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FUNGI SPOILAGE AND PROXIMATE COMPOSITIONS OF AFRICAN STAR APPLE (*Chrysophyllum albidum* G. Don) FRUITS IN PORT HARCOURT METROPOLIS, NIGERIA

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Abstract

This research was to evaluate fungi spoilage and proximate compositions of Chrysophyllum albidun fruits in Port Harcourt Metropolis, Nigeria. The experiment was carried out at the Mycology Unit, Department of Forestry and Environment, Rivers State University, Nkpolu-Oroworukwo and was laid out in a completely randomized design (CRD) and the treatment replicated (n=5,10) and the mean value separated using Duncan Multiple Range test at probability of 5%. The bio-deterioration of the fruits were caused by micro-organism associated with the infected fruits obtained from the three markets in Port Harcourt metropolis. The micro-organism isolated and identified are; Rhizopus stolonifer, Aspergilus niger and Pseudomonas aeruginosa. The results on the frequency occurrence of micro-organism in the three markets revealed fruits obtained that in Mile I market, R. stolonifer (4.0%±0.24) was highest followed by Mile 3 (2.0%±0.26). A. niger (5.0%±0.20) occurred more in the fruits obtained from Mile I and fruit garden markets. However, P. aeruginosa (5.0%±0.28) occurred more on the fruits obtained from Mile 3 market followed by fruit garden market. Pathogenicity test results revealed the that all the isolated micro-organisms from the infected fruits were pathogenic to wounded and unwounded C. albidum fruits. P. aeruginosa (8.2mm±0.23 – 15.0mm±1.25) caused significant (P<0.05) rot damage on both the wounded and unwounded fruit pulp followed by R. stolonifer (5.0mm±0.20 – 10.0mm±0.45) and the least rot damage was caused by A. niger (4.2mm±0.10mm – 6.0mm±0.25). Results on proximate composition of infected and uninfected fruits of C. albidum obtained in the three markets studied, moisture content (66.22%±3.20) of the infected fruits was higher than the uninfected (62.61%±2.05), ash content (1.51%±0.20) of the

infected C. albidum fruits significantly (P<0.05) differ from the uninfected (1.21%±0.01). It was generally observed that the protein, fats, crude fibre and carbohydrate content of uninfected were significantly (P<0.05) higher in values than the infected fruits of C. albidum obtained in the three markets. Results of this research therefore recommend that infected fruits are not good for consumption and increases the risk of some aliment among consumption. Proper washing of fruits with sodium chloride and water before consumption is necessary.
Key words: Fungi spoilage, proximate composition, *Chrysophyllum albidum*, micro-organisms.

INTRODUCTION

African Star Apple (*Chrysophyllum albidum*), is known by various tribal names in Nigeria as *agbalumo or Osan* (Yoruba), *udara* (Ibo, Efik and Ibibio), *ehya* (Igala) andagwaluma (Hausa). It is an indigenous plant, an edible tropical fruit, which is classified as a wild plant, and belong to the family *Sapotaceae* and classified under the genus Chrysophyllum [53]. *Chrysophyllum* is a genus of about 70-80 species of tropical trees native to tropical regions throughout the world, with the greatest number of species in the Northern South America and some parts of Africa [14]. The fruit is seasonal and glaborous when ripe, ovoid to subglobose, pointed at the apex and up to 6 cm long and 5 cm in diameter. The skin or peel is orange of golden yellow when ripe and pulp within the peel may be orange, pinkish, bricked or light yellow. African star apple fruit contains five large coffee-coloured seeds or sometimes fewer by abortion [38]. It is a tree with great potentials not only as a plantation species, but also in agroforestry system [48, 45]. In Nigeria, the fruit is gathered for household use or for sale in local markets during the months of December to April.

The pulp is reported as an excellent source of vitamins, iron, flavour to diets and raw materials to some manufacturing industries [8, 12]. However, [12] reported that the pulp goes bad after 5 days. Such postharvest losses have been minimized through processing of the fruit into value added products such as juice and jam [32]. In contrast, little research on the kernel exists in the literature. However, [32] showed that the seed is a good source of vegetable oil.

Other studies on the seed bordered on the antimicrobial properties [38] and the potential use of the seed as food ingredient. The seeds could be processed into flour and used in similar manner other flours such as orange seed [11] and mango kernel [11, 15] are used. This will, however, depend on the knowledge of its chemical composition and functional properties. Quality attributes of developed food products are generally affected by the functional properties of the flour.

The plant has in recent times become a crop of commercial value in Nigeria. The fleshy pulp of the fruits is eaten especially as snack and relished by both young and old [24]. The African star apple fruit has been found to have highest content of ascorbic acid with 1000 to 3,330 mg of ascorbic acid per 100gm of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew [17]. It is reported as an excellent source of vitamins, irons, flavours to diets and raw materials to some manufacturing industries [8, 21, 47, 57]. In addition, its seeds are a source of oil, which is used for

diverse purposes. The seeds are also used for local games [21]. The fruits also contain 90% anacardic acid, which is used industrially in protecting wood and as source of resin, while several other components of the tree including the roots and leaves are used for medicinal purposes [6, 21, 58].

Chrysophyllum albidum fruits are widely eaten in Southern Nigeria, being especially popular with children and women. The seeds, flesh and kernels have culinary and economic value locally, regionally and internationally [14, 46]. *C. albidum* forms an important part of the East and Central African nutrition, providing carbohydrate, protein and minerals. It has been reported that the fleshy pulp of the fruit is eaten especially as snack and relished by both young and old [8]. Apart from its culinary uses, *C. albidum* have been found to have other economic uses [53]. It medicinal properties, source of timber and industrial uses are widely reported [25, 38]. *C. albidum* is consumed in large quantities in South Western Nigeria where many households have this economic tree. The ultimate consumer, it is a measure against food insecurity and malnutrition while to the producers and marketers it is a source of income.

JUSTIFICATION

The post-harvest losses could discourage farmers from producing and marketing fresh produce, and limit the urban consumption of fresh fruits and vegetables. Hence, development of post-harvest technologies is believed to make great contribution to improve quality and use of these fruits. One of the limiting factors that influence the fruits economic value is the relatively short shelf-life period caused by spoilage organisms and pathogens' attack [55]. It is estimated that about 20-25% of harvested fruits are lost during post-harvest handling even in the developed countries [33, 60]. In developing countries however, post-harvest losses are often more severe due to inadequate storage and transportation facilities [55]. Fungal infections of fruits may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchase by consumers.

OBJECTIVE OF THE STUDY

This study was undertaken to investigate the fungi spoilage and proximate compositions of *Chrysophyllum albidum* fruits in Port Harcourt Metropolis Nigeria.

The specific objectives of this research were to;

1. isolate and identify microorganisms associated to African star apple *(Chrysophyllum albidum)* fruits.
2. determine the pathogenicity test of the isolated microorganisms on a relatively healthy fruits of *C. albidum.*
3. evaluate the proximate compositions of infected and uninfected fruits of *C. albidum.*

MATERIALS AND METHODS

Study Area

The study was carried out at the Laboratory of Forestry and Environment (Forest Pathology Unit), Rivers State University, Nkpolu-Oroworukwo, Port Harcourt and Department of Food Science and Technology Laboratory at Latitude 4.5^0N and

Longitude 7.0^{0}E on an elevation of 18m above sea level, a mean annual temperature of 27^{0}C and rainfall of 2000-2467mm [27].

Source and Collection of African Star Apple fruits

A total of one hundred and fifty (150) standardized ripe *Chrysophyllum albidum* fruits of average weight (20g) were purchased from the three markets; Mile 1, 3 and fruit garden in Port Harcourt Metropolis, Rivers State, Nigeria. The samples selected were at the peak of their freshness (fairly soft to touch, plump with orange-yellowish colour. The fruits were packaged with black polyethene bags and brought to the Forestry and Environment Laboratory (Mycology Unit) for further analyses and studies. The fruits were washed with potable water, sorted to distinguish infected and un-infected fruits. Each infected fruits from the three different markets (Plate 1).

Mile 1 **Mile 3** **Fruit Garden**

Plate 1: Infected Fruits

Isolation and Identification of Microorganisms

The infected fruits of *Chrysophyllum albidum* (Plate 2). The infected parts were cut into small pieces (3x4mm) size with the help of sterilized knife. The pieces were surface sterilized in 2% Sodium hypochorite solution for 3 minutes followed by washing with sterile water and then placed on three layers of moistened filter papers in Petri dishes replicated ten times. All Petri dishes were incubated at 28±2^{0}C for 3-5 days. The fungi were examined and the disease incidence (frequency) was calculated, the percentage of diseased fruits parts per total number of fruits incubated. The fruits were observed visually for rottening and microbial infection. Percentage disease incidence was calculated using the formula of [41, 27].

$$D_1 = \frac{Do}{D} \; x \; \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. equation\ (1)$$

Where;

D_1 = Disease incidence

D_o = Number of diseased fruits

D = Total number of fruits plated

However, Stereo binocular microscope was used to identify microorganisms by cultural and morphological descriptions with references to [22, 23, 59, 26]. For bacterial isolation, infected portions and transferred into sterile distilled water from which serial aliquot of 0.1ml of dilution was plated on nutrient agar (NA) and incubated at 37^{0}C for 24hours, discrete colonies were observed and further sub-cultured. The pure isolates were characterized and identified using the method of [37].

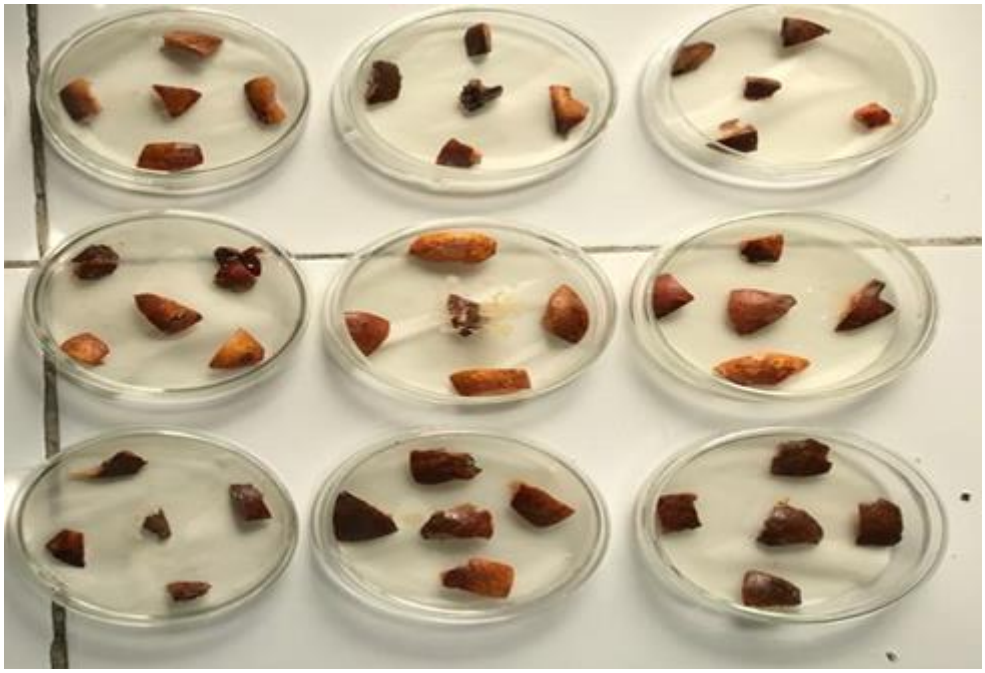


Plate 2: Infected Fruits Parts Incubated

Pathogenicity Test

Fresh uninfected ripe *Chrysophyllum albidum* fruits were surface sterilized with 70% ethanol and rinsed in several changes of sterile distilled water. The surface sterilized fruits were inoculated with a 7 days old culture of fungal and bacterial isolates into the wounded and unwounded fruits using 5mm Cork borer [29]. The extent of infection was determined according to the methods of [30]. The microorganisms obtained from the infections were re-isolated characterized and identified according to the method of [28].

Proximate composition of Infected and Uninfected *C. albidum* fruits Obtained from Port Harcourt Metropolis

The infected and uninfected fruits of *C. albidum* obtained from the three markets were kept in a dried clean containers, cut opened, deseeded and weighed. The freshy pulp was cut into pieces with sterile knife and dried in a dry cabinet at 60^0C for 5 days. The dried pulp was ground into powder and analysed for moisture, ash, protein, crude fibre, crude fat an carbohydrate content according to [18, 13, 19]. In determining moisture content, total ash, crude protein and crude fat, Standard Association of Official Analytical Chemistry [18, 13, 19].

Experimental Design and Statistical Analysis

The experiment was designed in a one-way analysis variance using SPSS-5 Version in a Completely Randomized Design (CRD). The treatments were replicated 10 times and mean separated using Duncan Multiple Range Test at probability of 5%.

RESULTS

Isolation and Identification of Micro-organisms from infected fruits of *Chrysophyllum albidum*

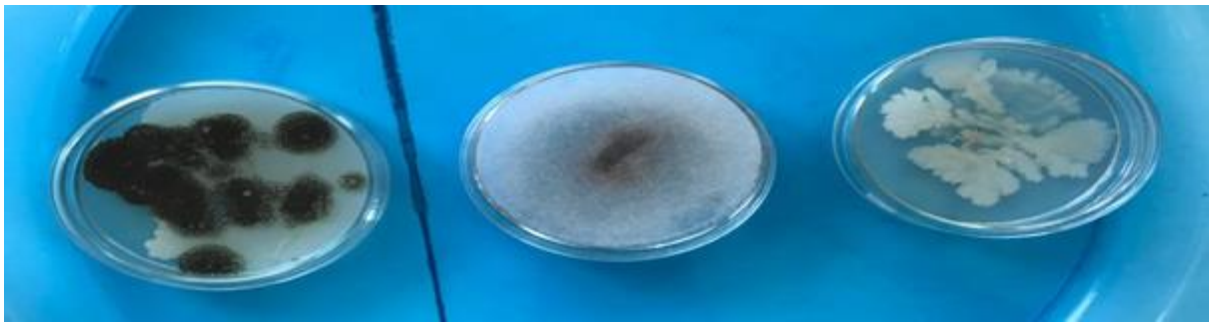
Results on the isolation and identification of micro-organisms obtained from infected fruits of *C. albidum* are presented in Table 1 and Plate 3. The results revealed three

microorganisms namely; *Rhizopus stolonifer, Aspergillus niger* and *Pseudomonas aeruginosa. A. niger* (5.0%±020) was predominantly present in the followed by *R. stolonifer* (2.0%±0.12 – 4.8%±0.24) and the least was *P. aeruginosa* (1.0%±0.20 – 2.0% ±0.28).

Table 1 Isolation and Identification of Micro-organisms from infected fruits of *Chrysophyllum albidum* Obtained from three markets in Port Harcourt Metropolis (n=10, Mean ±SD)

	Markets in Port Harcourt Metropolis		
Micro-organisms	**Mile I**	**Mile 3**	**Fruit garden**
Rhizopus stolonifer	4.0^{b}±0.24	3.0^{b}±0.26	2.0^{b}±0.12
Aspergillus niger	5.0^{a}±0.20	5.0^{a}±0.30	5.0^{a}±0.15
Pseudomonas aeruginosa	1.0^{c}±0.21	2.0^{c}±0.30	2.0^{b}±0.10
Grand total mean (%)	**33.3**	**33.3**	**30.0**

Mean values with the same superscripts (a,b,c..) in the same column are not significantly (P≤0.05) differently by DMRT (P≤0.05).



Aspergillus niger ***Rhizopus stolonifer*** ***Pseudomonas aeruginosa***

Plate 3: Pure Culture of the Microorganisms isolated

Pathogenicity test of the Microorganisms on the Fruit Rot of *C. albidum*

The results of the pathogenicity test of the isolated micro-organisms on the fruit rot of *C. albidum* are presented in Table 2 and Plate 4. Results indicated that the *Pseudonomas aeruginosa* (15.0mm±1.25) caused significant (P≤0.05) fruit rot damage on the wounded *C. albidum* fruits followed by *Aspergillus niger* (10.0mm±0.45) and the least was *Rhizopus stolonifer* (6.0mm±0.25). In the unwounded fruits of *C. albidum, P. aeruginosa* also caused the highest rot damage followed by *A. niger* and *R. stolonifer* caused the least fruit rot.

Table 2: Pathogenicity Test of the micro-organism on *C. albidum* (Mean ±SD)

	Rot Damage (mm)	
Micro-organisms	**Wounded fruits**	**Unwounded fruits**
Rhizopus stolonifer	6.0^{c}±0.25	4.2^{c}±0.10
Aspergillus niger	10.06^{b}±0.45	5.0^{b}±0.20
Pseudomonas aeruginosa	15.0^{a}±1.25	8.2^{a}±0.23

Mean values with the same superscripts (a,b,c..) in the same column are not significantly (P≤0.05) differently by DMRT (P≤0.05).

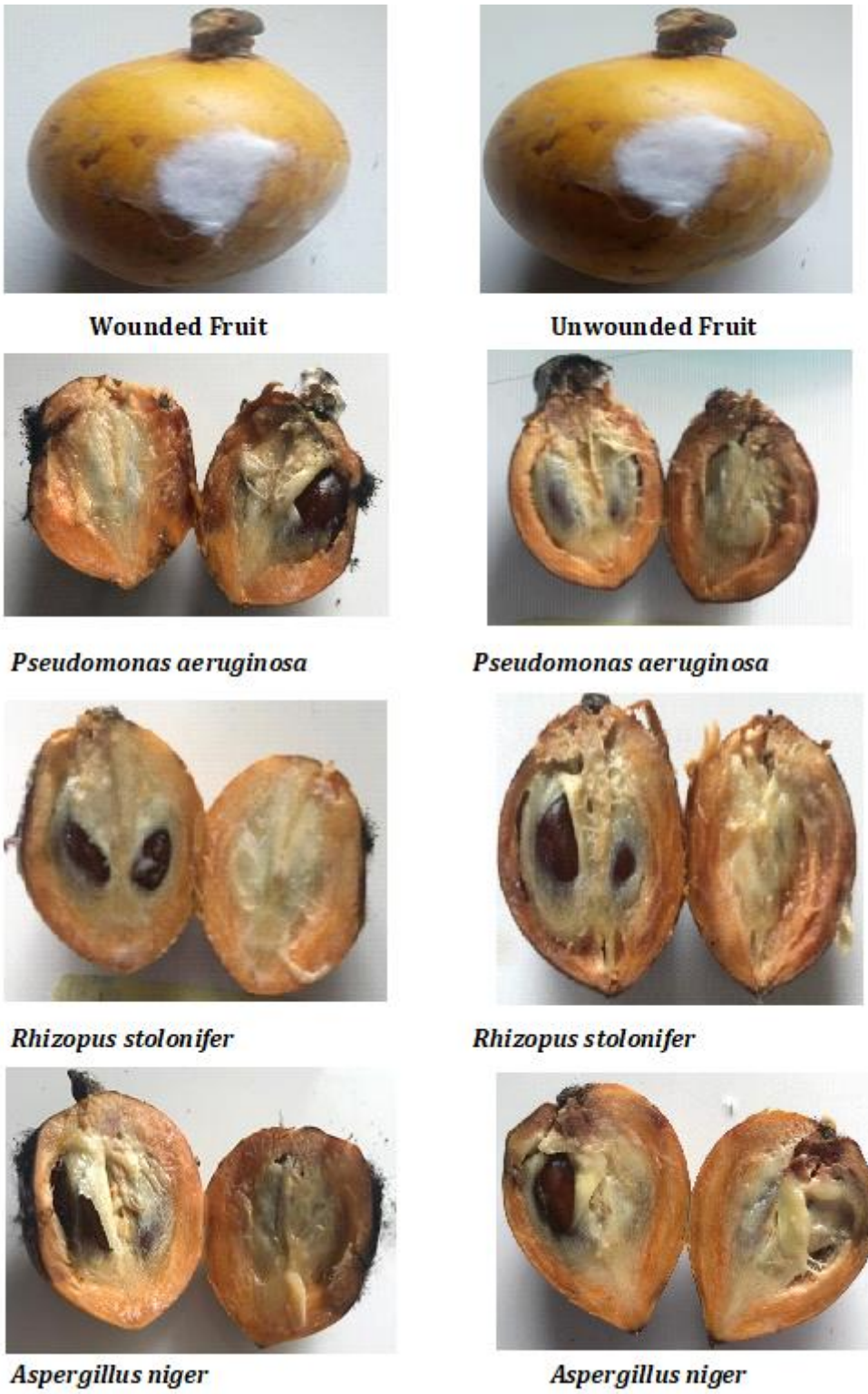


Plate 4. Pathogenicity test of the micro-organism

Proximate Compositions of Infected and Uninfected Fruits of *C. albidum* obtained from Markets in Port Harcourt Metropolis

Results on the proximate compositions of infected and uninfected fruits of *C. albidum* are presented in Table 3. Results revealed that moisture content (66.22%±3.20) of the infected fruits of *C. albidum* was significantly (P≤0.05) higher than the uninfected

(62.61%**±**2.05), the ash content (1.51%**±**0.20) of the infected fruits was higher than the uninfected fruits (1.21%±0.01). Conversely, there was significant percentage increase in protein (1.41%±0.20), fat (5.27%±0.24), crude fibre (3.27%±0.24) and carbohydrate (26.30%±2.04) contents of the uninfected fruits of *C. albidum*

Table 3 Proximate Compositions of Infected and Un-infected Fruits of *C. albidum* Obtained from Markets in Port Harcourt Metropolis (Mean±SD)

Sample *C. albidum* fruits	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Lipid (%)	Crude fibre (%)	Carbohydrate (%)
Uninfected	62.61^{b}±2.05	1.21^{b}±0.01	1.41^{a}±0.03	5.27^{a}±0.24	3.21^{a}±0.24	26.30^{a}±2.04
Infected	66.22^{a}±3.20	1.51^{a}±0.20	1.37^{b}±0.04	1.94^{b}±0.03	1.94^{b}±0.03	20.64^{b}±1.28

Mean values with the same superscripts (a,b,c..) in the same column are not significantly (P≤0.05) differently by DMRT (P≤0.05).

DISCUSSION

Microorganism associated with African star apple and their Biodeterioration

Two fungi and one bacterium were isolated from the deteriorating African star apple fruits obtained from three markets in Port Harcourt Metropolis, Nigeria. The fungi include *Rhizopus stolonifer*, *Aspergillus niger* and *Pseudonomonas aeruginsa.* However, *A. niger* was the most prevalent of all the isolates, followed by *R. stolonifer* and the least was *P. aeruginosa* (Table 1). The fruits were found associated with pathogenic fungal and bacterium deterioration with consequent is shrinking and obvious fungal mycelia growth. These micro colonised the deteriorating pulp. Most of these microorganisms which occurred in stored African star apple fruit has been reported to be the most important fruit rot pathogen in South-Eastern and South Western Nigeria [10, 28]. It was observed that these microorganisms caused significant reduction in cashew yield [51]. All the isolates were observed to be pathogenic to African star apple fruit notably *R. stolonifer* and *A. niger* are usually present in the air [59] aprobably secondary invaders. This study showed that (33%) of the fruits picked were infected. These infected fruits when packed with uninfected caused increased deterioration of African star apple in transit and storage [2, 16, 28]. The natural dropping of star apple fruits probably causes entry point for the fungi that were associated with fruit deterioration. It is also possible that insect vectors are involved in dissemination as reported by [4] indicating that fruit fly stings enhance the entry of *Colletotrichum gloesporoides* into African star apple fruits by their composition on the fruits.

Proximate composition of African star apple

[32] have reported 31.97% moisture content for the pulp fruit of *C. albidum* which was contrary to the present moisture (62-61%). Moisture content of foods is influenced by type, variety and storage condition [34]. The moisture content of African star apple kernel flour was within the acceptable limit of not more than 10% for long term storage of flour [49, 20]. The low moisture content of the flour would enhance its storage stability by preventing mould growth and reducing moisture dependent biochemical

reactions [49]. According to [50] any fruit with a moisture content > 15% is subject to deterioration from mold growth, heat, insects damage and sprouting. Therefore, this fruit should not be stored for a long period of time due to the high moisture content which is associated with rise of microbial activities during storage [36, 55].

The presence of *P. aeruginosa* in the infected fruits may be due to poor hygienic standard and improper handling of the fruits [30]. Some of these organisms namely *E. coli* and *P. mirabilis* have been implicated in gastroenteritis [43]. The incidence of *B. polymyxa,* and *B. cereus* in fruits are indicative of environmental contamination of most fruits as the fruits are constantly exposed to air, aerosols and dust particles during the course of selling the fruits. 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. [17] documented the richness in ascorbic acid of the African star apple, while [21, 57] separately attested to the excellent sources of vitamins, iron, flavours to diet, raw materials for some industries and essential minerals [12]. Thus, the fruit could serve as nutrient source for the isolated microorganisms. The incidence of *Pseudomonas aeruginosa* could be adduced to its nutritional versatility [30].

Ash is an indication of the mineral content of a food. Fruit pulps are not good sources of fat [49]. Similarly fat content was previously reported for the kernel [40].The crude fiber content was significantly higher than that of 0.6% for the pulp. The therapeutic effects of fiber in the prevention of heart diseases, colon cancer and diabetes and their role in the treatment of digestive disorders (diversticulosis) and constipation are widely documented [42].

The ash content is a measure of mineral content and was found to be low (1.5%) in the pulp of *C. albidum.* Generally, the ash content obtained from the fruit pulp of *C. albidum* indicates it is not good for seed cake compounding of animal feeds as the value is 2.5% [56, 42]. The percentage crude lipid content of the fruit pulp was 5.27%, though contrary to what was reported by [12] it is higher than the values reported in the fruits of *Dalium, guineanse, Tetrapleura tetraptera*, *Cola millenii* and *Parkia biglobosa* with values 0.175, 0.075, 0.15 and 0.25mg/g respectively [5]. The value was low compared to the value reported in *Parkia biglobosa* 22.47% [35]. Due to the low oil content of the fruit pulp it shows that the fruit pulp is not economical for commercial exploitation [36]. The crude fibre content of *C. albidum* fruit pulp was 3.21% which is similar to the crude fibre content of African bread fruit (3.3%) [1, 25, 31]. It was reported that, consumption of fruit containing high fibre content can reduce serum cholesterol level, colon, breast cancer, hypertension, enhance glucose tolerance and increase insulin sensitivity [52]. High fibre content in the diet at the same time reduces mineral, protein and carbohydrate bioavailability by hindering their hydrolytic breakdown [54, 42] The percentage crude protein (1.41%) obtained in *C. albidum* fruit was very low compared to 26.15% in *Dalium guineanse*, 18.75% in *Tetrapleura tetraptera* [5]. The percentage carbohydrate content in the fruit pulp was 26.30%. This value is comparable to the value reported for 25.00% *Balanite aegyptiaca* [44] This high percentage of carbohydrate makes the fruit to be a good source of energy.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The microorganisms isolated are no doubt involved in the infection, degradation and 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.

In the proximate analysis of the fruit pulps were determined using specific methods. The result obtained revealed that the fruit is a good source of nutrient. The fruit is prone to deterioration as a result of high moisture content of the pulp. The oil is also not economical for commercial exploitation due to its low content. The energy value obtained from the fruit pulp can contribute a lot of energy required for some of our daily activities.

Recommendations

Based on the findings the following are recommended:

1. Avoid picking fruits that are infected by micro-organisms for consumption.
2. Fruits/vegetable handling techniques should be adopted.
3. The incidence of *P. areuginosa* bacterium was clearly attested to human contamination during handling and can cause nausea, vomiting and diarrhea when injected in fruits, food and water.

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