Isolation of microorganisms from infected onions (*Allium cepa*) popularly consumed by low income earners in Ibadan, Nigeria

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ABSTRACT

Samples of three (3) different varieties of infected onion bulbs were collected from different locations at Oja-oba, Muslim, Oke-ado and Bode markets of Ibadan. Isolation of microorganisms from infected onions (*Allium cepa*) was carried out. The results revealed that the infected onion bulbs were associated with *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp., *Proteus* sp., *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Aspergillus fumigatus*. It was also observed that the market where samples were collected and the variety of onions have no significant effect on the level of bacterial infestation. The mean total bacteria count ranges from $2.2 \times 10^3$ to $8.5 \times 10^3$.

Furthermore, the market where samples were obtained and types of onions had no significant effect on the fungal infestation level. The level of infestation ranges from $1.1 \times 10^1$ to $3.1 \times 10^1$.

*E. coli* had the lowest bacterial prevalence of 8.3% in the study area when compared to *Klebsiella* sp. (33.3%), *Proteus* sp. (30.6%) and *Salmonella* sp. (27.8%). *Aspergillus niger* had the highest fungal prevalence of 44.8% while *Aspergillus flavus*, *Aspergillus fumigatus* and *Rhizopus stolonifer* had prevalence of 26.9, 14.9 and 13.4% respectively. It shows that the different varieties of onions purchased at the selected markets in Ibadan, Oyo State contained different pathogenic microorganisms. Adequate care should be taken in processing onions (*Allium cepa*) to prevent food poisoning.

Keywords: Markets, low income earners, infected onions, microorganism.

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INTRODUCTION

Onion (*Allium cepa*) is an important vegetable crop commonly grown in the world (Jay, 2005) for consumption and economic value to farmers. It belongs to the family *Alliacea* (Barth et al., 2009) and the crop is grown for its bulbs which are used daily in every home for seasoning and flavouring.

Onion is a valuable ingredient in the diet due to its content of sugars, vitamins and minerals (Ole et al., 2004). There are three (3) varieties of Onions: Red, White and Oriental. Most onion cultivars have been reported to have about 89% water, 4% sugar, 1% protein, 2% fibre and 0.1% fat (Denton and Ojeito, 1990). The consumption of onions in Nigeria has increased tremendously in the recent years probably due to increase in awareness on the health importance (Asagbara and Oyewole, 2002). It can be sautéed to dark brown and served to provide sweet and sour flavour to other foods (Ole et al., 2004).

Red onions sometimes called purple onions are cultivars of the onion with purplish red skin and white fleshy tinged with red. These onions tend to be medium to large in size and have a mild to sweet flavor. They are often consumed raw, grilled or lightly cooked with other foods, or added or as colour to salads. They tend to lose redness when cooked. Red onions are available throughout the year (Hung et al., 2004).

The red colour comes from Anthocyanidins and can be stored 3 to 4 months at room temperature. Red onions are available year round in summer and early fall, when they haven’t been in storage for long; they taste sweeter,
with sharpness intensifying through the winter months (Ole et al., 2004).

Oriental onion is an edible species of onion, native to China and cultivated in many other countries. Owing to its very mild and fresh taste oriental onion is often pickled and served as a side dish in Japan and Vietnam to balance the stronger flavour of some other component in a meal. It is used as a folk medicine in tonics to help the intestines and as a stomachic (Ole et al., 2004).

White onion is a type of dry onion that has a pure white skin and a sweet, mild white flesh. This onion is used in Mexican foods or complementing the flavours of other ingredients. Onions (Allium cepa) is used widely in seasoning, flavouring and sometimes cured and stored as spicing agent (Frazier and Westhoff, 2005). When improperly stored deteriorates and spoil during preservation which makes it unavailable for use all year round due to the effect of insufficient dryness of bulbs before storage or as a result of little quantity of storage microorganisms present on bulbs going into storage (Banwart, 2001; Charles, 2012).

Pathogens such as: Pseudomonas sp., Staphylococcus sp. and Erwinia sp., were identified with the crop (Dogondaji et al., 2005) and this often reduces germination, and yield of the crop. Pathogen attacks result in a decreased rate of plant photosynthesis (Kocal et al., 2008) and as a consequence yield loss (Berger et al., 2007). Pathogen infection often leads to plant death, the development of chlorotic and necrotic (Kim et al., 2010) lesions and to a decrease in photosynthetic assimilate production. Hence this study was carried out to evaluate the microbiological status of defected onions consumed by low income earners (Frazier and Westhoff, 2005; Banwart, 2001). The aim of this work is to isolate, identify and characterize the different micro-organisms that are associated with spoilage of onions and to categorize the pathogenic micro-organisms associated with the spoilage of onions.

**Aim and objectives**

The aim of this study is to isolate microorganisms from infected onions (Allium cepa) popularly consumed by low income earners in Ibadan, Nigeria with the under listed objectives:

i. To isolate the microorganisms causing spoilage in the selected onions
ii. To identify the microorganisms isolated in (i) above
iii. To characterize the microorganisms identified in (ii) above using biochemical test

**Justification**

It is an investigation to checkmate the public health significance on people by determining the risk posed to the consumers of spoilt onions and to suggest control measures and possibly solutions in avoiding the spoilage and its storage.

**MATERIALS AND METHODS**

**Sample collection**

Three different varieties of infected onions (Red, Oriental, and White) were randomly sampled from four different markets (Bode, Oja-oba, Oke-Ado and Muslim) all in Oyo State. The samples were kept in sterile labeled polythene bags and taken to the Laboratory for further analysis (Appendix).

**Culturing and isolation of organisms**

An appropriate section of 1.0 g were cut from each of the sample purchased from the markets with a sterile scalpel knife and was homogenized. The resultant homogenate was added to 10 ml of distilled water in a test tube and diluted serially. This was done to thin out the microbial load in the samples. Sterile pipettes were used to take 1ml of the serially diluted samples and plated on different media using spread plate method.

All culture media were prepared according to manufacturer’s instruction. MacConkey Agar (Mcc) and Nutrient Agar (NA); 25 g of the agar was weighed and dispensed into a sterile conical flask, 150 ml of distilled water was also measured and added to the agar. The mixture was homogenized and heated in a microwave for 10 min, then sterilized at 121°C for 15 min and allowed to cool before pouring. It was incubated at 35°C for 24 h for bacterial count and observes the morphological features of bacterial colony while Saboraud Dextrose Agar (SDA) for fungal growth was incubated at room temperature (25°C) for 72 h. Sub cultivating of different distinct colonies from bacteria growth was done on Nutrient agar and stored at 4°C for biochemical test.

All the bacterial isolates were identified on the basis of colony characteristics, morphological and biochemical reactions as described in Bergeys’ Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Fungi isolates were observed under the microscope using the wet mount method. A loop of the isolated colony was teased out onto a grease free slide and a drop of lacto phenol cotton blue was added and covered with cover glass and the fungus was examined microscopically. It was viewed under the microscope at ×40 objective lens. Colonies of fungi were observed on plate for colour, appearance and under the microscope for its spore type and nucleus which were compared with the diagram and structure of known fungi using identification keys of Ellis et al. (2007) and Campbell et al. (2013).

**RESULTS AND DISCUSSION**

From table 1, the mean total bacteria count for red onion, obtained at Bode, Oja-Oba, Oke-Ado and Muslim markets were not significantly different from each other (p ≤ 0.05). Also, the mean total bacteria count obtained on oriental onions at Bode, Oja-Oba, Oke-Ado and Muslim markets were not significantly different from each other (p ≤ 0.05). The mean total bacteria count obtained on white onions at Bode, Oja-Oba, Oke-Ado and Muslim markets
were also not significantly different from each other (p ≤ 0.05). These indicate that the market where onion samples were obtained has no significant effect on the level of bacterial infestation of onions.

The mean total bacteria count obtained from red onion, oriental onions and white onion at Bode, Oja Oba were not significantly different from each other (p ≤ 0.05). The mean total bacteria count for red onion, oriental onions and white onion obtained at Bode, Oke-Ado and Muslim markets respectively were not also significantly different from each other at each of the market (p ≤ 0.05). This implies that the variety of onions also has no effect on the level of bacterial infestation. From this Table 1, it could be observed that, neither the market nor species of onions has effect on the bacterial infestation level. The level of infestation ranges from $2.2 \times 10^3$ on white onion obtained at Bode market to $8.5 \times 10^3$ on red onion obtained at Oja-Oba market.

From Table 2, the mean total fungal count obtained reveals that, there were no significant differences (p ≤ 0.05) in the values obtained from different type of onions. Also, there was no significant difference (p ≤ 0.05) in the mean total fungal count at different market where samples were obtained. This implies that neither the market nor species of onions has effect on the fungal infestation level. The level of infestation ranges from $1.1 \times 10^1$ on red onion obtained at Oke-Ado market to $3.1 \times 10^1$ on Oriental onion obtained at Muslim market.

This agrees with the reports of other researchers (Muhammad et al., 2004; Dimka and Onuegbu, 2010) that fungi constitute a menace in the storage of many agricultural commodities including fruits, vegetables and nuts.

From Table 3, four bacteria isolates were identified; they are Klebsiella sp., Proteus sp., Salmonella sp., and Escherichia coli. Klebsiella sp. has the highest prevalence of 33.2% in the study area. The prevalence of Proteus sp. was 30.5%, Salmonella 27.9% while E. coli had the least prevalence of 8.3% in the study areas.

The prevalence of Klebsiella sp. was 8.3% at each market where samples were taken. Proteus sp. has prevalence of 5.6% at Bode market while having 8.3% in the other three markets respectively. Salmonella sp. has prevalence of 11.1% at Oke-Ado market while having 5.6% in the other three markets respectively. E. coli was isolated only at Muslim market; it was not isolated from Bode, Oke-Ado and Oja-Oba markets (Figures 1 and 3).

From Table 4, four fungi strain were isolated; Aspergillus flavus, Aspergillus fumigatus, Rhizopus stolonifer and Aspergillus niger. A. niger had the highest prevalence of 44.8% while Aspergillus flavus, A. fumigatus and R. stolonifer had prevalence of 26.9, 14.9 and 13.4%, respectively. Prevalence of each of the four isolated fungi species were lowest at Muslim market with Aspergillus niger having 7.5%, Aspergillus flavus 6.0%, while A. fumigatus, R. stolonifer had 1.5%, respectively (Figures 2 and 4).

Prevalence of each of the four isolated fungi species were highest at Bode market with A. niger having 17.9%, A. flavus 7.5%, A. fumigatus, 6.0% and R. stolonifer having 4.5%. This agreed with the findings of Adebayo and Diyaolu (2003).

R. stolonifer (4.47%) had the highest number of isolate from Bode market and Oke-Ado markets respectively as

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**Table 1.** Mean total bacterial count (cfu/g) isolated from different varieties of onion (*Allium cepa*) in different markets in Ibadan.

| Market     | Red (×10³) | Oriental (×10³) | White (×10³) |
|------------|------------|----------------|--------------|
| Bode       | 3.9 ± 0.5a³ | 3.2 ± 0.1a³ | 2.3 ± 0.2a³ |
| Oja-Oba    | 8.5 ± 0.1b³ | 5.3 ± 0.3b³ | 4.2 ± 0.2b³ |
| Oke-Ado    | 6.6 ± 0.2b³ | 5.8 ± 0.2b³ | 4.5 ± 0.3b³ |
| Muslim     | 4.3 ± 0.3b³ | 6.1 ± 0.3b³ | 4.2 ± 0.2b³ |

*a* indicates no significant difference down the column

Same lowercase superscripts indicate no significant difference across the row.

**Table 2.** Mean total fungal count (cfu/g) isolated from different varieties of onion (*Allium cepa*) in different markets of Ibadan.

| Market     | Red (×10) | Oriental (×10) | White (×10) |
|------------|-----------|----------------|-------------|
| Bode       | 2.5 ± 0.5ab | 2.4 ± 0.5ab | 1.8 ± 0.3ab |
| Oja-Oba    | 1.2 ± 0.2ab | 2.5 ± 0.1ab | 2.5 ± 0.2ab |
| Oke-Ado    | 1.1 ± 0.3ab | 2.2 ± 0.3ab | 1.9 ± 0.1ab |
| Muslim     | 2.2 ± 0.1ab | 3.1 ± 0.1ab | 2.4 ± 0.1ab |

Same lowercase superscripts indicate no significant difference across the row

Same uppercase superscripts indicate no significant difference down the column.
Table 3. Prevalence of bacteria isolates from different onion varieties (*Allium cepa*) in different markets in Ibadan.

| Isolates     | Total no. of isolates | Prevalence of isolates (%) | Prevalence of isolates at each markets (%) |
|--------------|-----------------------|-----------------------------|-------------------------------------------|
| *Escherichia coli* | 3                     | 8.3                         | 0 0 0 8.3                                 |
| *Salmonella sp.*    | 10                    | 27.9                        | 5.6 11.1 5.6 5.6                          |
| *Proteus sp.*       | 11                    | 30.5                        | 5.6 8.3 8.3 8.3                           |
| *Klebsiella sp.*    | 12                    | 33.2                        | 8.3 8.3 8.3 8.3                           |

Figure 1. Prevalence of bacterial isolates.

Figure 3. Pictorial prevalence of bacterial isolates.

reported by Dimka and Onuegbu, 2010.

From this result, it shows that onions purchased from Bode, Oke-Ado, Oja-Oba and Muslim markets in Ibadan were contaminated with bacterial and fungal isolates which were *Klebsiella sp.*, *Proteus sp.*, *Salmonella sp.*, *E. coli*, *A. flavus*, *A. fumigatus*, *R. stolonifer* and *A. niger*.

Different species of onions have effect on the level of both bacterial and fungal infestation. Also, location where samples were obtained did not also have effect on the degree of infestation of bacteria and fungi. However, Bode market has the highest prevalence of fungal infestation while Muslim market has the least. Therefore,
Table 4. Prevalence of fungi isolates from Onion (*Allium cepa*) in different markets in Ibadan.

| Isolates            | Total no. of isolates | Prevalence of isolates (%) | Prevalence of isolates at each markets (%) |
|---------------------|-----------------------|----------------------------|--------------------------------------------|
|                     |                       |                            | Bode | Oke-Ado | Oja-Oba | Muslim |
| Aspergillus niger   | 30                    | 44.8                       | 17.9 | 9.0     | 10.4    | 7.5    |
| Aspergillus flavus  | 18                    | 27.0                       | 7.5  | 6.0     | 7.5     | 6.0    |
| Aspergillus fumigatus | 10                | 15.0                       | 6.0  | 4.5     | 3.0     | 1.5    |
| Rhizopus stolonifer | 9                     | 13.5                       | 4.5  | 4.5     | 3.0     | 1.5    |

Figure 2. Prevalence of fungal isolates.

Figure 4. Pictorial prevalence of fungal isolates.

Table 5 described the fungi seen and isolated in the selected markets. *E. coli* had low prevalence of 8.3% in the studied area when compared to *Klebsiella* sp. (33.3%), *Proteus* sp. (30.6%) and *Salmonella* sp. (27.8%). This contamination could be as a result of poor packaging, transporting, and storage. Onions packed in nylon should immediately be spread out in air after
purchase to prevent spoilage caused by the heat generated from the polythene bags. Spoilt onions sold in the market should be avoided. However, adequate care should be taken when processing onions to prevent food poisoning. Further studies should be carried out on onion handling and quality control practices in the markets with a view to avert post-harvest losses and food poisoning.

CONCLUSION

From the results of this study, it can be concluded that all the onions purchased from different markets had high degrees of microbial isolates; conclusively, most of the samples were contaminated with micro-organisms; which is not quite suitable for the consumption of people. It also shows that onion bulbs could be a reservoir for microorganisms such as fungi and bacterial and it could also be as a result of exposing the onions to poor environment like poor packaging (which may increase its temperature and humidity) transporting, and storage.

RECOMMENDATION

It is therefore recommended that onions packed polythene bag should immediately be spread out in air for proper and good ventilation after purchase to prevent spoilage caused by the heat generated from the polythene bags. Good storage facility should be made available. Spoilt onions sold in the market should be avoided and its consumption discouraged. However, adequate care should be taken when processing onions to prevent food poisoning. Further studies should be carried out on onion handling and quality control practices in the markets with a view to avert post-harvest losses and food poisoning.

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| Organisms       | Colour                              | Appearance                      | Spore type                        | Nucleus               |
|-----------------|-------------------------------------|---------------------------------|-----------------------------------|-----------------------|
| Asperillus niger| Surface is black while the underneath is white to yellow | Filamentous with branching mycelia | Long, smooth, brown or yellow conidiospores | Multinucleated        |
| Rhizopus stolonifer| Dark or black with patches | Filamentous with cottony mycelia | Sporangiospore                    | Multinucleated        |
| Asperillus flavus| Yellow-green while the underneath is gold to red-brown | Filamentous with branching mycelia | Colourless rough conidiospores     | Multinucleated        |
| Asperillus fumigatus| Blue-green on its surface and white underneath | Filamentous with branching mycelia | Short, smooth grey-green conidiospores | Haploid               |

Table 5. Morphology description of isolated fungi from onion (Allium cepa) in different markets in Ibadan.
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APPENDIX

Red onion | White onion | Oriental onion