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MICROORGANISMS ASSOCIATED WITH THE SPOILAGE OF AVOCADO PEAR, *Persea americana* FRUITS

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Abstract

The microorganisms associated with the spoilage of Avocado pear, Persea americana fruits, purchased fresh from various markets in Benin City were investigated. The pour plate method was used for the isolation. A total of nine species of microorganisms were isolated and identified in this study. They comprise of seven bacterial and three

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fungal species. The seven bacterial species were: Bacillus, Enterobacter, Citrobacter, Acinetobacter, Klebsiella, Aeromonas and Micrococcus sp. Enterobacter sp., Bacillus sp. and Micrococcus sp. occurred in samples obtained from all the markets (Uselu, New Benin, Oliha, and Oba). Citrobacter sp. was isolated only from samples obtained from New Benin, Oliha, and Oba Markets. Klebsiella sp. was isolated from Uselu and Oba markets, while Acinetobacter sp. was isolated only from Oliha market. The three fungi isolates were: Mucor sp., Saccharomyces sp. and Geotrichum sp. whereas Geotrichum sp. and Saccharomyces sp. occurred in samples obtained from all the four markets surveyed. Mucor sp. was present only in samples bought from New Benin and Uselu markets. The bacterial counts range was 5.2 to 6.7 x 10^4cfu/g, while the fungal count range was 2.6-3.6x10^4cfu/g. Proper handling methods of Avogado fruits to ensure food safety are discussed.

Introduction

Persea americana commonly called Avocado pear is a member of the family Lauraceae, which are mainly shrubs and trees that yield resinous aromatic gum from their cut bark. 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 Cameroun, Equatorial Guinea, Gabon, Democratic Republic of Congo, Congo Brazzaville and Angola).

The fruit is a pome, characterized by a central core surrounded by edible fleshy layers (Barry, 2001). The Avocado fruit has a pulpy mesocarp of 3 to 9 mm^2 thickness, 7cm-20cm long, weighs 100g – 1000g and has a large central seed, 5cm – 6.4cm long. The skin texture is finely pebbled and dull green when ripe.

Avocado fruit is a major and cheap source of nutrients containing protein (2g), moisture (72.23g), fibre (6.7g), fat (14.66g) and carbohydrate (8.53g) and high energy value of 160 kcal per 100g. They are also rich in fatty acids, amino acids, potassium, B-vitamins,

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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). The oils from the pulps and seeds can be 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).

Avocado fruit used in commerce are picked hard and green and kept in coolers at 3.3^0 to 5.6^0C, until they reach their final destination. Once picked, avocados ripen in a few days at room temperature. The fruit has a very short shelf-life. It can averagely be stored 3-6 days before spoilage. The poor shelf life of the fruit has led to its high perishability, huge losses and market glut during harvest as noticed by large heaps of unsold rotten fruits in the refuse dumps of village and urban markets. These characteristics of Avocado fruits are serious setback for export market as well as industrial uses, as it does not offer flexibility throughout the market channels.

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 discoloration (Samson and Van Reenen-Hoekstra, 1988). Numerous species of microorganisms easily attack the fruit. 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.

Spoilage is always a concern to anyone who purchases fresh fruits. An increase in local acceptability of fresh fruits indicates that a proper understanding of factors of spoilage or prolonging freshness of fruits is of increasing importance to consumers. However limited studies exist regarding the microflora associated with spoilage of the popular avocado fruits sold in Benin City. This study was undertaken to obtain

relevant data on the microorganisms that are responsible for the high perishability of the ripe Avocado pear fruits; identify species that could pose a threat to food safety and health hazard to end consumers of spoilt fruits.

Materials and methods

Sample collection

Two samples each where purchased at four different markets: Oba, New-Benin, Oliha and Uselu in Benin-City. The avocado pear samples collected were fresh, undamaged, firm, healthy and ripe. The samples were dispensed into clean bags and then brought to the laboratory. The samples were left free of dust, insect and were under room temperature for between 5-6 days to undergo natural process of spoilage before being used in the study.

The media was prepared strictly according to manufacturer's instruction. They were sterilized by autoclaving at 121^0C for 15 minutes. After sterilization the agar was allowed to cool down to a temperature of 40^0C into appropriate Petri-dishes. Culture media used were: nutrient agar for bacteria and Sauboraud dextrose agar (SDA) for fungi.

Sample analysis

Samples were blended using a sterile blender. A homogenate of each sample was made by blending ten grams in 25ml of sterile water and then the sample was blended. Serial dilutions of up to 10^{-1}-10^{-5} was made in sterile test tubes by several transfers of 1ml of previously diluted samples from one dilution tube to 9ml of sterile water in another tube.

Inoculation and incubation

After preparation of serial dilution up to 10^{-1}-10^{-5}, then 1ml of serially diluted avocado pear sample was pipetted out to each serially marked petri-dish. Nutrient and sauboraud dextrose agar were poured into appropriately marked plates. The nutrient agar plate was then

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incubated at a temperature of 37^0C for 24hours, while the SDA was left at room temperature for 5 days.

Isolation of bacteria and fungi

Distinct colonies from the SDA and nutrient agar were sub-cultured into freshly prepared agar using aseptic techniques to prevent contamination. The plates were incubated at room temperature for 72 hours for the fungi and at 37^0C for 24 hours for bacteria. The developed colonies were counted and colonies forming units were calculated and recorded. The colonies were purified and then later stored in nutrient agar slant in refrigerator (4^0C) for characterization.

Cultural characteristics

Colour, margin and shapes of the bacteria on the media were observed and recorded.

Gram stain

The gram stain was carried out on 24 hours cultures. A smear of each of the bacterial isolates was made on clean grease free slide and heat-fixed using flame. Crystal violet stains (0.3%w/v) was added and allowed to stand for one minute. The stain was washed off with distilled water. Iodine (0.4%w/v), a mordant was added and allowed to stand for one minute before being rinsed off with distilled water. Ethanol (95%w/v), a decolouriser was then added and allowed to stand for 30 seconds before being rinsed off with distilled water and then counterstained with the secondary stain, safranin (0.4%w/v) and allowed to stand for one minute. This was then washed off with distilled water and allowed to dry. The stained smear was then observed under the microscope using oil immersion lens magnification (x100).

Biochemical tests

Indole test

The test organism was inoculated into a broth that contained tryptophan and incubated at 37^0C for 48 hours. Then 2ml of the broth

suspension was transferred to another test tube under aseptic conditions. About 0.5ml of Kovac's reagent was added to the broth. The mixture was shaken properly to ensure a thorough mixing and then observed for colour reaction. A positive result was indicated by a pink-coloured ring round the interface between the broth suspension and alcohol reagent which rose to the surface.

Results

The results of the total heterotrophic count of bacteria and fungi present in spoilt Avocado pear fruits purchased fresh from Oba, Uselu, New-Benin and Oliha markets are shown in Table 1. In all three sampling from New-Benin market, the total heterotrophic bacteria count, ranged from 4.8-6x810^{4}cfu/g, while the total heterotrophic fungal count ranged from 3.3 to 3.9x10^{4}cfu/g.

Oba market samples had a total heterotrophic bacterial count range of 5.6-8.6x10^{4}cfu/g while the total heterotrophic fungal count ranged from 2.1-4.810^{4}cfu/g. Also Uselu market had a total heterotrophic bacterial count range of 4.3-6.8x10^{4}cfu/g and the total heterotrophic fungal count also ranged from 2.4-4.4x10^{4}cfu/g. That of Oliha market had a total heterotrophic bacteria count range and total fungal count range of 4.9-5.5x10^{4}cfu/g and 1.9-3.2x10^{4}cfu/g respectively.

Table 1: The mean heterotrophic bacterial and fungal counts of avocado pear fruits purchased from different markets.

The highest mean bacterial count from all the sampled markets was at 6.7x10^{4}cfu/g while the lowest was 5.2x10^{4}cfu/g at Oba market respectively.

The mean fungal count of 3.6x10^{4}cfu/g was the highest and was found in Avocado pear fruit samples from New Benin market. The lowest mean count of 2.6x10^{4}cfu/g was obtained from Oliha market.

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Table 1: Heterotrophic Bacterial and Fungal Counts of Avocado Pear Fruits purchased from different Markets.

Sampling Location	**Total Heterotrophic Bacterial Count (After 48hr) (104cfu/g)**	**Total Heterotrophic Fungal Count (After 5days) 104cfu/g)**
NEW BENIN MARKET	4.8	3.9
A1	5.8	3.3
A2	6.8	3.8
OBA MARKET		
A1	8.6	2.1
A2	6.1	2.8
A3	5.6	4.8
USELU MARKET		
A1	6.8	3.2
A2	6.1	2.4
A3	4.3	4.4
OLIHA MARKET		
A1	5.5	2.8
A2	5.4	1.9
A3	4.9	3.2

Where A1 – A3 represents number of times sampling was done.

Table 2: The cultural, morphological and biochemical characteristics of the isolated bacteria, found in all avocado pear fruit samples purchased from all the markets. The probable bacteria species which were isolated include: *Micrococcus* sp., *Bacillus* sp., *Enterobacter* sp., *Acinetobacter* sp., *Citrobacter* sp., *Klebsiella* sp. *and Aeromonas* sp.

Table 2: Cultural, Morphological and Biochemical Characteristics of Bacteria Isolated

(1) Cultural Characteristics	**Description**						
Colour	Yellow	Creamy	Creamy	Purple	White	White	Creamy
Shape	Circular	Irregular	Circular	Circular	Circular	Large	Circular
(2) Morphological Characteristics							
Cell type	Cocci	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Curved	Single	Single	Straight
(3) Gram Stain	+	+	-	-	-	+	-
(4) Motility Test	-	+	+	-	+	+	+
(5) Biochemical Tests							
Catalase	+	+	-	-	+	+	+
Indole	-	-	-	-	-	+	NA
Urease	NA		-	-	+	-	NA
Oxidase	+	-	-	-	-	-	+
Citrate	-	+	+	+	+		NA
(6) Sugar Fermentation Test							
Glucose	-	A/G	A/G	AA	A/G	A/G	AA
Lactose	-	-	A/G	-	A/G	A/G	-
Probable identity	*Micrococcus* sp.	*Bacillus* sp.	*Enterobacter* sp.	*Acinetobacter* sp.	*Citrobacter* sp.	*Klebsiella* sp.	*Aeromonas* sp.

KEY: A/G: Acid and gas production, NA: Not Applicable, A/A: Acid production, +: Positive, -: Negative.

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Table 3: Morphological and Cultural Characteristics of Fungal Isolates. The fungal species isolated were: *Mucor*, *Saccharomyces* and *Geotrichum.*

Table 3: Morphological and Cultural Characteristics of Fungal Isolates

Isolates	Macroscopy	Microscopy
Mucor sp.	Cover agar surface. They are white and fluffy that later turned grey. Reverse side is white.	Sparsely septate, broad hyphae, sporangiophores, sporangia and spores were visualised.
Saccharomyces sp.	Colonies of *Saccharomyces* sp. grow rapidly. They are flat, smooth, moist, glistering, dull and cream to tannish cream in colour.	Unicellular, globose and diploid. Elongate in shape.
Geotrichum sp.	Colonies of *Geotrichum* sp. produced rapidly growing, white, dry, and powdery to cottony colonies resembling ground grass.	Unicellular, in chains, hyaline and result from fragmentation of undifferentiated hyphae by fission through double septa. They are rounded at the ends.

Table 4: The Sampling Location and Microbial Isolates Present

Enterobacter sp., *Bacillus* sp., *Micrococcus* sp., *Saccharomyces* sp and *Geotrichum* sp. were all isolated from samples purchased from New-Benin, Oba and Oliha markets. However, *Citrobacter* sp. was isolated only from fruit samples purchased from New-Benin and Oba markets. *Klebsiella* sp. was isolated in fruits obtained from Uselu and Oba markets, while *Acinetobacter* sp. was isolated only in fruits from Oliha market.

Table 4: Sampling Locations and Microbial Isolates Present

SAMPLING LOCATION (MARKET)	MICROBIAL ISOLATES PRESENT
NEW BENIN	*Bacillus* sp., *Enterobacter* sp., *Citrobacter* sp., *Micrococcus* sp., *Saccharomyces* sp., *Geotrichum* sp. and *Mucor* sp.
USELU	*Enterobacter sp., Klebsiella* sp., *Micrococcus* sp., *Saccharomyces* sp., *Geotrichum* sp. and *Mucor* sp.
OBA	*Micrococcus* sp., *Citrobacter* sp., *Enterobacter* sp., *Klebsiella* sp., *Bacillus* sp., *Geotrichum* sp. and *Saccharomyces* sp.
OLIHA	*Bacillus* sp., *Enterobacter* sp., *Acinetobacter* sp., *Micrococcus* sp., *Saccharomyces* sp. and *Geotrichum* sp.

Discussion

The rapid proliferation of both bacteria and fungi within the days of storage shows that adequate nutrients were available for microbial growth (see Table I). However, increase in storage time and biodegradation led to a fall of both bacterial and fungal populations in some samples, a phenomenon possibly due to exhaustion of nutrients for sustainability. Frazier & Weshoff (1998) reported that the availability of nutrients is crucial to increase or decrease of microbial numbers in fruits during spoilage.

Microorganisms are ubiquitous and they have been found to colonize Avocado pear fruits due to its high nutritional content that can support their growth and cause spoilage. The result from this research identified seven bacteria isolates namely; *Bacillus* sp., *Enterobacter* sp., *Citrobacter* sp., *Micrococcus* sp, *Acinetobacter* sp, *Klebsiella* sp. and *Bacillus* sp. Also, three fungi isolates, *Saccharomyces* sp., *Mucor* sp. and *Geotrichum* sp., were found in the samples collected from four different markets (Oba, Uselu, New-Benin and Oliha).

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The bacteria species, *Micrococcus* sp. and *Klebsiella* sp. (see Table 2), isolated from spoilt Avocado pear in this study were also identified in a similar investigation carried out by Ikenebomeh and Chikwendu, (1997). These organisms have been reported as the causative agents for bacterial soft rots. Like fungi, they can hydrolyse pectin giving rise to a soft mushy appearance or consistency (Liao *et al.*, 1993). Their presence is suggestive of contamination from soil, harvesting equipment, handling and storage facilities and on food-contact surfaces throughout the distribution chain.

Gram negative rod-shaped bacteria such as *Acinetobacter* sp. may also grow at chill temperatures and have been shown to contribute to the spoilage of post-harvest fruits and vegetables. The coliform/enteric bacteria e.g. *Citrobacter* sp. and *Enterobacter* sp. generally slower in growth at chill temperatures become more significant as the temperature rises above 5^0C. Their spoilage action is characterized by the production of gas, acid, slime, bitter flavors and faecal odours.

Bacillus sp. has been reported to be the most antagonist microorganism on post harvest Avocado pear fruit. This is in line with the findings of Korsten *et al*., (1993). Colonization of fruits and vegetables by the invading microorganism is a critical phase in the microbial spoilage of produce. The colonization process involves the ability of the microorganisms to establish themselves within the produce. This in initiated when the microorganisms (following adhesion and release of enzyme) degrade certain specific cell wall polymers such as protopectin, the cementing substance of the produce. The magnitude of the symptoms of the induced disease is a reflection of the extent of colonization (Chukwu *et al.*, 2008). Whereas both fruits and vegetables are highly susceptible to microbial spoilage, there is a variation in the susceptibility which is due largely to the differential chemical composition such as pH and moisture contents. Thus, the lower pH and moisture contents of the fruits make them more prone to fungal spoilage. Efiuvwevewere (2000), also reported that high moisture and relative humidity led to greater fungal growth

in agricultural produce and thus low storability of fruits and vegetables.

The prevalence of fungi as the spoilage organism of fruits and vegetables 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 (Effiuvwevwere, 2000). *Geotrichum candidum* isolated in this study are among the fungi responsible for post harvest rot of Avocado fruit. This finding is in agreement with the reports of previous investigations carried out by Akande (1975) and Onesira and Fatula (1976) in south western Nigeria. Chukwu *et al.,* (2008) also identified similar fungi from tomato and snake gourd in Rivers State, Nigeria. Guirrard (1958) and Franshauser (2005), earlier observed that the oil present in Avocado pear also encourages the metabolism of fungi. The fungal isolates in this study: *Geotrichum* sp. *Saccharomyces* sp and *Mucor* sp. probably grew due to the amount of oil found in Avocado pear as suggested from the reports of previous investigations. These organisms present a formidable challenge to commercial fresh fruit product operations from the farm to retail and wholesale outlets Liao *et al.*, 1993, 1997).

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. Furthermore, the incidence of infection is worsened by poor sanitary practices such as cross-contamination, contact infection during the transportation of product (Effiuvwevwere, 2000). The isolation of *Mucor* sp. and *Saccharomyces* sp. (see Table 3) from Avocado pear is this study is similar to the findings reported by the Chukwu *et al,* (2008), Pusey and Wilson (1984) and Effiuvwerewere (2000) who additionally reported that *Fusarium* sp, and *Rhizopus stolonifer* are responsible for the soft rot of tomato fruits. Ohr, *et al*., *(2003)* also reported that *Fusarium monilifome, Aspergillus niger and Rhizopus stolonifer* were isolated from rotten tomato fruits.

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As earlier stated, the presence of the fungi or their resistant spores is more likely to have originated from the farms where the fruits were harvested and some from the stores due to horizontal contamination by the already spoilt fruits. Johannessen *et al*., (2002) observed that most spoilage organisms may be present on fruits and vegetables from the farm, during harvest operations, and this may result in post harvest contamination and spoilage of these fruits and vegetables.

The present and subsequent spoilage due to these fungi, if not checked could lead to serious economic losses and possible health hazards when these fruits are consumed. Losses due to post harvest spoilage or pathological decay are a result either of latent infections in the field that become active following harvest or of cross-contamination during harvest, cleaning, storage and distribution. Therefore spoilage management should begin in the field by using an integrated strategy of good agricultural practice (GAP).

Conclusion

To reduce the rate of contamination which is turn leads to the spoilage of Avocado pear fruits and also lead to the ingestion of contaminated fruits by consumers, it is important that the sellers should be properly educated and sensitized on the need to improve their own personal hygiene which is one of the factors that affect post harvest of Avocado pear fruits, thereby introducing contaminants and spoilage then occurs.

This research has been able to isolate and identify microorganisms associated with the spoilage of avocado pear fruits and also present some steps to be taken in order to reduce the presence of microorganisms capable of causing harm to the consumer. It is suggested that proper handling would ensure a better quality of Avocado pear fruits being sold in our local markets.

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