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MICROBIAL QUALITY OF PROCESSED WATER MELON FRUIT SOLD IN KOFORIDUA MARKET IN GHANA

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ABSTRACT: *Most fresh-cut vendors on the streets are not formally educated on the hygienic way of food processing. It was suspected that fresh-cut water melon that has been processed by these vendors may be contaminated with microorganisms. Five fresh-cut water melon fruit vendors were randomly selected and samples were taken from them in three replicates. The samples were analyzed for fungi and bacteria. All samples were incubated aerobically at a temperature of 37°C for 24-48 hours. All bacteria counts were transformed to log_{10} colony forming units(cfu). The results were subjected to analysis of variance using Post Hoc Test. The results indicated that the fresh cut water melon contain fungi, and other bacteria such as Salmonella aureus, Enterobacter spp, Citrobacter, Klebsiella pneumonia, E. Coli, Aspergillus spp, salmonella, Shigella and Aspergillus spp. In conclusion fresh-cut water melon sold in Koforidua market could be a potential source of bacterial infection to consumers.*

KEY WORDS: watermelon, fresh-cut fruit, contamination and bacteria.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is known to be a popular staple summer fruit found in the world and it is mostly consumed as fruit salad, drinks (Alim-un-Nisa et al., 2012, Perkins-Veazie et al., 2013) or as a dessert (Blohm et al. 2020; Paris, 2020). Water melon has a natural source of antioxidants, Vitamin C and lycopene (Naz et al., 2014). Watermelon helps improve human health as a result of the presence of lycopene. It is known to control chronic diseases such as diabetes, cardiovascular events, and some forms of cancer (Figueroa et al., 2011). Similarly, Perkins-Veazie et al. (2001) reported that Lycopene, a carotenoid, has antioxidant properties that may reduce the incidence of certain cancers. Water melon has the ability to control hypertension, diabetes, cancer, and some coronary heart diseases (Maoto et al., 2019). Fresh-cut fruits can easily be contaminated and degraded due to the application of various preparation steps such as washing, peeling, cutting, and slicing. (Yousuf et al., 2019). Contamination and degradation of fresh-cut watermelon occur due to its low acidity and growing conditions (Wang et al., 2018; Wanwimolruk et al., 2015). Water melon is regarded as a potentially hazardous food. Eight different microbial isolates were obtained from the sliced watermelon samples, which include: *Escherichia coli, Klebsiella*

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aerogenes, Proteus mirabilis, Staphylococcus aureus, Lactobacillus spp., Saccharomyces cerevisiae, Rhizopus stolonifer and *Mucor spp* (Nwachukwu et al., 2008).

Edusei et al (2016), indicated that vendors washed the fruits before severing using water from tap, well, or borehole, the water was without disinfectant. In addition, they observed vendors using the same water to wash fruits several times without changing the water. It is suspected that such water may cause contamination. Generally, it is required that all fruits used for fresh cut be subjected to effective washing and rinsing using potable water which has been sanitized (Bhilwadikar et al., 2019). According to Edusei et al. (2016), most of the vendors had never acquired any formal training on food processing and personal hygiene particularly on fresh cut fruits. Base on the observation of Edusei et al (2016), this project seeks to specifically assess the microbial safety of fresh cut water melon sold in the koforidua market.

MATERIALS AND METHODS

Sampling Technique

Fresh-cut water melon fruits were randomly purchased from five vendors from the street of the Koforidua Central Market. Three different samples of watermelon were purchased from each street vendor. All samples were packaged in zip-lock rubber and placed on ice during its transportation to the laboratory. Microbial analyses were performed using a commercially available dehydrated media, manufacturer's instructions were followed.

Sample Preparation

The samples were analyzed in the microbial laboratory at the Koforidua Technical University. The rinsed watermelon samples were serially diluted four times (10^4) and aseptically plated on petri dishes after which molten agar (45%) was poured into them, swirled and allowed to solidify. All samples were incubated in an aerobic incubator at a temperature of 35±1°C for 24-48 hours. Sabouraud Dextrose Agar (SDA) was used for the enumeration of fungi at a temperature of 28 ±2.0°C for 3-5 days. MacConkey Agar (MCA) was used for the enumeration of coliforms at a temperature of 37°C for 24 hours. Following incubation, colonies developed were enumerated and transformed into colony forming units per millimeter (cfu ml^{-1}) of the samples. Also, 1ml of each sample was transferred aseptically into test tube containing 9ml of 0.1% peptone water. 10-fold dilutions were then prepared at 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilution, these were aseptically transferred into sterile plate count media using the pour plate method. The inoculated plates were incubated at 37^0C for 48. A series of biochemical tests were carried out to characterize and identify each isolate.

Catalase Test

A drop of 3% hydrogen peroxide (H_2 S) was placed on a glass slide and a bit of colony from the plate was taken with wire loop and emulsified with the hydrogen peroxide on the slide. A positive test was indicated by bubbling and fronting. b

Indole Test
The isolate was grown in 2ml peptone for 24 hours. After that, three to five drops of Kovac's sindole reagent were added and allowed to stand for five to ten minutes. A positive reaction was indicated by the development of a red color in the reagent layer above the broth while in the negative reaction, the indole reagent retained its yellow color.

Simmon's Citrate Agar Test
The isolate was inoculated into a Simmon's citrate agar slant in a bottle and incubated for 24 to48 hours at 37^0C. The development of deep blue color indicated positive reaction and when the color remained the same indicating (negative reaction).

Triple Sugar Iron (TSI)
The isolate was inoculated into a TSI agar slant and incubated for 24 to 48 hours at 37^0C. The development of yellow color with butt, slant, gas and H_2S indicating positive and black color indicated negative reaction.

Urease Test
The isolate was grown in 2ml of Urease agar for 24 hours for analysis. A positive reaction was indicated by the development of pink color in the reagent while in the negative reaction, the urease retained its color.

Isolation of Fungi
A sterile pipette was used to transfer the sample rinse in the peptone water into the petri dishes before pouring the prepared Saboraud's dextrose agar (SDA) into the petri dishes containing the sample. The spread sample was then incubated at room temperature (27-37^0C) for 7 days before identification.

Statistical Analysis
The microbial counts were subjected to Analysis of Variance (ANOVA) using the post Hoc Tests. The analyses that were used were Descriptive measures and repeated measures. The samples taking from the five vendors: Vendor A, B, C, D and E were analyzed for bacteria, yeast and molds. It was hypothesized that: H_0: There is no significant difference between the vendors in terms of hygiene. H_1: There is significant difference between the vendors in terms of hygiene. The results were tested statistically at an alpha = 0.05 and the decision rule was to reject H_0 if p-value is less than alpha, fail to reject otherwise.

RESULTS AND DISCUSSION

Bacteria and fungi are the common contaminants of our fruits and they could be easily transferred from the vendors to the processed fruits through mishandling (Asante et al., 2019). The consumption of ready-to-eat fruits directly from street vendors potentially increase the risk of food-borne illness. It is difficult to attest to the hygienic condition of these vendors during processing

and this could be a threat to human health (Asante et al., 2019). This research threw light on the microbial contamination of ready-to-eat vended fruits from vendors in Koforidua market.

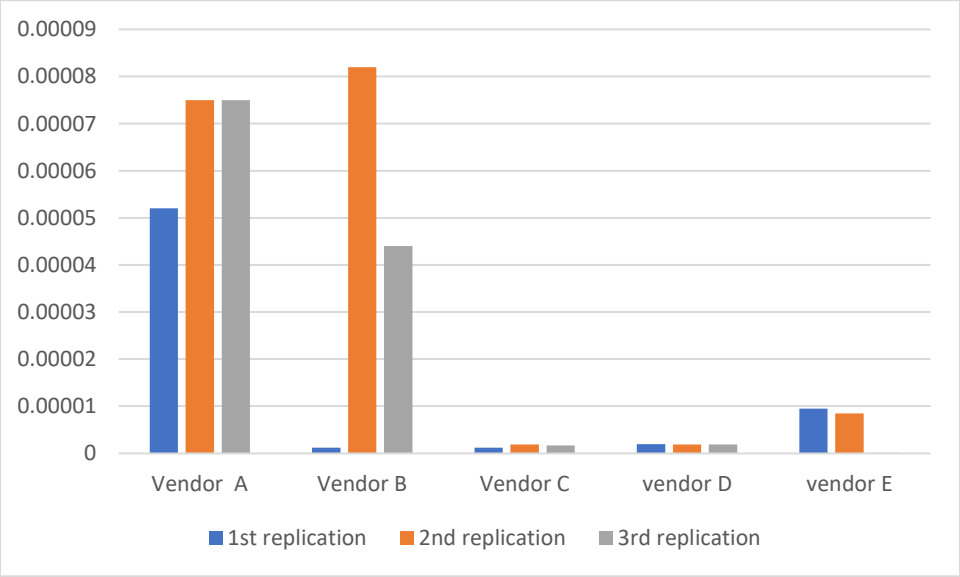

Figure 1: Comparing the replication of the five vendors to check the presence of bacteria Source: Field work, 2019

Figure 1 above indicates the present of coliforms in the water melon. When the sample collected from the venders were tested, in the first replication, there were about 5.2×10^3 cfu of coliform and 7.5×10^3 cfu of coliforms in the second and third replications for vendor A. Also, about 1.3×10^4 cfu of the coliform was found in the first replicate for vendor B, 8.2×10^3 cfu in the second replication and 4.4×10^3 cfu in the third replication for vendor B. Under vendor C about 1.2×10^4 cfu of coliform were found, while 1.9×10^4 cfu and 1.7×10^4 cfu were also found in the second and the third replications. Fresh-cut water melon collected from the vendor D, had 9.5×10^4 cfu of the coliforms, while 8.5×10^4 cfu and 1.2×10^5 cfu were found in the second and third samples respectively. Finally, the fifth vendor sample had 9.5×10^4 cfu of the coliforms were identified in the melon for the first replication, while 8.5×10^4 cfu and 1.2×10^4 cfu were also found in the second and third replication. This indicates that the highest coliforms counts were found in the fresh-cut water melon of the first vendor in all the three replications. Coliforms are indicators of some degree of potentially hazardous contamination (Luna-Guevara et al., 2019; Martin et al., 2016). Among the genera of bacteria isolated in the study, Staphylococcus spp was predominant in both the fruits samples. The contamination could be as a result of discharge into the atmosphere through sneezing or coughing or even the way in which the fruits processed by the fresh-cut vendors. It is required that these food vendors be properly trained on how to handle food in order not to transfer food borne illnesses to consumers.

E. coli count in fruits is widely used and accepted as indicators of fecal contamination (Johnston et al., 2005; Weldezgina and Muleta, 2016). Staphylococcus epidermis might have been introduced from handlers being a normal flora on the skin of human. These organisms are known to be associated with food poisoning or food infection. The presence of this microorganisms in fruits can be due to ecological and environmental influence since their survival in the atmosphere depends on a number of factors such as nature of microorganism, susceptibility to changes, resistance to new physical environment and the ability to form resistant strains (Haruta and Kanno, 2015).

Table 2: Descriptive Statistics

Replications	**Mean**	**Std. Deviation**	**N**
1st Replication	.00010675200	.000231038992	5
2nd Replication	.00003385600	.000040917560	5
3rd Replication	.00002453820	.000033751982	5

Source: Field work, 2019

Different bacteria species were isolated from a total fresh cut water melon samples analyzed and they include: *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella sp, Shigella sp* and *Escherichia coli* respectively. This is in agreement with a research conducted by (Del Ros ario and Beuchat, 1995). They reported that Escherichia coli has the ability to grow on water melon at a temperature of 25^{o}C within 36hours of storage. Similarly,(PenteadoMauro and Leitão, 2004) grew *Listeria monocytogens* on water melon fruit and reported that *Listeria moncytogens* can grow on water melon over a wide range of temperature. This is due to the low acidic nature of water melon. All the microorganisms isolated: Salmonella, *Esherichia coli* and *listeria monocytogenes* have been reported to cause food borne illness.(Yeni et al., 2015; Hagens and Loessner, 2007). The fresh cut watermelon sold in Koforidua market could be a potential source of foodborne illneses spread. The presence of these microorganisms can be associated with poor agricultural practices, unhygienic processing of the fresh cut fruits and the use of poor-quality water. However, these ~~v~~endors need education in order to produce quality fresh-cut water melon to consumers.

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Analysis of Microorganisms Presence

Table 5. Presence of microorganism in the sampled watermelon

Sample No.	**Indole**	**Melon, TSI or KIA**				**Simmon Citrate**	**Urease**	**Organism isolated/isolates**
		Butt	**Slant**	**H_2S**	**Gas**			
A_1	-	Y	Y	-	+	+	-	*Enterobacter spp, S. Aureus*
A_2	-	Y	Y	-	+	+	-	*Enterobacter spp, S. Aureus*
A_3	-	Y	Y	-	+	+	+	*Citrobacter, S. Aureus*
B_1	-	Y	Y	-	+	+	Slow +	*Klebsiella pneumonia, S. Aureus*
B_2	-	Y	Y	-	+	+	-	*Enterobacter spp, S. Aureus*
B_3	-	Y	R	Weak +	-	-	-	*Salmonella, S. Aureus*
C_1	-	Y	Y	-	+	+	+	*Enterobacter spp*
C_2	-	Y	Y	-	+	+	+	*Enterobacter spp, S. Aureus*
C_3	+	Y	Y	-	+	-	-	*E.Coli, S.Aureus, Aspergillus spp, fungi, SDA*

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D_1	-	Y	Y	-	+	Slow +	+	*Klebseilla spp*
D_2	-	Y	Y	-	+	+	-	*Citrobacter, S. Aureus*
D_3	-	Y	R	-	-	-	-	*Shigella, Aspergillus spp,* fungi, SDA
E_1	-	Y	R	+	-		-	*Salmonella*
E_2	-	Y	Y	-	+	+	+	*Klebseilla, S. Aureus*
E_3	-	Y	Y	-	+	+	+	*Klebseilla, S. Aureus*

Source: Field work

Further Analysis Using Repeated Measured of Analysis of Variances Techniques

Table 3: Mauchly's Test of Sphericity[a]

Measure: MEASURE

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b] Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Coliforms	.179	5.161	2	.076	.549	.602	.500

Source: Filed work, 2019

Mauchly's test of sphericity was to test whether the data was truly replicated. The p-value(sign) of 0.076 which is greater than 0.05 indicate that the data was significantly replicated for the experiment and it has met the assumptions of the test.

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Table 4: Tests of Between-Subjects Effects

Measure: MEASURE
Transformed Variable: Average

Source	Type II Sum of Squares	Df	Mean Square	F	Sig.
Intercept	8.531E-9	1	8.531E-9	3.223	.147
Error	1.059E-8	4	2.647E-9		

Source: Field work

Table 2 above indicates that the p-value = 0.147 which is greater than alpha = 0.05 we fail to reject the H_0 and conclude that there are no significant differences among the vendors in terms of hygiene. This means that there were microorganisms present in all the melon sample pick from the vendors for this research.

CONCLUSION

In conclusion, fresh-cut water melon sold on the streets of Koforidua market are not safe for consumption as it could be a potential source of bacteria spread to consumers.

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