Determination of the Nutrient Quality of Pulp and Seed of Long Shape Variety of *Persea americana (mill)* and their Associated Fungal Flora

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Abstract

The research on the determination of the nutrient quality of pulp and seed of long shape variety of Avocado pear and their associated fungal flora was carried out in Plant Science and Biotechnology Laboratory, Rivers State University, Portharcourt. Long shape variety of Persea americana was purchased from the fruit garden market in D. line, Portharcourt. The fruit was sent to Food Science and Technology Laboratory for nutrient and antinutrient analysis while while another fuit was kept for mycological studies. Result of proximate analysis revealed the presence of moisture, ash, lipid, carbohydrate, fibre and protein. It was observed that in all the parameters assessed, moisture (70.4 0.17), ash (3.3 ±0.06) and lipid (11.43±0.06) values were higher for the pulp samples except for fibre (4.57±0.06), protein (17.13±0.32) and carbohydrate (61.73±0.20) which were higher in the seed samples. The mineral contents indicated higher value for calcium (26.90±0.00), phosphorus (480.0±0.00), potassium (32.43±0.06) and magnesium (42.00±0.00) in the pulp samples. However, vitamin C (47.80±21.13), chlorophyll (77.03±1.67) and thiamine (6.87±0.11) were available in higher quantities in pulp samples while vitamin A was high in the seed sample. Antinutrient and phytochemical investigations revealed glycoside, oxalate, saponin, tannin, carotenoid, polyphenol, flavonoid and lignin to be present in both the seed and pulp of Peasea americana . Nevertheless, Candida sp. (100%) was the only fungal isolate identified from the long samples and are implicated to cause spoilage. In general, Peasea americana is rich in nutrients beneficial to humans but are also prone to fungal contamination.

Keywords: *Pear (Persea americana), variety, nutrient quality and fungal flora*

INTRODUCTION

The avocado tree, *Persea americana* is an evergreen tropical tree. According to Pamplona-Roger (2007), avocado is the only tree that produces comestible fruit among the laurel family. The fruits are extremely variable in shape, size, and colour. The shape may range from ellipsoid through pyriform and obovate to spheroid, with the skin of the fruit also varying in texture and colour. The colour of skin ranges from yellowish-green, reddish-purple, purple-black. The outer skin may also be lithy to woody or smooth to rough (Taah *et al.*, 2003). The uses of avocado depend on the varieties available. Because the tannins in avocado result in a bitter flavour when cooked over high heat, they are usually eaten raw (Purseglove, 1968; Morton, 1987). There are three races of avocado: Guatemalan, Mexican and West Indian which differ in their size, appearance, quality and susceptibility to cold (Nandwani, 2014), While each has unique features, cross-pollination permits the development of limitless varieties (Pamplona-Roger, 2007, Orhevba and Jinadu, 2011).

Avocados are an energy-rich food eaten in its fresh state. The nutrient content of avocados varies according to the cultivar, season, growing conditions and time of harvest (Naveh *et al.*, 2002). The time when an avocado is picked (i.e. the time of harvest) is crucial in the development of the fruit and therefore the nutrients present. Avocados mature on the tree and if harvested too early in its maturation stage the nutrient composition will be less than optimal. This is particularly the case for lipid content as the oil increases during maturation. (Lu *et al.*, 2009).

The seed of avocado is one of the under-utilized non-edible parts of the fruit, which are usually discarded as residues. Conducting a research on non-edible parts of fruits is an emerging trend, which may prove to be very profitable in the near future. Mostly, because it involves an important reduction in the production of wastes and the fact that the non-edible parts of many fruits like avocado have high levels of valuable bioactive compounds, particularly natural antioxidants (Lu *et al.*, 2009). The seed of avocado is redundant during the processing of the pulp. The seed waste may represent a severe ecological problem. However, at the same time, it may be of interest to industry as a source of bioactive compounds. Biological activities of the avocado seed such as antioxidant, antihypertensive, fungicidal, larvicidal, hypolipidemic, and recently amoebicidal and giardicidal activities have been reported (Noorul *et al.*, 2016).

MATERIALS AND METHODS

Sample collection

Samples of Avocado pear were purchased from the Fruit Garden Market at D. Line Port Harcourt and were allowed to ripen off the plant at room temperature within 3-4 days, to allow for optimum processing quality. It was then brought to of Plant Science and Biotechnology Laboratory for identification and isolation of fungal organisms present in the avocado samples to be studied. Samples for nutrient analysis were sent to Department of Food Science and Technology RSU, for analysis.

Nutrient analysis

The proximate composition was determined according to the method of AOAC (1990). Mineral contents were determined according to the methods of AOAC (2010).Analysis of Vitamin C was determined based on the method described by the Association of Analytical Chemist (AOAC, 1990).

Determination of Anti-nutrient

Saponin content of the samples was determined by the method described by Harborne (1973), oxalate was determined according to the method of Onwuka (2005), flavonoid and polyphenols was determined AOAC, (1990).

Preparation of mycological medium

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120^oC for an hour after washing with soap, while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Chuku, 2009). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminium foil. The conical flask containing the mycological medium was autoclaved at 121^o C and pressure of 1.1kg cm^{-3} for 15 minutes. The molten agar was allowed to cool to about 40 ^{o} C and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation of fungi

A threefold serial dilution was used in accordance to the method of Mehrotra & Aggarwal, (2003) where 1g of the various avocado samples were transferred into the first test tube containing 9mls of normal saline. 1ml of the solution was transferred to the second test tube and finally from the second to the third. 0.1ml aliquot from the second and third dilutions was plated onto Sabouraud Dextrose Agar in Petri dishes containing ampicillin to hinder the growth of bacteria and this was done in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25^o C ± 3^o C (Chuku, 2009). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates was obtained after a series of isolations. The set up was conducted monthly for a period of three months.

Identification of fungal organisms

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Barnett & Hunter, 1998).

Determination of percentage incidence

The percentage incidence of fungal occurrence was determined by the formular stated below (Chuku *et al.*, 2019):

$$\frac{X}{Y} \times \frac{100}{1} = \% \text{ incidence}$$

Where:

X= total number of each organism in a variety

Y= total number of all identified organism in a variety

Statistical Analysis of Data

Data obtained from this study were expressed as mean ± SEM. Analysis of group data was done by one-way analysis of the variance (ANOVA) followed by Duncan test for the establishment of significance differences. Statistical significance was set at ($p<0.05$).

Results and Discussion

Table 1: proximate ccompositio of seed and pulp of avocado pear (%)

Parameters	Avocado Pear Seed	Avocado Pear Pulp
Moisture	$12.57^a \pm 0.06$	$70.40^b \pm 0.17$
Ash	$2.50^a \pm 0.00$	$3.33^b \pm 0.06$
Lipid	$1.50^a \pm 0.00$	$11.43^b \pm 0.06$
Fiber	$4.57^a \pm 0.06$	$1.51^b \pm 0.01$
CHO	$61.73^a \pm 0.20$	$7.73^b \pm 0.06$
Protein	$17.13^a \pm 0.32$	$5.47^b \pm 0.03$

Table 2: Mineral content of seed and pulp of avocado pear (mg/100g)

Parameters	Avocado Pear Seed	Avocado Pear Pulp
Ca	$12.33^a \pm 15.48$	$26.90^b \pm 0.00$

Fe	2.53[a]±0.06	2.00[b]±0.00
Mg	35.00[a]±0.00	42.00[b]±0.00
P	350.67[a]±0.58	480.0[b]±0.00
K	274.33[a]±0.06	32.43[b]±0.06
Na	15.10[a]±0.00	5.00[b]±0.00

Table 3: Vitamin content of seed and pulp of avocado pear (mg/1000g)

Parameters	**Avocado Pear Seed**	**Avocado Pear Pulp**
Vit. C	0.00[a] ±0.00	47.80[b]±21.13
Vit. A	250.00[a]±0.00	200.00[b]±0.00
Thiamine	2.17[a]±0.01155	6.87[b]±0.11547
Chlorophyll	0.00[a]±0.00	77.03[b]±1.67

Table 4: Anti-nutrient & Phyto-chemical content of seed and pulp of avocado pear (%)

Parameters	**Avocado Pear Seed**	**Avocado Pear Pulp**
Glycoside	0.02[a]±0.01	0.00[b] ± 0.00
Oxalate	0.01[a]±0.00	0.01[a]±0.01
Saponins	0.51[a]±0.00	0.32[a]±0.19
Tannins	0.02[a]±0.00	0.01[a]±0.01
Carotenoid	2.06[a]±0.01	1.50[a]±0.43
Polyphenol	7.51[a]±0.01	7.40[a]±0.61

Flavonoid	4.89[a]±0.01	5.81[b]±0.00
Lignin	3.86[a]±0.02	4.05[b]±0.00

Table 5: Fungal percentage incidence

Isolates	Long variety
Candida sp.	100

The moisture content of avocado seed was 12.57±0.06 while that of the pulp was 70.40±0.17. Generally, increase level in seeds and pulp are known to encourage microbial spoilage (Oduro *et al.*, 2009). However, a high moisture content of the pulp of avocado pear observed in this study is an indication of the potency of the pulp to serve as diet for weight loss and an antioxidant that could boost the immune system thereby, preventing the accumulation of certain food materials in the body that could lead to serious health challenge (Chuku *et al.*, 2018). The moisture result is in line with Chuku *et al.*, (2019) on English pear, Wekhe *et al.*, (2020a) on *A. altilis* pulp.

The ash content of avocado seed was 2.50±0.00 while that of the pulp was 3.33±0.06. The ash content of avocado seed and pulp provides a measure of the total amount of minerals within a food (Obadina *et al.*, 2016). The ash content is in line with *Artocarpus heterophyllus* and *Treculia africana* reported by Wekhe *et al.*, (2021).

The lipid content of avocado seed was 1.50±0.00 while that of the pulp was 11.43±0.06. Fats are essential in diets as they increase the palatability of foods by absorbing and retaining flavors (Aiyesanmi and Oguntokum, 1996). The lipid content result is in line with *Artocarpus camansi, A. heterophyllus* and *T. africana* reported by Wekhe *et al.*, (2020b).

The fibre content of avocado seed was 4.57±0.06 while that of the pulp was 1.51±0.01. Crude fibre is the insoluble polymeric material of plant cell wall such as cellulose, hemicellulose, pectin and lignin that constitute the major part of dietary fibre (Johnston and Oliver 1982). High fibre intake has been linked with decreased chances of colon cancer and associated with reducing constipation.

The carbohydrate content of avocado seed was 61.73±0.20 while that of the pulp was 7.73±0.06 which had apparently the highest seed content. The seed carbohydrate content is in line with Wekhe *et al.*, 2020a on breadfruit varieties. Carbohydrates assist in the metabolism of fat and are known to supply quick and metabolizable energy in the food (Appiah 2011).

The protein content of the seed of avocado was 17.13±0.32 while that of the pulp was 5.47±0.03. It was evident that the protein content of the seed was higher than the pulp. These differences could be as a result of the soil nutrient levels since soil nitrogen level content could influence protein levels (Blumenthal *et al.*, 2008). Protein enhances the replenishment of lost cells (Andrew, 2008).

The calcium content of avocado seed was 12.33±15.48 while that of the pulp was 26.90±0.00. The calcium content of the avocado seed and pulp were higher than that of *T.*

africana and *A. camansi.* Calcium intake is important as it is known to reduce demineralization of bones (Greenberg, 1995).

The iron content of the avocado seed was 2.53±0.06 while that of the pulp was 2.00±0.00. Iron is known to be an important constituent of haemoglobin found in blood and contributes to the combat of anaemia (Devillota *et al.*, 1981).

The magnesium Content of avocado seed was 35.00±0.00 while that of the pulp was 42.00±0.00. The magnesium content of avocado pulp and seed is higher than groundnut paste preserved with some powdered botanicals reported by Chuku and Okogbule, (2017). Magnesium is essential in enzyme systems and helps maintain electrical potential in nerves (Ferrao *et al.*, (1987).

The seed of avocado has phosphorus content of 350.67±0.58 and pulp content of the avocado was 480.0±0.00. The phosphorus content of this study is higher than green pea (7.5± 0.005) reported by Chuku *et al.*, (2019).

The potassium content of avocado seed was 274.33±0.06 and the pulp content was 32.43±0.06. The potassium content is higher than green pea (98± 0.021) reported by Chuku *et al.*, (2019), three citrus species reported by Chuku and Akani, (2015) and groundnut paste preserved with some botanicals reported by Chuku and Okogbule (2017). Potassium is an important mineral which helps maintain electrolyte balance in humans and it's important in amelioration of hypertension also helps in building up of the body frame work (Whelton *et al.*, 1997).

The sodium content of avocado seed was 15.10±0.00 while that of the pulp was 5.00±0.00. The sodium content of this study is higher than breadfruit seeds (*A. camansi, A. hetrophyllus* and *T. africana*) reported by Wekhe *et al.*, (2020b). Sodium generally imparts flavour and enhances preservation of foods, but very high level poses serious health risks. The National Academy of Science (2004) recommends sodium intake of between 1.2g/day and 1.5g/day.

Vitamin c was absent in avocado seed while that of the pulp was 47.80±21.13. The vitamin c content of the pulp was lower than three citrus species (125.4±0.001, 130.5±0.004 and 87.9±0.001). The vitamin A content of avocado seed was 250.00±0.00 while that of the pulp was 200.00±0.00.The Vitamin A content in this study was higher than *Pisum sativa* (4.2mg/100g) and (2.5mg/100g) reported by Chuku *et al.* (2019). Vitamin A promotes good eye sight.

Thiamine is one of the B vitamins. The B vitamins are a group of water-soluble vitamins that are part of many of the chemical reactions in the body. The thiamine content of avocado seed was 2.17±0.01155 while that of the pulp was 6.87±0.11547. Thiamine (vitamin B12) helps the body's cells change carbohydrates into energy. The main role of carbohydrates is to provide energy for the body, especially the brain and nervous system. Thiamine also plays a role in muscle contraction and conduction of nerve signals and it's essential for the metabolism of pyruvate (Bender, 2003).

The glycoside content of avocado seed was 0.02±0.01 while that of the pulp is 0.00±0.00. Glycosides showed significant antioxidant activity, anticancer and antitumor activity, hepatoprotective activity, anti-inflammatory activity, anti-diabetes activity, antiviral activity, antibacterial and antifungal activity, and other biological effects.

The oxalate content of the avocado seed was 0.01±0.00 while that of the pulp was 0.32±0.19.Oxalates bind to calcium and prevent it's absorption in the human in the body

(Ekholm *et al.,* 2003). These phytochemical possess antimicrobial and anticarcinogenic activities (Campos-Vega *et al.,* 2010; Mathers, 2002; Martens *et al.,* 2017).

The tannins content of avocado seed was 0.02±0.00 while that of the pulp was as 0.01±0.01. This is in line with *M. dommestica* (0.2%) reported by Chuku and Emiri, (2019). Tannins has been reported to form complexes with proteins including enzymes resulting in reduced digestion and absorption. They are also known to bind iron (Fe) making it unavailable for absorption (Cornel University, 2008).

Carotenoid content in avocado seed was 2.06±0.01 while that of the pulp was 1.50±0.43. Carotenoids are beneficial antioxidants that can protect you from disease and enhance your immune system. Provitamin A carotenoids can be converted into vitamin A, which is essential for growth, immune system function, and eye health.

Polyphenol content in avocado seed was 7.51±0.01 while that of avocado pulp was 7.40±0.61.Polyphenol chelate metals such as iron and zinc and reduce the absorption of these nutrients (Karamac, 2009) but they also inhibit digestive enzymes and may also precipitate proteins (Adamczyk *et al.,* 2017).

Flavonoid content of avocado seed was 4.89±0.01 while that of the pulp was 5.81±0.00. The flavonoid content in this study is higher than *Pleurotus ostreatus* reported by Agbagwa *et al.,* (2020), and *P. sativum* (0.41%) reported by Chuku *et al.,* (2019). Flavonoids also chelate metals such as iron and zinc and reduce the absorption of these nutrients (Karamac, 2009) but they also inhibit digestive enzymes and may also precipitate proteins (Adamczyk *et Al.,* 2017).

The lignin content of avocado pear seed was 3.86±0.02 while that of the pulp was 4.05±0.00. According to Belyea and Ricketts (1993), lignin ties up cellulose indicating that higher concentrations of lignin result in reduced cellulose digestibility. These phytochemicals possess antimicrobial and anticarcinogenic activities (Campos-Vega *et al.,* 2010; Mathers, 2002; Martens *et al.,* 2017).

One fungal pathogen was isolated from the long shape specie of *Persea americana Mill*. The fungus identified was *Candida sp.* A wide range of fungi was isolated from avocado fruits in Port Harcourt. Some of these fungi are reported by several authors to be commonly implicated in the postharvest deterioration of many fruits and vegetables in the Tropics (Regnier *et al.,* 2010; Onyeani *et al.,* 2012; Oyetunjie *et al.,* 2012; Amadi *et al.,* 2014).

The mean isolation frequency of *Candida sp* was (100%). The origin of fruit contamination by fungi is difficult to determine. Generally, contamination of agricultural product is a function of many factors including infestation in the field prior to harvest, handling during harvesting and methods of packaging and transportation of the product to the market (Amadi *et al.,* 2014). Wounds are also known to be the major pre-disposing factor of fruits and vegetables to microbial attack both in transit and in storage (Amadi *et al.,* 2014).

CONCLUSION

It can be concluded that, the avocado seed is nutritionally valuable as the other parts of the plant based on the phytochemical and nutrients it constitutes. The consumption of avocado seed is recommendable since it has high nutritional contents that make it enough for possible dietary and ethno-medicinal use.

Persea americana fruit is endowed with vital nutrients which cut across proximate, mineral, vitamin, anti-nutritional and phytochemical divisions in the seed and pulp. But it is attacked and infected with spoilage organisms. Therefore, proper hygienic measures should be adopted by farmers and vendors. Consumers should properly wash fruits before consumption and diseased fruits should not equally be consumed.

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