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Isolation and Identification of Microbes Associated with Spoilage of African Star Apple (*Chrysophyllum albidum* Linn.) Sold in Markets in Benin City, Edo State, Nigeria

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ABSTRACT: African star apple (*Chrysophyllum albidum* Linn.) belong to the order Ebernale and family Sapotaceae. The study was aimed at isolating and identifying microbes associated with spoilage of Africa Star Apple (*Chrysophyllum albidum*) commonly sold at Oba, Uselu and Ikpoba Hill markets in Benin City, Edo State, Nigeria. Standard microbiological and biochemical techniques were used for the analysis. The results showed that viable bacterial counts of the spoilt cherry fruits ranged from 1.8×10^3 - 7.6×10^3 cfu/g, 8.8×10^2 -5.4×10^3 cfu/g and 8.7×10^2 -1.7×10^3 cfu/g for spoilt cherry obtained from Oba market, Uselu market and Ikpoba Hill market. The fungal counts of spoilt cherry fruits ranged from 3.1×10^3 - 9.4×10^3 cfu/g, 1.8×10^3 -6.0×10^3 cfu/g and 1.3×10^3 - 2.9×10^3 cfu/g for spoilt cherry obtained from Oba market, Uselu market and Ikpoba Hill market. The bacterial isolates obtained were *Klebsiella* spp., *Flavobacterium* spp., *Serratia* spp., *Staphylococcus aureus*, *Erwinia* spp., *Pseudomonas* spp. and *Escherichia coli*. The fungal isolates identified from the spoilt cherry fruits were *Saccharomyces* spp., *Aspergillus niger*, *Mucor* spp., *Penicillium* spp., *Fusarium* spp., *Aspergillus flavus* and *Geotrichum* spp. *Bacillus subtilis* had the highest percentage occurrence, while *Aspergillus niger* was the most occurring fungal isolate. The least occurring bacteria were *Staphylococcus aureus* and *Erwinia* spp., while the least occurring fungal isolates were *Geotrichum* and *Mucor* spp. This study showed high level of contamination of cherry fruits by microbes during preharvest and postharvest period.

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The African star apple (*Chrysophyllum albidum* Linn.) is an angiosperm belonging to the order Ebernales, family Sapotaceae (Ehiagbonare *et al*., 2008). It is known as "Udara' or Agbalumo in Nigeria. It is also popularly called cherry fruit. The plant has been reported to grow up to a height of 36.5 m and is known to occur in diverse ecological zones in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'Ivoire (Bada, 1997). It is commonly called African star apple fruit described as large beny containing 4 to 5 flattened seeds or fewer as a result of seed abortion (Keay, 1989). The seeds are also used for local games or discarded (Bada, 1997). A detailed description of the apple has been documented in the reports of Adebisi (1997) and Amusa *et al*. (2003). The fleshy edible pulp is consumed as relished by people and for the purpose of stopping irritation, loss of appetite and salivation. Studies have shown the fruit to be an excellent source of vitamins, iron, flavours to diet and raw materials to some manufacturing industries (Bada, 1997; Umelo, 1997; Adisa 2000). In addition to these qualities, it has an acceptable composition of moisture, ash, crude fibre, oil, protein, starch, sugars and ascorbic acid as cited in Adindu *et al*. (2003). The plant has in recent times become a crop of commercial value in Nigeria (Oboh *et al*., 2009). The fleshy pulp of the fruits is eaten especially as snack and its fruit has been found to have higher contents of ascorbic acid than oranges

and guava (Amusa *et al*., 2003). The fruits are not usually harvested from the trees, but left to drop naturally to the forest floor where they are picked up (Amusa *et al*., 2003). Fruiting season of the plant is usually in the months of December to April during which it is found both in rural and urban cities (Amusa *et al*., 2003). Allowing the fruits to drop before picking promotes fungal infections.

Recent market survey revealed that the fruits often deteriorate within a period of 5 days, with the deterioration starting with a change of colour from uniform orange to one with patches, and followed by shrinking of the fruit (Adebisi, 1997). Worth mentioning is the fact that the fruits in its season has engaged some petty traders from which they enjoy some level of economic gains. Therefore, the fruit is fast becoming a fruit of economic value. Hence the objective of the study was to isolate and identifying microbes associated with Spoilage of Africa Star Apple (*Chrysophyllum albidum*) commonly sold at Oba, Uselu and Ikpoba Hill markets in Benin City, Edo State, Nigeria.

MATERIALS AND METHODS

Fifteen (15) apple fruits were bought from three different markets, five each was bought from Oba market, Uselu market and Ikpoba Hill market all in Benin City, Edo State. The infected portions of the fruits were sliced into 2 mm^2 pieces, surfaced sterilized for 3 min with 1 % NaOCl and rinsed in four successive changes of sterile distilled water. The surface sterilized infected portions were then plated on sterile nutrient agar and potato dextrose agar using pour plate method. The nutrient agar plates were incubated at 36 ºC, while the potato dextrose plates were incubated for five days. The bacteria isolates were identified using standard microbiological techniques and by the use of Bergey's manual, while the fungal isolates were examined under a stereo binocular microscope, by the use of lactophenol cotton blue test; and their identities determined using cultural, morphological and descriptions in existing publications (Cheesbrough, 2005; Barnett and Hunter, 1972, Booth, 1971, Webster, 1980).

RESULTS AND DISCUSSION

The viable bacterial counts of the spoilt cherry fruits ranged from 1.8×10^3 - 7.6×10^3 cfu/g, 8.8×10^2 -5.4×10^3 cfu/g and 8.7×10^2 -1.7×10^3 cfu/g for spoilt cherry fruits obtained from Oba market, Uselu market and Ikpoba Hill market respectively. The cherry fruits obtained from Oba market had the highest bacterial counts while cherry fruits obtained from Ikpoba Hill market had the least bacterial counts as seen in Table 1. The fungal counts of spoilt cherry fruits ranged from 3.1×10^3 - 9.4×10^3 cfu/g, 1.8×10^3 - 6.0×10^3 cfu/g and 1.3×10^3 - 2.9×10^3 cfu/g for spoilt cherry obtained from Oba market, Uselu market and Ikpoba Hill market as shown in Table 1. The bacterial isolates were *Klebsiella* spp., *Flavobacterium* spp., *Serratia* spp., *Staphylococcus aureus, Erwinia* spp., *Pseudomonas* spp. and *Escherichia coli.* as shown in Table 2.

The fungal isolates identified from the spoilt cherry fruits were *Saccharomyces* spp., *Aspergillus niger, Mucor* spp., *Penicillium* spp., *Fusarium* spp., *Aspergillus flavus* and *Geotrichum* spp. as shown in Table 3. The distribution of the microorganisms isolated from the spoilt cherry fruits is shown in Table 4. The percentage occurrence of the microbial isolates is shown in Table 5. Among the bacterial isolates, *Bacillus subtilis* had the highest percentage occurrence of 18.0 %, while for the fungal *Aspergillus flavus* had the highest percentage occurrence of 24.53 %. The mean total microbial count of the samples was analyzed and the results shown in Table 1.

The result in Table 1 agrees with the report of Oyeleke and Oyewole (2005), were microbial load were observed after analysis on spoilt *Chrysophyllum albidum.* The report of Arotupin *et al.* (2016) agrees with this study were microbial loads similar to that of this study were observed. The microbes isolated from this research in Table 2 and 3 are similar with the microbes isolated from the research of Arotupin *et al*. (2016), Oranusi *et al*. (2015), Oyeleke and Oyewole (2005). The presence of these microbes could be because of the rich nature of the fruit, therefore supporting the growth and proliferation of these organisms. The seven fungi present in Table 3 in this study are similar to the eight fungi isolated from the research of Amusa *et al*. (2013).

Table 1: Mean total viable bacterial and fungal counts on spoilt Africa Star Apple (*Chrysophyllum albidum* Linn.)

Samples code	Mean bacteria counts (cfu/g)	Mean fungi counts (cfu/g)
A1	7.6×10^3	9.4×10^3
A2	3.4×10^3	5.5×10^3
A3	1.5×10^3	3.1×10^3
A4	4.4×10^3	7.7×10^3
A5	1.8×10^3	4.3×10^3
B1	3.0×10^3	4.8×10^3
B2	5.4×10^3	6.0×10^3
B3	2.2×10^3	3.0×10^3
B4	1.3×10^3	2.3×10^3
B5	8.8×10^2	1.8×10^3
C1	8.7×10^2	1.4×10^3
C2	3.3×10^3	2.9×10^3
C3	1.7×10^3	2.1×10^3
C4	1.2×10^3	1.7×10^3
C5	8.8×10^2	1.3×10^3

Keys: A = Oba market, B = Uselu market, C = Ikpoba Hill arket

Table 2: Cultural, Morphological and Biochemical Characteristics of the bacterial isolates

Characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Cultural	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Shape	Convex	Low convex	Convex	Convex	Flat	Low convex	Low convex
Margin	Smooth	Entire	Entire	Entire	Entire	Entire	Entire
Wetness/Dryness	Wet	Wet	Wet	Wet	Wet	Wet	Wet
Transparency	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Colour	Cream	Yellow	Red	Yellow	Cream	Green	Cream
Size	Medium	Medium	Medium	Small	Medium	Medium	Small
Morphology							
Gram stain	-	-	-	+	-	-	-
Cell type	Rod	Rod	Rod	Cocci	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Cluster	Single	Single	Single
Biochemical							
Catalase	+	+	+	+	+	+	+
Oxidase	-	+	-	-	-	+	-
Coagulase	-	-	-	+	-	-	-
Urease	+	+	-	+	-	-	-
Indole	-	-	-	-	-	-	+
Citrate	+	+	+	+	+	+	-
Sugar fermentation							
Glucose	AG	A(+)	A(+)	A(+)	A(+)	A(+)	AG
Lactose	+	-	-	-	+	-	+
Possible isolates	*Klebsiella* spp.	*Flavobacterium* spp.	*Serratia* spp.	*Staphylococcus aureus*	*Erwinia* spp.	*Pseudomonas* spp.	*Escherichia coli*

Table 3: Cultural and microscopic characteristics of the fungal isolates

Characteristic	1	2	3	4	5	6	7
Cultural	Moist milky colonies.	Black fluffy colony with reverse side yellow	White flat colony with reverse side cream	Colonies are first white, then powdery with blue-green pigment bothered in white	Cottony white colony with reverse side cream	Yellow green colony with reverse side yellow	Soft and creamy white colonies.
Microscopic							
Nature of hyphae	Pseudohyphae	Septate	Non-septate	Septate	Septate	Septate	Septate
Colour of spore	Colourless	Brown	Cream	Green	Cream	Yellow	Cream
Type of spore	Chlamydospore	Conidiospores	Sporangiospore	Conidiospores	Conidiospores	Conidiospores	Arthrospore
Appearance of special structure	Budding cells	Foot cells	Sporangium	Brush-like conidia	Micro and macro conidia	Foot cells	arthroconidia
Possible isolates	*Saccharomyces* spp.	*Aspergillus niger*	*Mucor* spp.	*Penicillium* spp.	*Fusarium* spp.	*Aspergillus flavus*	*Geotrichum* spp.

The natural dropping of star apple fruits and the poor hygienic standard in handling is responsible for some of the bacteria like *Escherichia coli* (Amusa, *et al.*, 2013; Nester *et al*., 2001).

The presence of *Bacillus subtilis* in this research like other researches is indicative of environmental contamination as the fruit is constantly been exposed to air, aerosols and dust particles (Gallo *et al*., 1992, Oyeleke and Oyewole, 2005).

Table 5 shows the percentage of occurrence of each of the bacterial and fungal isolates where *Bacillus subtilis* had highest percentage occurrence among the bacterial isolates recovered from the spoilt cherry fruits. *Aspergillus niger* was the most occurring fungal isolate, while the least occurring fungal isolates were *Geotrichum* and *Mucor* spp.

The research of Arotupin *et al*. (2016) showed *Aspergillus niger* with 20 % incidence which is lower than the 24.53 % of this study. Microorganisms causing rots in cherry fruits have been reported to produce a wide range of hydrolytic enzymes such as cellulases, pectinases, xylanases and proteases (Olivieri *et al*., 2004). These enzymes are responsible for tissue maceration and cell death, after which the microorganisms have access to the nutritional resources of the dead plant tissues (Aveskamp *et al*., 2008). The high bacterial and fungal counts portend a serious health implication. *Aspergillus* and *Penicillium* species produce mycotoxins involved in mycotoxicosis of humans and animals. *Staphylococcus* and *Bacillus* species produce potent toxins implicated in food borne illnesses, while the presence of *Escherichia coli* indicates faecal contamination. Proper handling during postharvest must be highly considered before these fruits are consumed.

Table 4: Distribution of the Microorganisms in the Spoilt Cherry Fruits

Bacterial isolates	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
Klebsiella spp.	+	+	-	-	-	+	+	+	-	+	-	-	+	-	-
Flavobacterium spp.	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+
Serratia spp.	+	-	+	+		-		-	+	-	+	-	+	-	-
Staphylococcus aureus	-	+	-	-	-	-	-	+	-	+	+	-	-	+	-
Erwinia spp.	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-
Escherichia coli	+	-	+	+	+	-	-	+	-	-	-	+	-	-	+
Pseudomonas spp.	+	+	-	+	+	-	+	-	-	-	-	+	+	-	+
Bacillus subtilis	+	+	-	-	+	-	+	-	+	-	+	+	-	+	+
Fungal isolates															
Saccharomyces spp.	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-
Mucor spp.	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
Penicillium spp.	+	-	-	+	+	+	+	+	+	-	-	-	+	-	+
Fusarium spp.	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-
Aspergillus flavus	+	-	+	-	+	-	-	-	-	+	+	+	-	+	-
Geotrichum spp	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-

Key: + = Present, - = absent

Table 5: Percentage Occurrence of the Microbial Isolates

Bacterial isolates	No. of isolates	Frequency (%)
Klebsiella spp.	07	14.0
Flavobacterium spp.	05	10.0
Serratia spp.	06	12.0
Staphylococcus aureus	04	08.0
Erwinia spp.	04	08.0
Escherichia coli	07	14.0
Pseudomonas spp.	08	16.0
Bacillus subtilis	09	18.0
Fungal isolates		
Saccharomyces spp.	11	20.75
Mucor spp.	04	07.54
Aspergillus niger	13	24.53
Penicillium spp.	09	16.98
Fusarium spp.	05	09.43
Aspergillus flavus	07	13.21
Geotrichum	04	07.54

Conclusion: The results of this present study showed that microorganisms are responsible for the deterioration of cherry fruits. Studies on this fruit is recommended to gain importance on wide industrial application. Local methods that are sustainable can be developed to reduce fruits losses. More-so, early harvesting of the fruits, prevention of over ripening and keeping the fruits dry and cool at all times should be ensured.

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