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Chemical composition and phyto-chemical factors of sorrel seeds (*Hibiscus sabdariffa L.)* boiled at varying durations

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***Target Audience**: Farmers and Researchers*

Abstract

This study was carried out to determine the chemical composition of sorrel seeds boiled at varying durations. Cleaned sorrel seeds were poured into three (3) litres of boiled water (at 100° C) per batch of 1kg (i.e. in ratio of 3:1) at different boiling durations of 0, 15, 30 and 45 minutes, respectively. The boiled sorrel seeds were sun dried by spreading on trays until well dried. Samples of the boiled sorrel seeds were analysed for chemical composition and phyto-chemical factors. The results showed that the duration of boiling significantly ($p< 0.05$) affected the crude protein, crude fibre and ether extract contents. Crude protein and ether extract values increased as the duration of boiling increased from 0 to 45 minutes. The lowest crude protein (28.00%) and crude fibre (12.80%) was obtained at 30 minutes duration of boiling while their respective values 29.22% and 14.50% were obtained from 45 minutes duration. Ether extract decreased with increased boiling duration. The values of anti – nutritional factor declined with increase in the duration boiling. There was also significant differences ($p<0.05$) in phytic acid and tannin levels.
The 45 minutes duration of boiling had the lowest value for phytic acid (0.19%), tannin (1.1%) and saponin (7.65%). It is concluded that processing of sorrel seed by boiling enhanced nutrient availability of crude protein, crude fibre and ether extract, and reduced anti-nutrients (phytic acid, tannin and saponin) to a tolerable level.

Keywords: Phyto-chemical factors; Boiling durations; Chemical composition; Sorrel seeds

Description of Problem

Sorrel (*Hibiscus sabdariffa*) seed is one of the alternative feed resources that have been found to thrive on a wide range of tropical soil conditions, and perform well on relatively poorer soil (1). *Hibiscus sabdariffa* is grown as border crop; the leaves are used for making soup and the calyxes for making the popular non-alcoholic beverage "Zoborodo" (2). Sorrel seeds contain a high amount of protein, dietary fibre, and minerals such as phosphorus (P), calcium (Ca) and magnesium (Mg) (3). The seeds contain about 35.90% crude protein (CP), 10.14% ether extract (EE), 10.09% ash and 15-17% crude fibre (CF) (4). It was reported by (5) that sorrel seeds contain 5.18% arginine, 16.5% CF, 13.5% EE and 38.57% CP. (6) reported 23.46% CP value, however, (7) reported that boiled sorrel seeds contain 22. 84% CP, 8.50% CF, 6.50% EE, 6.50% ash, 45.66% Nitrogen free extract (NFE) and 91.70% dry matter (DM). while (8) reported a value of 21.84% CP, 3.60% CF, 5.85% EE, 5.39% ash, 90.40% DM, 53.72% t NFE, 1.12% Calcium and 0.56% Phosphorus, respectively. Sorrel seeds are relatively cheap, readily available and less competitive between man and animals. A viable option of optimizing the productivity of animals and minimizing cost of protein is by the use of non-conventional

feedstuff such as sorrel seeds which is sold in Nigeria at less than two-fifth the cost of soybean and about half the price of groundnut cake, hence justifying the need to investigate its use in feeding animals. Sorrel seeds in their raw state are known to have a bitter taste which is attributed to anti-nutritional factors present in them. The unprocessed seed has been reported to contain total phenols, tannin and phytic acid as common anti-nutrients and these have been shown to have detrimental effects on the health and performance of animals (9 and 10). Effective utilization of sorrel seeds by non-ruminant animals will necessitate processing such as boiling, fermentation, sprouting, etc. to inactivate the anti-nutritional factors (11). Heat treatment also reduces anti-nutrients and increases the level of protein (12). This study was aimed at evaluating the chemical composition and anti–nutritional factors of sorrel seeds boiled at different minutes.

Materials and Methods

Study site

This study was carried out at the Rabbit Unit of the Department of Animal Science, Teaching and Research Farm, Ahmadu Bello University, Zaria. Zaria is within the Northern Guinea Savanna Zone of Nigeria, with Latitude 11° 09′ 01.78″ N and Longitude 7° 39′ 14.79″ E at an altitude of 671m above sea level (13). The mean minimum daily temperature is from 14° C - 24° C during the cold season while the mean maximum daily temperature is from 19° C - 36° C during the hot season. The mean relative humidity during dry and wet seasons is 21% and 72% respectively (14).

Sources and processing of sorrel seeds

The sorrel seeds used for this study were purchased from an open market in Yobe State during the harvest period. The raw sorrel seeds were sorted to ensure that cleaned grains were obtained. The method adopted by (15) was used for processing. The cleaned sorrel seeds were poured into three (3litres) of boiled water per batch of 1kg at 100° C (i.e. in the ratio of 3:1) at a different boiling duration of 0, 15, 30 and 45 minutes, respectively. The products were then sun dried by spreading on trays until well dried.

Chemical analysis

Samples of raw and boiled sorrel seeds were taken to the Biochemistry Laboratory, Department of Animal Science, Faculty of Agriculture, Ahmadu Bello Zaria, for chemical analysis. Dry matter, crude protein, ether extract, crude fibre and ash contents of the milled samples were determined using the method of (16). Protein was determined by Kjedahl procedure, ether extract was determined by subjecting the samples to petroleum ether extraction at 60-80° C using the soxhlet extraction apparatus. Dry matter of the samples was determined by oven drying at 100 ° C over 12-hours. Crude fibre was determined by boiling the sample reflux in weak sulphuric acid (0.255N H_2SO_4), then in a weak sodium hydroxide (0.312N NaOH) for 1 hour. The residue which consists of cellulose, lignin and mineral matter was dried and weighed. The ash content was determined by igniting a weighed sample in a muffle furnace at 500° C. The Nitrogen free extract (NFE) was obtained by the difference after the percentages of the other fractions have been separated from 100%.

Metabolizable energy (ME) was calculated from the proximate composition data using the formula of (17);

ME (kcal/kg) = 37 x % CP + 81.1 x % EE + 35.5 x % NFE

Phyto-chemical analysis

Raw and boiled sorrel seeds were taken to the Biochemical Laboratory for phyto–chemical analysis.

Determination of Phytates

The method used for phytate analysis was as described by (18).Two gram of Sorrel seed meal was weighed into a glass bottle. Fifty mililitres of 0.5N HCl was added and shaken on an orbital shaker at 2000rpm for about 3 hours to ensure homogeneity and maximum extraction of the phytic acid. The extract obtained was filtered using filter paper. Twelve milliliter was taken and neutralized with 12ml of 10N NaOH and 20% $Fecl_3$ solution was then added to the neutralized filtrate and then place in a boiling water bath for 15 minutes to precipitate ferric phytate. Then the solution was removed, cooled and centrifuged at 700rpm for 7 minutes, and the supernatant discarded. The precipitate was then washed with 3mls of 0.17N HCl and transferred into a beaker. The precipitate was then heated in a water bath at 80° C for 10 minutes; and 10ml of 0.5N NaOH added to the precipitate in ferric hydroxide and then converted to Sodium phytate. The precipitate was washed with hot water and centrifuged at 700rpm for 7 minutes again. The supernatant was discarded and transferred into a beaker using 5ml hot distilled water. 1ml of concentrated H_2S0_4 and 1.2 ml of 60 % per Chloric acid were added to the residue (filtrate) and the mixture was digested on a hot plate to evaporate the acids. The residue from the perChloric acid was removed by strong heating on Bunsen burner. It was then cooled and 10-20 ml of distilled water was added and neutralized with 10N NaOH (pH_7).

Determination of Tannin

The tannin of the raw and differently processed sorrel seeds was estimated using the method of (19). Tannin was determined using a slightly modified standard method described by (20). Two grams (2g) of the ground sample was defatted for 2 hours using a soxhlet apparatus. The residue was placed in an oven for 12 hours, retrieved and boiled at 100° C, with 300 ml of distilled water, diluted to 500ml in a standard volumetric flask and filtered through non-absorbent cotton wool. Volume of 25 ml of infusion was measured into a 2 litre porcelain dish and titrate with 0.1N potassium permanganate (0.1N potassium permanganate against 0.1N oxalic acid) until the blue solution changed to green. A few drops of 0.1N oxalic acid=0.006235 was poured to obtain the amount of tannin in the sample, 0.1N oxalic acid =0.006235 tannin.

Determination of Saponin

Ten gram ground sample was poured into a conical flask containing 100cm^3 of 20 % aqueous ethanol and agitated with a magnetic stirrer for twelve hours at 55^{o}C.The solution was filtered using Whatman number 1 filter paper and residue extracted with 200 cm^3 of 20 % aqueous ethanol. The extract was combined and reduced to about 40 cm^3 under vacuum. The extract and 200 cm^3 dethylether were transferred into a 250 cm^3 respiratory funnel and shaken vigorously. The process of purification continued until a colourless aqueous extract was obtained. The pH of the remaining aqueous solution was adjusted to 4.5 by adding 4 g NaCl, and the solution was then shaken successively with 60 cm^3 and 30 cm^3 portion of n-butanol. The butanoic extract was washed twice with 10 cm^3 of 5 % aqueous sodium chloride and evaporated to dryness in a fume cupboard to give the saponin. The value obtained was expressed in percentage according to the procedure reported by (21).

$$\% \text{ Saponin} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} \times 100$$

Statistical analysis

All data generated from the study were analysed using analysis of variance (ANOVA) using a General Linear Model (GLM) procedure of SAS (22) software package. The significant difference between treatment means was separated using Dunnett (23).

Table 1: Chemical composition of raw and boiled sorrel se ed at different durations

	Duration of boiled sorrel seed (minutes)					
Parameters (%)	RSS	15	30	45	SEM	LOS
Dry Matter	93.50	90.55	90.40	90.15	1.78	NS
Crude Protein	21.75^{d}	27.88^{c}	28.00^{b}	29.22^{a}	0.52	*
Crude Fiber	6.50^{b}	13.25^{a}	12.80^{a}	14.50^{a}	1.86	*
Ether Extract	26.79^{a}	14.33^{b}	12.10^{bc}	10.45^{c}	1.21	*
Ash	5.74	6.18	5.30	5.47	0.22	NS
Nitrogen Free Extract	39.22	38.36	41.80	40.36	0.98	NS

^{abc}: means with different superscript on the same row differ significantly at p<0.05
SEM: standard error of mean LOS: level of significance NS: not significant
RSS: Raw sorrel seeds

Table 2: Phyto-chemical Factors of Sorrel Seeds

	Duration of Boiled Sorrel Seeds (Minutes)					
Parameters (mg/100g)	RSS	15	30	45	SEM	LOS
Phytic acid	0.26^{a}	0.23^{b}	0.19^{c}	0.19^{c}	0.10	*
% Reduction in PCF	-----	11.53	26.92	26.92		
Tannin	3.15^{a}	2.08^{b}	1.9^{b}	1.1^{c}	0.28	*
% Reduction in PCF	------	33.97	39.70	65.08		
Saponin	8.66	8.39	7.7	7.65	0.36	NS
% Reduction in PCF	------	3.12	11.09	11.66		

^{abc}: means with different superscript on the same row differ significantly at p<0.05
SEM: standard error of mean LOS: level of significance NS: Not significant
PCF: Phyto-chemical factors RSS: Raw sorrel seeds

Results and Discussion

Chemical compositions of raw and boiled sorrel seeds

The result of the chemical composition of the raw and processed sorrel seed is shown in Table 1. The result showed that crude protein (CP) increased (21.75-29.22%) as the duration of boiling increased (0-45minutes). Similarly, crude fibre (CF) values of the boiled seeds were significantly (p<0.05) higher than the raw sorrel seeds. The values decreased from 15 – 30 minutes (13.25-12.80%) but increased at 45 minutes (14.50%) duration of boiling. The presence of an adequate level of fibre in the processed sorrel seed reveals that the seed can be utilized as a better source of fibre for animals. The ether extract (EE) levels of raw seeds (26.67%) was significantly (P<0.05) higher than the values for boiled sorrel seeds, though 45 minutes duration of boiling had the lowest value (10.45%). The EE levels were within the range given by FAO (24). This is non-comparable with the report of (25) that higher level of EE in the boiled sorrel seeds could be attributed to the decrease in the non-lipid component of the sorrel seeds during cooking. Ash and Nitrogen Free Extract (NFE) were not significantly affected (p>0.05) by the duration of boiling. The ash, NFE and CF values obtained in this study were slightly lower to the values reported by (26). The Ash value of 5.74% for raw seeds in this study agrees with the findings of (27) who reported that ash value for crushed seeds of sorrel ranged from 5.39-6.94%. The results here also showed that boiled seeds could also have high

NFE values as observed in 30 and 45 minutes duration of boiling. The result obtained in this study is in line with report of (28) who observed that the boiling, soaking or sprouting of sorrel seeds is accompanied by a significant increase in protein, fat and CF content while ash content is reduced.

Phyto-chemical factors of sorrel seeds

Table 2 shows the effect of different duration of boiling on the anti-nutritional factors of sorrel seeds. All parameters measured in the raw seeds showed higher values of anti-nutrients as compared to boiled seeds. There were significant ($p<0.05$) effect on the level of phytic acid found in the sorrel seed boiled at different duration though the values decreased as the duration of boiling increased. The tannin level recorded for the raw seed (3.15mg/100mg) was significantly ($p<0.05$) higher when compared to tannin level recorded for sorrel seed boiled at 15, 30 and 45 minutes (2.08, 1.9 and 1.1 mg/100mg), respectively. (29) reported that tannins can inhibit the activities of rumen microbes. Tannins have been found to affect digestibility and therefore the rate of utilization of dietary nutrients in both ruminants (30) and non-ruminants (31). Saponin was also significantly ($p>0.05$) reduced by increased boiling durations (8.39, 7.7, 7.65 mg/100g). According to (32), ruminants can break down saponins but monogastric cannot. The decreases in the levels of these anti-nutrients indicate their non-appearance to a minimum level and rendering the diet appetizing for the animals to consume. The percentage reduction in tannin, phytic acid and saponin observed in sorrel seeds subjected to boiling, agreed with the report of (33 and 34) who observed that processing of leguminous seeds either by cooking, soaking, autoclaving, roasting or fermentation significantly improved the nutritional and functional properties of legume seeds. (35) had also observed that most processing methods employed in improving the food value of non-conventional feedstuffs do not completely eliminate anti-nutritional factor substances but only reduce their concentration to tolerable levels in the feedstuff. (36) reported that if there are toxic elements in the feed, abnormalities in weights of liver and kidney would be observed. The abnormalities will arise because of increased metabolic rate of the organs in an attempt to reduce these toxic elements or anti-nutritional factors to non-toxic metabolites.

Conclusion and Application

1. It is concluded that boiling of sorrel seeds enhanced nutrient availability such as crude protein, crude fibre and ether extract.
2. Processing (to inactivate phyto–chemicals) of sorrel seeds by boiling significantly improved the nutritional and functional properties of the seeds.

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