

Effects of Fermentation on Nutritional and Microbial Qualities ~~Proximate and Antinutrient~~

~~2-Content~~ of Ash Neutralized Roselle (*Hibiscus* *sabdariffa* L.) Calyces ~~from Kwara and Ondo States,~~ ~~4-Nigeria~~

5

6 ABSTRACT

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~~This~~ The study was conducted to investigate the effects of fermentation on the nutritional and microbial qualities proximate and antinutrient content of ash-neutralized R_oselle calyces from Kwara and Ondo S_two states in Nigeria. Five calyx (Raw, 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, there fat content reduced and crude fibre and mineral contents varied significantly likewise the antinutrients (Tannin, Hydrogen cyanide, Phytate) significantly reduced by the fermentation process.

~~fermented naturally, fermented with coconut husk ash, fermented with cocoa pod ash and fermented with Gmelina tree ash) samples from each location were fermented. A total of 8 organisms were isolated during the fermentation. They include four (4) bacteria, one (1) yeast and three (3) moulds were isolated. These include Microorganisms isolated during fermentation include Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Mucor mucedo, Aspergillus niger, Aspergillus flavus. Moisture, ash, protein and carbohydrate contents increased significantly, fat content reduced and crude fibre and mineral contents varied significantly likewise the antinutrients (Tannin, Hydrogen cyanide, Phytate) significantly reduced by the fermentation process.~~

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9 ~~Keywords: /Roselle, fermentation, wood ash, proximate,~~ mineral, antinutrients~~Kwara, Ondo-~~

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11 1. INTRODUCTION

12 Malnutrition is an unhealthy condition that results from not eating enough food or not eating
13 enough healthy food-ref-. People are malnourished if their diet does not provide adequate calories
and protein for growth and maintenance or they are unable to fully utilize the food they eat due to
illness

15 (undernutrition) or when they consume too many calories (overnutrition) [1]. A condiment is a
spice,

16 sauce, or, preparation that is added to food to impart a particular flavor, to enhance its flavor, or in
17 some cultures, to complement the dish. Therefore, to alleviate malnutrition, the addition of food
18 condiments to our diet is also necessary.

19 Vegetables are agricultural products which are important sources of protective foods that are
20 highly beneficial for the maintenance of good health and prevention of disease [2]. Leafy
vegetables

21 represent an inexpensive but high quality nutritional source especially for the poor segment of the
22 population where malnutrition is wide spread.

23 The growth and development of plants are affected by environmental factors [3]. Previous studies
24 have shown that biological environment have effect on plant morphology, especially blade size,
texture,

25 thickness and plant morphology [4]. Others include climatic factors, soil factors, soil nutrients and

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fertilizers, the presence of heavy metals. Biological factors such as planting density, weeds, 26
diseases and

27 pests, soil microbes and so on [3] also play important roles.

28 Kwara is a State in Western Nigeria. Its capital is Ilorin. The primary ethnic group of Kwara State
29 is Yoruba, with significant Nupe, Bariba, and Fulani minorities. Kwara State consists of 16 Local

30 Government Areas. The State is also known as the State of Harmony. Its coordinates are 8° 30'N 5° 00'E.

31 Ondo State also known as the Sunshine State was created from the former Western State. Akure is the

32 State Capital. Ondo state has 18 Local Government Areas. Its geographic coordinates are 7° 10'N 5° 05' E.

33 E.

34 Ash is the residue powder left after the combustion of wood, for example, burning wood in a

35 home fire place or an industrial power plant. Ash contains calcium carbonate as its major component,

36 representing 25 or even 45 percent [5]. Less than 10 percent is potash, and less than 1

37 percent phosphate; there are trace elements of iron, manganese, zinc, copper and some heavy metals

38 [6]. However these numbers vary as combustion temperature is an important variable in determining

39 wood ash composition.

40 Roselle is an herbaceous upright plant growing up to 2 metres belonging to the family Malvaceae.

41 Two botanical varieties have been recognized and consumed over time, these are the red calyx

42 commonly used for juice and the green calyx varieties used to prepare soups, stews [7].

43 In West Africa, the green Roselle calyx variety is commonly steeped overnight in ash in water

44 solution or parboiled before it is been used to prepare soup.

47 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

49 2.1 SAMPLE COLLECTION AND PROCESSING

50 Green Roselle calyx was purchased from sellers at Oba market, Akure and from International

51 Market, Ilorin. Coconut husk was obtained from its sellers in FUTA community and ashed [8].

Cocoa pod

52 husk and *Gmelina* tree shavings was purchased from its sellers in Uso Town, Ondo state and were

all

53 transported to the laboratory in sterile polyethene bags.

54 The roselle calyx were sorted, cleaned and dried [9]. A 30g of ash was weighed and aseptically

55 poured into 2 litres of clean, sterile water in clean plastic containers with lid, followed by

homogenization

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and the addition of 150g of clean, sorted roselle calyx. The containers were labeled 56 FN: Fermented

57 naturally (samples fermented with water alone), FWCHA: Fermented with coconut husk ash, FWCPA:

58 Fermented with cocoa pod ash, FWGTA: Fermented with *Gmelina* wood ash. The raw roselle calyx was

59 the control. The samples were allowed to ferment at 30 ±2°C for 72 hours [7, 10].

61 2.2 Microbial analysis

62 Bacterial and fungal load were determined using standard methods as described by [11]. Nutrient

63 agar was used for bacteria while potato dextrose agar was used for fungal load using the pour

64 plating technique. The agar used was prepared according to the manufacturer's specification. Bacteria

plates

65 were incubated at 37°C for 18 hours while fungi plates were incubated at 25°C for 72 hours. The

number

66 of colonies growing in each plate was counted after incubation and classified by microscopic

examination

67 of colony morphology and biochemical tests after separation and growth on slants.

68 2.3 Proximate analysis

69 The proximate analysis of the samples was performed according to [12]. Procedures for ash,

70 crude fibre, fat, moisture and protein using a nitrogen to protein conversion factor of 6.25.

Carbohydrate

71 was determined by difference.

72 2.4 Anti nutrient content determination

73 Phytate was determined according to AOAC, 2013. Tannin content was determined according to
74 the method described by [13, 14]. Cyanide was determined by a modified [12, 15] method.

75 2.5 Sensory evaluation

76 The Organoleptic properties of Roselle calyx fermented naturally and with the ashes were
77 evaluated and compared with raw Roselle calyx. Organoleptic test was carried out by consumers
of
78 Roselle. The products were assessed on a 5 point Hedonic scale by regular Roselle consumers as
the

79 Panelists [16]. Roselle (*Hibiscus sabdariffa* L.) was prepared in five different ways: raw samples,
samples

80 fermented naturally, fermented with cocoa pod ash, fermented with coconut husk ash and samples
81 fermented with *Gmelina* tree ash were cooked with melon soup and served. The attributes tested
for are

82 aroma, color, texture and general acceptability. The mean scores were calculated.

83 2.6 Statistical analysis

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Statistics computed include mean $\pm$ Standard deviation, analysis of Variance 84 (ANOVA) and
85 Duncan New Multiple Range Test. All the data generated were analyzed statistically using mean $\pm$
86 standard deviation and analysis of variance statistical package for social sciences version 16.
87

88 3. RESULTS AND DISCUSSION

89

90 3.1 Microorganisms isolated during the fermentation of Roselle

91 A total of eight (8) microorganisms were isolated. They are *Bacillus subtilis*, *Pseudomonas*
92 *aeruginosa* and *Escherichia coli* as the only coliform, *Staphylococcus aureus*, *Saccharomyces*
cerevisiae,

93 *Aspergillus flavus*, *Aspergillus niger*, *Mucor mucedo*

94 3.2 Changes in the proximate composition during the fermentation of Roselle from Kwara and 95 Ondo States neutralized with different wood ash

96 Ash content was highest in Kwara samples fermented with *Gmelina* tree ash 7.76 ± 0.02 % and
97 least in Ondo samples fermented naturally 3.60 ± 0.05 %. Moisture was observed to be highest in
kwara

98 samples fermented naturally 21.13 ± 0.13 % and least in raw Ondo samples 10.56 ± 0.04 %. Fat was
highest

99 in raw Kwara samples 16.68 ± 0.33 % and least in ondo samples fermented with *Gmelina* tree ash
100 5.75 ± 0.04 %. Crude fibre was observed to be highest in Kwara samples fermented with coconut
husk ash

101 24.76 ± 0.08 and least in Ondo samples fermented with coconut husk ash 16.54 ± 0.04 %. Protein
was

102 highest in Ondo samples fermented naturally 13.68 ± 0.03 % and least in Ondo samples fermented
with

103 coconut husk ash 9.58 ± 0.02 %. Carbohydrate was highest in Ondo samples fermented with
coconut husk

104 ash 50.70 ± 0.01 % and least in Kwara samples fermented with cocoa pod ash 27.88 ± 0.05 %.

105 Table 1: Proximate composition (%) of fermented Roselle from Kwara neutralized with different 106 ash

Proximate Ash content Moisture
content

Fat content Crude fibre Protein Carbohydrate
content

RAW $4.63 \pm 0.09_b$ $12.46 \pm 0.06_b$ $16.68 \pm 0.33_e$ $18.96 \pm 0.09_a$ $9.82 \pm 0.06_a$ $28.78 \pm 0.11_b$

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FN $4.10 \pm 0.02_a$ $21.13 \pm 0.13_c$ $8.40 \pm 0.08_b$ $22.01 \pm 0.09_d$ $11.18 \pm 0.02_c$ $41.85 \pm 0.10_e$

FWCHA $6.76 \pm 0.12_c$ $13.35 \pm 0.02_c$ $9.15 \pm 0.14_c$ $24.76 \pm 0.08_e$ $10.17 \pm 0.04_b$ $35.81 \pm 0.03_c$

FWCPA $7.42 \pm 0.02_d$ $19.75 \pm 0.07_d$ $14.86 \pm 0.04_d$ $19.95 \pm 0.04_b$ $10.14 \pm 0.01_b$ $27.88 \pm 0.05_a$

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

Values represent means ± standard deviation of triplicate readings. Superscripts of the 107 same letter in a

108 column are not significantly different at P≤0.05.

109 Legend:

110 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa

111 pod ash; FWGTA: Fermented with *Gmelina* tree ash.

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

Proximat

e

Ash

content

Moisture

content

Fat content Crude fibre

content

Protein Carbohydrat

e content

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

6

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

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45.56±0.05_d

Values represent means ± standard deviation of triplicate readings. Superscripts of the 114 same letter in a

115 column are not significantly different at P≤0.05.

116 Legend:

117 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa

118 pod ash; FWGTA: Fermented with *Gmelina* tree ash.

119 **3.3 Changes in antinutrient content of fermented Roselle from Kwara and Ondo States neutralized**
120 **with different wood ash**

121 Phytates was observed to be highest in raw Kwara samples (14.42±0.20mg/g) and least in Ondo
122 samples fermented with *Gmelina* tree ash. Hydrogen cyanide was highest in raw Ondo samples
123 (107.87±0.09mg/kg) and least in Kwara samples fermented with coconut husk ash
(44.25±0.02mg/kg).

124 Tannins was observed to be highest in raw Kwara samples (1.43±0.06mg/g) and least in Kwara
samples

125 fermented with coconut husk ash (0.78±0.07mg/g).

126

127

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

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Antinutrients Phytates (mg/g) Glycosides
(mg/kg)

Tannins (mg/g)

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

Values represent means ± standard deviation of triplicate readings. Superscripts of the 129 same letter in a

130 column are not significantly different at P≤0.05

131 Legend:

132 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa

133 pod ash; FWGTA: Fermented with *Gmelina* tree ash

134 **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

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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

Values represent means ± standard deviation of triplicate readings. Superscripts of the 135 same letter in a

136 column are not significantly different at P≤0.05

137 Legend:

138 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa

139 pod ash; FWGTA: Fermented with *Gmelina* tree ash

140 **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

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Values represent means ± standard deviation of triplicate 141 readings

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

143 Legend:

144 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa

145 pod ash; FWGTA: Fermented with *Gmelina* tree ash.

146 **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

147 Values represent means ± standard deviation of triplicate readings

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

149 Legend:

150 FN: Fermented naturally; FWCHA: Fermented with coconut husk ash; FWCPA: Fermented with cocoa

151 pod ash; FWGTA: Fermented with *Gmelina* tree ash.

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The changes in the microbial population during fermentation revealed that the sample 152 fermented with

153 wood ash had a higher bacteria population while the sample fermented without wood ash had a higher

154 fungal population. The higher bacterial population recorded in the sample fermented with wood ash may

155 be due to alkaline nature of the wood ash which raised the initial pH that favoured their growth [17]. This

156 was in agreement with the result of [7,10].

157 *Saccharomyces cerevisiae* is a widely distributed spore former [18]. This may be due to

158 contamination from handling of the calyces in the process of fermentation since there are a lot of fungi

159 spores in the air. *Bacillus* sp has been reported to be responsible for the fermentation of African locust

160 bean to produce 'dawa dawa' [19] 'soumbala' [20], some Asian fermented foods [21,22]. Their presence

161 in these fermentations may be due to their ability to initiate fermentation of both nitrogenous and 162 carbohydrate products [23]. In addition, *Bacillus* sp. are known to produce a diversity of enzymes. Their

163 metabolic activities can contribute to flavour and aroma generating reactions [24].

164 *Staphylococcus aureus* occurrence agrees with the findings of [25] who reported the absence

165 of *Staphylococcus* in the 48 h fermentation period for 'dawadawa' and those of [26] and [27] who found

166 that *Staphylococcus* was present only at the early stages of the fermentation.

167 *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mucor mucedo* were found

168 only at the onset of the fermentation and thereafter could not be isolated. The possible

involvement of

169 these microorganisms in the fermentation is then ruled out. The microorganisms were presumed to be

170 probably contaminants obtained during the initial handling.
171 Of all the samples fermented, samples fermented naturally had the highest increase in protein
172 level (Table 2). The increase in protein content in the sample fermented naturally may be due to
173 increase in the mixture/ variety of microbial species involved which might have secreted some
174 extracellular enzymes (proteins) [28]. The increase in the microbial biomass in the form of single
cell
175 proteins may be another reason for the increase in the protein content [29].
176 The ash content of all the fermented samples (Table 1 and 2) shows significant decrease
177 ($P \leq 0.05$) in the sample fermented naturally. The high value of ash in the unfermented sample
shows that

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the calyx may have a reasonable quantity of mineral elements. Carbohydrate, moisture 178 levels
increased
179 while fat and crude fibre contents reduced significantly.
180 The results of the changes in the antinutrient of Roselle are as shown in Tables 3 and 4.
181 Fermentation of calyx brings about a significant decrease ($P \leq 0.05$) in the cyanide content of the
calyx
182 when compared with the unfermented calyx. However, the sample fermented with wood ash
recorded
183 the highest decrease in cyanide content with fermentation agrees with the report of [30] and [7]
that
184 cyanide content of the Roselle decreases with fermentation.
185 Fermentation brought about a significant decrease ($P \leq 0.05$) in the tannin content of the calyx
186 samples fermented naturally and with ash, however, *Gmelina* tree ash fermented samples had
the
187 lowest decrease. The decrease in the tannin may be because of the processing [29] and [31] that
188 samples were subjected to coupled with the activities of the microbial enzymes involved in the
189 fermentation process.
190 The phosphorus in the phytic acid interferes with Calcium, Iron, Magnesium and Zinc absorption
191 because of its ability to chelate divalent cationic minerals [32]. The results of the present study
revealed
192 that there was a significant decrease ($P \leq 0.05$) of the phytate content of fermented calyx samples.
193 However, the phytate content of the sample fermented with *Gmelina* tree ash was significantly
lower
194 ($P \leq 0.05$). The phytate content of the unfermented calyx was lower than that of tropical vegetables
195 (394.9-1692.3 mg/100g [33]). The decrease in the phytate content of the fermented calyx agrees
with the
196 report of [10] that phytate content decrease could be attributed to possible secretions of hydrolytic
197 enzymes (phytase) by the microorganisms. This enzyme is capable of hydrolyzing phytate.
198 The result of sensory evaluation as shown in Table 5 and 6 revealed that fermentation improves
199 taste, aroma, colour and texture of the calyx. Samples fermented with *Gmelina* tree ash had the
best
200 aroma with the raw sample having the least. In terms of colour, samples fermented naturally were
the
201 best with raw sample having the least. Samples fermented with *Gmelina* tree ash had the best
texture
202 with the raw samples having the least. For overall acceptability, samples fermented with *Gmelina*
tree
203 ash had the best with the raw samples having the least.

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205 4. CONCLUSION

206 The study therefore reveals that fermentation as a precooking method and the use of ash
especially
207 *Gmelina* tree ash can be used to reduce the antinutrient, improve the nutrient in Roselle. Also,
climatic

208 and environmental factors play a key role in the chemical composition of the plant
209

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