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Assessment of Some Anti-Nutritional Factors in Extruded Soybean and Aerial Yam Flour Blend

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Abstract

This study assesses the levels of some anti-nutritional factors (ANFs) in extrudates produced from the blend of soybean and aerial yam flours. The soybean and aerial yam flours were blended in the ratio of 3:1 (75% soybean flour, 25% aerial yam flour). The blended soybean and aerial yam flours was then extruded using a laboratory scale single-screw extruder at 33% feed moisture content; 110 °C barrel temperature and 130 rpm screw speed. The results of the laboratory analysis of the extrudates shows that the levels of anti-nutritional factors were 73.80 mg/100g of sample for oxalates; 1.81 mg/100g of sample for tannin; 25.39 mg/100g of sample for phytate; 2.22 mg/100g of sample for hydrogen cyanide (HCN); and 1.64% for alkaloids. This study has shown that the levels of the anti-nutritional factors in the extrudates produced from soybean and aerial yam flours blend are minimal, and are below the FAO/WHO recommended safe level of toxicity to human, and these anti-nutritional factors can be reduced to the level of non-toxicity, through extrusion processing technology.

Keywords: Anti-Nutritional Factors, Extrusion Processing, Single-Screw Extruder, Aerial Yam, Oxalate

Introduction

Anti-nutritional factors (ANFs) are biological components present in foods/food materials that can reduce nutrient utilization or food uptake, which leads to impaired gastrointestinal functions and metabolic performance. They exhibit outright anti-nutritional properties, and their presence in their native state cause acute or chronic toxicity after foods containing are ingested.

According to Iwe (2003), food extrusion has been practiced for more than sixty years. Extrusion cooking is an important and popular food processing technique classified as a high temperature/short time (HTST) process to produce fibre-rich products (Gaossong and Vasanthan, 2000). It is a process in which moistened, expansive, starchy and/or proteinacious food materials are plasticized and cooked in a tube by a combination of moisture, pressure, temperature and mechanical shear, resulting in molecular transformation and chemical reactions (Castells *et al.*, 2005). The process has found numerous applications, including increasing numbers of ready-to-eat (RTE) cereals; salty

and sweet snacks; co-extruded snacks; indirect expanded products; croutons for soups and salads; an expanding array of dry pet foods and fish foods; textured meat-like materials from defatted high-protein flours; nutritious precooked food mixtures for infants feeding; and confectionery products (Eastman *et al*., 2001).
According to Mukhopadhyay and Bandyopadhyay (2003), a single screw cooking extruder was used to reduce tannin in sesame oilseed meal. The process variables selected for the study were: extruder screw speed (63.18 to 96.80 rev min^{-1}); barrel temperature (63.18 to 96.80°C) and moisture content of raw oilseed meal (31.59 to 48.41%). Extrusion cooking technology is very effective in reducing the anti-nutritional factor, tannin from sesame meal (Mukhopadhyay and Bandyopadhyay, 2003).
Ajita and Jha (2017) reported that extrusion cooking also improves the nutritional quality of foods by destroying many natural toxins and anti-nutrients. A dilemma exists as to whether it is desirable to remove these compounds. Anuonye *et al*. (2012) reported a 78.18% reduction in the hydrogen cyanide (HCN) content of extruded pigeon pea and unripe plantain blends, as a result of extrusion processing. According to Arun kumar *et al*. (2018), extrusion process reduced the phytic acid content of sorghum-soya blends up to 57.48% as reflected by its lower content in the final product, compared to that in the feed material. The researchers inferred that the reduction in phytic acid content followed by extrusion might be due to the hydrolyzation of inositol into lower molecular weight forms like penta-, tetra and triphosphates.
El-Hady *et al*. (2003) also reported that higher extrusion temperature was more effective in reducing phytic acid in faba beans, chick peas, peas and kidney beans. Increase in feed moisture content has also been reported to result in proportional increase in phytic acid content. In other words, moisture arrests the destruction of phytic acid during extrusion (Arun kumar *et al*., 2018). Omosebi *et al*. (2018) reported that the extrusion process brings about significant reduction in the phytic acid content of extruded complementary diet from quality protein maize and soybean protein concentrate. It has been reported that though extrusion processing could not completely eliminate tannin, a considerable reduction is observed depending upon the process variables (Arun kumar *et al*., 2018). Negative influence of extrusion on tannin content has been reported to be attributed to the thermal degradation of molecules, changes in their chemical reactivity or the formation of insoluble complexes during the course of extrusion.

Problem Statement

There are some biological components that are present in foods/food materials that can reduce nutrients utilization or food uptake. This ultimately leads to impaired gastrointestinal functions and metabolic performance. This research aims to assess and identify the levels of some of these biological components, otherwise known as, anti-nutritional factors, in extruded food materials, with emphasis on ascertaining the wholesomeness or the extent of toxicity of the food material.

Limitation of the Study

This study is limited to the assessment of the levels of some anti-nutritional factors in extrudates produced from aerial yam and soybean flours blend. Future research in the area of other quality attributes of the extrudates is hereby suggested.

Research Gap

The production of food materials, with a minimal level of toxicity from the biological components (anti-nutritional factors) has been a thing of great concern to food processors. These biological components exhibit outright anti-nutritional properties and their presence in their native state cause acute or chronic toxicity after foods containing them are ingested. This present study attempts to bridge the gap and conceptualize an approach towards producing extruded food materials that contain minimal levels of anti-nutrients which can reduce nutrients utilization.

Blending of aerial yam and soybean, and or extrusion cooking of the blend, has not been adequately studied for its potential application in food products formulation. Processing it into stable blend, and subsequent extrusion processing to producing different wholesome products with minimal levels of anti-nutrients, will increase the visibility of the crop in food trade, thereby bringing to limelight its potential food uses to the food industry (Umoh and Iwe, 2022). In addition, the application of known methods of food processing, like extrusion cooking, in the utilization of aerial yam (*Dioscorea bulbifera*) would mean introducing new food processing technology and food products, thereby offering variety to consumers.

The aim/objective of this study is to assess the levels of some anti-nutritional factors contained in extrudates produced from the blend of aerial yam and soybean flours.

Materials and Methods

Collection of Soybean Seeds and Aerial Yam Bulbs/Sample Preparation

Soybean seeds and aerial yam bulbs used in this study were purchased from Uyo Urban market in Uyo Local Government Area, Akwa Ibom State, Nigeria.

The flour samples used in this research were prepared in the Food Processing Laboratory, Department of Food Science and Technology, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Preparation of Aerial Yam Flour

Aerial yam flour was prepared according to the method described by Umoh *et al*. (2021).The aerial yam bulbs (Plate 1) were cleaned and sorted to remove unwanted materials, before peeling with knife, washed with clean water and sliced to 10mm thickness using kitchen knife. The slices (chips) were then dried, using an oven at a temperature of 60 °C for 12 h. The dried slices (chips) were then milled using MF120 Hammer mill made in Italy, and sieved with laboratory sieve of 600μm aperture size. The flour obtained, shown in Plate 2, was packaged in a polyethene bag for subsequent use.

Plate 1: Photograph of Aerial yam bulbs

Plate 2: Photograph of processed Aerial yam flour

Preparation of Soybean Flour

Soybean flour was prepared according to the method described by Umoh *et al*. (2021). Seeds were screened to remove foreign materials, splits, and damaged beans. This was followed by washing and roll boiling at 100 °C for 30 minutes. It was then oven -dried at a temperature of 70 °C for 12h, and milled in a disc attrition mill. The milled full-fat soybean was sieved using a 100-mesh standard sieve. The flour obtained (Plate 3) was then stored in air- tight polyethene bag at room temperature for further use.

Plate 3: Photograph of processed full-fat soybean flour

Plate 4: Photograph of extrudate sample of aerial yam-soybean flour blend

Preparation of Sample Blend

The aerial yam–soybean flour blend was prepared in the ratio of 25:75 (1:3), expressed in percentage as 25% aerial yam flour and 75% soybean flour.

Extrusion Cooking

Extrusion cooking was carried out using a single-screw laboratory scale extruder in the Department of Food Science and Technology Laboratory, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Two hundred grams (200 g) of the flour blend (25% aerial yam flour, 75% soybean flour) was accurately measured and preconditioned to the desired moisture level of 33% (Strahm, 2000). The extruder was switched-on, the barrel temperature and screw speed of 110 °C and 130 rpm, respectively were set accordingly, and the raw material was fed, through the hopper, into the extruder. The extrudates were collected as they exit through the die (Plate 4), oven-dried, and packaged in air tight zip lock polyethylene bags for further laboratory analysis.

Determination of Anti-Nutritional Factors

The following anti-nutritional factors of extruded aerial yam and soybean flour blend were determined:

Oxalates

Determination of Oxalate which involves three major steps (digestion, oxalate precipitation and permanganate titration) was carried out according to the method described by Umoh *et al.* (2021).

Digestion:

Two and half grams (2.5g) of the sample was mixed with 95ml distilled water and 5ml 6N HCl in a 250ml beaker. The mixture was heated at 50°C for 2h using water bath, filtered and diluted to 125ml with distilled water.

Oxalate precipitation:

Four (4) drops of methyl red indicator was added to 50ml of the filtrate in a 100ml beaker, evaporated to 25ml volume, and filtered. The filtrate was treated with 5ml concentrated. NH_4OH, heated again to 90°C, and 10ml 5% $CaCl_2$ solution added with constant stirring. It was then cooled and left overnight at 5°C, centrifuged at 2500rpm for 5 minutes, supernatant decanted and the precipitate obtained, washed with 10ml 20% (v/v) H_2SO_4 solution and total volume diluted to 125ml distilled water.

Permanganate titration:

Aliquots of 125ml of the solution was heated to 90°C, titrated against 0.05N $KMnO_4$ solution to a faint pink colour, and the calcium oxalate content calculated thus: 10ml of 0.05N $KMnO_4$ = 2.2 mg oxalate.

Tannin

The Folin-Dennis spectrophotometric method as described by Umoh *et al.* (2021) was used in the determination of tannin. One gram (1g) of the sample was dispersed in 10ml distilled water, agitated and allowed to stand for 30 minutes at room temperature (23 °C), then centrifuged. Two and half millilitres (2.5 ml) of the supernatant, 2.5 ml of tannic acid solution were dispersed into a 50ml flask respectively. One millilitre (1 ml) of Folin Dennis reagent was added to each flask, followed by 2.5ml of saturated Na_2CO_3 solution. The mixture was then diluted to 50ml mark, and incubated for 90 minutes at room temperature.

The absorbance was measured at 250nm in a BENWAY Model 6000 electronic spectrophotometer. The tannin content was calculated using equation 2.1.

Tannin (%) = $\frac{An}{As} \times C \times \frac{100}{W} \times \frac{Vf}{Va}$ 2.1

Where:

An = Absorbance of test sample

As = Absorbance of standard solution

C = concentration of standard solution

W = weight of Sample

Vf = total volume of extract

Va = volume of extract analyzed

Phytic Acid (Phytate)

This was determined according to the method of Mecance and Widdowson as described by Umoh *et al.* (2021). Two and half grams (2.5 g) of the sample was extracted with 50 ml 3% TCA for 30 minutes, centrifuged and transferred into a 40 ml conical flask. Four millilitres (4 ml) of $FeCl_3$ solution was added, heated in a boiling water bath for 45 minutes, centrifuged and carefully decanted. The precipitate was washed with 20 ml 3% TCA, heated and centrifuged. Then dispersed in a few ml of water and 3 ml of 1.5 M NaOH added with mixing. The volume was brought to 30 ml with water, heated for 30 minutes, centrifuged and carefully decanted.

The precipitate was washed again with hot water, re-centrifuged and decanted. Then dissolved with hot 40 ml 3.2M HNO_3, and transferred into a 100 ml standard flask, cooled to room temperature and diluted to volume with distilled water. The iron (Fe) of the solution was determined, with an assumed 4:6 iron-phosphorus molecular ratio.

Calculation:

Determined Fe in the sample $= Xmg$

Convert Xmg to $mmole\ X = 5mmole = 55.85(mol$ Fe)

Therefore, phosphorus, P = $^{6}/_{4} \times X = 4mmole$ of P

Phytic acid, P.A $= 660.80 \times Y = Zmmole$ P.A

Where: 660.80 is the molar weight of P.A, Y is 6 $\times$ $molecula\ mass\ of\ P$

$\therefore mg$ P.A= $Z \times 660.80$, i.e,

Phytic acid $(mg/100g) = Zmmole \times molecular\ weight\ of\ P.A$

Hydrogencyanide (Cyanogenic Glycosides)

This was determined using the alkaline picrate method- by spectrophotometer as described by Umoh *et al*. (2021). Five grams (5 g) of the sample was made into paste. The paste was then dissolved in 50 ml distilled water in a conical flask and corked. The extraction process was allowed to stay overnight, then filtered. The filtrate was then used for cyanide determination. To 1ml of the filtrate in a corked test tube, 4 ml of alkaline picrate was added and incubated for 5 minutes in a water bath. After a reddish brown colour development, absorbance was read in the spectrophotometer at 490 nm.

The absorbance of the blank containing 1ml distilled water and 4 ml alkaline picrate solution was also read. The cyanide content was then extrapolated from the cyanide standard curve, and expressed as *mgHCN equivalent/100g* of sample.

Alkaloids

The gravimetric method of Harbone (1973) was used in the determination of alkaloids content in the flour sample. Five grams (5 g) of the sample was weighed into a 100 ml beaker; 50 ml of 10% acetic acid solution in ethanol will be added and stirred.

This was allowed to stand for 4 hours, filtered and the filtrate evaporated to ¼ of its original volume, and concentrated NH_4OH will be added drop wise to precipitate the alkaloids. The precipitate was filtered off using a weighed filter paper (W_1) and washed with 1% NH_4OH solution. The precipitate in the filter paper was oven dried at 60 °C for 30 minutes and weighed (W_2). The weight of alkaloid was determined by weight difference expressed as a percentage of the sample weight analyzed, using equation 2.2.

$$\text{Alkaloid (\%)} = \frac{W_2 - W_1}{W} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots \quad 2.2$$

Where:

w = weight of sample

w_1 = weight of empty filter paper

w_2 = weight of filter paper + precipitate.

Results and Discussion

The results of the anti-nutritional factors of extruded aerial yam and soybean flour blend are presented in Table 3.1.

Table 3.1: Anti-nutritional factors of extruded aerial yam and soybean flour blends

S/N	Anti-nutritional factor	Composition (mg/100g)
1	Oxalate	73.80±1.869
2	Tannin	1.81±0.016
3	Phytate	25.39±0.025
4	Hydrogen cyanide (HCN)	2.22±0.005
5	Alkaloids	1.64±0.001

Note: Values are mean ± standard deviation of triplicate determination

Oxalates

The result of the oxalate content of the extruded aerial yam-soybean flour blend is presented in Table 3.1. The oxalate content was 73.80 mg/100g of sample, which is within the range of 45.81 to 102.71 mg/100g, earlier reported by Umoh *et al.* (2021); 68.05 to 98.27 mg/100g for aerial yam and soybean flour (Umoh, 2020); lower than 167.20 to 256.90 mg/100g for processed false yam flour (Umoh, 2013). Oxalic acid can form soluble (potassium and sodium) or insoluble (calcium, magnesium and iron) salts or esters called oxalates, that are commonly found in plants or synthesized in the body.

In sensitive people, even small amount of oxalate can result in burning in the eyes, ears, mouth and throat; large amount may cause abdominal pain, muscle weakness, nausea and diarrhoea (Popova and Mihaylova, 2019).

Tannin

The tannin content of the extruded aerial yam-soybean flour blend was 1.81 mg/100g of sample (Table 3.1). The obtained value is within the range, earlier reported by Umoh *et al.* (2021), which varied between 0.93 and 5.46 mg/100g of sample, but higher than 0.03 and 0.04% for extruded meals of quality protein maize, soybean concentrate and cassava starch (Omosebi *et al*., 2018); 0.1942 to 0.4643 % tannic acid for extruded sorghum-soya blends (Arun kumar *et al*., 2018); 0.28 to 0.81mg GA/g – dry matter, for soybean hull (Tabibloghmany *et al*., 2020), but much more lower compared to 35.50 and 130.17mg/100g for sorghum-based extruded product supplemented with defatted soy meal flour (Tadesse *et al*., 2019).

Tannins exhibit anti-nutritional properties by impairing the digestion of various nutrients and preventing them from being absorbed by the body. Tannin can also bind and shrink proteins. Tannin-protein complex may result in digestive enzymes inactivation and protein digestibility reduction caused by protein substrate and ionizable iron interaction (Popova and Mihaylova, 2019; Ogunkoya *et al*., 2006).

Phytates

The extruded aerial yam and soybean flour blend recorded a phytate content of 25.39 mg/100g of sample (Table 3.1). The observed value is lower than 177.53 to 311.83 mg/100g for extruded sorghum-soya blends (Arun kumar *et al*., 2018); and 247.32 to 485.69 mg/100g for sorghum-based extruded product supplemented with defatted soy meal flour (Tadesse *et al*., 2019).

Phytates occur in several vegetable products. Seeds, grains, nuts and legumes store phosphorus as phytic acid in husks in the form of phytin or phytate salt. Their presence may affect bioavailability of minerals, solubility, functionality and digestibility of proteins and carbohydrates (Popova and Mihaylova, 2019).

Hydrogencyanide (HCN)

The result of the hydrogencyanide (HCN) content of the extruded aerial yam and soybean flours blend is presented in Table 3.1. The HCN content of the extrudate was 2.22 mg/100g of sample, which higher than 0.231 to 0.465 mg/100g of sample for processed false yam flour (Umoh, 2013); 0.11 to 0.13 mg/100g for extruded maize-soybean protein concentrate, earlier reported by Omosebi *et al*. (2018). However, the HCN content recorded from this study was lower than the recommended safe level of 10 mg HCN/kg, db (FAO/WHO, 1991; Bandna, 2012). This suggests that extrudates produced from aerial yam and soybean flours blend could be safe from the toxicity effect of hydrogen cyanide. Consumption of foods containing cyanogens could results in acute or chronic toxicity. So, it is pertinent to ensure that minimal levels are present in food for human consumption.

Alkaloids

The result of the alkaloids content of the extruded aerial yam-soybean flour blend is presented in Table 3.1. The alkaloids content of the extrudate was 1.64%, which is slightly lower than 2.19%, earlier reported by Umoh *et al.* (2021); 5.31 to 11.23% for aerial yam and soybean flour (Umoh, 2020); and 2.16% for processed false yam (Umoh, 2013). Some of the toxicological manifestations of potato glycol-alkaloids include gastro-intestinal upset and neurological disorders, especially in doses in excess of 20 mg/100g sample. Heat treatment removes the alkaloids present in most cultivated species of yam (Onwuka, 2018).

Conclusion

This study has shown that extrudate produced from soybean and aerial yam flour blend contains some levels of anti-nutritional factors, namely; oxalates (73.80 mg/100g of sample), tannin (1.81mg/100g of sample), phytates (25.39 mg/100g of sample), hydrogen cyanide (2.22 mg/100g of sample) and alkaloids (1.64 mg/100g of sample). The levels of these anti-nutritional factors are minimal, and are lower compared to extrudates from other related blended materials. They are also far below the recommended safe level of toxicity to human, and these anti-nutritional factors can be reduced to the level of non-toxicity, through extrusion processing technology.

Recommendations

The following are the relevant and actionable Recommendations for the study:

- In view of the difficulty encountered in sourcing for aerial yam bulbils in the course of carrying out this study, it is recommended that individuals, corporate organizations and government, at all levels should as a matter of exigency, venture into large scale cultivation of aerial yam. This is to ensure that aerial yam, as a food crop, does not go into extinction, and that the food crop is readily available and affordable for its potential food uses/applications.

- It is also recommended that other necessary food ingredients/spices be incorporated into the feed to further boost or improve the quality attributes of the extruded aerial yam and soybean flour blends.
- Other physical, chemical and organoleptic properties should be investigated. This is to ensure optimal exploration of the potential applications of aerial yam and its composites in food extrusion technology.
- The barrel temperature, screw speed and feed moisture content should be varied and another extrudates produced in order to identify the best extrusion process variables that would produce extrudates with a more reduced levels of toxicity.

Conflict of Interest

The authors of this article do hereby declare that no conflicts of interest exist.

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