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Nutrient Composition, Amino Acids Profile and Anti Nutritional Factors of Nixtamalized Soya Bean (*Glycine max*) using different alkali treatment

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Abstract

This study evaluates the effects of nixtamalization using different alkali treatment on the nutrient composition, amino acids profile and anti nutritional factors of soya bean for poultry feeds. Sodium Carbonate (Na_2CO_3), Potassium Carbonate (K_2CO_3), Sodium Hydroxide (NaOH) and soaking in water (control) were used in the process. Alkaline treatment effect was observed in Dry Matter (DM), Crude Protein (CP), Crude Fibre (CF), Ether Extract (EE), Total Ash, Nitrogen Free Extract (NFE), Ca and P of the nixtamalized soyabean. Similarly amino acid profiles (g/100 g protein) of the test soyabean varied with different alkaline treatments. Trypsin Inhibitor Activity (TIA) and phytic acid were inactivated by the different alkali salts. Anti-nutritional factors, protein quality assessors were significantly ($p<0.05$) correlated with alkali treatment. Preservation of protein quality of soyabeans and the inactivation of anti-nutritional factors in soyabeans for poultry feeds was observed to be better with 1% K_2CO_3 alkaline treatment.

Keywords: *Nixtamalization, Alkali, amino acids, anti nutritional factors, chemical composition, soyabeans*

Introduction

Soyabean (*Glycine max*) has remained the main vegetable protein source with major impact in the diet of both animal and humans on account of bioavailability of complete protein needed for human and animal health (Gu *et al*., 2010; Caprita *et al*., 2010). Raw soybean grain however contains Anti-Nutritional Factors (ANF) in variable amounts. This therefore calls for investigations into different methods of processing to help improve the functionality and nutrient quality of soyabean based diets.

Nixtamalization of both cereals and oilseeds using different alkali for nutritional improvement of feedstuff for human, poultry and livestock utilization has been reported (Addy *et al*., 1995; Ayanwale, 1999; Jackson et al., 2002; Wanjekeche *et al*., 2003; Fayomi, 2004; Sefa-Dedeh et al., 2004; Akande and Fabiyi, 2010; Oskoueian *et al*., 2011; Ari and Ayanwale, 2013).

Several types of plant proteins treatment with alkaline salts were earlier reported by DeGroot and Slump (1969) and Omueti *et al*. (1992). Ayanwale (1999) and Ayanwale (2003) reported the application of sodium sesquicarbonate (trona) in improving the nutritive values of soyabeans through the removal of polyphenols and destroying the trypsin inhibition factor. The use of sodium hydroxide and sodium hypo chloride in the

detoxification of a toxic variety of *Jatropha curcas,* a protein rich oilseed for animal utilization was also evaluated (Cano-Asseleih *et al*., 1989; Aregheore *et al*., 2003).
Vidal-Valverde *et al*. (1979) and Parsons *et al*. (1991) also reported the use of sodium bicarbonate solution, distilled water and potassium hydroxide in the removal of trypsin inhibitor activity in some oilseeds and particularly soyabeans. The use of blanching and nixtamalization processes using other alkali salts to improve feeding quality and reduction in intrinsic anti nutritional factors in feeds were also reported by Wanjekeche *et al*. (2003); Akande and Fabiyi (2010) and Oskoueian *et al*. (2011) while blanching was also reported by Nwosu (2011) to reduce Tyrpsin inhibitor activity and phytate.
Friedman and Master (1984) and Ayanwale (1999) however observed that alkaline treatment of soyabeans is dependent on the concentration levels of the alkaline used as treatment of soyabeans with strong alkaline resulting in decreased protein quality, loss of amino acids and the formation of amino acids lysinoalanine complex, which reduces lysine availability. This is supported by Oskoueian *et al*. (2011) who reported effects of different concentrations and types of alkaline salts in the inactivation of Tyrpsin Inhibitor Activity. The possibilities of alkaline salts to cause the release of bound minerals in nixtamalized feedstuff have also been reported (Ayanwale, 1999).
Alkali treatment of soyabeans was however observed (Moss *et al*., 1990) to have caused reduction in the values of dry matter, crude fibre and ash content compared to values of raw soyabeans while crude protein and either extracts values were higher than the values of raw soyabeans. A similar observation was reported by Wanjekeche *et al*. (2003). This development was ascribed to be as a result of the reduction in hemicelluloses content of residue. The aim of this experiment was to evaluate the effects of nixtamalization using different alkaline salts (sodium carbonate, potash and sodium hydroxide) on the proximate composition, amino acids profile and anti nutritional factors of soyabeans for utilization by broilers.

Materials and Methods

Experimental site

This study was conducted at the Livestock Complex of College of Agriculture, Doma Road, Lafia which is located between latitude 8^0 and 9^0 North and longitude 80^0 and 90^0 East. The minimum temperature is 21.9^0C and maximum temperature of 37.6^0C between January to June and the average annual rainfall is 823mm. The test ingredients were processed at the Livestock Complex and the Nutrition Laboratory of Faculty of Agriculture, Nasarawa State University, Lafia Campus.

Soyabean Collection, Processing and Diet Preparation:

Soyabeans seeds *(Glycine max)* were procured from a local market in Lafia metropolis of Nasarawa State, Nigeria. The raw soyabeans seeds were cleaned by winnowing and

hand picking of stones and debris before being subjected to the nixtamalization process using three (3) alkali treatments viz: Sodium Carbonate (Na_2CO_3) (T2), Potasium Carbornate (K_2CO_3) (T3) and Sodium Hydroxide (NaOH) (T4) and compared with the control treatment; soaking in water (H_20) (T1). The different alkali treatment processes are described as thus:

Soaking in Water (H_2O) - T1 (control)

The cleaned raw soyabeans were poured into a plastic container of cold water according to the methods reported by Ayanwale (1999). The soyabeans were allowed to soak by immersing the soyabeans in water at room temperature for 24 hours, this represents 0% alkaline. The soaked grains were drained, and sun dried by spreading on jute bags. The sun dried samples were milled and bagged.

Soyabeans Processed with Sodium Carbonate (Na_2CO_3) –T2

The method reported by Ayanwale (1999). The cleaned sample grains were soaked in 1% solution of sodium carbonate. The soyabeans sample was allowed to soak by immersing in the aqueous alkaline solution at room temperature for 24 h. The soaked grains were drained and sun dried by spreading on jute bags until stable weight was attained. The sun dried products were milled and bagged.

Soyabeans Processed with Potasium Carbornate (K_2CO_3) –T3

The method reported by Ayanwale (1999) for alkali treatment of soyabeans was applied. The cleaned sample grains were soaked in 1% solution of potassium carbornate (potash). The potash used was procured from local market in Lafia. The soyabean samples were allowed to soak by immersing the soyabeans in the aqueous solution at room temperature for 24 h. The soaked grains were drained and sun dried by spreading on jute bags until stable weight was attained. The sun dried samples were milled and bagged.

Soyabeans Processing with Sodium Hydroxide (NaOH) -T4

The cleaned samples grains were soaked in 1% solution of sodium hydroxide. The method reported by Ayanwale (1999) for alkali treatment of soyabeans was applied. The soyabeans samples were soaked in the aqueous solution of NaOH at room temperature for 24 h. The soaked and wet grains were drained, and sun dried by spreading on jute bags until stable weight was attained. The sun dried samples were milled and bagged.

Analytical procedures

Proximate analysis: Proximate composition of each of the nixtamalized soyabeans samples using alkali treatments were determined according to AOAC (2000) methods. The parameters determined include; moisture, crude protein, crude fibre, lipids and ash content, while nitrogen free extract was obtained by difference.

Determination of amino acid profile: The amino acid profile of the nixtamalized soyabeans samples using alkali treatments determined using the methods described by

Spackman *et al*. (1958). The soyabeans samples were dried to constant weights, defatted, hydrolyzed and evaporated in a rotary evaporator and then loaded into the Technicon Sequential Multisample amino acid analyzer (TSM).

Trypsin Inhibitor Activity (TIA): Trypsin inhibitor activities of the nixtamalized soyabeans samples using alkali treatments were determined according to the method described by Gupta and Deodhar (1975) and Hammerstrand *et al*. (1981). The methods shared the same principles of determining trypsin inhibitors in soyabeans products based on the trypic hydrolysis of synthetic substrate, Benzoyl-DL-Arginine-P-nitroanilide (BAPA).

Phytic acid determination: The phytic acid in the soyabeans samples subjected to the different processing methods was determined according to the modified method described by Wheeler and Ferrell (1971) and Steward (1974).

Protein Solubility Index (PSI): The protein solubility index method described by Araba and Dale (1990) was adopted to ascertain the protein quality of the raw and processed soyabeans subjected to the different alkali treatments.

pH methods of urease assay: The determination of pH of the nixtamalized soyabeans samples using alkali treatments was done using Urease assay as a measure of protein quality (Dudley-Cash, 2004). This was based on the hydrolysis of urea by the enzyme urease present in soyabeans to produce carbon dioxide and ammonia.

Results

The chemical composition of the nixtamalized soyabeans samples using alkali treatments (test ingredients) are presented in Table 1. The dry matter percentage ranged from 89.8 to 90.17% while crude protein percentage ranged from 38.34% to 40.27%. The crude fibre on the other hand had values ranging from 12.37 to 22.87%. The highest value of ether extract was obtained in 0% alkaline while the least (6.72%) was obtained in 1% K_2CO_3. The total ash percentages ranged from 3.61 to 5.29% while NFE percentages ranged from 21.08 to 25.34%. The highest Ca and P percentage values were 0.44 and 0.36% in 1% Na_2CO_3 and 0% alkaline respectively.

Table 2 presents the amino acid profiles (g/100g protein) of the nixtamalized soyabeans samples using alkali treatments. The values for lysine ranged from 4.79 to 5.78, while cystine had values ranging between 0.80 and 1.07. The methionine value were highest (1.23 and 1.30) in 0% alkaline and 1%NaOH while 1% Na_2CO_3 gave the least (0.90). The values for phenylalanine ranged from 3.78 to 4.42.

Table 1: Effect of Alkaline Treatment on the Chemical Composition of Nixtamaized Soyabean

	Chemical Composition (%)							
Alkaline Treatment	**Dry Matter**	**Crude Protein**	**Crude fibre**	**Ether Extract**	**Total Ash**	**NFE**	**Ca**	**P**
0% Alkaline	90.02	39.70	13.76	21.85	3.61	21.08	0.39	0.36
1% Na_2Co3	90.02	38.34	14.64	18.58	5.29	23.15	0.44	0.23
1% K_2CO_3	90.17	40.27	22.87	6.72	4.80	25.34	0.37	0.23
1% NaOH	89.83	39.64	12.37	21.31	5.05	21.63	0.42	0.23

Table 2: Effect of Alkaline Treatment on the Amino Acid Profile of Nixtamalized Soyabeans

	Amino Acids Profiles (g/100g Protein)			
Amino Acids	**0% Alkaline**	**1% Na_2Co_3**	**1% K_2CO_3**	**1% NaOH**
Lysine	5.23	5.50	5.78	4.79
Histidine	3.40	2.93	3.00	2.96
Arginine	5.50	5.82	5.82	4.80
Aspartic Acid	11.23	12.03	12.08	12.15
Threonine	3.18	3.24	3.48	3.16
Serine	2.59	2.66	2.78	2.64
Glutamic Acid	12.15	12.89	13.62	12.67
Proline	3.59	3.59	3.72	3.47
Glycine	3.00	3.45	3.49	2.68
Alanine	3.50	3.93	3.99	3.33
Cystine	1.07	0.80	0.88	0.96
Valine	3.37	2.75	2.80	3.69
Methionine	1.23	0.90	0.99	1.30
Isoleucine	3.10	2.53	2.65	2.78
Leucine	7.44	5.99	6.19	7.01
Tyrosine	3.18	2.91	2.77	2.63
Phenylalanine	4.42	3.78	3.79	4.26

The effect of alkaline treatment on the levels of anti-nutritional factors is presented in Table 3. The reduction in trypsin inhibitor activity ranged from 38.76% in the 0% alkaline treatment to 94.14% in 1% Na_2CO_3. Similarly, the percentage reduction of phytic acid ranged from 5.80% in 0% alkaline to 85.91% in NaOH. This value was closely followed by 85.86 and 85.87% in 1% potash and 1% $NaCO_3$. Urease assay values ranged from 0.03 to 0.09 (ΔpH) and the highest protein solubility index of 81.74% was obtained in 0% alkaline.

The correlation coefficients of anti-nutritional factors and assessment test factors are presented in Table 4. The following factors and assessors are significantly ($P<0.05$)

correlated: phytic acid and PSI, phytic acid and TIA, Phytic acid and reduction TIA, phytic acid and alkaline treatment, PSI and alkaline treatment, TIA and alkaline treatment, reduction in TIA and alkaline treatment, while TIA and PSI, reduction in TIA and PSI, TIA and reduction in TIA were also significantly ($P<0.01$) correlated.

Table 3: Effect of Alkaline Treatments on the Value of Anti-Nutritional Factors of Nixtamalized Soyabeans

Parameters	**Raw Soya**	**0% Alkaline**	**1% Na_2CO_3**	**1% K_2CO_3**	**1% NaOH**
Trypsin Inhibitor Activity TIA (mg/k)	15.35	9.40	0.90	1.20	1.15
Reduction in TIA (%)	0.00	38.76	94.14	92.18	92.51
Phytic Acid (mg/100g)	345.00	325.00	66.00	48.8	48.6
Reduction in PA (%)	0.00	5.80	80.87	85.86	85.91
Urease Assay (ΔpH)	0.03	0.07	0.09	0.03	0.04
Protein Solubility Index PSI (%)	85.74	81.95	76.44	76.65	74.6

Table 4: Correlation Coefficient between Anti-Nutritional Factors in Alkaline Treated Soyabeans

Parameters	**Correlation**	**P Value**
Phytic Acid v PSI	0.956	<0.05
Phytic Acid v TIA	0.956	<0.05
Phytic Acid v Reduction in TIA	0.956	<0.05
Phytic Acid v Alkaline Treatment	0.892	<0.05
TIA v PSI	0.984	<0.01
Reduction in TIA v PSI	0.984	<0.01
PSI v Alkaline Treatment	0.943	<0.05
TIA v Reduction in TIA	1.000	<0.01
TIA v Alkaline Treatment	0.886	<0.05
Reduction in TIA v Alkaline Treatment	0.882	<0.05

Discussion

The results of the chemical composition of alkaline treated soyabeans were consistent with the reports of HNIS, (1989); Ensminger *et al* (1990) and Ari *et al*. (2012) who observed variations in the proximate composition of soyabeans subjected to different processing methods. Similar variations were also reported by Ayanwale (1999) for

different concentration levels of sodium bicarbonate used in the processing of soyabeans. The dry matter, crude protein, ether extract, crude fibre, and total ash values recorded for alkaline treated soyabeans by Ayanwale (1999) were slightly different from the values obtained in this study.

The results obtained for amino acid profile indicates that processing methods affected the overall amino acid profile of soyabeans as earlier observed by OECD (2001). One percent (1%) K_2CO_3 presented the best overall amino acids profile. The differences observed in this study and that reported by DeGroot and Slump (1969) on the loss of amino acids resulting from the effect of potassium hydrogen phosphate (KH_2PO_4) on soyabeans seeds may be accounted for by the differences in concentration levels of the alkaline salts used.

The reduction in both TIA and phytic acid is attributed to treatment effects. This finding is supported by Akande and Fabiyi (2010); Nwosu (2011); Ocheme and Mikailu (2011) and Oskoueian *et al*. (2011) who reported reduction in anti-nutritional nutrients of oilseeds and cereals through blanching and treatment with different alkaline salts. The correlation between anti-nutritional factors and alkali treatments suggest strong effects of alkaline treatment in the removal of ANFs. This was earlier observed by Cano-Asseleih *et al*. (1989); Qin *et al*. (1996) and Vidal-Valverde *et al*. (1979). The changes observed in the nutrient composition of nixtamalized soyabeans is a function of the alkaline used. This is consistent with the findings of Sefa-Dedeh (2004) who observed the effect of nixtamalization on the chemical and functional characteristics of maize subjected to different alkaline treatments.

Conclusion

Nixtamalized Soyabeans processed with 1% K_2CO_3 is recommended from the findings of this study. This is on account of the protein quality preservation potentials and the inactivation of antinutritional factors in soyabeans required by the poultry feed industry.

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