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Potential Pathogens and Effects of Preservation on Microbial Loads of *Dacryodes edulis* (African Pear) Fruits.

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Abstract
The effects of preservation methods on the microbial loads of *D. edulis* (African Pear) fruits and the prevalence of potential human pathogens were investigated. Various treatments were used ranging from dipping in (1) 75 percent ethanol (2) 10 percent NaCl (3) 10 percent woodash solution (4) refrigeration while the control was left untreated. Refrigeration had the longest inhibitory effect on spoilage organisms followed by woodash and NaCl solution. The effects of ethanol only lasted waned off after the three days due to evaporation while the NaCl treated pfruits had gummy surface. Combination of refrigeration with woodash and NaCl respectively prolonged preservation up to 14 days. Statistical analysis showed significant influence of the treatment methods (P=0.05)on the microbial loads. Total heterotrophic bacterial counts were higher than total fungal counts which in turn were higher than total coliforms and total heamolytic bacterial counts. The last two groups og organisms had no significant difference all through the study period in all treatment methods Potential human pathogens observed were *Staphylococcus aureus, Baccillus, Pseudomonas* and *Enterobacter* species. The least prevalent was Enterobacter while the highest was Baccilus species. These organisms were both heamolytic and coagulase positive.

Key words: Preservation, spoilage, pathogens, harvested fruits.
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Introduction

Dacryodes edulis (Africa pear) tree belongs to the family of Buseraceae composed mianly of trees and shrubs. It bear, fruits which are pink when young but dark/deep blue when mature. The plant is common in the rain forest region of tropical Africa where it serves many dietary functions (Ejiofor and Okafor, 1997; Ajiwe *et al.* 1992). The plant is known by different names among the various tribes in Nigeria – Ube (Igbo), Eben (Efik) and Elemi (Yoruba).

African pear can be found within the tropical humid climatic zone of West Africa from Sierra Leone through Ghana, Benin, and Cameroun to Equatorial Guinea and Congo. The fruit is described as a pome with central hard made of several smaller ones and an edible mesocarp. It is a rich but cheap source of nutrients including vitamins, proteins, carbohydrate, fats and some essential elements (Onuorah *et al.*, 2001, Aiyelaagbe *et al*, 1998 Agrios, 1997). It is often eaten with maize (boiled, fried or roasted), yam (prepared in various ways) and even bread.

However, inspite of its usefulness and contributions to diet, *Dacryodes edulis* fruit easily undergoes microbial spoilage. This has reduced its availability and shelf-life to only the harvesting reasons. Both fungal and bacterial species have been implicated in the spoilage (Nwufo and Anyim., 1998 Harrigen, 1998).

This work was therefore designed to assess the effect of some local methods of preservation techniques on the shelf life and microbial survival periods in the *Dacryodes edulis* fruits. In addition the prevalence of potential pathogens in microbial deteriorating fruits was also determined.

Materials and Methods

The study area was Ogba/Egbema LGA, area of Rivers. The communities selected were only Omoku and Okwuzi as the entire local Government has very uniform climate condition.

Sample Collection

The fruits used in this section of the work were obtained directly from the tree when fully mature (deep/dark blue). They were taken to the laboratory within two hours of harvesting and subjected to different treatments.

Treatment of the Fruits

Healthy 100 *Dacryodes edulis* fruits were randomly divided into 5 groups of 20 each. Each group was subjected to a particular treatment only. The treatments were dipping into (i) Alcohol (Ethanol), (2) 10% NaCL solution, (3) 10% ash solution, (4) Refrigeration (5) control (no treatment) stored in ambient conditions. Each group was kept separate from each the for the entire duration of the experiment. **Estimation of Bacterial Loads**

Various culture media were used to obtained the various groups of organisms sort. These were Nutrient Agar for Total heterotrophic bacteria, McConkey Agar for coliforms, Blood Agar for Heamolytic bacteria and SDA for fungal counts. In each experimental period, three of the fruits in each treatment were collected and portions of their mesocarp cut out using sterile scalpel. The cut mesocarp parts were macerated and used to make serial dilution (ten fold). 0.2ml of 10^{-2} and 10^{-3} dilution were inoculated on the above media and incubated aerobically after spread plate inoculation. Observed colonies were expressed as cfu/g. This experiment was conducted for 3 weeks.

In the second part of the work only ash and NaCl treatments were used in conjunction with refrigeration ie each of the two treatments was carried out separately but combined with refrigeration. This time the experiment lasted for 14 days.

Characterization and Identification of the Potential

Human pathogens from the *Dacryodes edulis* fruits (African pear) was determined according to Chessbrough (2003) and characterized and identified by Cowan, and Steel, (1982). In this case 50 African pear fruits from farm (clean catch) and 50 healthy fruits from the markets were selected. These were kept without treatment but their mesocarp parts were macerated as in the former case and ten fold serial dilution was carried out on them. 0.2ml of 10^{-2} and 10^{-3} dilutions were spread plated in duplicates on Blood Agar. The plates were observed for heamolysis after twenty-four (24) hours aerobic incubation.

The fruits in this part of the experiment were stored in ambient conditions for 6 days.

Results;

The results of the various preservation treatments on the microbial loads found in D edulis fruits are presented in Table 1.The highest (best) preservative method was refrigeration as it inhibited the microbial growth more than other methds (16 days), followed by NaCl solution (14 days) and Woodash (12 days). Ethanol only lasted for 8 days while the control was only 6 days. The ethanol easily evaporated after a few days while the surface of the NaCl solution treated fruits were gummy.

Statistical analysis showed that the treatment methods influence the keeping quality of the D. edulis fruits (P=0.05).

The most prevalent organisms were the total heterotrophic bacteria, followed by the total fungal counts while the difference between the total coliforms and the total heamolytic bacterial counts were not significant (P=0.05) in all the treatment methods used all through the experimental period.

Table 2 shows that the keeping quality of all the fruits improved with combination of preservation methods. The combination lowered the microbial loads

significantly compared to the results in Table 1. Combination of NaCl solution with refrigeration however showed greater decrease in microbial loads than Woodash and refrigeration.The same trend of total heterotrophic bacterial counts being higher than total fungal counts and total coliforms being similar with total heamolytic bacterial counts persisted.Table 2).

Observations in Table 3 showed that heamolytic bacteria were quite few in the fruits obtained directly from the farm compared to the market samples. The prevalence of the organisms increased with keeping time showing multiplication in the African pear fruits. The most predorminant heamolytic bacteria were *Bacillus* species, followed by the *Pseudomonas* species before *Staphylococcus aureus* . The least was the *Enterobacter* species (Table 3).

Discussion

From the results obtained in this work, the storage of the *Dacryodes edulis* fruits resulted in increase of microbial loads of all the treatments used. However, statistical analysis showed that the level of microbial load was determined or influenced by the treatment.Every treatment lowered the microbial proliferation. This agrees with the finding of Adams and Moss (1995) and Fraizer and Westhoff (1995) who stated that the preservation process increased to shelf-life of the fruits. This is in agreement with the principle of preservation which is to retard the growth of spoilage organisms hence increasing the keeping quality of the item being preserved (Pelczar *et al*. 2003; Adams and Moss, 2000).

Observations in this work showed that the treatment method affected the rate of microbial growth inhibition. Observations showed that refrigeration had the best positive effect in the preservation process. This is because African pear fruits preserved by this method stayed without considerable spoilage till the 21 day of the experiment. It was observed that ethanol had very good effect only within the first 4 days, after which the effects waned off. This could be attributed to evaporation. Chessbrough (2003) and Pelczar *et al*., (2003) stated that ethanol is used as disinfectant. Inhibition of microbial growth is the reason for its usage as a disinfectant. However, its evaporation is a disadvantage in prolonged storage. Similarly, NaCl solution had higher inhibitory effects on spoilage organisms than ethanol. However, the solution made the surface of the pear fruits gummy, hence acted as a trap for dusts and microbial particles. However, the salt solution was able to inhibit most organisms because of its high osmotic potential which lasted more than ethanol. Some food items eg meat and fish have been preserved with NaCl. This is similar to the preservation of food items with sodium metabisulphate (Fraizer and Westhoff, 1995).

Ash solution has been used as a local preservative in hard epidermal fruits while in very succulent ones, it helps in their ripening (Bryant *et al,* 1988)). The use of ash is because it dissolves in the available moisture to create very high alkaline condition. This high alkalinity inhibits microbial growth. However, it does not penetrate much into the fruit because of its papery epidermal layer.

The preservation treatment reduced the rate of microbial proliferation. The effects of the treatment become evident when compared to the control (ambient condition), where the fruits spilt by on the 6th day. The reduced bacterial loads in the various preservation treatments indicated the strength of the preservation methods. The case of the refrigeration was because the low refrigeration temperature was not conducive for Africa pear fruit spoilage organisms as stated by Dawas and Kotze (1987). The low temperature did not allow the spoilage enzymes to operate optimally hence reduced microbial growth and spoilage.

The results obtained in this work showed that most predominant organism

were the total heterotrophic bacteria while the coliforms were not significantly different. The Fungal counts were higher than the bacterial group counts except the total hetertrotrophic bacteria. This is because the coliforms and heamolytic bacteria were included in the total heterotrophic bacterial counts. This type of situation had been explained severally by Pelcar *et al* (2008), Prescott *et al*, (2004) and Harrigan, (1998).

The assessment of spoilt *Dacryodes edulis* fruit as a potential source of human pathogens indicated higher prevalence in market samples. This is because most of the heamolytic bacteria which were equally coagulase positive were from the market samples. This could equally be attributed to contamination while in transit. However, the presence of the same organisms later in the farm samples could be attributed to contamination too as they were not initially there.

Several authors (Lewis *et al,* 2006; Mahovic *et al,* 2004, Pelczar *et al*, 2003, Chessbrough, 2003 Angie, 2001) have reported the implication of the isolated heamolytic organisms in human diseases.. This work therefore agrees that man can be infected by consuming spoilt or spoiling D. edulis fruits.

Observations showed that of time the fruits were kept, determined the loads of the potential pathogens as they kept multiplying the nutrients were exhausted. This was so till the 4th day when the bioloads began to decrease because of nutrient exhaustion. This could be so as most of the microbial utilizable nutrients in the African pear fruits had been exhausted.

In conclusion, this work showed that the best method to preserve the African pear was by combined salting and refrigeration. However in the absence of refrigerators, salting will help reduce microbial contamination and spoilage. In addition, spoilt pear fruits should not be consumed by human as they harbor pathogenic organisms.

Table 1: Total bacterial counts from variously treated *Dacryodes edulis* fruits (cfu/g).

		Ethanol	Salt solution	Refrigeration	Ash rubbing	Ambient condition
Day 1	THBC TCC THcBC TFC	0.4×10^1 0.2×10^1 0.1×10^1 0.4×10^1	0.4×10^1 0.1×10^1 - 0.4×10^1	0.6×10^1 0.3×10^1 0.2×10^1 0.5×10^1	0.9×10^1 0.4×10^1 0.2×10^1 0.6×10^1	1.2×10^1 0.6×10^1 0.4×10^1 1.3×10^1
Day 2	THBC TCC THcBC TFC	1.0×10^2 1.2×10^1 0.3×10^2 1.2×10^1	1.3×10^2 1.4×10^1 0.8×10^1 2.1×10^2	1.4×10^2 0.0×10^1 0.6×10^1 1.4×10^2	1.6×10^2 0.6×10^1 0.6×10^1 1.2×10^2	1.5×10^2 1.2×10 1.7×10^1 2.1×10^3
Day 4	THBC TCC THcBC TFC	2.4×10^4 1.2×10^2 1.6×10^2 1.4×10^3	$2.4. \times 10^3$ 1.3×10^2 1.0×10^2 1.4×10^2	2.3×10^2 1.0×10^1 1.1×10^1 1.4×10^3	2.6×10^3 1.0×10^1 1.2×10^1 1.2×10^4	2.7×10^4 1.4×10^2 1.6×10^2 1.3×10^4
Day 6	THBC TCC THcBC TFC	2.6 x 104 1.4 x 102 1.7 x 102 1.9 x 104	3.2 x 103 1.9×10^2 1.2×10^2 1.0×10^4	1.2×10^3 1.9×10^2 1.3×10^1 1.6×10^3	2.9×10^3 1.1×10^2 1.1×10^2 1.2×10^4	1.2×10^4 1.1×10^2 1.2×10^2 2.1×10^4
Day 8	THBC TCC THcBC TFC	2.6 x 104 1.6 x 102 1.9 x 102 2.1 x 104	1.1×10^4 1.2×10^2 1.2×10^2 1.9×10^4	1.1×10^4 1.4×10^2 1.4×10^2 1.9×10^3	1.4×10^4 1.6×10^2 1.4×10^2 1.6×10^4	Completely spoilt
Day 10	THBC TCC THcBC TFC	Completely spoilt	1.4×10^4 1.7×10^2 1.5×10^2 1.2×10^4	1.4×10^4 1.5×10^2 1.6×10^2 1.3×10^4	1.6×10^4 1.6×10^2 1.7×10^2 2.1×10^4	-
Day 12	THBC TCC THcBC TFC	-	1.9 x 104 1.9 x 102 1.8 x 102 1.8 x 104	1.6×10^4 1.6×10^2 1.6×10^2 1.6×10^4	2.2×10^4 1.9×10^2 1.8×10^2 2.4×10^4	-
Day 14	THBC TCC THcBC TFC	-	2.4×10^4 1.2×10^3 1.1×10^3 2.5×10^4	1.8 x 104 1.8 x 102 1.8 x 102 1.9 x 104	Spoilt	
Day 16	THBC TCC THcBC TFC	-	Spoilt	2.2 x 104 1.1 x 103 1.2 x 103	-	
Day 18	THBC TCC THcBC TFC	-	-	Spilt	-	

Table 2: Effect of combine refrigeration into salt and ash treatment on bacterial load of African pear fruits

		Salt solution	**Ash content**
1	THBC	0.2×10^1	0.4×10^1
	TCC	0.1×10^1	0.2×10^1
	THcBC	-	-
	TFC	0.2×10^1	0.3×10^1
2	THBC	1.4×10^1	1.1×10^2
	TCC	0.8×10^1	0.3×10^1
	THcBC	0.3×10^1	0.3×10^1
	TFC	1.1×10^2	1.0×10^2
4	THBC	1.2×10^2	1.4×10^2
	TCC	1.0×10^1	0.6×10^1
	THcBC	1.1×10^1	0.8×10^1
	TFC	1.4×10^2	1.2×10^2
6	THBC	2.4×10^2	2.6×10^2
	TCC	1.6×10^1	1.1×10^1
	THcBC	1.2×10^1	1.1×10^1
	TFC	2.1×10^2	2.3×10^2
8	THBC	1.2×10^3	1.4×10^3
	TCC	1.2×10^2	1.1×10^2
	THcBC	1.0×10^2	1.2×10^2
	TFC	2.3×10^2	2.6×10^2
10	THBC	1.9 x 103	2.1×10^3
	TCC	1.4 x 102	1.5×10^2
	THcBC	1.3 x 102	1.3×10^2
	TFC	1.2 x 102	1.3×10^3
12	THBC	2.4×10^3	2.6×10^3
	TCC	1.8×10^2	1.8×10^2
	THcBC	1.4×10^2	1.7×10^2
	TFC	1.6×10^3	1.4×10^3
14	THBC	3.1×10^3	3.3×10^3
	TCC	2.1×10^2	40×10^2
	THcBC	2.2×10^2	2.1×10^2
	TFC	2.1×10^3	2.1×10^3

Table 3: Prevalence of the Heamolitic bacterial species observed

Day	Organisms	Farm			Market		
		NTE	NTI	%	NTE	NTI	%
1	Escherichia coli	50	2	4	50	16	36
	Staphylococcus aureus	50	2	4	50	7	14
	Pseudomonas species	50	3	6	50	6	12
	Enterobacter species	50	-	-	50	3	6
	Bacillus species	50	10	20	50	21	42
2	Escherichia coli	50	8	16	50	22	44
	Staphylococcus aureus	50	6	12	50	15	30
	Pseudomonas species	50	4	8	50	10	20
	Enterobacter species	50	3	6	50	7	14
	Bacillus species	50	13	36	50	27	34
4	Escherichia coli	50	10	20	50	20	60
	Staphylococcus aureus	50	10	20	50	19	38
	Pseudomonas species	50	8	16	50	15	30
	Enterobacter species	50	5	10	50	13	26
	Bacillus species	50	20	40	50	30	66
6	Escherichia coli	50	15	30	50	25	50
	Staphylococcus aureus	50	12	24	50	11	22
	Pseudomonas species	50	11	22	50	9	18
	Enterobacter species	50	8	16	50	9	18
	Bacillus species	50	25	50	50	30	60
8	Escherichia coli	50	10	20	Spoilt		
	Staphylococcus aureus	50	8	16			
	Pseudomonas species	50	7	14			
	Enterobacter species	50	6	12			
	Bacillus species	50	21	42			

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