ISSN 2581 – 4346

Available Online at www.ijms.co.in
Innovative Journal of Medical Sciences 2021; 5(1):16-23

RESEARCH ARTICLE

A comparative study of bacteria and fungi associated with spoilage of banana and orange sold in Sokoto metropolis

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Received on: 01 December 2020; Revised on: 15 January 2021; Accepted on: 03 February 2021

ABSTRACT

Fruits are some of the essential elements of balanced diet required for good health maintenance. Banana and orange are the most extensively produced fruits in the world, which implies that they are the most widely consumed fruits and highly common. The comparative study of microorganism connected with spoilage of banana and orange in Sokoto metropolis was conducted to identify some of the microorganisms (bacteria and fungi) associated with the spoilage of fruits, with particular interest in banana and orange. Procedures involved in these investigations include collection of samples from the local market, preparation in the laboratory using standard procedure, and inoculation of samples into various Petri dishes containing nutrient agar and potatoes dextrose agar for bacteria and fungi, respectively. The prepared samples were incubated for 24 h at 34°C for the bacteria sample and for 5 days at room temperature for the fungal sample. The result shows orange to present a higher number of bacteria species than banana, whereas, the fungal isolate showed the same prevalence in both banana and orange, except for the frequency of occurrence of *Aspergillus* species which presented 33% appearance in orange and 50% appearance in banana. The result from this study shows that orange contains more microbes. This is associated with its high percentage of water content. The moisture content and requirement for the growth of these fruit encourage the growth and development of these organisms/pests. There is, therefore, the need to have proper understanding of some of the organisms, as this will inform consumers on the implications of eating some of the fruits when they begin to spoil.

Keywords: Bacteria, Banana, Fungi, Orange, Sokoto, Spoilage

INTRODUCTION

Fruits are some of the essential elements of balanced diet required for good health maintenance. This is because they serve as sources of essential vitamins and other elements essential for good health. Orange (*Citrus* spp.) and banana (*Musa paradisiaca*) are the most widely produced fruits in the world, orange being the most widely produced, and banana being the second most produced fruit. This naturally implies that they are the most widely consumed set of fruits, as they are largely available and highly common.

Banana

Banana is an herbaceous plant of the genus *Musa* and is one of the oldest plants cultivated. It is an essential produce of the tropical and subtropical regions of the world, with a total of about 8.8 million hectares used for the cultivation of banana across the globe. Compared to many other fruits, it is easy

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to eat and digest, having a digesting time of about 105 min compared to apple which has a digesting time of about 210 min. Banana is known to be a highly beneficial fruit. Study has shown that virtually all parts of a banana plant are of medicinal benefit or nutritional benefit. The sweetness of the fruit and its softness makes it a favorite fruit for most persons, hence, its high rate of consumption. However, despite these benefits, banana has been found to be easily plagued by pests, particularly nematodes, fungi, and bacteria. This is probably due to the moisture content and requirement for the growth of banana, which encourages the growth and development of these organisms/pests. There is, therefore, the need to have proper understanding of some of the organisms, as this will inform consumers on the implications of eating some of the fruits when they begin to spoil. Furthermore, a proper understanding of the common organisms responsible for the spoilage of these fruits will aid better preservation of the fruit and maximize the availability of healthy fruits for consumption.[1-11]

Orange

Orange (*Citrus sinensis*) is a fruit of the family Rutaceae. It is a fleshy bulb and does not rupture. It is categorized as a berry and usually has size ranging from 4 cm to 12 cm. Oranges, like bananas, are some of the most widely consumed fruits across the globe. It is known to have several nutritional and medicinal benefits. Part of its medicinal properties include antibacterial, antifungal, antidiabetic, cardioprotective, anti-cancer, anti-arthritic, anti-inflammatory, antioxidant, anti-tubercular, anti-asthmatic, and anti-hypertensive. Its phytochemical profile reveals the presence of such bioactive ingredients as limonene, citral, neohesperidin, naringin, rutin, rhamnose, eriocitrin, and Vitamin-C. According to Mohaptra *et al*. (2010), orange is the most widely produced fruit in the world. This is evident in the fact that orange as a fruit is found in virtually every town, city, and villages, in virtually every country of the world. Orange has also been found to be available all through the year, whether in season or out of season. This testifies to its adaptability through various seasons and climate. This adaptability, however, does not shield oranges from the effect of various diseases. Hence, they are vulnerable to various disease outbreaks. This vulnerability, according to Wu *et al*. (2014), is as a result of the "very narrow genetic diversity of cultivated citrus."

METHODOLOGY

Sample collection

One piece of orange and banana was purchased at Sokoto food market.

Equipment and materials

Distilled water, glass slides, cover slips, cotton wool, aluminum foil, conical flasks, beaker, measuring cylinder, Bunsen burner, autoclave, Petri dish/pour plate, bijou bottles, test tubes, wire loop, filter paper, microscope, incubator, forceps, and spatula were used.[12-20]

Reagent and chemicals used

Normal saline, distilled water, acetone, lugol's iodine, safranin, hydrogen peroxide, oxidase reagent, glucose solution, phenol red tryptophan, and Kovac's reagent were used.

Method

The following methods were used in the isolation and identification of spoilage of banana and mango.

Media preparation

Media (nutrient agar and potatoes dextrose nutrient agar) were prepared according to the manufacturer's instruction. 20–25 ml was aseptically poured into Petri dish to solidify.

Preparation of sample dilution

One gram of each spoilt sample (banana and mango) was aseptically taken and homogenized into 90 ml of sterile distilled water each. One

milliliter of each homogenate was transferred into test tube containing 9 ml distilled water. A decimal series of each homogenate was prepared down to a dilution 10^1–10^8 in sterile distilled water.

Enumeration of microorganisms

The media used is to isolate the different type of bacteria and fungi found in spoilt banana and orange. The enumeration of bacteria and fungi involves in the spoilage of banana and mango is carried out as follows; 0.1 ml aliquot from10^4, 10^6, and 10^8 dilution was inoculated into the designated media plate in duplicate. For bacteria, the plates were inoculated for 37°C for 24 h, while for fungi, the plates were inoculated at room temperature for 1 week.

Preparation of pure culture

A sterile wire loop is used to pick colonies of bacteria and streak onto new agar plates (nutrient agar medium) for purity. The plates were inoculated for 37°C for 24 h. Pure culture isolate of bacteria was maintained on appropriate agar slant (nutrient agar medium) and kept in a refrigerator and maintained at 40°C for identification.

Identification of bacterial isolate

The isolate was purified by subculturing onto appropriate slant agar bottle. The culture was identified by carrying out Gram stain, indole test, citrate test, catalase test, oxidase test, and motility test in accordance with the manufacturer's instruction.

Gram staining method

This is used to distinguish nearly all bacteria as Gram negative or Gram positive using crystal violent and subsequent with iodine (Gram-positive cell appears purple while Gram negative appears red). A thin smear of bacteria was made on a glass slide, air dried, and heat fix on a hot air oven for at least seconds. The smear was covered with crystal violate for 30–0 s, washed rapidly with clean water. The glass was tipped off all water and smear covered with lugol's iodine for 30–60 s. The iodine was washed off with clean water and decolorized with acetone and washed off immediately. The smear was covered with serafin for 1 min and washed off with clean water, the back slide was cleaned and placed in a rack to air dried. The dried smear was viewed with immersion oil microscopically using the (×10) objective lens microscope.[21-30]

Biochemical test

Colony from the slant bijou bottle was used to carry out the biochemical test for the bacteria identification such as catalase test, oxidase test, motility test, and indole test.

Indole test

Inoculate tryptone broth with the test organism and incubate for 18–24 h at 37°C, add 15 drops of Kovac's reagent down the inner wall of the tube.

Interpretation

Development of bright red color at the interface of the reagent and the broth within seconds after adding the reagent is suggestive of the presence of indole and is a positive test (Cheesbrough, 2006).

Catalase test

A loopful of each bacteria isolate was aseptically transferred to clean sterile glass slide. About 3% hydrogen peroxide was introduced on the slide containing the inoculum.

Interpretation

Evolution of effervescence as observes production of gas bubbles indicates a positive result (Cheesbrough, 2006).

Oxidase test

One gram of the oxidase reagent was dissolved in 10 ml of distilled water which was added in a filter paper in a Petri dish. The inoculum of test organism was smeared into the filter paper using a glass rod (Onyeagba, 2004).

Interpretation

Observation of a purple color indicates positive result.

Motility test

Stab motility media with inoculating needle.

Interpretation

If bacteria are motile, there will be growth going out away from the stab line, and test is positive. If bacteria are not motile, there will only be growth along the stab line. A colored indicator can be used to make the results easier to see.

Purification of fungal isolates

Colonies from potatoes dextrose agar (PDA) were aseptically picked with inoculating needle and streak unto new PDA and incubated for 30°C for 7 days. Pure colonies were stored in slant bottle in refrigerator until further identification.

Identification of fungal isolate

The various microscopic and macroscopic characteristics of fungal isolate were compared with an image analysis system using certain morphological features of fungi as were given by (Akinmusire, 2011). The microscopic features include color of morphology, color of colony, reverse colony color, and surface texture. A slide was prepared using methylene blue dye. A dye was dropped on a clean glass slide and fungi hyphae were aseptically removed from the subculture slant bottle with a wire loop and leased apart from the stain. The slide was carefully covered with cover slip with extension of air bubbles and observed under the low power (×40) lens of compound microscope.

RESULTS

The result shows the prevalence of bacteria and fungi found in spoilt banana and mango. Tables 1 and 2 describe the frequency and percentage distribution of the bacteria isolated. The bacteria isolate and their percentage distribution of orange in Table 1 indicate the following *Aeromonas* species 11.1%, *Serratia* species 11.1%, *Shigella* 22.2%, *Proteus* species 11.1%, *Staph* species 11.1%, and *Yersinia* species 33.3%. For banana, Table 2 indicates the following *Serratia* species 33.3%, *Staph* species 16.6%, and *Yersinia* species 50.0%. The result shows that orange has more bacteria isolate. Table 3 describes the biochemical test carried out, dilution factors for orange $10^4$3, $10^6$1, $10^6$2, and $10^6$3 and dilution factor for banana $10^6$1, $10^6$2, $10^8$1, and $10^8$2 show positive for catalase test, orange dilution factor $10^4$1 shows positive reaction for oxidase test. For motility test, dilution factor orange $10^4$2 and banana $10^4$1 $10^6$1 shows a positive result, for urease test, all tests positive except dilution factor orange $10^4$1. The result orange dilution factor has six bacteria isolate while banana has three bacteria isolate. Tables 4 and 5 describe the frequency and percentage distribution of fungal isolate. Tables 6 and 7 describe the macroscopic and microscopic view of mold and yeast isolate. Table 8 is a comparison that was done between fungi and bacteria that were isolated from the fruits under study and Table 9 shows the commonality of the of the species that were isolated both from the fruit samples while Table 10 presented comparison of fungi that were found among the bananas and oranges.

Table 1: Frequency and percentage distribution of bacteria isolates for spoiled orange sold in Sokoto market

Sample	Bacteria isolate	Frequency of occurrence	Percentage distribution
Orange	*Aeromonas* spp.	1	11.1
	Serratia spp.	1	11.1
	Shigella spp.	2	22.2
	Proteus spp.	1	11.1
	Staphylococcus spp.	1	11.1
	Yersinia spp.	3	33.3

Table 2: Frequency and percentage distribution of bacteria isolates for spoiled banana sold in Sokoto market

Sample	Bacteria isolate	Frequency of occurrence	Percentage distribution
Banana	*Serratia* spp.	2	11.1
	Staphylococcus spp.	1	16.6
	Yersinia spp.	3	50

Table 3: The results of biochemical tests on bacteria isolates for orange and banana from Sokoto market

Sample I.D	Gram reaction	Catalase	Oxidase	Motility	Urea	Indole	Slant	Butt	H_2s production	Gas production	Result
Orange $10^{-4}1$	–ve rod	–	+	–	–	–	R	Y	–	–	*Aeromonas* spp.
Orange $10^{-4}2$	–ve rod	–	–	+	+	–	R	Y	–	–	*Serratia* spp.
Orange $10^{-4}3$	–ve rod	+	–	–	+	–	R	Y	–	+	*Shigella* spp.
Orange $10^{-6}1$	–ve rod	+	–	–	+	+	R	Y	–	–	*Proteus* spp.
Orange $10^{-6}2$	+ve cocci	+	–	–	+	–	R	Y	–	+	*Shigella* spp.
Orange $10^{-6}3$	+ve cocci	+	–	–	+	–	R	Y	–	+	*Staph* spp.
Orange $10^{-8}1$	–ve rod	–	–	–	+	–	R	Y	–	–	*Yersinia* spp.
Orange $10^{-8}2$	–ve rod	–	–	–	+	–	R	Y	–	–	*Yersinia* spp.
Orange $10^{-8}3$	–ve rod	–	–	–	+	–	R	Y	–	–	*Yersinia* spp.
Banana $10^{-4}1$	–ve rod	–	–	+	+	–	R	Y	–	–	*Serratia* spp.
Banana $10^{-4}2$	–ve rod	–	–	–	+	–	R	Y	–	–	*Yersinia* spp.
Banana $10^{-6}1$	–ve rod	+	–	+	+	–	R	Y	–	–	*Serratia* spp.
Banana $10^{-6}2$	–ve rod	+	–	–	+	–	R	Y	–	–	*Yersinia* spp.
Banana $10^{-8}1$	–ve rod	+	–	–	+	–	R	Y	–	–	*Yersinia* spp.
Banana $10^{-8}2$	+ve cocci	+	–	–	+	–	R	Y	–	–	*Staph* spp.

Table 4: Frequency and percentage distribution of fungi isolates of spoiled orange sold in Sokoto market

Sample	Fungi isolates	Frequency of occurrence	Percentage distribution
Orange	*Aspergillus* spp.	2	33.3
	Cladosporium spp.	1	16.6
	Penicillium spp.	1	16.6
	Rhizopus spp.	2	33.3
Total		6	100

Table 5: Frequency and percentage distribution of fungi isolates of banana sold in Sokoto market

Sample	Fungi isolates	Frequency of occurrence	Percentage distribution
Banana	*Aspergillus* spp.	4	66.7
	Penicillium spp.	1	16.7
	Rhizopus spp.	1	16.7
Total		6	100

DISCUSSION

Six species of bacteria were isolated from the spoilt banana and orange obtained from Sokoto food market. They were identified as *Aeromonas* species, *Serratia* species*, Shigella* species, *Proteus* species*, Staph* species*,* and *Yersinia* species [Table 1]. *Yersinia* was the most predominant (33.3%) for orange and (50%) for the banana. Therefore, orange has more bacteria isolate compared to banana. This is so because of the high moisture content of water in orange. Orange and banana bacteria isolates have the following species similarity *Serratia* species*, Staph* species, and *Yersinia* species. The fungi isolated from the spoiled fruit are *Cladosporium* species, *Aspergillus* species, *Penicillium* species, and *Rhizopus* species identified. The highest occurring was *Aspergillus* species 33.3% in orange and 50% in banana. This result agreed with the report of Adebayo *et al*. on the study of microorganisms associated with spoilt fruit in Sokoto metropolis. Bacterial in spoilt orange is more predominant compared to banana. In fungal isolate, orange too appears higher probably due to high water content. The presence of these organisms in spoilt fruit is an indication that the fruits may have been exposed to fecal contamination from water of organic manure, improper handling, and storage. The absence of some organisms might be due to the nature or kind of the spoilt fruit samples or the methods of analysis used in this study. All the bacteria isolated in this work are inhabitants of the soil. *Serratia* species may come from the soil or from fecal contaminated water used for irrigation. *Serratia* species is an opportunistic pathogenic bacterium capable of causing diseases in diverse organisms including humans.

These microorganisms could possibly gain entrance into fruits through fecal contaminated water and

Table 6: Macroscopic and microscopic view of yeast isolate

Sample	Color obverse	Color reverse	shape	Elevation	Texture	Microscope
Orange 10^4	Dark green	Cream	Filamentous	Raised	Powder like	*Aspergillus* species
Orange 10^6	Cream	Cream	Irregular	Flat	Shiny	*Cladosporium* species
Orange 10^8	Green	Cream	Filamentous	Raised	Powder like	*Rhizopus* species
Banana 10^4	Pink	Cream	Circular	Flat	Shiny	*Penicillium* species
Banana 10^6	White	Cream	Filamentous	Raised	Powder like	*Aspergillus* species
Banana 10^8	Cream	Cream	Circular	Flat	Shiny	*Rhizopus* species

Table 7: Macroscopic and microscopic view of mold isolate

Sample	Color obverse	Color reverse	Shape	Elevation	Texture	Microscope
Orange 10^4	Dark green	Cream	Filamentous	Raised	Powder like	*Aspergillus* species
Orange 10^6	Cream	Cream	Irregular	Flat	Shiny	*Cladosporium* species
Orange 10^8	Green	Cream	Filamentous	Raised	Powder like	*Rhizopus* species
Banana 10^4	Pink	Cream	Circular	Flat	Shiny	*Penicillium* species
Banana 10^6	White	Cream	Filamentous	Raised	Powder like	*Aspergillus* species
Banana 10^8	Cream	Cream	Circular	Flat	Shiny	*Rhizopus* species

Table 8: Comparison between organisms present in orange and banana

Bacteria isolate	Orange	Banana	Commonality
Serratia spp.	+	+	+
Staphylococcus spp.	+	+	+
Yersinia spp.	+	+	+
Aeromonas spp.	+	–	–
Shigella spp.	+	–	–
Proteus spp.	+	–	–

Table 9: Comparison of outcome from biochemical test

Result	Orange	Banana	Commonality
Aeromonas spp.	+	–	–
Serratia spp.	+	+	+
Shigella spp.	+	–	–
Proteus spp.	+	–	–
Staph spp.	+	+	+
Yersinia spp.	+	+	+

Table 10: Comparison of fungi found in banana and orange

Fungi Isolates	Orange	Banana	Commonality
Aspergillus spp.	+	+	+
Cladosporium spp.	+	–	–
Penicillium spp.	+	+	+
Rhizopus spp.	+	+	+

manure, poor manufacturing practices (cultivating, harvesting, grading and packing), environmental contaminants, and poor sanitation in Sokoto market which can lead to food poisoning in that locality.

CONCLUSION

Six species of bacteria were isolated from the spoilt banana and orange obtained from Sokoto food market. They were identified as *Aeromonas* species*, Serratia* species, *Shigella* species, *Proteus* species, *Staph* species*,* and *Yersinia* species [Table 1]. *Yersinia* was the most predominant (33.3%) for orange and (50%) for the banana. Therefore, orange has more bacteria isolate compared to banana. This is so because of the high moisture content of water in orange. Orange bacteria isolates have the following species similarity *Serratia* species*, Staph* species, and *Yersinia* species. The fungi isolated from the spoiled fruit are *Cladosporium* species, *Aspergillus* species, *Penicillium* species, and *Rhizopus* species identified. The highest occurring was *Aspergillus* species 33.3% in orange and 50% in banana. This result is in consonance with the report of Adebayo *et al*. on the study of microorganisms associated with spoilt fruit. Bacteria are more predominant in spoilt orange compared to banana. In fungal isolate, orange too contains high water content. The presence of these organisms in spoilt fruit is an indication that it was exposed to fecal

contaminated water of organic manure, improper handling, and storage. The absence of the organism might be due to the nature or kind of the spoilt fruit samples or the methods of analysis used in this study. All the bacteria isolated in this work are soil dwellers. *Serratia* species may come from the soil or from fecally contaminated water used for irrigation. *Serratia* species is an opportunistic pathogenic bacterium capable of causing diseases in diverse organisms including humans. These microorganisms could possibly gain entrance into fruits through fecal contaminated water and manure, poor manufacturing practices (cultivating, harvesting, grading, and packing). Environmental contaminants and poor sanitation in Sokoto market lead to food poisoning in that locality.

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