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Changes in Microbiological Quality of Table Spreads Produced from African Pear (*Dacryodes edulis*) Pulp during Storage

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ABSTRACT

African pear (*Dacryodes edulis*) pulp was extracted and pasteurized. The pasteurized pulp was homogenized with different levels of food grade additives to form table spreads of samples A to H while sample I was left without preservative. The spreads were packed in sealed glass containers and stored at room temperature (28±20C) for 4 weeks to evaluate the changes in microbiological quality of table spread during storage period. Samples were collected in a weekly interval to study the microbiological assay of the spread starting from week zero to the last week. High total bacteria count of 1.8x107CFU/ml was seen in sample I (spread without preservative) at week zero, this increased significantly to 8.1x108CFU/ml after 3 weeks of storage and TNTC (too numerous to count) after 4 weeks of storage at 28±20C. The least growth were observed in samples A and C with bacteria counts of 8.1x107CFU/ml and 3.5x107CFU/ml, respectively. The least fungi count of 2.0x106CFU/ml was noted in sample C after 4 weeks of storage while the highest fungi count of 4.5x107CFU/ml was seen in sample I after 4 weeks of storage at room temperature (28±20C). The suspected microorganisms based on their morphology were; E. coli, Staphylococcus aureus, Salmonella specie while fungi were Penicillium specie and Aspergillus specie. Deterioration sets in significantly after two weeks storage as total bacteria and fungi counts rose above 1.0x107 and 1.5x106, respectively. The microbiological quality of the samples was stable up to the second week of storage except sample I (without preservative).

Keywords: Microbiological Quality, Vegetable Spreads, African Pear Pulp, Storage Temperature, Nutritional Quality, Preservatives

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1. Introduction

Food preservation is the science of extending the shelf life of food, maintaining its nutritional quality and avoiding the growth of unwanted microorganisms [1]. It is the process of treating and handling food to stop or slow spoilage which results to loss of quality, edibility or nutritional values and thus allow for longer storage. Preservation involves preventing the growth of bacteria, yeast, fungi and other microorganisms [2]. Vegetable spreads are spreadable product having at least 90% ingredients from nuts and pulps used in various forms, such as paste and slurry [3,4]. Vegetable spreads are used like the commercially available butter, they can be produced from almond, cashew, hazelnut, macadamia nut, peanut, pecan, pistachio and walnut [5]. Vegetable spread is popular and widely accepted by consumers due to its flavour, good nutritional values and suitability for consumption either alone or in combination with a variety of other foods. African pear pulp has great potential for vegetable spread production. The African pear tree (*Dacryodes edulis*) is a tropical *oleiferous* fruit tree that possesses enormous potential in Africa [6]. Various parts of the tree are used in traditional medicine[7,8]. The wood serves for firewood and carpentry [9]. The entire tree is used in Agro-forestry systems for soil conservation [10]. *Dacryodes edulis* fruit is popular in the diets of many Africans. It can be eaten raw, roasted or boiled in hot water and is eaten alone or used in garnishing cooked or roasted maize. It could also be used as butter to eat bread [8]. It has been found in Cameroon recipe that when the pulp of African pear is cooked and seasoned, it serves as spread [11]). According to Ayuku *et al*.[12], *D. edulis* has a potential to improve nutrition and food security. During the last three decades, more and more studies have been conducted on *D. edulis*, essentially the tree and its fruit. The scientific researches on *D. edulis* focused on the characterisation of propagation techniques of *D. edulis* tree [13], the nutritive value of its pulp and its oil [14] and the oil extraction processes[15]. These studies revealed excellent nutritional qualities of fruit pulp and interesting food processing properties of the oils extracted from the pulp and kernel safou [16]. These have also revealed the importance of this fruit nutritionally, therapeutically and in cosmetics. The pulp, the only edible part of the fruit is particularly rich in lipids, hence, it could be an important source of oil [17]. Besides lipids, *D. edulis* pulp contains substantial amount of many other nutrients including proteins, carbohydrates, minerals, vitamins and fibres[16]).

High content of protein and other essential nutrients in African pear spread makes it susceptible to microbiological contamination and growth. Thus, the objective of this work was to produce table spreads from African pear pulp treated with different levels of preservatives and to evaluate the effect of room temperature (28 ± 2^0C) storage on the microbiological quality of the product.

2. Materials and Method

2.1. Materials

Mature and good quality fruits of the African pear (*Dacryodes edulis*) were purchased from the fruit market in Port Harcourt and transferred to the Microbiological Laboratory in the Department of Food Science and Technology, Rivers State University, Port Harcourt, Nigeria. All apparatus and chemicals used for the study were obtained from the same Laboratory and were of analytical grade.

2.2. Extraction of African Pear Pulp and the Formulation of the Spread

The pulp was extracted using modified traditional method of pear roasting. African pear fruits were sorted and washed with tap water and sodium chloride solution, furthermore rinsed thoroughly. They were roasted at 60^0C for 4 min in a hot air oven (model QUB 305010G, Gallenkamp, UK). The roasted fruits were allowed to cool for 10 min, the thin bluish-black epicarp were gently removed and

discarded while the soft pulps were scraped off and recovered in sterile stainless steel plates. The extracted pulp was pasteurized by heating at 100^0C for 5 min in a stainless pot. The pasteurized pear pulp was treated with different levels of recommended food preservatives [18]. The mixture was homogenized properly using a laboratory stirrer (model JKL 2145, REMI Motors, India) to produce the African pear spread (APS) as presented in Figure 1 and Table 1.

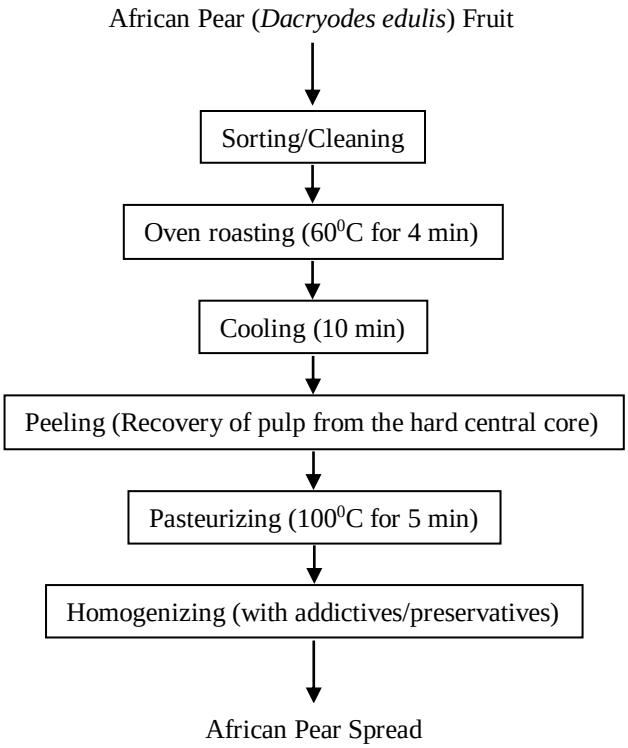

Figure1. Flow Chart for the Production of African Pear Spread

Table 1. African Pear Spread (APS) Formulations

SAMPLES	PEAR PULP	H_2O	PS (E200)	BHT (E321)	CA (E330)
	(g)	(g)	(g)	(g)	(g)
A	100	15	0.045		
B	100	15	0.035		
C	100	15		0.01	
D	100	15		0.005	
E	100	15			0.008
F	100	15			0.005
G	100	15	0.025	0.005	
H	100	15		0.005	0.008
I	100	15			

Key: PS = Potassium sorbate, BHT = Butylated hydroxytoluen, CA = Citric acid.

2.3. Storage of Samples

The produced spreads were stored in sealed glass containers at room temperature (28 ± 2^0C). Samples were collected at one-week interval for a period of four weeks for storage studies from week one, two, three and four using week zero as the control sample.

2.4. Microbiological Analysis

Storage stability of the spreads were conducted to study the changes in microbiological quality which included; total bacterial count, total fungi

count and an assay of the microorganisms associated with the spoilage of African pear spreads dusring storage.

2.4.1. Total Bacteria and Fungi Count

Microbial assay was carried out on each of the stored spread samples to test the presence of bacteria and fungi using nutrient agar (NA) and potato dextrose agar (PDA) for total bacteria and fungi counts, respectively. The procedure described by the International Commission on Microbiological Specification for Food [19] was adopted in this study.

2.4.2. Sterilization of Materials

All glassware used were sterilized by autoclaving at 121^{o}C for 15 min and allowed to cool before use.

2.4.3. Culture Media Preparation

The powder media were weighed and dissolved in distilled water in accordance to each dilution factor as follows: **NA**: 28g powder in 1000ml distilled water to obtain 2.8% (w/v) solution. **PDA**: 39g powder in 1000ml distilled water to obtain 3.9% (w/v) solution. The mixtures were stirred separately, properly and sterilized at 121^{0}C for 15 min in an autoclave. They were allowed to cool to 45^{0}C and then dispensed into dishes using the spread-plate technique.

For diluents' preparation, one tablet of phosphate buffer was dissolved in 100ml of distilled water. Using a 10ml syringe, 9ml each of the buffer solution were injected into five (5) separate test tubes. Each of the tubes were covered with cotton wool and aluminium foil and then, sterilized at 121^{0}C for 15 min in the Autoclave.

2.4.4. Serial Dilution and Inoculation

The spread sample was weighed (1g) and suspended in the first test tube (containing 9ml diluents), making the solution 10ml. Serial dilution was done; by pipetting 1ml from the primary suspension (10^{-1}) into the next tube (10^{-2}), 1ml from that 2nd tube into the next, the process continued to the last (fifth) tube. One ml from the last tube was removed and discarded. Prepared plates were inoculated using the spread plate (surface plating) method. The five tubes (10^{-5}) were used for inoculation. From each of these tubes, 0.1ml was pipette onto the surface of an agar plate and spread evenly using a sterile L-shaped glass spreader. The plates were incubated at 35^{0}C for 24 - 48 hr for NA and 30^{0}C for 96 hr for PDA. The colonies were counted using a colony counter and result recorded as colony forming unit per ml (CFU/ml).

2.4.5. Isolation and Identification of Microorganisms Associated with the Spoilage

This was done through sub-culture to obtain pure culture of isolates. Single colony of bacteria was randomly isolated from the mother culture onto a fresh prepared nutrient agar plate. It was inoculated using streak method and incubated at 37^{0}C for 24 hr for proper identification.

2.4.6. Gram Staining

A loop full of fresh culture was isolated and emulsified in a drop of distilled water and allowed to air-dry. The film was heat-fixed by passing it over a flame for 3 sec before covering with crystal violet for 60 sec. It was rinsed with running tap water, drained and covered with iodine for 60 sec, rinsed again with water and decolorized with ethanol for 30 sec, counterstained with safrain for 60 sec after rinsing off the ethanol. The film was rinsed finally and air-dried before viewing under the microscope.

2.4.7. Identification of Isolates

The following characteristics were used for identification of the isolates: size of colony, shape, edge, elevation, pigmentation, texture, opacity, consistency and odour of colony.

2.4.8. Biochemical Test

The following biochemical test were done for identification and confirmation of the isolates: indole test, motility, oxidase, catalase, methyl red and voges proskeur test.

3. Results and Discussion

3.1. Total Bacteria and Fungi Counts

As shown in Table 2, samples A, B, C and F did not show any bacteria growth at week zero. High total bacteria count of $1.8x10^7$CFU/ml was seen in sample I (spread without preservative) at week zero, this increased significantly to $8.1x10^8$CFU/ml after 3 weeks of storage and TNTC (too numerous to count) after 4 weeks of storage at $28±2^0$C. The least growth at week 4 were recorded in samples A and C with bacteria counts of $8.1x10^7$CFU/ml and $3.5x10^7$CFU/ml, respectively.

Fungal growth was not observed in all the samples at week zero. Also Samples C and F also showed no growth at week 1 as presented in Table 3. The least fungi count of $2.0x10^6$CFU/ml was noted in sample C while the highest fungi count of $4.5x10^7$CFU/ml was observed in sample I after 4 weeks of storage period at room temperature ($28±2^0$C). This implies that BHT preservative was effective in subduing the bacterial and fungal growth in the produced pear spread. The total plate count of all the spread samples were beyond the marginal acceptable count of 5.0×10^6CFU/ml, for table spreads and margarine [19].

Table 2. Total Bacteria Counts (CFU/ml) on APS Treated with Different Levels of Preservatives during Storage

Samples	Storage Time (Weeks)				
	0	1	2	3	4
A	NG	1.6×10^6	1.9×10^7	3.5×10^7	8.1×10^7
B	NG	1.0×10^6	1.6×10^7	3.3×10^7	1.0×10^8
C	NG	1.0×10^6	3.0×10^6	1.0×10^7	3.5×10^7
D	6.0×10^6	2.8×10^7	4.6×10^7	2.0×10^8	6.1×10^8
E	1.2×10^6	1.0×10^7	4.9×10^7	8.0×10^7	1.7×10^8
F	NG	2.0×10^6	5.6×10^6	1.2×10^7	1.0×10^8
G	1.0×10^6	6.0×10^b	2.9×10^7	1.1×10^8	2.0×10^8
H	3.0×10^6	1.9×10^7	2.9×10^7	8.1×10^7	1.5×10^8
I	1.8×10^7	4.7×10^7	8.9×10^7	8.1×10^8	TNTC

Table 3. Total Fungi Counts (CFU/ml) on APS Treated with Different Levels of Preservatives during Storage

Samples	Storage Time (Weeks)				
	0	1	2	3	4
A	NG	2.0×10^6	2.0×10^6	3.0×10^6	3.5×10^6
B	NG	1.1×10^6	2.0×10^6	2.8×10^6	3.0×10^6
C	NG	NG	1.0×10^6	1.5×10^6	2.0×10^6
D	NG	1.0×10^6	1.0×10^6	2.0×10^6	1.1×10^7
E	NG	2.0×10^6	2.0×10^6	8.0×10^6	1.7×10^7
F	NG	NG	1.0×10^6	2.0×10^6	3.0×10^6
G	NG	2.0×10^6	3.0×10^6	8.0×10^6	1.4×10^7
H	NG	1.0×10^6	1.1×10^6	2.0×10^6	1.0×10^7
I	NG	2.0×10^6	2.0×10^6	1.7×10^7	4.5×10^7

Key: APS = African pear spread; A = APS treated with potassium sorbate (0.045%);B = APS treated with potassium sorbate (0.035%); C = APS treated with BHT (0.01%);D = APS treated with BHT (0.005%);E = APS treated with citric acid (0.008%);F = APS treated with citric acid (0.005%); G = APS treated with BHT (0.005%) and potassium sorbate (0.025%); H = APS treated with citric acid (0.008%) and BHT (0.005%); I = APS without preservative; NG = no growth; TNTC = too numerous to count

3.2. Microorganisms Associated with Spoilage of APS and their Biochemical Reactions

The suspected microorganisms based on their morphology were; *E. coli* which was Gram negative (-), VP negative (-), Ind negative (-), MR negative (-), OX negative (-), with positive (+) motility as shown in Table 4. *Staphylococcus aureus* which was Gram positive (+), VP negative (-), Ind negative (-), OX positive (+) and catalase positive (+). *Salmonella specie* which was Gram negative (-), VP negative (-), Ind negative (-), OX negative (-) with positive (+) motility. *Penicillium specie* which its colonies grew in shades of green with broom-like head. *Aspergillus specie*, its colonies also grew in shades of yellowish-green with swollen vesicle and flasked-shaped phialides.

Table 4. Microorganisms Associated with Spoilage of APS and their Biochemical Reactions

Suspected Organism	Gram RXN	VP	Ind.	MR	Motility	OX	Catalase
E. Coli	-	-	-		+	-	.
Staph. Aureus	+	-	-			+	+
Salinonella sp	-	-	-		+	-	

Key:VP = voges proskeur, Ind = Indole, MR = methyl red, OX = oxidase,

4. Conclusion

The findings from this work showed that table spreads produced from African pear pulp and treated with 0.010% BHT preserved better than citric acid and potassium sorbate. The least bacteria counts were observed in samples A and C. In terms of fungi, least and highest counts were recorded in samples C and I, respectively after 4 weeks of storage period at room temperature (28 ± 2^0C). The microbiological quality of the table spreads treated with food grade preservatives was stable up to the second week of storage. The need to review the treatment content with cognisance to good manufacturing practice (GMP) is highly recommended.

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