Original Research Article

Microbiological Quality and Safety of fruits and vegetables from open market and shopping complex in Port Harcourt Metropolis

ABSTRACT

Aims: Physicochemical and microbiological quality of fruits and vegetables from open market and shopping mall in Port Harcourt metropolis were determined to ascertain the effect of the sales environment.

Methodology:pH and titratable acidity (TTA) of bananas (*Musa sapientum*), tomatoes (*Lycopersiconesculentum Mill*), lettuce (*Lactuca sativa L*) and cabbage (*Brassica oleracae var. capitata* L) were determined using standard analytical methods. Conventional microbiological method was used for the microbial analysis.

Results:pH (4.30 - 6.00) and TTA (0.05 - 0.28 % lactic acid) of samples from the two locations did not differ significantly (P>0.05). Aerobiccolony count (ACC), Coliform, *Escherichiacoli* and *Staphylococcus aureus*, ranged from 4.49 – 5.96, 3.70 – 4.59, 3.69 - 4.39 and 3.68 – 4.17 Log_{10} CFU/g respectively for the shopping mall samples, and 6.14 - 6.19, 3.66 – 5.23, 4.60 – 5.66, and 3.69 – 5.15 Log_{10} CFU/g respectively for the open market samples. *Salmonella* was detected at a level of 3.74, 4.30 and 4.65 Log_{10} CFU/g respectively in banana from the shopping mall; and lettuce and cabbage from the open market. Mould growth for the open market samples ranged from 4.01 - 4.40 Log_{10} CFU/g. Shopping mall samples had no mould and yeast growth except for mould count of 4.29 Log_{10} CFU/g in banana and yeast count of 4.30 and 3.69 Log_{10} CFU/g in tomatoes and lettuce. Banana and lettuce from the open market had no yeast count, but tomatoes and cabbage had counts of 4.30 and 4.46 Log_{10} CFU/g respectively.

Conclusion: Fruits and vegetables from the shopping mall generally had lower microbial count indicating the influence of the chilled storage environment. The detection of pathogens in the fruits and vegetables is unsatisfactory and can pose a health risk to consumers as well as the contamination of other produce.

Keywords:Banana, tomatoes, cabbage, lettuce, acidity, microbiological quality and safety

1. INTRODUCTION

Enumeration of the microbial indicators of quality and safety can help in determining the quality and safety of fruits and vegetables on display for sale. Vegetables are referred to as the edible portions of plants, excluding fruits and seeds, and are normally consumed as part of the main course of a meal. There are many classification of vegetables from leafy to fermented vegetables [1]. Fruit from culinary perspective, refers to an edible, juicy, and sweet entity derived from a flower on any flowering plant [2]. Fruits are botanically diverse, perishable, seasonal, and regional commodities that come in many forms, shapes and sizes, colours, flavours and textures. Edible fruits are grouped into four categories: fruits, fruits

used as vegetables, nuts, and cereals in culinary sense [3]. In food processing terms, fruits are nearly all acidic and are therefore called 'high acid' foods. The acidity naturally controls the type of organism that can grow in fruits, with yeasts and moulds being the predominant spoilage organisms to be found on fruit products. While vegetables have low acidity and are classified as 'low acid' foods. Low acid foods are more prone to deterioration by micro-organisms and can provide an ideal substrate for food poisoning organisms when in a moist environment.

Fruits and vegetables make great contribution to human nutrition through the provision of many vitamins and minerals and also impact positively on human health due to their unique phytochemical composition[4]. For these reasons, there is increase in the consumption of fruits and vegetables. Most of the fruits and vegetables are either eaten fresh or cooked or wasted to lack of storage facilities. These fruits and vegetables are readily available in the open markets and shopping malls. Leafy vegetables such as lettuce and cabbage and fruit vegetable such as tomatoes are regular constituents of vegetable salads which are minimally processes and eaten without heat treatment. Tomatoes is also a constituent of most ready-to eat foods such as burgers, suya, roasted plantain etc. Fruits such as bananas are consumed without heat treatment, though they are usually peeled before the consumption of the pulp, but without proper hygiene practices contaminants on the peel may indirectly contaminate the pulp during peeling and may be consumed.

In culinary sense, the quality factors of fruits are maturity, ripening and absences of defects. Some of these quality factors are also applicable to vegetables but quality issues with most vegetables is related to texture such as tenderness and firmness [3]. In microbiological terms, quality and safety are ensured by analysis of food for the presence of microbes. Fresh fruits and vegetables have natural microflora which are made up of diverse group of microorganisms such as bacteria, yeasts, moulds, viruses and parasites as well as insects and other plant pests which exist as harmless commensal [5]. They are also prone to microbial contamination through contact with soil, dust, irrigation and washing water, domestic livestock, handlers, processing equipment, and at home in the refrigerators [6], [7]. Even on display in shelves during sales several factors including temperature and handling by both sellers and buyers can contribute to the contamination of the fruits and vegetables. Agriopoulou et al. [8], acknowledged that eating of fresh fruits and vegetables is a healthy habit but their production in the external environment, poses the risk of infection with various pathogenic microorganisms, some of which cause serious foodborne illnessesPathogenic microorganisms such as *Salmonella, Shigella, Escherichia coli* O157:H7 have been isolated in fresh produce [9]. Salleh et al. [10], isolated different serotypes of *Salmonella* from raw vegetables. Fresh fruits and vegetables have been implicated in out breaks of foodborne diseases by pathogenic bacteria such as *Bacillus cereus*, *Campylobacter*, *E. coli* O157:H7, *Salmonella*, *Shigella* and *Staphylococcus aureus*; viruses (Norovirus and Hepatitis A) and parasite (*Cyclosporacayetanensis*) [11], [12], [13]. *Salmonella* outbreak have been associated with ready to eat vegetables like lettuce, fresh tomatoes and other fresh produce [12], [14], [15]. *Salmonella* have also been shown to survive even in cold storage at 4-20^{o}C [16].

The increase in demand of these food products has made it essential to know the quality and safety characteristics of these fruits and vegetables before consumption. Enumeration of the microbial load of produce can provide an insight into the quality as well as the related safety aspects of the produce and the effectiveness of the processing technique employed to kill spoilage microbes [3]. This study was therefore aimed at providing basic information by comparing the acidity and microbiological quality and safety of some fruits and vegetables sold in the open market and a major shopping mall in Port Harcourt metropolis.

2. MATERIAL AND METHODS

2.1. Purchases of fruits and vegetables

Unpackaged samples of bananas (*Musa sapientum*), tomatoes (*Lycopersiconesculentum* Mill), lettuce *Lactuca sativa L*) and cabbage (*Brassica oleracae var. capitata L*) were purchased from an open market and a popular shopping mall in Port Harcourt City. The samples in well labelled polyethylene bags were transported in chilled coolers to the laboratory for analysis.

2.2. Determination of pH and total totratable acidity (TTA)

pH and Titratable acidity (TTA) as % lactic acid was determined according to the standard AOAC [17] methods. Briefly, a digital pH meter (pH_S-2f Harris, England) after calibration using standard buffer of pH 4.0 and 7.0, was used to determine the pH of 10 ml of the homogenized and filtered sample in a beaker. Thereafter, the sample was titrated with a solution of 0.1N sodium hydroxide using 0.3 ml phenolphthalein as indicator and the acidity calculated as % Lactic acid using the formula:

$$\frac{Volume\ of\ NaOH\ \times \text{Normality of base} \times 0.09}{\text{Volume of sample} \times 100}$$

2.13 Microbiological analysis

The microbiological quality of the fruits and vegetables was carried out using conventional microbiological methods as described by Harrigan, [18]. Twenty-five (25 g) of each of the samples were homogenized in 225ml of sterile peptone water and serially diluted to 10^6 using same diluent. Aliquot (100 µL) of the dilutions were spread plated on appropriate media for each microorganism. The media were prepared following the manufacturers standard procedure. Coliform was enumerated on MacConkey Agar (MCA) incubated at 30^{o}C for 24-48 h. *Salmonella, Staphylococcus aureus,* total viable count and *Escherichia coli* were respectively, plated on Salmonella-Shegilla Agar (SSA),Mannitol Salt Agar (MSA), Nutrient Agar (NA) and Erosin Methylene Blue (EMB) incubated at 37^{o}C for 24 – 48 h. Potato Dextrose Agar (PDA) incubated at 25^{o}C for 72 h was used for mould and yeast enumeration. Microbial counts in colony forming units per gram (CFU/g) were obtained using the formula:

$$\frac{Number\ of\ colonies\ \times \text{Dilution factor}}{\text{Volume of inoculum}}$$

2.14. Experimental design and Statistical analysis

A completely randomized 2 x 4 × 7 full factorial experimental design was applied (location/samples/microorganisms). The microbial count in colony forming units per gram (CFU/g) were converted to Log_{10} CFU/g and then subjected to analysis using Minitab (Release 18.0) Statistical Software (Minitab Ltd., Coventry, UK). Statistical differences were obtained using analysis of variance (ANOVA) under the general linear model and Fisher pairwise comparison at 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. pH and total titratable acidity (TTA) as % Lactic acid of fruits and vegetables from open market and shopping mall in Port Harcourt Metropolis

The pH and TTA (% lactic acid) of the fruits and vegetables samples are shown in Figure 1 and Figure 2 respectively. The pH and TTA of the fruits and vegetables purchased from the two different point of sales did not differ significantly ($P>0.05$). pH of the fruits and vegetables ranged from 4.32 - 6.01 and 4.30 - 6.00 for the open market and the shopping mall respectively. Tomatoes had significantly ($P\leq0.05$) the least pH while lettuce had the highest. For the shopping mall, TTA ranged from 0.05 - 0.029 % lactic acid for banana and lettuce while the open market had TTA in the range of 0.05 – 0.28 % lactic acid for cabbage and lettuce. The TTA of lettuce from both locations was significantly ($P\leq0.05$) the highest. The variations in the acidity of the fruits and vegetables can be attributed to several factors such as variety and production conditions [19]. The variety of the lettuce, its production condition in addition to the presences of other acids may account for its higher in acidity level in comparison to fruit such tomatoes.pH of the tomatoes, banana was comparable with those reported by Ajayi and Olasehinde [20] and Maimunah, et al [21]. pH of the lettuce was higher than the proposed pH (5.90) for lettuce cultivars [22]. The cabbage samples had pH values that were lower than the report by Haque et al., [23] where nitrogen fertilizer was used in the production of cabbage. The pH of the selected fruits and vegetables were similar to the report by Jay [24], except for the banana that had higher pH. Although the atmosphere in the mall was a chilled condition while that of the open market was subject to the weather condition, the results indicated that the environment of sales had no significant ($P\geq0.05$) influence on pH and TTA of the individual fruits and vegetables. pH and TTA are important quality indicators in fruits and vegetables as they give the perception of sweetness or sourness. The low pH of the tomatoes is attributed to the presence of organic acids. Citric acid has been reported to be the major contributor of acidity in tomatoes followed by malic, glutamic and ascorbic acid [25], [26]. Lactic acid was used in the computation to ascertain whether there was any form of deterioration by fermenting microorganisms. The acidity levels which are in agreement with reports for fruits and vegetables is an indication that the fruits and vegetables are of good quality.

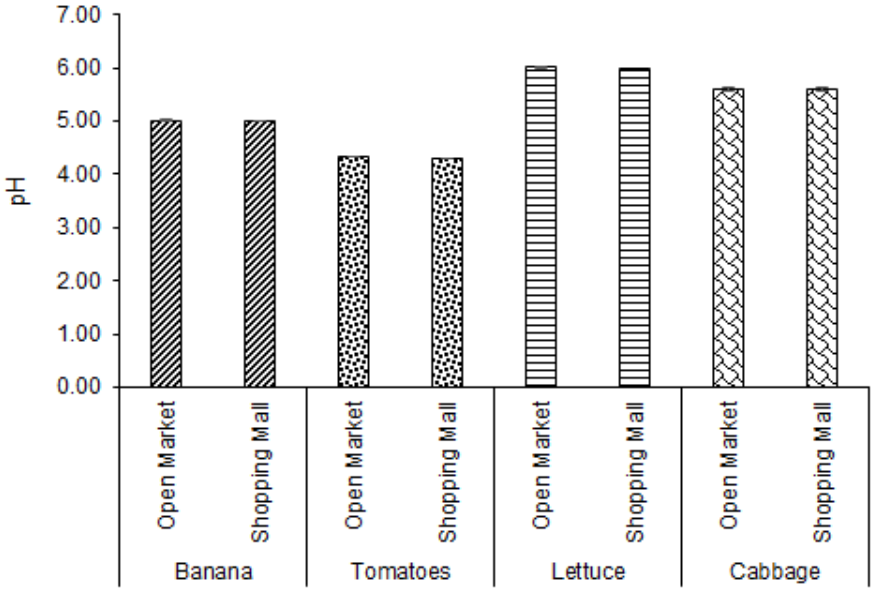

Fig. 1. pH of the selected fruits and vegetables from the open market and the shopping mall in Port Harcourt Metropolis

Bars and error bars are the mean and standard deviation of duplicate samples.
The means for each sample from the two locations did not varied significantly (P>0.05).

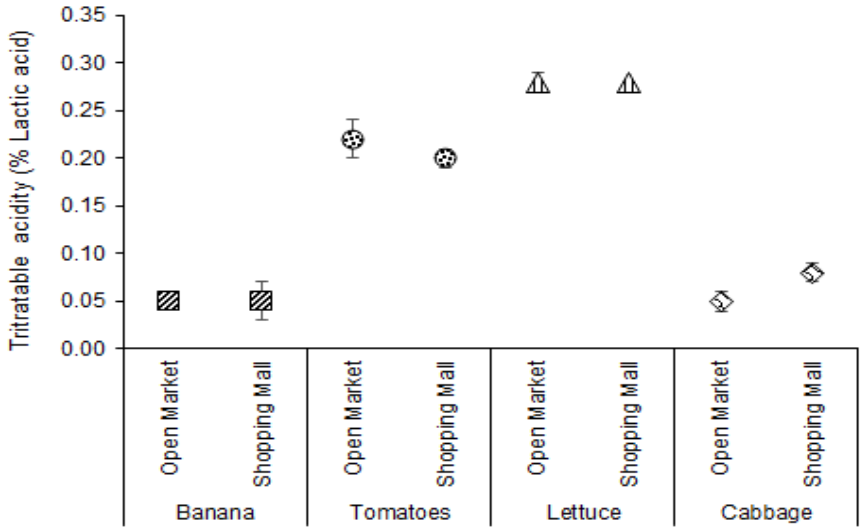

Fig. 2. Tritratable acidity (%Lactic acid) of the selected fruits and vegetables from the open market and the shopping mall in Port Harcourt Metropolis

Points and error bars are the mean and standard deviation of duplicate samples.
The means for each sample from the two locations did not varied significantly (P>0.05).

3.3 Microbiological quality of fruits and vegetables from open market and shopping mall in Port Harcourt Metropolis

The bacterial count(Log_{10} CFU/g) of the fruits(banana and tomatoes) and the leafy vegetables (lettuce and cabbage) are shown in Table 1, while the mould and yeast count are shown in Figure 3. The microbial load of the bananas varied significantly (P≤0.05). The values for the shopping mall ranged from 3.74 - 5.43 Log_{10} CFU/g for salmonella and ACC respectively. *Salmonella* and yeasts were not detected in the bananas from the open market, while the values for other microbes ranged from 3.69 - 6.19 Log_{10} CFU/g for *S. aureus* and ACC respectively. ACC, coliform and *E. coli* in the bananas from the open market was significantly (P≤0.05) higher than the shopping mall, the reverse was the case for *S.*

aureus and mound count. For the tomatoes samples from the open market, *E. coli* and *Salmonella* were not detected while values for others ranged from 3.66 - 6.14 Log_{10} CFU/g for *Coliform* and ACC respectively. *Salmonella,* mould and yeast were not detected in tomatoes samples from the shopping mall but had counts in the range of 3.68 - 5.96 Log_{10} CFU/g for respectively for *S. aureus* and ACC.There was no growth of *Salmonella*, mould and yeasts in lettuce from the shopping mall while in samples from the open market there was no growth of *S. aureus* and yeast. Bacterial counts in lettuce from the shopping mall varied from 3.69 - 4.49 Log_{10} CFU/g for respectively for *S. aureus and* ACC. For the open market, it varied from 4.30 - 6.16 Log_{10} CFU/g for *Salmonella* and ACC respectively. The bacterial load of lettuce from the open market was significantly (P≤0.05) higher than those from the shopping mall. Cabbages from the shopping mall, had no growth of *E. coli, Salmonella* and moulds. Both mould and yeast were detected in cabbage from the open market and the bacterial count varied from 4.65 - 6.18 Log_{10} CFU/g for *E. coli* and ACC respectively. Cabbage from the open market had significantly (P≤0.05) higher microbial load than those from the shopping mall.The new neutral pH and the low TTA of the samples should have encourage higher microbial growth, this was not the case and could be attributed other factors such as the initials microbial load of the displayed fruits and vegetables, contamination from other sources and the storage environment.

Table 1. Microbial count (Log_{10} CFU/g) of fruits and vegetables from open market and shopping mall in Port Harcourt metropolis

Samples	Location	Aerobic colony count (ACC)	Coliform	*E.coli*	*Salmonella*	*S. aureus*
Banana	Open Market	6.19±0.03^{a}	5.23±0.03^{a}	5.66±0.05^{a}	NG	3.69±0.01^{d}
	Shopping Mall	5.43±0.05^{b}	4.59±0.02^{c}	3.99±0.05^{d}	3.74±0.00^{c}	4.17±0.08^{b}
Tomatoes	Open Market	6.14±0.05^{a}	3.66±0.06^{e}	NG	NG	4.01±0.01^{c}
	Shopping Mall	5.96±0.03^{a}	3.70±0.01^{e}	3.69±0.02^{e}	NG	3.68±0.03^{d}
Lettuce	Open Market	6.16±0.06^{a}	4.77±0.01^{b}	4.60±0.01^{b}	4.30±0.03^{b}	NG
	Shopping Mall	4.49±0.01^{c}	4.04±0.05^{d}	4.39±0.01^{c}	NG	3.69±0.01^{d}
Cabbage	Open Market	6.18±0.06^{a}	5.09±0.01^{a}	4.66±0.01^{b}	4.65±0.01^{a}	5.15±0.01^{a}
	Shopping Mall	5.18±0.13^{c}	4.04±0.05^{d}	NG	NG	4.02±0.03^{c}

Values are means ± standard deviation of duplicate samples.
Means with the different superscript varied significantly (P≤0.05).
NG - No growth of the organism

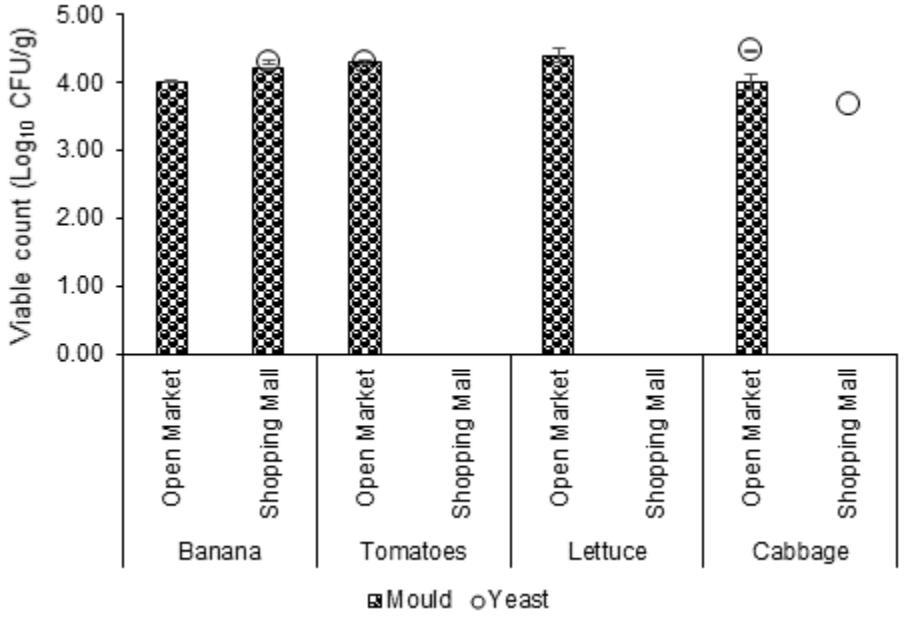

Fig. 3. Fungi (mould and yeast) count (Log_{10} CFU/g) of fruits and vegetables from open market and shopping mall in Port Harcourt metropolis

Bars and points represent the mean while the error bars are the standard deviation of duplicate samples

The result revealed that generally, the fruit and vegetable samples from the open market had higher microbial load than samples from the shopping mall. Aerobic colony count, coliform and *E.coli* are microbial indicators of quality andtheir presence in the food may not pose any health risks. In the fruits and the vegetables, aerobic colony count ranged from 4.49 – 5.96 and 6.14 - 6.19 Log_{10} CFU/g respectively for the shopping mall and the open market. Samples from the shopping mall had significantly (P≤0.05) the least aerobic count. Aerobic colony count also known as total viable counts is the total number of bacteria able to grow in moderate temperature under an aerobic condition. According to the guideline from Centre for Food Safety, [27], there are no applicable limits for satisfactory, borderlines or unsatisfactory levels for aerobic colony counts for fruits and vegetables. Aerobic colony count is not an indication of safety but of quality, providing useful information about the general quality and remaining shell-life of the food. It also highlights potential problem with storage and handling. The result of the total aerobic count in the selected fruits and vegetables therefore is not of priority risk. The lower values for samples from the shopping mall implythat the produce will have a longer shelf-life with the low temperature storage.

The coliform count in the fruits and vegetable samples varied significantly (P≤0.05). The values ranged from 3.70 – 4.59 and 3.66 – 5.23 Log_{10} CFU/g for samples from the shopping mall and the open market respectively. The counts for samples from the shopping mall was significantly (P≤0.05) lower for each of the samples except for the tomatoes where there was no significant difference. Tomatoes had significantly (P≤0.05) the lowest coliform count from both locations. The level of coliform in the vegetable were lower than the report by Abbass et al[28] for lettuce and cabbage. This showed that the level of contamination of the samples were low and an indication of good handling practices which may include the washing of the vegetables before display for sale.

E. coli is an indicator organism linked to direct or indirect contamination from food handlers. Its presence can also indicate the suitability of the food for human consumption as its presence has direct correlation with pathogens such as Salmonella [27].The fruits and vegetables had *E. coli* count in the range of Log_{10} CFU/g for the shopping mall and the open market respectively. There was no growth of *E. coli* in the tomatoes and cabbage from the open market and the shopping mall respectively. Banana from the open market had significantly the highest *E. coli* count. The *E. coli* count in this study is lower than values reported by Abbass[28]. The levels obtained were unsatisfactory compared to the guidelines ($<10^2$) for fruits and vegetables [27]. *E. coli* is a natural component of human gut flora, its presence in food is linked tocontamination of faecal originfrompoor hygiene [29]. This could be the case with highest level of *E. coli* count in the banana from the open market. However, the banana is usually peeled before consumption and peeling would reduce the microbial count.

Salmonella and *S. aureus* are pathogens that have been implicated in different outbreaks of forborne diseases, hence, their presence in food is more of a safety issue. *Salmonella* was detected at a level of 3.74, 4.30 and 4.65 Log_{10} CFU/g respectively in the banana from the shopping mall; and lettuce and cabbage from the open market. The presence of *salmonella* in fresh produce is unsatisfactory. According to the guidelines by Centre for Food Safety,[27],*Salmonella* is not to be detected in 25g of the fresh produce. *Salmonella* is an important food safety issue implicated in foodborne gastroenteritis. Although the virulence of the strain implicated and host constitution determines whether an infection with *Salmonella*spp will lead to disease [30]. *Salmonella* is often associated with foods of animal origin meat, poultry etc, its presence in these fruits and vegetables suggest contamination and can be a potential risk to human health.

The counts of *S. aureus* in the samples from the shopping mall and the open market varied respectively from 3.68 – 4.17 and 3.69 – 5.15 Log_{10} CFU/g. The level of *S. aureus* detected in the selected fruits and vegetables were within the borderline of 20 - ≤ 10^4 CFU/g for safety [25], except for cabbage from the open market with levels of 5.15 Log_{10} CFU/g. Unsatisfactory levels (> 10^4 CFU/g) of *S. aureus* might indicate enterotoxin production [31]. Lettuce from the open market had no growth of *S. aureus*. This is in line with the report by Eni et al.,[7]. The absence of *S. aureus* in the lettuce, met the satisfactory level and is an indication of a suitability of the produce for human consumption.

Mould and yeasts are mostly recognized as spoilage microorganisms, where they are not used as fermenters.Though the growth of mould may present the risk of contamination with mycotoxins which are known to be dangerous to health[32]. There was no mould growth in the samples from the shopping mall except for banana at a level of 4.29 Log_{10} CFU/g. Samples from the open market had mould count of 4.01 - 4.40 Log_{10} CFU/g. Tomatoes and lettuce from the shopping mall had no yeast count while counts of 4.30 and 3.69 Log_{10} CFU/g was respectively detected in banana and cabbage. Banana and lettuce from the open market had no yeast count, but tomatoes and cabbage had counts of 4.30 and 4.46 Log_{10} CFU/g respectively. The detected levels of mould and yeasts in the selected fruits and vegetables were higher than

counts reported by Abadias et al [33] for fresh, minimally-processed fruits and vegetables. The processing process may have led to the decrease. Yeasts and moulds are often implicated in spoilage, although the production of mycotoxins by moulds is an issue of public health concern.

4. CONCLUSION

The lower microbial counts in the fruits and vegetables from the shopping mall indicated the influence of the chilled storage environment of the mall. The presence of microbial indicators of quality above the satisfactory level indicates contamination which could be attributed to several factors and may shorten shelf-life of the produce. The detection of pathogens in the fruits and vegetables implies that these produce can lead to direct or indirect contamination of other produce and also be vehicles of transmission to humans. Hence, adoption of good manufacturing and hygienic practices are recommended. Though the several hands that touch the produce as buyers' raise and examine them before purchases may be difficult to control, modified atmosphere packaging will minimize direct contact with the produce.

CONSENT (WHEREEVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Not applicable
COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

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