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Research Article

Bacteriological Quality of African Pear (*Dacryodes edulis*) Fruits Retailed in Amai Market

Eboh OJ, Aghanenu AS and Onuoha T*

Department of Biological Sciences, Novena University, Ogume, Delta State, Nigeria

***Corresponding Author:** Onuoha T, Department of Biological Sciences, Novena University, Ogume, Delta State, Nigeria.

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Abstract

The bacteriological quality of fresh commercial African pear (*Dacryodes edulis*) from Amai was investigated using standard microbiological methods. A total of 30 fresh African pear fruits was sourced from Amai central market and transported to the microbiology laboratory of Novena University Ogume for examination. Total bacteria loads ranged from 5.0×10^2 to 2.5×10^5 cfu/ml. A total of 10 predominant bacterial isolates were identified to be *Escherichia coli* and *Staphylococcus aureus*. The percentage distribution was 60% for *Staphylococcus aureus* and 40% for *Escherichia coli.* The findings from this study suggest that African bush pear at local markets of Amai harboured pathogenic bacteria which makes it important for the implementation of proper hygienic measures during pre-and post- harvest stages of production and at the time of transportation, storage, selling, and consumption to safe guard public health.

Keywords: *Dacryodes edulis*; Economic value; *Escherichia coli* and *Staphylococcus aureus*

Introduction

Dacryodes edulis the African pear or safou is an evergreen tree indigenous to the central Africa and Gulf of Guinea region. The tree has a relatively short trunk and a deep, dense crown [1]. The preferential habitat of *Dacryodes edulis* is a shady, humid tropical forest. However, it adapts well to variations in soil type, humidity, and temperature and day length. The natural range extends from Angola in the South, Nigeria in the North, Sierra Leone in the West and Uganda in the East. It is also cultivated in Malaysia [1]. The main use of *D. edulis* is its fruit which according to Omogbai and Ojeaburu [1] can take various forms and sometimes reaching up 15 cm in length [2]. The fruit can be eaten either raw, cooked in salt water, roasted in hot ash or grilled in the oven. The major importance of the fruit is because of it lipid content richness [1,3,4]. *Dacryodes edulis* fruits possess medicinal properties and economic value. It is used as a perennial cure for a variety of aliment ranging from ear infection to fever and oral problems [4]. In Nigeria, the plant resin is used for treating parasitic skin disease and Jiggers, while the pulped bark is used to cicatrize wounds [4]. Recently, it was reported that the leaves were made into plaster to treat snake bite in Southwest Cameron, the stem exudates of the plant were reported to contain tannin, saponin and alkaloids [2].

In spite of its nutritional values, *D. edulis* is highly perishable and prone to microbial attack, as it is also rich source of nutrients to microorganism [1,4,5]. Research have shown that microorganisms penetrate the intact cuticle of the fruits through natural openings or wound during harvest resulting to microbial deterioration of the fruit [4]. The fruits are harvested and transported to the market within 24hours because of its very short shelf life. A huge quality fruits go to the waste due to lack of proper post- harvest preservation. Microorganisms penetrate the intact cuticle of the fruits through natural openings or wounds during harvest. Microbial deterioration of the fruits produce undesirable effect on fruit quality which not only effects the texture but the organoleptic properties,

resulting in spoilage [5]. The aim of this study was to investigate *Dacryodes edulis* fruit for their bacterial load sold in Amai market.

Materials and Methods

Study location

The study location was Amai town. Amai is located in Ukwuani Local Government area of Delta State, Nigeria. The geographical location of Amai is 06˚ 50' E and latitude: 05˚ 45' N, it is bounded on the west by Umuebu, to the east by Ogume, the south by Ezionum and Obiaruku in the northern. Amai has a central market, which is located close to Novena University Campus and opens every four days for trading.

Sample collection

A total of 30 bush pear fruits were sourced from various vendors within Amai market. The collected samples were immediately transferred into sterile polythene bags and transported to the laboratory for immediate analysis.

Sterilization of apparatus

All the apparatus used for this work including conical flask, petri- dishes, test-tubes, pipette, staining rack, bijou bottles, test tube racks, inoculating loop, inoculating needle and measuring cylinder were thoroughly washed with detergent and rinsed with distilled water. They were then sterilized in the autoclave at 121˚C for 15mins at 15Ibs. The inoculating loop and needle was flamed to red hot before use.

Media and reagents

The microbiological media used for the estimation of total heterotrophic bacteria include; Mannitol salt and MacConkey agar. The media was prepared according to manufacturer's instructions, using moist heat sterilization method with the aid of autoclave which operates at 121˚C for 15min.

Preparation of media

The dehydrated powder of mannitol salt, nutrient and MacConkey agar (27.78 g) each was dissolved in 250 ml of distilled water mixed thoroughly to aid adequate distribution of the agar and later subjected to sterilization at 121˚C for 15mins. After sterilization, the agar medium was allowed to cool before pouring into the petri dishes and after solidification of the agar. The media plates were inverted to prevent contamination of steam from the plate cover.

Enumeration of total bacterial counts and isolation

The spoilt parts of the pear fruits were cut out and mashed in a sterile mortar and 10 grams was added into 100ml of the sterile normal saline. The samples were serially diluted in ten folds. Total bacterial plate counts were determined using pour plate technique. Then the molten Nutrient agar, MacConkey agar and Mannitol salt agar at 45˚C were poured into the petri dishes containing 1 ml of the appropriate dilution for the isolation of the total bacteria coliforms respectively. They were swirled to mix and the total bacterial counts were counted using a colony counter after incubating the plates at 37˚C for 24h. Colonies was sub-cultured on slants for identification.

Preparation of pure culture

After the isolation and growth of the organism, a freshly prepared agar was made and poured into a petri dish and it was then allowed to cool. With the aid of a sterile inoculating loop which was flamed by the Bunsen burner a colony was picked and then it was streaked on the solidified agar plate at an angle. The inoculating loop was then flamed again and the plate was streaked again at another angle till all sides of the plate was covered. Then it was incubated for 24 hours at 37˚C.

Preparation of slant

This was done by preparing a fresh agar in a flask which was firstly heated (emulsification) to dissolve the agar, 5ml was then poured into a sterile Bijou bottle and it was autoclaved at 121˚C for 15min after which it was left in a slant position cool. The pure cultured organism was then transferred to it and allowed to incubate at 37˚C for 24 h, growth can be observed on the surface of agar slant.

Biochemical test for identification of bacteria isolates

Gram staining

A smear of bacterial isolate was made on a clean grease free slide with the aid of a sterile wire loop (inoculating loop). The smear was made by the addition of a loop full of distilled water and a loop full or a pick of the bacteria isolate on a clean grease free slide which was then heat-fixed and stained with crystal violet solution for 60 seconds which is the primary stain. Then the excess stain was poured off and it was washed with water, Lugol's iodine (Grams iodine) was added an allowed to react for 30 seconds. Iodine was washed off with water and was decolourized with acetone or 70%

alcohol, it was then rinsed immediately with water and counter-stained with safranin solution for 60 seconds. Finally, the slide was washed with water to remove any excess stain and it was blotted-dry and observed under the microscope using ×100 objective lens with oil immersion [6]. Motility test, Catalyses , Indole , Methyl red and Urease test was also used for characterizing the isolates

Results

Table 1 presents the total bacteria loads by the different retailers. The bacterial load was the highest in A, followed by samples C, samples D and samples B. The range of the TVC was from 5.0 $\times 10^2$ to 2.5 $\times 10^5$ cfu/ml.

Retailer	TVC (cfu/ml)
A	5.0×10^2
B	1.0×10^4
C	2.5×10^5
D	2.0×10^4

Table 1: Total viable count (TVC) of pear *(D. edulis*) sold at Amai market.

The bacterial colonies were characterized and identified using standard techniques. A total of 10 predominant bacterial isolates were recovered and this was distributed in both species of *Escherichia coli* and *Staphylococcus aureus.*

S/N Isolates	G	Ca	Cos	M	Ci	Ox	M	V	Novobiocin sensitive	Ind	TSI	S	B	Gas	H_2S	OP
1	GNB	+		+	-	+	+	-		-	+	Y	Y	YES	NO	*Escherichia coli* *Staphylococcus aureus*
2	GPC	+	+						+							
3	GNB	+		+	-	+	+			-	+	Y	Y	YES	NO	*Escherichia coli* *Staphylococcus aureus* *Staphylococcus aureus*
4	GPC	+							+							
5	GPC	+	+						+							
6	GNB	+		+	-	+	+	-		-	+	Y	Y	YES	NO	*Escherichia coli* *Staphylococcus aureus* *Staphylococcus aureus* *Staphylococcus aureus*
7	GPC	+	+						+							
8	GPC	+	+						+							
9	GPC	+	+						+							
10	GNB	+		+	-	+	+	-		-	+	Y	Y	YES	NO	*Escherichia coli*

Table 2: Biochemical Test on Isolates Obtained.

Key: + = Positive - = Negative; GPB = Gram Positive Bacilli; GPC = Gram Positive Cocci; GNB = Gram Negative Bacilli; + = Positive; - = Negative; Ca = Catalase; Co = Coagulase; M = Methyl Red; V = Voges Proskauers; Ox = Oxidase Test; Ind = Indole Test; OP = Organisms Present.

Table 3 shows the distribution of the bacterial isolates. *Staphylococcus aureus* occur more frequent in all the sample than *Escherichia coli*. The percentage distribution of *Staphylococcus aureus* was 60%, while that for *Escherichia coli* was 40%.

Bacterial isolates	Number of isolates	Percentage
Escherichia coli	4	40%
Staphylococcus aureus	6	60%
Total	10	100%

Table 3: Distribution of bacterial isolates in bush pear (*D. edulis*) sold at Amai Market.

Discussion

Consumption of fresh fruit is increasing significantly in the recent days. Fruits usually do not receive any treatment, as a result, they are prone to be contaminated with foodborne and antibiotic-resistant bacteria that may cause public health hazards. In this study, the total bacteria load of *Dacryodes edulis* fruits sourced from various vendors within Amai central markets ranged from 5.0×10^2 to 2.5×10^5 cfu/ml. This load is relatively high when compared to previous report on microbial load of fruits according to Akter., *et al.* [7] which ranged from 10^2 to 10^7 cfu/ml. A total of 10 predominant bacterial isolates were recovered and this was distributed in both species of *Escherichia coli* and *Staphylococcus aureus.* The percentage distribution of *Staphylococcus aureus* was 60%, while that for *Escherichia coli* was 40%. The results in this study is in line with the previous results of Gultie and Sahile in [8]. The high frequency level of isolates in this study is also in conformity with the previous results of Duru., *et al*. [3] who isolated pathogens from Southern Nigeria with a high frequency level. The high levels of these pathogens in this study might be attributed to the poor hygiene of the vendor, using microbial unsafe container, poor handling practice, and unsanitary market condition. In another investigation carried out by Omonigho in [9] also conforms with the results of this study. Who suggested that inadequate storage and handling of the edible products had enormous range of bacteria isolates

Conclusion

The findings from this study suggest that African bush pear at local markets of Amai harbours pathogenic bacteria which makes it important for the implementation of proper hygienic measures during pre- and post-harvest stages of production and at the time of transportation, storage, selling, and consumption of safe guard public health.

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