



**OYO STATE COLLEGE OF AGRICULTURE AND
TECHNOLOGY, IGBOORA (OYSCATECH)**



in Collaboration with

OYSCATECH GLOBAL CONSULT

PRESENTS

2ND BIENNIAL CONFERENCE

TAGGED:

OYSCATECH

2021

E-Proceeding

Theme:

**A SYNERGY BETWEEN AGRICULTURAL PRODUCTION,
TECHNOLOGICAL INNOVATION & MANAGEMENT EFFICIENCY:
A PANACEA TO ECONOMIC STABILITY
DURING PANDEMIC**

Date: Mon. 12th - Thur. 15th July, 2021

Venue: 800-Seater, Lecture Hall, OYSCATECH, Igboora

Editors:

Dr. W. B. BELLO, Dr. M. A. MOSOBALAJE

Dr. G. O. OYELERE, Mr. R. A. AJAO

Evaluation of Microbial Load Associated with the Spoilt Fruits of Pawpaw(*Carica papaya*) and Orange(*Citrus sinensis*).

Amusat, A.I.,¹ Amusat, M.A.,² Bamigboye, O.F.¹ and Ibraheem, A.A.¹

¹Department of Science Laboratory Technology, Oyo State College of Agriculture and Technology, Igboora., Emmanuel Alayande College of Education, Erelu, Oyo, Oyo State.

Corresponding Author E-mail: wumiamusat@gmail.com

Abstract

This study was carried out to isolate the bacteria and fungi that are associated with deteriorated Orange and Pawpaw. Samples of the Orange and Pawpaw were purchased at Towobowo market, Igboora, Oyo State. Analysis was carried out at Research Laboratory, Oyo State College of Agriculture and Technology, Igboora. Pour plate methods were used to investigate the microbial load of spoilt samples. Discrete Isolated colonies from these sources were sub-cultured to get purified culture for the study. The bacteria and fungi species were identified and investigated using standard methods of determination. The bacteria and fungi estimated for Orange includes; Total plate count (1.52×10^3 cfu/ml), Total yeast/mould count (5.26×10^2 cfu/ml) and Lactic acid bacteria (6.00×10^2 cfu/ml) respectively. Also, bacteria and fungi estimated in Pawpaw were; Total Plate count (1.85×10^3 cfu/ml), Total yeast/mould count (6.54×10^2 cfu/ml) and Lactic acid bacterial (7.12×10^2 cfu/ml) respectively. From the spoilt fruits, microorganisms examined showed that all the evaluated microbes were present in the two fruits, however, the microbial load of pawpaw are more than that of orange. Exposure of Orange and Pawpaw to air and poor handling gives room for deterioration by microorganisms as shown in the research work. Therefore fruits should be well protected from contaminant to reduce spoilage by microorganisms.

Key words: Microorganisms, Pawpaw, Orange, Bacteria, Fungi, Spoilage, Deterioration.

Introducton

Fruits and vegetables are vital source of nutrient to human beings. They give the body the necessary vitamins, fats, minerals and oil in the right proportion for human growth and development. Fruits and vegetables however, have serious challenges in their existence and this includes changes in climatic condition, pest, inadequate rainfall and fungal attack. Over the years, there had been an increase in the need to identify and isolate the fungi associated with their spoilage (Pau *et al.*, 1998). Spoilage refers to any changes in the condition of food in which the food becomes less palatable, or even toxic, these changes may be accompanied by attraction in taste, smell or texture (Pau *et al.*, 1998). Numerous microbial defects (Signs and symptoms) of agricultural crops are characterized by the types of microorganism responsible for deterioration; the process of infection in the case of fungal penetrating structure (aspensorium). Colonization of fungal is a critical phase in the microbial spoilage of post harvested fruits. The colonization processes involves the ability of the microorganism (Fungi) to establish following adhesion, and release of enzymes) (Akinmusire, 2011). Susceptibility of fruits and vegetables is largely due to differential chemical composition such as pH and moisture contents. The higher pH and moisture contents are associated with greater, predisposition to fungal spoilage. The occurrence of fungal spoilage of fruit is also recognized as a source of potential health risk as hazard to man and animals. This is due to production of mycotoxins (naturally occurring toxic chemical often of aromatic structure) compounds which are capable of including mycotoxicoses in man following ingestion or inhalation, but differ in their degree and manner of toxicity (Eni *et al.*, 2010). The contamination of fruits and vegetables by fungal could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Eni *et al.*, 2010). The preponderance of the isolated moulds, from orange (*Citrus sinensis*) belongs to *Aspergillius* species and this confirms their prevalence in foods and fruits and their by product.

Aspergillus spp., *Mucor spp.*, and *Fusarium spp.* were responsible for spoilt pawpaw (*Carica papaya*) in Awka, Anambra State (Onuorah *et al.*, 2014). Fungi as *Phytophthora cinnamon*, *Aspergillus niger* and *Candida* species fruits supply some necessary nutritional substances such as vitamins and essential minerals in human diet, this keeps the body in good and healthy condition (Ewekeye *et al.*, 2013) as discovered in their studies on postharvest deterioration of some fruits and vegetables in selected markets in Lagos State, Nigeria. It was also reported that consumption of fruits and vegetable products had dramatically increase in Nigeria by more than 40% during the past few decades. It is also estimated that about 30% of all fruits and vegetables produced are lost yearly due to spoilage.

Pawpaw (*Carica papaya*) fruits have been used for medicinal purposes for centuries as herbs and traditionally used for varieties of conditions related to respiratory, intestine, kidney and liver function. Orange (*Citrus sinensis*) also known as sweet orange is the most popular of the citrus fruits, it is widely cultivated in most regions of the world possessing a rich source of vitamin C, flavonoids, phenolic compounds and protein. Therefore, this research was conducted to evaluate the microbial load associated with the spoilt orange and pawpaw bought from Igboora so as to alert the society the risks and dangers associated with consumption.

Materials and methods

Sample Collection: Orange and Pawpaw fruits were purchased from Towobowo Market, Igboora, Oyo State. Four Samples (2 each) were collected from fruit vendors and kept in polythene bags for 72 hours for it to deteriorate by microorganisms.

Preparation of Media: Culture Media for this isolation were nutrient Agar (NA), Mac Conkey Agar (MAC), which was prepared by measuring 28g of their powder and mixed with 100ml of distilled water and mixed vigorously and incubated at 37°C for 48 hours and then kept inside refrigerator until use for bacteria isolation. 37.5g of Saboraud Dextrose Agar (SDA), and potato Dextrose Agar (PDA) powder measured, dissolved in 100ml/ 1 litre of distilled water and mixed vigorously, then incubated at 25°C for 3-5 days and then kept inside refrigerator until use for fungi isolation.

Isolation of Bacteria: The pour plate method according to Harigan and Mc Cane, (1990) was adopted, using Standard Microbiological Technique (Serial Dilution). A tenfold dilution of 1g of the sample was carried out in 9ml of sterile water (this was the aliquot) precisely, 1ml of the aliquot (supernatant) was pipetted and mixed in another 9ml of sterile water in a test tube. The test tube was shaken vigorously to homogenize. The exponential dilution continued to the fourth factor (10^{-4}) 1ml of the fourth factor was aseptically transferred and plated in duplicate set wing sterile molten lukewarm nutrient Agar (NA) which was amended with 1%. The pour plates were set and incubated at 37°C for 46 hours. Discrete colonies that developed after incubation were counted and enumerated as colony forming unit (Cfu/ml) after multiplying with the dilution factor (10^{-4}).

Isolation of Fungi (Yeast / Moulds): The pour plate method was used for the isolation of Fungi following the method of Barnett and Hunter, (1987). The BECTO Sabouraud Dextrose Agar was used, the diluents from the 4th test were aseptically transferred to sterile Petri dishes and about 15 – 20ml of sterile molten lukewarm SDA was pour into plate, allowed to set and incubated at room temperature ($28 \pm 2^\circ\text{C}$). Colonies that developed after incubation were counted and enumerated in colony forming unit per milliliter (Cfu/ml) sample.

Purification (Subculture) of Bacterial Isolates: Colonies from the primary plates were aseptically picked with sterile wire loop and transferred into freshly prepared sterile nutrient Agar plate, with a Streaking Technique such that discrete colonies appeared at the end of streaked line after incubation. The subculture plates were incubated at 37°C for 24hrs to 48hrs. Discrete colonies from the subculture plate were aseptically transferred and streaked on slant and incubated for another 24hrs at 37°C

Purification of Fungi Isolates: Colonies from primary plate were aseptically picked with sterile inoculation needle and transferred into a freshly prepared sterile SDA plate with streaking method and incubated for 5 – 7 days at temperature of 28 – 30°C. Discrete colonies were aseptically transferred and stickled on slant and incubated for 5days at 28°C to 30°C. Pure colonies were stored in the refrigerator at average of temperature of 10°C - 15°C until ready for Identification.

Results and discussion

Results

Table 1: Microbiological densities in deteriorated Orange fruits after four (4) days

Parameter Colonies forming unit per milliliter (Cfu/ml)
Total Plate Count 1.52×10^3
Total Yeast / Mould count 5.26×10^2
Lactic Acid Bacterial 6.00×10^2

Table 2: Microbiological densities in Pawpaw fruits deteriorated for four (4) days

Parameter Colonies forming unit per milliletr (Cfu/ml)
Total Plate Count 1.85×10^3
Total Yeast / Mould count 6.54×10^2
Lactic Acid Bacterial 7.12×10^2

The results on the isolation of Microorganism associated with deterioration of Orange and Pawpaw fruits describe as follows: the microbial loads or densities during the four (4) days deterioration process of Orange are shown in table 1. The total plate counts were found to be 1.53×10^3 . the yeast / mould densities were found to be 5.26×10^2 (Cfu/ml) and the total bacterial count were estimated to be 6.00×10^2 Cfu/ml. The microbial loads of Pawpaw revealed total plate counts to be 1.85×10^3 , the Yeast / Mould were counted to be 6.54×10^2 and the Lactic Acid Bacteria were counted to be 7.12×10^2 Cfu/ml. The total plate count, the yeast / moulds density and lactic acid bacterial found in Pawpaw was higher than that of the Orange. The results showed that the parameter evaluated revealed that the microorganism had access to Pawpaw than the Orange, this could be due to the presence of favourable conditions that made the microorganisms to thrived better such as (moisture content, moderate temperature and sugary content) unlike acidic medium in orange.

Discussion

The result revealed higher mould and lactic Acid Bacteria in both samples compared to the result of Angela *et al.* (2010) that reported 3.8×10^6 and 2.9×10^7 for carrot in samples (vendor A & B) respectively. While sample C (7.8×10^6) had values higher than the present work, Angela *et al.* (2010) also reported 1000 colony counts for pineapple in vendor A (1.3×10^6) and vendor C (4.6×10^6) respectively. The high bacteria counts observed in the present study are similar to those obtained in previous studies (Uzeh *et al.*, 2009 and Bukar *et al.*, 2010). Although lower plate counts was 1.52×10^3 . The present result showed high bacteria count for Pawpaw (7.12×10^2) this result deviated from the report of Mbajiuka *et al.* (2014) who reported (4.10×10^3) for tomato fruit in Abia State. They also reported 5.00×10^3 bacteria count for Pawpaw and this is also lower compared to the present results.

This finding revealed total yeast/mould count to be 6.54×10^2 for Pawpaw which is higher compared to the value reported by Mbajiuka *et al.* (2014) for Pawpaw (5.00×10^4) while reported values for tomato was higher (9.8×10^7) compared to the present study.

Conclusion

From the results obtained, it was discovered that organisms encountered are food borne pathogens. It was also revealed that some spoilage microorganism gained access into these fruits during the process of cultivating, harvesting, packing and environmental contaminants which had in one time or the other been involved in food poisoning.

The frequency of occurrence of bacterial was higher than that of fungi in both fruits. The high prevalence of bacteria and fungi demands that appropriate control measure against infection should be employed. Adequate microbiological knowledge and handling practice of these produce would help minimize waste due to deterioration. It is therefore, important that both the farmer who harvests the fruit into bags for transportation, the marketers and consumers take necessary precaution in preventing fruit contamination. This, enhance reduction in the risk of microbial toxins that are deleterious to human health.

References

- Akinmusire, O.O. (2011). Fungal Species Associated with the Spoilage of Some Edible Fruits in Maiduguri Northern Eastern Nigeria. *Advances in Environmental Biology*, 5, 157-161.
- Akintobi, A.O., Okonko, I.O., Akano, O.R., Agunbiade, S.O. And Onianwa, O. (2011). Isolation and identification of fungi associated with the spoilage of some selected fruits in Ibadan, South Western Nigeria. *Academia Arena*, 3(11): 1-12.
- Angela O.E., Ibukunoluwa, A.O. and Oranusi, U.S (2010) Microbial Quality of Fruits and Vegetables Sold in Sango Ota, Ogun State, Nigeria. *African Journal of Food Science*, 4(5): 291-296.
- Bukar, A., Uba, A., Oyeyi, T.I. (2010). Occurrence of some Enteropathogenic Bacteria in some minimally and fully processed read-to-eat foods in Kano metropolis, Nigeria. *African J.Food Sci.* 4(2):32-36.
- Bernelt, H. and Hunter, B.B. (1987). Illustrated general of imperfect fungi 4thedn Macmillan publishing company, New York USA.PP 106-130.
- Eni, A.O., Ibukunoluwa, A.O., and Oranusi, U.S., (2010). Microbial Quality of Fruits and Vegetables sold in Sango Otta, Nigeria. *Afr. J. Food.Sci.* 4(5):291-296.
- Ewekeye, T.S., Oke, O.A., Quadri, A.I., Isikalu, A.O., Umenwaniri, M.O. and Durosini, M.L. (2013). Studies on Post-harvest Deterioration of Some Fruits and Vegetables in Selected Markets in Lagos State, Nigeria. *American Journal of Research Communication*, 1pp, 209-22
- Harigan, E.F. and McCane, M.E, (1990). Laboratory Methods in food and Dairy microbiology Academic. Press London 5. Pp 12-14.



- Pau, A.T., Lund, B.M., Baird-parker, A.C. and Gould. (1998). The Microbiological Safety and Quality of Foods “. Aspen Publishers, Inc. Gaithersburg, Maryland, U.S.A. 1885pp.
- Uzeh, R.E., Alade, F.A. and Bankole, M. (2009). The Microbial Quality of Pre-packed mixed Vegetable Salad in some Retail outlets in Lagos, Nigeria. *Afr. J. Sci.* 3(9):270-272.
- Mbajiuka, S., Chinedu, and Enya, E. (2014). Isolation of Microorganisms Associated with Deterioration of Tomato (*Lycopersicon esculentum*) and Pawpaw (*Carica papaya*)Fruits. *Inter.J. Curr.Micro. Appl.Sci.* 3(5):501-512.