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Nutritional Evaluation of Fermented Roselle (*Hibiscus sabdariffa*) Calyx

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Abstract: Roselle (*Hibiscus sabdariffa*) Calyx was fermented traditionally and by controlled fermentation using puer strains of *Aspergillus niger*, *Aspergillus flavus* and Saccharomyces cerevisiae. The proximate composition shows that there was significant increase (P=0.05) in protein content (14.7±2.5-10.8±1.1%) of the fermented samples compared to the unfermented (4.8±1.3%). The antinutritional composition of the samples showed a significant decrease (P=0.05) in the phytate and tannin content (488.8±3.7-752.4±7.5 mg/100g and 1.21±0.1-1.92±0.0%) of the fermented samples in comparison to the unfermented 2143.6±0.8 mg/100g and 5.30±1.1%). The nutritional evaluation revealed that there was a decline in growth in the albino rats on all the treatments except for control diet where there was increase in growth. Serum aspertate amino transferase (AST) and serium alanine amino transferase (ALT) activities of the rats fed with the calyx samples were higher (268.0±11.5-409.3±12.4 iμ L^{-1} and 19.0±1.1-23.5±1.7 iμ L^{-1}) than the control (208.0±406 and 16.4±11.8 iμ L^{-1}).

Key words: Roselle, fermentation, nutritional, evaluation

INTRODUCTION

Roselle (*H. sabdariffa*) an important base fibre crop is a member of the malvaceae family[1]. It is indigenous to Asia but is now cultivated in several other countries of the world[2].

In many homes in south western Nigeria. It is prepared traditionally either by steeping over night or parboiling with wood ash to neutralize the antinutrient before using it to prepare soup, stew and sauces [3]. Finot[4] stated that processing of foods can improve nutrition, safety and occasionally lead to the formation of antinutritional and toxic compounds.

Roselle is a good source of iron, niacin, riboflavin and β carotene which is up to 285 μg[2]. It has been suggested that increased intake of carotenoid especially β carotene is inversely related to cancer risk[2,5].

However no attempt has been made to study this herb from nutritional point of view. The purpose of this study was to evaluate with rats the nutritional quality of fermented Roselle calyx.

MATERIALS AND METHODS

A 400g of dry green Roselle Calyx was collected form Oja Oba Market Akure. The chemicals used were of analytical grade and glass distilled was used.

Sample preparation: The Roselle Calyx was divided into four portions of 100 g each. The first portion was fermented traditionally while the remaining portions were inoculated singly with pure strains of *Aspergillus niger, Aspergillus flavus* and *Saccharomyces cerevuscae* isolated and characterized from the fermentation. They were allowed to ferment at room temperature (27±2°C) for three days

Sample analysis: The proximate composition (ash, fat, moisture and crude fibre) of fermented calyx were determined using standard AOAC[6] method and the protein was determined using the micro-kjeldhal method (Nx6.25). The tannin content was determined using the method of Makkar et. al.[7] while the phytate content was determined using Wheeler and Ferrel[8] method.

Nutritional evaluation: Twenty four albino rat (Wistar strain) aged four weeks were obtained from Department of Biochemistry, University of Ilorin. The rats were fed on commercial diet purchased from Bendel feed, Edo State for 3 days before the feeding experiment. The fermented and unfermented calyx samples were used to formulate diets based on the method reported by Aletor[9] and Anning et. al.[10].

The diets contained 60% of samples at the expense of the maize in the basal diet as shown in Table 1 below.

After 21day experiment, performance indices calculated include daily weight gain/loss (g/rat/day), daily feed intake (g/rat/day) and feed: gain ratio (g/rat/day).

Biochemical assay: The rats were anaesthetized with diethyl/ether and blood samples were collected into EDTA bottles.

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Table 1: Composition of basal diet

Sample	Quality
Supplemented Caesin	10.0
Vitamin premix	1.0
Mineral premix	2.4
Mineral Oil	10.0
Maize	76.6
Total	100.0

Reflotron M0.6.02<06.00 (Boehringer Mannhein Company Germany) was used for the analyses of some major serum biochemical markers of fed calyx samples on the rats. The biomarkers assayed for were aspartate amino transferase (AST) and alanine amino transferase (ALT). Standardized amount of the sample were automatically pipetted and applied on the test strip. The strip was inserted into the test chamber and result was displayed after some seconds on the computer monitor. The test were carried out at 25°C.

Analysis of data: The data were analyzed using mean±SD and analysis of variance[11].

RESULTS AND DISCUSSION

The results of the proximate composition is shown in Table 2. The sample fermented traditionally had the highest protein content (14.7±2.5%). The increase in protein in the sample fermented traditionally may be due to increase in the mixture/variety of microbial species involved which might have secreted some extracellular enzymes (protein)[12,13].

There was a decrease in the ash content of the fermented samples compared to the unfermented sample. Microorganisms might have use some of the minerals presen t in the ash for their metabolic activities[14].

There was no considerable difference in fat, fibre and carbohydrate content. This therefore supports the fact that the protein increase could be as a result of hydrolyses of starch to glucose and they were used by the same organisms as a carbon source to synthesize microbial biomass rich in protein[15].

The results of the antinutritional analysis revealed that they were significantly lower compared to the unfermented samples (Table 3).

Tannin affects nutritive value of food by forming complex with protein (both substrate and enzyme) thereby inhibiting digestion and absorption[16]. The decrease in tannin may be as a result of the processing which the samples were subjected to coupled with the activities of microbial enzymes involved in the fermentation[16,17].

The complexing of phytic acid with nutritionally essential minerals and the possibility of interference with proteolytic digestion have been suggested as responsible for the antinutritional activity[16]. Zokiti[25] earlier reported decrease in phytate content during fermentation of Roselle seeds.

Nutrient utilization and performance of albino rats fed fermented calyx samples are shown in Table 4. There was a significant difference (P=0.05) between the daily weight and feed: gain ratio of albino rats fed diet

Table 2: Proximate composition of calyx samples (%) fermented with indigenous isolates (mean ± SD*)

Sample	Moisture	Ash	Crude Fibre	Protein	Fat	Carbohydrate
Aspergillus niger	$12.5^{c}\pm3.1$	$5.0^{c}\pm0.2$	$10.1^{b}\pm0.1$	$12.6^{a}\pm1.1$	$5.3^{a}\pm1.0$	$54.4^{a}\pm2.5$
Aspergillus flavus	$12.5^{c}\pm3.5$	$4.9^{c}\pm0.1$	$9.5^{b}\pm0.3$	$11.8^{a}\pm1.0$	$5.8^{a}\pm1.5$	$55.3^{a}\pm3.1$
Saccharomyces cerevisiae	$12.8^{c}\pm3.5$	$5.0^{ab}\pm0.2$	$9.0^{bc}\pm0.6$	$10.8^{b}\pm1.1$	$5.7^{a}\pm1.7$	$56.6^{a}\pm0.6$
Unfermented	$11.1^{ab}\pm1.2$	12.8±2.7	$11.3^{ab}\pm0.7$	4.8±1.3	$3.9^{b}\pm1.1$	$55.9^{a}\pm2.3$

* Values represent means of triplicate determinations, Means with the same subscript (letter(s)) along the same column are not significantly different (P>0.05)

Table 3: Antinutrient contents of calyx samples fermented with indigenous isolates after 72 h (mean±SD*)

Sample	Phytate (mg/100g)	Cyanide (mg kg^{-1})	Tannin (%)
Aspergillus niger	488.8 ± 3.7^{a}	$0.7^{c}\pm0.0$	$1.92^{ac}\pm0.0$
Aspergillus flavus	752.4 ± 7.5^{bc}	$1.3^{d}\pm0.0$	$1.32^{bcd}\pm0.1$
Saccharomyces cerevisiae	564.1 ± 1.1^{ab}	$1.2^{d}\pm0.0$	$1.79^{ac}\pm0.0$
Unfermented	2143.6 ± 0.8^{d}	$3.5^{b}\pm0.0$	5.30 ± 1.1^{a}

* Values represent means of triplicate determinations, Means with the same subscript (letter(s)) along the same column are not significantly different (P>0.05)

Table 4: Nutrient utilization and performance of albino rats fed with fermented calyx samples (mean±SD*)

Sample	Daily weight gain/loss (g/rat/day)	Daily feed intake	Feed: gain/loss ratio
Control	$1.5^{a}\pm0.4$	$4.2^{ab}\pm0.5$	$3.2^{a}\pm0.8$
***Aspergillus niger*	$-1.8^{b}\pm0.5$	$4.1^{ab}\pm0.1$	$-2.3^{b}\pm0.4$
Aspergillus flavus	$-0.7^{c}\pm0.1$	$4.2^{ab}\pm0.2$	$-5.2^{bcd}\pm0.7$
Saccharomyces cerevisiae	$-1.1^{bc}\pm0.4$	$4.2^{ab}\pm0.4$	$-3.9^{bc}\pm1.1$
**Unfermented	$-1.9^{b}\pm0.4$	$3.9^{b}\pm0.3$	$-2.2^{b}\pm0.5$

* Values represent means of triplicate determinations, ** Some rats on this diet died within 15 days, Means with the same subscript (letter(s)) along the same column are not significantly different (P>0.05)

Table 5: Serum levels of Aspertate transferace and Alanine amino transferase of albino rats fed fermented calyx samples (mean±SD*)

Sample	Aspertate transferace	Alanine amino transferase
Control	$208.0^{a} \pm 4.6$	$16.4^{ab} \pm 1.8$
Aspergillus flavus	$397.0^{a} \pm 13.2$	$19.0^{abc} \pm 1.1$
Saccharomyces cerevisiae	$409.3^{a} \pm 12.4$	$22.2^{bc} \pm 1.9$
Fermented without wood ash	$268.0^{a} \pm 11.5$	$23.5^{c} \pm 1.7$

Means with the same subscript (letter(s)) along the same column are not significantly different (P>0.05)

containing calyx samples and the control diet. There was no significant difference (P=0.05) between daily feed intake of fermented calyx samples and those of the control diet. The results indicated that there was a decline in growth in the albino rats on all the treatments except for the control diet where there was increase in growth. The higher growth recorded in rats fed with control diet may be due to higher feed consumption in addition to the better nutritional quality of the feed. Gain in weight, good feed conversion efficiency and overall performance is a good indicator that a diet is of high quality [18].

The low growth observed in the feed containing the calyx samples could be attributed to poor nutritional quality as well as low feed intake[10].

The serum asparatate amino transferase (AST) activity of the albino rats fed calyx samples were higher and significantly different from the control (Table 5) the serum alanine amino transferase (ALT) activity of the albino rats fed calyx samples were also significantly higher (P=0.05) than the control.

AST is an enzyme that increases in activity in diseases such as severe bacterial infections, malaria, pneumonia, pulmonary infacts and tumours of organs such as heart and muscle (Cheesbrough, 1991). ALT is principally found in the liver and is regarded as being more specific than AST for detecting liver cell damage [20].

The implication of the results is that there is a pronounced toxicological effect in rats fed with the calyx samples compared to the control. The high level of AST and ALT reported in rats fed with the calyx samples could be due to the level of antinutrients (tannin and phytate) present in the samples. Emmanuel[21] reported that papaya seed containing 2.83% of tannin as tannic acid equivalent significantly depressed the rate of growth and death of rats. High tannin in feeds causes reduction in nitrogen retention and reduction in protein digestibility[22,23]. Roberts and Yudins[24] reported convulsive fit and paralysis as signs of phytate intoxication in experimental rats. There is the need for more research on how to reduce the antinutritional factors in the Roselle calyx.

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