EFFECT OF PROCESSING METHODS ON PROXIMATE AND ANTI-NUTRITIONAL FACTORS OF ROSELLE (*HIBISCUS SABDARIFFA* L.) SEEDS

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ABSTRACT
Roselle seeds were analyzedto assess the effect of different processing methods on their proximate and antinutritional factor composition. The seeds were divided into four treatments containing unprocessed, soaked, sprouted and boiled roselle seeds. Each treatment was replicated three times. Effect of processing methods on proximate composition revealed that boiling and sprouting significantly (P<0.05) increased ether extract but decreased nitrogen free extract. Crude fiber content was significantly (P<0.05) depressedby soaking in water.Crude protein, however, was unaffected (P<0.05) by the processing methods. Sprouting significantly (P<0.05) increased calcium and depressed phosphorus, boiling on the other hand significantly (P<0.05) reduced calcium in the roselle seeds. Boiling was most effective (P<0.05) at depressingtrypsin inhibitor activity and tannin content of roselle seeds, and phytate was significantly (P<0.05) depressed bysprouting.
Key words: Roselle, processing methods, proximate, antinutrients

INTRODUCTION
The search for cheaper alternative unconventional feedstuffs to remedy the competition between man and livestock for the available conventional ingredientscannot be over emphasized. Roselle (*Hibiscus sabdariffa* var. *sabdariffa),* is an annual, herbaceous, shrub (Alagbejo, 2000). It is cultivated in India, East Indies, and parts of South East Asia, the semi – arid environments of West and North-East Africa, and to some extent in tropical America (Morton, 1987). In Nigeria, roselle is found mainly in the Guinea and Sudan savanna vegetational zones, and it is produced in large quantities (Alagbejo, 2000). The calyces have been the most utilized part of the plant; processed to produce jam, jelly, soup and the popular "*zobo*" beverage in Nigeria (Schippers, 2000; Kwari*et al.,* 2010). There are two basic types; the green and the red or brown. Seed yields of 400 – 600 kg for the green type, and 1000 kg per hectare for the red type is achievable (Schippers, 2000). Previous studies have shown that roselle seeds contain high amounts of protein, dietary fiber and minerals such as phosphorus, magnesium and calcium (Ismail *et al.,* 2008; Tounkara*et al*., 2011).The seeds are fermented to make a cake referred to as sorrel 'meat' or '*iyu*' as it is called among the *Taroh* people of Plateau State, Nigeria.Hainida*et al.,* (2008) found that lysine to arginine ratio was low in roselle seeds (0.4 – 0.5), implying reducedartherosclerotic tendency (Morita *et al*., 1997).Roselle seed is reported to contain anti-nutritional factors that have been associated with a reduction in food digestibility and a decrease in nutrient bioavailability.These include tannin (105.50mg/100g), phytic acid (57.13mg/100g), protease inhibitors (285.27TIA/100g) (Abdu *et al*., 2008), and total polyphenols (878.33mg/100g) (Yagoub*et al*., 2008). Effective utilization of roselle seeds bynon-ruminant livestock will necessitate processing to reduce or inactivate anti-nutrient activity. This study is intended to evaluate the nutritive and anti-nutritive tendencies of differently processed roselle seeds.

Materialsand Methods
Source and Preparation of Roselle Seeds
Roselle seeds were purchased from the market in Mangu, Plateau State. The samples were winnowed and sieved, and then pooled. 1kgeach of the differently processed roselle seeds was used. The first part was milled without processing of any kind (URS). The second part was soaked in 2litres of water for 24 hours, drained, and sun-dried until crisp (SRS) before milling. The third part was soaked in 2 litres of water for 24 hours, drained and packed into jute sacks and left at room temperature for 48 hours to sprout. The sprouted seeds were sun-dried until crisp (SPRS). The fourth part was added to 4 litres of water already boiling over fuel wood and cooked for 30 minutes according to method described by Kwari*et al*. (2010). The water was drained and the seeds were sun-dried until crisp (BRS), then milled.

Treatments

Experimental seed samples drawn from pooled roselle seeds were randomly assigned to fourtreatments: unprocessed roselle seeds (URS),soaked roselle seeds (SRS),sprouted roselle seeds (SPRS), andboiled roselle seeds (BRS),respectively. Each treatment had three replications.

Chemical Analysis

Proximateand spectrophotometry analysis

Moisture contentand dry matter were determined by placing the sample in a 105°C oven for 20 hours (at constant weight) (AOAC, 1980). Ash determination was performed by burning the sample at 550°C in a muffle furnace for 3 hours (AOAC, 1980). Crude Protein was determined by the Kjeldahlprocedure (AOAC, 1980).It was estimated by multiplying the nitrogen value obtained with a conversion factor of 6.25 (Galyean, 2010). Ether extract was determined by Soxhlet extraction method andcrude fiber content was determined by digestion method (AOAC, 1980).Nitrogen free extract (NFE) was calculated by difference;

$$NFE (\%) = (100 - \%H_2O - \%Ash - \%CP - \%EE - \%CF)$$ (Galyean, 2010).

Calcium and phosphorus contents were analysed using spectrophotometric method as outlined by AOAC (1980).

Determination of anti-nutritional factors

Phytic acid was determined by the method described by Reddy *et al*. (1982). 5.0cm^3 of 0.3% potassium thiocyanate was added to 25cm^3 of the filtered sample soaked in 2% HCl for 5 hours. The mixture was titrated with a standard solution of 1.04% ferric chloride ($FeCl_3$) until a brownish yellow colour persisted for 5 minutes. Concentration of phytate phosphorus was determined, and phytic acid calculated on the assumption that it contains 28.20% phosphorus by weight.Tannin content was determined by the method described by Allen *et al.* (1974). The sample was prepared by weighing 0.5g oven dried ground sample into a 100ml conical flask. 60cm^3 of distilled water was added and allowed to boil for 1 hour, and filtered while still warm into a volumetric flask, and then made to mark. The extract was prepared using 0 – 3ml aliquot of tannic acid. 3.0cm^3 aliquot of the sample was pipette into a 50cm^3 volumetric flask. 2.5% folin-denis reagent was added and the mixture was refluxed for 2 hours, followed by the addition of 17% Na_2CO_3 solution. The optical density (absorbance) readings were taken at 760nm wade length using water as reference. Tannin value was then calculated as

$$\text{Soluble tannins (mg/g)} = \frac{\text{Concentrate (mg) x extract volume (cm)}}{\text{10 x aliquot volume (cm}^3\text{) x sample weight (g)}}$$

Trypsin inhibitor activity (TIA) was determined using the AOAC (1980) method. The sample was determined by extracting with 10 volumes of petroleum ether. The extract was prepared by sustaining 1g of the determinate sample in 20ml of 0.05 NH_4Cl. A standard was also prepared by adding 1.0ml of trypsin solution, 2ml 2.0% casein and 1ml 0.12N PO_4 into 4.0ml phosphate buffer, with pH 7.6.

Data Analysis

Data generated were analysed for variance (ANOVA) using SPSS (2012) statistical software. Significant differences in treatment means were separated using the Duncan's Multiple Range Test (DMRT) as outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Effect of processing methods on proximate, calcium, and phosphorus contents of roselle seeds.

Crude protein values (29.90 – 32.53%) obtained are higherthanreported by Aliyu *et al*. (2021) for raw boiled seeds, and lower than reported by Kwari*et al*. (2011) for soaked, sprouted, fermented and boiled roselle seeds. Crude protein did not differ ($P<0.05$) among treatments, corroborating the report of Kwari*et al*. (2011) and Duwa*et al*. (2012) who had found no significant ($P<0.05$) effect of soaking on crude protein of processed roselle seeds. Abdul *et al*. (2008) found no significant ($P<0.05$) difference in crude protein levels as a result of boiling roselle seeds, though Aliyu *et al*. (2021) reported significant ($P<0.05$) increase.Halimatul*et al.* (2007) reported similarity in protein quality of dried (unprocessed) roselle seeds androselle seeds boiled at 100^{o}C for 30 minutes.

Boiling and sprouting significantly ($P<0.05$) increased ether extract in the seeds, probably as a result of decrease in non-lipid component of the seeds (Duwa*et al.,* 2012). Boiling significantly ($P<0.05$) lowered nitrogen free extract, which might have been attributed to leaching of soluble components into the boiling water. Yagoub*et al.* (2008) and Duwa*et al*. (2012) had reported significant ($P<0.05$)

reduction in soluble carbohydrates in boiled roselle seeds.Contrary to the findings of this study, Aliyu *et al*. (2021) reported decreased (P<0.05) ether extract as a result of boiling.

Table 1: Effect of processing methods on nutrient and mineral content of unprocessed and processed roselle seeds.

Nutrient (%)	URS	SRS	SPRS	BRS	SEM
Crude protein	29.90	32.53	30.63	30.28	3.21^{NS}
Ether extract	14.26^{b}	15.25^{b}	17.94^{ab}	20.24^{a}	1.73^{*}
Crude fiber	16.51^{ab}	13.83^{b}	15.07^{ab}	19.28^{a}	1.97^{*}
Ash	4.18	4.62	4.07	4.69	0.45^{NS}
Nitrogen free extract	30.80^{a}	30.17^{a}	29.39^{ab}	22.25^{b}	3.17^{*}
Macro - minerals (%)					
Calcium	0.26^{b}	0.21^{bc}	0.36^{a}	0.19^{c}	0.03*
Phosphorus	0.18^{ab}	0.17^{ab}	0.15^{b}	0.20^{a}	0.02^{*}

URS=Unprocessed roselle seed; SRS=Soaked roselle seed; SPRS=Sprouted roselle seed; BRS=Boiled roselle seed. a, b, c, means within the same row bearing different superscript differ significantly (P<0.05); SEM=Standard Error of Means; NS=Not significant (P>0.05); *=Significant difference (P<0.05).

Boiling significantly (P<0.05) increased crude fiber.This may be attributed to the loss of some soluble nutrient fractions in the boiling water. Decrease in nitrogen free extract (NFE) corresponded to increase in crude fiber.Increased crude fiber level in roselle seeds due to boiling was also reported by Kwari*et al.* (2011). Soaking, however, significantly(P<0.05) depressed crude fiber.Crude fiber values range between 13.83 and 19.28%. Slightly higher crude fiber levels than obtained in this study were reported by Kwari*et al*. (2011) and Duwa*et al*. (2012). Depressed NFE due to boilingis inversely supported byhigh crude fiber and high fat levels in the seed(Lykke and Padonou, 2019).

No significant (P<0.05) difference was observed in ash content among the various treatments. Total mineral (ash) content did not differ significantly (P<0.05), indicating no important mineral loss. Ash content of sprouted and boiled roselle seeds were similar to that reported by Yagoub*et al.* (2008) and Tounkara*et al.* (2011) respectively, Anhwange*et al*. (2006) found that roselle seeds are a good source of Ca, P and Mg.

Calcium and phosphorus levels varied significantly (P<0.05). Boiling significantly (P<0.05) decreased calcium, corroborated by the findings of Yagoub*et al*. (2008) who had reported similar decrease in calcium content of soaked and boiled roselle seeds, but contradicting Abdurrahaman*et al*. (2021) who reported increased calcium by boiling. The findings of this study may beattributed to leaching out of minerals into the water medium.

Sprouting significantly (P<0.05) decreased phosphorus in the seeds, conforming to Kwari*et al.* (2011) who reported similar reduction in phosphorus level of sprouted roselle seeds. On the contrary, Yagoub*et al*. (2008) had found no significant (P<0.05) difference in phosphorus level of 48 hours sprouted roselle seeds. The decrease in phosphorus may be due to increased demand for nutrients, particularly phosphorus by the sprouted seeds.

Effect of processing methods on antinutritional factors

Inhibitors of trypsin prevent this enzyme from performing a crucial role in the digestion of proteins by neutralizing it, causing additional trypsin secretions from the pancreas (Lyman and Lepkovsky, 1957). Trypsin contains a large amount (15 – 22%) of important sulfur amino acids, methionine and cystine (Hwang *et al.,* 1986). Soaking, sprouting, and boiling decreased trypsin inhibitor activity (TIA) by 11.54, 7.69, and 69.23%, respectively in the roselle seeds. Reduction in TIA due to soaking of roselle seeds was also reported by Halimatul*et al.* (2007). Investigations with some legume seeds such as *Phaseolus vulgaris* (Desphande and Sheryan, 1983), African oil bean seed (*Pentaclethra macrophylla*Benth) (Kingsley, 1995), and mucuna seeds (Tuleun and Igba, 2008; Mugendi*et al*., 2010) had reported decreased TIA due to soaking. Since trypsin inhibitors are low molecular weight proteins, their extraction from seeds to the soaking medium is quite possible. Sprouting had the least reduction of trypsin inhibitor activity. 69.23% reduction in TIA obtained due to boiling supported the report of Abdu *et al.* (2008) who reported similar decrease in TIA of roselle seeds subjected to different boiling periods. TIA is heat labile, therefore, heat degradation should be responsible for destroying the TIA during boiling (Ismail *et al.,* 2008; Tuleun and Igba, 2008; Mugendi*et al.,* 2010). Boiling was the most effective in reducing TIA.

Table 2: Effect of processing methods on anti-nutritional factors of roselle seeds

Anti-nutritional factors (mg/100g)	URS	SRS	SPRS	BRS	SEM
Trypsin inhibitor activity	0.52[a]	0.46[a]	0.48[a]	0.16[b]	0.03*
Percent reduction	-	11.54	7.69	69.23	-
Phytic acid	51.00[a]	47.02[a]	23.84[b]	50.21[a]	4.05*
Percent reduction	-	7.80	53.26	1.55	-
Tannin	0.31[a]	0.26[ab]	0.28[a]	0.22[b]	0.02*
Percent reduction	-	17.36	9.33	29.90	-

URS=Unprocessed roselle seed; SRS=Soaked roselle seed; SPRS=Sprouted roselle seed; BRS=Boiled roselle seed. a, b, c, means within the same row bearing different superscript differ significantly (P<0.05); SEM=Standard Error of Means; *=Significant difference (P<0.05).

Approximately 60 – 80% of phosphorus in cereal grains and oil seeds are bound in phytate complexes, thereby, resulting in reduced availability of minerals. Soaking, sprouting, and boiling of roselle seeds reduced phytic acid content by 7.80, 53.26, and 1.55% respectively, in agreement with the findings of Yagoub*et al.* (2008). Sprouting was responsible for thehighest percentage reduction in phytic acid. This may be attributed to the fact that phytic is a water-soluble salt which may be leached into the soaking water (Kakati*et al.* 2010), and to increased phytase activity accompanied by a corresponding decrease in phytate content during sprouting (Hafez *et al.,* 2000; Akande and Fabiyi, 2006; Mugendi*et al.,* 2010). Hafez *et al.* (2000) and El-Adawy*et al.* (2002) had also reported decreased phytic acid content due to sprouting in soybeans and chickpeas respectively. Effect of boiling on the phytic acid content of the roselle seeds was not significant (P>0.05), corroborated by Yagoub*et al.* (2008) who reported that phytic acid content of roselle seeds was unaffected by cooking. However, Aliyu *et al.* (2021)reported that cooking significantly (P<0.05) decreased phytic acid.

Dietary tannins adversely affect the digestion and absorption of protein, and to a lesser extent carbohydrate, by forming complexes with protein and carbohydrates resulting inthe formation of insoluble enzyme - resistantcompounds, leading to growth depression in animals. Soaking, sprouting, and boiling of roselle seeds decreased tannin by 17.36, 9.33, and 29.90%, respectively. Soaking and boiling significantly (P<0.05) decreased tannin. This is corroborated by the findings of Kwari*et al.* (2011) and Duwa*et al.* (2012) who reported reduced tannin content in roselle seeds due to soaking. Tannin is both water soluble and thermo-labile,therefore. Tannin levels obtained in this study are lower than reported by Kwari*et al.* (2011), Duwa*et al.* (2012), and Aliyu *et al.* (2021) for different categories of processed roselle seeds.This agrees with the findings of Kwari*et al.,* (2011) and Duwa*et al.,* (2012). Sproutingnot affect (P<0.05) tannin content.

CONCLUSION

Sprouting and boiling were most effective at deactivating trypsin inhibitor, phytic acid and tannin of roselle seeds.The significant (P<0.05) reduction of anti-nutritional factors obtained by the processing methods may improve nutrient utilization, digestibilityand performance of animals in feeding trials.

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