**Study on the correlation between some physical and microbial quality characteristics of peeled sweet orange (*Citrus sinensis*)**


## Abstract

The consumption of ready-to-eat fruits such as oranges has increased in recent times. This study assessed the correlation between some physical and microbial quality characteristics of peeled sweet orange (*Citrus sinensis*) sold in Yenagoa, the capital of Bayelsa State, Nigeria. A total of thirty peeled oranges were purchased, three from each location. Standard microbiological methods were applied for the analysis. Results showed that the in-situ characteristics and microbiological load of peeled oranges sold in Yenagoa, Nigeria were the range of 1.86 to 3.15 Log CFU/g (total fungi), 1.56 to 2.86 Log CFU/g (total heterotrophic bacteria), 3.14 to – 4.19 (pH), 1.84 to 3.03 ppt (salinity), 3.27 to 5.00 mS/cm (conductivity) and 2.56 to 4.10 ppt (total dissolved solid). There was statistical deviation ($p<0.05$) for the in situ characteristics only. There was a positive significant association between the total dissolved solids and conductivity, and total heterotrophic bacteria and total fungi. Salinity negatively correlates with total heterotrophic bacteria and total fungi. This indicates that diverse factors affect the density and in-situ characteristics of the orange. The microbial isolates found in the samples include *Staphylococcus aureus, Escherichia coli, Entrobacter, Bacillus, Proteus, and Micrococcus species, as well as Aspergillus, Penicillium, Mucor, Fusarium, Saccharomyces cerevisiae*, and *Trichoderma* species. Some of the bacterial diversity is known to cause diseases and conditions when ingested. The majority of the microbial isolates found in this study were contaminants from the environment and therefore said to be opportunistic. However, with improved handling and hygiene levels among orange vendors, the microbial load could be reduced.


**Comment [1]:** This statemant does not match what has been carried out in research.

**Comment [2]:** The meaning of this statement is not clear.

**Comment [3]:** This statement is not the essence of the research.


**Keywords:** Fruits, Microbes, Orange, Public Health


## 1.0 Introduction

The growth, development, and maintenance of the body's important systems depend on the essential elements found in food for human survival [1,2]. There are several criteria used to classify food, but one of the most common one is based on how simple it is to eat [1]. The other food groups require processing before consumption, while foods that are ready to eat do not. In a number of public places, such as markets, streets, roads, and hospitals, ready-to-eat foods are sold for purchase. Consumption of ready-to-eat food has increased due to its portability and capacity to handle only a few simple domestic duties [1]. According to Izah et al. [3], ready-to-eat food consumption is on the rise due to its accessibility, affordability, and convenience.

The ready-to-eat foods are further grouped into two categories: uncooked foods such as fruits like Pawpaw (*Carica papaya*), Watermelon (*Citrullus lanatus*), Pineapple (*Ananas comosus*), Orange (Citrus species), Apple (*Malus domestica*), and Cucumber (*Cucumis sativus*), and cooked

foods such as nutritive food drinks like fruit juice and refined alcoholic and non-alcoholic beverages [3]. Most of these fruits are sold whole or sliced when fresh [1]. The increased consumption of these fruits could be due to their nutritional value. In recent times, so many fruits have been blended into fruit drinks or fruit juices and packaged in a more secure way. In addition, some fruits are also used as additives in the preparation of nutritive drinks. For instance, Nwachukwu et al. [4] reported that sugar cane, pineapple, and orange can be used to sweeten the sharp, sour taste of raw zobo (Hibiscus) drink extract. Agwa et al. [5] reported industrially made uses of orange to include Fanta Orange Drink, SoyGood, Chi Happy Hour, Chivita Premium, Bobo Orange, Caprisonne, FanDango Citrus, Yojus Orange Drink, Piko, and Jove Orange Drink. Typically, about 90% of Nigeria's total fruit is processed into fruit juice [6]. Also, Lateef et al. [7] stated that oranges account for about 90% of total fruit production in southern Nigeria.

Freshly sliced fruits are consumed in several parts of Nigeria, including urban and rural areas. Some fruits that are usually sold in sliced forms include pineapple, papaya, and watermelon. While other such as apple, cucumber and carrots are sold as whole fruits. Fruits such as oranges meant for ready consumption in street outlets and public places are usually sold with their rinds or peels removed using knives.

Generally, most ready-to-eat fruits sold are prone to microbial contamination, which occurs at several stages of processing, including handling, storage, and distribution [8,9]. This is probably due to the poor sanitary conditions in which these fruits are handled and displayed for sale [10]. The poor hygienic conditions could expose consumers to the risk of foodborne diseases. Some microbes such as *Escherichia coli, Staphylococcus aureus, Enterobacter, Streptococcus, Bacillus, Micrococcus, Proteus, Pseudomonas, Corynebacterium, Klebsiella, Salmonella* (bacteria), *Saccharomyces cerevisiae, Aspergillus, Penicillium, Mucor, Fusarium,* and *Rhizobacter* species (fungi) have been widely reported in ready-to-eat foods [1, 11-20]. Babalola et al. [21] also stated that *Salmonella breandercup, Salmonella enteritidis, Streptococcus pyogenes, Clostridium welchii, Clostridium botulinum, Vibrio parahaemolyticus,* and *Bacillus cereus* are common food poisoning bacteria. Some bacteria, most notably those from the Enterobacteriaceae family, are extremely toxic and can cause illness, especially in those with compromised immune systems.

In oranges, especially *Citrus sinensis,* the peels inhibit the infestation and growth of some microbes [8]. Though there is a natural opening in the rinds near the navel and stem-end vascular tissue where infiltration of solution is possible [8]. Orange is typically stored after harvest prior to sale. Polydera et al. [22] stated that during storage, orange juice undergoes a number of deteriorative reactions, resulting in quality degradation. The spoilage or decay could be due to their sugar contents. Several microbes are usually known to ferment sugary substrates. Some of the microbes that infiltrate the orange through the rind could cause spoilage, leading to a reduction in their shelf life. The vital factors affecting the spoilage of juices include pH, oxidation reduction potential, water activity, nutrients, antimicrobial effects, and competing microflora [23]. Of all these factors, pH and water activity are the two most prominent parameters affecting juice spoilage [23]. Therefore, the association between pH and some other in-situ characteristics such as conductivity, salinity, and total dissolved solid microbial density (total fungi and total heterotrophic bacteria counts) is important.

In research, correlational analysis is a statistical method used to identify the relationship between two variables and assess the strength of their linear relationship. Thus, it is used to measure the magnitude of change in one variable as a result of the change in the other. Therefore, it will be important to apply this tool to show whether a change in pH and other in-situ characteristics will statistically affect the microbial population.

Therefore, this study was designed to [1] assess the concentration of the in-situ characteristics of orange juice, [2] enumerate the density and identify the microorganisms found in oranges with rinds removed sold in public places in Yenagoa metropolis, Nigeria, and [3] show the association between the in-situ characteristics of orange juice and the microbial density of the orange. The findings of the study will help orange consumers and the general public.

## 2.0 Materials and Methods

### 2.1. Field Sampling

Freshly peeled orange samples were purchased from ten areas: Igbogene, Akenfa, Agudama-Epie, Edepie, Okutukutu, Opolo, Kpansia, Amarata, Onopa, and Swali in the Yenagoa metropolis, Nigeria. The orange was purchased from thirty fruit vendors, three from each sampling area. The oranges were packaged in sterile Ziploc bags and stored in an ice pack. Analysis was done approximately 3 hours after sample collection.

### 2.2 Sample preparation

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

### 2.3 Enumeration of microbial counts

Three media (Nutrient Agar, Sabouraud Dextrose Agar, and Salmonella-Shigella Agar) were used to enumerate the microbial population. The media was prepared according to the manufacturer's instructions. The pour-plate method previously described by Pepper and Gerba [24] and Benson [25] was used in the study. About 1 ml of the serially diluted peeled orange sample was plated in the media. The agar plates meant to enumerate total heterotrophic bacteria, Salmonella Shigella counts, and total fungi using Nutrient Agar, Salmonella-Shigella Agar, and Sabouraud Dextrose Agar were incubated inverted at room temperature for 24-48 hours and 3-5 days, respectively. The colonies that grew on the agar places were counted and expressed as colony forming units (CFU)/g of the peeled orange sample and the colonies were isolated into pure culture.

### 2.4 Identification of microbial diversity

The total heterotrophic bacteria isolates were identified based on biochemical tests (gram reaction, citrate, catalase, oxidase, Indole, coagulase, motility, methyl red, urease) using the guide of Cheesbrough [26] and Benson [25]. Thereafter, the resultant characteristics were compared with those of known taxa using the scheme of Cheesbrough [26] and Bergey's Manual of Determinative Bacteriology by Holt et al. [27]. In addition, the isolates that grew on the Nutrient Agar plates were streaked in Blood Agar, Mannitol Salt Agar, Levine's eosin Methylene Blue and incubated for 24 hours. The interpretations were done following the guide provided by Benson [25] and Cheesbrough [26].

Microscopic and macroscopic methods were employed for the identification of the fungi. The yeasts were identified following traditional microbiological methods based on their cultural, morphological, and physiological/biochemical characteristics (using carbon fermentation and assimilation tests, and growth based on temperature using glucose-peptone-yeast extract broth) as described by Kurtzman and Fell [28], APHA [29], and Benson [25], and used by Iwuagwu and Ugwuanyi [30], Okoduwa et al. [31], and Izah et al. [32-35].

For the moulds, the physical observation of the fungal isolates was compared with the guide provided by Benson [25], while the microscopic morphology was determined using Lactophenol cotton blue stain as described by Pepper and Gerba [24] and Benson [25] as applied by Izah and Ohimain [36]. The resultant microscopic characteristics for both mould and yeast were compared with the schemes of Pepper and Gerba [24], Ellis et al. [37], and Benson [25].

**Comment [4]:** This item is not shown in the result of research?

**Comment [5]:** The results of the determination with this methods are not visible in the result!

**Comment [6]:** The methods for determining pH, Salinity, total dissolves solid, conductivity are not explained in the method!, please explained!

### 2.5 Statistical Analysis

SPSS software version 20 was used to carry out the statistical analysis. The microbial density data was transformed to a logarithmic scale. The data was presented as mean ±standard. A one-way analysis of variance at P = 0.05 was carried out for all parameters (in-situ characteristics and microbial counts), and Waller Duncan test statistics were used to discern the source of the observed variations. Further, a box plot was carried out, showing the minimum, maximum, median, lower interquartile, upper interquartile, and interquartile range for each of the parameters. The Pearson correlation was used to show the degree of association between the parameters at p = 0.05 and p = 0.01.

## 3.0 Result and Discussion

Table 1 shows the in-situ characteristics and microbiological load of peeled oranges sold in Yenagoa, Nigeria. Total fungal counts ranged from 1.86 (at Kpansia) to 3.15 Log CFU/g (at Okutukutu). There were no significant differences at p=0.05. The box plot showed that samples from Agudama-Epie, Akenfa, Okutukutu, and Onopa have very high deviations however, the samples from Kpansia have a lower deviation from the median mean (Figure 1).

Total heterotrophic bacteria counts ranged from 1.56 (at Kpansia) to 2.86 Log CFU/g (at Onopa). There were significant variations (p=0.122). However, multiple comparisons showed that the deviation observed is a result of the values recorded in Kpansia, Swali, and Onopa. The box plot

showed that the values in the samples from Amarata and Okutukutu have higher and lower deviations from the median mean (Figure 1).

pH ranged from 3.14 (at Opolo) to 4.19 (at Agudama-Epie), being statistically different at $p<0.05$. The mean separation showed that the deviation observed is a result of the values recorded in Onopa, Okutukutu, and Agudama-Epie, as well as Opolo and Kpansia. The box plot showed that the values in the samples from Kpasia and Opolo have higher and lower deviations from the median mean (Figure 2).

Salinity ranged from 1.84 (at Opolo) to 3.03 (at Kpansia), being statistically different at $p<0.05$. Multiple comparisons showed that the statistical deviation observed is a result of the values recorded in Kpansia and Swali and Opolo and Amarata. The box plot showed that the values in the samples from Opolo and Okutukutu have higher and lower deviations from the median mean (Figure 2).

The conductivity ranged from 3.27 (at Agudama-Epie) to 5.00 mS/cm (at Swali). There were statistical variations at $p<0.05$. Multiple comparisons showed that the deviation observed is a result of the values recorded in Kpansia, Igbogene, Agudama-Epie, and Swali. The box plot showed that the values in the samples from Opolo and Edepie have higher and lower deviations from the median mean (Figure 2).

Total dissolved solid ranged from 2.56 (at Agudama-Epie) to 4.10 ppt (at Swali). There were statistical variations at $p<0.05$. Multiple comparisons showed that the deviation observed is a result of the values recorded in Onopa, Opolo, Agudama-Epie, and Swali. The box plot showed that the values in the samples from Opolo and Edepie have higher and lower deviations from the median mean (Figure 2).

Table 1: In-situ characteristics and Microbiological load of peeled orange sold in Yenagoa metropolis, Nigeria

| Locations | pH | Salinity, ppt | Total dissolved solid, ppt | Conductivity, mS/cm | Total Heterotrophic Bacteria, Log cfu/g | Total coliform, Log CFU/g | Samonella-Shigella, Log CFU/g |
|---|---|---|---|---|---|---|---|
| Akenfa | 3.90±0.10ab | 2.11±0.12abc | 3.21±0.15cd | 4.12±0.19cde | 2.52±0.10ab | 2.71±0.77a | ND |
| Igbogene | 3.49±0.19ab | 2.05±0.05abc | 3.01±0.11bc | 3.82±0.14bc | 2.05±0.47ab | 2.96±0.36a | ND |
| Agudama-Epie | 4.19±0.16b | 1.95±0.12ab | 2.56±0.13a | 3.27±0.17a | 2.23±0.55ab | 2.85±0.90a | ND |
| Edepie | 3.44±0.22ab | 2.33±0.06bc | 3.20±0.10bcd | 4.11±0.11cde | 2.55±0.13ab | 2.08±0.43a | ND |
| Okutukutu | 4.24±0.15b | 2.05±0.05abc | 3.10±0.33bc | 3.97±0.34cd | 2.47±0.03ab | 3.15±1.08a | ND |
| Opolo | 3.14±0.11a | 1.84±0.56a | 3.61±0.34d | 4.52±0.42ef | 2.18±0.53ab | 2.73±0.96a | ND |
| Kpansia | 3.17±1.04a | 3.03±0.22d | 2.79±0.18ab | 3.45±0.20ab | 1.56±0.05a | 1.86±0.13a | ND |
| Amarata | 3.56±0.39ab | 2.46±0.27c | 3.35±0.13cd | 4.14±0.16cde | 2.34±1.13ab | 2.16±0.53a | ND |
| Onopa | 4.07±0.25b | 1.88±0.28ab | 3.56±0.22d | 4.34±0.26de | 2.86±0.64b | 2.52±0.49a | ND |
| Swali | 3.80±0.60ab | 2.98±0.14d | 4.10±0.25e | 5.00±0.30f | 1.70±0.25a | 2.09±0.32a | ND |

The same alphabet along the column indicates not significantly different at $P>0.05$ according to the Waller-Duncan test statistics; Each value is expressed as mean ± standard deviation (n =3); ND= Not Detected.

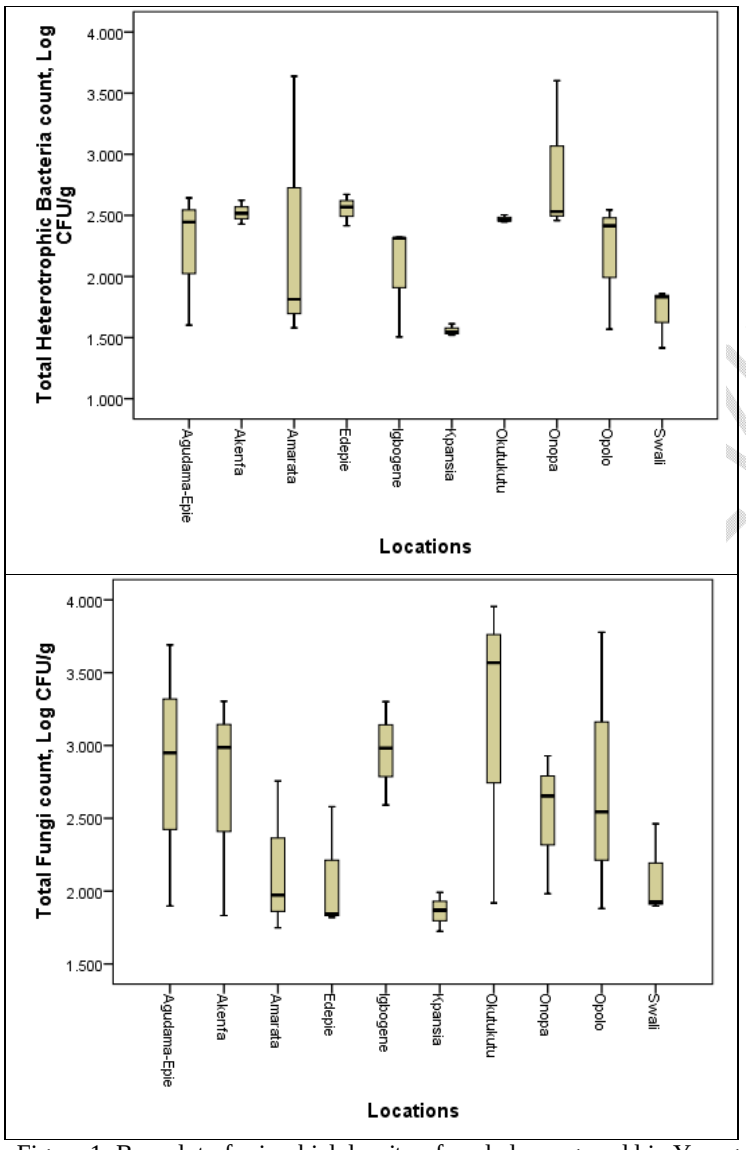


Figure 1: Box plot of microbial density of peeled orange sold in Yenagoa metropolis, Nigeria

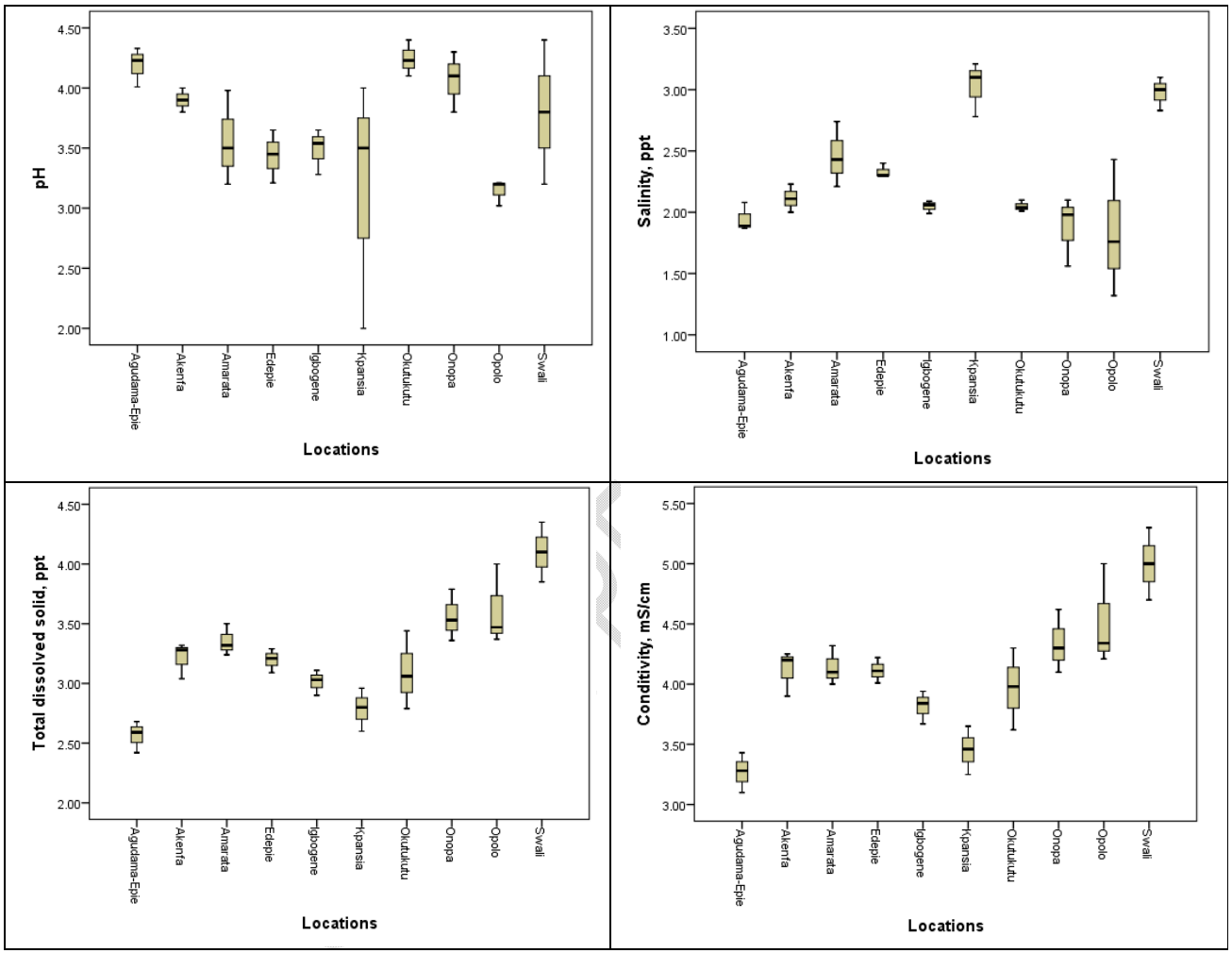


Figure 2: Box plot of the basic in-situ characteristics of peeled orange sold in Yenagoa metropolis, Nigeria

Salmonella-Shigella counts were not found in any of the samples. Furthermore, lack of statistical variation in the density of total heterotrophic bacteria and total fungi counts could be attributed to similar peeling and marketing strategies employed by fruit vendors in the study area. Most of the vendors peel the oranges with knife and slice it open upon request by customers. The peeled oranges are often displayed in trays which lack covering. Sometimes, the peeled oranges are displayed in the sun, which makes them dry. The findings of this study are comparable to previous reports on some fruit juices. Odu and Adeniji [6] reported the microbial counts of packaged orange juice sold in Port Harcourt, Nigeria, in the range of 0.35–7.1 x $10^3$cfu/mL (total heterotrophic bacteria) and 1.5–2.5 x $10^2$ CFU/mL (total fungi). Agwa et al. [5] reported the microbial population from orange juices processed industrially (Fanta Orange Drink, SoyGood, Chi Happy Hour, Chivita Premium, Bobo Orange, Caprisonne, FanDango Citrus, Yojus Orange Drink, Piko, Jove Orange Drink) and locally sold in Port Harcourt Metropolis, Nigeria, ranged from 1.0–4.0 x $10^3$ CFU/ml and 1.1–5.0 x $10^5$ CFU/ml, respectively (total heterotrophic bacteria) and 1.0 – 6.0 x $10^2$ CFU/ml and 1.0 – 7.0 x $10^2$ CFU/ml, respectively (total fungi). The population of microbes found in this study is slightly lower than the result from orange juices previously reported. Lateef et al. [7] reported the microbial load of orange juices produced locally in Ogbomoso, Nigeria, in the range of 3.5 x $10^4$ – 2.15 x $10^5$ CFU/ml (bacterial) and 7.5 x $10^4$ – 1.25 x $10^5$ CFU/ml (yeasts). Generally, the microbial population reported from orange fruit juice is within the range of $10^2$-$10^5$ CFU/mL, as reported by Lateef et al. [7].

Generally, the low population of microbes, especially the bacteria found in the freshly peeled orange, may be due to the pH, which is acidic (Table 1). Low pH (i.e., acidic) could inhibit the growth of pathogenic bacteria [23-39] and encourage the growth of lactic acid bacteria and acid-tolerant molds and yeasts. Furthermore, the pH recorded in this study is within the range 3.0-4.5 that has been reported in different fruit juices [23-39].

Conductivity is one of the parameters used to show early changes in a medium. Conductivity and total dissolved solids are in-situ quality parameters that are commonly used to describe salinity levels [40]. The two parameters are often correlated. Total dissolved solids provide information about all dissolved inorganic and organic substances in a medium, which can be ionized, colloidal, etc. The statistical deviation observed across the different locations suggests variation in the in-situ characteristics of the orange. These differences could be due to the ripeness of the orange and the level of deterioration in biochemical compositions. These parameters are highly unstable and can be influenced by quite a number of conditions, including temperature. Similar trends have been reported in the fermentation media of maize for ogi production [41] cassava for the production of fufu [42].

Table 2 shows the Pearson Correlation of some in-situ and microbial characteristics in peeled oranges sold in the Yenagoa metropolis, Nigeria. Salinity showed a strong negative and significant ($p<0.05$) association with total heterotrophic bacteria and total fungi. Total dissolved solids showed a very strong positive statistical correlation ($p<0.01$) with conductivity, an indication that they are influenced by similar conditions. Generally, total dissolved solids provide information about the inorganic salts and small amounts of organic matter in a medium, while conductivity shows the ability of the orange juice to conduct electrical current. Total

heterotrophic bacteria count strongly correlates ($p<0.05$) with total fungi, an indication that they are influenced by similar conditions. The correlation between total fungi and total heterotrophic bacteria is evident. The negative shows that they are from different sources. This is because high salinity is supposed to prolong the lag time and reduce the microbial communities, but due to the fact that many of the microbes are opportunistic pathogens and may have entered the fruit due to handling, therefore, the density is a function of the hygienic condition of the vendors as well as the status of the peeled oranges prior to purchase. Generally, studies have shown that parameters that correlate are from similar sources [43-52]. Therefore, diverse parameters are influence the salinity, total heterotrophic bacteria, and total fungi.

Table 2: Pearson Correlation of some physical and microbial characteristics in peeled orange sold in Yenagoa metropolis, Nigeria

| Parameters | pH | Salinity | Total dissolved solid | Conductivity | Total heterotrophic bacteria | Total fungi |
|---|---|---|---|---|---|---|
| pH | 1 | | | | | |
| Salinity | -.238 | 1 | | | | |
| Total dissolved solid | -.030 | .119 | 1 | | | |
| Conductivity | -.019 | .074 | .991** | 1 | | |
| Total heterotrophic bacteria | .302 | -.363* | .055 | .089 | 1 | |
| Total fungi | .183 | -.444* | -.082 | -.050 | .379* | 1 |

*. Strong statistical association at the 0.05 level; **. Very Strong statistical association at the 0.01 level

.

Table 3 presents the microbial isolates of freshly peeled oranges sold in Yenagoa, Nigeria. The bacterial diversity includes *Staphylococcus aureus, Escherichia coli, Entrobacter, Bacillus, Proteus* and *Micrococcus* species, while the fungal isolates are *Aspergillus, Penicillium, Mucor, Fuarium Saccharomyces,* and *Trichoderma* species. The similarity in the microbial diversity in this study could be associated to similar handling procedures practiced by the fruit vendors and same life style and climatic conditions. The microbial isolate is similar to a previous study from orange juice. Lateef et al. [7] isolated *Saccharomyces cerevisiae, Bacillus cereus, B. subtilis, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Micrococcus,* and *Rhodotorula* species from locally processed pasteurized orange fruit juices sold in Ogbomoso, Southwest Nigeria. Agwa et al. [5] reported *Bacillus, Micrococcus, Staphylococcus, Enterococcus* species, *Escherichia coli* (bacteria), *Aspergillus, Penicillium, Trichoderma* species, and *Saccharomyces cerevisiae* from industrially and locally processed orange juice sold in Port Harcourt, Nigeria. Bello et al. [39] reported *S. aureus, Klebsiella, Salmonella* species, *Aspergillus niger, and Saccharomyces cerevisiae* as microbial contaminants found in orange juice sold in Sagamu, Ogun state, Nigeria. The occurrence of these fungi and *Lactobacillus*

species in the orange could be attributed to the acidic conditions, which enhance their growth. Some species of Lactobacillus are beneficial to human health; hence, they can be classified as probiotics. Again, *Micrococcus*, and *Bacillus* species cause major spoilage in citrus products [6]. Basically, lactic acid bacteria such as lactobacillus counts are responsible for the sour taste of orange that is undergoing spoilage. Hence, the population of lactobacillus species in this study could depend on how ripe the oranges are prior to sampling. The presence of bacteria such as *Staphylococcus aureus, Escherichia coli,* and *Streptococcus* species could be associated with contamination from handling processes, such as the water used in washing oranges prior to peeling. Sometimes, fruit vendors sell more than one type of fruit and use the same water for washing, which could lead to cross-contamination. This is because microbes such as *Staphylococcus aureus* hardly thrive under acidic conditions, but yet they are isolated from the peeled oranges, hence their presence could be from cross contamination. *E. coli* was found in all the orange samples. Aneja et al. [23] stated that *E. coli* can survive in the acidic conditions of fruit juices due to the acid stress response. The species of bacteria found in the peeled oranges could cause foodborne diseases. For instance, Lateef et al. [7] stated that *B. subtilis* and *E. coli* could produce heat stable enterotoxins, which are responsible for diarrhea and food poisoning. Basically, fungi such as *Penicillium* and *Aspergillus* species produce toxins in food products. Dubey and Maheshwari [53] stated that *Aspergillus* species produce aflatoxins and ochratoxin B, and *Penicillium* species produce citrinin and cyclopiazonic acid. Generally, this fungus rarely causes disease conditions except for individuals with immunocompromised conditions. The potential diseases cause by these fungi that produces toxin includes Aspergillosis (*Aspergillus* species) and hyalohyphamycosis (*Penicillium* species). The infections from these fungi are classified as opportunistic systemic mycoses. Hence, the occurrence of these pathogens in oranges, especially the bacteria, is a source of public health concern.

Table 3: Microbial isolates from peeled orange sold in Yenagoa Metropolis, Nigeria

| Locations | Microbial isolates | |
|---|---|---|
| Akenfa | Bacteria | Fungi |
| Igbogene | *Staphylococcus aureus*, *Escherichia coli*, *Bacillus*, and *Micrococcus* species | *Saccharomyces Aspergillus* and *Trichoderma* species |
| Agudama-Epie | *Staphylococcus aureus*, and *Bacillus* species | *Saccharomyces*, *Aspergillus* and *Penicillium* species |
| Edepie | *Staphylococcus aureus* and *Escherichia coli* | *Aspergillus* and *Saccharomyces* species |
| Okutukutu | *Staphylococcus aureus*, *Entrobacter* and *Bacillus* species | *Aspergillus, Fuarium* and *Penicillium* species |
| Opolo | *Staphylococcus aureus*, *Escherichia coli* | *Aspergillus* and *Saccharomyces* species |
| Kpansia | *Staphylococcus aureus*, *Bacillus* and *Micrococcus* species | *Aspergillus, Penicillium* and *Saccharomyces* species |
| Amarata | *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* species | *Aspergillus, Saccharomyces* and *Mucor* species |
| Onopa | *Staphylococcus aureus*, *Escherichia coli*, | *Saccharomyces* and |

| | | |
|---|---|---|
| | *Proteus* species | *Trichoderma* species |
| Swali | *Staphylococcus aureus, Escherichia coli, Bacillus,* and *Micrococcus* species | *Aspergillus, Penicillium* and *Saccharomyces* species |

The microbes listed in each location were identified in at least of the triplicate samples of the orange

## 4.0 Conclusion

Fruits are sources of vitamins and minerals. This study evaluated the correlation between some physical and microbial densities of peeled sweet oranges sold in Yenagoa, Bayelsa State, Nigeria. Conductivity, total fungi, and total heterotrophic bacteria displayed a positive, statistically significant association. Salinity and total heterotrophic bacteria and total fungi populations are inversely correlated. This demonstrates how a number of variables affect the density and in-situ characteristics of the orange. The microbial load was in the range of $10^1$–$10^3$ CFU/g depending on the type of microbes, i.e., total heterotrophic bacteria and total fungi. The occurrence of most bacterial isolates indicates contamination from the environment, probably due to poor handling and hygienic conditions. Most of the microbes identified are causative agents of common human diseases. With improved hygienic conditions and good packaging, the number of microbial isolates could be reduced. The use of clean water for the washing of oranges prior to peeling and the use of show glasses for displaying oranges during sale or distribution could minimize contamination. Also, frequent hand washing by orange vendors could reduce microbial contamination.

**Comment [7]:** This statemant does not match what has been carried out in research.

**Comment [8]:** This is not an object study in this reseach. So it needs to be eleminated.

## References


1. Izah SC, Aseiba ER and Orutugu LA (2015). Microbial quality of polythene packaged sliced fruits sold in major markets of Yenagoa Metropolis, Nigeria. Point Journal of Botany and Microbiology Research, 1(3): 30 – 36.
2. Izah, S.C., Inyang, I.R., Angaye, T.C.N. and Okowa, IP (2017). A review of heavy metal concentration and potential health implications in beverages consumed in Nigeria. Toxics, 5 (1): 1-15. doi:10.3390/toxics5010001.
3. Izah SC, Etebu EN, Aigberua AO, Odubo TC, Iniamagha I (2022). A meta-analysis of microbial contaminants in selected ready-to-eat foods in Bayelsa State, Nigeria: Public Health implications and risk-reduction strategies. Hygiene and Environmental Health Advances. 4:100017. https://doi.org/10.1016/j.heha.2022.100017.
4. Nwachukwu, E., Onovo, O.M. and Ezeama, C.F. (2007). Effect of lime juice on the bacterial quality of Zobo drinks locally produced in Nigeria. Research Journal of Microbiology, 2(10): 787 – 791.
5. Agwa, O.K., Ossai-Chidi, L.N. and Ezeani, C.A. (2014). Microbial Evaluation of Orange Fruit Juice Sold in Port Harcourt, Nigeria. *American Journal of Food Science and Nutrition Research*, 1(5): 28-33.
6. Odu, N.N. and Adeniji, A. O. (2013). Microbiological analysis of some packed fruit juices sold in Port Harcourt Metropolis, Nigeria. *Nature and Science*, 11(4): 30 –40.

7. Lateef, A., Oloke, J.K., and Gueguim-Kana, E.B. (2004). Antimicrobial resistance of bacterial strains isolated from orange juice products. African Journal of Biotechnology, 3 (6):334-338.
8. Allafi, A. and Busamri, S. (2011). Microbiology of fresh oranges after storage at room and refrigeration temperature conditions. International Journal of Food Safety, Nutrition, Public Health and Technology, 3(1): 1 – 3.
9. Daniel, A.A., Danfulani, S., Barnabas, B.B., Peter, G. and Ajewole, A.E. (2014). Microbiological quality of sliced fresh fruits sold in Bida, Nigeria. Global Journal of Biology, Agriculture and Health Science, 3(3): 178 – 180.
10. Nwachukwu, E., Ezeama, E.F. and Ezeanya, B.N. (2008). Microbiology of polyethylene-packaged sliced watermelon (*Citrullus lanatus*) sold by street vendors in Nigeria. African Journal of Microbiology Research, 2: 192-195.
11. Izah, SC, Kigigha LT, Anene EK (2016). Bacteriological Quality Assessment of *Malus domestica* Borkh and *Cucumis sativus* L. in Yenagoa Metropolis, Bayelsa state, Nigeria. British Journal of Applied Research 01(02), 05-07.
12. Kigigha LT, Izah SC and Kpea TB (2015). Microbiological quality of fermented *Cassava Flakes* (Gari) sold in Yenagoa, Metropolis, Nigeria. *Bulletin of Advanced Scientific Research* 01 [07]: 157 – 160
13. Kigigha LT, Ovunda HO and Izah SC (2015). Microbiological quality assessment of suya sold in Yenagoa Metropolis, Nigeria. Journal of Advances in Biological and Basic Research 01[05]: 106 – 109
14. Kigigha LT, Samson GA, Izah SC, Aseibai ER (2018) Microbial Assessment of Zobo Drink Sold in Some Locations in Yenagoa Metropolis, Nigeria. EC Nutrition 13(7): 470-476.
15. Kigigha, L. T., Opusunju, I.C. and Izah, S.C. (2017). Assessment of Bacteriological Quality of Puff-Puff Sold in Amassoma, Bayelsa State, Nigeria. Bulletin of Trends in Biological Sciences, 1(1): 18 -22.
16. Kigigha, L.T., Berezi, J. and Izah, S.C. (2017). Bacteriological Quality Assessment of Meat Pie Sold in Yenagoa Metropolis, Nigeria. EC Nutrition, 6(6): 189-195.
17. Kigigha, L.T., Igoya, U.O.S. and Izah, S.C. (2016). Microbiological Quality Assessment Of Unpeeled Groundnut Sold in Yenagoa Metropolis, Nigeria. International Journal of Innovative Biochemistry & Microbiology Research, 4(4):11- 22.
18. Seiyaboh EI, Izah SC (2020). Assessment of Microbial Characteristics of Processed Palm Weevil "*Rhynchophorus phoenicis*" Larvae Sold in some Market Areas in Bayelsa State, Nigeria. *Journal of Advanced Research in Medical Science & Technology*; 7(1): 24-29.
19. Orutugu LA, Izah SC. and Aseibai (2015). Microbiological quality of Kunu drink sold in some major markets of Yenagoa Metropolis, Nigeria. Continental Journal of Biomedical Science. 9(1): 9 – 16.
20. Ineyougha ER, Orutugu LA and Izah SC. (2015). Assessment of Microbial Quality of Smoked *Trachurus trachurus* sold in some Markets of Three South-South States of Nigeria. International Journal of Food Research, 2: 16 – 23.

21. Babalola OO, Fagade OE, and Gopane RE. 2011. Microbiological quality control study of some processed fruit juices by conventional approach. Life Science Journal. 8(S2):18-24.
22. Polydera, C., Stoforosb, N.G. and Taouki, P.S. (2005). Quality degradation kinetics of pasteurised and high pressure processed fresh Navel orange juice: Nutritional parameters and shelf life. Innovative Food Science and Emerging Technologies 6: 1 – 9.
23. Aneja, K.R., Dhiman, R., Aggarwal, N.K, Kumar, V. and Kaur, M. (2014). Microbes Associated with Freshly Prepared Juices of Citrus and Carrots. International Journal of Food Science, http://dx.doi.org/10.1155/2014/408085.
24. Pepper, I.L. and Gerba, C.P. (2005). Environmental microbiology. A laboratory manual. Second edition. Elsevier academic press.
25. Benson H.J (2002) *Microbiological Applications:* Laboratory Manual in General Microbiology. complete version, 5th edition. McGaraw-Hill, New York.
26. Cheesbrough, M. (2004). *District Laboratory Practice in Tropical Countries.* Low price Edition part 2. Cambridge press, England.
27. Holt, J.G., Kneg, N.R., Sneath, P.H.A., Stanley, J.T. and Williams, S.T. (1994). *Bergey's Manual of Determinative Bacteriology*. William and Wilkins Publisher, Maryland. New York.
28. Kurtzman CP, Fell JW (1998). *The Yeasts: A Taxonomic Study*. 4th edition, Elsevier Science, Amsterdam, The Netherlands.
29. APHA (American Public Health Association) (2006). Standard Methods for the Examination of Water and Wastewater, 22nd ed.; American Public Health Association: Washington, DC, USA.
30. Iwuagwu JO, Ugwuanyi J O (2014). Treatment and Valorization of Palm Oil Mill Effluent through Production of Food Grade Yeast Biomass. Journal of Waste Management, http://dx.doi.org/10.1155/2014/439071.
31. Okoduwa SIR, Igiri B, Udeh CB, Edenta C and Gauje B (2017). Tannery Effluent Treatment by Yeast Species Isolates from Watermelon. Toxics 5, 6; doi:10.3390/toxics5010006.
32. Izah SC, Bassey SE, Ohimain EI (2017) Removal of Heavy Metals in Cassava Mill Effluents with Saccharomyces cerevisiae isolated from Palm Wine. MOJ Toxicology, 3(4): 00057.
33. Izah S.C., Bassey S.E., and Ohimain E.I., 2017. Assessment of Some Selected Heavy Metals in Saccharomyces cerevisiae Biomass Produced from Cassava Mill Effluents". EC Microbiology 12(5): 213-223.
34. Izah S.C., Bassey S.E., and Ohimain E.I., 2017. Changes in the treatment of some physico-chemical properties of cassava mill effluents using Saccharomyces cerevisiae, Toxic, *5*(4), 28; doi:10.3390/toxics5040028. PMID:29051460.
35. Izah S.C., Bassey S.E., and Ohimain E.I., 2017. Assessment of heavy metal in cassava mill effluent contaminated soil in a rural community in the Niger Delta region of Nigeria. EC Pharmacology and Toxicology, 4(5): 186-201.

36. Izah SC, Ohimain EI. (2013). Microbiological quality of crude palm oil produced by smallholder processors in the Niger Delta, Nigeria. Journal of Microbiology and Biotechnology Research, 3(2): 30 – 36.
37. Ellis, D., Davis, S., Alexiou, H., Handke, R., Bartley, R. (2007). Descriptions of Medical Fungi. Second Edition. Printed in Adelaide by Nexus Print Solutions, Underdale, South Australia.
38. Uzeh RE, Alade FA, Bankole M (2009). The microbial quality of prepacked mixed vegetables salad in some retail outlets in Lagos, Nigeria. African Journal of Food Science, 3(9): 270-272.
39. Bello, O.O., Bello, T.K., Fashola, M.O. and Oluwadun, A. (2014). Microbiological quality of some locally-produced fruit juices in Ogun State, South western Nigeria. E3 Journal of Microbiology Research, 2(1): 001-008.
40. Rusydi AF (2018). Correlation between conductivity and total dissolved solid in various type of water: a review. IOP Conf. Series: Earth and Environmental Science 118: 012019 doi :10.1088/1755-1315/118/1/012019
41. Izah, SC, Kigigha LT, Okowa IP (2016). Microbial quality assessment of fermented maize *Ogi* (a cereal product) and options for overcoming constraints in production. Biotechnological Research, 2(2): 81-93.
42. Izah SC (2018). Variations in microbial density and in-situ water quality characteristics of cassava fermentation medium for fufu production. MOJ Toxicology, 4(6):386–389.
43. Okowa IP, Kigigha LT, Izah, SC (2016). Variation in physicochemical water quality parameters during fermentation of maize for *Ogi* production. Biotechnological Research, 2(3): 125-131.
44. Aghoghovwia O.A., Umoru O.D., and Izah S.C., 2018, Physicochemical characteristics of nun river at Gbarantoru and Tombia Axis in Bayelsa State, Nigeria, Bioscience Methods, 9(1): 1-11
45. Aghoghovwia, O.A., Miri, F.A. and Izah, S.C. 2018. Impacts of Anthropogenic Activities on Heavy Metal Levels in Surface Water of Nun River around Gbarantoru and Tombia Towns, Bayelsa State, Nigeria. Annals of Ecology and Environmental Science, 2(2): 1 – 8.
46. Izah SC, Aigberua AO, Ogwu MO (2022). Trace element composition of Gallus gallus domesticus eggs and health risks associated with their consumption in Port Harcourt, Nigeria. Journal of Food Safety and Hygiene, 8(3): 202-222
47. Izah SC, Aigberua AO, Richard G. (2022). Concentration, Source, and Health Risk of Trace Metals in Some Liquid Herbal Medicine Sold in Nigeria. Biological Trace Element Research, 200, 3009–302.
48. Izah SC, Uzoekwe SA, Aigberua AO (2021). Source, geochemical spreading and risks of trace metals in particulate matter 2.5 within a gas flaring area in Bayelsa State, Nigeria. Advances in Environmental Technology, 7(2): 101-118
49. Izah S.C., Bassey S.E., and Ohimain E.I., 2017. Assessment of heavy metal in cassava mill effluent contaminated soil in a rural community in the Niger Delta region of Nigeria. EC Pharmacology and Toxicology, 4(5): 186-201.
50. Aigberua AO, Izah SC, Richard G. (2021). Hazard Analysis of Trace Metals in Muscle of *Sarotherodon melanotheron* and *Chrysichthys nigrodigitatus* from Okulu River, Rivers

State, Nigeria. Journal of Environmental Health and Sustainable Development. 6(3): 1340-1356.

51. Ogamba EN, Charles EE, Izah SC (2021) Distributions, pollution evaluation and health risk of selected heavy metal in surface water of Taylor creek, Bayelsa State, Nigeria. Toxicology and Environmental Health Sciences, 13(2): 109 – 121. DOI: 10.1007/s13530-020-00076-0.
52. Uzoekwe SA, Izah SC, Aigberua AO (2021) Environmental and human health risk of heavy metals in atmospheric particulate matter ($PM_{10}$) around gas flaring vicinity in Bayelsa State, Nigeria. Toxicol Environ Health Sci. 13(4), 323-335 https://doi.org/10.1007/s13530-021-00085-7.
53. Dubey, R.C. and Maheshwari, D.K. (2013). A textbook of Microbiology. 2013 Revised edition. S. Chad and Company LTD. Ram Nagar, New Delhi.