CARD International Journal of Medical Science and Applied Biosciences (IJMSAB)
ISSN: 2536-7331 (Print): 2536-734x (Online)
Volume 2, Number 1, March 2017
http://www.casirmediapublishing.com

Isolation of Micro-Organisms Associated with Rot Diseases of Fruits using Pawpaw and Shaddok as Case Study

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ABSTRACT

This research study dwelt on Microbial Isolation of Organisms Associated with Rot Disease of Fruits using Shaddok and Pawpaw as Case Study. This was necessitated due the fact that food spoilage is a complex process and excessive amount of food is lost due to microbial spoilage of food, even with the modern day preservation techniques. Based on this knowledge, more detailed sensory, chemical and microbiological analysis is usually carried out on food materials to assess their consumption safety. Two samples (pawpaw and shaddock) were considered in the present study, to determine the total bacterial count and the actual specific organisms responsible for their spoilage; whilst, the chemical and physical parameters are the main determining factors for detection of spoilage microorganisms. From the results obtained, the pH values of the two samples before spoilage were 6.50 (for pawpaw) and 4.72 (for shaddock), while after spoilage, their pH reduced as follows 3.05 and 4.38 respectively. It was, also, noticed that pawpaw spoiled faster than shaddock. Some microorganisms identified in the screening include staphylococcus specie with ($1.05x10^5$tcfu/g), proteus spp (with $7.2x10^4$tcfu/g), E. coli (with $5x10^2$tcfu/g), klebsiella (with $8x10^2$tcfu/g), penicillium specie (with $3.0x10^2$tcfu/g), coccidioides immitis (with $2.0x10^2$tcfu/g), saccharomyce specie (with $1.87x10^2$tcfu/g), and these all have negative effect on human health. It is therefore recommended that food that ready to be eaten should be stored in the refrigerator to reduce the chances of microbial spoilage and also, food should be properly cooked or re-heated before consumption to avoid ingesting microorganism into the body that are injurious to health.

Keywords: Isolation, Microorganism, Rot Disease, Fruits

INTRODUCTION

Food spoilage refers to various changes in which the food becomes less palatable or even toxic to consumers. These changes may be accompanied by alterations in taste, smell, appearance or texture. Numerous microbial defects of agricultural crops are characterized by the type of micro-organisms responsible for their deterioration (Akinmusire, 2011; Baiyewu, 1994). Fruits and vegetables are very important, and have high dietary and nutritional qualities. Studies have evaluated the association of fruits and vegetables consumption with the reduction of risk of specific diseases (Diaz-Cinco *et al* 2005; Baiyewu, 1994). Their consumption has drastically increased by more than 30% during the past few decades (Barth *et al* 2009). Fresh fruit and vegetables consumption increase by 25.8% and 32.6%, and far exceeded the increases observed for processed fruit and vegetable products respectively. It is also estimated that about 20% of all fruits produced is lost each year due to spoilage (Barth *et al* 2009). Jay *et al* (2000) reported that 20 new human fungal pathogens are documented each year. It was, also, estimated that about 20-25% of the harvested fruits are decayed by pathogens during post harvest handling even in developed countries (Daries and Albrigo, 1994).

Deterioration of foods generally is attributed to two main causes, which are natural degradation due to activities of enzymes and growth of micro-organisms (bacteria, molds and yeasts). Though micro-organisms can result in useful products through their activities, particularly during fermentation of foods (such as wine and cheese), the negative effects of their activities result in decay, rottening and poisoning of foods; whence the basis of microbial food spoilage occurs when these micro-organisms release their own enzymes into the foods and absorb the nutrients, thereby changing the physical and chemical states of the food; this, of course, results to lowering of the nutritional value. Bacteria and fungi may also produce waste products which act as poisons or toxins, thus causing unwarranted ill-effects (Balla and Farkas 2006).

However, Paw-paw and shaddock are among the fruits cultivated worldwide that have great nutritional value. Because shaddock is a seasonal fruit, its microbiological study has been made necessary so as to identify its spoilage organisms. Added to their nutritive value is their medical importance, inspectional effect, as well as economic importance (Crane *et al,* 2007). Paw-paw and shaddock fruits are consumed raw and fresh. They are consumed worldwide, but there is knowledge of the spoilage organism in only developed countries, and these organisms are detrimental to human health.

MATERIALS AND METHODS

Sample Collection and Glass Ware Sterilization

A fresh sample A (pawpaw) and sample B (shaddock) was carefully harvested from a farm at St. Patrick's Catholic Church Mgbirichi (a town close to Imo state polytechnic Umuagwo) in the Ohali/Egbema L.G.A. of Imo State, Nigeria. The analytical procedures were conducted at the New Concept Laboratory, Obinze-Owerri.

Dry heat method was employed, in a hot air oven. The glass wares include: Pipettes, test-tubes, McCartney bottles and Durham tubes. The sterilization was conducted at 160°C for 1hour. Pipettes were dried and kept in a canister before the sterilization. Test-tubes and scalpels were wrapped in aluminum foils, while the beakers, measuring cylinder and conical flasks were plugged with cotton wool before the oven treatment.

PREPARATION OF THE CULTURE MEDIA

Nutrient Agar (NA): 28.0gm of the NA was dissolved in 1000ml of distilled water, and was gently heated to dissolve the media completely. Sterilization was done in an autoclave at 15psi (121^{O}C) for 15minutes. The medium was, then, dispensed into the plate.

Mac-Conkey Agar (MA): 52.5g of the Mac-Conkey agar powder was dissolved in 1000ml of distilled water. Controlled heating was applied to facilitate the dissolution. Sterilization was then conducted by means of an autoclave for 15minutes at 121^{O}C; it was removed and transferred to a Petri dishes.

Eosin Methylene Blue Agar (EMBA): 36gms of EMBA was suspended in 1000ml distilled water. Heating was applied to dissolve the medium completely. It was then dispensed and sterilized in an autoclave at 15lbs pressure (121°C) for 15minutes. The final product was then transferred to a Petri dish (Plate).

Sobouraud Dextrose Agar (SDA): This was prepared by dissolving 65gms of the media in 1 litre of distilled water. Heating was applied in a controlled manner to dissolve the medium completely at 121°C (15lbs) for 15minutes.

P^{H} Determination

The pH of the samples was determined using a *PHS-3C* digital pH meter in a solution of sample-to-water ratio of 1.10 was used. In other words, 200ml of distilled water was added to 20g of each sample. The pH electrode was dipped into the solution, and the meter deflection (result) was observed and recorded accordingly.

Isolation of the Organism

1gram of each of the samples was weighed into a test tube containing 9ml of sterile water as diluents. Then 10folds of serial dilution of the water were prepared using sterile water as diluents as well. 0.1ml aliquot (10^{-2}, 10^{-3}) of each sample was inoculated on Nutrient agar plate and MacConkey agar plate using spread-plate method. These were then incubated at 37°c for 24 hours (for bacterial count) and 25°C for 88hours (for fungi count). After the respective incubation period, the calories formed were observed on different plates and counted accordingly.

Through the morphological expression, the Most Probable Organism, *MPO* (*MPB* for bacteria and *MPF* for fungi) was identified and noted.

RESULTS AND DISCUSSION

The results of pH analysis (before and after spoilage) for both samples are presented in *Table 1,* while those of the plate counts (Bacteria and Fungi), before and after spoilage, are respectively presented in *Tables 2* and *3*.

Table 1: Result of pH Analysis

Fruit sample	Before spoilage	After spoilage
Pawpaw	6.50	3.05
Shaddock	4.72	4.38

Table 2: Total Bacterial Count and Morphological Expression of Organisms Isolated from the Samples.

(a) Before Spoilage

Sample	Activity	Colour	Elevation	Size	Shape	Tcf/g	M P B
Sample A (Pawpaw)	Total NA	Dull Creamy colony	Raised	1-2	Irregular	$1.05x10^5$	Staphylococcus spp.
	Total MA (Coliform ount)	No growth	No growth	No growth	No growth	No growth	
	Total EMBA (faecal Coliform Count)	No growth	No growth	No growth	No growth	No growth	
Sample B (Shaddok)	Total NA	Dull Creamy colony	Raised	1-2	Regular	$1.5x10^4$	Staphylococcus spp
	Total MA (Coliform Count)	Pinkish & colourless colony	Raised	1-3	Irregular	$8x10^2$	Klebsiella spp
	Total EMBA (faecal Coliform Count)	Greenish metallic sheen	Flat	1-3	Irregular	$5x10^2$	Escherichia coli

(b) After Spoilage

Sample	Activity	Colour	Elevation	Size	Shape	Tcf/g	M P B
Sample A (Pawpaw)	Total NA	Creamy	Raise	1-3	Irregular	$1.05x10^5$	Staph. spp
	Total MA (Coliform ount)	Pink & colourless	Flate	1-3	Irregular	$7.2x10^4$	Proteus spp
	Total EMBA	Pink & grey	Raise	1-3	regular	$4.6x10^3$	Escherichia coli

	(faecal Coliform Count)						
Sample B (Shaddok)	Total NA	Creamy	flat	1-3	irregular	$1.24x10^5$	Staph. spp
	Total MA (Coliform Count)	Pink & colourless	raise	1-3	regular	$9.4x10^4$	Klebsiella spp
	Total EMBA (faecal Coliform Count)	Pink & grey	raise	1-3	regular	$5.7x10^3$	Escherichia coli

Table 3: Total Fungi Count and Morphological Expression of Organisms Isolated from the Samples.

(a) Before Spoilage

Sample	Activity	Occurence	Cultural	Microscopic	Tcf/g	M P F
Sample A (Pawpaw)	Saboraud Dextrose Agar Count	3	White fluffy Colony, that later turns to green shades, as pigmented spores are produced	Hyphae, hyaline are separate conidiophores; forms branched phialdes, and then a brush.	$3.0x10^2$	Staph. spp
Sample B (Shaddok)	Saboraud Dextrose Agar Count	2	Whitish colony at early stage, that turns brown later.	Separate Hyphae, whose arthrosphores are barrel in shape	$2.0x10^2$	Coccidioides immitis

(b) After Spoilage

Sample	Activity	Occurence	Cultural	Microscopic	MPF	Tcf/g
Sample A (Pawpaw)	Saboraud Dextrose Agar Count	175	Moist dull creamy colony that turns to tannish creamy later.	Unicellular, globose in shape, without hyphae.	$1.87x10^2$	Saccharo-myces spp
		12	At early stage, the colony is whitish but at later stage, it turns to brown.	Hyphae are separate, with barrel-shaped arthrosphores.		Coccidio-ides immitis
Sample B (Shaddok)	Saboraud Dextrose Agar Count	110	Moist dull creamy colony that turns to tannish creamy at later stage	Unicellular, globose in shape, without hyphae.	$1.10x10^2$	Saccharo-myces spp

From the result of the analysis conducted, the pH of the Samples decreased (*6.50 to 3.05 for Pawpaw;* and *4.72 to 4.38 for Shaddok*), becoming more acidic as the fruit samples undergo degradation by the causative microorganisms. This is traceable to the fact that when microorganisms act on food substances, they inject acidic toxins in the foods which result to the sour taste observed when foods spoil (Bracket, 1994). Also, the degradation level was more established in the Pawpaw than in the Shaddok. The reason could be likened to the stronger epicapal protection of fruit juice in the shaddock, restricting to some extent, free microbial penetrations; this is in recalcitrance to the case of pawpaw, which has a very soft epicap.

The total bacterial count, *TBC* showed reasonable level of growth, in the presence of the nutrient algar, for both samples (unlike in the total coliform count, *TCC* and total faecal coliform, *TFC* for pawpaw), which increased as the fruits undergo spoilage. This reaffirms that there is a level (important) bacteria resident on every living tissue, including fruits and vegetables (Chukwuka *et al,* 2010). It could, also, be observed that the *TBC* in both samples (*1.05X105tcf/g* for pawpaw and *9.40X104tcf/g* in the shaddock) was more pronounced when compared with the *TCC* and *TFC* for the study samples. This is justified by the fact that bacteria grow very rapidly in the presence of nutrient, utilizing the varying forms growth (descript cell, flamentus and filamentous cell growths) to form great deal of biomas (Nwokeke, 2014).

Generally, the results show that microorganisms (especially bacteria and fungi) have great capacity of degrading food samples (including fruits and vegetables).

CONCLUSION

The present study reaffirms that food spoilage is caused by microorganisms, in which acidic toxins are injected into the foods by the causative organisms. Sample A (Pawpaw) was more predominantly

affected than the shaddock by the activities of the microbes. The *TBC* (in the presence of *NA*) showed higher level growth than the *TCC* and *TFC* (in the presence of *MA* and *EMBA* respectively).

However, the organisms isolated (and identified) during the study include: *Staphylococcus specie, Klebsiella specie, Escherichia Coli, Proteus specie, Penicillium specie, Coccidioides Immitis* and *Saccharomyce specie*.

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