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Evaluation of Proximate Qualities and Fungal Isolates Associated with the Spoilage of *Persea americana* Fruit

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***Abstract*: The study examined the proximate composition and fungal isolates associated with the spoilage of *Persea americana* (avocado pear). A total of ten ripped-spoilt avocado pears (five each from Ogbete market, Enugu State and Eke market, Afikpo), were randomly purchased and sent to the lab for analysis. Using 75% alcohol as a sterilizer, contaminants were removed from samples. With the aid of sterile blade, the spoilt portions were scoped of and inoculated onto aseptically prepared isolating media. The plates were incubated for fungal isolation at room temperature for 5 days. Isolates were identified using conventional laboratory procedures. Proximate analysis showed that the avocado pear is made up of 18.77% fats, 4.9% carbohydrates, 32.13% dry matter, 19.76 grams of protein, and 64.68% moisture. Five species of fungi, including Mucor sp., *Geotrichum* sp., *Fusarium* sp., *Aspergillus flavus*, and *Aspergillus niger*, were recovered *Aspergillus niger* (26.0%) and *Mucor* sp. (29.6%) were found to have the highest percentage of fungi. As a result of the aforementioned, we advise against eating spoiled avocados despite the fruit's high nutritional value.**

***Keywords*: Evaluation, Proximate, Avocado, Fungi.**

1. Introduction

Persea Americana, also known as the avocado pear, is a tree that is native to Mexico and Central America. It is a member of the Lauraceae family, which primarily consists of shrubs and trees that produce resinous aromatic gum from their cut bark (Chen et al., 2008). (Wogu & Ighile, 2014). In the tropical and subtropical rain forest zone of the Southern regions of West Africa, it is one of the well-known native fruit trees (Akpoka *et al*., 2020). A large berry with a single seed is called an avocado, also known as an alligator pear (Wogu & Ighile, 2014). Avocados easily ripen after being harvested and have a fleshy, green-skinned body that can be spherical or pear-shaped. To maintain a predictable fruit quality and quantity, the trees, which are partially self-pollinating, are frequently propagated through grafting (Eze & Chimaeze, 2014).

Many people adore avocado fruit, and it contributes significantly to diets by addressing food insecurity issues in developing nations. Additionally, it is available throughout the year, including key times when traditional staples that are challenging to store are scarce (Wogu and Ighile, 2014). The oils from the pulps and seeds are used in the production of foods, medications, and cosmetics in addition to many other industrial applications. They are comparable to other vegetable oils currently in use and are high in monounsaturated fatty acids. In 2013, Dreher noted that clinical research on avocado diets had consistently demonstrated their beneficial effects on heart health, including blood lipid profiles. This is primarily due to the fruit's effects as a whole food and its high nutrient content. Like other plant-based foods, avocados mostly contain beneficial monounsaturated fats. The monounsaturated fats in avocados not only supply fat-soluble vitamins but also aid in regulating cholesterol production and may lower the risk of cardiovascular disease (Schwingshackl, 2012). It has also been demonstrated that eating a diet high in monounsaturated fats, like the Mediterranean diet with olive oil and nuts, lowers the risk of cardiovascular disease (Mente, 2009; Estruch, 2013). Consuming avocados as part of a balanced diet may reduce total and LDL (bad) cholesterol.

Avocados are picked when they are hard and green and kept in coolers between 3.30 and 5.6 degrees Celsius until they reach their destination. Avocados ripen in a few days at room temperature after being picked. The fruit can typically be stored for 3-6 days before it spoils and has a very short shelf life. The fruit's short shelf life has contributed to its high perishability, significant post-harvest losses, and market glut during harvest, as evidenced by the abundance of unsold, rotting fruit that can be found in the village and urban markets' refuse piles. These qualities of avocado fruits are a serious hindrance to both the export market and industrial uses, as they do not provide flexibility across all market channels (Wogu & Ighile, 2014).

One-fourth of all avocado fruits harvested are thought to go bad before being eaten. Fresh avocado fruit spoilage, unlike that of many other fruits, typically happens during storage, transportation, and while waiting to be processed (Eze & Chimaeze, 2014). The avocado fruit can become spoiled due to bacterial, viral, and fungal diseases. The fruit may be harmed by disease and microbes, which can result in spotting, rotting,

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cankers, pitting, and discoloration. Numerous microorganism species can easily attack the fruit. The avocado fruit's structure affects the type of spoilage that is most likely to occur (Eze & Chimaeze, 2014). Due to the avocado fruit's high nutritional value and high rate of spoilage, a variety of microorganisms may be involved in its deterioration. It is against this backdrop following its high acceptability that this study is set to examine the proximate qualities and microorganisms associated with the spoilage of *Persea americana* fruit sold in Ogbete and Afikpo.

2. Materials and Methods

A. Sample Collection

A total of ten (Five each from each market) ripped and damaged samples avocado pears fruits were randomly purchased from Ogbete market in Enugu State and Eke Market in Afikpo Ebonyi State. The samples were put in sterile bags and sent to the laboratory for proximate and microbial analysis.

B. Proximate Quality Determination

1) Moisture content

The air-oven method was used to determine the moisture content of the samples. The Petri-dishes were first washed, dried in the oven, allowed to cool in a desiccator and the weight noted. 12g of each of the sample was measured, transferred into the petri-dishes and weighed. The petri-dishes containing the samples were then dried in an oven at 1050C for 3 hours. The petri-dishes were removed from the oven, allowed to cool in the desiccator and the weight was noted (A.O.A.C 2000).

This process was continued until a constant weight was obtained (A.O.A.C. 2000). The loss in weight during drying in percentage was taken to be the percentage moisture content.

$$\% \text{ moisture} = \frac{\text{loss in weight due to drying} \times 100}{\text{Weight of sample taken}}$$

$$\% \text{ moisture} = \frac{W3 - WA \times 100}{W2}$$

Where W3 = Initial weight of the sample,
WA = constant weight after drying and
W2 = weight of sample taken.

2) Ash content

It is the weight of residue obtained after burning a weighed quantity of avocado in an open crucible at 750°C in a muffle furnace till a constant weight is achieved.

$$\% \text{ Ash in avocado} = \frac{\text{weight of residue ash formed x 100}}{\text{Weight of avocado initially taken}}$$

3) Fat content

250ml flask was dried in an oven at 1000c, allowed to cool in a desiccators and weighed. 5g of the samples were put inside a thimble and this was plugged with wool, the thimble was placed into extractor for extraction period of 60minutes afterwards, the thimble was removed. The flask was then disconnected and was placed in an oven at 1000c for 2hours after which it was cooled and weighed.

$$\text{Fat} = \frac{\text{Increase in mass X 100}}{\text{Mass of sample used}}$$

4) Protein content

Total protein was determined by the kyeldahl method as modified by Williams (1964). The analysis of a compound of its protein content by Kyeldahl method is based upon the determination of the amount of reduced nitrogen present. About 38g of sample was weighed into a fitter paper and put into a Kyeldahl flask, 5 tablets of Na_2SO_4 were added with 1g of $CUSO_4$ respectively. Twenty milliliter (20ml) of concentrated H_2SO_4 were added and then digested in a fume cupboard until the solution becomes colorless. It was cooled overnight and transferred into a 500ml flat bottom flask with 200ml of water. This was then cooled with the aid of packs of ice block. About 60 to 70ml of 40% of NaOH were poured into the conical flask which was used as the receiver with 50ml of 4% boric acid using 3days of screened methyl red indicator. The ammonia gas was then distilled into the receiver until the whole gas evaporates. Titration was done in the receiver with 0.1m NH_2S0_4 until the solution becomes colorless.

$$\frac{Vs - V_b \text{ X } 0.1401 \text{ X N acid } (6.25) \text{ X } 100}{\text{Original Wt of sample used}}$$

Where Vs = vol (ml) of acid required to titrate sample, vb = vol(ml) of acid required to titrate blank, N acid = normality of acid.

5) Crude fiber content

The bulk of roughages in food is referred to as fiber and is estimated as crude fiber. Twenty gram (20g) of the sample was defatted with diethyl ether for 8hours and boiled under reflux for filtered through cheese cloth on a flutter funnel. This was later washed with boiling water to completely remove the acid. The residue was then boiled in a round bottomed flask with 200ml of 1.25% sodium hydroxide (NaCH) for another 30minutes and filtered through previously weighed couch crucible. The crucible was then dried with samples weighed. This was later incinerated in a muffle furnace at 600^0c for 2 to 3 hours and later allowed to cool in desiccators and weighed.

$$\text{Cal.} = \frac{\text{Weight of fiber} = (C_2 - C_3)}{\text{Weight of original sample}}$$

$$\% \text{ fiber} = \frac{C_2 - C_3 \text{ X } 100}{\text{Wt of original sample}}$$

6) Carbohydrate content

Available carbohydrate (%) = 100 – (protein (%) + moisture (%) + Ash (%) + fiber (%) + Fat (%). Here, the avocado pear sample was digested with perchloric acid and hydrolyzed starch. The soluble sugar was determined calorimetrically by amthrone method. Duplicate standards were pipette out using 1ml dilute glucose standard solutions and 5ml of freshly prepared anthrone reagents pipette rapidly to all the tubes and

mixed thoroughly. These covered tubes were placed in boiling water bath for exactly 12minutes to allow change in color following which sample was allowed to cool room temperature. The readings of the two cuvettes containing anthrone reagents and deionizer distilled water were measured and difference in readings note for correction in the calculations. The prepared solutions were transferred to one glass cuvette and their absorbance read at 630nm against a blank containing 1ml of deionized distilled water and anthrone reagents. Concentration of the test samples were obtained via interpolation of the absorbance readings. The mass of the glucose was obtained by calculation of the concentration and dilution made. The mass of starch was obtained by calculating the mass of glucose and a factor of 0.9.

C. Fungal Analysis

1) Sample Preparation

All the samples were sterilized with 75% alcohol to remove any dust and contaminating microbes on the surface order than the spoilt portion. Sterile samples were thereafter allowed to air dry.

2) Sample Inoculation

With the aid of sterilized blade, spoilt portions of each of the samples were scooped of. Placed on each of the prepared isolating media (Saburoid Dextrose Agar), and using sterilized wire – lop, the sample was streaked over the surfaces of the media. The plates were then incubated at room temperature for five days.

3) Identification of Isolates

Isolates where identified based on the colonal and cellular features.

4) Pathogenicity Test

Pathogenicity test was conducted on the isolated colonies by inoculating the isolates on a health avocado pear and incubated for seven days. This was done by boring a hole on a healthy fruit and with the aid of sterilized wire loop, loopful of isolates were inoculated on the holes, sealed and the incubated for seven day to detect deterioration.

3. Result

Table 1
Proximate composition of avocado pear

Parameters	Ogbete Samples composition (%)	Afikpo Samples composition (%)
Fat content	18.77	19.01
Carbohydrate	4.90	5.7
Dry matter	32.13	29.11
Ash content	1.05	3.2
Crude fibre	4.65	5.9
Protein	19.76	19.02
Moisture content	18.74	18.06

Proximate analysis of the sample revealed that avocado pear is reached composed with nutrients. Samples from Ogbete Enugu had 18.77% fats, 4.8% carbohydrate, 32.13% dry matter, 1.05% ash content, 4.65% crude fibre, 19.76% protein and 64.68% moisture content (tab.1). however, samples from Afikpo contained 19.01% fat, 5.7% carbohydrates, 29.11% dry matter, 3.2% ash, 5.9% crude fibre, 19.02% protein and 18.06% moisture (table 1). Samples from Afikpo had higher fat content (19.01%) than samples from Ogete Enugu (18.77%). However, protein content of samples from Ogbete Enugu was slightly higher (19.76%) than those obtained from Samples from Afikpo (19.02%) (table 1).

Table 2
Cultural and cellular characteristics of fungal isolates

Top	reverse	growth	microscopy	suspected fungi
White then grey	white	fluffy covering plate	Sparsely septate, broad hyphae, Sporangiophores	*Mucor* sp.
Dry white	white	rapidly growing, powdery to cottony	Unicellular, in chains, hyaline undifferentiated hyphae	*Geotrichum* sp.
yellow-green			yellow Slightly roughed varying sizes of conidia with, unbranched none septate conidiophore	*A. flavus*
Red	white	cottony aerial Mycelium	lots of microconidia and ellipsoidal macroconidia	*Fusarium* sp.
Black	yellow		branched septate conidiophore	*A. niger*

Cultural and cellular characteristics of fungal isolates indicates the isolation of five different species of fungi with varying pigmentation and microscopic features. The isolates included *Mucor* sp., *Geotrichum* sp., *Fusarium* sp. *Aspergillus flavus* and *Aspergillus niger* (table 2).

Table 3
Percentage occurrence of Fungal isolates accounting to sample source

Fugal isolate	Number of isolate		Percentage isolate	
	Ogbete sample	Afikpo sample	Ogbete sample	Afikpo sample
Mucor sp	8	10	29.6	34.5
Aspergillus niger	7	5	26.0	17.2
Fusarium sp.	5	3	18.5	10.3
Aspergillus flavus	4	7	14.8	24.1
Geotrichum sp.	3	4	11.1	13.8
	27	29		

A total of five species of fungi were recovered from the samples which included *Mucor* sp., *Aspergillus niger*, *Fusarium* sp., *Aspergillus flavus* and *Geotrichum* sp. (tab. 3). Afikpo sample recorded the highest number of isolates (29) while Ogbete samples recorded 27 isolates. *Mucor* sp. Isolated from Afikpo had the highest percentage occurrence of 34.5%. *Aspergillus niger* isolated from Ogbete had the highest percentage occurrence of 26.0% and so was *Fusarium* sp. (18.5%). However, Aspergillus *flavus* and *Geotrichum* sp. Isolated from Afikpo samples were higher than those isolated from Ogbete samples. As they recorded 24.1% and 13.8% occurrences respectively (table 3).

4. Discussion of Findings

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The percentage of crude protein and fats found in this study is consistent with findings from a related study by Nwaogu *et al*. (2008), who found that crude protein had a mean of 18.55±1.26 and fats had a mean of 18.53±0.26, respectively. The present study's carbohydrate content also differs from that of the Nwaogu *et al*. study (2008). While Nwaogu *et al*. (2008) recorded a higher percentage of 47.35±3.24, this study presented here recorded a low percentage of carbohydrates. The crude protein found in this study, however, was higher than that found by Egbuonu *et al*. (2018), who found 2.640.01 crude protein. Additionally, the amount of carbohydrates in this study was different from what Egbuonu *et al*. reported (2018). While the percentage of carbohydrates in this study was 4.9%, other studies have found levels as high as 80.12±0.15.

Moreover, the moisture content of this present study disagrees with the result obtained by Nnaji and Okereke (2016) in the study in Umuahia Nigeria. Their mean moisture content was as high as 69.25±8.11a in one of their avocado varieties. The percentage carbohydrate content of this present study agrees with their result of carbohydrate from varieties of Brogdon Russel Choquette which were 4.90±1.07a 8.64b±2.05b 7.96±1.38b respectively.

Our result on percentage composition of protein (19.76 and 19.02 for Ogbete and Afikpo respectively) in this present study agrees with the high result recorded by Emelike & Barber, (2018), in their study on the Assessment of Food Quality and the Associated Mycoflora of Okpa, a Local Recipe from Bambara Groundnut. They had a parentage protein content as high as of 15.60%. But our result on Carbohydrate disagrees with that of their study. While were recorded as low as 4.90% and 5.7% for Ogbete and Afikpo respectively, Emelike & Barber, (2018), recorded high value of 25.25%.

The number of fungi recovered in this study is greater than that noted by Nwogu *et al* (2014). In contrast to Nwogu *et al*. (2014), who only found three fungal isolates, this study found six. Some of the isolates found in this study, like *Mucor* sp. and *Geotrichum* sp., match those found by Nwogu *et al* (2014). However, the total number of fungi isolated from this present study is less than that isolated by Kebede and Belay (2019) in Ethiopia. While we isolated six fungal species, Kebede and Belay (2019) in their study on Fungi Associated with Post-harvest Avocado Fruit Rot at Jimma Town, Southwestern Ethiopia recovered 30 species of fungi.

Since microorganisms are present everywhere, it is possible that the high concentrations of fungi found in this study originated from the surroundings, such as the farm, store or market. Due to the fact that these fruits are preserved without the risk of contamination. Our stance is consistent with that of Buck *et al*. (2003), who, after analyzing the findings of their study, hypothesized that the presence of bacteria and fungi was largely due to the fruit's outer surface being exposed to the environment indiscriminately at farms and in markets. Our argument was strengthened by Wogu and Ighile (2014), who noted that the presence of the fungi or their resistant spores is more likely to have come from the farms where the fruits were harvested and some from the stores due to horizontal contamination by the already spoiled fruits. When claiming that pre-harvest and post-harvest factors, including farm soil type, storage conditions, and handling practices, may be the likely source of these microbes in their respective studies, researchers like CFS (2006) and Leff & Fierer (2013) made similar allusions to this fact. Furthermore, it wouldn't be inappropriate to suggest that the pulp's high nutritional value could also contribute to microbial contamination and the decline of the pear. Our argument is well supported by Dreher and Davenport's (2013), contention that this indiscriminate exposure and the avocado pear's high nutritional content frequently led to an increased likelihood of contamination that was spread by flies, airborne dust, unhygienic human contact, and damage to the fruit's outer surface.

The presence of *A. niger* in this present study may not be of serious health concern though care most still be taken as this organism is known to be an opportunistic fungus especially in immunocompromosed individuals. Our position is in line with the remark made by Schuster *et al.* (2002), when they concluded that "*A. niger* is generally regarded as a non-pathogenic fungus widely distributed in nature. Humans are exposed to its spores every day without disease becoming apparent. Only in few cases has A. niger been able to colonise the human body as an opportunistic invader and in almost all these cases the patients have a history of severe illness or immunosuppressive treatment."

The ability of the fungal isolates in this present study as recorded in their parthogenicity test is in line with the result obtained be Emelike and Barber, (2018).

5. Recommendations

a) There is need for avocado consumers to ensure that it is properly washed in clean water before it is eaten as it has been found to be contaminated with pathogenic bacteria and fungi.
b) There is a need for sensitization to educate people on the need to have the plant in their homes to ensure a steady supply of avocado fruit.

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