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Isolation and Identification of Microorganisms Involved in the Spoilage of Pawpaw Fruit (*Carica papaya*) Enugu Metropolis, Nigeria.

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ABSTRACT

This project work was carried out to isolate and identify the microorganisms involved in the spoilage of pawpaw fruit (Carica papaya). Microorganisms associated with spoilage of the fruit were studied using standard microbiological method. The serial dilution method was employed using nutrient agar, Mac Ctonkey agar, CLED agar and Sabouraud dextrose agar. A total number of 10 samples of spoilt pawpaw fruits (Carica papaya) were examined by culturing them on nutrient agar, CLED agar and MacConkey agar for bacteria and sabouraud agar for fungi. The bacterial isolates were *Escherichia coli* (27.6%) which had the highest occurrence, *Enterobacter spp.* (24.4%), *Staphylococcus aureus* (29.6%) with *Pseudomonas* (18.6%) having the least occurrence .The fungal isolates obtained were *Aspergillus niger, Aspergillus flavus* and *Fusarium spp.* The fungal and bacterial loads were high enough to cause food spoilage or food infection. The presence of the isolates may be due to improper hygienic practices from the point of harvesting, transportation and storage by handlers. Above all, the high moisture content of pawpaw fruit (Carica papaya) makes it highly perishable as its supports the growth of microorganisms.

Keywords: Microorganisms, Spoilage, Pawpaw Fruit (*Carica papaya*).

INTRODUCTION

Fruits are very important and have dietary and nutritional qualities. Consumption of fruit products has dramatically increased by more than 30% during the past few decades [1]. During the period 1870 - 2004, US per capita consumption of fruits increased by 19.9%, to 694.3 pounds per capita per year. Fresh fruit and vegetable consumption increased by 25.8 and 32.6%, respectively, and far exceeded the increases observed for processed fruit products. It is also estimated that about 20% of all fruits produced is lost each year due to spoilage [2].

[3] reports that 20 new human fungal pathogens are documented each year. Most microorganisms that are initially observed on whole fruit surface are soil inhabitants. Vectors for disseminating these microbes include soil particles, airborne spores, and irrigation water.

A fruit is the edible part of a mature ovary of a flowering plant. It is usually eaten raw. Fruits could also be described as the succulent or fleshy covering of a nut which is pulpy, often juice in character. As they were developed from the flower of a plant, they consist of ripened seed or seeds with some tissues attached [4]. Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health.

Fruits are widely distributed in nature. One of the limiting factors that influence the fruits economic value is the relatively short shelf-life period caused by pathogens attacked. It is estimated that about 20-25 of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries [5].

Increasing interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decisions about their use [6]. Fruits, apart from being taken as

food also have some medicinal importance. The latex from the trunk of the pawpaw tree is applied externally to speed the healing of wounds, ulcers, boils and warts. The seed is also used to expel worm and the flower may be taken in an infusion to induce menstruation. In the southern part of Nigeria, fruit such as pawpaw production has improved the diet of the local people, whose diet generally consisted of starch staples lacking essential vitamin and minerals [7]. These fruits were usually displayed on benches and in baskets for prospective customers in the open markets until sold, thereby exposing them to further microbial infection beside those associated with the fruit, surface and those from adjacent infected fruits.

The primary cell wall of fruit is composed of approximately 10% proteins and 90% polysaccharides, which can be divided into three groups: cellulose, hemicelluloses and pectin [8], Numerous cell wall degrading enzymes can be secreted by pathogens to breach and use the plant cell walls as nutrient sources that reduced post-harvest life and finally lead to develop inedible, undesirable quality and soft rot spoilage. In developing countries, post-harvest losses are often more severe due to inadequate storage and transportation facilities. Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decayed [9]. Studies by [10] had shown that fungi can survive and/or grow on fresh produce and that the nutrient content (carbohydrate, protein and fat) of fresh produce support pathogens.

Fruits are however, affected by a wide array of microorganisms causing its decay. These microorganisms, under the influence of environmental factors, pose a serious threat to fruits production. Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture [11]. Spoilage fungi that typically produce more diverse and greater amounts of extracellular depolymerases successfully attack and spoil both fruits and vegetables [12]. Fungi in particular produce an abundance of extracellular pectinases and hemicellulases that are important factors for fungal spoilage. Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue [13].

Spoilage microorganisms can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and post-harvesting handling, or during storage and distribution. Those same types of soil-borne spoilage microbes that occur on produce are the same spoilage microorganisms that are present on harvesting equipment, on handling equipment, in the packing house, in the storage facility and on food contact surfaces throughout the distribution chain. Therefore, early intervention measures during crop development and harvesting through the use of good agricultural practices (GAP) will provide dramatic reductions in yield loss due to spoilage at all subsequent steps in the food-to-fork continuum [14]; [15]. Although available literatures reveal that the importance of fruit is increasing daily, the incidence of microbial attack on this fruit demands attention. Over the years, there has been an increase in the need to identify and isolate the fungi associated with their spoilage. The aim of this study was to isolate and identify the fungi that are associated with the spoilage of orange, pawpaw and banana fruits sold in some selected markets in Eastern Nigeria.

AIM OF THE STUDY

This was designed to isolate and identify the microorganisms involved in the spoilage of pawpaw fruit (Carica papaya).

MATERIALS AND METHODS

Materials

The materials used in this study were of analytical standards

Methods

Collection of Sample

The pawpaw fruit was purchased from Ogbete Main Market Enugu State Nigeria. These samples were placed in separate sterile plastic bags and transported to the laboratory for microbial analysis.

Preparation of Culture Media

The media (Nutrient, MacConkey, Sabouraud Dextrose Agar and CLED) for culturing were prepared according to the manufacturer's directives and autoclaved at 121° C for 15 minutes for 15 Ibs pressure. The media were allowed to cool for 45 degrees, then swirled well before aseptically poured into Petri-dishes and allowed to solidify.

Sample Preparation

About one grams (Ig) of fruit pawpaw was weighed out using a mechanical weighing balance, homogenized into 90 ml of sterile distilled water using a sterile blender. Ten fold serial dilutions of the homogenates were made using sterile pipettes.

Laboratory Isolation of Bacteria Organism and Fungi Associated with Pawpaw

From the 10-fold dilutions of the homogenates 0.lml of 10^{-2}, 10^{-3} and 10^{-4} dilutions of the homogenates was plated method. The plates were swirled clock wisely and anticlockwise, allowed to solidify and then incubated at 37°C for 24 -48 hours. MacConkey agar and nutrient agar was used for bacteria isolation while SDA was used for fungi isolation. Total viable aerobic bacteria count was performed in nutrient agar. At the end of the incubation periods colonies were expressed as colony forming unit of the suspension. Desired colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Pure isolates of the resulting growth were then stored at 40°C.

Biochemical Tests carried for Identification of Bacteria Isolates

Catalase Test

A smear of a small portion of a colony under test was placed into a tube containing about 2 ml of hydrogen peroxide. Catalase positive strains caused effervescence (air bubbles) while catalase negative does not.

Coagulate Test

A drop of normal saline was placed on a clean slide. About one or two colonies of the test organism were picked with a sterile loop and emulsified in the drop of saline to form a smooth milky suspension. The inoculating wire-loop was dropped into undiluted plasma obtained by centrifuging human blood to which sodium oxalate an anticoagulant, has been added to a concentration of 0.2 - 0.3 percent. Coarse clumping become visible to the naked eye within 5-10 seconds indicated a positive result while no reaction indicated negative result.

Indole Test

The peptone water medium was inoculated and incubated for 48 hours at 37°C. After 48 hours of incubation, 3 drops of Kovac's reagent was added and was shook very well and allowed to stay for 15 minutes (in each tube). The red ring on the surface of the peptone water indicates positive result, while yellow ring indicates negative result.

Sugar Fermentation Test

This test was carried out using a media called triple sugar iron agar, containing dextrose (simple sugar), lactose and sucrose. The medium was prepared according to the manufacturer's directive which is to dissolve 65gm into 100ml of distilled water, after which 10ml of the prepared medium was added into test

tubes, 3 drops of phenol red was added. Durham's tubes were inserted in an inverted position (for detection of gas production) making sure it touches the medium. The tubes were pluged with non-absorbent cotton-wool and sealed with aluminum foil before being sterilized with autoclave at 105°C for 15 minutes at 15Ib pressure. After sterilization, each tube were inoculated with specific colony and incubated for 24 hours at 37°C and unincubated tubes serve as control.

Acid production was indicated by a change in colour from orange to yellow colour, indicating that acid has been produced and gas production was indicated by the presence of air spaces between the Durham tubes and the medium used presence of air bubbles also indicated presence of gas interring that gas has been produced,

Note
After solidification, the plates were inverted and incubated for 24 hours at 37°C. After incubation, the grown colonies were observed and there morphologies were recorded.

RESULTS

Table 1: Plate Count of Viable Bacterial Organisms Isolated from Pawpaw

Sample Number	Mean Bacterial Count per ml Pawpaw
1	50
2	81
3	72
4	64
5	60
6	37
7	66
8	80
9	49
10	73
Average	68.4

Table 2: Standard Plate of Different Colonial Form Isolated from Pawpaw

Sample Number	Code of Colonial Forms Pawpaw			
	A	B	C	D
1	10	25	9	6
2	20	15	30	16
3	10	2	40	20
4	4	10	20	30
5	15	16	9	20
6	11	9	10	7
7	30	3	21	12
8	20	21	30	9
9	16	5	10	18
10	39	10	8	16
Average	17.5	11.6 18.7		15.4

Key:
A = *Escherichia coli*, B = *Pseudomonas spp*, C = *Staphylococcus spp* and D = *Enterobacter spp*

Table 3: Morphological and Biochemical Characteristics of Bacterial isolates

Morphology	Gram Reaction	Glucose	Lactose	Sucrose	Catalase	Coagulase	Indole	Identified organisms
Rod	-ve	-	+	-	+	-	+	*Escherichia coli*
Rod	-ve	AG	-	AG	-	-	+	*Pseudomonas spp*
Cocci	+ve	AG	AG	AG	+	+	-	*Staphylococcus spp*
Cocci	-ve	AG	-	+	-	-	+	*Enterobacter spp*

Key:
-ve = Gram negative test
+ve = Gram positive test
A = Acid production during sugar fermentation
G = gas production during sugar fermentation
+ = positive
- = Negative

Table 4: Percentage Distribution and Bacterial Isolated from Spoiled Pawpaw fruit

	Name of Bacterial Isolated	Mean Count of Distribution Bacterial organism Pawpaw	Percentage Pawpaw
A	*E. coli*	17.5	27.6
B	*Pseudomonas spp*	11.6	18.4
C	*Staphylococcus spp*	18.7	29.6
D	*Enterobacter spp*	15.4	24.4
	Total	63.2	100

Table 5: Identification of Fungal Isolates

Morphology	Microscopic Examination with Lactophenol	Fungi Identified
1. Pinkish shining smooth in front view and pink colour at the reverse view	Simple branched aseptate hyphae with conida lined at the tips of each hyphae	*Fusarium spp*
2. Black colouration in front and creamish in reverse view	Aseptate hyphae with rough head of pigment	*Aspergillus flavus*
3. Colonies with loose white to yellow mycelium, rapidly turning dark brown and eventually black on the development of conidia	Vesicles were light, yellow brown. Phialides growing radially along the periphery of vesicles. Primary phialides and secondary phialides are bogh brown	*Aspergillus niger*

DISSCUSION

The findings of this study showed that *Aspergillus flavus, Aspergius niger, Fusarium spp.* were found in fruits sold in Ogbete Market Enugu, Eastern Nigeria. All the three organisms isolated were confirmed to be pathogenic on the fruit but in varying degrees. It showed that of all the isolated fungi, *Aspergillus niger* was highly pathogenic. All the organisms were successful taking part in the decay and are thus confirmed as the causal organism of fruit decay [11]; [12]. Generally, spoiling fungi are considered toxigenic or pathogenic. Toxigenic fungi have been isolated from spoiling fruits. During refrigeration some moulds may produce mycotoxins [10].

The fungi isolated in this study have been reported to produce secondary metabolites in plants tissues. These secondary metabolites are potentially harmful to humans and animals. A good example is Aflatoxin which has been associated with cancer of the liver and also with acute hepatitis in humans, especially in the developing world. Pathogenic fungi on the other hand, could cause infection [8]. *Aspergillus spp.* are known to produce several toxic metabolites such as malformins and they can produce a mycotoxin which is a very important toxin worldwide because of the hazard it poses to human and animal health thus extra care should be taken during personnel handling of these fruits, such as harvesting, cleaning, sorting, packaging, transport and storage [9].

The bacterial isolates identified in this study include *Escherichia coli, Pseudomonas spp, Enterobacter spp* and *Staphylococcus aureus.* This is consistent with the findings of previous studies. Microorganisms most commonly found in fruits generally involve *Pseudomonas* and *Staphylococcus aureus.* Sufficient moisture, abusive temperature and adequate time well ensure a continuing

increase in the bacteria population. They are all associated with plant where they are known to cause plant diseases of the rot. *E. coli* are indicator of feacally contaminated products. Therefore, food processors may be sources of this microbial chance of inoculation, microbial food poison, food intoxication and food spoilage hence, food processor or seller of fruits may be counterproductive by being responsible for public health hazard and loss of revenue [7].

Most of the organisms found in this study are those commonly found in soil and water. But the presence of other indicator organism like *Enterobacter spp* may be as a result of possible contamination during sales or unhygienic handling of the fruits. In this study, the fungi isolated were *Fusarium spp., Aspergillus flavus* and *Aspergillus niger*.

CONCLUSION

This study detected the profile of spoilage fungi and bacteria which caused pathogenecity of some local fruits. It also showed that fungi and bacteria were involved in the spoilage of many fruits. Mechanical injuries such as bruises or cut that occur during harvesting or post-harvesting, grading and packing could provide infection sites for spoilage pathogens. Fruit spoilage however can be controlled by the following practices, washing of harvested fruit with clean or pure water, proper cleaning and sanitation of warehouses and disinfection of packaging and transit containers, proper handling of the fruit during harvest to prevent bruises and scars or other mechanical injuries.

It is therefore important that both the fanner who harvests the fruits into bags for transportation, the marketers and consumers take necessary precaution in preventing contamination and eating of contaminated fruits. This will however enhance reduction the risk of mycotoxins that are deleterious to human health

REFERENCES

1. Akinmusire, O.O. (2011). Fungal Species Associated with the Spoilage of some Edible Fruits in Maiduguri Northern Eastern Nigeria. *Advances in Environmental Biology,* 5(1): 151- 161.
2. Al-Hindi, R.R., Al-Najada, A.R. and Mohammed, S.A. (2011). Solution and Identification of some Fruit Spoilage Fungi: Screening of Plant Cell Wall Degrading Enzymes. *African Journal of Microbiology Research,* 5(4): 5-10
3. Barth, M., Hankinson, T.R., Zhuang, H. and Breidt, F. (2009). Microbiological Spoilage of Fruits and Vegetables. W.H. Sperber, M.P. Doyle (eds), Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety. C. Springer Science + Business Media; pp 135-183.
4. Chukwuka, K.S., Okonko, I.O. and Adekunle, A.A. (2010). "Microbial Ecology of Organisms causing Pawpaw Fruit Decay in Oyo State, Nigeria.'[1] *American Eurasian Journal of Toxicological Sciences.* 2(1):43-50.
5. Droby, S. (2006). Improving Quality and Safety of Fresh Fruits and Vegetables after Harvest by the Use of Biocontrol Agents and Natural Materials." *African Journal of Microbiology Research,* 709:45-51 .
6. Eckert, J.W. and Ogawa, J.M. (2008). "The Chemical Control of Post-harvest Diseases: Deciduous Fruits, Berries, Vegetables and Root/Tuber Crops." *Annual Review Phytopathology*. 26:433-469.
7. Monso, E.M. (2004). "Occupational Asthma in Greenhouse Workers." *Curr. Opin. Pulm. Med.* 10:147-150.
8. Nagy, S. and Shaw, P.E. (2009). *Tropical and Subtropical Fruits: Composition, Properties and Uses.* 5

Edition. New York: Avi Publishing Company.
9. Nathalie, J. (2006). Plant Protein Inhibitors of Cell Wall Degrading Enzymes." *Trends Plant Sci.* 11:359-367.
10. Oduola, T., Adeniyi, F.A.A., Ogunyemi, E.O., Bello, I.S., Idowu, T.O. and Subair, H.G. (2007). "Toxicity Studies on an Unripe Carica Papaya Aqueous Extract: Biochemical and Haematological Effects in Wistar Albino Rats. " *Journal of Medicinal Plants Research.* l(l):l-4.
11. Raven, P.H., Evert, R.F. and Eichhorn, S.E. (2005). 14-Fungi: Biology of Plants. 7th Edition, New York: WH Freeman, pp. 105, 186, 290.
12. Singh, D. and Sharma, R.R. (2007). *Post-harvest Diseases of Fruit and Vegetables and their Management.* In: Prasad, D. (Ed.), Sustainable Pest Management. India: Daya Publishing House, New Delhi.
13. Tango, U.M. (2010). Chains of Fruits. *Journal for the Study of Chains of Fruits in Africa.* 18(6): 600-603.
14. Tournas, V.H, and Katsoudas, E. (2005), "Mould and Yeast Flora in Fresh Berries, Grapes and Citrus Fruits. *International Journal of Food Microbiology,* 105: 11-17.
15. Tournas, V.H. and Stack, M.E. (2001). Production of Alternariol and Glternariol Methyl Ether by Alternaria Alternata Grown on Fruits of Various. *Food Prot* 64:528-532.