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Research Article

MICROORGANISMS ASSOCIATED WITH ONIONS (RED AND WHITE ONIONS) STORED AT DIFFERENT STORAGE CONDITIONS

***Omorodion nnenna J. P. and Wokoma Victoria A.**
University of Port Harcourt, Department of Microbiology PMB5323,
River State Nigeria
**Author for Correspondence: nnennaomorodion@gmail.com*

ABSTRACT
Onion (*Allium cepa* L) is one of the oldest vegetables cultivated for food. An important crop in Nigeria based on consumption and is grown mainly in the north, during the dry season (Octoberto April). This study is aim at determining the organisms associated with onion stored at different storage conditions, two species sampled (red and white onions). Evaluation was doneusing total heterotrophic bacterial count, total *Staphylococcus* count, total coliform count, total fungi count, total *Salmonella/Shigella* count and physiochemical analysis. Total heterotrophicbacterial count of the onions samples ranged from 1.5 x 10^{-2} to 4.6 x 10^{-8} CFU/g. Percentage occurrence of bacteria isolated were; *Bacillus* sp 35.5%, *Micrococcus* sp 12.5%, *Klebsiella* sp 13.5%, *Pseudomonas* sp 15.5% and *Staphylococcus* sp 23%. *Bacillus* sp had the highest occurrence for both samples while *Micrococcus* sp had the lowest occurrence in red onion sample and *Klebsiella* sp had the least occurrence in white onion samples. Total fungi count ranged from 1.9 x 10^{-2} to 7.9 x 10^{-4} CFU/g. Percentage occurrences of fungi isolated were; *Saccharomyces* sp 6%, *Aspergillus niger* 38.5%, *Aspergillus flavus* 6%, *Candida* sp 10%, and*Penicillum* sp 29%, *Rhizopus* 20%, and *Mucor* sp 13%. Red onions had a higher pH and moisture content than the white onion. Contamination was as a result of poor handling and storage and temperature of storage. Some of the organisms isolated are pathogenic, this makesit a potential for the spread of food borne infections. The consumers' needs to be educated on the health implications of pathogenic microorganisms associated with onions.

Keywords: *Red Onion, White Onion, Storage conditions, pH and Moisture Content*

INTRODUCTION
Onion (*Allium cepa L*) is an important vegetable crop in Nigeria based on consumption and economic value to farmers. It is ancient in nature and also one of the oldest vegetable cultivatedby man for food. In the world onion build are found in ancient Egypt, India, China and Europeduring the middle ages, and in the late sixteenth or early seventeenths centuries, it spread to thenew world by the Spaniards. The crop is now wide spread in both temperate and tropical regions with Europe as the largest producers (Cobley and Steele, 1976). Onion is a valuable ingredient in the diet due to its content of sugars, vitamins and minerals (Ole *et al*., 2004).

In the tropic most onions are grown with irrigation during the relatively cool dry season in semi-arid region, they are not good crop for hot wet tropic. Onions can be grown under a widerange of climatic condition but they succeed best in a mild climate without excessive rainfall or great extremes of heat and cold. They are not suitable to regions with heavy rainfall in the low and humid tropics. Cool conditions with an adequate moisture supply are most suitable forearly growth followed by warm drier condition for maturation, harvesting and curing. They canbe grown on a variety of soils, but the soil should be retentive of water, non – packing and richin nutrient, a good fertile loamy soil usually gives the best result. They may be grown successfully on peat soils. The soil optimum pH is about 6.0 – 7.0 (alkaline) (Carl and Hall, 1986). In Nigeria the crop is grown mainly in the north, during the dry season (October to April). Onions (*Allium cepa L*., from the Latin word *cepa* "onions"), is a monocotyledonous plant used as

vegetable and spice across the world in the daily preparation of meals for its nutritional value. Its distinctive taste enhances the flavor of meals while contributing to the human health with benefits through its vital source of nutrients. Onions can be consumed eitherraw, cooked or as salad dressing. It is one of the worlds most cultivated and used vegetable. They are grown in different varieties and are usually available in different shapes, sizes and colors. Onions is usually grown with three common color varieties, White onion, Red onion, and Yellow onion (Brown). Onion is known for being a good natural source of flavonoids mainly represented by the flavonoid - quercetin and kaempferol, which are present as their glycosides (Viuda and Fernández, 2008). These flavonoid are beneficial as they exhibit antiallergenic, anti- inflammatory, cardio protective, vasodilatory, anti- carcinogenic and antioxidant properties, antibacterial and anti-fungal properties (Shon *et al*., 2004). The antibacterial activity of onion juice can be attributed to the presence of flavonoids and polyphenols which have been reportedto have a broad spectrum of antibacterial activity (Hendrich, 2006). That is why onions is being considered as a good source of natural additives to retard food deterioration (Navas *et al*., 2006).

Contamination of onions by microorganisms could also be as a result of poor handling and processing practices, in food chain supply, poor storage conditions, and distribution to local market sellers, marketing practices and transportation. Locally, onions are stored in jute bags and baskets which are handmade with materials from palm, bamboo and fibrous jute trees. Storage losses are a function of storage environment as well as the condition and cultural practices used during the growing season. Proper control of storage environment can however,significantly extends the storage season from that which would result from storage environment that were not matched to the condition of the onions when placed in storage (Carl and Hall, 1986).

The aims of this research project are; to isolate, identify and characterize the different micro-organisms that are responsible for the spoilage of onions stored at different storage conditions.

MATERIALS AND METHODS

Study Area

The study was conducted in the General Microbiology laboratory of the Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria.

Sample Collection

Two species of onions (White and Red) samples were bought from different sellers in Port Harcourt, packaged in sterile polytene bags and then taken to the laboratory immediately for analysis.

Sample Preparation

The eight onion bulbs (four each) were weighed and then six were cut into four sections each, with a sterile knife under aseptic conditions to produce a total of eight sections. The two speciesof onion were labeled as:

R=For red specie of onion

W=For white specie of onion

They were further labeled and stored under different conditions below and were later analyzedon day 0, 1, 3, 5, and 7 after storage.

A= Whole onion stored at ambient temperature for both specie of onions.

B=Cut onion stored at ambient temperature

Four sections each from the two species of onions cut were placed in a sterile plastic tray andstored at ambient temperature for 7 days.

C=Cut and stored in the refrigerator

Four sections each from the two species of onions cut were placed in a sterile Ziploc bag and closed properly to prevent contamination. They were stored in the refrigerator for 7 days.

D=Cut and stored in the freezer

Four sections each from the two species of onions cut were placed in a sterile Ziploc bag andstored in the freezer for 7 days.

Sample Preparation

70% of alcohol was used to sterilize the surface of the onion bulb. About 10g of the onion fruit was cut using a sterile knife from each of the sample and it was transferred separately into a sterile crucible (motor and pestle), sterile distilled water was added into the motor, it was then crushed. 1ml of the sample was further diluted by pipetting 1ml of the stock into 9ml of normal saline contained in a test tube to get dilution (10^{-2}), and was further diluted up to dilution (10^{-6}). While culturing the media, 0.1ml of the solution gotten from the test tubes with a dilution factor of 10^{-2} and 10^{-4} were dispensed on the plate count agar in duplicates. 0.1ml of the solution with dilution factor 10^{-2} and 10^{-3} were dispensed on the selective media for bacteria isolation.

Isolation of Microorganism (Bacteria and Fungi)

From the aliquot, dilution factors of 10^{-2} and 10^{-4} 0.1ml was plated on the plate count agar which was used for total viable heterotrophic count. 0.1ml from the dilution factor of 10^{-1} and 10^{-2} of the aliquots was used on Mannitol salt agar for *Staphylococcus* sp count, on MacConkey agar for coliform count, on Salmonella-Shigella agar strictly for the detection of *Salmonella* sp and *Shigella* sp. The plates were incubated at 37ºC for 24 hours for bacteria growth.

For total fungi cell count, 0.1ml of the diluent was plated on potato dextrose agar and incubated at 25-27ºC for 2-5 days. The spread plate method was used for this technique and was aseptically inoculated into the five (5) agar plates using sterile hockey sticks. The plates were done in duplicates and labeled properly in respect to dilutions. After incubation the colonies are counted and recorded. The presence of colonies on media is indicative of viable cells and they were selected from those dilution yielding 30 to 300 colonies per plate and the viable count per ml was determined by multiplying the average number of colonies of the duplicate plate by corresponding dilution factor. Bacterial and fungal count was carried out on each of the plate to determine the number of bacterial and fungal growth. The total heterotrophic bacteria count of the sample was obtained from the plate count agar culture after 24 hours of incubation and the numbers of colonies obtained were expressed as a colony forming unit (CFU/g). This was obtained by counting the whole plate and using the number obtained to multiply by the dilution factor.

Characterization and identification of the colony isolates was achieved by initial morphological examination of the colonies in the plate (macroscopy) for colonial margin, shape, size, elevation, surface, texture, edge, consistency, and opacity on the growth medium and results recorded. The isolates were identified and characterized based on their cultural characteristics, gram staining. Single colonies of bacteria growth were picked from the different bacteriological media previously incubated based on their morphological characteristics. These isolates were sub-cultured on freshly prepared nutrient agar media using streak plate method and incubated for 24 hours at 37ºC so as to get pure cultures of distinct colonies.

The isolated bacteria colonies sub-cultured were transferred with the aid of a sterile inoculating wire loop from the nutrient agar media to agar slants prepared in bijou bottles and labeled for easy identification of the isolate. This was done by dispensing 10ml of nutrient agar solution into the bijou bottles. The medium was sterilized by autoclaving at 121ºC for 15 minutes at 15psi. The bottles were slanted and allowed to solidify and then isolates from pure culture were inoculated on the surface of the agar slant. It was incubated at 37^0c for 24 hours and later preserved in the refrigerator for further identification to genera level by microscopy and biochemical test. Isolates were identified based on colonial morphology and cultural characteristics on growth media which include: colony size, color, opacity, consistency, colony pigmentation, elevation, odor, swarming, identification materials, reagents and protocols. Examination of Fung was done based on the colonial morphology (color, size, texture) and the cell morphology (mycelium, hyphae) of the fungi using lactophenol blue. A piece of mycelium from the petri dishes was mounted on a clean grease free slide using a sterile wire loop and was covered with a cover slip. A drop of lactophenol cotton blue was added and allowed to sit for a few minutes before examining under the microscope for the presence of hyphae and arthrospores using x40 objective.

Physiochemical Analysis

Determination of pH

Each specie sample of the onions was weighed and 70% of ethanol was used to sterilize the surface of the

onion bulb to reduce the introduced micro-organism in the onions when it was kept at room temperature for 1 to 3 days. About 10g of the spoiled onion fruit was cut from each of the onion specie (Red and White onion) using a sterile knife and transferred separately into a sterile crucible and crushed. It was then filtered to extract the juice (filtrate) and the pHwas checked immediately using a pH meter.

Determination of Moisture Content

Each specie sample of the onions was weighed and 70% of ethanol was used to sterilize the surface of the onion bulb to reduce the introduced micro-organism in the onions when it was kept at room temperature for 1 to 3 days. Two sterile petri dishes were weighed and labeled appropriately. About 10g of the spoiled onion fruit was cut from each of the onion specie (Redand White onion) using a sterile knife and transferred separately into the petri dishes. The onion samples were put into the incubator to dry for 10 minutes at 105ºC. After the time elapsed they were taken out, weighed and results recorded. This was done periodically for an hour until all the moisture was dried up from the samples and they had a constant weight when weighed.

It was calculated using the formula below

$$\% \text{ Moisture content} = \frac{Weight\ of\ fresh\ sample - weight\ of\ dried\ sample}{Weight\ of\ sample\ used} x \frac{100}{1}$$

Statistical analysis

All analysis was done in duplicates for each of the samples, and data were reported for duplicate analyses of the same samples. All statistical analyses were carried out using analysis of variance (ANOVA). Significance of the differences was ascribed at the 0.05 level for ANOVA

RESULTS

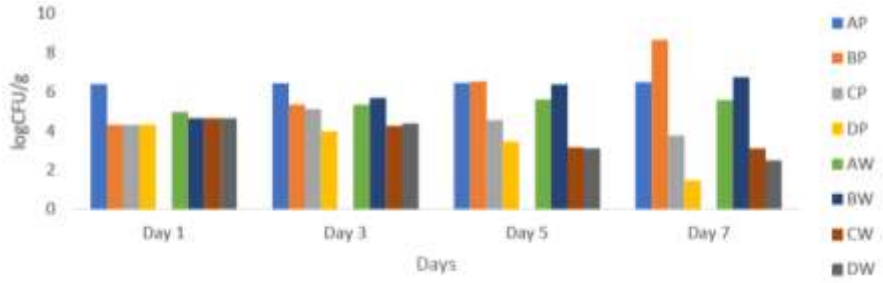

Figure 1: Total viable count of the white and red onion sample at different storage temperature

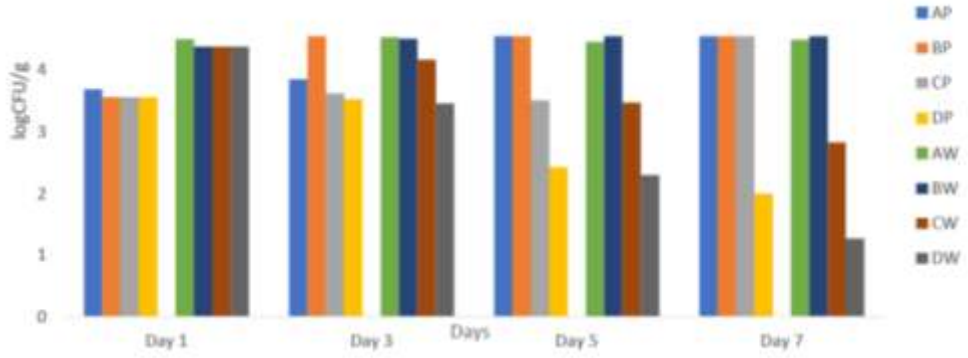

Figure 2: Total coliform count of the white and red onion sample at different storage temperature

Keys: AR=Whole red onion at room temperature, BR= Cut red onion and stored at room temperature, CR=red onion cut and stored in the fridge, DR=red onion cut and stored in the freezer; AW=whole white onion at room temperature, BW: Cut white onion at room temperature, CW= white onion cut and stored in the fridge, DW= white onion cut and stored in the freezer

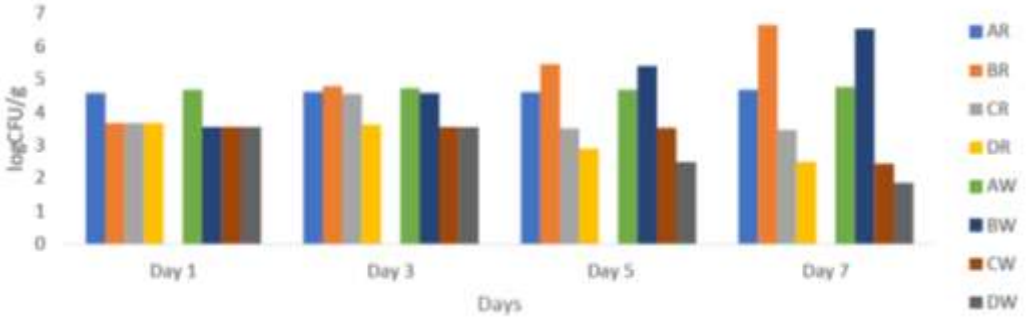

Figure 3: Total staphylococcal count of the white and red onion sample at different storage temperature

Keys: AR=Whole red onion at room temperature, BR= Cut red onion and stored at room temperature, CR=red onion cut and stored in the fridge, DR=red onion cut and stored in the freezer; AW=whole white onion at room temperature, BW: Cut white onion at room temperature, CW= white onion cut and stored in the fridge, DW= white onion cu-+t and stored in the freezer

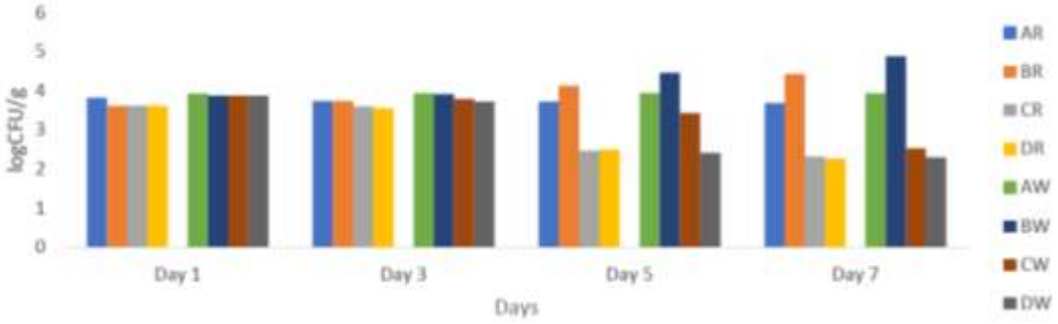

Figure 4: Total fungal count of the white and red onion sample at different storage temperature

Keys: AR=Whole red onion at room temperature, BR= Cut red onion and stored at room temperature, CR=red onion cut and stored in the fridge, DR=red onion cut and stored in the freezer; AW=whole white onion at room temperature, BW: Cut white onion at room temperature, CW= white onion cut and stored in the fridge, DW= white onion cut and stored in the freezer

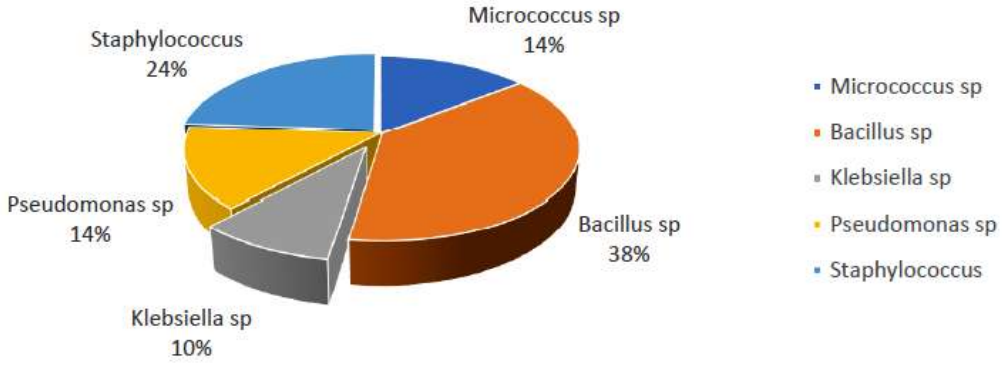

Figure 5: Frequency of occurrence of the bacterial isolates from white onions sam

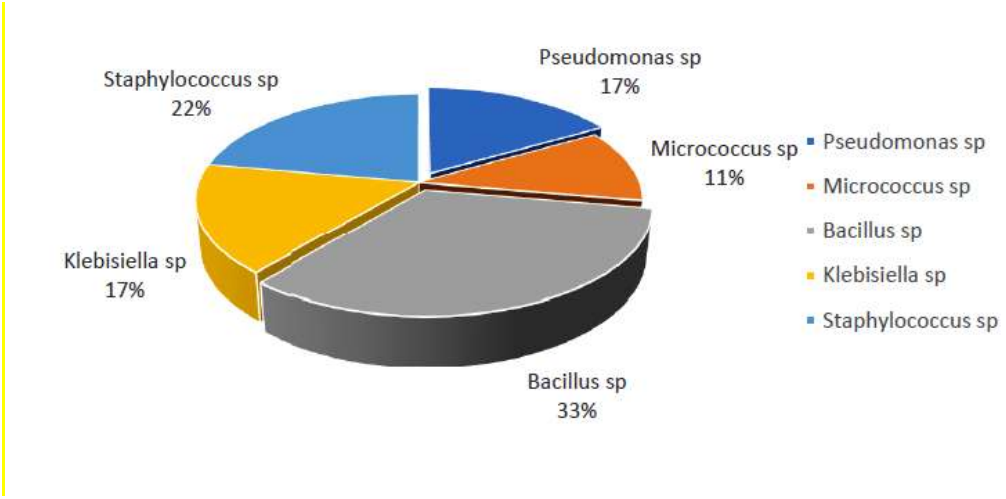

Figure 6: Frequency of occurrence of the bacterial isolates from red onions sam

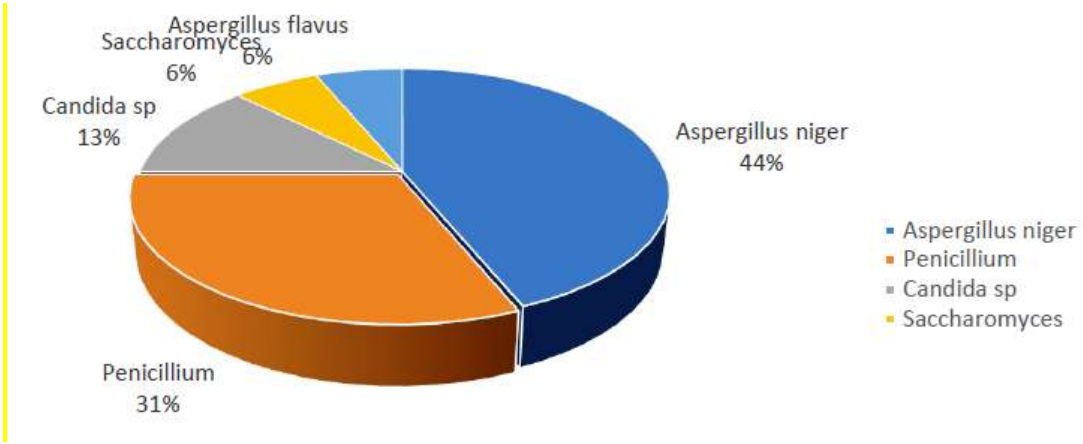

Figure 7: Frequency of occurrence of the fungal isolates from white onions samples

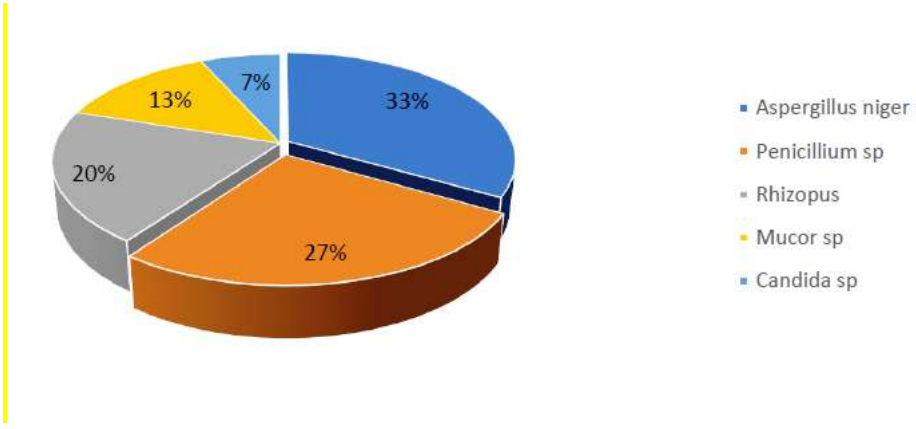

Figure 8: Frequency of occurrence of the fungal isolates from red onions sample

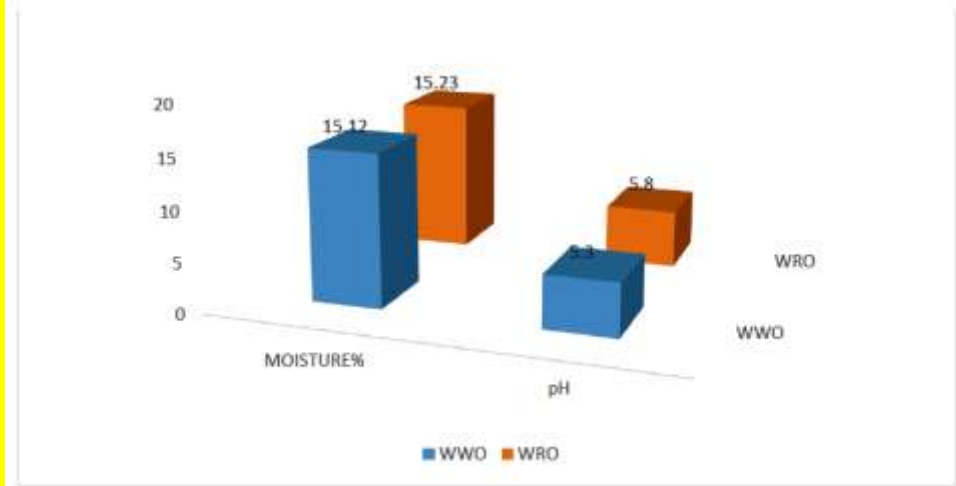

Figure 9: Mean pH and moisture content of the onion samples
Key: WWO - Whole white onion, WRO -Whole red onion

DISCUSSION

The study was to analyze different storage conditions of whole and used (cut-onions) onions. Thestorage conditions of cut and kept at ambient temperature, freezing temperature and refrigerating (low temperature) was used in the course of the study. The heterotrophic bacteria count of the whole-red onion (AR) at Ambient temperature ranged from 2.55×10^6 (6.40) on the day 1 to (3.1×10^6 (6.49) on the day 7 while the count of heterotrophic bacteria of cut-red onion at Ambient temperature (BR) ranged from 2.12×10^4 (4.32) on the day 1 to 4.6×10^8 (8.66) on day 7. The heterotrophic bacterial count obtained is higher than the count of heterotrophic bacteria count reported by Salami *et al.*, (2019). Among the cut-red onion stored at low temperature, the total heterotrophic bacterial count reduced ranging from 2.12×10^4 CFU/g (4.32logCFU/g) on the day 1to 30CFU/g (logCFU/g) on the day 7 with the cut-red onion at freezing temperature (DR) having the least reduced heterotrophic bacterial counts. The heterotrophic bacterial count of the whole- white onion stored at ambient temperature (AW) increased from 9.5×10^4 on the day 1 to 3.9×10^5 on the day 7 while the cut onion sample stored at ambient temperature (BW) had heterotrophic bacteria count increased from 4.5×10^4 CFU/g (4.65logCFU/g) to 5.8×10^6 (6.76logCFU/g) on the day 7. Among the cut white onion stored at low temperature, the total heterotrophic bacterial count reduced ranging from 4.5×10^4 CFU/g (4.65log CFU/g) on the day 1 to 1.5×10^2 CFU/g (2.49 log CFU/g) with the cut-white onion at freezing temperature (DW) having the least reduced heterotrophic bacterial count. Significant growth of heterotrophic bacteria was observed in the redonion compared to the white onion ($p<0.05$) and the cut onions as shown in this study resulting to increases in the count of heterotrophic bacteria when stored at ambient temperature. This might be as a result of increase in the surface for attachment of the nutrient rich onion-bulb as cut onion leaves the inner tissues of the onion bulb exposed (Salami *et al*., 2019).

The total coliform of the whole-red onion stored at ambient temperature (AR) ranged from (4.9×10^3 CFU/g (3.69logCFU/g) to 3.6×10^4 (4.55logCFU/g) while the cut red onion at room temperature (BR) increase from 3.7×10^3 (3.56logCFU/g) on day 1 to 2.4×10^6 CFU/g (6.38 log CFU/g) on the day 7. The count is similar to the count of staphylococcus as recorded in the findings of Oricha *et al*. (2019). Among the cut red-onion stored at low temperature, the coliform count reduced to 1.0×10^2 CFU/g (2.0 log CFU/g) with cut red-onion at freezing temperature having themost reduced coliform count at day 7. The coliform count of the whole-white onion at room temperature (AW) ranged from 3.2×10^4 CFU/g (4.50 logCFU/g) on day 1 to 3.9×10^4 CFU/g (4.59logCFU/g) on the day 7 while the coliform count of the cut-

white onion stored at room temperature (BW) increased from 2.4×10^4 CFU/g (4.38logCFU/g) to 3.4×10^5 (5.53 logCFU/g). Among the cut-white onion sample at lower temperature, the coliform count reduced from 2.4×10^4 (4.38 logCFU/g) to 190 CFU/g (1.29logCFU/g) with the cut-white onion stored at freezing temperature(DW) having the least coliform count. The coliform count of the samples increased in the cut-onion samples stored at ambient temperature and cut sample at freezing temperature resulted in the reduction of coliform growth. Temperature as shown in this study is essential in the preservation of cut onions from coliform thus making it available for subsequent used (Willey, 2011).
The staphylococcal count of the whole-red onion ranged from 3.9×10^4 CFU/g (4.59logCFU/g) to4.9×10^4 CFU/g (4.69logCFU/g) while the staphylococcal count of the cut-red onion at ambient temperature increased from 4.8×10^3 CFU/g (3.68 logCFU/g) to 4.6×10^6(6.66 logCFU/g). Amongthe cut red onion at lower temperature, the staphylococcal count of the samples reduced from the highest count of 4.8×10^4 CFU/g (3.68 logCFU/g) on day 1 to 3.2×10^2 CFU/g (2.50log) CFU/g on day 7 with the lowest staphylococcal count observed in cut-red onion (DR) at freezing temperature.The staphylococcal count of the whole white onion (AW) ranged from, 4.9×10^4 CFU/g (4.69 logCFU/g) on day 1 to 5.9×10^4 CFU/g (4.77 logCFU/g) on the day 7 while the staphylococcal count of the cut white onion (BW) stored at ambient temperature increased from 3.7×10^3 CFU/g (3.56logCFU/g) to 3.5×10^6 (6.54 logCFU/g). Among the cut white onion sample at lowertemperature, the staphylococcal count reduced from 3.7×10^3 CFU/g (3.56 logCFU/g) to 740 CFU/g (1.86logCFU/g) with the cut-white onion sample stored at freezing temperature (DW) having the least staphylococcal count. The presence of Staphylococcus in Onion was also revealed by the findings of Salami *et al.,* (2019) although the growth count was lower than count observed in this study. *Staphylococcus* is commensal and presence could be as a result of cross contamination during harvesting, transportation and sales. The growth or Staphylococcus is reduced by freezing temperature (Willey, 2011).
The fungal count of the whole red onion stored at ambient temperature (AR) ranged from 4.9×10^3CFU/g (3.69logCFU/g) to 6.8×10^4 CFU/g (3.83logCFU/g) while the fungal count of cut red onion at room temperature (BR) increased from 4.2×10^3 CFU/g (3.62 logCFU/g) to 2.7×10^4 (4.43 logCFU/g). Among the cut red onion at lower temperature, the fungal count of the samples reducedfrom the highest count of 4.2×10^3 CFU/g (3.62 logCFU/g) on day 1 to 1.9×10^2 CFU/g (2.27log) CFU/g on day 7 with the lowest fungal count observed in cut red onion at freezing temperature (DR). The fungal count of the whole white onion (AW) ranged from, 8.7×10^3 CFU/g (3.93 logCFU/g) on day 1 to 8.8×10^8 CFU/g (3.93 logCFU/g) on the day 7 while the fungal count of thecut-white onion stored at room temperature (BW) increased from 7.6×10^3 CFU/g (3.88 logCFU/g)to 7.9×10^4 (4.89 logCFU/g). Among the cut-white onion sample at lower temperature, the fungal count reduced from the highest count of 7.6×10^3 CFU/g (3.88 logCFU/g) to 2.0×10^2 CFU/g (2.30 logCFU/g) with the cut-white onion (DW) stored at freezing temperature having the least fungal count. No growth of *Salmonella* and *Shigella* was recorded in the course of the study.
The different bacteria identified were *Bacillus* sp, *Micrococcus* sp, *Klebsiella* sp, *Pseudomonas* sp, and *Staphylococcus* sp. *Saccharomyces* sp *Aspergillus niger, Aspergillus flavus, Mucor* sp, *Candida* sp, *Rhizopus* sp *and Penicillum* sp . These organisms are similar to the microorganisms identified by Salami *et al.* (2019) and Oricha, (2019) as being the major spoilage organisms of onion bulbs.
Among the bacteria isolated for both the red and white onion, *Bacillus* sp had the highest frequency of occurrence while *Micrococcus* sp had the lowest frequency of occurrence in red onion sample and *Klebsiella* sp had the least frequency of occurrence of the white onion samples. *Aspergillus niger* was also of the highest fungi abundant in both red and white onion. *Bacillus* sp form spores which enable them to survive in an unfavorable condition like hot and cold temperature (Willwy *et al.,* 2011). Generally spoilage fungi are known to toxigenic or pathogenic under favorable conditions (Adebayo *et al*., 2012). Al-Hindi *et al.,* (2011) isolated toxigenic fungi from spoilingfruit, pathogenic fungi are cable of causing infections. Aspergillus spp are known to produce several toxic metabolites (Samuel and Ifeanyi 2015). (Oricha, 2019), and it was also identified as the most abundant fungi among others isolated from onion (Salami *et al*., 2019) (Nkem *et al*., 2020).
Moisture content was observed to be higher in red onions (15.23%) sample compared to the moisture

content of white onion sample which had (14.4%). The pH of the onion samples were observed to be lower in white onion sample (5.3) compared to the red onion sample with pH value of 5.8. Moisture content and pH are important factors influencing food spoilage (Wiley *et al.*, 2011). Storage of food is an important consideration in the preservation and availability of food and onionis an important vegetable in food preparation and spicing of food. As revealed by this study, onion bulb cut and kept can result in increase in the microbial load of onions because of their exposed tissues however; freezing to a large extent can ensure the preservation. Therefore, it is best recommended for cut onions to be stored for later use.

The result has shown that some of the organisms isolated from the onions are pathogens and as much makes it a potential source for the spread of food borne pathogens. Damage of onions at harvest or during packing and temperature of storage also affect the spoilage of onions. Also improper environmental conditions during harvesting, transit, storage and marketing may favor these microorganisms causing spoilage.

RECOMMENDATION

1. Farmers should follow good farming procedures and storage technique to avoid contamination of the onion vegetable by microorganisms.
2. Proper sanitation practices should be maintained during harvesting, processing, handling transportation and storage before distribution to local market sellers.
3. Retailers/sellers of the onion vegetable should maintain good storage conditions to prevent contamination by microorganisms.
4. In household's onions should be stored in baskets and kept in a cool, dry and well-ventilated area. Onions kept in the fridge or freezer should be properly sealed in an airtight container.
5. There is need to educate consumers on the health implications of pathogenic organisms associated with onions so as to avoid food borne infections.

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