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Nutritive Evaluation of Boiling Duration on the Anti-nutrients and Amino Acids Composition of Roselle Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Author GSB designed the study. Author AOA performed the statistical analysis. Author FOA wrote the protocol. Author SD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted to examine how cooking Roselle seeds (*Hibiscus sabdarrifa L.*) in 100°C at different lengths of time (from 10 to 60 minutes) affects the contents of antinutrients and amino acid composition, to select the most optimal cooking time span for these seeds. The Roselle seeds (*Hibiscus sabdarrifa L.*) were boiled at 100°C for 10, 20, 30, 40, 50 and 60 minutes and a raw sample was assayed. There were seven treatment groups in a completely randomized design (CRD). Data collected were analysed using the Analysis of Variance procedures of S.A.S. 9.0 and significant difference ($P<0.05$) in means among the treatments was separated using a Tukey test. The result of the laboratory trial showed that there were significant ($P\leq0.05$) differences in antinutritional factors and amino acid composition of Roselle seeds boiled at different durations. Boiling of Roselle seeds for 60 minutes significantly ($P\leq0.05$) reduced the anti-nutritional factors (TIA, Tannin, Phytic acid, and Oxalate). Boiling for 60 minutes increased histidine, threonine, serine, glutamic acid and proline composition of Roselle seeds (*Hibiscus sabdarrifa L.*). The

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chemical composition, micro and macro minerals, amino acid composition of boiled Roselle seeds for 60 minutes revealed the overall suitability of Roselle seeds as an alternative feed ingredient in poultry nutrition. It is therefore recommended that boiling Roselle for 60 minutes can be used to replace maize in the diets of broiler birds.

Keywords: Roselle seeds; amino acids; antinutritional factors; processing methods.

1. INTRODUCTION

The poultry industry occupies a unique position in the Livestock sector of the economy because they are highly prolific and are a good converter of feeds [1,2]. The best solution to Nigeria's protein intake particularly from animal origin is to increase poultry production [3]. The cost of feeding is attributed to the high cost and scarcity of conventional feed ingredients like maize, groundnut cake, soybean meal and fish meal. Soybean which is a major protein source in poultry diets has several food and industrial uses.

The major factor militating against an increase in poultry production is non-availability of feed at the economic price. Most of the protein concentrates are very expensive since they are also stapled food for our ever-increasing human population. Given the above, attempts have been made by poultry nutritionists to explore cheaper and safe alternative protein sources to replace soybean cake and groundnut cake in poultry diets. One of such alternative sources is Roselle seeds.

Alternative plant protein sources and their nutritional potentials in the diets of broiler birds in developing countries like Nigeria have been highlighted [5].

Roselle seed is presently sold in Nigeria at two fifths of the cost of soybean meal and about half the price of groundnut cake, thus justifying the need to investigate its use in feeding broiler chickens. Roselle seeds in 100°C at different lengths of time (from 10 to 60 minutes) affects the contents of antinutrients and anti-amino acids, in order to select the most optimal cooking time span for these seeds – one in which a large loss of antinutrients would take place, but at the same time, a good composition of amino acids would be preserved. This would allow substitution of corn in chicken feed and would have an economic impact. From the standpoint of economics, availability and nutritional value, Roselle seed represents an attractive alternative in broiler chicken diets.

The usefulness of a protein feedstuff for poultry depends upon its ability to supply sufficient amount of the essential amino acids (EAA) that the bird requires, as well as the protein digestibility and the level of toxic substances associated with it [4].

The high demand that outweighs the supply of grains especially soybean cake and groundnut cake that are the main sources of protein in animal feeds, stapling food for Nigerians and Agro-allied industries has resulted in a prohibitive cost of livestock feeds. Efforts are now geared towards alternative protein sources that are cheap and readily available with less competition with humans. This will reduce the cost of feed and animal products.

Therefore, this study was designed to evaluate the duration of boiling on antinutritional and amino acids composition of Roselle seeds.

2. MATERIALS AND METHODS

2.1 Source and Processing of Roselle Seeds

The Roselle seeds (*Hibiscus sabdarrifa L.*) that were used for the study were purchased from the open market at Potiskum, Yobe State, Nigeria and were bulked before processing. The seeds were thoroughly cleaned by winnowing and sieving. The seeds were processed as follows:

Batches of 250 g Roselle seeds were subjected to various boiling time/durations: 0 (Raw), 10, 20, 30, 40, 50 and 60 minutes respectively. Each duration of boiling time represented as a treatment. For each boiling time, 1 liter of water was first brought to the boiling point in a 4 - liter aluminum capacity pot. A batch of 250 g Roselle was then poured into the boiling water. From this point, the specified time for boiling was taken. At the end of the period of boiling, excess water was drained off. The boiled seeds were then sun-dried for 5 days.

2.2 The Effect of Duration of Boiling on the Concentration of Anti-nutritional Factors and Minerals of Roselle (*Hibiscus sabdariffa*) Seed

Samples of differently boiled Roselle seeds (*Hibiscus sabdarrifa L.*) (0, 10, 20, 30, 40, 50 and 60 minutes) were further subjected to quantitatively phytochemical analysis for Anti-nutrients by the standard methods described by [4] at the Biochemical Laboratory of the National Research Institute for Chemical Technology (NARICT), Massawa, Zaria. Kaduna State, Nigeria.

2.3 Determination of Tannin

Tannin was determined using the standard method described by [6]. Two grams of the sample of Roselle seed Meal (RSM) was boiled with 300 ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non- absorbent cotton wool. Twenty-five mls of the distilled water was measured into a 2-liter porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution turned green, then few drops of 0.1N potassium permanganate was added. The titer was multiplied by 0.0066235 to obtain the amount of tannin in the sample.

2.4 Determination of Phytate

Phytate was determined using the standard method described by [6]. A known weight (5 g) of a ground sample of RSM was soaked into 100 ml of 2% HCl for 5 hours and filtered through a filter paper, and 25 ml of the filtrate was taken and 50 cm^3 of 0.3% potassium thiocyanate solution was added in a conical flask. The mixture was titrated using a standard solution of ferric chloride ($FeCl_{3)}$ until a brownish – yellow color persisted for 5 minutes. The concentration of the $FeCl_3$ was 1.04% w/v

Calculation:

Mole ratio of $FeCl_3$ to Phytate = 1:1
The concentration of Phytate phosphorus

$$= \frac{Titre\ value}{1000} \times \frac{0.064}{} \times weight\ of\ sample$$

2.5 Determination of Oxalate

The method of [6] was used to determine the oxalate content in the RSM. The total oxalic acid of the powdered samples was determined by weighing 2 g into a 250 ml flask. Then 190 ml distilled water and 10 ml of 6M hydrochloric acid were added. The mixture was heated for 1hour in boiling water bath, cooled, then transferred into a 250 ml volumetric flask, and diluted to volume and filtered. Four drops of methyl red indicator were added followed by concentrated ammonia till the solution turned faint yellow. It was then heated to 100°C and allowed to cool and filtered to remove precipitate containing ferrous ions. The filtrate was boiled, and 10 ml of 5% calcium chloride was added with constant stirring. It was then allowed to stand overnight. The mixture was filtered through Whatman No 40 filter paper. Then the precipitate was washed several times with distilled water and transferred to a beaker and 5 ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80°C and titrated against 0.5% potassium permanganate until the pink color persisted for approximately one minute. A blank was also run for the test sample from the amount of potassium permanganate used, the oxalate content of the RSM sample was calculated using the formula below:

1 ml of potassium permanganate = 2.24 mg oxalate.

2.6 Determination of Trypsin Inhibitor

The method outlined by [6] was used in the determination of trypsin inhibitor content of RSM. A known weight (1 g) of ground sample was weighed and dispersed into 50 cm^3 of 0.5 M NaCl solution. The mixture was stirred for 30 minutes at room temperature and centrifuged. The supernatant was filtered through Whatman No 41 filter paper. The filtrate (extract volume) was used for the assay.

Preparation of standard trypsin and substrate in 0.1 M phosphate buffer pH 7.7 was prepared, and 1 cm^3 of trypsin inhibitor was added into 2 cm^3 of trypsin standard solution and incubated for 10 mins at 37°C, and then 5 cm^3 of substrate and 2 cm^3 of trypsin was added into a test tube and incubated at 37°C for 10 minutes. A blank of 5 cm^3 substrate was prepared in a test tube (without trypsin inhibitor extract added). The contents of the test- tube was left for 10 minutes, and the reaction was stopped by adding 3 cm^3 5% of the blank substrate. It was then filtered and then measured spectrophotometrically at 410 nM. The trypsin inhibitor activity was expressed as the number of trypsin unit

incubated (TUI) per unit weight of the sample analyzed.

Calculation:

$$\frac{TUI}{mg} = \frac{(b - a)}{0.1} \times 1$$

Where b = absorbance of test sample solution
a = absorbance of the blank

$$T = 1 \times \frac{V_f}{V_a} \times D \times \frac{1}{W}$$

Where:

W = weight of the sample
V_f = total volume of extract used in the assay
D = dilution factor
V_a = volume of standard solution
T = Trypsin Inhibitor

2.7 Estimation of Amino Acids Content of Both the Raw and Boiled Roselle Seeds

The Amino Acid profile in the known sample was determined using methods described by [7]. The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technical sequential Multi-Sample Amino Acid Analyser (TSM).

2.7.1 Defatting sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in Soxhlet extraction apparatus [7].

2.7.2 Nitrogen determination

A small amount (200 mg) of ground sample was weighed, wrapped in Whatman filter paper (No. 1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulfate (Na_2SO_4), copper sulfate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in the standard volumetric flask. An aliquot (10 ml) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing four drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standardizing 0.01 N hydrochloric acid to grey colored

$$Nitrogen \quad (\%) = \frac{(a - b)x0.01x14\,xVx\,100}{WxC}$$

Where:

a = Titre value of the digested sample
b = Titre value of the blank sample
v = Volume after dilution (100 ml)
W = Weight of dried sample (mg)
C = Aliquot of the sample used (10 ml)
14 = Nitrogen constant in mg.

2.7.3 Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. 7 ml of 6N HCl was added, and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis, e.g., methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C± 5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip, and the content was filtered to remove the humans. It should be noted that tryptophan is destroyed by 6N HCl during hydrolysis.

The filtrate was then evaporated to dryness in a hot air oven. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

2.7.4 Loading of the hydrolysate into tsm analyzer

The amount loaded was between 5 to 10 microliters. This was dispended into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysate and period of the analysis lasted for 76 minutes.

2.7.5 Data analysis

All data obtained from this study were subjected to analysis of variance (ANOVA) using the general linear model procedure of [8]. Significant levels of differences among treatment means

were separated using Duncan's Multiple Range test [9]

3. RESULTS AND DISCUSSION

The result of the effect of duration of boiling of Roselle seeds on the level of some antinutritional factors (ANFs) is subsumed in Table 1. A significant decrease ($P \leq 0.05$) in trypsin inhibitors (TIA), phytic acid and oxalate activity, was observed as the duration of boiling increased. Processing methods significantly ($P<0.05$) reduced antinutritional factors as compared to the control which had higher concentrations in Table 1. The levels of phytic acid, trypsin inhibitor, and oxalate activity were reduced when raw Roselle seed was subjected to boiling at different durations. The observation agrees with [10], who confirmed that there is a reduction in anti-nutritional content through leaching in water when legume seeds are subjected to boiling.

Liener [11] reported that processing of leguminous seeds either by soaking, cooking or fermentation significantly improved the nutritional and functional properties of legume seeds. Munro and Bassir [12] reported a significant reduction of phenolic compound in soaked, cooked, sprouted and fermented Roselle seeds compared to raw Roselle seeds. The percentage reduction in levels of tannin, phytate, saponin and trypsin inhibitor in Roselle seed boiled at varying durations also agreed with the findings of [13] who confirmed that processing of legume seeds (soaking, autoclaving, fermentation and roasting) led to a reduction in some anti-nutritional content. The hydrothermal treatment of seeds was, however, more effective in reducing the anti-nutrients contents because of the hydrolysis of the chemical compounds that make up the antinutrients. The effectiveness of reducing anti-nutrients by boiling has been reported for other seeds such as soybeans [14].

The effect of boiling durations of Roselle seeds on the level of amino acids (AAs) profile. There were significant differences ($P \leq 0.05$) across different boiling durations in the seventeen (17) AAs (Lysine, Valine, Methionine, Isoleucine, Leucine, phenylalanine, Cystine, Arginine, Aspartic acid, serine, Glycine, Alanine, Tyrosine, Histidine, Threonine, cysteine**,** and Glutamic) detected as shown in Table 2. The highest concentration of amino acids was recorded in raw Roselle seed (0% boiling time) in lysine (5.68 g/100 g), arginine (5.61 g/100 g), aspartic acid (11.01 g/100 g), glycine (4.51 g/100 g), alanine (3.99 g/100 g), cystine (1.41 g/100 g), valine (5.55 g/100 g), methionine (2.72 g/100 g), isoleucine (3.51 g/100 g), leucine (6.81 g/100 g), tyrosine (3.50 g/100 g) and phenylalanine (5.20 g/100 g) which differed significantly ($P<0.05$) from the processed Roselle seeds. Boiling Roselle seeds at 30, 40, 50 and 60 minutes were not significantly different ($P>0.05$) with the highest values recorded in histidine which differed significantly ($P<0.05$) from the values recorded from raw (1.86 g/100 g), 10 (1.92 g/100 g) and 20 minutes (2.01 g/100 g) boiling of Roselle seeds. The reduction in the concentration of amino acids in the boiled Roselle seed could be associated with denaturing at high temperature which may lead to loss of toxic substances, particularly of the proteinaceous toxins such as trypsin inhibitors and haemagglutinins [15] and as heating progress, protein quality decreases and consequently reduce due to Maillard browning reaction which causes reduction in lysine content [11,15]

The results of amino acid composition in Roselle seed showed the possibility of using Roselle seeds especially as a source of protein and amino acid composition for human nutrition. These findings conform with the reports of [10] which showed that Roselle seeds could be used as a strong source of protein and the protein quality depends on the amino acid composition.

The increased amino acids (lysine, arginine, aspartic acid, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine) of boiled Roselle seed meal sample could be attributed to one of the catabolic process which involves the breakdown of proteins into amines and amino acids during boiling as reported by [13]. Variations in the raw and processed seeds of Roselle may be linked to trans-amination and deamination reactions. Boiling Roselle seeds for 10-60 min at 100 ^{o}f reduces some of the amino acid compositions (Histidine, Threonine, serine, glutamic acid and proline) of the seeds as compared to the control. These reductive effects of boiling on some of the amino acid compositions of the seeds may be attributed to amadori rearrangements which led to the production of glucose phenylosazone from glucose phenylhydrazone as a result of high and prolonged heat.

Table 1. Antinutritional factor content of Roselle seeds boiled at 100°C for varying time durations and percentage of destruction (mg/100 g)

Parameters	Raw (0)	10	20	30	40	50	60	SEM
TIA	12.35[g]	11.73[f]	10.24[e]	10.02[d]	9.01[c]	8.02[b]	7.16[a]	0.11
%destruction of TIA		5.02	17.09	25.65	36.5	51.81	69.1	
Tannin	2.45[g]	2.41[f]	2.09[e]	1.54[d]	1.46[c]	1.31[b]	1.06[a]	0.02
%destruction of Tannin		3.55	17.01	44.01	68.18	83.07	106.62	
Phytic Acid	1.41[e]	1.40[e]	1.35[e]	1.24[d]	1.15[c]	1.05[b]	0.77[a]	0.04
%destruction of Phytic Acid		0.71	4.26	12.06	18.44	25.53	45.39	
Oxalate	1.17[g]	1.11[f]	1.09[e]	1.06[d]	1.02[c]	0.98[b]	0.87[a]	0.01
%destruction of Oxalate		5.13	6.84	9.40	12.82	16.24	25.64	

[abcdefg]*Means with different superscripts within the same row are significantly(P<0.05) different. SEM: standard error of mean*

Table 2. Effect of duration of boiling on amino acids profile of Roselle seeds at 100^{0}C for varying time durations (g/100g protein)

Parameters (g/100 g protein)	Raw (0)	10	20	30	40	50	60	SEM
Histidine	1.86[d]	1.92[c]	2.01[b]	2.14[a]	2.15[ab]	2.15[a]	2.17[a]	0.01
Arginine	5.61[a]	5.26[b]	5.11[c]	5.04[d]	5.01[e]	4.83[f]	4.62[g]	0.01
Aspartic Acid	11.01[a]	10.85[b]	10.47[c]	10.08[d]	10.01[e]	9.93[f]	9.61[g]	0.01
Threonine	2.81[f]	2.96[e]	3.11[d]	3.14[d]	3.20[c]	3.42[b]	3.51[a]	0.02
Serine	2.93[d]	3.01[c]	3.04[c]	3.04[c]	3.08[bc]	3.13[b]	3.21[a]	0.02
Glutamic acid	13.30[d]	13.80[c]	13.92[c]	14.79[b]	14.90[b]	15.11[a]	15.20[a]	0.08
Proline	2.97[d]	3.01[c]	3.02[c]	3.06[c]	3.18[b]	3.23[b]	3.36[a]	0.05
Glycine	4.51[a]	4.29[b]	4.14[c]	4.01[d]	4.00[d]	3.87[e]	3.53[f]	0.01
Alanine	3.99[a]	3.96[a]	3.78[b]	3.49[c]	3.32[d]	3.35[d]	3.27[d]	0.03
Cystine	1.41[a]	1.22[b]	1.20[b]	1.19[b]	1.05[c]	0.98[d]	0.93[d]	0.01
Valine	5.55[a]	5.33[b]	5.04[c]	4.95[d]	4.94[d]	4.61[e]	4.20[f]	0.02
Methionine	2.72[a]	2.28[b]	2.19[c]	2.11[d]	2.05[e]	2.01[f]	1.98[f]	0.02
Lysine	5.68[a]	5.45[b]	5.26[b]	5.11[c]	4.87[d]	4.61[e]	4.25[f]	0.19
Isoleucine	3.51[a]	3.38[b]	3.21[c]	3.17[c]	3.08[d]	2.91[e]	2.73[f]	0.01
Leucine	6.81[a]	6.71[a]	6.50[b]	6.31[c]	6.16[d]	5.88[e]	5.50[f]	0.02
Tyrosine	3.50[a]	3.31[b]	3.15[c]	3.14[c]	2.99[d]	2.99[d]	2.36[e]	0.02
Phenylalanine	5.20[a]	4.95[b]	4.82[c]	4.59[d]	4.54[e]	4.39[f]	4.13[g]	0.02

[abcdefg]*Means with different superscripts within the same row are significantly(P<0.05) different. SEM: standard error of mean*

4. CONCLUSIONS

The amino acid composition of boiled Roselle seeds for 60 minutes revealed the overall suitability of Roselle seeds as an alternative feed ingredient in poultry nutrition and may serve as a potential source of functional ingredients. Roselle seeds could be used as a supplement food or as diet enrichment especially in the low protein diets due to increased histidine, threonine, serine, glutamic acid and proline composition of Roselle seeds.

5. RECOMMENDATIONS

The boiled Roselle seeds up to 60 minutes could be a biomarker for alternative feed ingredient in poultry diet.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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