Original Article

Journal of Food Safety and Hygiene

journal homepage: http://jfsh.tums.ac.ir

Microbiological, nutritional quality and antioxidant activity of fermented Delonix regia seeds

Olaniyi Oladipo Oladiti

Department of Microbiology, Federal University of Technology, Akure, Nigeria

ARTICLE INFO

Article history:
Received 11 May. 2018
Received in revised form
23 Sep. 2018
Accepted 04 Oct. 2018

Keywords:
Antioxidant;
Delonix regia;
Fermented foods;
Proximate;
Physicochemical parameters

ABSTRACT

This study evaluated the microbiological, nutritional quality and antioxidant profile of fermented *Delonix regia* seeds. Isolation and characterization of microorganisms was performed by standard microbiological and biochemical methods, while proximate and antioxidant contents of fermented substrates were determined by standard chemical methods. The total aerobic and lactic acid bacterial and fungal counts increased from 2.0 to 4.4 cfu/ml, 0.7 to 18.9 cfu/ml and 0.7 to 2.4 cfu/ml respectively. The microorganisms isolated and identified from the samples were *Bacillus subtilis*, *Staphyloccocus aureus*, *Serratia marcescens*, *Micrococcus luteus*, *Streptomyces greceus*, *Lactobacillus plantarun*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Aspergillus flavus*, *A. fumigatus*, *Fusarium spp.*, *Penicillium notatum*, *Penicillium italicum*, *Rhizopus japonicum* and *Saccharomyces cerevisiae*. The moisture, fat and protein contents of the raw samples increased from 11.10, 1.66 and 19.99 % to 18.25, 4.67 and 32.16 % after fermentation respectively. The antioxidant properties (FRAP, TAC, Flavonoid and DPPH) were remarkably higher than the unfermented D. *Regia* seeds. The appreciable increase in nutritional and antioxidant properties of fermented *D. regia* seeds suggest that it might be a good and cheap source of ingredient which can be integrated into human diet and animal feed.

Citation: Oladiti OO. **Microbiological, nutritional quality and antioxidant activity of fermented Delonix regia seeds.** J Food Safe & Hyg 2018; 4(3-4): 69-75.

1. Introduction

Fermented products via fermentation combined with other food processing techniques constituted integral part of diets in many homes around the world mostly in African and Asian countries. Different traditional food processing techniques such as cooking, smoking, sprouting/germination and less often toasting are commonly practiced in most of the African countries. However, fermented foods have gained popularities and well accepted by all in these countries due to lots of nutritional benefits accrued to them (1,2). They are produced by the use of microorganisms and hydrolytic enzymes with lead to desirable quality that are quite different from raw or unfermented organic substrates (3). Their beneficial effects from the nutraceutical point of view include immune-modulatory, antiallergenic, antihypertensive and anti-tumourigenic (4,5,6). Some metabolites released on them during fermentation by consortium of microorganisms involved in the process also improved their nutritional status and extended their shelf life (7). Their thiamine, nicotinic acid, riboflavin and perhaps, protein content are enhanced as a result of microbial activity. Other dietary benefits of fermented foods include improvement of food safety, minerals bioavailability and vitamins, toxins reduction and improved digestibility and industrial utility (Jimoh et al., 2012).

The principal key player in food fermentation process is lactic acid bacteria (LAB); they help in the production of antimicrobial metabolites such as

* Corresponding author. Tel: +2348068054636
E-mail address: microladit@gmail.com

bacteriocins, reuterins, hydrogen peroxide, diacetyls and organic acids. The presences of these antimicrobial compounds in fermented foods either inhibit or kill unwanted food borne pathogens and spoilage microorganisms. A number of LAB genera to be precised *Enterococcus spp.*, *Leuconostoc spp.*, *Lactobacillus spp.*, *Pediococcus spp.*, *Lactococcus spp.* and *Streptococcus spp.* have been screened and confirmed to produce these inhibitory substances during the course of fermentation (8). Some LAB are known as good producers of antioxidants such as flavanoids, benzoquinones, alkaloids, terpenoides, xanthones, phenols, tetralones and steroids (9). Fermented foods enriched with antioxidants protect consumers' body against oxidative damage (9).

Dietary supplements that contain antioxidants help in disease prevention and maintenance of good health. Antioxidants prevent human body from diseases such as cancer and coronary heart disease. Carcinogenic and toxic nature of synthetic or commercially available antioxidants has led to search for natural antioxidants (10). Previous investigations have revealed that plant foods such as vegetables, fruits, spices, tea and wines are proven sources of biological antioxidants (11). Many plant parts such as flowers, root, seed, stem and leaves contain arrays of antioxidant compounds which are considered as safe and inexpensive source of antioxidant from natural origin (12). However, antioxidants within the plant tissues are either lockup or available in inactive forms. Thus, microbial activities and their enzymes are required to make these compounds free in utilizable forms for use in traditional and modern medicine (13).

In folk medicine, *D. regia*, an average tall flowering plant has been used effectively for the treatment of ailments such as rheumatism, hemiplagia, leucorrhoea, inflammation and constipation (14). Its therapeutic potential is attributed to the fact that it has antioxidant, antimicrobial, anti-diarrhoeal, anti-ulcer and anti-inflammatory activity (15). In search of underutilized plant material with desirable nutritional and pharmacokinetic properties, *D. regia* seed was therefore considered a plant part of choice and hence subjected to submerged state fermentation with a view to unlock its phyto-constituents. This research assessed the microbiological, nutritional quality and antioxidant profile of fermented *D. regia* seeds.

2. Materials and methods

2.1. *Delonix regia* seeds collection and preparation for microbiological and chemical analyses

Microorganisms associated with fermented *D. regia* seeds were isolated according to the method of Chelule et al. (16). Two hundred grams (200 g) milled seeds of *D. regia* was suspended in a clean transparent screw capped glass jar containing two liters of sterile distilled water. The mixture was incubated in static condition for three days at room temperature (28±2°C), and at interval of 24 h, isolation of microorganisms were carried out using pour plating technique with suitable dilution factors and incubated at 30°C for 24 and 72 h for bacteria and fungi respectively. The resultant bacterial and fungal colonies were counted and expressed as CFU per millimeters and SFU/ millimeters respectively. Pure bacterial isolates were presumptively identified via morphological and some biochemical identification. Thereafter, Bergey's Manual of Determinative Bacteria was consulted as a standard reference to tentatively identify the bacterial isolates (17). Pure fungal isolates were identified on the basis of cultural characters as well as microscopic structure (18).

2.3. Proximate composition of fermented *D. regia* seeds

The method of AOAC (19) was adopted in the determination of the proximate composition of the fermented samples. The proximate parameters considered were moisture content, ash content, fat content, crude fiber and crude protein content, carbohydrates and moisture contents.

2.4. Total titratable acidity and pH determination

Total titratable acidity (TTA) and pH of the fermenting substrate were determined as described by Akharayi and Omoya (20). TTA was determined by diluting fermented slurry with sterile distilled water, and the mixture with the addition of (few drops) 1% phenolphthalein was titrated against 0.1 M NaOH. The pH was determined by dipping a calibrated pH meter (Crison Basic Model 20) into the fermented samples; the readings were taken and recorded.

2.5. Antioxidant profile of fermented *D. regia* seeds

The antioxidant parameters evaluated from the fermented *D. regia* seeds were total flavonoid, ferric reduction property (FRP), free radicals scavenging (FRS) ability and total antioxidant activity.

2.5.1. Total flavonoid determination

The colorimeter assay was used to determine the total flavonoid content in the fermented *D. regia* seeds

and the absorbance was read at 510 nm against the reagent blank. Total flavonoid content in the samples was expressed as mg/g (21).

2.5.2. Determination of FRP

FRP of the fermented *D. regia* seeds was determined according to Pulido et al. (22). Fermented *D. regia* seeds (0.3 ml) was diluted with equal volume of 200 mM sodium phosphate buffer pH 6.6 and 0.25 ml of 1% KFC. The mixture in a test tube was incubated at 50°C for 20 min, followed by the addition of 0.25 ml of 10% TCA and centrifuged at 2000 rpm for 10 min. After centrifugation, 1.0 ml of the clear supernatant was diluted with 1 ml distilled water and 0.1% $FeCl_3$ and the reading was taken at 700 nm.

2.5.3. Determination of FRS

The FRS of the fermented *D. regia* seeds was determined against DPPH (1,1-diphenyl-2-picryhydrazyl) as described by Gyamfi et al. (23). One ml from the fermentation medium was mixed with 1 ml of 0.4 mM methanolic solution of the DPPH, the mixture was left in the dark for 30 min and the absorbance was measured at 516 nm.

2.5.4. Total antioxidant activity determination

The determination of total antioxidant activity (TAA) was based on the reduction of Mo (VI) - Mo (V) by the fermented *D. regia* seeds and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (24). TAA of the fermented samples was determined by adding different concentrations of samples with 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) followed by the incubation of the reaction mixture in water bath at 95°C for 1 h 30 min. The mixture was cooled to room temperature against blank and the absorbance was measured at 695 nm. The TAA was expressed as garlic acid equivalent.

2.6. Statistical analysis

Data obtained were analyzed by analysis of variance and significant differences between means were compared using Duncan multiple range test.

3. Results

Table 1 shows the total bacterial and lactic acid bacterial (LAB) counts from fermented *D. regia*. Total bacterial counts from fermented *D. regia* increased from 1.3×10^9 cfu/ml at 0 h to 4.4×10^9 cfu/ml at 48 h, and beyond, a reduced total bacterial load was recorded. LAB counts from fermented *D. regia* recorded an appreciable increase all through the fermentation time.

Table 1. Total bacterial and lactic acid bacterial count from fermented *D. regia*

Fermentation time (hour)	Bacterial count ($\times10^9$ cfu/ml)	Lactic acid bacterial count ($\times10^9$ cfu/ml)
24	2.1	0.9
48	4.4	10.9
72	3.1	18.9

Table 2 shows the total fungal counts from the fermented *D. regia*. The fungal loads increased on PDA and SDA all through the fermentation time.

Table 2. Fungal counts from fermented *D. regia*

Fermentation time (hour)	Fungi count on PDA ($\times10^6$ cfu/ml)	Fungi count on SDA ($\times10^6$ cfu/ml)
24	1.4	1.3
48	2.2	2.0
72	2.4	2.2

Key: PDA: Potato dextrose agar, SDA: Sabraud dextrose agar

Table 3 shows the morphological and biochemical characterization of bacteria isolated from the fermented Delonix regia seeds. The bacterial strains were identified as *Bacillus subtilis, Staphyloccocus aureus*, *Serratia marcescens*, *Streptomyces greceus*, *Micrococcu luteus*, *Lactobacillus plantarum* and *Lactobacillus casei* respectively.

Table 3. Morphological and biochemical characterization of bacterial isolates from fermented *D. regia* seeds

Isolate	Cell shape	Colony shape	Cell appearance	Cell orientation	Gram	Motility	Catalase	Coagulase	Indole	Citrate	H.	Sugar fermentation					Probable organisms
												Lactose	Mannitol	Sucrose	Maltose	Glucose	
A	R	LR	Creamy	Rough	+	+	+	-	-	+		Ag	AG	Ag	Ag	Ag	*Bacillus subtilis*
B	C	SR	Creamy	Smooth	+	+	+	+	-	+		Ag	AG	Ag	Ag	Ag	*Staphyloccocus aureus*
C	R	LR	Pink	Convex	-	+	+	+	-	+		Ag	Ag	AG	Ag	AG	*Serratia marcescens*
D	C	R	White	Raised	+	-	+	+	-	+		Ag	Ag	Ag	Ag	Ag	*Streptomyces greceus*
E	C	R	Creamy	Smooth	+	-	+	-	-	-		Ag	Ag	Ag	Ag	Ag	*Micrococcus luteus*
F	R	SR	creamy	Smooth	+	-	-		-	-	+	Ag	Ag	Ag	Ag	Ag	*Lactobacillus plantarum*
G	R	LR	creamy	Smooth	+	-	-		-	-	-	Ag	Ag	Ag	Ag	Ag	*Lactobacillus casei*

+ = Positive, - = Negative, AG = Acid and Gas production; Ag = Acid production without Gas; LR = Long rod; SR = Short rod; R = Rod; C = Cocci

Table 4. Cultural and microscopic identification of fungi isolated from the fermented *D. regia* seeds

Isolates code	Cultural characterization and microscopic observation	Probable organisms
A11	Powdery, yellow to green colony and the reverse was yellowish. Radiated conidia head with hyaline, long, rough walled conidiophore and globose to subglobose finely roughened to echinulate conidia	*Aspergillus flavus*
A12	Powdery blue–green colonies with pale yellow reverse. Septate hyphae with smooth walled conidiophores, ending in vesicle and subglobose shape.	*Aspergillus Fumigates*
A13	Sparse mycelium, whitish with a purple reverse. Septate hyphae with conidia moderately curved and pointed at both end	*Fusarium species*
A14	Grayish green with reverse pale yellow color.	*Penicillium notatum*
A15	Colony whitish and dark brown to black-brown.	*Rhizopus japonicum*
A16	Bluish green with reverse yellow brown. Septate hyphae with smooth walled conidiophore with ellipsoida to cylindrical conidia	*Penicillium italicum*
A17	Creamy color, oval shape with branched cell.	*Saccharomyces cerevisiae*

In Table 4, the cultural characterization and microscopic observation of fungal isolates associated with fermented *D. regia* seeds is revealed. The fungi consisted of *Aspergillus flavus*, *A. fumigatus, Fusarium spp., Penicillium notatum, Penicillium italicum, Rhizopus japonicum* and *Saccharomyces cerevisiae*, respectively.

Table 5 shows the TTA and pH value of the fermented *D. regia* seeds. The total titratable acidity increased with corresponding pH decrease with increase in the fermentation time from 24 to 72 h. The total titratable acidity of the fermented sample increased from 3.3 at 24 h to 18.1 at 72 h, while the pH decreased from 6.8 to 4.1.

Table 5. Total titratable acidity (TTA) and pH value of fermented *D. regia* seeds

Fermentation time (days)	TTA	pH
24	3.3	6.8
48	14.1	4.9
72	18.1	4.1

Table 6 shows proximate composition of the raw and fermented *D. regia* seeds. The moisture, fat and protein contents of the fermented samples increased from approximately 11, 2 and 20% in raw samples to 18, 5 and 32%, respectively in the fermented samples. There was remarkable reduction in the fiber and carbohydrate content by approximately 79 and 16% in the fermented samples when compared with unfermented samples.

The antioxidant component of the raw and fermented *D. regia* seeds is shown in Table 7. The FRAP, TAC, flavonoid and DPPH in the unfermented samples increased from approximately 26 mg/g, 9 mg/g, 1 mg/g and 10% to 44 mg/g, 32 mg/g, 3 mg/g and 10% respectively in the fermented samples.

Table 7. Antioxidant profile of the raw and fermented *D. regia* seeds

Antioxidant properties	Unfermented	Fermented
Ferric reducing antioxidant property (FRAP) (mg/g)	$26.10^{a} \pm 1.73$	$43.61^{b} \pm 0.05$
Total antioxidant capacity (TAC) (mg/g)	$9.06^{a} \pm 0.20$	$32.34^{b} \pm 0.35$
Flavonoid (mg/g)	$0.95^{a} \pm 0.02$	$2.98^{b} \pm 0.04$
DPPH (%)	$10.21^{a} \pm 2.61$	$46.29^{b} \pm 0.25$

Data are represented as mean ± standard deviation. Means with the same superscript across the column are not significantly different from each other ($p<0.05$)

4. Discussion

Array of microorganisms were isolated during the fermentation of *D. regia* seeds. Isolation of different microorganisms from fermented plant materials has been reported (25). The establishment of co-metabolism or microbial synergy between fungi and bacteria during the fermentation of plant materials is well documented (26,27). Lactic acid bacteria (LAB) create acid environment for the proliferation of fermentative fungi, and in return, vitamins, utilizable simple sugars and other growth factors are produced by fungi (28). The occurrence of different bacterial and fungal isolates such as LAB, *S. aureus*, *S. marcescens*, *B. subtilis, S.*

Table 6. Proximate composition of raw and fermented *D. regia* seeds (%)

Sample	Moisture Content	Ash Content	Fat Content	Fiber Content	Protein Content	Carbohydrate Content
Raw	$11.10^{a} \pm 0.02$	$2.53^{c} \pm 0.11$	$1.66^{a} \pm 0.21$	$16.13^{c} \pm 0.01$	$19.99^{a} \pm 0.01$	$48.59^{b} \pm 0.01$
DF	$16.57^{b} \pm 0.01$	$2.02^{b} \pm 0.12$	$3.84^{b} \pm 0.01$	$11.34^{b} \pm 0.04$	$25.09^{b} \pm 0.03$	$41.14^{a} \pm 0.03$
AF	$18.25^{c} \pm 0.03$	$0.65^{a} \pm 0.11$	$4.67^{c} \pm 0.02$	$3.4^{a} \pm 0.02$	$32.16^{c} \pm 0.15$	$40.87^{a} \pm 0.02$

Data are represented as mean ± standard deviation. Means with the same superscript down the row are not significantly different from each other ($p>0.05$).Key: DF: During fermentation, AF: After fermentation.

greceus, *A. flavus*, *A. fumigatus*, *Fusarium spp.*, *P. notatum* , *R. japonicum*, *P. italicum* and *S. cerevisiae* during the fermentation of *D. regia* seeds suggest that they might be classified either as natural microflora or contaminating microorganisms emanated from the substrates meant for fermentation, soil, utensil used or fermentation vessels, aerosols, rodents and the personnel involved (29,30). Duangjitcharoen et al. (31) and Gunawan et al. (32) isolated LAB from fermented plant beverages and *Xylocarpus moluccensis* seeds respectively. The presence of *A. flavus*, *A. fumigatus* and *Fusarium spp*. signal great health concern since these fungi are known for mycotoxins production. Some strains of *A. flavus* produced carcinogenic toxic chemical called aflatoxins, while certain strains of *A. fumigatus* and *Fusarium spp*. secrete toxic fumonisins and zearalenone, respectively (30).

The fermentation of *D. regia* seeds was characterized by a decrease in pH and corresponding rise in TTA. Increase in TTA and decrease in pH during the spontaneous fermentation of plant seeds has been reported (33). The decrease in pH with a corresponding increase in TTA during fermentation has been reported by some researchers who had done similar investigation. Ayo (34), Ojokoh et al. (27), Amankwah et al. (36) and Ojokoh et al. (27) reported a decrease in the pH and a rise in the TTA of the fermenting medium for millet flour, millet-acha based kunun zaki (beverage), breadfruit-cowpea blend flours, maize flour and pearl millet and acha flour blends, respectively. Decrease in pH might be due to the presence of organic acids such as lactic acid by LAB involved in the fermentation of carbohydrate contents of the fermenting substrates (27,29).

The increase in the protein content of fermented *D. regia* seeds in this study agrees with the reports of Sade (37), Chukwu and Abdul-kadir (38) and Ojokoh et al. (27). For instance, Sade (37) reported an increase in the protein content of raw millet from 14.0 to 17.5% in the fermented millet, while Chukwu and Abdul-kadir (38) reported an increase in the protein content from 6.9 to 10.6% in acha. Similarly, Ojokoh et al. (27) reported an increase in the protein content of fermented pearl millet with acha by approximately 50% when compared with unfermented sample. The increase in the crude protein contents of the fermented *D. regia* seeds might be due to the secretion of hydrolase enzymes rich in protein and excess nitrogen entrapment by the consortium of microorganisms involved (39,40). The crude fiber decreased consistently all through the fermentation period. Many researchers have reported similar reduction in the amount of crude fiber in fermented plant seeds or cereals. In their studies, Sade (37) and Amankwah et al. (36) reported a reduction in the crude fiber content of fermented millet and maize flours respectively. Similar result was reported by Ojokoh et al. (27) when pearl millet with acha was subjected to fermentation. The decrease in the crude fiber content of the fermented *D. regia* seeds might be connected with the ability of LAB to produce crude fiber-degrading enzymes in the course of fermentation (27,40). There was significant increase in the moisture content of fermented *D. regia* seeds when compared with the unfermented substrate. The finding from this study was in agreement with the reports of some researcher who had carried out similar experiment. There was an increase in the moisture content of fermented maize flour from 14.2 to approximately 20% (41). In another study by Abiola and Ekunrin (42), moisture content in the raw melon husk increased from 2.46 to 5.84% after 72 h of fermentation. Also, Zakari et al. (43) reported that the moisture content of pearl millet-bambara groundnut blend flours increased from 12.0 to 12.9% after fermentation. The reduced moisture content in the fermented samples might be due to the utilization of moisture by the microorganisms for various metabolic activities and this reduction might contribute to the longer shelf life and prevent microbial spoilage of the end product (44).

In this study, there was significant increase in the antioxidant profile of fermented *D. regia* seeds. Hunaefi et al. (45) reported an increase in the total flavonoids and flavonols of *orthosiphon aristatus* (Java Tea) when series of LAB fermentation methods was exploited. The increase in the DPPH content of the fermented *D. regia* seeds agrees with the report of Oliveira et al. (46) who reported an increase in the DPPH of fermented rice (*Oryza sativa*) bran from 32 to 59% after 96 h of solid state fermentation. Fermentation of black soybean by *Bacillus subtilis* was found to increase its total phenolic and flavonoid contents as well as its antioxidant activities of anti-DPPH radicals and ferric reducing antioxidant power (FRAP) (47). The significant increase in the antioxidant components evaluated might be attributed to the involvement of LAB in the fermentation (45). LAB have the ability to produce exopolysaccharides that power bank for antioxidant components. These exopolysaccharides produced by LAB demonstrated potential in vitro antioxidant properties (1,1 diphenyl-2- picrylhydrozyl radical scavenging activities, chelation of ferrous ion, inhibition of linoleic acid peroxidation and reducing power) during an oral administration of their strain (45).

5. Conclusion

In conclusion, the fermented *D. regia* seeds had higher nutritive value and better antioxidant activity than the unfermented samples. The antioxidant properties of *D. regia* seeds could enhance through food grade LAB fermentation. Hence, fermented *D. regia* seeds can be exploited as feed ingredient and used as substitute to essential ingredients known to be expensive in infant food nutrition.

Conflict of interest

There is no conflict of interest.

Acknowledgements

The technical supports from the Department of Microbiology, Federal University of Technology Akure, Nigeria is highly appreciated.

References

1. Holzapfel WH. Appropriate starter culture technologies for small-scale fermentation in developing countries. Int J Food Microbiol 2002; 75: 197-12.
2. Rolle R, Satin M. Basic requirements for the transfer of fermentation technologies to developing countries. Int J Food Microbiol 2002; 75: 181-87.
3. Ogunshe AAO, Ayodele AE, Iheanyi OO. Microbial studies on Aisa: a potential indigenous laboratory fermented food condiment from *Albiziasaman* (Jack). Mull. Pak J Nutr 2006; 5: 51-58.
4. Osuntoki A, Korie I. Antioxidant activity of whey from fermented milk, fermented with *Lactobacillus* species isolated from Nigerian fermented foods. Food Technol & Biotechnol 2010; 48: 505-11.
5. Wu J, Guo Y, Liu Y, et al. Rapid determination of oil content in seed of *Jathropha curcas* by NMR. J Biobased Material & Bioenergy 2010; 4: 436-39.
6. Agarry OO, Nkama I, Akoma O. Production of *Kunun-zakia* (A Nigerian fermented cereal beverage) using starter culture. Int Res J Microbiol 2010; 1: 18-25.
7. Jimoh SO, Ado SA, Ameh JB, et al. Yeast in locally fermented beverages, characteristics and diversity of yeast in locally fermented beverages sold in Nigeria. World J Eng & Pure Appl Sci 2012; 2: 40.
8. Leroy F, De Vuyst L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci & Technol 2010; 15: 67-78.
9. Tejesvi MV, Tamhankar SA, Kini KR, et al. Phylogenetic analysis of endophytic *Pestal otiopsis* species from ethnopharmaceutically important medicinal trees. Fungal Divers 2009; 38:167-83.
10. Aljadi AM, Kamaruddin MY. Evaluation of the phenolics contents and antioxidant capacities of two Malaysian floral honeys. Food Chem 2004; 85: 513-18.
11. Kumar A, Chattopadhaya S. DNA damage protecting activity and antioxidant potential of pudina extracts. Food Chem 2007; 100: 1377-84.
12. Anwar F, Jamil A, Iqbal S, et al. Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. Grasas Y Aceites 2006; 57: 189-97.
13. Tung YT, Wu JH, Kuo, YH, et al. Antioxidant activities of natural phenolic compounds from *Acacia confuse* bark. Bioresist Technol 2007; 98: 1120-23.
14. Sumitra S, Sonia NK. A review: Introduction to *Delonix regia*, World J Pharm Sci 2014; 3: 8-50.
15. Vivek MN, Sachidananda HC, Manasa M, et al. Antimicrobial and antioxidant activity of leaf and flower extract of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum*. J Appl Pharm Sci 2013; 3: 064-71.
16. Chelule PK, Mokoena MP, Gqaleni N. Advantages of traditional lactic acid bacteria fermentation of food in Africa. Curr Res Technol Education Topics in Appl Microbiol & Microbial Biotechnol 2010; 1160-67.
17. Holt JG, Kneg NR, Sneath PH, et al. 1994. Bergey's manual of determinative bacteriology. Wilkins Publishers, Baltimore. 136-41.
18. Pitt JI, Hocking AD. 1997. Fungi and food spoilage, 2th ed. Black Academic, London, U.K. 414.
19. AOAC. Official methods of Analysis. 17th end. Association of Official Analysis Chemists, Washington DC. 2012; 2200 pp.
20. Akharaiyi FC, Omoya FO. Effect of processing methods on the microbiological quality of liquid pap "ogi" prepared from maize. Trends Appl Sci & Res 2008; 3: 330-34.
21. Bao JY, Cai M, Sun G, et al. Anthocyanins, flavonoid and free radical scavenging activity of thines bayberry (*Myrial rubia*) extracts and their colour properties and stability. J Agric & Food Chem 2005; 53: 2327-32.
22. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agri & Food Chem 2000; 48: 3396-02.
23. Gyamfi MA, Yonamine M, Aaniya Y. Free radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguine* on experimentally induced liver injuries. General Pharm 1999; 32: 661–67.
24. Abhishek M, Satish KV, Santosh KS, et al. Investigation of the antimicrobial, antioxidant and anti-inflammatory activity of compound isolated from *Murraya koenigii*. Int J Appl Biol & Pharm Technol 2011; 2: 470-77.
25. Kebede A. Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of *borde*, an Ethiopian cereal beverage. Int J Food Nutr & Sci 2014; 1: 7-15.
26. Fayemi OE, Ojokoh AO. The Effect of different fermentation techniques on the nutritional quality of the

cassava product (fufu). J Food Process Preserv 2014; 38: 183-92.
27. Ojokoh AO, Fayemi OE, Ocloo FCK, et al. Effect of fermentation on proximate composition, physicochemical and microbial characteristics of pearl millet (*Pennisetum glaucum* (L.) R. Br.) and acha (*Digitaria exilis* (Kippist) Stapf) flour blends. J Agri Biotechnol & Sustain Develop 2015; 7: 1-8.
28. Ana ATC, Rosinea AP, Hilaro CM, et al. Inhibition of *Listeria monocytogenes* by lactic acid bacterium isolated from Italian salami. Food Microbiol 2006; 23: 213-19.
29. Olaniyi OO. Microbiological quality assessment of some NAFDAC approved fruit juices sold in Ilorin metropolis, Nigeria. Afr J Food Sci 2013; 7: 222-26.
30. Olaniyi OO, Saanu AB, Akinyele BJ, et al. Microbiological evaluation and antibiotic susceptibility pattern of bacteria associated with '*burukutu*', a non-alcoholic beverage. J Food Resource Sci 2018; 7: 1-7.
31. Duangjitcharoen Y, Kantachote D, Ongsakul M, et al. Selection of probiotic lactic acid bacteria isolated from fermented plant beverages. Pak J Biol Sci 2008; 11: 652-55.
32. Gunawan S, Darmawan R, Nanda M, et al. Proximate composition of *Xylocarpus moluccensis* seeds and their oils. Ind Crops Prod 2013; 41: 107–12.
33. 33. Kunene NF, Geornaras I, Von HA, et al. Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. J Appl Environ Microbiol 2000; 66: 1084–92.
34. Ayo J. Effect of acha (*Digitaria exilis* staph) and millet (*Pennisetum typhodium*) grain on *kunun zaki*. Brit Food J 2004; 106: 512-19.
35. Ojokoh AO, Fayemi EO, Ocloo FCK, et al. Proximate composition, anti-nutritional contents and physicochemical properties of breadfruit (*Treculia africana*) and cowpea (*Vigna unguiculata*) flour blends fermented with *Lactobacillus plantarum*. Afric J Microbiol Res 2014; 8: 1352-59.
36. Amankwah E, Barimah J, Acheampong R, et al. Effect of fermentation and malting on the viscosity of maize-soyabean weaning blends. Pak J Nutr 2009; 8:1671-75.
37. Sade FO. Proximate, anti-nutritional factors and functional properties of processed pearl millet (*Pennisetum glaucum*). J Food Technol 2009; 7: 92-97.
38. Chukwu O, Abdul-kadir AJ. Proximate and chemical composition of Acha (*Digitaria exilis* and *Digitaria iburua*) grains. J Food Technol 2008; 6: 214-16.
39. Sallam KI, Ahmed AM, Elgazzar MM, et al. Chemical quality and sensory attributes of marinated pacific saury (*Cololabis saira*) during vacuum-packaged storage at 4ºC. Food Chem 2008; 102: 1061-70.
40. Olaniyi OO, Bankefa EO, Ibitoye OF, et al. Nutrient enrichment of mannase-treated cassava peels and corn cob. Res J Microbiol 2015; 10: 533-41.
41. Marina CNA, Théodore ND, Marina KC. Effect of fermentation process on nutritional composition and aflatoxins concentration of doklu, a fermented maize based food. Food Nutr Sci 2013; 4: 1120-27.
42. Abiola C, Ekunrin MF. Effect of fermentation on the microbial, nutrient and anti-nutrient contents of melon (*Cucumis melo* L.) husk. Microbiol J 2016; 6: 9-14.
43. Zakari UM, Hassan A, Abbo ES. Physico-chemical and sensory properties of "*Agigi*" from pearl millet (*Pennisetum glaucum*) and bambara groundnut (*Vigna subterranean*) flour blends. Afric J Food Sci 2010; 4: 662-67.
44. Azeez L, Adeoye MD, Ganiyu OT, et al. Influence of microbial contamination on the antioxidant composition and free radical scavenging effects of fresh and decaying spices. Fountain J Natural & Appl Sci 2012; 1: 55–64.
45. Hunaefi D, Akumo D, Riedel H, et al. The effect of *Lactobacillus plantarum* ATCC 8014 and *Lactobacillus acidophilus* NCFM fermentation on antioxidant properties of selected *in vitro* sprout culture of *Orthosiphon aristatus* (Java Tea) as a Model Study. Antioxidants 2012; 1: 4-32.
46. Oliveira MS, Cipolatt EP, Furlong EP, et al. Phenolic compounds and antioxidant activity in fermented rice (*Oryza sativa*) bran. Ciênc Tecnol Aliment Campinas 2012; 32: 531-37.
47. Dajanta K, Janpum P, Leksing W. Antioxidant capacities, total phenolics and flavonoids in black and yellow soybeans fermented by *Bacillus subtilis*: A comparative study of Thai fermented soybeans (*Thua nao*). Int Food Res J 2013; 20: 3125-32.