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EFFECTS OF PROCESSING METHODS ON ANTINUTRITIONAL CONTENTS OF SANDBOX (*Hura crepitans*) SEED PROTEIN CONCENTRATE AND ISOLATE FOR ECONOMIC DEVELOPMENT

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Abstract

The antinutritional contents of protein concentrate and isolate produced from sandbox (Hura crepitans) as influenced by different processing methods were studied with a view to increasing the utilization of the seed as food and/or feed ingredient, coupled with continuous increases in prices of staple animal protein which encouraged sourcing for substitute protein nourishment from the plant origin. Though, the aftermath lethal effect of consuming some of these plants has led them to being neglected and under-utilized, however for economic advantages, processing these ranges of protein-rich plants into flours and protein products is a good step in the right direction. The effects of different processing methods on the antinutritional content were investigated in this study. Whole sandbox seeds were collected, decorticated and divided into three portions. Samples of defatted flours, protein concentrate and protein isolate were prepared from sandbox seed using standard processing procedures of cooking, soaking, fermenting, defatting, solubilization and lypholization to significantly ($p<0.05$) reduced certain anti-nutrient contents (tannin, saponin and oxalate). The results shows sandbox seed protein concentrate (1.02 mg/100 g, 0.11 mg/100 g and 1.47 mg/100 g) and protein isolate (0.07 mg/100 g, 0.04 mg/100 g and 1.04 mg/100 g) and had the least and nutritionally stable values for the antinutrient contents amongst other samples for tannin, saponin and oxalate, respectively. Based on the findings of this study, sandbox seed protein products can be utilized as an alternative protein source in human food as well as in the fortification of starchy livestock feedstuff.

Key words: *sandbox, antinutrients, concentrate, isolate, economic development.*

INTRODUCTION

One of the most deleterious challenges facing developing tropical countries is scarcity of food coupled with the unprecedented rise in human population and the alarming drop in food production particularly in the last decade (FAO, 2010). The continual search for readily available alternative sources of foods had been inevitably embarked upon, has population growth increased despite the alarming economic crisis which threatens world health and welfare (Fowomola and Akindahunsi, 2010). The widening gap between estimated protein requirement and actual protein consumption in many developing tropical countries including Nigeria is as a result of lack of basic information and or improper harnessing of available and abundant non-conventional functional feed which has limited their usage in balanced and economical rations for fishes and livestock (FAO, 2010). Today, most people cannot afford the marked prices of preferred protein sources for animals and aquatic. The documented report of Aletor and Aladetimi (1989) amongst several other facts not published; provided information on the nutritional potentials of some locally available under-utilized leguminous plant seeds, also Fowomola and Akindahunsi (2008) reported on the nutritional quality of sandbox tree, an under-utilized plant in Nigeria, but the occurrences of anti-nutritional factors had aborted or rather limited the utility of most these plants with food potentials.

It is noteworthy, scientifically, that the balance between nutrients and anti-nutrients in some plant foods can only be evaluate when the concentrations of anti-nutritional factors (AFNs) are known, as it had been reviewed and documented that the toxicities of some ANFs are either unaffected or minimally removed by some treatments employed in food processing (Souci et al., 2000; Aberoumand and Deokule, 2009). Although, these treatments (including soaking, dehulling, sprouting, ordinary cooking, pressure cooking and fermentation) are expected to improve palatability and give nutritional benefits, however verified investigations revealed that the content, physicochemical and functional properties of food plant samples are altered, depleted and/or rather absent (Rao and Deosthale, 1982; Siljestrom et al., 1986).

Further improvements on the nutritional quality and effective utilization of some legumes, oil seeds and some under-utilized seeds to their full potential, as food has been gaining prospects in research and development by adopting economically sustainable processing methods in the inactivation and removal of anti-nutritional factors using less expensive, physical and biochemical techniques including soaking, cooking, selective filtration, irradiation, enzymatic treatments, germination and fermentation (Ghadge et al., 2008). It is therefore worthwhile to ensure that the processing methods employed do not destroy the functionality of ingredients in food plants.

Cooking as a simple and common domestic practice inactivates heat sensitive anti-nutritive factors such as trypsin and chymotrypsin inhibitors and other volatile compounds. The process of decanting or discarding cooking water may be necessitated for, but some other vital soluble compounds would be loosed together with. Carlini and Udedibie (1997) report revealed that boiling jackbean for 2 h completely eliminated trypsin inhibitor activity in, while a further boiling for 3 h totally removed lectin from jackbean.

Soaking as a traditionally employed method in food processing is about the most simplified technique to eliminate soluble anti-nutritional factors, however, a study documented that some metabolic reactions takes place during soaking which affects some of the rheological and functional compounds (Vidal-Valverde et al., 1992).

Fermentation which has been widely used was documented to improve the nutritional quality of *Hura crepitans* seeds by increasing the concentrations of the essential amino acids and vitamins, while reducing the anti-nutrients levels concentrations of alkaloids, oxalate, saponins, tannins, phytate and cyanide significantly, thereby improving the protein digestibility was reported by Fowomola and Akindahunsi (2008).

A number of chemical treatments had also been employed with the sole aim to improve the nutritional significance of legumes, oil seeds and other under-utilized plants with food potentials. Ologhobo et al. (1993) reports on the extraction of jackbean flour using selected solvents resulted in removal of portions of varying toxicities, based on the solubility (or otherwise) attributes in the extracting medium. It was further reported that higher concentrations of anti-nutritional factors in the base-soluble portion than in the other portions, indicating a better extraction ability of anti-nutritional factors by alkali rather than by acid, ether or alcohol. An archive report of D'Mello and Walker (1991) also achieved substantial results in extraction employing potassium bicarbonate (a base) to inactivate ANFs in jackbeans.

Sandbox (*Hura crepitans*) is a promising seed its high protein and oil contents (Abdulkadir et al., 2013). Research efforts on sandbox (*Hura crepitans*) seeds have focused majorly on its protein quality, nutritional, toxicological, effects of fermentation on anti-nutrients and nutrients contents, and anti-microbial studies (Fowomola and Akindahunsi, 2005; Fowomola, 2006; Fowomola and Akindahunsi, 2008).

The production of flours and protein products from sandbox (*Hura crepitans*) seeds devoid of any anti-nutrients will be a step in the right direction and for this study, simple, economical and feasible methods were employed with the purpose of removing and/or reducing the anti-nutritional substances to the minimum level Generally Recognized As Safe (GRAS) by combination cooking, soaking, fermenting, defatting, solubilizing and lypholizing (freeze drying).

MATERIALS AND METHODS

Sample collection

Dried sandbox (*Hura crepitans*) pods were gathered from sandbox trees at Parks and Gardens in Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The whole seeds were broken out of the pods, moistened with tap water in order to soften the shell and decorticated manually, air dried and stored in tight moisture-free polythene bags at -10°C for further use. All chemicals used were of analytical grades and were acquired from Fisher Scientific (Oakville, ON, Canada) and Sigma-Aldrich (St. Louis, MO, USA).

Preparation of untreated and treated flours

The seeds were further divided into three portions and subjected to different processing treatments. The first portion was kept without processing (untreated), while the second and third portions were prepared using slightly modified method of Ayanwale and Kolo (2001), and a combination of Nwosu (2010) and Ayanwale and Kolo (2001), respectively. While the second portion of seeds was cooked (100±2°C) with water (1:3 w/v) at atmospheric pressure for 2 h, the third portion was soaked in water (1:5 w/v) in *lieu* of cooking for 24 h. The second and third portions were drained and transferred into a calabash, uniformed lined with clean plantain leaves (up to 5 layers) and allowed to undergo fermentation for 72 h in the incubator (Memmert, IN30, Germany). All samples were dried at 50°C in a hot air oven (Gallenkamp oven, OVL570 010J, United Kingdom) for 12 h, cooled, ground in a warring blender (Binatone, BLG-450, China) set at high speed to obtain homogenous flours of untreated sandbox flour (USF), cooked fermented sandbox flour (CFS), and soaked fermented sandbox flour (SFS).

Preparation of defatted flour

Defatted untreated sandbox flour (DUS), defatted cooked fermented sandbox flour (DCF), and defatted soaked fermented sandbox flour (DSF) were prepared using a modified method of Sathe (1994). The full-fat flour was defatted using cold (4°C) acetone (flour to solvent ratio 1:5 w/v) with constant stirring for 4 h with a magnetic stirrer (Compact Magnetic Stirrer, United Kingdom). The defatted flour was placed inside a fume cupboard for 6 h to dry and to remove any trace of residual acetone. The flakes were then ground again and sieved (Endecotts sieve, United Kingdom) through a sieve size mesh of 150 μm to obtain fine powder, packed in plastic tubes and stored at -10°C.

Preparation of protein concentrate

Sandbox protein concentrate (SPC) was prepared as described by Cheftel et al. (1985) from defatted soaked fermented sandbox flour (DSF).

Preparation of protein isolate

The method described by Chavan et al. (2001) was adopted to prepare sandbox protein isolate (SPI) from defatted soaked fermented sandbox flour (DSF).

Determination of tannin

Tannin content of sandbox seed flours and proteins were determined employing the spectrophotometric procedure described by Bainbridge et al. (1996).

Determination of saponin

The solvent extraction gravimetric method was employed in the saponin content determination of the sandbox seed flours and proteins (Harborne, 1973).

Determination of oxalate

Oxalate contents of sandbox seed flours and proteins were determined by the method of Munro and Bassir (1969).

RESULTS AND DISCUSSIONS

The results of the anti-nutrient contents (tannin, saponin and oxalate) of the examined samples are shown in Table 1. The values varied between 0.07-1.85 mg/100 g, 0.04-0.23 mg/100 g, and 1.04-25.57 mg/100 g for tannin, saponin and oxalate, respectively.

Table 1. Anti-nutrient levels of sandbox seed flours and protein products

Sample	Anti-nutrient levels (mg/100 g)		
	TANNINS	SAPONINS	OXALATE
USF	1.85	0.23	25.57
DUS	1.75	0.2	8.35
CFS	1.54	0.16	18.37
DCF	1.36	0.14	7.78
SFS	1.77	0.19	24.48
DSF	1.51	0.16	7.91
SPC	1.02	0.11	1.47
SPI	0.07	0.04	1.04

USF: Untreated sandbox flour; DUS: Defatted untreated sandbox flour; CFS: Cooked fermented sandbox flour; DCF: Defatted Cooked fermented sandbox flour; SFS: Soaked fermented sandbox flour; DSF: Defatted Soaked fermented sandbox flour; SPC: Sandbox Protein Concentrate; SPI: Sandbox Protein Isolate

Cooking, soaking, fermentation, defatting, solubilizing and lypholizing were employed as processing techniques to reduce anti-nutrients in the samples. Phillips and Abbey (1989) earlier reported that the soaking of seeds induced the leaching out of water soluble anti-nutrients such as glycoside, alkaloids, phytates, oligosaccharides and tannins, thus made soaking successful for the removal of the soluble anti-nutrients in the sandbox seed flours and proteins.

Cooking has been reported to be effective in reducing most of the anti-nutrients also including inactivating protease inhibitors and reducing trypsin inhibitor activity and phytic acid content (Wang et al., 1997), hence making sandbox seed flours and proteins adequately free. Enechi and Odonwodu (2003) also revealed that the effect of cooking was significant in the reduction of the toxic effects of oxalate, phytate and tannin.

This study also revealed that the fermentation significantly reduced the levels of tannin, saponin and oxalate from 1.85 to 1.54 mg/100 g, 0.23 to 0.16 mg/100 g and 25.57 to 18.37 mg/100 g, respectively. These result compared favourably with the work of Fowomola and Akindahunsi (2008) who reported that fermentation significantly reduced the toxic levels of anti-nutrients (alkaloids 25.6 ± 0.2- 5.0 ± 0.3, tannins 18.6 ± 1.5-5.8 ± 1.3, saponins 8.5 ± 0.1-1.4 ± 0.1, oxalate 23.7 ± 0.25-3.6 ± 0.12, phytate 18.1 ± 0.31-7.8 ± 0.13 and cyanide 1.66 ± 0.1-0.6 ± 0.03 mg/100 g) present in sandbox seed, and also with the report of Ibukun and Anyasi (2012) which revealed reductions in the oxalate, tannin and saponin levels of fermented samples of sesame (2.57 to 0.36 mg/g, 0.019 to 0.008 mg/g and 2.68 to 1.01 mg/g respectively), musk melon (2.1 to 0.27 mg/g, 0.007 to 0.004 mg/g and 5.1 to 2.8 mg/g, respectively) and white melon (1.35 to 0.14 mg/g, 0.008 to 0.005 mg/g and 3.5 to 1.9 mg/g, respectively). Mubarak (2005) reported that the activities of indigenous enzymes as well as processing techniques employed could have been responsible for degrading the anti-nutrients.

The report of Enujiugha and Agbede (2000) revealed that tannin usually forms insoluble complexes with proteins, thereby interfering with their bioavailability and poor palatability had been generally attributed to high tannin contents in diets. Baumann et al. (2000) reported that saponin causes hemolytic activity by reacting with the sterols of erythrocyte membrane.

The report of Soetan and Oyewole (2009) revealed that oxalates and phytates, bind to minerals like calcium and magnesium and interfere with their metabolism, which leads to muscular weakness and paralysis.

According to Makkav and Becker (2000), the combined effect of soaking, cooking and fermentation processes exhibited significance in reducing most of the anti-nutrients in plant food to a minimal level Generally Recognized As Safe (GRAS) that may have less or no adverse effects on human and animals when ingested.

The defatting and solubilization effects however emphasized a remarkable reduction in the anti-nutrient contents of the full fat flours compared to the final values in defatted flours and its proteins.

CONCLUSIONS

The novelty of sandbox seed to be considered as edible food by man and animals is steadily gaining acceptability through ongoing further research and development.

The need to focus on other advanced scientific methods to further eliminate the hindering anti-nutritional factors in sandbox seeds, which had caused its neglect due to the lethal and harmful effects associated to the consumption of the untreated whole seed, gathered from previous reports resulting to partial blindness, nausea, vomiting, purging, burning in the throat, suffocating and headache which could have further limit the utility of the high protein content and the nutritional value it projects to confer in the diets of man and animal.

The results of this study however revealed that soaking, cooking, fermenting and defatting, together with solubilizing could be employed independently, but preferably in combination for the processing of sandbox seed to significantly reduce the anti-nutrients to the minimum level Generally Recognized As Safe (GRAS) and improve its utility for further food processing.

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