Chemical composition of roselle (*Hibiscus sabdariffa* L.) seeds fermented at varying durations

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Abstract

A study was carried out to determine the chemical composition of roselle seeds fermented at varying durations. One kg cleaned Roselle seeds were soaked in 3L of water for 8 hours. The soaked seeds were drained and fermented for 0, 24, 48, 72 and 96 hours as treatments. Samples of fermented roselle seeds at different duration of fermentations were analyzed for chemical composition including anti-nutritional factors. The results showed that there was significant ($P < 0.05$) effect of fermentation duration on chemical composition and anti-nutritional factors of roselle seeds. Dry matter and crude protein content increased as fermentation duration increased from 0 to 96 hours. The highest dry matter (91.87%) and crude protein (35.05%) were obtained at 96 hours while crude fibre and ether extract decreased with increased fermentation duration. Crude fibre (6.55%) and ether extract (26.79%) were highest at 0 hours. Ash and nitrogen free extract content ranged from 5.41 – 5.78 % and 30.62 – 39.19 %, respectively. Anti-nutritional factor contents declined with increased fermentation duration. The lowest values for saponin (2.02%), tannin (0.15%), phytic acid (5.44%) and trypsin inhibitor (8.93%) were obtained at 96 hours fermentation. It can be concluded that roselle seeds fermented for 96 hours enhanced nutrient availability and significantly reduced tannin, phytate, saponin and trypsin inhibitor anti-nutritional factors.

Keywords: Anti-nutritional factor, chemical composition and fermentation.

Introduction

Roselle (*Hibiscus sabdariffa* L.) is a leguminous shrub that is well adapted to the Guinea and Sudan savannah vegetation belts of Nigeria (Alagbajo, 2000). It belongs to the family *Malvaceae*. It is believed to have originated from tropical Africa (Mc-Clean, 1973) but now cultivated in many countries of the world (Kalyane, 1986). *Hibiscus sabdariffa* L. is suitable for tropical climate with well distributed rainfall of 1500 – 2000 mm yearly, from sea level to about 600 m altitude. In Nigeria, roselle is grown as a border crop with the calyces used for the production of a popular non-alcoholic beverage ('zobo') as well as sauce, jams, jelly and coloring materials for food, wines and drugs (Tsai *et al.*, 2002). The leaves

form a popular vegetable in most regions of the world (Ojokoh *et al.*, 2006). There are several reports on the chemical composition of roselle seeds (RS). Studies by Animal Production Research Centre APRC (1997) reported that RS contain 28% crude protein, 19.9% ether extract, 5.5% ash and 18% crude fibre. In other studies, Dashak and Nwanego (2002) and Isidahomen *et al.* (2006) reported 35.19 and 25.92% crude protein, respectively and 15% crude fibre in RS.

The RS also contain high amount of mineral such as calcium, magnesium and phosphorus and can supplement other expensive conventional protein sources in the diet of poultry, thereby reducing the cost of production and the competition between man and livestock for this feed sources. In

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spite of the numerous advantages, RS in their raw state are known to have bitter taste which is attributed to its anti-nutritional factors. The unprocessed seed has been reported to contain tannin, hibiscin and hydroxyl flavone as major anti-nutritional factors (Mahadevan *et al.*, 2009). However, the most commonly reported anti-nutritional factors are total phenols, tannins and phytic acid, and these have been shown to have detrimental effects on the health and performance of animals (Diarra *et al.*, 2011; Kayembe, 2011). Effective utilization of Roselle seeds by poultry will necessitate processing to inactivate anti-nutritional factors (Soetan and Oyewole, 2009). Processing methods such as fermentation have been reported to be effective in doing this. This study is aimed at evaluating the chemical composition and anti-nutritional factors of RS fermented at varying durations.

Materials and methods

Experimental site

The experiment was carried out at the Teaching and Research Farm, Department of Animal Science, Ahmadu Bello University, Zaria. Zaria is located in the Northern Guinea savannah zone of Nigeria, longitude, 11° 09' 01.78" N and latitude, 7° 39' 14.79" E at an altitude of 671mm above sea level. The climate is characterized by a well-defined dry and wet season. The climate is tropical with annual temperature of 24.9°C and rainfall averages 1050mm (Ovimap, 2014).

Sources and processing of roselle seeds

The RS used for these studies were purchased from an open market in Yobe state during the harvest period. The RS were sorted and thoroughly cleansed of dirt by winnowing and sieving to ensure clean grains.

Fermentation method

One (1) kg cleaned RS were soaked in 3L of water for 8 hours. The soaked seeds were

drained and placed in an air tight polythene bag and allowed to ferment for 24, 48, 72 and 96 hours. The RS were spread on a tarpauline sheet, sun dried for three days and crushed in a 1.5 mm sieve size.

Chemical analysis

Samples of fermented RS at different levels of fermentation (24, 48, 72 and 96 hours) were taken to the Animal Science Biochemical Laboratory for proximate analysis using the method described by A.O.A.C. (2005). Metabolizable energy (ME) were calculated from the proximate composition data using the formulae described by Van Soest (1991); $ME = 37 (\% CP) + 81.1 (\% EE) + 35.5 (\% NFE)$

Anti-nutritional analysis

Determination of Phytic acid

Method used for phytic acid analysis was described by A.O.A.C (2005). Briefly, 2gram of RS meal was weighed into a glass bottle. Fifty millilitres 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 12 mL of 10N NaOH and 20% FeCl₃ 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 seven minutes, and the supernatant discarded. The precipitate was then washed with 3 mL of 0.17N HCl and transferred into a beaker. The precipitate was then heated in 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 seven minutes again. The supernatant was discarded and transferred into a beaker using 5mL hot distilled water. 1mL of concentrated H₂SO₄ 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 per chloric 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 (PH₇).

Determination of Tannin

Tannin acids of the raw and differently processed RS were estimated using the method of Earp *et al.* (1981). Tannin was determined using a slightly modified standard method described by A.O.A.C. (2005). Two grams of the ground sample was defatted for two hours using 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 500 mL in a standard volumetric flask and filtered through non-absorbent cotton wool. A volume of 25 mL of infusion was measured into a 2L 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 100cm³ of 20 % aqueous ethanol and agitated with a magnetic stirrer for twelve hours at 55°C. The solution was filtered using whatman number 1 filter paper and residue extracted with 200 cm³ of 20 % aqueous ethanol. The extract was combined and reduced to about 40 cm³ under vacuum. The extract and 200 cm³ dethylether were transferred into a 250 cm³ 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 cm³ and 30 cm³ portion of n-butanol. The butanoic extract was washed twice with 10 cm³ of 5 % aqueous sodium chloride and evaporated to dryness in fume cupboard to give the saponin. The value obtained was expressed in percentage according to procedure reported by Hudson and El-difrawi (1979).

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

Determination of Trypsin inhibitors

The trypsin inhibitor activity was estimated and determined by the method of Kakade *et al.* (1974). In this method, 1 g of fine sample was extracted with 50 mL of NaOH for 1 hour. The PH of the suspension was adjusted to between 9.5 and 9.8 with 0.01N HCl and kept overnight at 4°C in a refrigerator. Two portions of each extract was pipetted into a 50 mL volumetric flask and made up to mark with distilled water. In determining trypsin inhibitor level. The solution in the tubes were thoroughly mixed and heated to 37°C in a water bath for 10 minutes. Five percent of BAPNA solution (40mg N-a-Benzoyl-DL- Arginine P-nitroanilid hydrochloride) was added to all tubes at 37°C. The reaction was stopped after 10 minutes by the addition of 1 mL 30% acetic acid solution to tubes. The solution in the tubes were thoroughly mixed and titrated through whatman No.42 filter paper. The trypsin inhibitor in mg/g was calculated using the formula.

$$\text{Trypsin inhibitor} = \frac{\text{A standard} - \text{A sample}}{\text{Dilution factor}}$$

$$0.1 \times \text{sample wt in g} \\ 1000 \text{ sample size}$$

Results and discussion

Chemical composition of Roselle seeds fermented at varying durations

The result of the chemical composition of RS fermented at varying durations is presented in Table 1. The result showed that durations of fermentation significantly

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($P < 0.05$) affected the chemical composition of RS. The crude protein increased (21 to 35%) as duration of fermentation increased (0-96hrs). Conversely, there was decreased in crude fibre as fermentation period increased. Crude fibre was significantly ($P < 0.05$) higher in the raw RS (6.55%) compared to RS fermented at varying durations. Ether extract was higher in the raw RS (26.79%), while RS fermented for 96 hours had the lowest value (21.74%). There were significant ($P < 0.05$) differences in the ash content of RS fermented at varying durations. NFE significantly ($P > 0.05$) decreased at varying durations of fermentation, raw RS had the highest value (39.19%) while RS fermented for 48 hours had the least value (30.62%).

The observed increased in crude protein of RS fermented for 0-96 hours when compared with raw RS, tends to show a buildup of more microorganisms responsible for fermentation, which are also proteinous in nature. This agrees with the findings of Walter (1984) who reported an upgrade in nutrient composition of fermented material. Udensi *et al.* (2008)

also reported that processing by soaking and fermentation increased the availability of some nutrients. The values obtained for crude protein of RS fermented at varying durations in this study were within the range of 21-35%. Dashak and Nwanegbo (2002) and Kwari *et al.* (2011) reported that RS contained 35.90%, crude protein, while Abu El-Gasim *et al.* (2008) reported that raw RS contained 21-35% crude protein.

The lower crude fibre value observed in RS fermented at varying durations as compared to raw RS might be due to leaching of some fibre content during soaking. Fibre helps to maintain the health of the gastrointestinal tract. The presence of an adequate level of fibre in both raw and processed RS reveals that the seed can be utilized as better source of fibre for animals. There was decrease in ether extract content of RS fermented at varying durations. This could be as a result of leaching of some ether extract content during soaking before fermentation of the RS or as a result of energy been utilized by fermentation microbes. The varied ash content values in raw the RS and fermented RS, shows addition of mineral in the course of fermentation.

Table 1: Chemical composition of roselle seeds fermented at varying durations

Parameters (%)	(T1) Raw	(T2) 24hrs	(T3) 48hrs	(T4) 72hrs	(T5) 96hrs	SEM
Dry matter	89.44 ^c	87.22 ^d	90.22 ^b	90.07 ^b	91.87 ^a	0.09
Crude protein	21.74 ^c	33.79 ^d	34.23 ^c	34.55 ^b	35.05 ^a	0.02
Crude fibre	6.55 ^a	5.66 ^d	6.29 ^b	6.28 ^b	5.99 ^c	0.05
Ash	5.73 ^a	5.56 ^b	5.70 ^a	5.41 ^c	5.78 ^a	0.06
Ether extract	26.79 ^a	24.07 ^b	23.08 ^c	22.22 ^d	21.74 ^e	0.07
Nitrogen Free Extract	39.19 ^a	30.79 ^d	30.62 ^e	31.61 ^b	31.40 ^c	0.02

^{abcde}: means on the same row with different superscript are significantly different ($p < 0.05$), sem: standard error of means

Anti-nutritional factors of Roselle seeds fermented at varying durations

The effects of varying durations of fermentation on anti-nutritional factors are presented in Table 2. There were significant ($P < 0.05$) effects on the level of tannin found in RS fermented at varying durations. The level of tannin decreased as fermentation duration increased from raw RS (0.28

mg/100g) and RS fermented for 24 hours (0.27mg/100g), 48 hours (0.20 mg/100g), 72 hours (0.15 mg/100g) and 96 hours (0.15mg/100g). The phytic acid level recorded in raw RS (6.95 mg/100g) was significantly ($P < 0.05$) higher when compared to phytic acid level recorded in RS fermented at 24, 48, 72 and 96 hours (6.22, 5.92, 5.65 and 5.44 mg/100g),

respectively. The level of saponin decreased significantly ($P>0.05$) at varying durations of fermentation from 24, 48, 72 and 96 hours (2.20, 2.19, 2.14 and 2.04 mg/100g), respectively, while raw RS had the highest level of saponin (2.45 mg/100g). Trypsin inhibitor was also significantly ($P>0.05$) reduced by the increase in durations of fermentation (10.10, 9.79, 9.21 and 8.93 mg/100g respectively). The raw RS had the highest level of trypsin inhibitor (10.15 mg/100g). The observed reduction in tannin, phytate, saponin and trypsin inhibitor in RS subjected to fermentation, agrees with the report of Abeke (1997), who confirmed that there is reduction in anti-nutritional content through leaching in water when legume seeds is soaked before fermentation. Yagoub and Abdalla, (2007) also observed

that processing of leguminous seeds either by soaking, cooking or fermentation significantly improved the nutritional and functional properties of legume seeds. Abu El-Gasim *et al.* (2008) reported a significant reduction of phenolic compound in soaked, cooked, sprouted and fermented RS compared to raw RS. The percentage reduction in levels of tannin, phytate, saponin and trypsin inhibitor in RS fermented at varying durations also agreed with the findings of Udensi *et al.* (2008) who confirmed that processing of legume seeds (soaking, autoclaving, fermentation and roasting) led to reduction in some anti-nutritional content. A preliminary study by Afolabi *et al.* (1985) showed that even though leguminous seeds were high in crude protein, they were toxic to broilers when fed raw.

Table 2: Anti-nutritional factors of roselle seeds fermented at varying durations

Parameters (%)	(T1) Raw (0)	(T2) 24hrs	(T3) 48hrs	(T4) 72hrs	(T5) 96hrs	SEM
Saponin	2.45 ^a	2.20 ^b	2.19 ^b	2.14 ^b	2.02 ^c	0.04
Tannin	0.28 ^a	0.27 ^a	0.20 ^b	0.15 ^c	0.15 ^c	0.01
Phytic acid	6.95 ^a	6.22 ^b	5.92 ^c	5.65 ^d	5.44 ^e	0.05
Trypsin inhibitor	10.15 ^a	10.10 ^a	9.79 ^b	9.21 ^c	8.93 ^d	0.06

^{abcde}: Means on the same row with different superscripts are significantly different ($P<0.05$), SEM: Standard error of means.

Conclusion

The study showed that roselle seeds fermented for 96 hours enhanced nutrient availability and significantly reduced anti-nutritional factors (Tannin, Phytate, Saponin and Trypsin inhibitors).

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