Microbiological Evaluation of Shelf-life Indices of Fermented African Locust Bean Cake Stored Under Preservative Treatment

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Abstract: Daddawa also known as iru, among the Yorubas in South-west Nigeria, is a popular condiment used as taste and flavour enhancer in soup and dishes in Africa. Daddawa is traditionally produced from locust beans *(Parkia biglobosa)* seeds. This work was carried out on the preservation of fermented *Parkia biglabosa* seeds sourced from local producers and laboratory produced. The fermented laboratory control and local samples were treated with *1%* salt w/w. A control experiment was left untreated. The samples were stored at ambient temperature (34°C and 30°C) for 30 and 120 days. The analysis consisted of aerobic and anaerobic mesophilic count, Staphylococcus count and Fungal count, detection of *Escherichia coli, S.aureus, Salmonella* sp, *Mucor* sp and *Rhizopus* sp. Contaminant isolates were identified from laboratory treated, untreated (Control) and locally produced, using standard procedure. The proximate analysis and organoleptic assessment of the laboratory treated, untreated (control) and purchased locust bean cake were carried out using standard procedure. The treated locust bean cake indicated microbial log reduction at120 days due to the effects of the preservatives; the Control shows microbial Log increase in the untreated locust bean cake. Isolated and characterized bacterial isolates at 30 and 120 days in the treated and untreated locust bean cake was *E.coli* only isolated in Kwanaryan daddawa, *Staphylococcus aureus* were isolated in all the purchased Locust bean cake. *Salmonella* sp was not detected and a predominant fungal genera were *Mucor* sp and *Rhizopus* sp. The result indicated 40% elimination of contaminants at Gude, Jogana and Kwanaryan daddawa. The mean proximate composition at 30 and 120 days shows nutritional quality of treated locust bean cake. The Organoleptic assessment indicated that the judges rejected control daddawa due to off flavor.

Keywords: African locust bean cake, Salt, Microbial log reduction, Nutritional quality.

Introduction

Parkiabiglobosa seed is one of the major sources of plant protein in African diet (Ademola *et al,*2013). Daddawa also known as iru, among the Yorubas in South-west Nigeria, is a popular condiment used as taste and flavour enhancer in soup and dishes in Africa. Daddawa is traditionally produced from locust beans (*Parkia biglobosa*) seeds (Farinde*et al*., 2017).Odunfa (1981) stated that fermented locust bean seed is commonly consumed in Ghana, Nigeria, Sierra-Leone and Togo. In Nigeria it is called iru in Yoruba, dawadawa in Hausa and ogiri `igala in Igbo. It is also referred to as kinda in Sierra-Leone and kpalugu in Ghana. Preservation and preservatives are designed to inhibit/control the activities of spoilage causing organisms in food, a process also referred to as sanitization. Spoilage causing organisms due to their growth and metabolic activities produce by-products, which change the texture, taste, flavor and the aroma of the food. Preservatives by their nature are intended to keep the food devoid of these changes. Preservatives act on both Gram positive and Gram negative food spoilers (Jay, 2000). The general idea of products having preservatives is to increase shelf life and prevent items from spoiling. The more shelf life a product has, the more marketable it becomes.

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more marketable it becomes. The use of preservatives makes products stay fresher, longer and give more time for products to be used (Bumpres, 2010). Shelf life is the period of time during which the food product remain safe, retain desired microbiology, physical, chemical and sensory characteristics (Ademola *et al.*, 2013). This study is aimed at determining the microbiological evaluation of shelf-life indices of fermented African locust bean cake stored under preservative treatment.

Materials and Methods

Sourcing of Locally Produced Daddawa: The purchased daddawa were obtained from Gude (sample C), Jogana (sample D) and Kwanaryan daddawa (sample) at the point of production.

Laboratory production of Daddawa: Controlled fermentation of *P.biglobosa* seeds using mixed Bacillus species *Bacillus subtilis* and *Bacillus pumilus* as inocula. The fermentation process was set up using Bacillus spp. The organisms were inoculated into 300g of unfermented seeds of *P.biglobosa* and were wrapped with sterile aluminum foil and placed in an earthen pot with cover. Fermentation was allowed to progress at room temperature (28 ± 20C) in the laboratory, Department of Microbiology, Bayero University Kano. After mashing using a sterilized blender the African locust bean cake was dried in an oven at 55°C for 48 hours by modifying the earlier work of Gberikon *et al.*, (2015).

Application of Treatment: The laboratory fermented (sample A) and local samples were treated with 1% w/w Table Salt, the laborarory control (sample B) was left untreated (Omafuvbe *et al.*,2006; Ademola *et al*,2013).Microbiological Quality: The microbiological quality (Aerobic mesophiliccount, Anaerobic mesophiliccount, Staphylococcus count and Fungal count) were determined as follows:

Aerobic Mesophilic Bacterial Count (APC)

From the serially diluted samples, 1ml was transferred into duplicate petri dish. This was followed by pouring 9ml Plate Count Molten agar (PCA) (Kept at 45+1°C in a water bath). The plates were swirled and allowed to solidify and incubate, at 37°C for 24 hours. After which the incubated plates, with count between 30 - 300 colonies, were counted, and the average number of colonies obtained was multiplied by dilution factor to get the number of colony forming unit per gram (FAO, 1992).

Enumeration of Viable Anaerobic Count

This is similar to the method applied for the enumeration of viable aerobic bacterial count. In this case, Nutrient agar was used for culturing anaerobic bacteria. Also, the cultured plates were placed in the anaerobic jar together with 0.5g of Sodium borohydride ($NaBH_2$) and 0.5g of sodium trioxocarbonate ($NaCO_3$). The jar was incubated for 24 hrs. At 37°C. The plates with colonies between 30-300 (and above), were counted and the average was multiplied by the dilution factor. The mean of the triplicate plates was taken and multiplied by the dilution factor (Cheesebrough, 2000).

Staphylococcus Count

The preparation of Daddawa homogenate and dilutions was done as described under aerobic plate count and 1ml of the dilutions of homogenate was pipetted onto the surface of previously dried Baird - parker agar plates and spread with a sterile bent glass rod. Triplicate plates were prepared from each dilution. The plates were inverted and incubated at 37°C for 24hrs. After 24hrs, plates with 30 - 300 (and above) separate colonies were counted and the average total numbers of the colonies were multiplied by the dilution factor. Slants of the isolates were made for further studies (FAO, 1992).

Enumeration of Moulds and Yeast

Antibiotic (chloramphenicol) was added to Mycological media (mycophil or malt agar) to inhibit susceptible bacterial growth. The stock solution was prepared by dissolving 0.1g chloramphenicol in 40ml distilled water and by adding this solution to 960ml medium mixture after autoclaving. An aliquot of 1.0ml of each dilution was pipetted into each of the appropriately marked triplicate petri dishes. About 9ml of mycophil or malt agar tempered to 45°C was poured into each petri dish. After solidifying, the plates were inverted and incubated at 20 - 25°C for 5 days. The counting was reported as mould count, times the dilution factor (FAO, 1992).

Isolation and Characterization of Microorganisms Associated with Daddawa Product

Detection of *Escherichia coli* isolates

This was carried out in accordance to the guidelines provided by FAO (1992). A loop-full from homogenate was incubated into lactose peptone broth containing Durham tubes following 24hours incubation. The tubes were observed for gas and acid production. Tubes that change colour from blue to yellow with bubbles of gas were recorded as positive. A loop-full of inoculum from gas positive tubes were streaked onto Eosine Methylene blue (EMB) agar (oxoid) plate and incubated for 48 hours at 37°C. Following incubation, colonies which form bluish-black colour with a green metallic sheen, and reddish colonies were noted and isolated on agarslants for further biochemical test (catalase test and indole test) to confirm the presence of the species. The test organism was inoculated in a Bijour bottle containing 3ml of sterile trytone water, and was incubated at 37°C for 48 hours. Indole was treated by adding 0.5ml of Kovac's reagent and shaken gently. As a result red colour in the surface layer was examined within 10 minutes. Red surface layer indicated positive indole test (Cheesebrough, 2000).

Detection of *Staphylococcus aureus*

This was carried out according to Cheesebrough's (2002) method. Plates of prepared mannitol salt agar were inoculated with 1 ml homogenate and incubated at 37°C for 24hours. Following incubation, mannitol fermenting organisms, which showed a yellow zone surrounding their growth, were incubated on nutrient agar slants for biochemical test. Gram staining, catalase test and coagulase test were carried out on the suspected organisms.

Detection of *Salmonella* sp

This was carried out according to FAO, 1992. The homogenate was incubated in peptone water at 37°C for 20 hrs. After incubation, Ten milliliter (10ml) was transferred into 100ml Selenite cysteine enrichment medium and incubated at 37°C for 24 hours. Loopful from the enrichment medium was streaked onto Deoxycholate Citrate Agar (DCA, Oxoid) plates and incubated at 37°C for 24-48 hrs. Pale colours with black centres were presumed to be *Salmonella* sp. The colonies were Gram-stained and tested for motility. Black colonies indicated the presence of *Salmonella* sp. Gram-negative motile rods were inoculated onto nutrient agar slants for subsequent biochemical tests.

Detection and Characterization of *Yeast* and *Moulds*

One millilitre of homogenized Daddawa was pipetted into each of the appropriate marked duplicate petri dish. An Antibiotic (Chloramphenicol) was added to 9ml of Potato Dextrose Agar (PDA) at 45°C and poured into each petri dish and allowed to solidify. The plates were inverted and incubated at room temperature for 5 - 7 days (FAO, 1992). After five days of incubation, growth was identified and proper species examination was done. The colour and the cultural characteristics were observed. The microscopic examination was done by using a sterile forcept to pick a single outgrowth (colony) from the culture plate. These were followed by placing it on a microscope slide, then a drop of lactophenol cotton blue was added, covered with covered slip, and examined using x 10 objective followed by x 40 objective. The various species were independently identified due by the colour, septate or unseptate of their hyphae as well as nucleated sporangiophore and conidia (Cheesebrough, 2002)

Proximate Analysis: The proximate analysis of African locust bean cake was conducted according to the procedure of AOAC (2000).

Sensory Evaluation: The Sensory Quality of the products were determined according to 9 – point Hedonic scales (David, 2005).

Results and Discussion

It was observed that there was microbial log reduction in the treated African locust bean cake at 120 days and microbial log increase in the untreated (Control) Daddawa Statisticallyat 0.05 level of significant diffrence (figure 1-4). This is actually due to the fact that the samples treated with 1% experience moisture reduction which discourage microbial growth and proliferation. The 1% table salt reduced the bacterial load of the stored daddawa samples.These findings are consistent with the works of Ademola, *et al.*, (2013) who also observed microbial log reduction in the treated locust bean cake and microbial log increase in the untreated sample of Daddawa. Isolated and characterized bacterial isolates before treatment were *E.coli, Staphylococcus aureus, Mucor*sp and *Rhizopus* sp while *Salmonella* sp was not present (Table 1). Isolated and characterized bacterial isolates at 30 and 120 days in the treated and control fermented African locust bean cake were *E.coli*only isolated in Kwanaryan daddawa. The occurrence of *E. coli* in Kwanaryan daddawa might be attributed to use of recent feacally contaminated water in the Daddawa preparation or could be due to unhygienic activities of the Daddawa/Food handlers Bukar *et al.*, (2009) reported that 5(10.0%) out of 50 food handlers in three small scale industries in Kano Metropolis investigated carried *E.coli*on their hands. *Staphylococcus aureus* (was isolated in all the locally purchased Locust bean cake. The *Salmonella* sp was not detected in the Laboratory treated, control (laboratory untreated) and purchased Daddawa. *Salmonella* specie has been reported to be transmitted via water and salmonella carriers as food handlers (Bukar*et al.*, 2012). At 30 days *Mucor* sp and *Rhizopus* sp were the predominant fungal species, this is in line with the findings of Rabi *et al.*, (2013), at 120 days *Mucor* sp and *Rhizopus* sp were detected only in Control Daddawa. The result indicated 40% elimination of contaminants at Gude, Jogana and Kwanaryan daddawa (Table 2). The proximate composition at 120 days shows improvement in the nutritional quality of treated locust bean cake (Table 3). The organoleptic assessment shows that the judges rejected Laboratory untreated daddawa (Control) as a result of what the panelists termed as "unpalatable taste" due to off flavor (Table4) while the 1% treated daddawa was relished by panelists (Omafuvbe *et al.*, 2006). Recently, the use of more natural preservatives has become more popular than the synthetic antimicrobials and antioxidants (Ahn *et al.*, 2007).

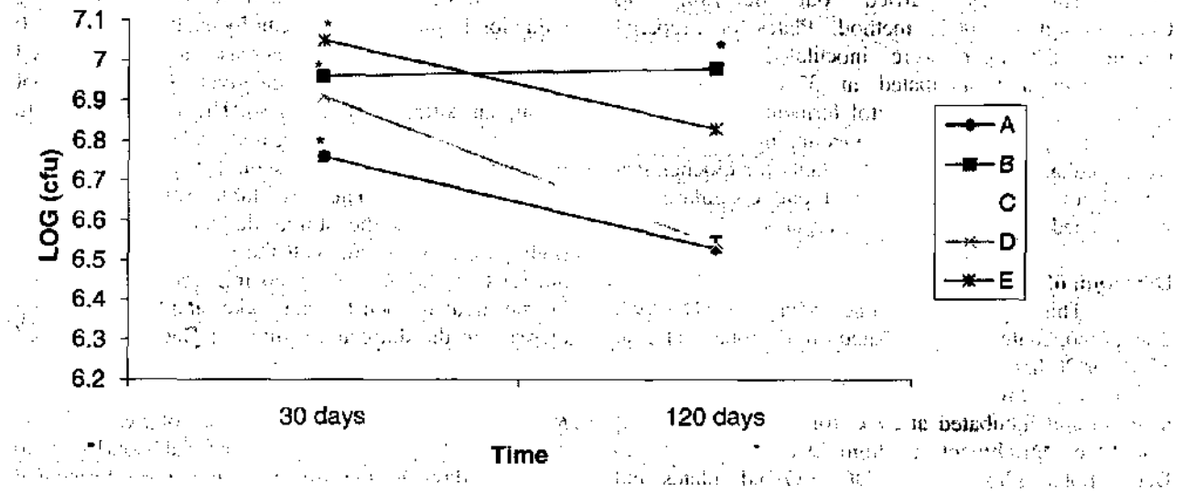

Figure 1: Aerobic mesophilic count reduction of 1% Table Salt treated Daddawa

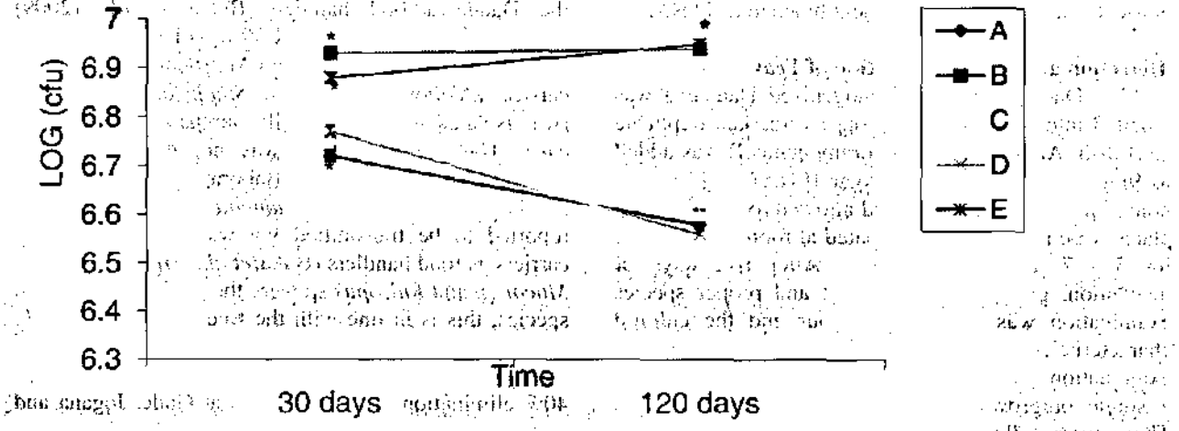

Figure 2: Anaerobic mesophilic count reduction of 1% Table Salt treated Daddawa

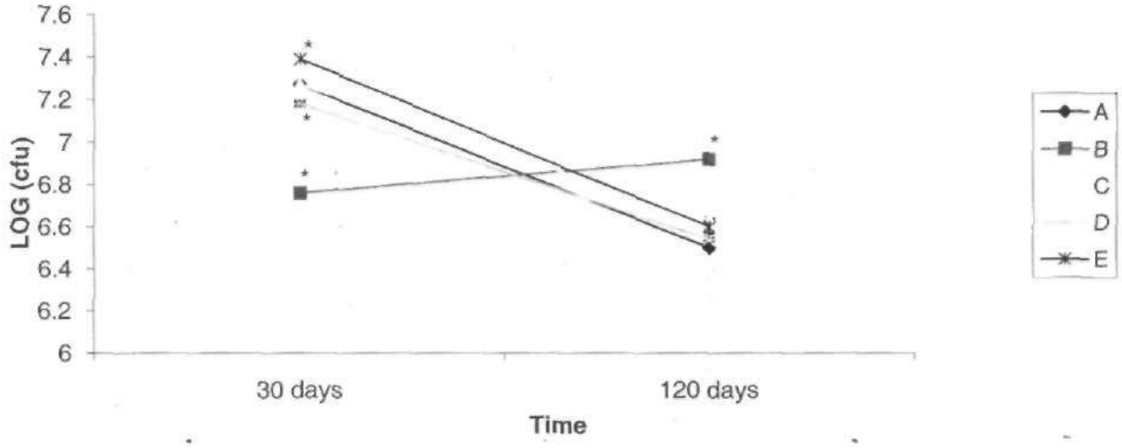

Figure 3: Staphylococcus count reduction of 1% Table Salt treated Daddawa

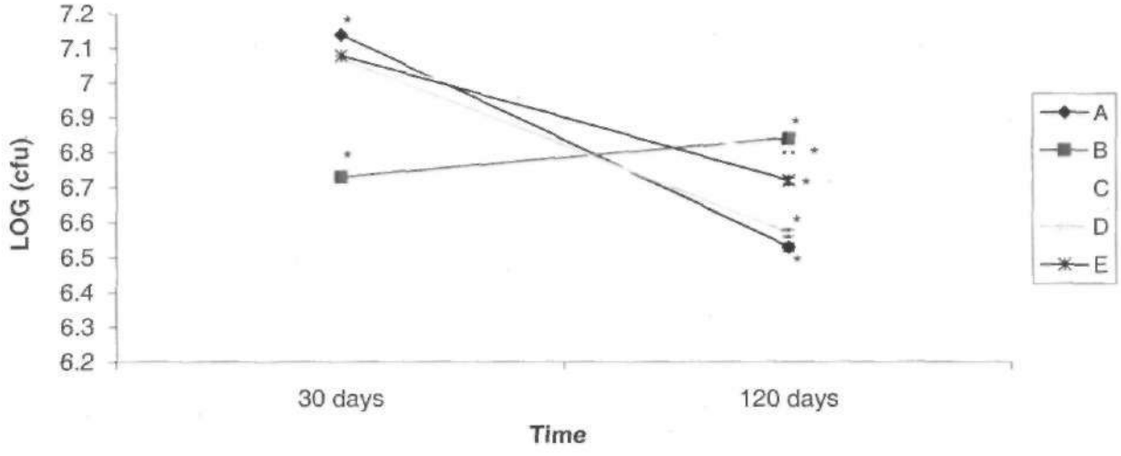

Figure 4: Fungal count reduction of 1% Table Salt treated Daddawa
* Line Graphs with asteric are significantly different at 0.05 level, values are means of three replicates ± SE

KEY: A= Laboratory Treated; B= Laboratory Untreated (Control); C= Gude; D= Jogana; E= Kwanaryan daddawa

Table 1: Detection of Microbial species of 1% Salt treated locust bean cake at 30 and 120 days.

S/N	Site	Salt treated samples at 30 days					% recovery 30 days	Salt treated samples at 120 days					% recovery 120 days	% eliminate
		E.coli	*Staphylococcus aureus*	*Salmonella* spp	*Mucor* sp	*Rhizopus*sp		*E.coli*	*Staphylococcus aureus*	*Salmonella spp*	*Mucor*sp	*Rhizopus*sp		
1	Laboratory treated daddawa	-	-	-	-	-	0	-	-	-	-	-	0	0
2	Laboratory Untreated Daddawa (Control)	-	-	-	+	+	40	-	-	-	+	+	40	0
3	Gude	-	+	-	+	+	60	-	+	-	-	-	20	40
4	Jogana	-	+	-	+	+	60	-	+	-	-	-	20	40
5	Kwanaryandaddawa	+	+	-	+	+	80	+	+	-	-	-	40	40

KEY: +=positive; -=negative; M=mucor; R-rhizopus.

Table 2: Mean Proximate Composition of Salt (1%) treated African Locust Bean Cake at 30 and 120 days

Sample	Time (Day)	Moisture %	Ash %	Crude Fat %	Crude Protein %	CHO %	Crude Fibre %
	30						
A		4.18	9.26	14.18	40.2	23.37	9.11
B		9.09	7.84	26.37	28.21	20.06	8.73
C		4.05	16.25	21.31	32.58	26.09	4.96
D		5.82	20.41	12.09	36.39	21.5	4.72
E		4.18	9.26	14.18	40.2	23.37	9.11
	120						
A		2.51	10.06	10.1	38.53	27.06	9.92
B		10.78	6.8	31.37	25.83	18.83	6.69

C	3.06	17.06	15.16	34.93	25.25	4.84
D	3.15	17.26	15.06	30.07	29.19	5.57
E	3.07	10.31	13.06	31.18	34.52	8.16

KEY: A= Lab treated; B= Lab untreated (Control); C=Gude; D= Jogana; E= Kwanaryan daddawa

Table 4: Distribution of Responses on HEDONIC SCALE for 1% Salt Treated and Untreated African Locust Bean Cake at 120 Days.

Option	Scale	Laboratory Treated A	Untreated B	Purchased Gude C	Jogana D	Kwanaryan daddawa E
Like extremely	9	2		3		4
Like very much	8	3		3	3	2
Like moderately	7	2			1	1
Like slightly	6	3		2	5	3
Neither like nor dislike	5		4	2	1	
Dislike slightly	4		2			
Dislike moderately	3		1			
Dislike very much	2		3			
Dislike extremely	1					
Mean ± Standard deviation		7.4±1.11	9.25±7.46	7.3±1.55	6.5±10.34	7.7±1.27
Total response		10	10	10	10	10
% Dislike		0	45.94	0	0	0

KEY:A= Gude; B=Jogana ; C= Kwanaryan daddawa; D= Laboratory Treated ;E= Laboratory Untreated (Control)

Conclusion

African locust bean condiment was purchased from local manufacturers at the point of production. Control standard Daddawa was produce in the Laboratory. The fermented laboratory control and local samples were treated with the preservatives and control experiment was left untreated. The quality control indicated microbial log decrease in the treated daddawa while untreated daddawa shows microbial log increase. Contaminant isolates were identified from laboratory treated, untreated (Control) and locally produced African locust bean cake. The proximate analysis and organoleptic assessment of the laboratory treated, untreated (control) and purchased locust bean cake indicated the effect of Salt which tends to improve the shelf life of processed *P.biglobosa* seeds by reducing the number of microbial load on the treated samples which could have been agents of deterioration or spoilage and reduce the shelf life.

Recommendation

However, further studies are needed for preservation of processed *Parkiabiglobosa* seeds using more natural preservatives in order to extend the shelf life of the product.

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