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Microbial Contamination of Peeled Oranges Sold Along Roadsides in Rumuolumeni, Port Harcourt, Rivers State, Nigeria

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Abstract

Microbial contaminations of the peeled oranges for sale were investigated vis-à-vis the microbial air quality at the points of sale. Sixty (60) oranges were purchased from high, low and less vehicular /human traffic sales points and analyzed using standard microbiological procedures. Analysis involved the spread plate and plate exposure techniques, Results of the analysis showed that the mean total microbial (bacteria and fungi) load on the surface of peeled oranges at the points of sale were: 1.0 x 10 4 , 1.4 x 10 4 , 1.4 x 10 4 cfu/ml for less, low, and high human and vehicular movement points of sales respectively, thus shows no significant difference existed at P>0.05. The counts for Escherichia coli, Staphylococcus aureus, Salmonella and heterotrophic bacteria on the peeled orange surfaces were; 1.5 x 10 3 , 2.7 x 10 3 , 1.8 x 10 3 , and 6.4 x 10 3 cfu/ml respectively, with results showing a significant difference at P > 0.05. Pearson's correlation coefficient showed 33% and 55% for bacterial and fungal spores on the peeled orange surfaces respectively were dependent on time. A total of 29 microbial isolates belonging to five genera (Escherichia, Staphylococcus, Salmonella, Candida and Aspergillus) were identified. The result showed that the outer surfaces of peeled oranges are contaminated with microbes of medical importance irrespective of the points of sale. Escherichia coli, Salmonella and Staphylococcus aureus counts satisfied the set limit of bacteria in ready-to-eat foods. However, the presence of these bacteria is an indicator of poor sanitary practice of orange vendors and of the surrounding air quality. Hence, orange vendors should receive appropriate training in hygiene matters that are in line with their work ethics and they should cover the peeled oranges displayed for sale.

Keywords: microbes, outer-surface, peeled-oranges, roadsides, points of sale, vendors,

INTRODUCTION

Oranges are round, orange-colored citrus fruits that grow on trees (Amerine *et al.,* 2007; Idise, 2011). There are many different varieties of orange, some are sweet and some sour. Common types include; sweet oranges, grape fruits, lemons, limes and hybrids (O'Neil *et al.*, 2012). Oranges first originated in Southern China and it is grown commercially, worldwide in tropical and warm temperate regions, thus, making it the most widely planted fruit tree in the world (Nicolosi *et al.,* 2020). Oranges contain very good amount of vitamin A, vitamin C and other flavonoid antioxidants such as *Alpha* and *Beta* carotenes, *Beta*-Cryptoxanthin and Zeaxanthin (Tsuda *et al.,* 2004; Ehler, 2011). Regular intake of orange juice, reduces viral infections, protects cells from damage, boosts immune system and helps eliminate cancer-causing free radicals (Tsuda *et al.,* 2004). Oranges are processed into juice, which can be consumed directly, or into concentrate, both used in cocktail drinks, punches, orange ades and liqueurs (Amerine *et al.,* 2007). Oranges are prepared or processed by handling with bare hands and peeling off the outer surface (pericarp) with the aid of a knife (O'Neil *et al.*, 2012; Idise, 2011). With proper hand hygiene, microbes are warded off and the peeled orange put up for sale in open markets for consumers to make purchase. Following this, the contact surfaces or juice sac of these peeled oranges are important and considered a microbial harbor unit because more often, consumers cut-open the juice sac with the mouth directly to obtain the juice and by this practice, come in contact with the microbes or the surfaces. The hand of the processor that comes in contact with these oranges can as well be a route of transmission of microbes. Hand hygiene, however, cannot be expected to break the

chain of microbial contamination in air environment that is heavily polluted with soot; disturbed market environment associated with human and vehicular movements accompanied with fumes and consumers unguarded touch on the displayed oranges (Anakwe, 2017). Again, air turbulence and aerosolized moisture already polluted environment (Anakwe, 2017). Unfortunately, the mechanism for evaluating the quality of fruit cleaning regimes is limited. The only method used in assessing fruit cleanliness is visual observation, which does not necessarily correspond with the microbiological risk (Jeddi *et al*., 2014). Peeled oranges put up for sale sometimes left unattended or uncovered along the roadside for long periods of time. This act thus exposes the eating surface (Juice sac) to potential environmental hazards. This study, therefore, evaluated the permissible levels of microbial contaminations of the outer surface of peeled oranges sold along roadsides with a view to determining the ambient microbial air quality at the sales points.

2.0 MATERIALS AND METHODS

2.1 Purchase of Peeled Oranges

Sixty (60) samples of peeled oranges were purchased from three (3) different points of sale (less, moderate and high human and vehicular movement points) in street of Rumuolumeni Community of Port Harcourt, Rivers State, Nigeria. The sale of peeled oranges along the street of Rumuolumeni is noted to have increased greatly, as traders record high profits. Twenty (20) samples each from the sales point were purchased and transported in a sterile bag to the Diagnostic and Demonstration Laboratory in Rivers State College of Health Science and Management Technology, Port Harcourt, for microbiological analyses.

2.2 Physical/Sensory Examination of the Orange Samples

The peeled orange samples were virtually and physically examined before purchased as carried out by Bonvissuto (2020). The color, smell, surface area, taste, and shapes were determined by physical/sensory observation.

2.3 Microbial Air Quality Determination

The air quality with respect to bacteria and fungi micro-spore were determined at 20-, 40- and 60-minutes interval exposure time on freshly prepared Nutrient agar media and Potato Dextrose agar media respectively, at the points of sale. The air quality was determined as carried out as described by Anyanwu *et al.* (2019).

2.4 Microbiological Examination of Peeled Oranges

Microbial analysis employed standard microbiological procedures as adopted by Public Health England (2017), where several streaks of swab were taken from the contact surface of the peeled oranges and inoculated into normal saline for 12 hours. Thereafter, a 10-fold serial dilution was carried-out on the sample, and a dilution factor of 10^{-2} from which a 0.1 inoculum volume was inoculated on freshly prepared nutrient agar, potato dextrose agar, mannitol salt agar, eosin methylene blue agar and *Salmonella/Shigella* agar as modified and adopted by Wemedo *et al.* (2016). The media plates were incubated at 37^{o}C for 24 hours for microbial growth. Growth on media were counted as colonies and recorded as colony forming unit per milliliters.

2.5 Microbial Characterization and Identification

The colonial appearances of the isolates were aseptically, examined carefully and the colonies sub-cultured in nutrient agar for biochemical identification. The microbial isolates were identified on the bases of morphological and biochemical scheme as described by Benson *et al.* (2018).

2.6 Statistical analysis

This involved determining the average of the colony counted and thereafter analyzing their differences in each batch. The least significant difference test was done as documented by Ogbeibu (2005) and data presented graphically. The data were presented in tables of frequency and analyzed

using mean and percentage while Analysis for variance and t-test were used to test the hypothesis at 0.05 significance level using Statistical Package for Social Sciences (SPSS) software.

3.0 RESULTS

3.1 Physical/Sensory Examination of the Peeled Orange Samples

Table 1, shows the physical and sensory observation of the investigated oranges. All oranges purchased and observed had similar physical/sensory properties. They all had sweet taste, same juice sac coloration, similar surface areas and shape.

Table 1: Physical/Sensory Examination of the Peeled Orange Samples at their Respective Points of Sale

Points of Sale	Smell	Surface Area	Juice -sac Color	Taste	Shape
F1	Non	Smooth	Off-white	Sweet	Spherical
F2	Non	Smooth	Off- white	Sweet	Spherical
F3	Non	Smooth	Off-white	Sweet	Spherical

Keys: F3= Less human and Vehicular Movement Sales Point, F2= Moderate Human and Vehicular Movement Sales Point, F1= High Human and Vehicular Movement Sales Point

3.2 Contact Surface (Juice Sac) Microbial Loads

Table 2 shows faecal bacteria counts of 1.1×10^3, 1.6×10^3 and 1.7×10^3cfu/ml, for peeled oranges sold at road side with less, moderate and high human and vehicular movement points respectively. No significant difference exists at $P>0.05$. Similarly, no statistical difference exists at $P>0.05$ in loads of *Staphylococcus aureus* counts at 1.6×10^3, 3.8×10^3 and 3.2×10^3cfu/ml for peeled oranges sold at road side with less, moderate and high human and vehicular movement points respectively. *Salmonella/Shigella* reported a bacteria population of 1.3×10^3 cfu/ml for less human and vehicular movement point, 2.2×10^3cfu/ml for moderate human and vehicular movement points and 2.0×10^3cfu/ml for high human and vehicular movement points. All *Salmonella/Shigella* bacterial isolated were significantly not different at $P>0.05$. Heterotrophic counts obtained were not significantly different at $P>0.05$ as obtained, with less human and vehicular movement points reporting a bacteria count of 6.4×10^3cfu/ml, followed by points with moderate and high human and vehicular movement points reporting $6.1 \times 10^3$and 6.6×10^3cfu/ml respectively, with no significant difference ($P>0.05$). The total microbial load at the sales point on the peeled oranges were 1.04×10^4, 1.37×10^4 and 1.35×10^4cfu/ml for peeled oranges sold at road side with less, moderate and high human and vehicular movement points respectively.

Table 2: Contact Surface (Juice Sac) Bacterial Loads

Bacterial Isolates	F3 (n=20) cfu/ml	F2 (n=20) cfu/ml	F1 (n=20) cfu/ml	Total Bacterial cfu/ml	WHO Permissible Limit (cfu)
Escherichia coli	1.1×10^3	1.6×10^3	1.7×10^3	1.5×10^3	<10
Staphylococcus aureus	1.6×10^3	3.8×10^3	3.2×10^3	2.7×10^3	<100
Salmonella/Shigella	1.3×10^3	2.2×10^3	2.0×10^3	1.8×10^3	0
Heterotrophic Bacteria	6.4×10^3	6.1×10^3	6.6×10^3	$6.4. \times 10^3$	<100
Total Bacterial Load	1.04×10^4	1.37×10^4	1.35×10^4		

Keys: F3= Less human and Vehicular Movement Sales Point, F2= Moderate Human and Vehicular Movement Sales Point, F1= High Human and Vehicular Movement Sales Point, n = number of orange sample, cfu = coliform forming unit

3.3 Air Bacteria Spore

The regression coefficient of the bacterial spore and time of exposure of the peeled orange as shown in Figure 1 reported a negative slope of -0.485 and a positive intercept of 67.34. The result implied that for every minute of exposure of the peeled oranges, there is a -0.5 bacterial spore dispersed on the peeled orange surface. Similarly, the correlation coefficient value $R^2$of these relationship which is

0.327 implies that 33% of the bacterial spore so dispersed on the juice sac is dependent on time of exposure.

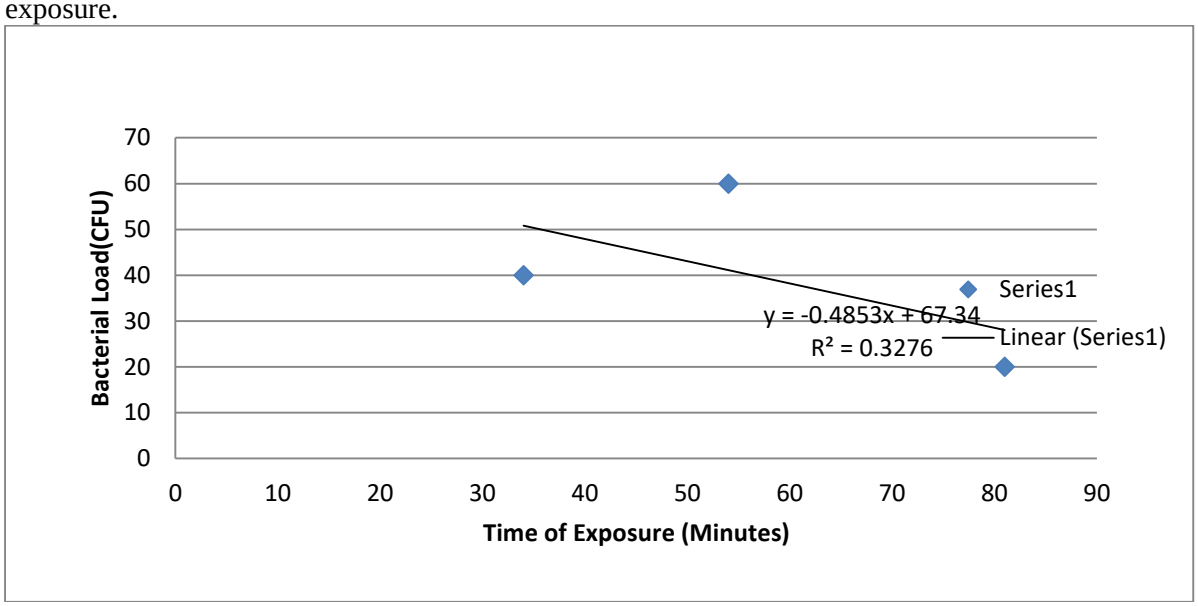

Figure 1. A regression curve showing bacterial load and time of exposure of peeled oranges

3.4 Arial Fungal Spore

Figure 2 shows the regression coefficient of the fungal spore and time of exposure on the peeled oranges. A negative slope (-1.645) and a positive intercept of 76.20 result implied that for every minute of exposure of the peeled oranges, there is a -1.6 fungal spore colony dispersed on the orange surface. Similarly, the correlation coefficient value $R^2$of this relationship which is 0.534 implied that 53% of the fungal spore so dispersed on the juice sac is dependent on time of exposure.

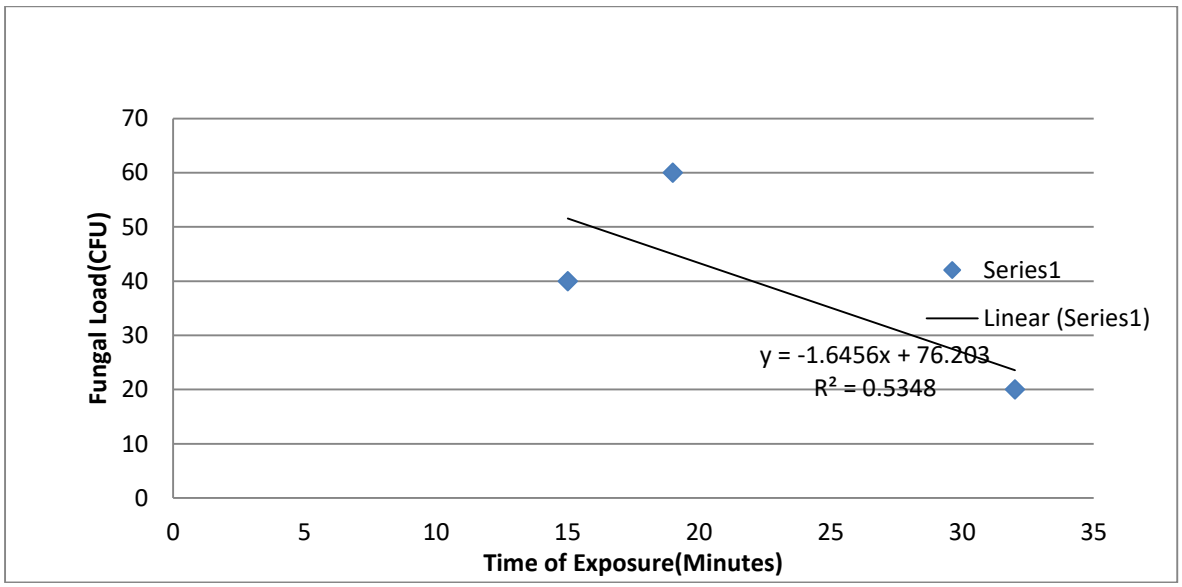

Figure 2: A regression curve showing fungal load and time of exposure of peeled oranges

3.5 Characteristics of the Isolates

Tables 3 and 4 show the bacterial morphology, biochemical reactions and fungal morphology of the isolates recovered from the orange juice sac surface from the points of sale. Phenotypic feature revealed green metallic sheen-colored colonies that are round and tiny with a positive chemical reaction to lactose sugar. *Salmonella* was identified on Salmonella/Shigella agar with a grey colony that is catalase negative. A positive coagulase test was used to identify *Staphylococcus aureus* which

reacted positive to sugar fermentation. Table 4 shows some fungi recovered. They are *Aspergillus niger* and *Mucor* sp. with 19 and 26 percentage frequency respectively as noted in Table 5. Furthermore, Table 5, shows sales point with moderate human and vehicular movement points with greater microbial density on their peeled oranges, though not significantly different from the other sales point. *Mucor* sp. was the most occurred microbes in the study; with *Escherichia coli* and *Salmonella* sp. the least occurred microbes in the study.

Table 3: Bacterial Morphology and Biochemical Reactions

Colour	Shape	Size	Coagulase	Sucrose	Lactose	Catalase	Bacteria
Grey	Round	Tiny	-	-	+	-	*Salmonella* sp.
Yellow	Round	Large	+	+	+	+	*Staph. aureus*
Green Metallic Sheen	Round	Small	-	-	+	+	*Escherichia coli*

Table 4: Isolated Fungal Macroscopic Features

Macroscopic Features	**Probable Fungi**
Black powdery growth	*Aspergillus niger*
Whitish with black spore	*Mucor* sp.

Table 5: Frequency Distribution and Percentage Occurrence of the Identified Isolates

Microbes	F1(n=20)	F2 (n=20)	F3 (n=20)	Frequency of Occurrence	Percentage (%) of Occurrence
Escherichia coli	1	2	2	5	16
Staphylococcus aureus	2	3	2	7	23
Salmonella	2	2	1	5	16
Mucor sp.	4	2	2	8	26
Aspergillus sp.	1	2	3	6	19
Total Microbes	10	11	10		

Keys: F3= Less human and Vehicular Movement Sales Point, F2= Moderate Human and Vehicular Movement Sales Point, F1= High Human and Vehicular Movement Sales Point, n = number of samples

4.0 DISCUSSION

Faecal coliforms count on the orange juice sac at the points of sale showed an insignificant difference in the loads, however, counts obtained does not satisfy National Agency for Food and Drug Administration and Control (NAFDAC) permissible limit for ready-to-eat food in Nigeria. NAFDAC states that the maximum limit of faecal bacteria on food should be less than or equal to 10 coliforms forming unit. Muyanja *et al.* (2011) raised a serious issue on the dangerous abuse of street foods. This study identified high load of faecal coliforms in the sales points with special observation on high human trafficking point with vendors' choice attributed to quick sales. The presence of faecal matter on the surface of peeled orange may be attributed to non-practice of hygienic exercise amongst vendors, given situations where Muyanja *et al.* (2011) reported the absence of toilet facilities on vending sites which could lead to dispersal of *Escherichia coli*. Situations were vendors use nearby bushes in place of toilet and clean-up with sheets of paper. Accordingly, Staphylococcal load on the juice sac were significantly not different at the points of sale basically because the hand is a common factor used in processing/preparing the orange in all sales point, thus the hand harbors *Staphylococcus aureus* as a normal flora of the body. So contamination by Staphylococcal on the peeled orange surfaces may have occurred during hand peeling by the vendors (Umoh & Odoba, 1999). Umoh and Odoba (1999) shared Ghosh *(*2004) study who reported that foods that are handled frequently during preparation are prime targets of Staphylococcal contamination. *Staphylococcus aureus* have reportedly been present in more than 13% of street food surveyed in Zaria, Nigeria (Umoh & Odoba, 1999). Consequently, the presence of Staphylococcal organism in this study has no threat to consumer or public health, as loads observed were below the set permissible limit of Staphylococcal in ready to

eat food. *Salmonella* counts on the orange juice sac were significantly not different at the points of sale probably, because report has it that *Salmonella* sp. can survive on human hands for more than three hours (Mensah *et al.*, 2000; Halker & Blaser, 1988). Thus, the presence of it on surface of peeled orange is unquestionable. There has been report of *Salmonella* food-borne outbreak resulting from consumption of orange juice. Heterotrophic bacteria load on the peeled orange surfaces were significantly not different, with the highest load observed in sales point with high human and vehicular movement points that are permissible, according to NAFDAC. Even at urban areas, exposure to high risk of heterotrophic bacteria on ready to eat food due to high commercial activities whereas low activities of humans and vehicular movement may have control heterotrophic bacteria contamination on the surfaces of the orange. Air quality has an impact on the peeled oranges for sale, this impact on the orange can likely have an effect on human health. The occurrence of fungal spore, specifically, *Aspergillus niger*, in this study have also been isolated from nasal swabs of occupants from market space environment (Obi *et al.*, 2018). Hence, the composition of arial microbiome with respect to bacterial and fungal spore dispersal is dependent on time.

4.1 CONCLUSION

The microbial contaminations of the outer surface of peeled oranges sold along roadsides in streets of Rumuolumeni are heavy on sales points with high human and vehicular movement points. *Staphylococcus aureus*, *Escherichia coli*, *Mucor* sp., *Aspergillus* sp. and *Salmonella* sp. are some microbes isolated from the peeled orange surfaces and thus present public health risk, given the microbes' elevated levels.

4.2 RECOMMENDATION

Purposeful hygienic practices are recommended during orange preparation and sampling for sale. Additionally, consumers are advised to cut and dispose the targeted contact surface before ingesting the oranges. Orange vendors should hence, receive appropriate training in hygiene matters that are in line with their work ethics and they should cover the peeled oranges displayed for sale.

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