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Nutrient Composition, Amino Acids Profile and Anti Nutritional Factors of Alkali Treated Soya Bean (*Glycine max*)

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Abstract: This study evaluates the nutrient composition, amino acids profile and anti nutritional factors of alkali treated soya bean for poultry feeds using sodium carbonate, potash, sodium hydroxide and compared with soaking in water. 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. 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. Treatment was observed to be better with 1% potash.

Key words: Alkali treatment, 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 of however contains several Anti-Nutritional Factors (ANF) in variable amounts. The use of alkali in nutritional improvement of feedstuff for poultry and livestock utilization has been reported (Addy *et al*., 1995; Ayanwale, 1999; Wanjekeche *et al*., 2003; Fayomi, 2004; Akande and Fabiyi, 2010; Oskoueian *et al*., 2011).

The treatment of several types of plant proteins with alkaline salts was 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 by 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 oilseeds and soyabeans. The use of blanching and other alkali salts to improve feeding quality and reduced 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 alkal salts in the inactivation of Tyrpsin inhibitor activity. The possibilities of alkaline salts to cause the release of bound minerals in feedstuff have 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 processing 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.

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MATERIALS AND METHODS

Seeds collection and processing: Soyabeans seeds *(Glycine max)* were procured from a local market in Lafia metropolis of Nasarawa State, Nigeria. The collected seeds were cleaned by winnowing and hand picking of stones and debris.

Experimental treatment: The raw soyabeans were subjected to three alkali treatments viz: sodium carbonate, potash), sodium hydroxide. Each of these alkali treatments served as experimental treatment groups to be compared with soaking in water. The different soyabean processing with alkaline are described as thus.

Soaking in water: The raw soyabeans were sorted to ensure homogeneous cleaned grains. The cleaned 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 hrs. The soaked grains were drained and sun dried by spreading on jute bags. The sun dried products were milled and bagged.

Soyabeans processed with sodium carbonate: The method reported by Ayanwale (1999) was adopted. The raw soyabeans were sorted to ensure homogenous cleaned grains. The cleaned grains were soaked in a 1% solution of sodium carbonate.

The sodium carbonate salt used was manufactured by Avondale Laboratories, England. The one percent (1%) aqueous solutions of the powdered sodium carbonate salt were prepared by dissolving 10.0 g (sodium carbonate) in 1,000 milliliters of water at room temperature.

The soyabeans was allowed to soak by immersing the soyabeans in the aqueous solution at room temperature for 24 hrs. 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 potash: The method reported by Ayanwale (1999) for alkali treatment of soyabeans was adopted. The raw soyabeans were sorted to ensure homogenous cleaned grains. The cleaned grains were soaked in a 1% solution of potash. The potash used was procured from local market in Lafia. The one percent (1%) aqueous solution of the potash was prepared by dissolving 10.0 g (potash) in 1,000 milliliters of water at room temperature.

The soyabeans were allowed to soak by immersing the soyabeans in the aqueous solution at room temperature for 24 hrs. 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 processing with sodium hydroxide: The raw soyabeans were sorted to ensure homogenous cleaned grains. The cleaned grains were soaked in a 1% solution to sodium hydroxide. The method reported by Ayanwale (1999) for alkali treatment of soyabeans was adopted.

The sodium hydroxide salt used was manufactured by Fisher Scientific Comp., New Jersey USA. The one percent (1%) aqueous solution of the powdered sodium hydroxide sample was prepared by dissolving 10.0 g (sodium hydroxide), in 1,000 milliliters of water at room temperature.

The soyabeans were alone to soak by immersing the soyabeans in the aqueous solution at room temperature for 24 hrs. The soaked and wet grains were drained and sun dried by spreading on jute bags until stable weight was attained. The sun dried products were milled and bagged.

Analytical procedures

Proximate analysis: Proximate composition of each of the alkali treated soyabeans samples 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 alkali treated soyabeans was 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 raw and soyabeans subjected to alkali treatment 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 differently processed soyabeans samples was

Table 1: Effect of alkaline treatment on the proximate composition of 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% $NaCO_3$	90.02	38.34	14.64	18.58	5.29	23.15	0.44	0.23
1% Potash	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 soyabeans

	Amino acids profiles (g/100 g protein)			
Amino acids	0% Alkaline	1% $NaCO_3$	1% Potash	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

Table 3: Effect of alkaline treatments on the value of anti-nutritional factors of soyabeans

Parameters	Raw soya	0% Alkaline	1% $NaCO_3$	1% Potash	1% NaOH
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/100 g)	345.00	325.00	66.00	48.80	48.60
Reduction in PA (%)	0.00	5.80	80.87	85.86	85.91
Urease assay ( ) pH)	0.03	0.07	0.09	0.03	0.04
PSI (%)	85.74	81.95	76.44	76.65	74.60

TIA = Trypsin Inhibitor Activity; PSI = Protein Solubility Index

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

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 alkaline treated soya beans (test ingredient) is presented in Table 1. The Dry Matter (DM) percentage ranged from 89.8-90.17% while Crude Protein (CP) percentage ranged from 38.34-40.27%. Crude Fibre (CF) had values ranging from 12.37-22.87%. The highest value of Ether Extract (EE) was obtained in 0% alkaline while the least (6.72%) was obtained in 1% potash. The total ash percentages ranged from 3.61-5.29% while Nitrogen Free Extract (NFE) percentages ranged from 21.08-25.34%. The highest Ca and P percentage values were 0.44 and 0.36% in 1% $NaCO_3$ and 0% alkaline respectively.

Amino acid profiles (g/100 g protein) of the alkaline treated soyabean are presented in Table 2. The values for lysine ranged from 4.79-5.78, while cystine had values ranging 0.80-1.07. Methionine values were highest (1.23 and 1.30) in 0% alkaline and 1% NaOH while 1% $NaCO_3$ gave the least (0.90). The values for phenylalanine ranged from 3.78-4.42.

Table 3 presents effect of alkaline treatment on the levels of anti-nutritional factors. The reduction in Trypsin Inhibitor Activity (TIA) ranged from 38.76% in the 0% alkaline treatment to 94.14% in 1% $NaCO_3$. Similarly, the percentage reduction of phytic acid ranged from 5.80% in 0% alkaline to 85.91% in NaOH. These values were 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 ( ) pH) 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.

DISCUSSION

The results of the proximate composition of alkaline treated soyabeans were consistent with the reports of HNIS (1989) who observed variations in the chemical composition of soyabeans subjected to different processing methods. Similar observations were reported by Ayanwale (1999) for different concentration of alkaline on soyabeans and Oskoueian *et al*. (2011) for

oilseeds. DM, CP, CF and total ash values observed for alkali treated soyabeans by Ayanwale (1999) were slightly different from the values obtained in this study.

Amino acid profile results of this study is in consonant with the observation of OECD (2001) who reported variations due to treatment effects on the amino acid composition of soyabeans. 1% potash gave the best overall amino acids, differences observed between this study and that reported by DeGroot and Slump (1969) on the loss of amino acids resulting from the effects of potassium hydrogen phosphate (KH_2PO_4) on soyabeans seeds may be accounted for by differences in concentration levels of the alkali 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).

Conclusion: Alkali treatment with 1% potash is recommended on account of the preservation of protein quality of soyabeans and the inactivation of anti-nutritional factors in soyabeans for poultry feeds.

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