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ORIGINAL RESEARCH

Bacteriological Quality of Paw-Paw (*Carica papaya*) vended in Amassoma, Bayelsa State, Nigeria

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ABSTRACT
This study investigated the bacteria quality of paw-paw vended in Amassoma, Bayelsa state. Triplicate samples were collected from six locations. Standard bacteriological procedures were used for determining the bacteria isolates and population. Results showed that total heterotrophic bacteria counts, total coliform and total *Staphylococci* counts ranged from 0.57 – 6.22 x 10^4 cfu/g, 0.64 – 5.37 x10^2 cfu/g and 47 – 72 cfu/g. Analysis of variance showed that there were no significance differences (P>0.05) among the various locations for each of the parameters. The bacteria density was within the tolerable limits of the International Commission on Microbiological Specification for Foods which is 10^4cfu/g. Tentative bacteria identified included *Staphylococcus aureus, Escherichia coli, Enterobacter, Bacillus, Proteus, Pseudomonas, Streptococcus, Micrococcus* species. The percentage similarity between the various locations with respect to the bacteria isolates ranged from 44.44 – 85.71%. Among the isolates, *Staphylococcus aureus* and *Escherichia coli* had the highest occurrence frequency. This could be associated to handling processes. However, with improved hygiene, the bacteriological quality of the paw-paw could be improved.

KEY WORDS: *Food quality, Microbes, Paw-paw*

Introduction

Food is a vital substance required by all organisms for the sustenance of life, and its associated functions such as growth, development and maintenance of the body (Kigigha *et al*., 2017; Iweala *et al*., 2014; Izah *et al*., 2015a, 2016, 2017). Food materials are majorly from vegetation/plants (such as fruits, vegetables, cereals, tuber, grains etc.) and animals (Izah *et al*. 2016, 2017). Food provides essential nutrients required by the body for growth and development. Most food is sold in public places. According to Izah *et al*. (2016b), most food vended in public places does not require further processing prior to consumption. As such they are ready for consumption at selling points (Iwegbue *et al*., 2013). The patronage and consumption of ready-to-eat food has increased in recent time (Izah *et al*., 2015b, 2016).

Ready to eat food are of different class including beverages (Izah *et al*., 2017; Orutugu *et al*., 2015), food sold in restaurant and fruits. Basically fruits are a source of vitamins and essential minerals for human body. The type of fruits consumed depends on the locality. However, some fruits are consumed everywhere (including urban and rural areas) such as orange, pawpaw, water melon, pineapples, grape-fruit, carrots, cucumber, apple etc (Izah *et al*., 2015a, 2016). These fruits are extraordinary source of nutrients, micronutrients, vitamins and fiber essential for health and wellbeing of human (Oranusi and Olorunfemi, 2011).

Pawpaw (*Carica papaya*) is one of the major fruit consumed worldwide. Paw-paw is cultivated in tropical and subtropical

regions of the world. Till date, Nigeria is the second largest producer of paw-paw globally. Paw-paw has several varieties with different shape and color. According to Izah *et al*. (2015a), the shape, color of the endocarp can be used to distinguish some of the varieties. Some of the common noticeable color of the endocarp include red, orange, yellow etc. (Izah *et al*., 2015a). According to Adedeji and Oluwalana (2013), paw-paw contain vitamins (including A and C), and other nutrients including potassium, calcium and iron.

Beside mere consumption as food, paw-paw has other food related uses. For instance, it is used in the preparation of jam (Adedeji and Oluwalana, 2013). The paw-paw fruit, seed, leaves and part have medicinal uses. The uses vary from location to location and locality to locality. Some of the medicinal uses have been documented by Chukwuka *et al*. (2010), Anibijuwon and Udeze (2009), Afolayan (2003).

Probably due to health benefits of paw-paw including vitamins, minerals, weight control etc., it's consumed by wide range of people irrespective of their socioeconomic status or gender. The usual practice is that the paw-paw fruit is sold as whole or sliced and packaged in transparent polythene bag and sold at small bit. This trend is usually occurred in several part of the country (Izah *et al*., 2015a).

Fruits are known to harbor natural non-pathogenic microorganisms from their environment and or during deterioration. However, they could be contaminated via handling and packaging processes (Chukwu *et al*., 2010). International Commission of Microbiological Specification in Food has specified level of microbial contamination acceptable in food. But regulatory agencies seldom monitored the quality of this fruit which could be source foodborne disease (Chukwu *et al*., 2010) especially gastrointestinal tract disorder (Bello *et al*., 2014). This is because some of these fruits are processed in poor sanitary condition and processes lacking quality control. As such, this study aimed at investigating the bacteriological quality of paw-paw sold in Amassoma, Bayelsa state.

Materials and Methods

Field Sampling

Triplicate sample of sliced paw-paw were purchased from six different vendors at different location in Amassoma. As such a total of 18 samples were obtained. The samples were packaged in an ice box and sent to laboratory for analysis. Analysis were carried out <12 hours after sample collection

Sample preparation

The samples were prepared according to the method previously described by Izah *et al*. (2015a), Ineyougha *et al*. (2015), Kigigha *et al*. (2016, 2017). About 20g of the sample were blended in 180 ml of sterile water. Prior to re-use, the blender was washed with sterile water.

Enumeration bacteria counts

The bacteria density in the various samples was enumerated using pour plate method previously described by Pepper and Gerba (2005), Benson (2002). About 0.1 ml of the samples was aseptically plated in different media including Nutrient Agar Mannitol Salt Agar, MacConkey agar and Salmonella-Shigella Agar. The various media was prepared according to manufacturer's instruction. The agar plates were incubated at 37°C for 24-48 hours. After incubation, the colonies that grew on the various medium were counted and expressed as colony forming units (cfu)/g of the paw-paw samples.

Tentative identification of the bacteria isolates

Different colonies that grew on the MacConkey agar was streaked in Levine's eosin Methylene Blue (EMB) Agar and incubated at 37º C for 24 hours. The presence of small nucleated colonies with greenish metallic sheen indicates *E. coli*, while absence of the sheen with large nucleated colonies indicate *Enterobacter* sp. (Pepper and Gerba, 2005; Benson, 2002). Growth on the Nutrient Agar was streaked in Mannitol Salt Agar and incubated inverted at 37°C for 24 hours. The presence of yellow pigment indicates *Staphylococcus aureus*. Also, the bacterial pure culture were streaked in Blood Agar, the presence of hemolytic properties indicates *Streptococcus* species. While growth with swarming characteristics suggests *Proteus* species. Biochemical test including gram reaction, citrate, catalase, oxidase, Indole, coagulase, motility, methyl red were carried out using the scheme of Cheesbrough (2004) and Benson (2002). The resultant microbial species from biochemical test in this study was compared with those of known taxa using scheme of Cheesbrough (2004) and Bergey's Manual of Determinative Bacteriology by Holt *et al*. (1994).

Statistical Analysis

Statistical Package for Social Sciences software version 20 was used for the statistical analysis of the bacteria parameters. Descriptive statistics i.e. mean and standard error values were expressed. A one-way analysis of variance

was carried out at P = 0.05 and Tukey HSD Test was used for Post hoc. Sorenson qualitative index was used to determine the bacteria diversity similarity between samples from the different location at critical level of significance = 50% (Ogbeibu, 2005). The similarity chart was plotted using Microsoft excel.

Results and Discussion

Table 1 presents the bacteria density of paw-paw sold in Amassoma, Bayelsa state. The total heterotrophic bacteria counts, total coliform and total Staphylococci counts ranged from 0.57 – 6.22 x 10^4 cfu/g, 0.64 – 5.37 x10^2 cfu/g and 47 – 72 cfu/g. Typically there was no significance difference (P>0.05) among the various locations for each of the bacteria parameters. Salmonella-Shigella counts were not present in any of the samples.

No significance difference (P>0.05) in the various location for each of the bacteria population studied. This could be due to similarity in handling processes, hygienic level and processing methods (peeling and packaging) by the sellers or vendors (Izah *et al*., 2016; Kigigha *et al*., 2015a,b). The bacteria density of the paw-paw is within the acceptable and tolerable limits for total aerobic bacteria. This limits are ≤10^3 (acceptable), 10^4 to 10^5 (tolerable) as specified by International commission of Microbiological Specification in Food (ICMSF, 1996; Olopade *et al*., 2014; Kigigha *et al*., 2015a,b; Izah *et al*., 2016). But the occurrence of coliform suggests the presence of contaminants. Authors have variously reported allowable limits of 0.00 for coliforms in food. As such, the paw-paw falls short of quality based on microbiological specifications.

The occurrence of coliform could be due to handling processes, water used for washing the fruit prior to slicing, cellophane used for wrapping the sliced fruit. The knife used for slicing could also be a source of contaminants of the sliced paw-paw. The general environment especially soil could be the source of bacteria contaminants in the fruit.

Daniel *et al*. (2014) reported the microbial counts of sliced pawpaw packaged in polythene bags in sold Bida, Nigeria and report bacterial counts in the range of 3.2-3.7 x10^3cfu/g. Oranusi and Olorunfemi (2011) reported microbial density of sliced paw-paw fruits sold in Ota Ogun State, Nigeria in the range of 10^6, 10^5 to 10^6 cfu/g for total heterotrophic bacteria and total coliform respectively. Izah *et al*. (2015a) reported total heterotrophic bacteria and total coliform counts in pawpaw sample vended in Yenagoa metropolis in the range of 4.457 - 5.439 Log cfu/g and 3.124 - 4.140 Log cfu/g respectively. The slight variation with previous findings from this study could be attributed to hygienic level of fruit handlers, season of study, nature of the marketing environment etc. (Izah *et al*., 2015a).

The bacteria isolated from the paw-paw samples are presented in Table 2. The isolates include *Staphylococcus aureus*, *Escherichia coli, Enterobacter, Bacillus, Proteus, Pseudomonas, Streptococcus, Micrococcus* species. Figure 1 presents the bacteria diversity similarity between locations based on Sorenson qualitative index. The similarity interaction between the various locations with respect to bacteria diversity ranged from 44.44 – 85.71%. Most of the bacteria interaction at the samples from the different location were above the similarity critical level of significance = 50%. However, instance of dissimilarity were observed between Location A and Location B and Location B and F. The high significant interaction i.e. similarity of the bacteria isolates from the various location suggest similarity in handling processes and hygiene level of the processors.

The various bacteria tentatively identified in this study had some similarity with the work of previous authors. For instance, Izah *et al*. (2015a) reported the occurrence of *Staphylococcus aureus, Escherichia coli, Enterobacter aerogene Bacillus, Proteus* and *Streptococcus* species in paw-paw vended in Yenagoa metropolis, Bayelsa state. Chukwu *et al*. (2010) reported *Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Salmonella* species, *Proteus* species in pre-cut Pawpaw vended in Kano metropolis. Daniel *et al*. (2014) reported that *Escherichia coli, Staphylococcus* species and *Bacillus* species in paw-paw sold in Bida, Nigeria. Oranusi and Olorunfemi (2011) reported that *Bacillus subtilis, Klebsiella pneumonia, Escherichia coli* in paw-paw sold in Ota, Ogun state. Bello *et al*. (2014) reported *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* cereus*, Klebsiella, Enterobacter*, *Proteus*, *Streptococcus* paw-paw based locally produced juice sold in Ogun state, Nigeria.

Most studied have reported *E.coli* and *Staphylococcus aureus* as the predominant bacteria isolates found in paw-paw. The occurrence of these microbes in the fruits is a result of contamination during handling processes and sales

(Chukwu *et al*., 2010; Izah *et al*., 2015a).

The occurrence of *Escherichia coli* and *Enterobacter* species in the samples suggests the occurrence of fecal contamination in the paw-paw samples (Izah *et al*., 2015a). This may have occurred through the use of contaminated water in washing the fruits prior to slicing, improper washing of the fruit prior to cutting. Furthermore, *Staphylococcus aureus* is found in the nasal passage, hands and skin of humans as normal flora (Izah *et al*., 2015a).

As such they could have entered the fruit handling as well (Chukwu *et al*., 2010). The occurrence of *Pseudomonas* species at few instances could be from inadequate washing as well. As such, the bacteria would have entered the fruit from the soil. Typically, authors have attributed the presence of microbes in fruit to poor hygiene, handling, and use of contaminated materials in fruit preparation (Chukwu *et al*., 2010; Nwachukwu *et al*., 2008; Nwachukwu and Ezejiaku, 2014; Daniel *et al*., 2014; Izah *et al*., 2015a). Contamination could also occur during transportation and storage processes (Izah *et al*., 2015a).

Table 1: Bacteria density of Pawpaw vended in Amassoma, Bayelsa state, Nigeria

Location	Total Heterotrophic Bacteria, x 10^4 cfu/g	Total coliform, x10^2 cfu/g	Total Staphylococci counts, cfu/g	Salmonella-Shigella, Log cfu/g
A	4.09±0.89a	4.37±1.68ab	72.00±17.39a	ND
B	5.79±2.13a	4.65±2.23ab	59.00±14.18a	ND
C	4.07±2.43a	8.53±0.28b	70.00±12.50a	ND
D	0.57±0.23a	0.64±0.26a	56.33±10.73a	ND
E	3.19±1.31a	4.89±2.26ab	47.33±6.64a	ND
F	6.22±1.78a	5.37±0.75ab	55.00±9.54a	ND

Each value is expressed as mean ± standard error (n = 3); The same alphab*et al*ong the column is not significantly different at *P*>0.05 according to the Tukey HSD Statistics

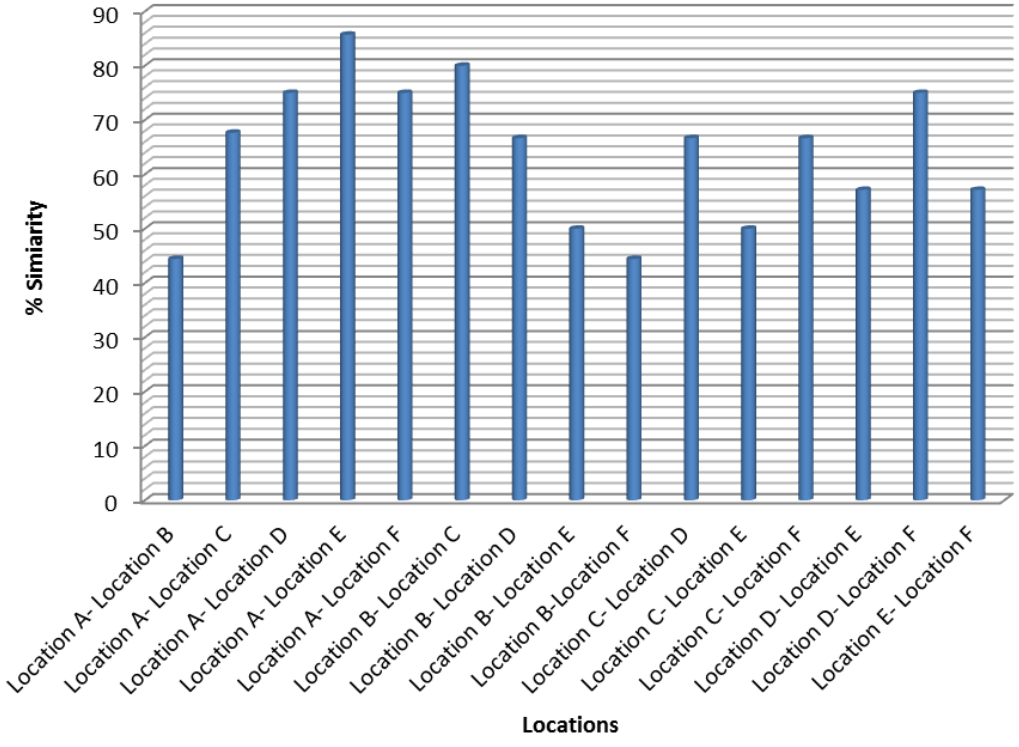

Figure 1: Similarity index of bacteria diversity found in the sliced paw-paw samples between each of the location in Amassoma

Table 2: Tentative bacteria isolates from paw-paw vended in Amassoma

Bacteria isolates	Locations					
	A	B	C	D	E	F
Escherichia coli	+	+	-	+	+	+
Enterobacter species	-	+	+	-	-	-
Streptococcus species	-	-	-	-	+	-
Bacillus species	+	-	+	+	-	+
Staphylococcus aureus	+	+	+	+	+	+
Micrococcus species	-	+	-	+	-	-
Proteus species	-	-	-	-	-	+
Pseudomonas species	-	+	+	-	-	-

Some isolates are found in one out the three replicate samples per location

Furthermore, most house flies are vector. Through inappropriate cover and packaging, flies could contaminate the fruit with all manners of materials including microbes in its environment via perching processes. In addition to flies, sometime cockroaches could also perch on the polythene used for the packaging of the fruit (Oranusi and Olorunfemi, 2011).

Conclusion

This study evaluated the bacteria quality of paw-paw vended in Amassoma. The study found that the total aerobic bacteria density is within tolerable level as recommended by International Commission of Microbiological Specification of Food. However, the occurrence of coliform in the fruits suggests contamination. Many of the bacteria tentatively identified are microbes of public health importance. Handling process and hygiene level of vendors could be major source of contamination.

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