



Effect of Fermentation on the Nutritional Composition of Roselle Calyx Obtained from Ekiti and Benue State, Nigeria

Anthony Okhonlaye Ojokoh¹, Oluwabunmi Olabisi Adaja¹
and Ayomide Olubunmi Omojokun^{1*}

¹Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author A.O. Ojokoh designed the study, author OOA performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and author A. O. Omojokun managed the analyses and literature searches of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/42314

Editor(s):

(1) Dr. P. Rama Bhat, PG Biotechnology, Alva's College, Karnataka, India.

Reviewers:

(1) R. Prabha, Animal and Fisheries Sciences University, India.

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(3) Sherif Mohamed El-Kadi, Damietta University, Egypt.

Complete Peer review History: <http://www.sciencedomain.org/review-history/25778>

Original Research Article

Received 10th May 2018
Accepted 16th July 2018
Published 3rd August 2018

ABSTRACT

Aim: The study was conducted to investigate the effect of fermentation on the nutritional qualities of Roselle calyx from two States in Nigeria.

Study Design: Roselle calyx was obtained from various locations and grouped into four: fermented naturally (FN), fermented with coconut husk ashes (FWCHA), Fermented with cocoa pod ashes (FWCPA), Fermented with *Gmelina* tree ashes (FWGTA), while unfermented samples served as control.

Place and Duration of Study: Fermentation of samples and isolation of microorganisms were carried out in the department of Microbiology while chemical analysis was carried out in Department of Chemistry, Federal University of Technology Akure, Ondo State, Nigeria between February 2015 and October 2016.

*Corresponding author: E-mail: fagbemi.o.ayomide@gmail.com;

Methodology: Roselle calyx was fermented naturally (FN), fermented with coconut husk ashes (FWCHA), Fermented with cocoa pod ashes (FWCPA), Fermented with *Gmelina* tree ashes (FWGTA) separately for 72 hours. The nutrient, antinutrient and mineral compositions of raw and fermented calyx were determined using standard procedures.

Results: For samples obtained from Ekiti, protein and carbohydrate increased, crude fibre, fat and moisture decreased and ash content increased except in FN when compared to raw samples. Samples obtained from Benue showed an increase in protein, fibre and ash contents, an increase in carbohydrate content except for FWCPA while moisture and fat decreased significantly. Mineral content of samples obtained from Ekiti revealed a significant increase in sodium, calcium, magnesium and iron, a decrease in potassium and no significant difference in zinc content. Samples collected from Benue showed an increase in calcium, decrease in potassium except in FWCHA, magnesium content varied, and there was no significant difference in sodium and zinc. All antinutrients analyzed (phytate, glycoside, tannin, and phenol) decreased significantly in the samples obtained from both Ekiti and Benue state.

Conclusion: The nutritional composition and antinutrient content of the roselle calyx fermented with different methods varied significantly.

Keywords: Fermentation; nutritional; antinutrient; Roselle; ashes.

1. INTRODUCTION

Vegetables are plants that are consumed by humans as food. Leafy vegetables are plant leaves that are eaten as vegetables. They can be eaten raw or cooked. Vegetables play an important role in human nutrition, although they contain antinutrients which interfere with nutrient absorption in the body system.

Two varieties of Roselle calyces are well known in Nigeria. The red calyx used for making a delicious drink known as zobo and the green calyx popularly used to prepare soup and stew.

Roselle (*Hibiscus sabdarifa* L.) leaves serve as a vegetable in Africa. Parts of the plant such as leaves and seeds are of medicinal importance. The calyx of roselle (green) is very rich in vitamin

C and riboflavin with some significant mineral present [1,2]. Rosselle calyces are used as a digestive and purgative agent and a folk remedy for abscesses, billows, cancer, hypertension etc [1]. Calyces of roselle contain nine times more vitamin C than Citrus [3,4]. The seed of roselle is rich in protein, calorie, fat, and fibre [3,5]. The leaves are used as diuretic and sedative. They also help in reducing hypertension [3, 6].

It is popularly prepared by soaking it overnight in water and wood ash or by parboiling with wood ash. This is to neutralize the antinutrients and also reduce its acidity before it is used to prepare soup [7]. Although different research has been done on this plant, this research was conducted to compare the effect of fermentation on *Hibiscus sabdarifa* obtained from two geographical locations in Nigeria.



Fig. 1. Dried roselle calyx
Source: afrolems.com



Fig. 2. Fresh roselle calyx
Source: www.medicinalplantsinnigeria.com

2. MATERIALS AND METHODS

2.1 Sample Collection and Processing

Fresh roselle calyces were purchased from Ado-Ekiti, Ekiti State and Owukpa, Benue State. Coconut husks were obtained from coconut sellers around the Federal University of Technology, Akure (FUTA) community. Cocoa pods and *Gmelina* tree shavings were obtained from Uso Town, Ondo State. The Coconut husk, cocoa pod, and *Gmelina* tree shavings were ashed according to the method described by Gaines. The roselle calyx was sorted, cleaned and dried. A 30 g of each ash was weighed into 2 liters of sterile water in separate clean plastic containers with lid, followed by homogenization and the addition of 150 g of dried roselle calyx. These samples were left to ferment for 72 hours. Samples fermented naturally without the addition of ash served as control. The containers were labeled FN: Fermented naturally (samples fermented with water alone), FWCHA: Fermented with coconut husk ashes, FWCPA: Fermented with cocoa pod ashes, FWGTA: Fermented with *Gmelina* wood ashes.

2.2 Microbial Analysis of the Samples

Bacterial and fungal load were determined using standard methods as described by [8]. Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while de Man Rogosa Sharpe agar was used to isolate lactic acid bacteria. Techniques were enumerated by using appropriate serial dilution and pour plate techniques. The bacterial culture was incubated at 37°C for 18 to 24 hours, fungal plates were inverted and incubated at 24°C for 48 to 72 hours. de Man Rogosa sharpe agar plates were incubated at 32°C for 18- 24 hours anaerobically. The organisms were characterized based on biochemical and morphological observations according to the methods of [9,10].

2.3 Proximate Analysis

The proximate analysis of the samples was determined according to the AOAC [5] procedures for crude fibre, ash, moisture, fat, protein using (N* 6.25) and Carbohydrate content was determined by difference.

2.4 Anti Nutrient Content Determination

Phytate was determined according to the method of AOAC [11]. Tannin content was determined

according to the method described by Makkar et al. and Doss, et al. [12,13]. Glycoside was determined by a modified method of AOAC and Onwuka [11,14].

2.5 Determination of Mineral Composition

The mineral analysis was done by the method of AOAC [11]. Minerals analyzed include sodium, potassium, calcium, magnesium, zinc, and iron.

2.6 Statistical Analysis

All analyses were performed in triplicates. The data obtained were subjected to one-way analysis of variance (ANOVA) while differences in mean were determined using Duncan's New Multiple Range Test (DMRT). All data analyses were done with SPSS 16.0 version.

3. RESULTS AND DISCUSSION

A total of ten (10) microorganisms were isolated during the fermentation process. *Escherichia coli* were the only coliform present on the first day of fermentation. The total viable bacteria were *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The only yeast isolated was *Saccharomyces cerevisiae*, while the molds isolated are *Geotrichum albidum*, *Penicillium chrysogenum*, *Aspergillus niger* and *A. flavus*.

Changes in the proximate composition of fermented Roselle from Ekiti and Benue states neutralized with different wood ashes are shown in Tables 1 and 2. The protein content of the samples obtained from both Ekiti and Benue states increased compared to their raw samples. The carbohydrate contents also increased except Benue sample fermented with cocoa pod ashes. Moisture and fat contents of all the fermented samples decreased. The crude fibre content of samples gotten from Ekiti decreased while those from Benue increased after fermentation. The ash content of the samples increased after fermentation except for those fermented naturally.

Changes in antinutrient content of fermented Roselle from Ekiti and Benue states neutralized with different wood ashes are shown in Tables 3 and 4. It was observed that all the antinutrients decreased after fermentation by the different methods.

Table 1. Proximate composition (%) of fermented Roselle samples from Ekiti neutralized with different ashes

Proximate	Ash content	Moisture content	Fat content	Crude fibre content	Protein content	Carbohydrate content
RAW	5.08±0.14 ^b	18.30±0.21 ^e	13.24±0.14 ^e	20.47±0.15 ^d	10.70±0.22 ^a	32.21±0.20 ^a
FN	3.17±0.05 ^a	11.00±0.04 ^a	6.44±0.04 ^b	20.42±0.01 ^d	14.17±0.19 ^c	44.80±0.15 ^e
FWCHA	5.38±0.20 ^c	14.19±0.03 ^d	7.28±0.06 ^c	18.57±0.07 ^c	10.86±0.02 ^a	43.72±0.09 ^c
FWCPA	7.79±0.04 ^e	12.80±0.10 ^b	8.99±0.60 ^d	14.80±0.01 ^a	12.49±0.10 ^b	43.13±0.07 ^b
FWGTA	7.40±0.06 ^d	13.09±0.01 ^c	17.08±0.09 ^a	17.08±0.03 ^b	12.36±0.04 ^b	44.48±0.14 ^d

Table 2. Proximate composition (%) of fermented Roselle samples from Benue neutralized with different ashes

Proximate	Ash content	Moisture content	Fat content	Crude fibre content	Protein content	Carbohydrate content
RAW	4.50±0.07 ^b	20.95±0.17 ^e	16.72±0.35 ^e	19.36±0.04 ^a	9.84±0.07 ^a	28.63±0.12 ^b
FN	4.35±0.03 ^a	12.57±0.04 ^a	8.46±0.06 ^b	21.93±0.05 ^d	11.23±0.15 ^e	41.46±0.14 ^e
FWCHA	6.66±0.14 ^c	13.48±0.05 ^c	9.18±0.14 ^c	24.63±0.07 ^e	11.25±0.03 ^c	34.80±0.04 ^c
FWCPA	7.01±0.01 ^d	20.32±0.03 ^d	14.60±0.05 ^d	19.81±0.02 ^c	10.21±0.02 ^b	28.05±0.04 ^a
FWGTA	8.03±0.04 ^e	12.92±0.05 ^b	8.30±0.01 ^a	19.58±0.04 ^b	12.12±0.02 ^d	38.86±0.02 ^d

Table 3. Antinutrient content of fermented Roselle from Ekiti neutralized with different ashes

Samples	Phytates (mg/g)	Glycosides (mg/kg)	Tannins (mg/g)	Phenols (mg/g)
RAW	10.97±0.08 ^e	105.17±0.05 ^e	1.25±0.06 ^c	28.00±0.06 ^e
FN	10.42±0.04 ^d	84.06±0.18 ^c	1.00±0.03 ^b	25.50±0.12 ^d
FWCHA	8.06±0.02 ^b	66.11±0.08 ^b	0.75±0.04 ^a	19.57±0.08 ^b
FWCPA	10.19±0.08 ^c	97.14±0.04 ^d	0.82±0.02 ^a	22.45±0.15 ^c
FWGTA	5.80±0.08 ^a	61.09±0.09 ^a	0.75±0.03 ^a	18.87±0.02 ^a

Table 4. Antinutrient content of fermented Roselle from Benue neutralized with different ashes

Antinutrients	Phytates (mg/g)	Glycosides (mg/kg)	Tannins (mg/g)	Phenols (mg/g)
RAW	14.83±0.15 ^e	82.78±0.18 ^e	1.42±0.07 ^d	35.01±0.15 ^e
FN	13.75±0.01 ^d	73.51±0.02 ^c	0.93±0.01 ^b	23.94±0.08 ^c
FWCHA	7.67±0.06 ^a	53.63±0.06 ^a	0.81±0.06 ^a	19.67±0.04 ^a
FWCPA	8.61±0.07 ^b	80.25±0.04 ^d	1.05±0.03 ^c	24.93±0.03 ^d
FWGTA	8.95±0.03 ^c	60.31±0.13 ^b	0.91±0.06 ^b	21.80±0.06 ^b

Changes in the mineral composition of fermented roselle from Ekiti and Benue states neutralized with different wood ashes are shown in Figs. 3 and 4. For samples obtained in Ekiti, potassium content decreased, sodium and calcium increased, zinc had no significant difference, the iron content varied while magnesium content increased except for sample fermented with cocoa pod ashes. As for samples obtained from Benue state, zinc and sodium had no significant difference, potassium content decreased except for sample fermented with coconut husk ashes, calcium content increased except for sample fermented naturally, iron content increased in all

the fermented samples, magnesium content increased in sample fermented with coconut husk ashes and *Gmelina* tree ashes while decrease was observed in the samples fermented naturally and fermented with cocoa pod ashes.

The presence of *Escherichia coli* in the fermenting sample on the first day of fermentation could be as a result of the water used for fermentation; it could be from the soil during harvesting. Other organisms present could be from the soil while some are normal flora of the sample.

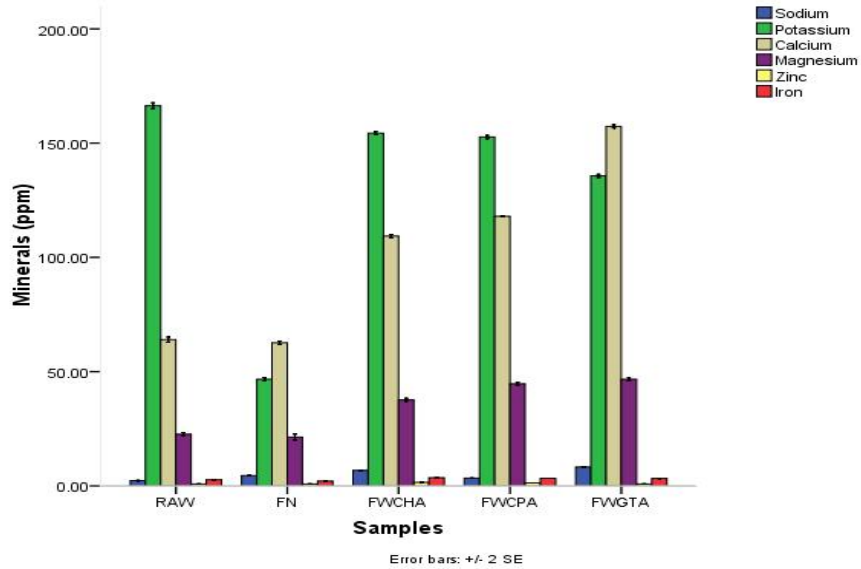


Fig. 3. Mineral content (ppm) of fermented Roselle from Ekiti neutralized with different ashes

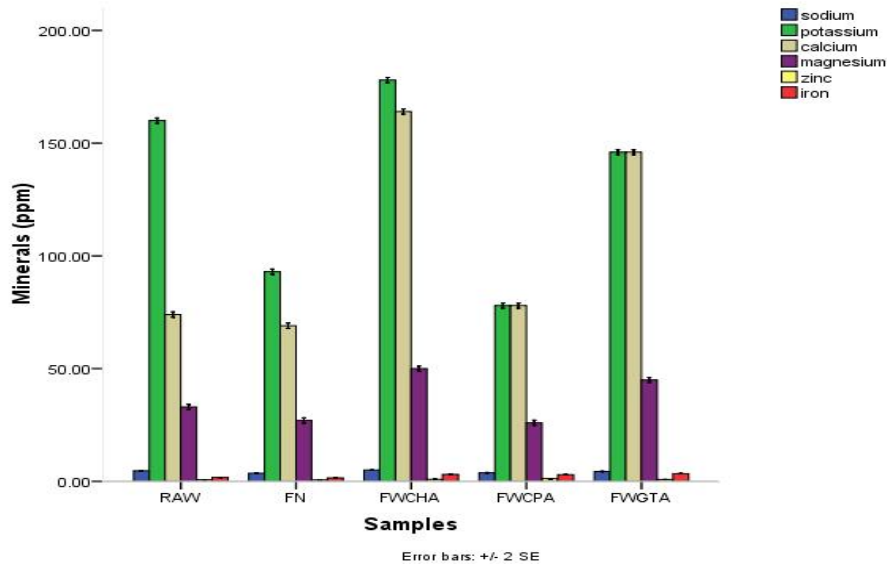


Fig. 4. Mineral content (ppm) of fermented Roselle from Benue neutralized with different ashes

Increase in the protein content of the fermented samples obtained from both states could be as a result of extracellular enzymes secreted by microbial activities during fermentation [15]. It could also be due to the increase in microbial biomass in the form of single cell proteins [16].

The decrease in moisture content of the fermented samples could be as a result of oven drying followed by proper packaging which prevented it from absorbing moisture from the

atmosphere. The result is in agreement with that observed for Roselle calyces neutralized with Trona by Ojokoh, et al. [17].

A decrease in fat content could be due to its utilization by some of the microorganisms during fermentation. Fermented samples recorded increased carbohydrate content compared to the raw samples. The result obtained for carbohydrate could be as a result of the metabolic activities that occurred during the

fermentation process. Increase in ash content after fermentation could imply an appreciable increase in the quantity of minerals present.

Variations recorded in the proximate composition of samples observed from both Ekiti and Benue States before and after fermentation could be as a result of varying environmental factors in these locations. These factors may include soil, water, air, insect and pest activities as well as animals.

The varying result obtained in the mineral composition of fermented calyx could be due to different factors such as the source of ashes as well as the environmental factors of the states where the samples were collected from.

A decrease in antinutrient composition of all the samples could be due to the activities of microbes during fermentation. The decrease in antinutrients conforms to the findings of Ojokoh et al. [17]. Reduction in phytate also agrees with the report of Zokti [18] that phytate content decrease could be attributed to possible secretions of hydrolytic enzymes (phytase) by the microorganisms.

4. CONCLUSION

The research reveals that fermentation with different wood ashes improved the nutritional content of the sample while antinutrients were decreased at various levels. It was also discovered that the nutrient and antinutrient contents of the sample vary with geographical location.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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