

Effects of Fermentation on Nutritional and Microbial Qualities of Ash Neutralized Roselle (*Hibiscus sabdariffa* L.)

ABSTRACT

The study was conducted to investigate the effect of fermentation on the nutritional and microbial qualities of Roselle calyces from two States in Nigeria. Roselle calyx was fermented naturally (FN), fermented with coconut husk ash (FWCHA), Fermented with cocoa pod ash (FWCPA), Fermented with *Gmelina* tree ash (FWGTA) separately for 72 hours. The chemical and antinutrient compositions of raw and fermented calyx were determined using standard procedures. The microbial assessment of the samples was also determined. Moisture, ash, protein and carbohydrate contents increased significantly in fermented samples compared to raw calyx, however, the fat content reduced and crude fibre varied significantly likewise the antinutrients (Tannin, Hydrogen cyanide, Phytate) significantly reduced by the fermentation process ($\alpha=0.05$). A total of 8 organisms were isolated during fermentation, which included four (4) bacteria, one (1) yeast, and three (3) moulds. Microorganisms isolated during fermentation include *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* (Bacteria) *Saccharomyces cerevisiae* (Yeast), *Mucor mucedo*, *Aspergillus niger*, *Aspergillus flavus* (Mold)

Keywords: [Roselle, fermentation, ash, proximate, mineral, antinutrient]

1. INTRODUCTION

Malnutrition is an unhealthy condition caused by not eating enough healthy food. People are malnourished if unable to fully utilize the food they eat (undernutrition) or when they consume too many calories (overnutrition) [1].

Vegetables are agricultural products which are beneficial to the body; maintain good health and helps in the prevention of diseases [2]. Leafy vegetables are affordable and also possess high quality nutritional source which can aid in alleviating malnutrition rampant among the less wealthy population .

Roselle is an herbaceous upright plant growing up to 2 metres belonging to the family Malvaceae. Two varieties of Roselle has been recognized and also consumed, these are green calyx variety used to prepare soups, sauces and stews while the red calyx variety is commonly used for juice[3].

In West Africa, the green Roselle calyx variety is commonly steeped overnight in ash in water solution or parboiled before it is been used to prepare soup. Ash is the residue powder left after the combustion of wood. The major component of ash is calcium carbonate, about 25 or even 45 percent [4]. Less than 1 percent is phosphate, and less than 10 percent potash [5].

Environmental factors have an enormous effect on the growth and development of plants [6]. Previous studies have shown that plant morphology, most importantly, blade size, texture, thickness and plant morphology are affected by biological environment [7]. Others include fertilizers, climatic factors, soil nutrients, soil factors. Biological factors such as pests, soil microbes, planting density, weeds and diseases [3] also play important roles.

The traditional fermentation of foods serves as an improvement of diet with the change in the aroma, flavour and texture in food[8]. The nutritional benefits of Roselle are however limited without fermentation. The specific objective of the research is to determine the influence of climatic and environmental factors on the chemical composition of Roselle calyx and the neutralizing efficacy of ash on Roselle calyx.

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND PROCESSING

Kwara is a State in the Middle belt zone of Nigeria with the woodland and tall grass savanna vegetation. The State Capital is Ilorin. The State is also known as the State of Harmony. Its coordinates are 8° 30'N 5° 00'E. Ondo State, however, is in the South western region of Nigeria with the tropical rain forest vegetation, it is also known as the Sunshine State. Akure is the State Capital. Its geographic coordinates are 7° 10'N 5° 05' E.

Green Roselle calyx was purchased from sellers at Oba market, Akure and from International Market, Ilorin. Coconut husk was obtained from coconut sellers in Federal University of Technology, Akure (FUTA) community [9]. Cocoa pod husk and *Gmelina* tree shavings were purchased from Cocoa and *Gmelina* sellers in Uso Town, Ondo state and were all transported to the laboratory in sterile polyethene bags.

Dry ashing technique was used to prepare ash from Coconut husk, cocoa pod and *Gmelina* tree shavings according to the method described by Gaines. Coconut husk, cocoa pod and *Gmelina* tree wood shaving were charred separately using an open flame. A 100g each of the charred sample was weighed in separate porcelain crucibles labeled 1, 2, 3 respectively and placed inside the muffle furnace

at 500°C until all the carbon was oxidized. The ash residue obtained was then weighed and cooled in a desiccator.

The Roselle calyx was sorted, cleaned and dried [10]. A 30g of ash was weighed and aseptically poured into 2 litres of clean, sterile water in clean plastic containers with lid, followed by homogenization and the addition of 150g of clean, sorted roselle calyx. The containers were labeled FN: Fermented naturally (samples fermented with water alone), FWCHA: Fermented with coconut husk ash, FWCPA: Fermented with cocoa pod ash, FWGTA: Fermented with *Gmelina* wood ash. The raw roselle calyx was the control. The samples were allowed to ferment at $30 \pm 2^\circ\text{C}$ for 72 hours [3, 11].

2.2 Microbial analysis of the samples

Bacterial and fungal load were determined using standard methods as described by Cheesbrough. Nutrient agar was used for bacteria while potato dextrose agar was used for fungal load. A 1g of the sample aliquot was measured using a sterile syringe into 9 mls of clean, sterile water followed by homogenization (10^{-1}), another 1ml was measured from 10^{-1} into 10 mls of sterile water and then homogenized (10^{-2}). The serial dilution was done to (10^{-5}) followed by pouring already prepared molten agar in an aseptic environment. Microbial analysis was carried out at 24 hours interval throughout the 72 hour fermentation process. The agar used was prepared according to the manufacturer's specification. Bacteria plates were incubated at 37°C for 18 hours while fungi plates were incubated at 25°C for 72 hours. The number of colonies grown in each plate was counted after incubation and classified by microscopic examination of colony morphology and biochemical tests after separation and growth on slants.

2.3 Proximate analysis

The proximate analysis of the samples was performed according to the Association of Official Analytical Chemists (AOAC) 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 AOAC. Tannin content was determined according to the method described by Makkar *et al*, Doss *et al*. Cyanide was determined by a modified AOAC, Onwuka method.

2.5 Sensory evaluation

The Organoleptic properties of Roselle calyx fermented were evaluated and compared with raw Roselle calyx. Organoleptic test was carried out by consumers of Roselle. The products were assessed on a 5 point Hedonic scale by regular Roselle consumers as the Panelists [17]. Roselle (*Hibiscus sabdariffa* L.) was prepared in five different ways: raw samples, samples fermented naturally, fermented with cocoa pod ash, fermented with coconut husk ash and samples fermented with *Gmelina* tree ash were cooked with melon soup and served. The attributes tested for were aroma, color, texture and general acceptability.

2.6 Statistical analysis

Statistics computed include mean $\pm$ Standard deviation, analysis of Variance (ANOVA) and Duncan New Multiple Range Test. All the data generated were statistically analyzed using mean $\pm$ standard deviation and analysis of variance statistical package for social sciences version 16.

3. RESULTS AND DISCUSSION

3.1 Microorganisms isolated during the fermentation of Roselle

A total of eight (8) microorganisms were isolated. They are *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* as the only coliform, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Aspergillus flavus*, *Aspergillus niger*, *Mucor mucedo*. The changes in the microbial population during fermentation revealed that the sample fermented with ash had a higher bacteria population while the sample fermented without ash had a higher fungal population

3.2 Changes in the proximate composition during the fermentation of Roselle from Kwara and Ondo States neutralized with different wood ash

Table 1: Proximate composition (%) of fermented Roselle from Kwara neutralized with different ash

Proximate	Ash content	Moisture content	Fat content	Crude fibre	Protein	Carbohydrate content
RAW	4.63±0.09 ^b	12.46±0.06 ^b	16.68±0.33 ^e	18.96±0.09 ^a	9.82±0.06 ^a	28.78±0.11 ^b
FN	4.10±0.02 ^a	21.13±0.13 ^e	8.40±0.08 ^b	22.01±0.09 ^d	11.18±0.02 ^c	41.85±0.10 ^e
FWCHA	6.76±0.12 ^c	13.35±0.02 ^c	9.15±0.14 ^c	24.76±0.08 ^e	10.17±0.04 ^b	35.81±0.03 ^c
FWCPA	7.42±0.02 ^d	19.75±0.07 ^d	14.86±0.04 ^d	19.95±0.04 ^b	10.14±0.01 ^b	27.88±0.05 ^a
FWGTA	7.76±0.02 ^e	12.15±0.08 ^a	8.08±0.04 ^a	20.42±0.06 ^c	11.51±0.03 ^d	39.87±0.14 ^d

109 Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a
110 column are not significantly different at P≤0.05.

111 Legend:

112 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa
113 pod ash; FWGTA: Fermented with *Gmelina* tree ash.

114 **Table 2: Proximate composition (%) of fermented Roselle samples from Ondo neutralized with**
115 **different ash**

Proximate	Ash content	Moisture content	Fat content	Crude fibre content	Protein	Carbohydrate content
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RAW	5.08±0.05 b	10.56±0.04 a	13.34±0.04 e	20.59±0.08 d	10.86±0.32 b	31.73±0.02 ^a
FN	3.60±0.05 a	11.12±0.01 b	6.55±0.04 ^b	22.82±0.02 e	13.68±0.03 e	42.05±0.04 ^b
FWCHA	5.82±0.01 b	17.20±0.10 c	6.69±0.00 ^c	16.54±0.04 a	9.58±0.02 ^a	50.70±0.01 ^e
FWCPA	7.12±0.01 c	11.28±0.02 c	6.96±0.05 ^d	17.70±0.17 c	12.91±0.01 d	43.84±0.03 ^c
FWGTA	7.06±0.05 c	13.06±0.05 d	5.75±0.04 ^a	17.05±0.04 b	11.34±0.03 c	45.56±0.05 ^d

116 Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a
 117 column are not significantly different at P≤0.05.

118 Legend:

119 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa
 120 pod ash; FWGTA: Fermented with *Gmelina* tree ash.

121 3.3 Changes in antinutrient content of fermented Roselle from Kwara and Ondo States neutralized 122 with different wood ash

123 **Table 3: Antinutrient content of fermented Roselle from Kwara neutralized with different ash**

Antinutrients	Phytates (mg/g)	Glycosides	Tannins (mg/g)
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		(mg/kg)	
RAW	14.42±0.20 ^e	82.49±0.10 ^e	1.43±0.06 ^d
FN	13.18±0.04 ^d	72.63±0.01 ^c	0.95±0.03 ^{bc}
FWCHA	7.83±0.08 ^a	44.25±0.02 ^a	0.78±0.07 ^a
FWCPA	8.45±0.06 ^b	80.59±0.08 ^d	1.00±0.04 ^c
FWGTA	9.48±0.06 ^c	60.82±0.17 ^b	0.88±0.05 ^b

124 Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a
 125 column are not significantly different at P≤0.05

126 Legend:

127 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa
 128 pod ash; FWGTA: Fermented with *Gmelina* tree ash

129 **Table 4: Antinutrient content of fermented Roselle from Ondo neutralized with different ash**

Samples	Phytates (mg/g)	Glycosides (mg/kg)	Tannins (mg/g)
RAW	12.97±0.08 ^e	107.87±0.09 ^e	1.20±0.03 ^d
FN	11.12±0.04 ^d	83.66±0.18 ^c	1.05±0.03 ^c

FWCHA	8.65±0.02 ^b	68.17±0.08 ^a	0.79±0.02 ^a
FWCPA	10.51±0.06 ^c	98.19±0.04 ^d	0.87±0.05 ^b
FWGTA	6.80±0.08 ^a	71.09±0.09 ^b	0.79±0.03 ^a

130 Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a
131 column are not significantly different at P≤0.05

132 Legend:

133 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa
134 pod ash; FWGTA: Fermented with *Gmelina* tree ash

135 **Table 5: Sensory evaluation profile of the Roselle samples from Kwara**

Samples	RAW	FN	FWCHA	FWCPA	FWGTA
AROMA	1.00±0.00 ^a	1.66±0.57 ^b	3.00±0.00 ^c	3.36±0.57 ^d	5.00±0.00 ^e
COLOUR	0.66±0.57 ^a	5.00±0.00 ^d	2.00±0.00 ^{bc}	1.66±0.57 ^b	2.66±0.57 ^c
TEXTURE	1.00±0.00 ^a	2.00±0.00 ^b	3.66±0.57 ^c	3.33±0.57 ^c	4.66±0.57 ^d
OVERALL ACCEPTABILITY	1.00±0.00 ^a	2.33±0.57 ^b	3.00±0.00 ^c	2.33±0.57 ^b	4.50±0.70 ^d

136 Values represent means ± standard deviation of triplicate readings

137 Superscripts of the same letter in a column are not significantly different at P≤0.05

Legend:

FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa pod ash; FWGTA: Fermented with *Gmelina* tree ash.

Table 6: Sensory evaluation profile of the Roselle samples from Ondo

Samples	RAW	FN	FWCHA	FWCPA	FWGTA
AROMA	1.33±0.60 ^a	1.85±0.10 ^b	2.80±0.20 ^c	3.30±0.15 ^d	4.83±0.25 ^e
COLOUR	0.50±0.10 ^a	4.90±0.50 ^d	2.25±0.57 ^{bc}	1.50±0.20 ^b	2.50±0.00 ^c
TEXTURE	1.25±0.57 ^a	1.90±0.20 ^b	3.85±0.10 ^c	3.51±0.50 ^c	5.00±0.50 ^d
OVERALL ACCEPTABILITY	1.08±0.00 ^a	2.00±0.15 ^b	3.01±0.20 ^d	2.31±0.33 ^c	4.65±0.60 ^e

Values represent means ± standard deviation of triplicate readings

Superscripts of the same letter in a column are not significantly different at P≤0.05

Legend:

FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa pod ash; FWGTA: Fermented with *Gmelina* tree ash.

The higher bacterial population recorded in the sample fermented with ash may be due to alkaline nature of the ash which raised the initial pH that favoured their growth [18]. This was in agreement with the result of [3,11].

Saccharomyces cerevisiae is a widely distributed spore former [19]. This may be due to contamination from handling of the calyces in the process of fermentation since there are a lot of fungi spores in the air. *Bacillus* sp has been reported to be the primary microorganism for the fermentation of African locust bean to produce 'dawa dawa' [20] 'soumbala' [21], some Asian fermented foods [22, 23]. Their presence in these fermentations may be due to their capability to start the fermentation of nitrogenous as well as carbohydrate products [24]. In addition, *Bacillus* sp. is known to produce a diversity of enzymes. Their metabolic activities can contribute to flavour and aroma generating reactions [25].

Staphylococcus aureus occurrence agrees with the findings of [26] who reported the absence of *Staphylococcus* in the 48 h fermentation period for 'dawadawa' and those of [27] and [28] who found that *Staphylococcus* was present only at the early stages of the fermentation.

Klebsiella pneumoniae, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mucor mucedo* were found only at the onset of the fermentation and thereafter could not be isolated. The possible involvement of these microorganisms in the fermentation is then ruled out. The microorganisms were presumed to be probably contaminants obtained during the initial handling.

Of all the samples fermented, samples fermented naturally had the highest increase in protein level (Table 2). The increase in protein content in the sample fermented naturally could be as a result of an increase in the different species of microorganisms involved which may have secreted some extracellular enzymes (proteins) [29]. The increase in the microbial biomass in the form of single cell proteins may be another reason for the increase in the protein content [30].

The ash content of all the fermented samples (Table 1 and 2) shows significant decrease ($P \leq 0.05$) in the sample fermented naturally. The high value of ash in the unfermented sample shows that the calyx may have a reasonable quantity of mineral elements. Carbohydrate, moisture levels increased while fat and crude fibre contents reduced significantly.

The results of the changes in the antinutrient of Roselle are as shown in Tables 3 and 4. Fermentation of calyx brings about a significant decrease ($P \leq 0.05$) in the cyanide content of the calyx

when compared with the unfermented calyx. However, the sample fermented with wood ash recorded the highest decrease in cyanide content with fermentation. This however agrees with the report of [31] and [3] that cyanide content of the Roselle decreases with fermentation.

Fermentation brought about a significant decrease ($P \leq 0.05$) in the tannin content of the calyx samples fermented naturally and with ash, however, *Gmelina* tree ash fermented samples had the lowest decrease. The decrease in the tannin may be because of the processing [30] and [32] that samples were subjected to coupled with the activities of the microbial enzymes involved in the fermentation process.

The phosphorus in the phytic acid interferes with Calcium, Iron, Magnesium and Zinc absorption because of its ability to chelate divalent cationic minerals [33]. The results of the present study revealed that there was a significant decrease ($P \leq 0.05$) of the phytate content of fermented calyx samples. However, the phytate content of the sample fermented with *Gmelina* tree ash was significantly lower ($P \leq 0.05$). The phytate content of the unfermented calyx was lower than that of tropical vegetables (394.9-1692.3 mg/100g [34]. The decrease in the phytate content of the fermented calyx agrees with the report of [10] that phytate content decrease could be attributed to possible secretions of hydrolytic enzymes (phytase) by the microorganisms. This enzyme is capable of hydrolyzing phytate.

The result of sensory evaluation as shown in Table 5 and 6 revealed that fermentation improves taste, aroma, colour and texture of the calyx. Samples fermented with *Gmelina* tree ash had the best aroma with the raw sample having the least. In terms of colour, samples fermented naturally were the best with raw sample having the least. Samples fermented with *Gmelina* tree ash had the best texture with the raw samples having the least. For overall acceptability, samples fermented with *Gmelina* tree ash had the best with the raw samples having the least.

4. CONCLUSION

The study therefore reveals that fermentation as a precooking method and the use of ash especially *Gmelina* tree ash can be used to reduce the antinutrient, improve the nutrient in Roselle. Also, climatic and environmental factors play a key role in the chemical composition of the plant

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