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An assessment of the chemical and nutritional quality, trypsin inhibitor activity and protein digestibility of soybean products in Nigeria

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Target Audience: *Monogastric nutritionists and researchers, Research and Development (R & D), and feed processors*

Abstract

The varied processing protocols used to process soybean results in soybean products with a variety of nutritional properties and anti-nutritional components, hence this study was conducted to evaluate the nutritional properties and residual trypsin inhibitor content of soybean products processed in Nigeria. Soybean products (soybean meal and full fat soy meal) were sourced from processing plants across Nigeria and assayed for their crude protein content. Based on their crude protein content, soybean products were classified as either low protein (44-46% crude protein), "type 48" (47-49% crude protein), "type 50" soybean meal (≥ 50% crude protein), or full fat meals (< 40%). They were further assayed for moisture, ether extract, crude fibre, ash, gross energy, amino acid profile, trypsin inhibitor activity, protein dispersibility index (PDI), KOH protein solubility and in vitro protein digestibility, and metabolizable energy for poultry was estimated. This study showed that "type 50" soybean meal was significantly highest in crude fibre (7.33 ± 0.71%), moisture (11.42 ± 0.21%), and ash (7.97 ± 0.50%), "type 48" soybean meals were significantly higher in in vitro protein digestibility (49.15 ± 6.26%), while full fat meals were significantly higher in ether extract (18.01 ± 0.54%), Gross energy (5.28 ± 0.007 kcal/g) and trypsin inhibitor activity (9.20 ± 1.57 TIU mg/g). While only the "Type 50" soybean meals were within the tolerance range for trypsin inhibitors [4 mg/g] in broiler chickens. This study has provided baseline data for the variability that could be expected for soybean products originating from Nigeria.

Keywords*: Anti-nutritional factors; Serine proteases; Protein digestion; Heat processing*

Description of problem

Soybean (*Glycine max*) is the most important protein feedstuff used in livestock feeding the world over. Based on data from 2017-2019, about 76% of soybean produced globally is fed to livestock, with about 37% and 20.20% fed to poultry and swine respectively, in form of protein-rich soybean meal or cake, and 7% fed as full fat meals to livestock (1).

The relatively balanced amino acid profile and high protein digestibility attributed to soybean products when fed to monogastric species is only attained after a heat pre-treatment of soybean seeds. A heat pre-treatment removes anti-nutritional factors found in soybean, particularly serine protease inhibitors like Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitor (BBI) and chymotrypsin inhibitors which inhibit endogenous trypsin and chymotrypsin digestion. While soybean seeds are subjected to heating and either mechanical pressing or solvent extraction to obtain soybean cake/meals, they may be extruded in the preparation of full fat meals. The heat pre-treatment of soybean seeds involves controlled variables such as moisture, temperature, and heating time that differ from processing plant to processing plant, and result in soybean products with varying nutritional characteristics and protein quality. For example, overheating of soybean seeds could result in excessive denaturation of soybean proteins and destruction of amino acids from the reaction between the ε-NH_2 group of amino acids and the carbonyl group of reducing sugars (2), creating Maillard reaction products that impair protein digestibility and nutrient utilization. On the other hand, under-processing of soybean seeds results in soybean products with significant residual trypsin inhibitors, lectins, and low molecular weight oligosaccharides, which may:

1. induce hypertrophy or hyperplasia of the pancreas, a consequence of compensatory secretion of digestive enzymes to offset the effect of ingested trypsin inhibitors (3–5)
2. impair gut mucosa integrity and induce gastrointestinal disorders (6,7)
3. reduce amino acid digestibility in poultry and swine and reduced growth performance (8–10).

Hence, the dire implications of soybean product quality for growth and performance of livestock informed this investigation into the chemical and nutritional quality, trypsin inhibitor activity and protein digestibility of locally produced soybean products; soybean meal and full fat soy meals, available for livestock feeding in Nigeria.

Materials and methods

Soybean product sourcing and sampling

Soybean products were collected from 12 commercial processing plants (not feed mills, each processing plant represents a commercial soybean product brand sold on the Nigerian market) located in Oyo, Kaduna, Abuja, Lagos, Kano, and Ogun States. Soybean meal (n = 12) and full-fat soya meal (n = 2) were ground in a laboratory mill and aliquot samples stored in airtight jars at room temperature until analysis.

Chemical analyses

The proximate composition and amino acid evaluations of all meal samples were done in quadruplicates by reflectance spectroscopy in the near-infrared (NIR) region, and metabolizable energy estimated. Protein dispersibility index, PDI, and KOH solubility was determined using the method of (11) with slight modifications. For PDI, 5 g samples were added to 75 ml of distilled water, homogenized in a blender at 8,500 rpm for 10 minutes, filtered and centrifuged at 1000 × g for another 10 minutes. For KOH solubility determination, 1.5 g samples were added to 75ml of 0.2% KOH solution and homogenized in a blender at 8500 rpm for 20 minutes. Thereafter, 50 ml aliquots were centrifuged at 2,500 × g for 15 minutes and supernatant separated. The nitrogen content of the supernatants was then determined by the micro-Kjeldahl method, and results expressed as a percentage of the total nitrogen content of the sample.

Residual trypsin inhibitor activity was assayed in two steps; extraction and enzymatic assay, based on the procedure of (12) with slight modifications. Firstly, trypsin inhibitor was extracted from 1g sample in 50ml of 0.01N NaOH (pH 11.0) for 3 hours with constant shaking at 624 × g. and pH of supernatant was adjusted to 8.4 – 10. In the second step, aliquots of a chromogenic substrate for trypsin; N_α-Benzoyl-D,L-arginine p-nitroanilide hydrochloride (DL-BAPNA B-4875, Sigma Aldrich, Saint Louis, USA) were incubated with the supernatant and trypsin from porcine pancreas (T-4799, Sigma Aldrich, Saint Louis, USA; (13)), and enzyme activity estimated as the p-nitroaniline released by the hydrolysis of D,L-BAPNA at the bonds between arginine and p-nitroaniline moieties by colorimetric analysis at 405 nm.

Protein digestibility was estimated *in vitro* by a method mimicking protein digestion in chickens (14). Briefly, aliquots of meals were hydrolyzed with 6M HCl, the nitrogen content of the digest estimated by the ninhydrin method and protein content of the digest was calculated as N × 6.25. Thereafter 500 mg crude protein equivalents of each meal were weighed in 5 replicates, pre-incubated in 10 mM HCl-KCl buffer then incubated in 1.5 mL pepsin solution for 30 mins (gastric digestion), and in 5 mL pancreatin solution for 180 mins (intestinal digestion). Prior to the intestinal phase, the digest was corrected to pH 7.5 with a 1M NaOH and sodium acetate buffer. At the end of the digestion, each tube was centrifuged at 600 rpm, and aliquots drawn and assayed for free amino acids and dipeptides by the ninhydrin method against a lysine standard solution ranging from 0-2 μg/ml at 568 nm.

Calculations and statistics

Trypsin inhibitor activity/ml was calculated as the change in the absorbance of the control (containing no inhibitor extract/supernatant) and inhibited reaction mix per /minute.

$$Trypsin\ inhibitor\ unit/ml = \frac{\Delta A_{405nm/min}\, control - \Delta A_{405nm/min}\, inhibited}{(9.96)(ml\ trypsin\ in\ reaction\ mix) \times dilution\ factor}$$

$$Trypsin\ inhibitor\ unit/mg = Trypsin\ inhibitor\ unit/ml \div mg\ solid/ml$$

$$Trypsin\ inhibitor\ unit(mg/g) = Trypsin\ inhibitor\ unit/mg \div 1.9$$

Where ; 9.96 is the millimolar extinction coefficient of p-nitroanilide at 405nm.

In vitro protein digestibility was calculated as:

$$protein\ digestibility\ (\%) = \frac{OD_{568nm}\ of\ digested\ sample}{OD_{568nm}\ of\ 6M\ HCl\ hydrolyzed\ sample} \times 100$$

All soybean meals were grouped based on their crude protein content; low crude protein (44-46% crude protein), “type 48” (47-49% crude protein), and “type 50” (≥ 50% crude protein), and full fat soya (<40% crude protein) group was included (15). All data obtained were subjected to analysis of variance (ANOVA) with the crude protein groups as treatments, and significant means separated by Tukey honestly significant difference (HSD) test at 95% probability in JASP (version 0.16.1) statistical package.

Results

Thirty three percent of the soybean meals used in the current study were of “type 50”, 25% were either “type 48” or “low protein”, and 17% were full fat soya meals with crude protein content < 40% (Table 1). “Type 50” soybean meals had the highest moisture content, ash, and crude fibre ($p<0.05$), and the least ether extract content, while the low protein and type 45 meals had similar composition ($p>0.05$). Total phosphorus composition and gross energy values were similar ($p>0.05$) for all soybean meal types in this study. Full fat meals had a minimum of 5 times the ether extract of all soybean meals tested, and a significantly higher ($p<0.05$) gross energy content. The carbohydrate fractions of the soy products (measured in nitrogen free extract, NFE) was highest ($p<0.05$) in the low protein and “type 48” meals followed by the full fat meals, and least in the “type 50” meals. Apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen (AMEn) were significantly higher in “type 48” soybean meals, compared to the low protein and "type 50" groups.

The amino acid profile of soybean and full fat meals assessed in this study are shown in Table 2. Generally, higher concentrations of the amino acids assayed were observed in full fat meals except for lysine, tryptophan, proline, cystine, and glycine. The highest lysine content ($p<0.05$) was recorded in "type 48" meals, with no significant variation in threonine and tyrosine content in all meals assayed. Variations in the amino acid profile amongst soybean meals were only observed for tryptophan, histidine, and alanine, where the least ($p<0.05$) concentrations were observed in low protein meals. The protein quality assessments conducted (Table 3) showed that trypsin inhibitor activity ranged from 2.27 – 9.2 mg/g and was found to be significantly higher in the full fat meals, low protein, and "type 48" meals, , and least in "type 50" meals. KOH protein solubility was highest in "type 48" and full fat soy meals, and least in "type 50" and low protein meals, while protein dispersibility index (PDI) was least in low protein meals, and highest in "type 50" soybean meals. Low protein and "type 48" soybean meals had the highest *in vitro* protein digestibility which compared favourably with protein digestibility in the full fat soy samples, and least in "type 50" soybean meals.

The output of correlations between the indices of soybean protein quality; KOH solubility and protein dispersibility indices, trypsin inhibitor content, crude protein, and ether extract of the soybean products in the current study are shown in Table 4. Moderate negative correlations were observed between protein dispersibility index and *in vitro* protein digestibility ($r_= -0.69$, $p<0.001$), protein dispersibility index and residual trypsin inhibitor ($r_= -0.51$, $p=0.01$), and residual trypsin inhibitor and crude protein ($r_= -0.65$, $p<0.001$), with a positively weak correlation between protein dispersibility index and crude protein ($r_= -0.43$, $p=0.04$). Moderate positive correlations were also observed between KOH solubility and *in vitro* protein digestibility ($r_= 0.46$, $p=0.03$), residual trypsin inhibitor and in vitro digestibility ($r_= 0.51$, $p=0.001$), *residual* trypsin inhibitor and ether extract ($r_= 0.57$, $p<0.001$). A strong negative correlation was also observed between crude protein and ether extract ($r_= -0.93$, $p<0.001$).

Discussion

The goal of this work was to create a reference database of the nutritional composition, protein quality, residual trypsin inhibitor activity, and *in vitro* protein digestibility of soybean products (soybean and full fat soy meals), available as feedstuffs in Nigeria. The proximate and amino acid compositions of soy products from the current study were consistent with values observed for soybean and full fat meals assesses in previous published reports (16–24). The chemical constituent of soybean meal particularly the anti-nutritive factors, carbohydrate composition, and protein/amino acids are known to be influenced by processing conditions amongst other variables (24-27). According to (28) arginine was the essential amino acid with the highest concentration in extruded full fat soya (EFFS) and methionine was the essential amino acid with the least concentration in raw and differently processed soybeans, followed by solvent extracted soybean (SES).

A precise balance between good protein digestibility and appropriately reduced anti-nutritional bio-actives determines the protein quality of soybean products. (29). KOH protein solubility and the protein dispersibility index are two of the most used quality control indices used to measure soybean protein quality in the animal feed industry. The KOH protein solubility test indirectly determines the degree of protein denaturation due to heat processing, by measuring the solubility of soy-globulins, which are the major storage proteins in soybeans. Before processing, the KOH protein solubility value of soybean seeds is above 90-95% (i.e., more than 90-95% of the protein in soybeans is soluble) and continues to decline with heating. According to (30), adequately processed soybeans should have a KOH protein solubility value ranging from 78-85%, while (31) quote a 67-77% KOH protein solubility value as adequate for properly processed soybean products. Studies have shown a decline in performance of chicks when fed diets containing soybean meal with KOH solubility values ranging between 70-74% (3,11), and KOH solubility values > 85% or < 70% are indicative of under-processed and over-processed soybean products, respectively. These reports suggest that about 75-100% of the soybean and full fat soy meals available for animal feeding in Nigeria are overprocessed, which reduces their protein digestibility. In an effort to deactivate anti-nutritional factors, heat processing of soybeans for longer periods of time or at higher temperatures frequently results in over processing.

On the other hand, the protein dispersibility index measures the solubility of soy-globulins in water and is used to determinate the degree of under-processing that soybean meals may have been subjected to. This index is considered more reliable, consistent, and sensitive than the urease index or KOH protein solubility and a protein dispersibility index value of 15-30% is considered appropriate for adequately processed soybean meal (30). The protein dispersibility index values observed in this study were consistent with those found in studies using soybean seed that had been adequately processed (30). Residual trypsin inhibitors in soybean and full fat soy meals reduce protein digestibility by a dual action; increased formation of inactive complexes between trypsin inhibitors, and increased digesta viscosity (32). Earlier studies have ascribed about 40% of growth inhibition seen in rats fed raw soybeans to trypsin inhibitors (33). Only the trypsin inhibitor activity values recorded for "type 50" soybean meals in this study were within the tolerance range reported for broilers (34). In a similar study conducted to assay for trypsin inhibitors in soybean meal products from different world areas, 45.5% of soybean products obtained from Africa was adjudged to contain less than 3 trypsin inhibitor units, (TIU) (mg/g), while 6.8% and 47.7% containing 3-4TIU(mg/g) and greater than 4 TIU (mg/g), respectively (35). Although *in vitro* protein digestibility may not always correlate perfectly with *in vivo* digestion, studying factors that affect in vitro digestibility provide valuable insights into their potential impact on protein utilization. Factors such as antinutrients, degree of heat treatment, processing techniques and genetic factors influence protein digestibility of soy products (18, 36). The higher protein digestibility recorded for "low protein" and "type 48" soybean meals compared to the "type 50" and full fat soya meals in the current study could be attributed to processing conditions (18 ,37).

Conclusions and Applications

The following conclusions can be drawn as regards soybean products; soybean and full fat soy meals available for livestock feeding in Nigeria:

1. The trypsin inhibitor values of the soybean products ranged between 2.27 – 9.2 mg/g with full fat soy meals showing the highest residual trypsin inhibitor activity.

2. Based on crude protein content, 33% of soybean meals are type 50 (≥50% CP), 25% are either type 48 (47- 49% CP) or low protein (44-46% CP), while the full fat soy meals have crude protein content of < 40%.
3. "Type 50" Soybean meals are higher in crude fibre, ash, and protein dispersibility index, and have the least residual trypsin inhibitor activity.
4. "Type 48" Soybean meals have higher *in vitro* protein digestibility and KOH protein solubility values than "Type 50" soybean and full fat soy meals.
5. Full fat soy meals possess higher fat content, gross energy value, and residual trypsin inhibitor activity than soybean meals.
6. About 75-100% of soybean and full fat soy meals are over processed, are high in residual trypsin inhibitors, and have moderate-low *in vitro* protein digestibility.
7. The correlations with greater practical interest among the variables studied were observed among the *in vitro* protein digestibility, residual trypsin inhibitor activity and KOH protein solubility.
8. Residual trypsin inhibitor activity was positively correlated with KOH protein solubility and *in vitro* protein digestibility.
9. Modifications to the processing conditions adopted by the various processing plants can be made to address variations in the nutritional composition and trypsin inhibitor concentrations of soybean and full-fat soy meals in Nigeria. This will ensure that the products are adequately processed and that there are few variations in their nutritional quality.

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Table 1: Proximate and chemical compositions of soybean products; soybean meal and full fat soya meal obtained from different commercial processing plants in Nigeria

All samples n = 12	Low protein n = 3 (25%)	"type 48" n = 3 (25%)	"type 50" n = 4 (33%)	Full fat soy meals n = 2 (17%)
Crude protein (%)	46.59 ± 0.39[b]	47.34 ± 0.36[b]	51.42 ± 0.82[a]	37.88 ± 0.56[c]
Ether extract (%)	3.50 ± 1.18[b]	3.66 ± 1.69[b]	1.55 ± 0.36[c]	18.01 ± 0.54[a]
Crude fibre (%)	5.58 ± 0.24[bc]	5.17 ± 0.24[c]	7.33 ± 0.71[a]	6.07 ± 0.13[b]
Moisture content (%)	9.90 ± 0.50[b]	10.17 ± 0.14[b]	11.42 ± 0.21[a]	8.89 ± 0.10[c]
Ash content (%)	7.28 ± 0.16[b]	7.05 ± 0.16[b]	7.97 ± 0.50[a]	5.21 ± 0.06[c]
Gross energy (kcal/g)	4.14 ± 0.01[b]	4.15 ± 0.02[b]	4.13 ± 0.01[b]	5.28 ± 0.01[a]
Apparent metabolizable energy (AME, kcal/kg)	1968.33 ± 27.78[b]	2026.67 ± 50.64[a]	1963.50 ± 46.56[b]	ND
Apparent metabolizable energy corrected for nitrogen (AMEn, kcal/kg)	1852.78 ± 31.01[b]	1911.44 ± 43.43[a]	1834.50 ±47.54[b]	ND
Nitrogen free extract (NFE, %)	27.14 ± 27.14[a]	26.62 ±.1.79a	20.31 ± 1.69[c]	23.93 ± 0.84[b]
Total phosphorus (%)	0.54 ± 0.02	0.53 ± 0.02	0.55 ± 0.01	ND

Means on the same row with different superscripts are significantly different (P<0.05); ND – not determined

Apparent metabolizable energy (AME, kcal/kg) and Apparent metabolizable energy corrected for nitrogen (AMEn, kcal/kg) were calculated from WPSA equation (WPSA (World´s Poultry Science Association). 1989. European Table of Energy Values for Poultry Feedstuffs).

Table 2: Amino acid profile of soybean products; soybean meal & full fat soya obtained from different commercial processing plants in Nigeria

All samples n = 12	**Low protein n = 3 (25%)**	**"type 48" n = 3 (25%)**	**"type 50" n = 4 (33%)**	**Full fat soy meals n = 2 (17%)**
Essential amino acids (g/100g)				
Leucine	5.12 ± 1.39^{b}	5.49 ± 1.65^{b}	4.76 ± 0.32^{b}	7.79 ± 0.28^{a}
Lysine	5.39 ± 0.50^{ab}	5.85 ± 0.34^{a}	5.53 ± 0.94^{ab}	4.45 ± 0.14b^{c}
Isoleucine	2.92 ± 0.81^{ab}	3.26 ± 1.01^{ab}	2.65 ± 0.10^{b}	3.93 ± 0.24^{a}
Phenylalanine	2.16 ± 1.36^{b}	2.65 ± 1.70^{ab}	1.47 ± 0.17^{b}	4.30 ± 0.32^{a}
Tryptophan	1.20 ± 0.03^{b}	1.30 ± 0.06^{a}	1.35 ± 0.04^{a}	1.16 ± 0.07^{b}
Valine	4.09 ± 0.62^{b}	4.39 ± 0.54^{b}	4.12 ± 0.22^{b}	5.15 ± 0.18^{a}
Methionine	1.32 ± 0.11^{b}	1.48 ± 0.06^{b}	1.57 ± 0.15^{bc}	1.90 ± 0.06^{a}
Arginine	6.44 ± 0.45^{b}	6.76 ± 0.48^{ab}	6.90 ± 0.58^{ab}	7.49 ± 0.11^{a}
Threonine	3.42 ± 0.13	3.51 ± 0.13	3.71 ± 0.32	3.38 ± 0.17
Histidine	2.09 ±0.14^{b}	2.35 ± 0.08^{a}	2.36 ±0.22^{a}	2.28 ± 0.06^{ab}
Non-essential amino acids (g/100 g)				
Tyrosine	2.12 ± 0.83	2.38 ± 1.02	2.10 ± 0.38	3.18 ± 0.10
Proline	3.21 ± 0.05^{a}	3.38 ± 0.08^{a}	3.59 ± 0.26^{ab}	3.17 ± 0.15^{a}
Cystine	1.31 ± 0.08^{ab}	1.53 ± 0.19^{a}	1.60 ± 0.18^{a}	1.20 ± 0.03^{b}
Alanine	3.59 ± 0.34^{c}	4.10 ± 0.09^{b}	4.25 ± 0.23^{b}	4.80 ± 0.32^{a}
Glutamic acid	12.03 ± 0.15^{b}	12.25 ± 0.32^{b}	12.49 ± 0.52^{b}	13.60 ± 0.33^{a}
Glycine	4.17 ± 0.34^{a}	4.68 ± 0.26^{a}	4.87 ± 0.71^{a}	4.02 ± 0.16^{ab}
Serine	3.20 ± 0.47^{ab}	3.52 ± 0.57^{a}	3.26 ± 0.14^{ab}	3.92 ± 0.16^{a}
Aspartic acid	7.34 ± 1.10^{b}	7.78 ± 1.53^{ab}	7.08 ± 0.33^{b}	9.49 ± 0.18^{a}

Means with different superscripts are significantly different (P<0.05)

Table 3. Protein quality assessment; trypsin inhibitor activity, protein solubility, protein dispersibility index and *in vitro* protein digestibility of soybean products obtained from different commercial processing plants in Nigeria.

All samples n = 12	**Low protein n = 3 (25%)**	**"type 48" n = 3 (25%)**	**"type 50" n = 4 (33%)**	**Full fat soy meals n = 2 (17%)**
Trypsin inhibitor units, TIU (mg/g)	6.21 ± 2.38^{a}	7.32 ± 0.49^{a}	2.27 ± 2.63^{b}	9.20 ± 1.57^{a}
KOH protein solubility (%)	49.86 ± 2.90^{b}	68.55 ± 14.91^{a}	50.21 ± 2.78^{b}	54.84 ± 2.51^{ab}
Protein dispersibility index (%)	13.39 ± 1.66^{c}	17.19 ± 1.43^{b}	21.10 ± 1.62^{a}	16.95 ± 1.76^{b}
In vitro protein digestibility(%)	48.98 ± 8.57^{a}	49.15 ± 6.26^{a}	32.59 ± 4.26^{b}	40.45 ± 2.97^{ab}

Means on the same row with different superscripts are significantly different (P<0.05).

Table 4. Correlation between soybean meal protein quality indices, residual trypsin inhibitor activity, crude protein and ether extract of soybean products collected from different commercial processing plants in Nigeria

	KOH solubility	*In vitro* protein digestibility	Residual trypsin inhibitor activity	Crude protein	Ether extract
Protein dispersibility index (PDI)	r = 0.004 p = 0.01	r = -0.687 p<0.001	r = -0.508 p = 0.011	r = 0.430 p = 0.036	r = -0.227 p = 0.285
KOH solubility		r = 0.457 p = 0.025	r = 0.295 p = 0.161	r = -0.076 p = 0.723	r = -0.114 p = 0.597
In vitro protein Digestibility			r = 0.512 p = 0.001	r = -0.272 p = 0.109	r = 3.784e-4 p = 0.998
Residual trypsin inhibitor activity				r = -0.651 p<0.001	r = 0.570 p< 0.001
Crude Protein					r = -0.932 p<0.001

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