International Journal of Biochemistry Research & Review
16(3): 1-8, 2017; Article no.IJBCRR.30168
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

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

Ojokoh Anthony Okhonlaye[1] and Adaja Oluwabunmi Olabisi[1*]

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

Authors' contributions

This work was carried out in collaboration between both authors. Author AOO designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Author OAO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/30168
Editor(s):
(1) Fatih Oz, Food Engineering Department, Agriculture Faculty, Ataturk University, Turkey.
(2) Hector M. Mora Montes, Department of Biology, University of Guanajuato, Mexico.
Reviewers:
(1) Makinde Folasade Maria, Bowen University, Nigeria.
(2) Lennox, Josiah Asime, University of Calabar, Calabar, Nigeria.
(3) Mercy Badu, Kwame Nkrumah University of Science and Technology, Ghana.
(4) Atamgba A. Asuk, Cross River University of Technology, Nigeria.
(5) Elly Kurobuza Ndyomugyenyi, Gulu University, Uganda.
(6) Phyu Phyu Myint, Sittway University, Sittway, Myanmar.
Complete Peer review History: http://www.sciencedomain.org/review-history/18513

Original Research Article

Received 21st October 2016
Accepted 9th February 2017
Published 5th April 2017

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

**Corresponding author: E-mail: oluwabunmiolabisi@gmail.com;*

content reduced and crude fibre varied significantly likewise the antinutrients (Tannin, Hydrogen cyanide, Phytate) significantly reduced by the fermentation process (α=0.05). A total of 8 organisms were isolated during fermentation, which included four (4) bacteria, one (1) yeast, and three (3) moulds. *Pseudomonas aeruginosa* was isolated before fermentation. *Staphylococcus aureus, Escherichia coli* were isolated before and during fermentation at 24 hours and could not be isolated after. *Bacillus subtilis, Saccharomyces cerevisiae Aspergillus flavus and Aspergillus niger, Mucor mucedo* were isolated throughout the fermentation. Microorganisms isolated during fermentation include *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa. Saccharomyces cerevisiae, Mucor mucedo, Aspergillus niger, Aspergillus flavus.*

Keywords: Roselle; fermentation; ash; proximate; mineral; antinutrient.

1. INTRODUCTION

Malnutrition is an unhealthy condition caused by not eating enough healthy food. Also, 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 help 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 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].

The traditional fermentation of foods serves as an improvement of diet with the change in the aroma, flavour and texture [6]. The nutritional benefits of Roselle are however limited without fermentation [3,9]. 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

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 [7]. 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 100 g each of the charred sample was weighed in separate porcelain crucibles labeled 1, 2, 3 respectively and placed inside the muffle furnace at 500℃ 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 [8]. A 30 g 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 150 g 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 ± 2℃ for 72 hours [3,9].

2.2 Microbial Analysis of the Samples

Bacterial and fungal load were determined using standard methods as described by Cheesbrough [10]. Nutrient agar was used for bacteria while potato dextrose agar was used for fungal load. A 1 g of the sample aliquot was measured using a sterile syringe into 9 mls of distilled sterile water followed by homogenization (10^{-1}), another 1 ml was measured from 10^{-1} into 9 mls of sterile water and then homogenized (10^{-2}). The serial dilution was done upto (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℃ for 18 hours while fungi plates were incubated at 25℃ for 72 hours. The numbers of colony in each plate were counted after incubation. The isolated colonies were sub cultured to purify. The colonies were then characterized based on cultural, morphological, biochemical characteristics.

2.3 Proximate Analysis

The proximate analysis of the samples was performed according to the Association of Official Analytical Chemists (AOAC) [11] 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. [12], Doss et al. [13]. Cyanide was determined by a modified AOAC, Onwuka method [14].

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 [15]. 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 ± Standard deviation, analysis of Variance (ANOVA) and Duncan New Multiple Range Test. All the data generated were statistically analyzed using mean ± 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

The result of the changes in the proximate composition of Roselle samples from Kwara and Ondo States are shown in Tables 3 and 4.

3.3 Changes in Antinutrient Content of Fermented Roselle from Kwara and Ondo States Neutralized with Different Wood 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 [16]. This was in agreement with the result of [3,9].

Saccharomyces cerevisiae is a widely distributed spore former [17]. 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' [18] 'soumbala' [19], some Asian fermented foods [20,21]. Their presence in these fermentations may be due to their capability to start the fermentation of nitrogenous as well as carbohydrate products [22]. In addition, *Bacillus* sp. is known to produce a diversity of enzymes. Their metabolic activities can contribute to flavour and aroma generating reactions [23].

Table 1. Changes in microbial population during fermentation of Roselle from Kwara

Samples	Bacterial load (cfu/ml) x 10^5				Fungal load (sfu/ml) x 10^5			
	0 HR	24 HR	48 HR	72 HR	0 HR	24 HR	48 HR	72 HR
RAW	1.52±0.00^a	2.15±0.00^b	3.08±0.00^c	3.42±0.01^d	5.83±0.02^a	6.24±0.01^b	7.17±0.01^c	8.03±0.01^d
FN	2.56±0.01^a	3.14±0.05^b	3.87±0.01^c	4.14±0.01^d	4.85±0.03^a	5.61±0.01^b	6.08±0.02^c	8.13±0.02^d
FWCHA	4.51±0.01^a	5.07±0.01^b	8.34±0.01^c	8.55±0.01^d	1.32±0.02^a	2.17±0.02^b	2.60±0.01^c	2.94±0.03^d
FWCPA	4.72±0.01^a	5.42±0.05^b	8.63±0.01^c	8.94±0.00^d	0.83±0.01^a	1.34±0.01^b	1.78±0.02^c	2.37±0.01^d
FWGTA	5.60±0.05^a	6.04±0.05^b	8.85±0.01^c	9.38±0.01^d	1.24±0.02^a	1.75±0.02^b	2.35±0.00^c	2.81±0.02^d

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

Table 2. Changes in microbial population during fermentation of Roselle from Ondo

Samples	Bacterial load (cfu/ml) x10^5				Fungal load (sfu/ml) x 10^5			
	0 HR	24 HR	48 HR	72 HR	0 HR	24 HR	48 HR	72 HR
RAW	1.15±0.00^a	2.32±0.01^b	2.84±0.02^c	3.39±0.01^d	5.77±0.01^a	6.17±0.01^b	7.23±0.01^c	7.58±0.00^d
FN	1.58±0.01^a	3.52±0.02^b	4.18±0.02^c	4.75±0.01^d	4.71±0.00^a	5.69±0.00^b	6.04±0.01^c	6.32±0.01^d
FWCHA	5.24±0.02^a	5.63±0.02^b	6.27±0.01^c	7.32±0.00^d	1.14±0.01^a	1.95±0.01^b	2.57±0.01^c	2.74±0.00^d
FWCPA	5.28±0.02^a	6.39±0.01^b	7.09±0.01^c	7.74±0.01^d	0.74±0.01^a	1.14±0.01^b	1.76±0.01^c	2.32±0.00^d
FWGTA	5.32±0.01^a	6.51±0.00^b	7.95±0.02^c	9.39±0.02^d	1.65±0.00^a	1.77±0.00^b	2.37±0.01^c	2.75±0.01^d

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

Table 3. 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

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

Table 4. Proximate composition (%) of fermented Roselle samples from Ondo neutralized with different ash

Proximate	Ash content	Moisture content	Fat content	Crude fibre content	Protein	Carbohydrate 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
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

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

Table 5. Antinutrient content of fermented Roselle from Kwara neutralized with different ash

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 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. 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}

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

Table 7. 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}

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

Table 8. 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

Staphylococcus aureus occurrence agrees with the findings of [24] who reported the absence of *Staphylococcus* in the 48 h fermentation period for '*dawadawa'* and those of [25,26] who found that *Staphylococcus* was present only at the early stages of the fermentation.

Escherichia coli, Pseudomonas aeruginosa 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 4). 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) [27]. The increase in the microbial biomass in the form of single cell proteins may be another reason for the increase in the protein content [28].

The ash content of all the fermented samples (Tables 3 and 4) shows significant decrease (P≤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 5 and 6. Fermentation of calyx brings about a significant decrease (P≤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 [29,3] that cyanide content of the Roselle decreases with fermentation.

Fermentation brought about a significant decrease (P≤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 [28] and [30] 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 [31]. The results of the present study revealed that there was a significant decrease (P≤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≤0.05). The phytate content of the unfermented calyx was lower than that of tropical vegetables (394.9-1692.3 mg/100 g [32]. The decrease in the phytate content of the fermented calyx agrees with the report of [9] 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 Tables 7 and 8 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Unicef. United Nations International Children's Emergency Fund.
   Available:http://www.unicef.org/media/files/jme_2015_edition_sept_2015.pdf
   (Accessed July 2016)
2. Shade A, Ashley D, Kent S, Jones E, Ryan J, Newton E, Katherine DM. Interannual dynamics and phenology of bacterial communities in Eutrophic Lake. Linno Ocean. 2004;52:487-494.
3. Ojokoh AO, Adetuyi FA, Akinyosoye FC, Oyetayo VO. Fermentation studies on roselle (*Hibiscus sabdariffa* L.) calyces neutralized with trona. J. Food Tech. 2002;7(3):75-78.
4. Hume E. Wood ashes: How to use them in the garden. Ed Hume Seeds; 2006.
   Available:http://www.humeseeds.com/ashes.html
5. Lerner RB. Wood ash in the garden. Purdue University, Department of Horticulture and Landscape Architecture; 2006.
   Available:http://www.hort.purdue.edu/ext/woodash.html
6. Steinkraus KH. Indigenous fermented foods involving an alkaline fermentation.

In: Handbook of Indigenous Fermented Foods, Steinkraus, K.H. (Ed.). 2nd Edition, Marcel Dekker, Inc., USA. 1996;349-62.

7. Sullivan DM, Carpenter DE. Proximate and mineral analysis. In D.M. Sullivan and D.E. Carpenter, eds. Methods of Analysis for Nutritional Labeling, USA. 1993;105–9.
8. Joslyn MA. Methods in food analysis. 2nd ed., Academic Press New York, London; 1990.
9. Zokti JA. Effect of fermentation on proximate composition and physico-chemical properties of *Hibiscus sabdariffa* (Roselle). M. Tech Thesis. Federal University of Technology, Akure. 2003;77.
10. Cheesbrough. Biochemical test to identifying bacteria: Health and safety in district laboratories. 2nd ed. Cambridge University Press. 2005;75.
11. AOAC. Official method of analysis of the Association of Official Analytical Chemists International, 12th ed. Washington, DC. 1970;345-62.
12. Makkar HP, Blummel NK, Beckhen K. Determination of tannins and their correlation with chemical and protein precipitation method. J. Sci Food Agric. 1993;61:161-85.
13. Doss A, Pugalenthi M, Vadivel VG, Subhashini G, Subash RA. Effects of processing technique on the nutritional composition and antinutrients content of under-utilized food legume *Canavalia ensiformis* L. DC. Int. Food Res. J. 2011;18(3):965–70.
14. Onwuka GI. Food analysis. Instrumentation, Theory and Practice, Napthali Prints. 2005;210.
15. Munoz AM, King SC. (eds.). International consumer product testing across cultures and countries. ASTM International, MNL. 2007;55.
16. Cooke RC, Coursey DG. Cassava, a major cyanide containing food crop: Cyanide metabolism in FAO of the United Nations, Rome. 1981;56-62.
17. Neiman AM. Sporulation in the budding yeast, *Saccharomyces cerevisiae*. Gene. 2011;189(3):737-765.
18. Aderibigbe EY, Odunfa SA. Growth and extracellular enzyme production by strains of Bacillus isolated from fermenting African locust bean, 'iru'. J. App Bact. 1990;69: 662-71.
19. Ouoba LI, Diawara B, Amoa-Awua WB, Traore AS, Lange Moller P. Genotyping of starter cultures of *B. subtilis* and *B. pumilis* for fermentation of (*P. biglobosa*) to produce 'Soumbala'. Int. J. Food Microbiol. 2004;90:197-205.
20. Sarkar PK, Cook PE, Owens JD. Bacillus fermentation of soybeans. Journal. Microbiol. Bio. 1993;9:295-299.
21. Wang J, Fung DY. Alkaline fermented foods: A review with emphasis on 'pidan' fermentation. Crit. Rev. Microbiol. 1996;22: 101-38.
22. Omafuvbe BO. Natural fermentation of soybean (*Glycine max*) with added salt for 'soy-daddawa' production. J. Microbiol. Bio. 1994;10:479-80.
23. Leejeerajumnean A, Duckham SC, Owens DJ, Ames JM. Volatile compounds in bacillus-fermented soybeans. J. Sci. Food Agric. 2001;81:525-29.
24. Ogbadu L, Okagbue RN. Fermentation of African locust bean (*P. biglobosa*) seeds: Involvement of different species of Bacillus. Food Microbiol. 1988;5:195-9.
25. Omafuvbe BO, Shonukan OO, Abiose SH. Microbiological and biochemical changes in the traditional fermentation of soybean for 'soy-daddawa'- A Nigerian food condiment. Food Microbiol. 2000;17:469-74.
26. Achi OK. Microorganisms associated with natural fermentation of *Prosopis africana* seeds for the production of 'okpiye'. Plant Foods Hum. Nut. 1992;42:297-304.
27. Odetokun SM. Effects of fermentation on some physiochemical properties, antinutrients, and *in vitro* multienzyme digestibility of selected legumes. Ph.D Thesis. Federal University of Technology, Akure. 2000;148.
28. Okafor N. An integrated bio-system for disposal of cassava waste, integrated bio-systems in Zerol emission applications. Proceedings of the Internet Conference on Integrated Bio-system; 1998.
29. Ogundana SK, Fagade OE. Nutritive value of some Nigerian edible mushrooms. Food Chem. 1992;8:263-268.
30. Aletor VA. Allelochemicals in plant food and feedstuff 1. Nutritional, biochemical and physiopathological aspects in animal production. Vet. Hum. Toxicol. 1993;35:57-67.

31. Oboh G, Akindahunsi AA, Oshodi AA. Dynamics of phytate-zinc balance of fungi fermented cassava products (Flour and 'Garri'). Plants Food Hum. Nutri. 2003;58:1-7.

32. Akindahunsi AA, Oboh G. Effects of some post-harvest treatment on the bioavailability of zinc from some selected tropical vegetables. La Rivista Italiana Delle Sostanze Grasse. 1999;76:285-7.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18513