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Public Health Implications of Microbial Spoilage of *Chrysophyllum al.* (African star apple) from Egbema, Rivers State – Nigeria.

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Abstract
The effects of microbial deterioration on nutritional quality and public health implications of *Chrysophyllum albidum* (Africa star apple) were investigated using standard analytical methods. Market samples of the fruit were significantly more infected than the forest/farm samples with fungal species being more predominant (P = 0.05). Microbial deterioration showed significant decrease (P = 0.05) in all the nutritional properties assessed – moisture content, crude protein, crude fibre, ash, carbohydrate and fats/oil contents. The pathogenicity tests carried out showed that *Botryoidoplodia, Cladosporum, Fusarium, Penicillium, Rhizopus* and *Aspergillus* species were the major fungal pathogens while *Geotrichum, Trichoderma* and *Saccharomyces* species were mainly contaminants. *Erwinia, Pseudomonas* and *Bacillus* species were the main bacterial pathogens of the fruits. Other bacterial species observed include *Lactobacillus, Salmonella, Escherichia, Klebsiella* and *Staphylococcus* species. Total heterotrophic bacterial, Total Coliform and Potential bacterial pathogens (PBP) including total fungal counts increased with storage time. However, Potential Pathogenic Bacteria Count were not observed in the early days (1-2) of storage. Results obtained showed that microbial spoilage of the *C. albidum* fruits posed potential health dangers to consumers of the infected fruits as nutritional value decreased with an increase in likely human bacterial pathogen. Consumption should be within 0-3 days of harvesting.

Key words; Nutritional quality, spoilage, health, microbial, Foods
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Introduction

Chrysophyllium albidum (African star apple) belongs to the family Sapotaceace and is primarily forest tree found in West, East and Central African countries (Amusa *et al.*, 2003; Houessou *et al.*, 2012 Amusa *et al, 2003*). The fruit is a berry, green when young but yellow to orange when mature for consumption. It is a fruit relished by both the adult and the young when in season especially late November to April in both rural and urban settlements (Adepoju and Adeniji, 2012; Chukwumalume *et al.*, 2012). The fruit tastes slightly sour when harvested forcefully from the trees but slightly sweet and good when allowed to drop naturally off the tree. It is commonly called *Udara* or *Udala* in Igbo and *Osan* or *Agbalumo* in Yoruba.

The *C. albidium* tree grows to a height of 25-35 metres and 1.5 to 2m in girth with numerous branches. Each fruit has 4-6 or less, brown seeds which are flattened side ways and properly covered with cream coloured endocarp. The African star apple has been found to be

high in vitamins (especially C), calcium, potassium while the oil from the seeds serve various domestic and industrial purposes (Houessou *et al., 2012;* Adepoju and Adeniji, 2012; Amusa *et al.,* 2023). The fruits also contain anarcordic acid (resin) and gum used for different purposes. The fibre from the fruit has been said to be a good roughage (Zubbair, 2009).

Some researchers have stated that African star apple has nutritional components like protein, carbohydrate, and fats/oil. It is a good source of dietary fibre as stated earlier (Eni *et al.*; 2010; Chukwumalume, *et al.*; 2010; Odugbemi *et al.*, 2007). Inspite of all these uses, *C. albidium* fruits have short-shelf life as it is a good medium for microbial proliferation.

The microbial spoilage of *C. albidum* fruits is well-known but the effect on the nutritional quality has remained uninvestigated. Again, the presence of potential pathogens during storage has also remained unknown with the likely health implications of consuming deteriorated fruits. This work therefore was designed to bridge these gaps.

Materials and methods

Study area

The study area was Egbema, in Ogba/Egbema/Ndoni Local Government Area of Rivers State, Nigeria. Egbema is at the northern apex of Rivers State at the boundary with Imo State. It is in the typical tropical rainforest zone of Nigeria, well-known for the availability of the *C. albidium* (African Star apple) in its season.

Some *C. albidium* fruits were bought from the local markets in the area while others were obtained from the forest trees of the same plant in the same study area. Only very healthy fruits without injuries were used in the study as injured or infected ones were promptly discarded.

Microbiological analysis;

The fruits were placed in groups of 10 and in each sampling day, any three were used. There were 8 groups for each set (market and forest samples sets) The flesh mesocarp of the randomly selected fruits was aseptically cut out and ground into pulp asceptically too. 1.0g of the pulp was used for a tenfold serial dilution using sterile saline as diluent. 0.2ml of 10^{-2} -10^{-4} dilutions were inoculated on various culture media using the spread plate techniques as described by Chessbrough (2005) in duplicates. Nutrient agar was used for total heterotrophic bacteria (THBC), McConkey agar for total coliform count (TCC), and Sabouround dextrose agar for total fungal count (TFC). The potential pathogenic bacterial count (PPBC) was determined by the heamolytic activities of the organisms on blood agar and coagulase test results (Harrigan, 1998). The work was carried out for eight days beginning with the first day as zero day. The number of microbial colonies observed was recorded as colony forming units per gram of the fruit for each day of storage. Representatives of the observed colonies were further sub-cultured by streaking technique to obtain pure isolates which were characterized and identified according to Chessbrough (2005) and Holt *et al.*, (1994) for bacterial isolates. The fungal isolates were identified following the description of Tsuneo (2002) and Barnett and Hunter (2000).

Nutritional quality analysis;

Determination of nutritional properties of the *C. albidium* (African star apple) fruits was done according to AOAC (2011) methods. The moisture content was assessed by the weight loss technique by drying at 105°C for 8hrs to

a constant weight and reweighing to obtain the difference. The ash content was determined after heating the ground pulp till it turned gray –white at 800°C (in muffle furnace (Gallenkemp Hotspot oven). The difference in weight between the initial and final weights was taken as ash content after cooling to room temperature. Determination of the crude fibre was done following the protocols of AOAC (2011). The fruit pulp (ground) was boiled in 1.25% H_2SO_4 for 30 minutes and washed in distilled water after filtration in muslin cloth. It was again boiled in 1.25% NaOH for 30 minutes before filtering and washing in distilled water again. The insoluble portion of the fruit pulp was then washed in ethanol and later petroleum ether before heating at 105°C for 5-10 minutes to arrive at a constant weight. The remaining pulp was incinerated at 600°C for 2 hours and allowed to cool. The difference between the weight after the ethanol and ether treatment from the final weight was taken as the crude fibre.

The crude fat/oil content was determined following AOAC (2011) procedure. 2.0g of the pulped mesocarp was wrapped in fat-free Watman (ashless) paper and placed in soxhlet extractor. Petroleum ether was used as the extractant while heating at 60°C for 7hours. The sample was then dried in Gallenkamp Hot air oven to complete driness. The difference in weight was taken as fat/oil content.

Determination of crude protein was also done by the same AOAC (2011) procedure using the Kjeldahl digester (Eclipse Scientific Model K 285). The distillate obtained after the catalytic digestion was titrated against 0.1 N HCL. Colour change from green to pink was the end point. The difference between test and blank results was multiplied by 6.25 (constant) to give the crude protein content of the *C. albidum* fruit.

Determiantion of the carbohydrate content was done by the formular stated by Bryant *et al.*, (1988) as follows:

Carbohydrate = 100 – (protein % + fat % + moisture content % + ash % + crude fibre %).

The processes were done in duplicates and the results were subjected to standard deviation statistical tool to test the significance of result obtained.

Results

Assessment of microbial prevalence in the macerated *C. albidum* fruit mesocarp showed the presence of both bacterial and fungal speices. Organisms belonging to 9 bacterial genera were observed. These were *Bacillus, Pseudomonas, Erwinia, Staphylococcus*, and *Lactobacillus*. Others include *Escherichia, Salmonella* and *Klebsiella.* While *Klebsiella, Salmonella and Staphylococcus aureus* were not observed in the forest fruit samples, all the organisms had higher prevalence in the market samples (Table 1). A similar trend was observed in the fungal species where the highest fungal species was the *Aspergillus* (30%), followed by *Rhizopus* (20%) while the least was *Saccharomyces (10%)* and *Geotrichum* species(8%) in the forest *C. albidum* fruits. In the market fruits, only *Geotrichum* species had a 20% prevalence while others had between 22 and 40% prevalence.

Table 2 shows that all parameters assessed had the least values by the 6th and 7th days which were statistically different from values from other days of storage. Observations showed that values obtained within 0-3 days were not statistically different. However,

significant changes were prominent after the 4th day of storage.

Moisture contents ranged from 73.3 to 85.5%, Crude protein had 1.6-4.2mg/g and ash content ranged between 0.8 – 2.2mg/g. The Crude fibre was from 2.4 – 4.6mg/g, fat/oil was between 2.4 to 5.3mg/g and carbohydrate content ranged from 9.2 to 4.7mg/g. In each case, lowest values were observed in the 6th and 7th days of storage. Values from the 6th and 7th days were not significantly different. Highest values were obtained in the 0-3 days of storage with exception of moisture content.

Microbial loads increased significantly with storage time. Lowest loads were observed in the early days of storage but the loads increased significantly after the 3rd day of storage ($P = 0.05$) Potential bacterial pathogens were not observed in the 0-4 day of storage in the forest fruits but occurred by the 3th day in the market samples.

Generally, THBC had significantly higher values than other bacterial groups, followed by the fungal counts while the least were the potential bacterial pathogens in both forest and market fruit samples. However, market fruits had higher bacterial loads than the forest fruits. Again, on each storage day, more microbial and fungal loads were observed in the market fruit samples ($P = 0.05$).

Observations on the pathogenicity tests showed that *Bacillus, Pseudomonas* and *Erwinia* species were the main bacterial pathogens of *C. albidum* fruits while *Geotrichum, Saccharomyces* and *Trichoderma* species were less pathogenic among the fungi. Statistical analysis showed significant differences on pathogenicty of the microbial isolates ($P = 0.05$).

Table 1: Prevalence of bacterial and fungal isolates from the C. albidium fruits examine

Organisms	Farm/forest fruits			Market fruits		
Bacteria	**NFE**	**NFI %**	**%**	**NFE**	**NIF**	**%**
Bacillus	50	10	20	50	20	40
Pseudomonas	50	6	12	50	11	22
Erwinia	50	6	12	50	10	20
Staphylococcus	50	5	10	50	19	38
Lactobacillus	50	4	8	50	14	28
Escherichia	50	6	12	50	10	20
Salmonella	50	-	-	50	4	8
Klebsiella	50	-	-	50	5	10
S. auerus	50	-	-	50	3	6
Fungi						
Fusarium	50	-	-	50	12	24
Geotrichum	50	4	8	50	10	20
Cladosporum	50	5	10	50	16	32
Penicillium	50	6	12	50	10	20
Aspergillus	50	11	22	50	20	40
Saccharamyces	50	5	10	50	10	20
Trichodemia	50	5	10	50	11	22
Rhizopus	50	10	20	50	5	10
Botryodylodia	50	-	-	50	16	32

Table 2: Variations in nutritional contents and microbial loads with days of storage (mg/g)

Days	Mc	Cp	Ash	Cf	Fat/oil	Cho
1	73.9 ± 0.2^{a}	4.1 ± 0.3^{a}	2.1 ± 0.1^{a}	4.6 ± 0.3^{a}	5.3 ± 0.1^{a}	9.2 ± 0.1^{a}
2	74.4 ± 0.3^{a}	4.3 ± 0.2^{a}	2.0 ± 0.2^{a}	4.3 ± 0.4^{a}	5.2 ± 0.2^{a}	9.0 ± 0.3^{a}
3	75.3 ± 0.2^{a}	$0 + 0.4^{a}$	1.8 ± 0.2^{b}	3.7 ± 0.3^{b}	4.8 ± 0.4^{b}	8.7 ± 0.3^{b}
4	77.4 ± 0.4^{a}	3.6 ± 0.7^{b}	1.5 ± 0.2^{c}	3.6 ± 0.5^{b}	4.2 ± 0.3^{c}	7.3 ± 0.2^{c}
5	79.7 ± 0.3^{b}	2.1 ± 0.5^{c}	1.2 ± 0.4^{d}	3.0 ± 0.3^{c}	13.6 ± 0.5^{d}	6.7 ± 0.4^{d}
6	82.5 ± 0.5^{b}	1.6 ± 0.6^{d}	1.0 ± 0.5^{c}	2.9 ± 0.5^{c}	3.2 ± 0.3^{e}	5.4 ± 0.3^{e}
7	85.8 ± 0.3^{c}	1.3 ± 0.4^{d}	0.8 ± 0.4^{d}	2.4 ± 0.6^{d}	2.4 ± 0.4f	4.7 ± 0.2^{f}
0	73.3 ± 0.3^{a}	4.1 ± 0.3^{a}	2.1 ± 0.1^{a}	4.5 ± 0.2^{a}	5.3 ± 0.2a	9.1 ± 0.2^{a}

- Figures followed by the same alphabets are not significantly different while those followed by different alphabets are significantly different.

  Keys; Mc = Moisture content; Cp = Crude protein; Cf = Crude fibre and Cho = carbohydrate

Table 3: Spoilage potentials of the isolated micro-organisms in African Star apple

Organism	No of days for spoilage signs to appear						
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Bacillus species	-	X	X	X	X	X	X
Erwinia caratovora	-	X	X	X	X	X	X
Escherichia species	-	-	-	X	X	X	X
Pseudomonas putida	-	X	X	X	X	X	X
Staphylococcus epidemidis	-	-	-	X	X	X	X
E.coli	-	-	-	X	X	X	X
Lactobacillus species	-	-		X	X	X	X
Klebsiella species	-			X	X	X	X
Salmonella species	-			X	X	X	X
Fungi							
Botrydoplodia species	-	X	X	X	X	X	X
Geotrichum species	-	-	-	X	X	X	X
Aspergillus species	-	X	X	X	X	X	X
Fusarium species	-	X	X	X	X	X	X
Clodosporium species	-	X	X	X	X	X	X
Saccaromyces species		-	-	X	X	X	X
Penicillum species	-	X	X	X	X	X	X
Trichoderma species	-		-	X	X	X	X
Rhizopus species	-	X	X	X	X	X	X

X = Spoilage signs observed

Table 4: Counts of the various bacterial group determined (cfu/g)

Bacterial group	Farm/Forest fruits sample Days of storage							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
THBC	0.4×10^1	0.9×10^1	1.2×10^2	1.1×10^3	1.6×10^3	2.1×10^4	$2.6 \times 10+$	2.5×10^4
TCC	0.1×10^1	0.2×10^1	0.3×10^1	1.0×10^1	0.9×10^1	1.3×10^2	1.0×10^2	1.9×10^2
PPBC	-	-	-	0.2×10^1	1.1×10^1	1.1×10^1	1.3×10^1	1.6×10^1
TFC	0.3×10^1	0.5×10^1	1.2×10^1	1.7×10^1	1.5×10^2	1.3×10^3	2.2×10^4	4.4×10^4
	Market fruits samples							
THBC	0.9×10^1	1.4×10^1	1.3×10^3	1.9×10^3	2.4×10^4	2.8×10^4	3.7×10^4	3.2×10^4
TCC	0.3×10^1	0.7×10^1	1.2×10^1	1.9×10^1	1.1×10^2	1.6×10^2	1.1×10^3	1.9×10^3
PPBC	-	-	0.4×10^1	0.8×10^1	1.1×10^1	1.1×10^2	1.3×10^2	1.8×0^2
TFC	0.6×10^1	1.1×10^1	1.9×10^1	1.5×10^2	2.4×10^1	1.9×10^3	2.7×10^4	6.1×10^4

Key;
THBC = Total heterotrophic bacterial count; PPBC = Potential Pathogenic Bacterial Count
TCC = Total Coliform Counts TFC = Total Fungal Count.

Discussion

In this investigation, nine bacterial and nine fungal species were observed. The bacteria were *Bacillus, Pseudomonas, Erwinia, Staphylococcus, Lactobacillus* and *Escherichia* species. Others were *Staphylococucs aureus, Klebsiella* and *Salmonella* species. The fungal isolates were *Fusarium, Cladosporium, Penicillium, Aspergillus, Geotrichum* and *Saccharomyces* species. Others were *Trichoderma, Rhizopus* and *Botryodioplodia* species.

Joy (2000), Prescott *et al* (2005) and Pelczar *et al*., (2005) reported that several microorganisms can grow on/in fruits because of the high degradable components of fruits. Similarly Agrios (1997) , Adebisi (1997) and Angie (2001) reported that while some of these organism are the main plant pathogens (primary invaders), others are merely contaminants or secondary invaders.

Some of these organisms are well known plant pathogens which include organisms like *Erwinia, Bacillus* and *Pseudomonas* species among the bacteria. Known fungal plant pathogens observed in the study include *Fusarim, Cladosporum, Botryodioplodio, Rhizopus, Aspergillus* and *Penicillum* species. The nature of the harvesting pattern of *C. albidum* fruits (dropping from tree to the ground) exposes it to contamination by various organisms (Adebisi, 1997, Adeloja, 1997). The soil has been described as home to several organisms (Prescott *et al*., 2005). It is therefore possible that any of these organisms can gain access into the fruit. Some of the fruits could be infected while in transit as the market fruits were significantly more infected than the forest samples Various containers are used in the carriage of fruits to the markets, in addition to careless display and selection during buying and selling in the markets. Zubbair (2009), Eni *et al*., (2010) and Chukwumalume *et al*., (2012) had earlier reported a similar situation.

The pathogenicity tests carried out showed that only three of the observed nine bacterial isolates (*Bacillus, Erwinia* and *Pseudomonas* were mainly pathogens of *C. albidum* fruit. Among the fungi, *Saccharamyces, Geotrichum* and *Trichodema species* were not actually pathogenic. The discolouration observed could have come from the physical injury to the fruits while carrying out the tests. These organisms, unable to induce considerable spoilage early could be either contaminants or late secondary invaders, which agrees with Angie (2001).

The presence of carbohydrate, proteins and fats/oil in *C. albidum* fruits has been reported (Oyebade *et al*., 2011; Akpabio *et al*., 2012; Adepoja and Adeniji 2012). Ash and fibre were also observed while water is a natural component in all berries. Values obtained in these parameters fall within those reported by Adepoju and Adeniji (2012), Chukwumalume *et al*., (2010) and Oyebode *et al*., (2011). These components are microbial nutrients hence the high proliferation of the microorganisms in the fruits.

The microbial deterioration of the fruits caused significant decrease in protein, carbohydrate, fats/oil, fibre and ash contents. The components were degraded to ensure utilization and proliferation of the microorganisms. This extensively decreased their contents in the infected fruits. However, the observed increase in the moisture content was due to the liquefaction of the fruit components especially protein, during the metabolic processes. The

microbial metabolism of fruit components released additional moisture resulting in the apparent increase observed. Oranusi and Braide (2012) and Adebisi (1997) also agrre that fruits are good media for microbial growth. Adeloja, (1997), Amusa *et al*., (2003), Eni *et al*., (2010) and Zubbair (2009) had reported microbial deterioration of *C. albidum* fruit components. Observations in this study showed that the rate of deterioration increased with time of storage. It further showed that without preservation, *C. albidum* fruits should be consumed within 0-3 days of harvesting. Within these few days, the nutritional values are high with less microbial loads too.

Again, microbial loads of the various bacterial groups increased significantly by the 4th day of storage. This positively correlated with the loss, of nutritional components. The microorganisms metabolized the nutrients, hence making the fruit less nutritious for human consumption. The microbial analysis also showed that the THBC were much higher than the TCC and P PBC. TCC and PPBC are integral fraction of THBC. This observation agrees with that of Etok *et al.,* (2012a) working on *Dacryodes edulis.* In the same vein, Etok *et al*., (2012b) reported the presence of potential human bacterial pathogens in *D. edulis* which were stored in various ways, as also observed in this work.

Results further showed that there could be some health risks if the *C. albidum* fruits are consumed after the 3rd – 4th day of storage. The presence of PPB and the growth of some aflatoxin-producing fungal species (*Fusarium* and *Aspergillus* species) makes consumption of the fruits after the 4th day a health risk. Jay (1997) and Frazier and Westhoff (2001) reported that aflatoxin produced by *Aspergillus* and *Fusarium* species are anti-health metabolites. The observation of *S. aureus, Salmonella* species and Coliforms give the impression that the *C. albidium* fruits should be thoroughly washed before consumption.

In conclusion, the consumption of *C. albidum* fruits could be said to be best within 0-3 days of harvesting as nutritional values begin to depreciate and microbial infections likely to occur..

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