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BIOCHEMICAL CHARACTERIZATION OF BACTERIA AND FUNGI ISOLATES ASSOCIATED WITH POST-HARVEST SPOILAGE OF AVOCADO PEAR (*PERSEA AMERICANA)* SOLD IN TWO FRUIT MARKETS IN THE BENIN CITY METROPOLIS, NIGERIA

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ABSTRACT

Persea americana is a major and cheap source of nutrients-containing protein fruit and commonly referred to as Avocado pear. It is a green-skin, fleshy body and may be spherical or pear-shaped and ripens easily after harvest, significantly reducing its shelf-life. The average storage time before spoilage is 3-6 days. The fruit is highly cherished by many and as such a significant dietary contribution, in developing countries. However, the poor shelf-life of the fruit has led to its high perishability, huge post-harvest losses and market glut during harvest. In this study, fresh, undamaged, firm, healthy-looking, ripe avocado fruits purchased from Oba Market and New-Benin market were left free of dust and insects under room temperature for between 5-6 days to undergo a natural process of spoilage. A homogenate of each of the sample was achieved by blending 25 grams of the sample in 225 ml of sterile 1.5 % peptone water with a sterile glass blender. Serial dilutions of up to 10^{-1} - 10^{-5} were made and 1 ml of each of the dilutions were transferred into sterile Petri dishes and respectively mixed with 15 ml of an appropriate sterile media and incubated at a temperature of 37 ºC for 48 hours, while the Sauboraud dextrose agar (SDA) was left at room temperature for 5 days. After incubation, bacterial and fungal colony-forming units were counted and used to determine the total aerobic viable counts (TAVC), total coliform counts (TCC), *Escherichia coli* counts (EC), *Staphylococci* counts (SC) and total fungi counts (FC). Representative colonial isolates were subsequently subcultured on nutrient agar slants and stored at a temperature of 4 ºC prior to characterization. Phenotypic identification of microbes was performed according to standard methods. The present study revealed that the coliform bacteria (TCC = 2.42 x 10^3 cfu/g and 2.06 x 10^3 cfu/g) accounted for a significant fraction of the total bacterial population (TAVC = 2.75 x 10^4 cfu/g and 9.68 x 10^3 cfu/g) isolated from spoiled pear produce. Hence, *Erwinia and Klebsiella aerogenes* of genus of Enterobacteriaceae, were the main spoilage bacteria; while *Phytophthora*s species (FC = 1.73 x 10^4 cfu/g and 1.02 x 10^4 cfu/g) was the main spoilage fungus of pear produce sold in the two Nigerian markets. The isolation of pathogenic organisms also calls for a public health concern.

Keywords: Avocado Pear, Microbial, Post-Harvest Spoilage, *Persea americana*, Biochemical Characterization

INTRODUCTION

Persea americana commonly called Avocado pear is a tree native to Mexico and Central America **(Chen, Moriell, Ashworth, De La Cruz, & Clegg, 2008**) and a member of the family Lauraceae, which are mainly shrubs and trees that yield resinous aromatic gum from their cut bark (**Wogu & Ighile, 2014**). It is among the well-known indigenous fruit trees in the tropical and subtropical rain forest zone of the Southern regions of West Africa (extending eastward from Sierra Leone to Nigeria and Western regions of Central Africa, which includes Cameroon, Equatorial Guinea, Gabon, Democratic Republic of Congo, Congo Brazzaville, and Angola. Avocado or alligator pear which also refers to the fruit is a large berry that contains a single seed (**Wogu & Ighile, 2014**).

Avocados have a green-skin, fleshy body that may be pear-shaped or spherical and they easily ripen after harvesting. The trees are partially self-pollinating and are often propagated through grafting to maintain a predictable quality and quantity of the fruit (**Eze & Chimaeze, 2014**). Avocado fruit is a major and cheap source of nutrients containing protein (2 g), moisture (72.23 g), fibre (6.7 g), fat (14.66 g) and carbohydrate (8.53 g) and high energy value of 160 kcal per 100 g. They are also rich in fatty acids, amino acids, potassium, B-vitamins, vitamins K and E. Avocado fruit is much cherished by many people and it makes a significant dietary contribution, as it improves the food problems in developing countries. Besides, it is available at most seasons including strategic periods of the year when conventional staples that are difficult to store are scarce (**Okafor, 1975 in Wogu and Ighile, 2014**). The oils from the pulps and seeds are used in foods, pharmaceuticals and cosmetics manufacturing as well as numerous industrial uses. They are rich in monounsaturated fatty acids and are comparable to other currently used vegetable oils (**Lopez, *et al.*, 1996)**.

Avocados are commercially valuable and are picked hard and green and kept in coolers at 3.30 to 5.6 ºC until they reach their destination. Once picked, avocados ripen in a few days at room temperature. The fruit has a very short shelf-life and can averagely be stored 3-6 days before spoilage. The poor shelf life of the fruit has led to its high perishability, huge post-harvest losses and market glut during harvest as noticed by large heaps of unsold rotten fruits in the refuse dump of the village and urban markets. These characteristics of Avocado fruits are a serious setback for export market as well as industrial uses, as it does not offer flexibility throughout the market channels **(Wogu & Ighile, 2014**). It is estimated that one-fourth of all Avocado fruits harvested are not consumed before spoilage. Spoilage of fresh avocado fruits usually occurs during storage and transport and while waiting to be processed unlike many other fruits (**Eze & Chimaeze, 2014**).

The avocado fruit is vulnerable to bacterial, viral, and fungal diseases which lead to its spoilage. Disease and microorganisms can affect the fruit causing spotting, rotting, cankers, pitting and discolouration **(Abbott, 1999**). Numerous species of microorganisms easily attack the fruit. The composition of the avocado fruit influences the likely type of spoilage (**Eze & Chimaeze, 2014**). The high spoilage rate of Avocado fruit coupled with its high nutritional contents pre-supposes that an array of microorganisms may be involved in its spoilage of Avocado fruits.

The issue of food safety cannot be overemphasized when it comes to the consumption of food or fruits. Hence, in the purchase and consumption of fresh fruits, spoilage is often an issue of concern. Also, consumption of fruits spoilt by spoilage microorganism can lead to foodborne diseases such as infections and intoxications. Also, depending on the type of spoilage microorganism and the severity of the foodborne disease, death may occur.

Although, some researchers have worked on the microbiological and nutritional qualities of avocado pear, however, limited studies exist regarding the characterization of bacteria and fungi isolates associated with the post-harvest spoilage of avocado fruits sold in two popular fruit markets in the Benin City Metropolis. Hence, this research on the characterization **of bacteria and fungi Isolates associated with** Post-Harvest Spoilage **of Avocado fruits.**

MATERIALS AND METHODS

Sample Collection

Two samples each were purchased from two different markets (Oba Market and New-Benin Market). The avocado pear samples collected were fresh, undamaged, firm, healthy and ripe. The samples were dispensed into sterile bags and then brought to the laboratory. The samples were left free of dust, insect and

kept under room temperature for between 5-6 days to undergo a natural process of spoilage before being used in the study.

Isolation and Enumeration of Bacteria and Fungi

A homogenate of each sample was made by blending 25 grams of the sample in 225 ml of sterile 1.5 % peptone water with a sterile glass blender (**Public Health England, 2014**). Serial dilutions of up to 10^{-}-10^{-5} were made and 1 ml of each of the dilutions were transferred in to sterile Petri dishes and were respectively mixed with 15 ml of sterile molten nutrient agar medium, MacConkey agar medium, eosin methylene blue (EMB) agar medium, mannitol salt agar medium, and Sauboraud dextrose agar (SDA) medium amended with entrancing antibiotic. The inoculated nutrient, MacConkey, EMB, and mannitol salt agar Petri plates were then incubated at a temperature of 37 ^{o}C for 48 hours, while the SDA was left at room temperature for 5 days. After incubation, bacterial and fungal colony-forming units were counted and counts were used to deducing the total aerobic viable counts (TAVC); total cruciform counts (TCC), *Escherichia coli* counts (EC), *Staphylococci* counts (SC), and total fungi counts (FC).Representative colonial isolates were subsequently subcultured on nutrient agar slants and stored in the refrigerator at a temperature of 4 ^{o}C for characterization (**Bergey's Manual, 1984;1986**).

Identification and Characterization of the Microbial Isolates

Phenotypic identification of microbes was performed according to standard methods **(Bergey's Manual, 1984; Bergey's Manual, 1986)**.Morphological traits examined include the orientation, size, margins, shapes, and pigmentation (colour) which were performed by visual examination of microbial isolates on culture media, as well as cell wall characteristics which were performed by Gram staining of the isolates. The biochemical tests employed include: the production of coagulase enzyme (coagulase test);the production of catalase enzyme (catalase test),the ability of isolates to produced cytochrome oxidase (oxidase test),the production of urease enzyme (urease test), biodegradation of tryptophan to produce indole (indole test), utilization of citrate as a sole carbon source (citrate test), production of stable acids from glucose fermentation (methyl red test), production of acetoin as the main end product with small quantities of mixed acids from glucose metabolism (Voges Proskauer test), fermentation of mannitol (Mannitol test), the fermentation of lactose (Lactose test) and Microdase a modified oxidase test to that detects the enzyme oxidase.

RESULT

Microbial Load Obtained in Avocado Produce

Table 1 represents the microbial concentration of microbes associated with Avocado spoilage obtained from two markets in Benin City metropolis. The pear produce obtained from Oba market had the highest total aerobic viable counts (TAVC) ($2.75x10^4$cfu/g), while the lowest TAVC value was recorded in the pear produce obtained from New Benin Market ($9.68x10^3$) cfu/g). Total coliform counts were $2.42x10^3$ cfu/g and $2.06x10^3$ cfu/g for Oba and New Benin Market respectively No *Escherichia coli* count (ECC) was recorded for both New Benin and Oba Markets. *Staphylococcus* count (SC) was lowest ($0.7x10^1$cfu/g) in the New Benin Market and highest ($2.4x10^1$cfu/g) in the Oba market. Fungi count (FC) was also lowest ($1.02x10^4$cfu/g) in Oba market.

Table 1 Concentration of Microbes Associated with Avocado spoilage

Parameter	Sample (Oba Market)	Microbial Load (cfu/g)	Mean Microbial Load (cfu/g)	Sample (New Benin)	Microbial Load (cfu/g)	Mean Microbial Load (cfu/g)
TAVC	1	$4.5x10^4$	$2.75x10^4$	1	$3.25x10^3$	$9.68x10^3$
	2	$3.65x10^3$		2	$3.95x10^3$	
	3	$3.45x10^3$		3	$3.25x10^4$	
	4	$4.65x10^4$		4	$4.00x10^3$	
	5	$3.90x10^4$		5	$4.70x10^3$	
TCC	1	$4.7x10^3$	$2.42x10^3$	1	$3.50x10^2$	$2.06x10^3$
	2	$3.15x10^3$		2	$3.15x10^3$	
	3	$4.70x10^2$		3	$4.70x10^2$	
	4	$4.70x10^2$		4	$3.30x10^3$	
	5	$3.30x10^3$		5	$3.05x10^3$	
ECC	1	0	0	1	0	0
	2	0		2	0	
	3	0		3	0	
	4	0		4	0	
	5	0		5	0	
SC	1	$1.50\ x10^1$	$2.4x10^1$	1	$0.50x10^1$	$0.7x10^1$
	2	$3.50\ x10^1$		2	$0.00x10^1$	
	3	$4.50x10^1$		3	$2.00x10^1$	
	4	$1.50x10^1$		4	$1.00x10^1$	
	5	$1.00x10^1$		5	$0.00x10^1$	
FC	1	$1.75x10^4$	$1.73x10^4$	1	$1.20x10^4$	$1.02x10^4$
	2	$9.00x10^3$		2	$9.00x10^3$	
	3	$2.30x10^4$		3	$1.50x10^4$	
	4	$2.15x10^4$		4	$6.50x10^3$	
	5	$1.55x10^4$		5	$8.50x10^3$	

Key: TAVC: Total Aerobes Viable count; TCC: Total Colony Count; ECC: Escherichia Coli Count; SC: Staphylococcus Count; FC: Fungi Count; cfu/g: Colony Forming Unit per gram.

Morphological Identification of Microbial Isolates

Table 2 represents the phenotypic characterization of microbial isolates using morphological and biochemical methods. Six bacteria colonies with unique characteristics were respectively isolated from pear produce collected from Oba and New Benin markets respectively. Phenotypic characterizations identified *Erwinia* species, *Bacillus cereus* and *Staphylococcus aureus* as spoilage bacteria in the pear produce collected from both Oba and New Benin markets. *Pseudomonas aeruginosa* was seen only in pear produce obtain from Oba market. *Phytophthora* species was the main spoilage fungi in the pear produce collected from both Oba and New Benin markets.

Table 2 Phenotypic Characterization of Microbial Isolates Obtained from **Associated with The Spoilage of Avocado Pear**

Location	Microbial type	Isolates	Colonial characteristics	Microscopic characteristic	Biochemical tests											Probable Organism
					Co	Ca	Ox	Ur	Ci	In	Mr	Vp	Ma	La	Mi	
Pear produce from Oba market	Bacteria	1	Spreading mucoid colony on nutrient agar plate	Gram negative rods	-	+	-	-	+	-	-	+	-	+	Np	*Erwinia species*
		2	Seriated dry colony on nutrient agar plate	Gram positive rods	-	+	-	-	+	-	-	+	-	-	Np	*Bacillus cereus*
		3	Yellow pigmented colony on nutrient agar plate	Gram positive cocci in pairs and tetrads	-	+	+	+	-	-	+	+	-	+	+	*Micrococcus Species*
		4	Yellow pigmented colony on nutrient agar plate	Gram positive cocci in clusters	+	+	-	+	-	-	+	+	+	+	-	*Staphylococcus aureus*
		5	Mucoid colony on MacConkey agar plate	Gram negative rods	-	+	+	-	+	-	-	-	-	-	Np	*Pseudomonas aeruginosa*
		6	Yellow mucoid colony on mannitol salt agar plate	Gram positive cocci in clusters	+	+	-	+	-	-	+	-	+	-	-	*Staphylococcus aureus*
	Fungi	1	White fluffy and spreading colony that turned black with age	Coenocytic hyphae with intercalating chlamydospore	Np	Np	Np	Np	Np	Np	Np	Np	Np	Np	Np	*Phytophthora species*
Pear produce from New Benin market	Bacteria	1	Yellow mucoid colony on mannitol salt agar plate	Gram positive cocci in clusters	+	+	-	+	-	-	+	-	+	-	-	*Staphylococcus aureus*
		2	Spreading mucoid colony on nutrient agar plate	Gram negative rods	-	+	-	-	+	-	-	+	-	+	Np	*Erwinia species*
		3	Seriated dry colony on nutrient agar plate	Gram positive rods	-	+	-	-	+	-	-	+	-	-	Np	*Bacillus cereus*
		4	Pink mucoid colony on MacConkey plate	Gram negative rods	-	+	-	-	+	-	-	+	+	+	Np	***Klebsiella aerogenes*
		5	Yellow pigmented mucoid colony on nutrient agar plate	Gram positive cocci in clusters	+	+	-	+	-	-	+	-	+	-	-	*Staphylococcus aureus*
	Fungi	1	White fluffy and spreading colony that turned black with age	Coenocytic hyphae with intercalating chlamydospore	Np	Np	Np	Np	Np	Np	Np	Np	Np	Np	Np	*Phytophthora species*

Key: Co: coagulase; Ca: Catalase; Ox: Oxidase; Uri: Urease; Ci: Citrate; In: Indole; Mr: Methyl Red; Vp: Voges Proskauer; Ma: Mannitol; La: lactose; Mi: Microdase Test; Np: Not Performed; **previously *Enterobacter aerogenes*

DISCUSSION

The high concentration of bacteria and fungi in the spoilt avocado pear samples (Table 1) indicates that these microbes were the main cause of spoilage. Bacteria and fungi were found in high numbers mainly because of the indiscriminate exposure of the fruit's outer surface to the environment at the farms and in the market **(Buck, Walcott, & Beuchat, 2003**). This indiscriminate exposure coupled with the high nutritional content of the pear often resulted in an increased likelihood of contamination of the avocado pear that was propagated by flies, airborne dust, unhygienic human contacts, and damages to the fruit's outer surface (**Dreher & Davenport, 2013)**. The implicated spoilage bacteria and fungi were *Erwinia* species, *B. cereus*, *P. aeruginosa*, *S. aureus*, *Micrococcus* species, *Phytophthora* species and *Klebsiella aerogenes* (Table 2). **Eze & Chimaeze (2014)** also reported the presence of some of these spoilage organisms in avocado pear. The presence of *staphylococcus aureus* in virtually all the pear produce examined is an indication of human contamination of the pear produce from handling. *S. aureus* has been shown to produce enterotoxins that are extremely potent gastrointestinal toxins that can cause symptoms of intoxication when ingested. The wide spectrum of bacteria found in the spoilt avocado pear compared to fungi could be due to the low carbohydrate and high moisture contents of the pear (**Ihekoronye & Ngoddy, 1985**). Pre-harvest and post-harvest factors, consisting of the farm soil-type, storage conditions, and handling practices, maybe the likely source of these microbes **(CFS, 2006; Leff & Fierer, Bacterial Communities Associated with the Surfaces of Fresh Fruits and Vegetables, 2013**).

CONCLUSION

The present study revealed that the coliform bacteria accounted for a significant fraction of the total bacterial population isolated from spoiled pear produce. Hence *Erwinia and Klebsiella aerogenes*, of the genus of Enterobacteriaceae, were the main spoilage bacteria; while *Phytophthora* species was the main spoilage fungus of pear produce sold in the Nigerian markets. The isolation of several other pathogenic organism is a huge public health concern.

Author Contributions

O.A. designed the study and wrote the manuscript; O.S. conducted the experimental work and critical revisions; T. E Contributed to the manuscript

writing and Revisions; M.U contributed to revisions of the manuscript and study design.

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