furthermore, IT95K-1072-57 recorded low levels of tannins, but comparatively high contents of phenols and polyphenols, while in IT06K-149-1 there was high contents of tannins, phenols and polyphenols. Although IT93K-452-1 recorded low contents of phenols, there were high contents of tannins and polyphenols.

REFERENCES

Amata, I.A and Lebari, T.A. (2012). Comparative evaluation of the amino acid profile and anti-nutritional content of the leaves of four selected browse plants in the tropics. *Int. J. Plant Anim. Environ. Sci.* 2(1): 107-111.

A.O.A.C. (2005). Official methods of analysis 18th Edition, Association of Official Analytical Chemists, Washington D.C.

Bakr, A.A. (1996). Effect of Egyptian cooking methods of faba beans on its nutritive value,dietary protein utilization and iron deficiency anemia.1. The role of main technological pretreatments. *Plant Foods Hum. Nutr.* 49: 83-92.

Chinma, C.E., Alemede, I.C. and Emelife, I.G. (2008). Physiochemical and functional properties of some Nigerian cowpea varieties. *Pak. J. Nutr.,* 7(1):186-190.

Chang, M.J., Bailey, J.W and Collins, J.L. (1994). Dietary tannins from cowpeas and tea transiently alter apparent calcium absorption but not absorption and utilization of protein in rats. *J. Nutr.* 10: 283-288.

Djocgoue, P., Boudjeko, T., Mbouobda, D., Nankeu, I., Hadrami, El and Omokolo, N. (2007). Heritability of phenols in the resistance of *Theobroma cacao* against *Phytophthora megakarya,* the casual agent of black pod disease. *J. Phytopathol.* 155: 519-525.

Enwere, N.J. (1998). Foods of Plant Origin. 1stEdn. Afro-Obis Publ. Ltd., Nsukka, Nigeria. 301 p.

Fokom, R., Nana, Wakam L., Tchameni S and Nwaga, D (2010). Arbuscular Mycorrhizal Fungi (AMF) colonization and rhizobial nodulation of cowpea as affected by flavonoids application. *Res. J. Agric. Biol. Sci.* 6(6): 1015-1021.

Herbs (2000). Tannins. A nutritional guide for nutritional and traditional health. Retrieved from *www.herbs2000.com/h-menu/tannins.htm*

Kalidass, C. and Mohan, V.R. (2012). Biochemical composition and nutritional assessment of selected under-utilized food legume of the genus *Rhynchosia. Int. Food Res. J.* 19 (3): 977-984.

Khokhar, S. and Apenten, R.K.O. (2003). Antinutritional factors in food legumes and effect of food processing. In. The Role of Food, Agriculture, Forestry and Fisheries in Human Nutrition.(ed. Squires, Victor R) *Encyclopedia of Life Support Systems* (EOLSS) pp 82-116.

Lattanzio, V., Cardinali, A., Linsalata, V., Perrino, P and Ng, Q.N. (1997). Flavonoids HPLC finger print of wild Vigna species. *In.* Singh, B (ed): *Advances in Cowpea Research* .pp 66-73.

Mahalakshmi, V., Ng, Q., Lawson, M and Ortiz, R. (2007). Cowpea (*Vigna unguiculata*) (L.) Walp.) : Core collection defined by geographical, agronomical and botanical descriptors. *Plant Genetic Resources: Characterization and Utilization* 5 (3): 113-119.

Nana, W.L., Nwaga, D., Fokom, R., Oneya, S and Ngakou, A. (2002). *Variation des composes phenoliques chez (Vigna unguiculata* (L.) Walp. *(Legumineuse) et influence des rhizobia et des mycorhizessurleur biosynthese. Afr. J. Sci. Technol.* 3: 127-135.

Navia, J. (1998). Could tannins explain classic migraine triggers? Retrieved from *www.widowmaker.com/juvia/tannins/tannexpl.htm on 3/11/15.*

Oboh, H.A., Muzquiz, M., Burbano, C., Cuadrado, C., Pedrosa, M.M., Ayet, G. and Osagie, A.U. (2000). Effect of soaking, cooking and germination on the oligosaccharide content of selected Nigerian legume seeds. *Plant Foods Hum. Nutr.*55: 97-110.

Okpara, D.A and Oshilim, A.F. (2001). Response of vegetable cowpea *(Vigna unguiculata* [L.] Walp. Subspecies *unguiculata*) to planting date and fertilizer nitrogen in the humid tropics. *J. Applied Chem. Agric. Res.,* 7: 95-103.

Okwu, D.E and Orji, B.O. (2007). Phytochemical composition and nutritional quality of *Glycine max* and *Vigna unguiculata* [L.] Walp. *Am. J. Food Technol.* 2: 512-520.

Pandey, K.B. and Rizvi, S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity.* 2 (5) 270 - 278.

Patte, H.E. (1985) (ed.). *Evaluation of Quality of Fruits and Vegetables.* AVI Publishing, Westport, CT. pp 315 -328.

Preet, K. and Punia, D. (2000). Antinutrients and digestibility (*in vitro*) of soaked, dehulled and germinated cowpeas. *Nutr. Health,* 14: 109-117.

Price, M.L., Van Scooc, S. and Buttler, L.C. (1978). A critical evaluation of the vanillin reaction as an assay for tannins and sorghum grain. *J. Agric. Food Chem.,* 26: 12-14.

Ramakrishna, V., Jhansirani, P and Ramakrishna Rao, P. (2006). Anti-nutritional factors during germination in Indian bean (*Doliches lablab* L.) seeds. *World J. Dairy Food Sci.* 1 (1): 06-11.

Salunkhe, D.K., Jadhar, S.J., Kadam, S.S and Chavan, J.K. (1982). Chemical, biochemical, and biological significance of polyphenols in cereals and legumes. *CRC Crit. Rev. Food Sci. Nutr.* 17: 277-305.

Singh, B.B., Cambliss, O.I. and Sharma, B. (1997). Recent advances in cowpea breeding. In: Singh, B.B., Mohan Raj, D.R., Dashiell, K. and Jackai, L.E.N. (eds.). *Advances in cowpea research.* Ibadan. IITA. pp 30-49.

Soris, P.T and Mohan, V.M. (2011). Chamical and nutritional assessment of two less known pulses of genus *Vigna. Tropical and Subtropical Agroecosystems.* 14: 473-484.

Suarez, F.L., John, S., Julie Furne, K., Troy, T.L., Kerr, P.S. and Levitt, M.D. (1999). Gas production in humans ingesting soybean flour derived from beans naturally low in oligosaccharides. *Am. J. Clin. Nutr.,* 69: 135-139.

Urbano, G., Aranda, P., Goo'mez-Villalba, E., Freijnagel, S., Porres, J.M and Frias, J. (2003). Nutritional evaluation of pea (*Pisum sativum* L.) protein diets alter mild hydrothermal treatment and with and without added phytase. *J. Agric. Food Chem.,* 51: 2415-2420.

Vijayakumari, K., Siddhuraju, P. and Janardhanan, K. (1996). Effects of different post-harvest treatments on anti-nutritional factors in seeds of the tribal pulse, *Mucuna pruriens (L.)* DC. *Int. J. Food Sci. Nutr.* 47: 263-272.

Vadivel, V and Pugalenthi, M. (2008). Removal of antinutritional / toxic substances and improvement in the protein digestibility of velvet bean (*Mucuna pruriens)* seeds during processing. *J. Food Sci. Technol.* 45(3): 242-246.

Wang, T.L., Domoney, C., Hedley, C.L., Casey, R. and Grusak, M.A. (2003). Can we improve the nutritional quality of legume seeds? *Plant Physiol.*, 131: 886-891.