Bacteriological Quality Assessment of *Malus domestica* Borkh and *Cucumis sativus* L. in Yenagoa Metropolis, Bayelsa state, Nigeria

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Abstract:

The study evaluated the bacteriological quality of apple (*Malus domestica* Borkh) and cucumber (*Cucumis sativus* L.) sold in some selected markets in Yenagoa metropolis, Bayelsa state, Nigeria. For each type of fruit/vegetable three samples were bought from each market. Bacteriological studies on the samples were carried out using standard microbiological procedure. Results showed that total heterotrophic bacteria and total coliform ranged from 3.640 - 5.584 Log cfu/g and 2.993 - 4.962 Log cfu/g respectively for cucumber and 43.873 - 5.382 Log cfu/g and 3.242 - 3.931 respectively for apple. Analysis of variance showed that there was no significance difference (P>0.05) among the various markets. The bacteria diversity in both fruit samples were *S. aureus, P. aeruginosa*, *E. coli, Enterobacter, Proteus* and *Bacillus* spp. *S. aureus.* Of the isolates, coliforms (*E. coli* and *Enterobacter*) were the most predominant microbial isolates for both fruits. This suggests that contamination of the fruits was mainly by handling processes. Therefore, with improve hygienic condition of vendors, the bacteria quality enhanced and the risk of food borne diseases could also be minimized.

Article Information:

1. Introduction

Food is a vital substance required by the body for the sustenance of life [1]. Most food materials are mainly from biological diversity including plants (fruits, vegetables, cereals, tuber, grains etc) and animals (including pisces, reptiles, mammals, etc). Eni *et al*. [2], Oranusi and Olorunfemi [3] described fruits and vegetables as extraordinary dietary sources of nutrients, micronutrients, vitamins and fibers which are essential for healthy growth and development. Typically, food can be classified into ready to eat food and foods that required processing before consumption. Ready to eat food are grouped into minimally, fully processed [4] and no processed food prior to consumption. Food that required no processing before consumption are mainly fruits such as apple, cucumber, carrot etc. Oranusi and Olorunfemi [3] described ready to eat fruits as fruits that are bought directly from vendors (in markets, streets and other public places such as schools, hospital, motor parks etc) and are eaten almost immediately without having to cut, peel, or rinse /wash.

However, with increased awareness about potential food borne diseases, most consumers of ready to eat fruits now prefer to wash/rinse the fruits prior to consumption. But significant number of people still consumes the fruits as processed/prepared by the vendor. Fruit vendors are found in several locations in Nigerian including urban and rural areas. The type of fruits that are consumed by individuals depend mainly on the locality and income of the families. For instance, in rural areas where vast land is available, fruits such as oranges, pears, banana, cucumber, carrot, guava, coconut are cultivated. This depends on the prevailing environmental conditions that encourage the growth of the fruits trees. In some areas, fruits are used them to beautify compounds. But to a large extent, most families that plant fruits and used it for beautification purposes mainly consume them with their household and sell little or none. However, some individuals plant fruits trees for commercial purposes.

In Nigeria, some fruits are predominantly cultivated in some region of the country probably due to the favourable environmental requirement of such plants, while several others do not thrive in Nigeria. For instance, cucumber (*Cucumis sativus* L.) are mainly cultivated in several region of Nigeria, unlike apple (*Malus domestica* Borkh) that is predominantly imported into Nigeria. These fruits get contaminated by microbes in unhygienic or unsanitary environment through damages that occur in the fruits during harvesting, transportation and even handling. Again, fruits can get contaminated through the use of contaminated water for washing/rinsing, poorly washed tray/basin/bowl/wheelbarrow in displaying fruits prior to selling. Microorganisms are said to be ubiquitous and therefore found in air, soil, water, foods and on humans [5]. Some microorganisms have the tendency to move with dust as propagules can deposit in fruits displayed for sales in busy public places especially during the dry season.

Most fruits are rich in sugar which makes them desirable to several microorganisms to thrive especially carbohydrate fermenters which produce alcohols, organic acids, aldehydes and other desirable and undesirable products. For instance, Oyewole and Isah [6] listed *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Penicillium, Saccharomyces* as microbes that cause fermentation in some locally fermented foods in Nigeria. The products that are produced depend mainly on the type of microorganism that causes fermentation in the fruits. During fermentation, the microbes cause spoilage of the fruits, thus making them loose their nutritional, and biochemical attributes.

Food borne diseases affects the gastrointestinal tract and can be transmitted through ingestion of contaminated foods and drinks [7]. Akhigbemidu et al. [8] described food as a chemically complex matrix that contains adequate nutrients to enhance microbial growth. Mahamud *et al*. [9] also noted that the occurrence of bacteria in vegetables/fruits is a source of potential health hazard to man and animals that consumes such product. In Nigeria, several types of ready-to-eat foods and flavored drinks are sold by street food vendors and consumed by millions of people [7]. However, microbial quality of apple (*Malus domestica* Borkh) and cucumber (*Cucumis sativus* L) have been variously reported from some retail outlets in Lagos [10], some market in Kaduna [9], some location in Sango Ota [2], Ota [3], Owerri [11]. Presently, there is little or no existing literature on the bacteriological quality of apple and cucumber in Yenagoa metropolis, Bayelsa state, Nigeria; the need for this study.

2. Materials and Methods

2.1. Field Sampling

Cucumber and apple displayed for sale was purchased from five markets (including Igbogene, Akenfa, Agudama-Epie, Tombia and Swali) in

Yenagoa metropolis, Bayelsa state. The sample were obtained from fifteen dealers for a particular fruit, three being for each markets. The fruits were packaged in sterile Ziploc bag and analysis was carried out approximately three hours after collection.

2.2. Sample Preparation

About 20 grams of the sample were blended (BLG-450, Binatone, Nigeria) in 180 ml of sterile water. The blender was washed and rinsed sterile and de-ionized water prior to re-use.

2.3. Enumeration of Microbial Counts

Nutrient Agar, MacConkey Agar, Salmonella-Shigella Agar were used to enumerate the total heterotrophic bacteria, total coliform and Salmonella-Shigella counts. Each media was prepared according to manufacturers instruction. The pour plate method previously described by Pepper and Gerba [12] and Benson [13] was used in the study. About 0.1 ml of the serial diluted samples was plated and incubated inverted at 37°C for 24-48 hours. Thereafter, the colonies that grew on each medium were counted and expressed as colony forming units (cfu)/g of the sample and the colonies were isolated into pure culture.

2.4. Identification of Microbial Diversity

The pure cultures from MacConkey agar colonies were streaked in Levine's eosin Methylene Blue (EMB) Agar and incubated at 37° C for 24 - 48 hours. The presence of small and large nucleated colonies with greenish metallic sheen indicates *E. coli* and *Enterobacter* sp respectively [12, 13]. The growth were also streaked in Blood Agar and presence of swarming properties indicate the presence of *Proteus* sp. Growth from Nutrient Agar was streaked in Mannitol Salt Agar and yellow pigmentation indicates the presence of *Staphylococcus aureus*. All the different pure culture were streaked in nutrient Agar from where the biochemical test (citrate, catalase, oxidase, Indole, coagulase, motility, methyl red) were carried out using the scheme of Cheesbrough [14] and Benson [13]. The resultant characteristics were compared with those of known taxa using scheme of Cheesbrough [14] and Bergey's Manual of Determinative Bacteriology by Holt *et al*. [15].

2.5. Statistical Analysis

SPSS software version was used to carry out the statistical analysis of the log transformed microbial counts. A one-way analysis of variance at $\alpha = 0.05$ was carried out and Duncan Multiple Range Test was used for multiple comparison.

3. Results and Discussion

Table 1 presents the bacteria population of cucumber sold in five markets of Yenagoa metropolis in Nigeria. The total heterotrophic and total coliform counts ranged from 3.640 - 5.584 Log cfu/g and 2.993 - 4.962 Log cfu/g respectively. Salmonella-Shigella counts were not detected in the cucumber samples (i.e. 0.00). There was no significance variation (P>0.05) among the markets.

Table 1: Bacteria population of cucumber sold in some major markets in Yenagoa metropolis, Bayelsa state

Markets	Total Heterotrophic Bacteria counts, Log cfu/g	Total coliform counts, Log cfu/g	Salmonella-Shigella counts, Log cfu/g
Igbogene	5.584±0.861a	4.500±0.434a	ND
Akenfa	3.640±0.750a	2.993±0.372a	ND
Agudama-Epie	5.515±0.383a	4.499±0.434a	ND
Tombia	5.126±0.810a	4.962±0.577a	ND
Swali	4.858±0.615a	4.621±0.600a	ND

Each value is expressed as mean ± standard error (n = 3); the same letters along the column is not significantly different at *P*>0.05 according to Duncan Statistics; ND= Not Detected

The bacteria density of apple samples sold in some market of Yenagoa metropolis is presented in Table 2. The values ranged from 3.873 - 5.382 Log cfu/g and 3.242 - 3.931 Log cfu/g for total heterotrophic and total coliform counts respectively, being not significantly different (P>0.05) among the markets. Salmonella-Shigella counts were not detected in the samples.

Table 2: Bacteria population of apple sold in some major markets in Yenagoa metropolis, Bayelsa state

Markets	Total Heterotrophic Bacteria counts, Log cfu/g	Total coliform counts, Log cfu/g	Salmonella-Shigella counts, Log cfu/g
Igbogene	4.659±0.476a	3.931±0.736a	ND
Akenfa	5.382±1.087a	3.276±0.998a	ND
Agudama-Epie	3.888±0.465a	3.694±0.416a	ND
Tombia	3.873±0.658a	3.242±0.525a	ND
Swali	4.206±0.920a	3.470±0.469a	ND

Each value is expressed as mean ± standard error (n = 3); the same letters along the column is not significantly different at P>0.05 according to Duncan Statistics; ND= Not Detected

Based on the results of this study, the high bacteria counts indicate contamination of both type of fruit/vegetable (cucumber and apple) samples by bacteria. Eni *et al*. [2] attributed high microbial counts in fruits and vegetables to storage conditions and how long these produce were kept before they were obtained for analysis. Similar trend could have happened in this present study. Again, bacteria in the samples could also have occurred through cross contamination by the microbes in the washing water and storage materials [2]. Oluwafemi *et al*. [5] have linked microbiological quality of foods to the quality of the water used by the vendors for the food preparation. The aerobic bacteria density was within the acceptable and tolerable limit of $\leq 10^3$ and 10^4 to 10^5 respectively as recommended by ICMSF [16] cited in Olopade *et al*. [17]. While the coliform more than <10 per gram of ready to eat vegetable as recommended by FAO [18] cited in Abdullahi and Abdulkareem [19]. However, the findings of this study had some similarity with previous study by Oluwafemi *et al*. [5], who reported total viable bacteria and total coliform of 2.0 x10^3 cfu/g and 3.0 x10^3 cfu/g respectively as the bacteria load found in cucumber sold in some Nigeria cities. But slightly different from the work of other authors including; Uzeh *et al*. [10] who reported 5.6 x10^6 cfu/g as bacteria density found in cucumber sold in some retail outlets in Lagos, Lagos state; Oranusi and Wesley [11] reported bacteria density of apple fruit sold in Owerri, Imo state in the range of 3.4 x10^5 – 4.5 x 10^7cfu/g and 2.2 x10^4 – 3.4 x 10^6 cfu/g respectively for total aerobic plate counts and total coliform; Abdullahi and Abdulkareem [19] reported total aerobic plate counts and total coliform of cucumber sold in retailer in Sagbon-gari, Zaira in the range of 1.3 x 10^5 - 1.9 x 10^6 cfu/g and 8.0 x 10^5 – 1.6 x 10^7 cfu/g respectively; Oranusi and Olorunfemi [3] reported total aerobic plate counts and total coliform of apple sold in Ota, Ogun state as 6.0 x10^4 –7.0 x 10^6 cfu/g and 2.2 x 10^5 - 3.4 x 10^6 cfu/g respectively. The variation in this study with some previous study could be due to handling processes. According to Oluwafemi *et al*. [5], sanitary handling of street foods varies significantly and may be contributory factor in samples with unacceptable microbial quality.

Apart from *Proteus* sp. which was only detected in Tombia markets, other bacteria including *S. aureus, P. aeruginosa*, *E. coli, Entrobacter* and *Bacillus* sp were detected in both vegetables (i.e. cucumber and apple) (Table 3). *S. aureus* was found in all the samples across the markets for both fruits. This trend was closely followed by *E. coli*. Both microbes representing the highest occurring bacteria found in both type of vegetable under study.

The bacteria diversity found in this study has some similarity with previous reports. Muhamud *et al*. [9] reported *S. aureus, Klebsiella* and *Citrobacter* spp. from cucumber sold in some markets in Kaduna, Kaduna state, Nigeria. Uzeh *et al*. [10] reported *P. aeruginosa, S. aureus* and *Proteus vulgaris* from cucumber sold in some retail outlets in Lagos, Lagos state. Abdullahi and Abdulkareem [19] reported *S. aureus, Bacillus* sp. and coliforms as bacterial diversity found in cucumber sold by retailers in Sagbon-gari, Zaira. Oranusi and Olorunfemi [3] reported *Klebsiella pneumonia, P. aeruginosa, B. subtilis, S. aureus* and *Bacillus* sp. as bacteria diversity found in apple sold in Ota Ogun state. Eni et al. [2] reported the

presence of *S. aureus* from cucumber and *S. aureus* and *Salmonella* sp. from apple samples sold in some locations in Sango-Ota, Ogun state. Oranusi and Wesley [11] reported *Klebsiella, Shigella, Bacillus, Staphylococcus, Pseudomonas, E. coli, Enterococcus, Salmonella, Micrococcus, Aeromonas, Proteus, Enterobacter* and *Aerococcus* spp. as bacteria isolates found in apple fruits sold in some locations in Owerri, Imo state.

Table 3: Bacteria isolates of cucumber and apple sold in Yenagoa metropolis, Bayelsa state, Nigeria

Microorganisms	Igbogene	Akenfa	Agudama-Epie	Tombia	Swali
Bacillus sp	(-)	(+)	(+)	(+)	(-)
	[+]	[-]	[-]	[+]	[+]
Staphylococcus aureus	(+)	(+)	(+)	(+)	(+)
	[+]	[+]	[+]	[+]	[+]
Pseudomonas aeruginosa	(-)	(+)	(-)	(+)	(+)
	[-]	[-]	[+]	[ ]	[-]
E. coli	(+)	(+)	(+)	(+)	(-)
	[-]	[+]	[+]	[+]	[+]
Proteus sp	(-)	(-)	(-)	(-)	(-)
	[-]	[-]	[-]	[+]	[-]
Entrobacter sp	(-)	(-)	(-)	(+)	(-)
	[-]	[-]	[-]	[+]	[-]

Note: Each of the bacteria occurred at least once in each of the three samples obtained for a particular vegetable in each market; - = absence; + = present; ( ) = cucumber [ ] = apple;

Some of these microbes identified in both cucumber apples could be linked to spoilage. The extent of spoilage depends mainly on the microorganisms involved [9]. The trend of *S. aureus* being the most prevalence found in both vegetables is in agreement with the work of Mahamud *et al*. [9] that reported that *S. aureus* has the tendency to proliferate and compete successfully in respect of the other microbes. Typically, *S. aureus* is found in the nasal passage, hands and skin of humans as normal flora [20]. Hence the high occurrence of this organism is highly associated with handling processes. The occurrence of *S. auerus*, is a pathogenic organism of public health importance because it causes food poisoning [2, 10, 21]. Some species of Bacillus can cause bacteremia/septicemia, endocarditis and respiratory tract [20, 21]. The occurrence of *P. aeruginosa* could be contamination from the environment. Uzeh *et al*. [10] reported that *Pseudomonas* spp. inhabitant of soil and water, and are responsible for angular leaf spot diseases of cucumber. Other bacteria could have entered the apples and cucumber from environmental materials including soil and water.

4. Conclusion

Fruits and vegetable constitute a significance part of human diets. This study evaluated the bacteriological quality of cucumber and apples sold in Yenagoa metropolis, Nigeria. The study found that both fruits are within the acceptable and tolerable limit of total aerobic bacteria for ready to eat foods. But higher than the limit for coliforms set by Food and Agricultural Organization. *S. aureus* and coliforms were the prevalent microbes found in both samples. Their occurrence could be linked to handling processes and contaminant from the environment including soil and water. Therefore, there is the need to practice improve hygienic condition in the handling, storing, distribution and marketing of this fruits i.e apples and cucumbers.

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