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RESEARCH ARTICLE

Isolation and Characterization of Microorganisms Involved in the Postharvest Loss of *Carica papaya* (Papaya) and *Mangifera indica* (Mango) in Awka, Southeastern Nigeria

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ABSTRACT

Isolation and characterization of microorganisms associated with post-harvest loss of *Carica papaya* and *Mangifera indica* fruits sold in the four major markets of Awka metropolis, Anambra State were conducted. Isolation of associated fungi and bacteria from the fruits were carried out on Sabouraud dextrose agar (SDA) and Nutrient agar respectively. The organisms were isolated and characterized on the basis of their colonial, morphological and biochemical features. A total of six (6) fungi were isolated from papaya *viz*. *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Candida* sp., *Geotrichum* sp. and *Saccharomyces* sp; while a total of four (4) fungi were isolated from *Mangifera indica viz*. *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp., and *Candida* sp. Also, a total of six (6) of bacteria were isolatad from papaya *viz*. *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp. and *Erwinia* sp.; while a total of four bacteria were isolated from *Mangifera indica viz*. *Lactobacillus* sp., *Micrococcus* sp., *Streptococcus* sp. and *Pseudomonas* sp. Fungal counts of up to 9.8 x 10^5 cfu/g and 3.0 x10^5 cfu/g were recorded in papaya and mango respectively. Also, bacterial counts of up to 3.6 x 10^5 cfu/g and 8.0 x 10^5 cfu/g were recorded in papaya and mango respectively. Some of the organisms isolated are human pathogens which can cause harm to people upon consumption. Therefore, fruits washing and storage at proper sanitary conditions, and refrigeration are necessary to increase shelf-life and reduce the risks of diseases to human health upon consumption.

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INTRODUCTION

Carica papaya, commonly known as papaya, is grown throughout the tropics and subtropics for its melonlike fruit, which is usually eaten fresh. The acropetally produced fruits are clustered near the top of small (2-8m), single-stemmed, herbaceous trees. New flowers are formed continuously; thus, a single hermaphroditic tree will have flowers and fruit in all stages of development. In eastern Nigeria, papaya is domestically and richly grown. Harvesting begins about 12-15 months after seeding and proceeds until the trees become too tall (5-6 m) for efficient harvesting. Commonly eaten parts of the plant include the fruit and leaves. The edible portion of papaya is composed mostly of water (86.8%), carbohydrates (10.82 g/100g), fat (0.26 g/100g), protein (0.47 g/100g), vitamins and trace metals. Traditionally, juice from the papaya fruit and root has been used as medicinal and dietary supplements (Aravind *et al*., 2013).

Mango (*Mangifera indica* L.), the most vital fruit in Anacardiaceae family, is a tropical fruit with high nutritional and medicinal values. It originated from the Asia Indo-Burmese region and is now commercially grown in more than 87 countries (Tharanathan *et al*., 2006). Morphologically, it belongs to drupe, where the pericarp is distinguished into smooth exocarp (peel), fleshy mesocarp (pulp), and stony endocarp (kernel). Fully ripe mango is famous for its aroma, intense peel coloration, pleasing taste, high amount of bioactive compounds such as phenolic compounds, β-carotene, vitamin C, and minerals. The chemical composition of mango varies with the location of cultivation, variety, and stage of maturity (Ma *et al*., 2011; Manthey and Perkins-Veazie, 2009).

Spoilage in papaya and mango may be referred to as rot or decay and can be extensive around the surfaces of

the fruits. These changes may be accompanied by alterations in taste, smell, appearance or texture. Numerous microbial defects of agricultural crops are characterized by the types of microorganisms responsible for their deterioration (Akinmusire, 2011). Spoilt papaya and mango fruits are characterized by tissue softening, formation of rot and mycelia, moisture loss, unpleasant odour, sour taste, and shrinkage. The spoilage may be caused by microbes, attacks by insects and rodents, physical injury such as bruising as well as chemical breakdown of the fruit may also lead to deterioration in fruit quality. The occurrence of spoilage in fruits by microorganisms depends on the types of organisms present and whether the fruit under its existing condition of storage can support the growth of any or all of them. However, only certain species out of all the organisms present in a fruit will be able to thrive well and spoil it. Susceptibility of fruits is largely due to differential chemical composition such as pH and moisture contents and are associated with greater predisposition to microbial spoilage (Barth *et al*., 2009).

In developing countries, postharvest deterioration of fruits and vegetables are often more severe due to inadequate storage and transportation facilities; and fruits generally, are affected by a wide array of microorganisms causing its decay. Therefore, this research was embarked upon to identify the postharvest bacteria and fungi associated with the loss of *Carica papaya* and *Mangifera indica* fruits sold in Awka metropolis of Anambra State, Nigeria.

MATERIALS AND METHODS

Sample collection

A total of 8 samples of fruit were collected (i.e. 2 fruits per market for both papaya and mango) from 4 markets (Eke-Awka, Nkwo Amaenyi, Amawbia and Oye Ifite markets). These markets are the 4 major markets in the metropolitan area of Awka, Nigeria. Summarily, 4 papaya and 4 mango fruits were collected. Spoilt/infected ripe fruits were purchased from fruit vendors at the 4 major markets. These fruits were seen displayed on benches and in baskets in the open for buyers to purchase. The fruits were collected into sterile polythene bags and brought to the laboratory for the study.

Sample labeling

The samples were labeled as thus:

Papaya

- Eke-Awka market - eP
- Nkwo Amaenyi market - nP
- Amawbia market - aP
- Oye Ifite market – oP

Mango

- Eke-Awka market – eM
- Nkwo Amaenyi market – nM
- Amawbia market - aM
- Oye Ifite market – oM

Inocula preparation

The fruit samples were washed with sterile distilled water and surface-swabbed with 70% alcohol. The portions on the samples showing spoilage symptoms were cut off with sterilized scalpel. The cut portions were homogenized into separate pulps using a sterilized laboratory mortar and pestle. The homogenized pulps were transferred immediately into sterile beakers, wrapped with aluminum foil and stored under aseptic condition for subsequent use.

Bacterial isolation

Plating: Nutrient agar was prepared according to manufacturer's specification. The pour-plate method was adopted using 1 g of the homogenized pulp. After plate counting had been done, colonies from the primary plates were aseptically picked with a sterile wire loop and transferred unto freshly prepared sterile nutrient agar plate. The plates were then incubated at 37^{o}C for 24 hours to 48 hours. Discrete colonies from the subculture plates were aseptically transferred and streaked on slants and were incubated for another 24 hours at 37^{o}C.

Characterization and identification of bacterial isolates: All bacterial isolates were characterized and identified based on their cultural, morphological, and biochemical characteristics. Staining and biochemical tests that were conducted include the following: Gram stain, catalase test, oxidase test, motility test, citrate test, urease test, and sugar fermentation test.

Fungal isolation

Plating: Sabouraud Dextrose Agar was used and prepared according to the manufacturer's specification. Bacterial contamination was inhibited using chloramphenicol at a concentration of 0.05mg/ml of the sterile medium before pouring into sterile plates. One gram (1 g) of the homogenized pulp was transferred aseptically onto solidified SDA. The plates were incubated at 27^{o}C temperature for 72 to 96 hours.

Isolation: The types of colonies on the plates were observed and recorded at the end of the incubation period. Representative colonies were randomly selected and subcultured until pure isolates were obtained. The pure cultures when obtained were maintained on agar slants which were kept as stock cultures under refrigeration (4^{o}C) for later use.

Characterization and identification of fungal isolates

The isolates were characterized based on their colonial and cellular morphology. The colonial morphology of the isolates was observed macroscopically and characteristics such as colour of mycelia and spores, shape and surface texture were recorded. The isolates were then observed microscopically under light microscope for their cellular morphology using wet mounting (on a drop of distilled water on a glass slide, a small mycelia portion was introduced with an inoculating wire needle and then stirred with the needle. Two drops of methylene blue were added, covered with a cover slip and observed under x40 objective lens for hyphal nature and arrangement of fruiting structures). Appropriate drawings were made together with photographic images taken, and the different characteristics of the fungal isolates were recorded. Then, the various fungal isolates were compared using a fungal atlas.

Table 1: Fungal counts of the spoilt papaya and mango fruits

Sample	count (cfu/g)
Pawpaw	
eP	6.3×10^5
nP	9.8×10^5
aP	1.0×10^4
oP	8.8×10^4
Mango	
eM	5.0×10^4
nM	1.0×10^4
aM	2.8×10^5
oM	3.0×10^5

Table 2: Bacterial counts of the spoilt papaya and mango fruits

Sample	count (cfu/g)
Pawpaw	
eP	3.6×10^5
nP	3.1×10^5
aP	2.5×10^5
oP	3.6×10^5
Mango	
eM	8.0×10^4
nM	8.0×10^5
aM	7.0×10^5
oM	3.6×10^5

Plate count for both fungi and bacteria

Using standard microbiological technique, a ten-fold serial dilution of 1g of the homogenized pulp was done. One milliliter (1ml) of the aliquot of the fourth factor (10^{-4}) was aseptically transferred and plated in duplicate sets in sterile molten lukewarm nutrient agar and Sabouraud dextrose agar (amended with chloramphenicol at a concentration of 0.05mg/ml to inhibit bacterial growth). The plates were allowed to set and incubated at 37^{o}C for 48 and 72 hours respectively. The discrete colonies that developed after incubation were counted and enumerated as colony forming unit (cfu/g).

RESULTS AND DISCUSSION

The spoilt fruits examined revealed that both bacteria and fungi were implicated in the degenerative effects. For the papaya fruits, the bacterial and fungal counts ranged from 2.5×10^5 to 3.6×10^5 and 10^4 to 9.8×10^5 respectively; also, for the mango fruits, the bacterial and fungal counts ranged from 8.0×10^4 to 8.0×10^5 and 10^4 and 3.0×10^5 respectively (Tables 1 and 2). The colonial and morphological characteristics of the fungal isolates from papaya and mango are presented in Table 3 and were identified as thus from pawpaw *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Candida* sp., *Geotrichum* sp. and *Saccharomyces* sp.; from mango *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp. and *Candida* sp. (Table 4). The bacterial isolates from pawpaw are *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp. and *Erwinia* sp.; and from mango *Lactobacillus* sp., *Micrococcus* sp., *Streptococcus* sp. and *Pseudomonas* sp. (Table 5). The staining and biochemical tests for both the bacterial and fungal (yeast) isolates are presented in Tables 6 and 7).

Studies on the fungi implicated in the spoilage of pawpaw and mango fruits showed that the fruits contained a teeming population of fungi and bacteria. The overall probable genera of fungi isolated were *Aspergillus, Penicillium, Rhizopus, Candida, Geotrichum* and *Saccharomyces* and bacterial genera included *Staphylococcus, Streptococcus*, *Bacillus, Pseudomonas, Micrococcus, Erwinia* and *Lactobacillus.* The presence of the bacteria and fungi isolated from the spoilt fruits of papaya and mango points to the health risk posed by the presence of those organisms in vended fruits. However, the presence of these organisms in fruits may be linked to a number of factors, such as improper handling and processing of fruits, use of contaminated water during washing, cross contamination from other fruits or the use dirty processing utensils and unhygienic storage conditions. When the pathogenic forms of these bacteria and fungi are being consumed in such quantity that is deleterious to the human health, then food borne diseases with characteristic symptoms as nausea, vomiting, abdominal pain and sometimes fever may be the outcome (Poubol and Izumi, 2005).

On the other hand, the prevalence of fungi as one of the groups that brings about spoilage is due to a wide range of factors which are encountered at each stage of handling from pre-harvest to consumption and is related to the physiological and physical conditions of the produce as well as the extrinsic parameters to which they are subjected (Akintobi *et al*., 2011). Damage inflicted on produce at the time of harvest is a major cause of infection since most of the spoilage microorganisms invade the produce through such damaged tissues. Similarly, the extent of deterioration is influenced by the depth of the wound. The incidence of infection is worsened by poor sanitary practice such as cross contamination and contact infection during the transportation (Singh *et al.,* 2002). The microbial spoilage of pawpaw and mango fruits can

Table 3: Colonial and morphological characteristics of the fungi isolated from the spoilt papaya and mango fruits

Isolates	Colonial Characteristic	Characteristics	Probable organism
1	Green colonial with white edges with velvety texture and creamy underside.	Septate aerial conidiophores that are peperndicular to and walled off from which they arise.	*Penicillium* species
2	Green dry colonial with white edges and creamy underside.	Vegetative mycelium consisting of septate branching	*Aspergillus* species
3	Whitish, growing rapidly and filled the petri dish with dense cottony mycelium and changes brown black with age.	Non-septate mycelia, sporangiospores are oval in shape and are directly opposite to the branched rhizoids. Sporangia and the columella are subglobose. Sporangiophores are smooth walled.	*Rhizopus* species
4	Creamy white, smooth colonies with ground-glass appearance.	Budding, spherical to elongated cells, forming pseudomycelia.	*Candida* species
5	Moist milky colonies.	Budding cells with pseudomycelia.	*Saccharomyces* species.
6	Soft and creamy white colonies.	Cells with hyaline hyphae which fragments into arthroconidia.	*Geotrichum*

Table 4: Occurrence of the isolated fungi in the spoilt papaya and mango fruits

Sample	*Penicillium*	*Aspergillus*	*Rhizopus*	*Candida*	*Saccharomyces*	*Geotrichum*
eP	-	+	-	+	-	+
nP	-	+	+	-	-	-
aP	-	-	+	+	+	-
oP	+	+	+	+	-	+
eM	-	+	-	+	-	-
nM	-	+	-	+	-	-
aM	+	-	+	+	-	-
oM	-	+	+	+	-	-

"+" = isolated; "-" = not isolated

Table 5: Occurrence of the isolated bacteria in the spoilt pawpaw and mango fruits

Sample	*Staphylococcus*	*Streptococcus*	*Bacillus*	*Pseudomonas*	*Micrococcus*	*Erwinia*	*Lactobacillus*
eP	+	+	-	-	+	-	-
nP	+	-	-	-	+	+	-
aP	+	-	+	-	-	+	-
oP	+	+	+	-	-	-	-
eM	-	-	-	-	-	-	-
nM	-	-	-	-	-	-	-
aM	-	-	-	-	-	-	+
oM	-	+	-	+	+	-	-

"+" = isolated; "-" = not isolated; "smpl" = sample

Table 6: Biochemical tests of the bacterial isolates

Probable isolates	Gram's reaction	Motility	Catalase	Methyl red	VP	Citrate	Urease	Glucose	Mannitol
Staphylococcus	+ cocci	-	+	-	-	+	+	-	-
Streptococcus	+ cocci	-	-	+	-	-	+	-	-
Bacillus	+ rods	+	+	+	-	+	+	A	A
Pseudomonas	- rods	+	+	-	-	+	+	A	-
Micrococcus	+ cocci	-	+	+	-	+	+	-	-
Erwinia	- rods	+	+	+	-	+	+	AG	-
Lactobacillus	+ rods	+	-	-	-	-	+	A	A

"-" = negative; "+" = positive; AG = acid and gas; A = acid; VP = vogues-proskauer

Table 7: Biochemical tests of the yeasts isolated

Probable isolates	Germ tubes	Glucose	Mannitol	Maltose	Lactose	Sucrose	Urease
Candida	+	AG	-	-	-	A	+
Saccharomyces	-	AG	-	AG	-	AG	+
Geotrichum	-	A	-	-	-	-	-

"-" = negative; "+" = positive; AG = acid and gas; A = acid

be controlled by adequate hygienic practice by mango fruits handlers and good storage of the harvested fruits. These will help reduce the health risk posed to the consumers of such important fruits.

Conclusion

The most limiting factor that influences the economic values of papaya and mango fruits is the relatively short shelf life caused by spoilage. Therefore, it is paramount that spoilage of fruits in general be reduced especially in regions with no or limited storage facilities, which is the case in most rural areas where most of the fruits are grown before being transported to urban markets. Proper and adequate handling practices of these produce would help minimize wastes due to deterioration. In view of the health hazards associated with the consumption of spoilt fruits containing these microorganisms, pawpaw and mango fruits must be adequately handled and processed hygienically. Also, routine inspection of fruits and vegetables by food regulatory agencies is highly recommended.

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