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Research Article

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Chemical and Microbial Compositions of Unprocessed and Processed Soybean as Fish Feed Ingredient

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Abstract

Some of fish farm diseases have been linked to feeds and could have link with the ingredients used in compounding them. In this study, 5 kg of soybean seed were purchased and divided into five equal parts. Each part was processed by roasting, toasting, solid state fermentation (SSF), sprouting and other part unprocessed all in triplicate. The SSF and sprouting processing were carried out in a dark room for seven days and pH and Temperature were recorded twice daily. Each of the samples was tested for proximate compositions, phytochemical analysis, microbial load aflatoxin B1 and B2. The results of the study showed that the toasted and roasted soybean were at 120℃ at a time interval of 32-45 minutes. The pH range of 4.0 -5.5 were recorded for soybean during SSF while pH 5.0 to 6.0 for sprouting. Highest coliform counts of 7.79x10³ were from fermented soybean while lowest of 4.81 x10³ were from toasted soybean. The qualitative and quantitative results showed saponin, tannin, oxalate, alkaloid and glycosides were present though varied according to the processed methods. The proximate compositions varied with the processing methods. The lowest Aflatoxin B1 and B2 of 0.76mg/100g from toasted and 0.46mg/100g from fermented soybean. Based on this toasting method is recommended for processing soybean to reduce phytochemicals, microbial loads, Aflatoxin and increase nutrient compositions.

Keywords: Soybean, Processing, Phytochemical, Proximate, Aflatoxin, Microbial Load

Introduction

Mycotoxins are toxic chemicals produced by certain species of moulds usually belonging to the Aspergillus, Penicillium or Fusarium genera [1]. The importance of mycotoxins to aquaculture and livestock industry first became apparent during the early 1960s with outbreaks of aflatoxicosis in young Turkeys in the United Kingdom and hatchery-reared rainbow trout (*Onchorynchus mykiss*) in the United States [1]. In both cases, the origin of aflatoxicosis was aflatoxin-contaminated feed (peanut meal for turkeys and cottonseed meal for rainbow trout). Other mycotoxins described since then include ochratoxin A, deoxynivalenol, T-2 toxin, zearalenone, moniliformin, cyclopiazonic acid and fumonisin [1]. Of course, failure to provide adequate storage facilities for grains and other feedstuffs can increase the levels of aflatoxin and other types of mycotoxin contamination in field crops infected with a toxigenic fungal organism [2]. Aflatoxins in feeds or feed ingredients are usually a mixture of four aflatoxins with only slightly different chemical structures. The most prevalent and most toxic to animals is AFB1 constituting about 75 percent of the total fumonisin mixture in contaminated corn [3].

Some moulds that can produce mycotoxins, such as *Fusarium species* which are more active during periods of prolonged moisture from excessive rainfall, as was experienced during the late summer and fall of 2009 over widespread areas of the southeastern and mid-western U.S [2].

Materials and Methods

Experimental location

Adamawa state is located within the climate of Northern Guinea Savannah Zone and lies between latitude 8° and 11° N and Longitude 11.5° and 13°E and climate is tropical with two distinct seasons which are dry and wet seasons. The research was carried out at the Department of Fisheries, Moddibo Adama University of Technology wet laboratory, Yola in Adamawa state.

Experimental design

This is a factorial experiment arranged in completely randomized design which has three ingredients were processed using four different methods and replicated thrice.

Experimental set-up

The experimental setup was contained four processing methods which were Toasting, Roasting, Solid fermentation and Sprouting and replicated thrice. The unprocessed (raw) ingredients acted as the control.

Experimental procedures

Processing methods

5 kg of soybean seeds were purchased from feedstuff market at Jambutu. The ingredient was divided into five equal portions and each was processed and the other unprocessed using Sogbesan methods [4, 5].

Toasting

1 kg of each of the soybean was toasted following the methods of Sogbesan [4, 5]

Roasting

1kg of each of the soybean was roasted following the methods of Sogbesan [4, 5]

Solid State Fermentation

The soybean seeds were fermented in triplicate. 1% of sodium hypochlorite solution was added to sterilize the ingredients. Then the mixture of 0.1M of Acetic Acid and 0.1M of Sodium ethanoate was used to prepare buffer solution of pH 4-6 which created an enable medium for fungi growth. 200g of each of the ingredients in powdered form was mixed with 900ml of distilled water and collected into transparent covered 5liters plastic in a mimicked dark and room temperature of 27°C according to the method Sogbesan *et al*. [6]. 10ml of the buffer solution was mixed with each of the treated soybeans. Solid fermentation was carried out for seven days in a controlled laboratory under room temperature.

Sprouting

1kg of each of the soybean was sprouted following the methods of Sogbesan [4, 5].

Mycological and Quantitative Analysis of Mycotoxins

Mycological analysis

Prepared 70% ethanol was used to wash the ingredients twice for five minutes, and then rinsed in 20ml of sterilized distilled water according to Adriau and Dehant. Then soybean were inoculated with Ampicillin modified (PDA) and incubated for 7 days at 28ºc. Then the plates were checked for fungi presence. Each fungus was sub-cultured for pure colony of each of the fungi colony species. Each pure colony was identified morphologically and microscopically to species level. 65 plates were used for the analysis.

Quantitative estimation of mycotoxins by high performance liquid chromatograph

All chemicals and mycotoxins standards were purchased from Sigma-Aldrich, USA. Mycotoxins (1.0 mg of each mycotoxin in capped amber bottles) were used. The working solutions were prepared according to AOAC by injecting 1mL of acetonitrile into each via to dissolve the mycotoxins [7]. Working solutions was stored at 8 °C. To estimate mycotoxins levels, Hassan method with minor modifications as follows: 100 µL of chloroform was added to the sample extract or to the mycotoxins working standards, mixed well for 30 seconds, and then filtered using a No.4 Whatman filter paper [8]. Then, 900 µL of water: acetonitrile (9:1 v/v) was added and mixed well for 30 seconds.

Thirty microliters of this mixture were injected into a high-performance liquid chromatography (HPLC) column which had been preconditioned with 5 mL methanol and 5 mL acetonitrile: water (9:1 v/v) for 15 min. The HPLC system consisted of an Agilent Technologies Pump Model 1200 Series, G1321A FLD system. A fluorescence detector was used for the quantitation under the following conditions: FLD at 295 nm (excitation) and 330 nm (emission); Column Zorbax Eclipse Plus C18 Analytical 4.6 × 250 mm, 5-Micron; Post Column UVE LC Tech, Photochemical Post Column Derivatizer UVC 254 nm. All HPLC analyses were carried out under isocratic conditions using a mobile phase of acetonitrile: methanol: water (30:15:55 v/v/v) and the flow rate will be fixed at 1.0 mL/min. The mixture was filtered using a membrane filter and degassed in an ultrasonic bath for 25 min prior to use. The injected volume was 30 µL.

Phytochemicals Qualitative and Quantitative investigation of Soybean

2 g each of soybean collected were used for Qualitative and Quantitative investigations were carried out to assess Phytate, Oxalate, Alkaloid, Saponin, Tannin and Glycosides on each of the treatments following the methods of AOAC and Sogbesan [7, 4].

Proximate Analysis of the Soybean

The samples were analyzed for dry matter, Crude protein, Crude lipid, Ash, Crude fibre, Nitrogen free extract and Gross energy following the methods of Association of Official Analytical Chemists a stated in AOAC [7].

Statistical Analysis

All data collected was subjected to One-way analysis of variance (ANOVA). Standard deviation and standard error were calculated to identify the range of means and error respectively. Least Significance difference (LSD) was used to determine the level of significance at the probability of 5% among treatments using SPSS 16.0 and Graphpad Instat (DATASET 1) Statistical Packages for Windows 2000.

Results

Proximate Composition of Unprocessed and Processed Soybean

The proximate compositions of the unprocessed and processed Soybeans are presented on table 1. The crude protein of the processed ingredient was higher than that unprocessed.

Table 1: Proximate Composition of unprocessed and Processed Soybean

Treatments	Dry matter %	Crude protein %	Crude lipid %	Crude fibre %	Ash %	Nitrogen free extract %	Gross energy Kcal/g
Raw	90.10±2.65^{a}	36.63±1.35^{c}	17.53±1.01^{b}	5.00±0.18^{ab}	5.72±0.11^{a}	25.51±1.31^{b}	403.03±20.04^{b}
Toasted	90.75±2.72^{a}	39.13±1.88^{ab}	19.26±1.09^{a}	6.16±0.07^{a}	6.00±0.09^{a}	20.21±1.03^{c}	471.32±21.04^{a}
Roasted	90.20±2.87^{a}	38.15±1.53^{b}	15.20±1.11^{c}	4.95±0.02^{ab}	5.70±0.10^{a}	26.20±1.12^{ab}	374.35±20.14^{c}
Fermented	90.65±2.66^{a}	40.38±1.22^{a}	15.13±1.03^{c}	3.95±0.01^{b}	5.65±0.08^{a}	29.54±1.17^{a}	479.48±20.31^{a}
Sprouted	90.25±3.25^{a}	38.19±1.27^{b}	13.40±1.05^{d}	4.00±0.09^{b}	5.75±0.12^{a}	28.91±1.13^{a}	466.68±20.15^{a}

Means on the same column with different superscripts are significantly different ($p<0.05$)

Phytochemical Compositions of Unprocessed and Processed Soybean

Table 2 showed the quantitative analysis results of the raw and processed soybeans seeds. There was significant difference ($p<0.05$) in most of the values for the phytochemical comparing the raw to the processed.

Table 2: Phytochemical compositions of Unprocessed and Processed Soybean

Treatments	Tannins	Saponins	Alkaloid	Glycoside	Oxalate
Raw	27.15±1.22^{b}	15.23±0.22^{a}	42.34±1.21^{a}	12.46±0.82^{b}	44.63±1.72^{a}
Roasted	35.22±2.02^{a}	16.36±0.22^{a}	34.76±0.02^{b}	17.43±0.61^{a}	31.26±1.53^{b}
Toasted	21.04±0.12^{b}	10.05±0.42^{b}	22.11±0.11^{c}	3.26±0.01^{c}	28.34±1.02^{b}
Fermented	25.16±0.03^{b}	17.43±0.03^{a}	40.16±1.42^{a}	11.03±0.52^{b}	43.23±0.92^{a}
Sprouted	23.14±0.82^{b}	14.45±0.12^{a}	40.34±1.37^{a}	11.78±0.42^{b}	40.20±0.92^{a}

Means on the same column with different superscripts are significantly different ($p<0.05$)

Microbial Assessment of Soybean

Tables 3 showed the microbial load of the identified fungi from soybean seeds processed using roasting, toasting, solid state fermentation and sprouting respectively. The highest coliform counts were recorded in fermented soybean and lowest values in toasted soybean seeds and there were significant differences ($p<0.05$) between these values.

Table 3: Identification of Fungi in Unprocessed and Processed Soybean

Treatments	Coliform count fu/ml	Isolated Fungi
Raw	5.72±0.01×10^{3b}	*A. flavus, A. niger, A. fumigatum, A. parasiticum*
Toasted	4.81±0.02×10^{3b}	*A. flavus, A. niger, A. fumigatum, Microspora andoni*
Roasted	5.5±0.02×10^{3b}	*A. niger, A. flavus, A. fumigatum*
Fermented	7.79±0.03×10^{3a}	*A. niger, A. paraciticum, A. flavus, Trycophyton rubrum*
Sprouted	6.99±0.03×10^{3a}	*Fusarium solam, C. albicon, A. flavus, A. niger, A. parasiticum*

Means on the same column with different superscripts are significantly different ($p<0.05$)

Aflatoxins Composition of Processed Soybean

Table 4 presented the Aflatoxin B1 and B2 for Raw and processed Soybean seeds. From the table, it can be deduced that toasted soybeans aflatoxin B1 were significantly ($p<0.05$) higher than others.

Table 4: Aflatoxins composition of Unprocessed and Processed Soybean

Treatments	AF.B1	AF.B2
Raw	1.96±0.01^{d}	2.03±0.03^{b}
Roasted	2.73±0.03^{b}	2.15±0.01^{a}
Toasted	0.76±0.02^{e}	0.73±0.01^{d}
Fermented	2.94±0.01^{a}	0.46±0.01^{c}
Sprouted	2.46±0.05^{c}	0.98±0.01^{c}

Means on the same column with different superscripts are significantly different ($p<0.05$)

Discussion

The different processing method employed in this study are among those methods for processing animal feed ingredients as reported by Sogbesan *et al* and Sogbesan [5, 6]. The proximate compositions of these ingredients varied with the processing method in comparable to the unprocessed. In his report, Sogbesan on the processing of Mucuna reported that Mucuna seed processed reported better crude protein, ash and lower fibre compared with the raw [9]. It has also been reported by Pontes *et al*. that the proximate composition of ingredients used in fish feed must be known because their chemical and nutritional qualities are relevant for their efficiency [10]. Among the various factors that are essential in fish nutrition, is the composition of the ingredients and studies have shown that diet influences behavior, structural integrity, health, physiological functions, reproduction and growth of fish. For that reason, the determination of qualitative and quantitative requirements of essential nutrients is of fundamental significance for an adequate formulation of diets for fish [11].

The protein and lipid composition of feed ingredients in fish feed are important because Brett and Groove have identified these two nutrients as the primary sources of metabolic energy rather than carbohydrate and lipid [12]. Protein of either plant or animal origin

in fish feed acts as both structural and energy component the quality and quantity have the decisive influence on the growth rate of fish provided that all other physiological requirements are satisfied [12, 13]. Banrie *et al*. demonstrated that the rate of absorption of amino acids from protein-based diet in the gut affects protein synthesis, catabolism and oxidation within the body since the diets contains required essential amino acids for muscle anabolism [14]. Dietary lipid has been identified as steady source of metabolizable energy and essential fatty acids [15, 16].

The fungi identified from this study are among those reported as fungi species that produce Aflatoxin according to [17, 18]. However, only *A. flavus* and *A. parasiticus* are of economic importance. And from this study, these two fungi are reported in the raw and processed. The fact that microbial growth was reported in all ingredients studied despite their compositions and processing methods corroborate with the report of Osho *et al*. that no feed was completely free of fungi contamination [19].

The occurrence of *Aspergillus spp.* is significant in public health. *A niger* and *A. flavus* had been reported as the common agents of food spoilage most especially in the tropics where their spores are widely distributed [19]. Some species are known to secrete toxins known as aflatoxin which cause food poisoning and are carcinogenic to man. While some when ingested, affect the liver and no effective therapeutic treatment has yet been known. *Aspergillus spp* caused "Aspergillosis" (a disease of the lungs) [20]. Many human and animal diseases such as mycotic abortion, aflatoxin poisoning, allergic reaction, systemic infections are attributed to mould and fungi ingestion [20]. *Penicillum spp* and *Fusarium spp* are also capable of secreting toxins like ichra toxins and penicillic acid that are dangerous to human health. Various lung diseases in farmers are associated with mould and grain dust. Aflatoxins, even at diminutive dietary levels have been established to decrease growth rate and feed conversion efficiency in animals fed such feed [21].

The presence of Aflatoxin B1 and B2 in soybean despite the different processing methods is an indication that this mycotoxin cannot be fully eliminated from an ingredient with heat or water treatments. Although toasting and solid state fermentation methods prove better in reducing the quantities of Aflatoxin B1 and B2. Processing technology has a direct impact on feed quality in terms of physical properties and nutrient digestibility. Aflatoxins are fluorescent compounds, they are chemically classified as difurocoumarolactones and their biosynthesis by the producing fungi is via polyketide pathway [22, 23]. Aflatoxins are the most well-known mycotoxins and extensive research has been done about these mycotoxins. This reported corroborate with the work of Olorunfemi *et al*. who reported highest incidence of fish feed contaminations for fuminosin B1, fumonisin B2, aflatoxin B1 and ZEN with a mean of 900.9, 220.6, 103.0 and 4.5ug/kg from the southwest Nigeria [24]. In Central Europe, Pietsch *et al*. reported presence of FBs and ZEN in 11 samples of commercial fish feeds [25-27]. Hashimoto et al. reported the presence of AFB1 (1313 µg/g) and FBs (1112, 2 µg/kg) in 42 fish feed samples in Brazil [8]. These are evidences that fish feeds are carriers of multi-mycotoxins as they are majorly formulated from plant sources that are good substrates for mould proliferation and occurrence of Aflatoxin B1 found in grains from countries from Middle East, Asia and Africa is very high and worrying.

The presences of toxins, inhibitors and anti-growth factors in soybean remain a challenge that limits their maximum utilization and inclusion in fish diets [28]. This report corroborates with the findings from the study, where lowest weight gain and feed utilization was reported in the control which contains both phytotoxin and mycotoxin hence *Clarias gariepinus* couldnt utilize the nutrient in each of the ingredients for growth.

In conclusion, lowest phytochemicals in terms of tannins, saponin, glycosides, alkaloids and oxalate were recorded in ingredients processed by toasting along with better proximate values. Aflatoxin B1 and B2 had lowest values in ingredients that were toasted in comparison to raw, roasted, sprouted and SSF. Lowest fungi coliforms were recorded in ingredients that were toasted compared to raw, roasted, SSF and Sprouting [29-37].

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