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# Microorganisms associated with African star apple (*Chrysophylum albidum*) and their hydrolases

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**Microorganisms associated with African star apple (*Chrysophylum albidum* Linn), their quality characteristics and hydrolases were investigated. The bacteria species were *Bacillus cereus, B. polymyxa, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus,* while the fungi species were *Aspergillus flavus, A. fumigatus, A. niger, A. repens, Fusarium* sp*., Mucor mucedo, Trichoderma viride* and *Rhizopus stolonifer.* Pathogenicity tests revealed that all the isolates were pathogenic on the fruits. Proximate analysis showed that microbial infections significantly reduced the carbohydrate, crude fibre, protein, moisture content and fat. However, mineral analysis accounted for an increased K, Ca, Mg, Na, Zn and P in an infected fruits compared with the apparently healthy fruits. The pH values ranged from 5.05 to 5.23. Massive infection leading to the deterioration of the fruits that could ultimately affect its quality posing health risk could be reduced by the early consumption of the fruits. All the microbial isolates produced the hydrolases which could be responsible to their enhanced abilities to deteriorate the fruit.**

**Key words:** African star apple, fruits, hydrolases, pathogenic, infections, microorganisms.

## INTRODUCTION

The African star apple (*Chrysophylum albidum* Linn.) is an angiosperm belonging to the order Ebernales, family Sapotaceae (Ehiagbonare et al., 2008). The plant has been reported to grow up to a height of 36.5 m and are known to occur in diverse ecological zones in Nigeria, Uganda, Niger Republic, Cameron 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). 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 the people (CENRAD, 1999) and for the purpose of stopping irritation, loss of appetite and salivation.

Asenjo (1946) reported that star apple fruit edible pulp is very rich in ascorbic acid even with about 100 times richer than of oranges and 10 times than that of cashew or guava. Studies have shown the fruit to be an excellent source of vitamins, iron, flavours to diet and raw materials to some manufacturing industries (Okafor and Fernandes,

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1987; Bada, 1997; Umelo, 1997; Adisa 2000). In addition to these qualities, is an acceptable com-position of moisture, ash, crude fibre, oil, protein, starch, sugars and ascorbic acid as cited in Adindu et al. (2003).

Recently, mineral analysis implicated the presence of K, P, S, Ca, Mg, Al and Zn (Chukwuemeka, 2006). The seed is also known to be an important source of oil, for diverse purposes (Amusa et al., 2003). The seeds are used for local games (Bada, 1997). The seed shell of the fruit has been adjudged to be an efficient adsorbent alternative material for the removal of heavy metals and organic matters from water and waste waters (Amuda et al., 2007; Oboh et al., 2009). However, the fruit is reported to contain 90% anacardic acid, used industrially to protect wood and wood materials, and a source of resin, while the leaves, roots and stem bark of the tree have medicinal purposes (Adewusi, 1997; Bada, 1997).

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). The fruits are not usually harvested, but left to naturally fall to the forest floor from where they are picked which tend to predisposes the fruits to microbial infections (Amusa et al., 2003). 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. This study focused on the isolation of microorganisms associated with African star apple fruits with the aim of establishing their quality, safety and extracellular hydrolases.

## MATERIALS AND METHODS

### Source of samples

Apparently fresh African star apple (*C. albidum* Linn.) fruits were obtained from Oja-Oba (Market), Akure, Nigeria. The samples were brought to the Microbiology Laboratory, The Federal University of Technology, Akure, Nigeria for further analyses and studies.

### Sample treatments and isolation of associated microorganisms

The samples were surface sterilized with 75% ethanol, rinsed in several changes of sterile distilled water and placed inside sterile Petri dishes until symptoms of infections were noticed. For bacterial isolation, infected portions of the fruits were sliced into pieces, transferred into sterile distilled water from which serial dilutions were carried out. An aliquot of 0.1 ml of dilution $10^5$ was plated on nutrient agar (NA) and incubated at 37°C for 24 h. Discrete colonies were observed, counted, recorded as colony forming unit per gram (cfu/g) and further sub-cultured to obtain pure cultures. The pure isolates were characterized and identified using the methods described by Holt et al. (1994).

A slight modification of the method of Amusa et al. (2003) was used for the isolation of associated fungi. In this case, a 0.1 ml aliquot of dilution $10^3$ was dispensed on sterilized potato dextrose agar (PDA) in Petri dishes and incubated for seven days at ambient temperature. The fungal isolates were counted as spore forming unit per gram (sfu/g) and pure cultures of the fungi were examined with a stereo binocular microscope and identified by cultural, morphological and detailed descriptions in the references standard of Booth (1971), Barnett and Hunter (1972) and Webster (1980).

### Pathogenicity test

Freshly harvested ripe African star apple fruits were surface sterilized with 75% ethanol and rinsed in several changes of sterile distilled water. The surface sterilized fruits were inoculated with $10^2$ of 24 h old culture of bacterial isolates with sterile needle and syringe and incubated at 37°C. The spores of 48 h old fungal isolates were injected into the fruits and incubated at 28°C. The control fruits were injected with injection water. The extent of infection was determined according to the method of Amusa et al. (2003). The bacteria obtained from the infections were re-isolated, characterized and identified according to the methods earlier described, while wet mounts of fungal structures from the infected portions were stained with lactophenol in cotton blue and viewed under microscope for the presence of pathogen used in the inoculation.

### Determination of nutrient composition

The fruits were initially kept in dried clean containers, cut opened, deseeded and weighed. The fleshy pulp was cut into pieces with sterile knife and dried in a dry cabinet at $60^oC$ for 5 days. The dried pulp was ground into powder and analysed for moisture, ash, protein, crude fibre, crude fat and carbohydrate content according to AOAC (2005) techniques. The mineral contents were determined using the standard method of AOAC (1990).

### Determination of the pH

The pH of the African star apple fruit was determined using Jenway 3015 pH meter. The pieces of the fruit were homogenized for 30 min at 5 min interval inside a clean glass beaker with sterile distilled water. The electrode of the standardized pH meter was inserted into the homogenates and reading was taken and recorded.

### Determination of the viscosity of African star apple fruits

The viscometer was cleansed with appropriate solution, rinsed in distilled water and drained dry. Ten milliliters of distilled water was added to the viscometer at 20°C. Using suction to draw water above the upper mark let the liquid level to fall and the timing started with a stop watch as meniscus passes the upper mark until it got to the lower mark. The viscometer was then rinsed and the samples applied and the time required for its passage between meniscuses was determined which was used to calculate the viscosity with the formula:

Viscosity (CP) = flow time of sample solution at 20°C x specific gravity of the sample solution x 1.002 / Flow rate of water at 20°C.

### Determination of hydrolases from the microbial isolates

The amylase of the microbial isolates was determined according to the methods described by Alves et al. (2002) and Carrim et al.

**Table 1**. Microbial counts in days of African star apple (*Chrysophylum albidum*) fruits.

| Day | Bacteria (cfu/g) | Fungi (sfulg) |
|---|---|---|
| 2 | $3.40 \times 10^5$ | $5.60 \times 10^2$ |
| 4 | $3.20 \times 10^5$ | $4.20 \times 10^2$ |
| 6 | $1.60 \times 10^3$ | $6.00 \times 10^2$ |
| 8 | $2.50 \times 10^4$ | $3.80 \times 10^2$ |
| 10 | $4.00 \times 10^5$ | $2.80 \times 10^2$ |
| 12 | $2.00 \times 10^4$ | $4.50 \times 10^2$ |
| 14 | ND | $2.00 \times 10^2$ |
| 16 | ND | $4.00 \times 10^2$ |

Key: cfu = colony forming unit; / = per; g = gram; sfu = spore forming unit; ND=not detectable.

**Table 2.** Incidence and pathogenicity of microorganisms associated with African star apple Fruits

| Microbial isolates | Incident rate (%) | Infection (Diam. mm) |
|---|---|---|
| **Bacteria** | | |
| *Bacillus cereus* | 28 | 15 |
| *B. polymyxa* | 35 | 20 |
| *Escherichia coli* | 30 | 20 |
| *Proteus mirabilis* | 20 | 15 |
| *Pseudomonas aeruginosa* | 35 | 25 |
| *Staphylococcus aureus* | 18 | 08 |
| **Fungi** | | |
| *Aspergillus flavus* | 45 | 25 |
| *A. fumigatus* | 40 | 28 |
| *A. niger* | 20 | 12 |
| *A. repens* | 30 | 25 |
| *Fusarium* sp. | 38 | 22 |
| *Mucor mucedo* | 60 | 15 |
| *Trichoderma viride* | 20 | 10 |
| *Rhizopus stolonifer* | 65 | 19 |

% = percentage; Diam. = diameter; mm = milliliter.

(2006), while lipase was by the methods of Carrim et al. (2006) and Damaso et al. (2008). The method of Onyeocha and Ogbonna (1983) was used for protease and pectinase by Carrim et al. (2006), while cellulase was determined using the method of Nwodo et al. (2010).

## RESULTS AND DISCUSSION

The highest bacterial count ($4.0 \times 10^5$ cfu/g) was recorded on day 10 while day 6 recorded the least counts ($1.60 \times 10^3$ cfu/g) (Table 1). These counts may not be unconnected with the nature of the associated bacteria. The highest fungal count ($6.0 \times 10^2$ sfu/g) was recorded on day 6 while day 14 recorded the least counts ($2.0 \times 10^2$ cfu/g) (Table 1). These counts could be a direct consequence of the fungi to easily utilize the nutrients in the fruits for growth. Fourteen microorganisms were isolated from the deteriorating African star apple fruits investigated. These included *Bacillus cereus, B. polymyxa, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* for bacteria. The fungi isolated were *Aspergillus flavus, A. fumigatus, A. niger, A. repens, Fusarium* sp*., Mucor mucedo, Trichoderma viride* and *Rhizopus stolonifer* (Table 2). The array of these microorganisms could be due to the nutrient rich nature of the fruits, thus supporting the growth and proliferation of the organisms. Asenjo (1946) documented the richness in ascorbic acid

of the African star apple, while Bada (1997) and Umelo (1997) separately attested to the excellent sources of vitamins, iron, flavours to diet, raw materials for some industries and essential minerals (Amusa et al., 2003). Thus, the fruit could serve as nutrient source for the isolated microorganisms. The incidence of *Pseudomonas aeruginosa* could be adduced to its nutritional versatility (Oberhardt *et al*., 2008). Also, *Pseudomonas aeruginosa, E. coli, Proteus mirabilis* and *Staphylococcus aureus* were human pathogens cum flora (Awe et al., 2009), while *B. cereus* and *B. polymyxa* were associated with air and soil (Gravens et al., 1975; Awe et al., 2009). Therefore, the presence of the former group of bacteria could be traced to human contamination, while the later could have resulted from air and soil contamination.

The eight fungi isolated in this study were similar to isolates from the deteriorating African star apple fruits in Ibadan, Nigeria by Amusa et al. (2003) with the exception of *Aspergillus tamari, Penicillum sp.* and *Botryodiplodia theobromae.*

*B. polymyxa* and *P. aeruginosa* were the most frequently encountered bacteria and *Staphylococcus aureus* was least prevalent (Table 2). The poor hygienic standard and improper handling of the fruits could be accountable for the observed bacteria (Awe et al., 2009). Some of these organisms namely *E. coli* and *P. mirabilis* have been implicated in gastroenteritis (Nester et al., 2001). The incidence of *B. polymyxa,* and *B. cereus* are indicative of environmental contamination of the fruits as the fruits are constantly exposed to air, aerosols and dust particles during the course of selling the fruits which in most cases take days to weeks. Therefore, faulty foods and fruits handling techniques especially in between sales during which the fruits are stored at improper temperature (Gallo et al., 1992) and possibly in contact with contaminated surfaces could be adjudged for the incidences of *B. polymyxa* and *P. aeruginosa. Staphylococcus aureus* in fruits clearly attested to human contamination during handling. This singular organism is known to be associated with enterotoxin characterized by short incubation period, violet nausea, vomiting and diarrhea (Awe et al., 2009) when injested in foods, water, fruits and vegetables.

The predominant fungus was *R. stolonifer* followed by *M. mucedo*, *A. flavus, A. fumigatus,* while *A. niger* and *T. viride* least occurred. *A. niger* and *R. stolonifer* isolated in this study have been associated with field infection (Amusa et al., 2003). In addition, *Aspergillus* and *Rhizopus* species in this study and *Penicillum* species had been reported to play significant roles in melon pod rotting for seeds recovery (Uffonry and Achi, 1998; Kehinde and Ikenga, 2010). The micromycetes nature of the isolated fungi from the African star apple fruits tend to be a prelude to their ease of adaptation to changing environmental conditions and to infect and deteriorate different plants substrates hinged to specific biological properties (Luganskas, 2005). Aluded to this is the fact that many micromycetes are not only known as plant pathogens, but are sources of vital mycotoxins of interest in animal and human health (Placinta et al., 1999). These fungi isolated from the fruits were in no small measure indigenous to soil environment (Aboloma et al., 2009; Awe et al., 2009; Kehinde and Ikanga, 2010) from where they probably own their origin.

**Table 3.** Viscosity (%), nutrient composition (%), pH, titratable acidity (TTA) and mineral content (%) of African star apple fruits.

| Parameter | Apparently healthy fruits | Spoilt fruits |
|---|---|---|
| Viscosity | 12.32 | 4.25 |
| Moisture content | 66.45 | 40.14 |
| Crude fibre | 44.33 | 26.26 |
| Crude protein | 9.83 | 12.28 |
| Ether extract (fat) | 14.96 | 9.24 |
| Ash | 6.67 | 10.28 |
| Carbohydrate | 26.22 | 12.36 |
| pH | 5.25 | 6.62 |
| TTA | 1.65 | 1.24 |
| Fe | 1.28 | 0.45 |
| Ca | 46.25 | 52.12 |
| Mg | 38.34 | 46.35 |
| Na | 2.56 | 3.26 |
| K | 6.15 | 8.25 |
| P | 2.26 | 1.12 |
| Zn | 3.34 | 4.22 |

The nutrient analysis of the apparently healthy African star apple fruits lucidly had viscosity of 12.32%, moisture content of 66.45%, crude fibre of 44.33%, crude fat of 14.96% and carbohydrate of 26.22% that were higher than the spoilt fruits with 4.25, 40.14, 26.26, 9.24 and 12.36% for viscosity, moisture content, crude fibre, crude fat and carbohydrate, respectively (Table 3). However, crude protein of 12.28% and ash content of 10.28% were more in spoilt fruits than in apparently healthy fruits with 9.83 and 6.66% crude protein and ash content respectively (Table 3). In any case, Adelaja (1997) discovered lower carbohyradate content of 29.90%, crude protein of 8.80% and crude fat content of 17.10%. Similarly, Amusa et al. (2003) reported carbohydrate content of 29.60%, crude protein of 8.75%, crude fat content of 16.20% and moisture content of 42.10%. These observed changes could have been a function of time of harvest of the fruits as well as the environment where the plants are grown. Also, the nature of the nutrient content of the apparently healthy fruits compared with the spoilt fruits may not be unconnected with the activities of the microorganisms in the fruits cum time. Ajayi (2011) asserted the immense activities of microorganisms in the fermentation of African yam bean seeds that resulted in its nutritional composition.

**Table 4.** Degradative enzymes production in halo diameter (mm) of microbial isolates from African star apple fruits.

| Microbial Isolate | Hydrolase activity (mm) | | | | |
|---|---|---|---|---|---|
| | Protease | Amylase | Cellulase | Lipase | Pectinase |
| *Bacillus cereus* | 3.10 | 10.26 | 0.00 | 1.18 | 0.00 |
| *B. polymyxa* | 0.00 | 12.00 | 4.40 | 0.00 | 6.50 |
| *Escherichia coli* | 4.20 | 6.28 | 0.00 | 0.00 | 0.00 |
| *Proteus mirabilis* | 5.25 | 4.46 | 0.00 | 1.26 | 3.45 |
| *Pseudomonas aeruginosa* | 8.62 | 14.00 | 10.00 | 3.20 | 6.15 |
| *Staphylococcus aureus* | 4.26 | 2.15 | 0.00 | 0.00 | 0.00 |
| *Aspergillus flavus* | 1.85 | 8.20 | 6.10 | 2.18 | 5.56 |
| *Aspergillus fumigatus* | 2.15 | 5.62 | 3.66 | 3.22 | 6.26 |
| *Aspergillus niger* | 3.21 | 8.05 | 8.05 | 4.26 | 5.66 |
| *Aspergillus repens* | 2.26 | 8.66 | 8.66 | 3.15 | 5.14 |
| *Fusarium sp.* | 0.06 | 3.25 | 4.40 | 0.00 | 2.40 |
| *Mucor mucedo* | 0.41 | 6.15 | 0.46 | 5.25 | 1.42 |
| *Trichoderma viride* | 3.32 | 4.25 | 2.40 | 3.50 | 4.64 |
| *Rhizopus stolonifer* | 0.00 | 5.50 | 1.15 | 6.45 | 1.00 |

The average pH values obtained show that the apparently healthy fruits are moderately acidic with pH of 5.25, while the spoilt fruits tend toward neutral with pH of 6.62. This in no small measure that indicated that the fruits could permit and tolerate the growth of bacteria and fungi (Frazier and Westerhof, 1967).

In addition, mineral content analysis revealed iron and phosphorous to be 1.28 and 2.26% respectively in the apparently healthy fruits more than the spoilt fruits which had iron of 0.45% and phosphorous of 1.12% (Table 3). This study also showed higher calcium of 52.12%, magnesium of 46.35%, sodium of 3.26%, potassium of 8.25% and zinc of 4.22% in spoilt fruits than healthy fruits which recorded calcium of 46.25%, magnesium of 38.34%, sodium of 2.56%, potassium of 6.16% and zinc of 3.34% (Table 3). The availability of these minerals in the fruits is an indication of the rich nature of the fruits with the essential elements. The more of these minerals in the healthy fruits as opposed to the spoilt and vice visa could have resulted from the concomitant uptake and released of these minerals in the course of the metabolic activities of the associated microorganisms. In their deductions from the previous studies, Amusa et al. (2003) posited that infection and deterioration of the fruits by pathogens might have lead to an increase in mineral content and decrease in metabolic synthetases of African star apple fruits.

The degradative enzymes production values of the microorganisms are illustrated in Table 4. Notably, all the bacterial isolates elaborated amylase, while *B. cereus, E. coli, P. mirabilis* and *Staphylococcus aureus* did not produce detectable cellulase. Also, all the bacterial isolates with the exception of *B. polymyxa, P. mirabilis* and *P. aeruginosa* were positive for lipase. In this study, all the fungal isolates expressed detectable hydrolases except *R. stolonifer* and *Fusarium* sp. without protease and lipase respectively. Therefore, the elaboration of these hydrolytic enzymes by the microbial isolates could be responsible for their proliferation in the fruits. This finding agreed with the fact that microorganisms are rich sources of enzymes (Akpan, 2004). Amylasaes are one of the most important enzymes used in biotechnological processes (Ajayi, 2011), particularly in starch hydrolysis. Cellulases have wide applications in textile, paper pulp as well as the feed industries (Nwodo et al., 2010). The protease and pectinase would have acted on protein content and cementing materials of the fruits, thus reducing the viscosity of the fruit juice. Microbial pectinases were reported to account for 25% of the global food enzymes sales (Jayani et al., 2005). The microorganisms from the fruits could be vital sources of these economically viable enzymes.

## Conclusion

The microorganisms isolated are no doubt involved in the infection, degradation and cum deterioration of the African star apple fruits. Hence, the deteriorative activities of the microorganisms tend to exact its influence on the nutritive value of the fruits. The versatility of the array of enzymes elaborated by the microbial isolates could find their usefulness in techno-industrial applications if properly harnessed.

## Conflict of interests

The authors have not declare any conflict of interest.

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