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Comparative analysis of the physicochemical, phytochemical and mineral composition of avocado (*Persea americana* mill.) pulp and seed

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Abstract
An analysis on Some Physicochemical, Phytochemical and Elemental Composition of Avocado (Persea americana Mill.) Pulp and Seed were carried out. Avocado fruit is a tropical fruit and is one of the most productive plants per unit of cultivated area. Avocado stands out for its high nourishing value and its usefulness to growth and development. It has also been the subject of intense and varied use in the past not only for its importance in food but also for medicinal needs. The length, thickness, width, roundness, sphericity, mass, true density, bulk density, porosity, angle of repose, static coefficient of friction were all investigated. Moisture contents of 78.39±0.17% and 57.65±0.27% (DB) for the pulp and seed was determined respectively which revealed that the seed contained more of moisture than fat and fibre. The pulp of avocado has the highest content of all proximate analysis considered except in carbohydrate where the seed has 12.71±3.41. It was stated that the pulp has the highest amount of total carbon of 83.20±0.014%. These values were analyzed using AOAC methods. The pulp of the avocado proved to be rich in Saponins (0.15±0.02mg/100g), phenolic compounds (3.04±0.11mg/100g), 0.13±0.02 mg/100g Tannins, 0.01±0.00 mg/100g Cyanogenic glycosides, 0.15±0.00 mg/100g Alkaloids, 2.08±0.19 mg/100g Steroids, 5.11±0.11 mg/100g Phytates and flavonoids (4.05±0.12mg/100g). The seed was superior to the pulp in the contents of all phytochemical compounds with 18.91±2.01mg/100g of Saponins, 7.00±1.18 mg/100g of total phenols, 0.26±0.22mg/100g Tannins, 0.04±0.02mg/100g Cyanogenic glycosides, 0.70±0.12mg/100g Alkaloids, 7.00±1.18mg/100g Phenols and 44.3±3.1 mg/100g of flavonoids but lesser in steroids and phytates with 0.08±0.00mg/100g and 3.31±0.19mg/100g respectively. This research work was carried out in the Nutrition Laboratory, Department of Animal Production and Health. Federal University of Technology, Akure, Nigeria.

Keywords: Physicochemical, Phytochemical Composition, Flavonoids, Cyanogenic Glycoside, Persea Americana, cardiovascular.

Introduction

The Persea americana Mill. tree belongs to the Lauraceae family, genus Persea and is originated from Central America. Apart from its usage as food, avocado pear is a chief source of fiber which lowers the risk of cardiovascular disease, hypertension, cancer, hypoglycemic and anti-viral as well as obesity. The seed has a varied application in ethno-medicine, ranging from treatment of dysentery, diarrhoea, ulcer, toothache, decreasing cholesterol, as well as beautification of the human skin [18] and [23]. Persea americana is one of the 150 varieties of pear. The tree is extensively cultivated in tropical and subtropical areas with a height of about 60-80 feet [23] and [32]. It has a green to black knotted egg shaped fruit with a leathery skin and a large seed of about 5-6cm. The leaves are evergreen and the flowers unisexual [32].

The World Health Organisation (WHO) Alma Ata (1978) recent declaration in primary Health care made way for the official recognition of traditional medicine as a source of primary healthcare [27]. Since then, healthcare delivery has immensely value-added with the combination of phytomedicine and traditional medicine in most part of the world, especially in Asia, Africa, Middle East, etc. Persea americana, is of no doubt among the plants contributing to health improvement and contains some of these important chemicals. As a result of the importance of this plant in orthodox medicine, there is need to determine the plant chemicals that may be accountable for these health benefits as well as design machines that' can handle it in terms of processing, handling and packaging. The present study investigated and compared the presence of Physicochemical, Phytochemical and chemical Composition of Avocado (Persea americana Mill.) Pulp and Seed.

Methodology

Collection of sample and preparation

Samples of Persea americana used for this analysis were obtained from Uchi Market in Auchi, Estako West Local Government Area of Edo State, Nigeria. The fruits were thoroughly screened to remove the bad ones. The plant materials (pulp and seed) were identified and authenticated by Mr. Oguntokun O. Michael of the Nutrition Laboratory, Department of Animal Production and Health. Federal University of Technology, Akure, Nigeria. The identified pulp and seed were cleaned, peel removed and de-stoned. They were subjected to conditioning. In conditioning these seeds, they were sorted in group of two and three depending on availability. Each group was labeled accordingly. The fruits at the ripening stage were cut open to obtain their pulp and seeds. The pulp and seed were sliced and oven dried for 24 hours at 50°C. After oven drying, the samples were then grounded to fine powder using Thomas-Wiley milling machine. The grounded samples were stored in air tight bottles till when needed for analysis (Figure 1 a, b, c and d).

Fig 1: Ripe avocado pear **(a)** Cross section of Avocado pulp **(b)** after de-skinning and de-stoning **(c)** and Avocado pulp after blending **(d)**

Physical Properties Assayed

The procedures adopted by Nwaokobia [14] was used to determine the Length, width, thickness, sphericity, roundness, porosity, mass, true and bulk densities determined in this study. A sample of 100 pulp and seed were randomly selected following the procedure adopted by Dutta [10]. A Vernier caliper reading to 0.01mm accuracy (Stainless Steel Electronic LCD Digital Vernier Caliper Gauge Micrometer 0-6" Range) was used to measure the length, width, Shape, diameter and thickness of each sample.

Water displacement method as described by Aviara [5]**.** was used to obtain true density. The size distribution for the fruit was determined by classifying the fruits into large, medium and small after which the percentage average length, width, mass and pulp thickness in each class. Bulk density was determined using the [16] and [1] recommended method. Color was determined by reflectivity and absorptive character using electromagnetic radiation.

Sphericity was calculated by using the data on geometric mean diameter and the length of the bean.

$$Ø= \frac{(\mathbf{LWT})^{\mathbf{1/3}}}{\mathbf{L}} \text{- - - - - - - - - - - -} \quad (1)$$

The porosity (ε) was calculated using the values of bulk density and true density as stated by Jain [29]

$$\text{Porosity} = \frac{\text{True Density}-\text{Bulk Density}}{\text{True Density}} \times 100 \text{- - - - - - -} \quad (2)$$

$$\text{Roundness}= \frac{A\rho}{AC} \text{- - - - - - - - - - - -} \quad (3)$$

Where, A_P =Largest projected area of object in natural rest position,
A_C = Area of smallest circumscribing circle.

Frictional Properties

The frictional properties of avocado pulp and seed determined in this study were repose angle and static coefficient of friction. Angle of repose was determined using the procedure employed by Aviara, [6]. This involved filling an open ended box of 150×150×150mm in size having removable front panel with sample. The front panel was swiftly detached to allow sample to slide and assumed its natural slope in bulk. The angle of repose was calculated from the depth of the free surface of the sample to the horizontal distance from the side having the free surface. Static coefficient of friction of the avocado pulp and seed was determined on four different structural surfaces namely, galvanized iron sheet, glass, hessian bag, plywood with wood grains perpendicular to the direction of movement and plywood with wood grains parallel direction of movement.

Analysis of Persea americana pulp and seed

In analyzing Persea americana pulp and seed, four basic methods were involved; proximate and ultimate analysis, Phytochemical and chemical composition. The proximate analysis covers the fixed carbon, volatile matter and moisture content and ash percentages. The ultimate analysis indicates the range of rudimentary chemical constituents such as Carbon, Hydrogen, Oxygen, Sulphur and Nitrogen. It is useful in determining the quantity of air required for combustion and the volume and composition of the combustion gases [17]**.** Phytochemical analysis assayed the presence of saponins, tannins, flavonoids, cyanogenic glycosides, alkaloids, Phytates, phenols and steroids were carried out using the procedures described by Onwuka [20]**.**

Proximate Analysis

In the proximate analysis studied, moisture content was determined by weighing two grams (2g) of the fresh sample of crude extract of pulp and seed of avocado and placing them in the crucible and heated at 105˚C until a constant weight was attained. The moisture contents of the samples were calculated as loss in weight of the original sample and expressed as percentage moisture content. Crude protein was determined by the Kjeldahl method with slight modification [17]**.** Crude fat was estimated using the Soxhlet extraction method according to A.O.A.C standard [15]**.** Crude Fiber estimation was done using the A.O.A.C standard method [15]**.** Ash Content was also done using the A.O.A.C standard method [15]**.**

Volatile Matter was determined by deducing the loss in weight of moisture free Powdered avocado pulp and seed when heated in a crucible fitted with cover in a muffle furnace at 950°C for 7min. and estimated in percentage using:

$$\% \text{ Volatile matter} = \frac{\text{Loss in weight of Moisture free sample}}{\text{Weight of moisture free sample}} \, 100 \ldots (4)$$

Fixed Carbon wasdetermined indirectly by deducting the sum total of moisture, volatile matter and ash percentage from 100%

$$\text{i.e Fixed carbon of sample} = 100 - (\% \text{ moisture} + \% \text{ volatile matter} + \% \text{ ash}) \text{ -- } \quad (5)$$

The energy value (kcal) of the samples was estimated by multiplying percentage crude protein, crude lipid and carbohydrate by the recommended factor (3.44, 8.37 and 3.57 respectively) used in vegetable and seed analysis [2].

Ultimate Analysis

In the Ultimate analysis studied, Carbon and Hydrogen was estimated using the Liebig's method for the estimation of carbon and hydrogen [17]**.** Total carbon was determined using near infrared spectroscopy method as described by Bernard [8]**.**

Sulphur was determined by heating 10g of avocado pulp and seed were heated with Eschka mixture (which consists of 2 parts of MgO and 1 part of anhydrous NaCO3) at 800°C. The sulphate formed was precipitated as BaSO4 (by treating with $BaCl_2$) and the Sulphur in avocado was computed as follows:

32g Sulphur in the sample pod and bean will give 139g and 256g BaSO4 respectively.

Amount of sulphur in the seed and pulp = $\frac{32y}{533} = 0.06y$ and $\frac{32y}{800} = 0.04y$ respectively

$$\% \text{ of sulphur in the seed and pulp } = \frac{100}{x} \times 0.06y \text{ and } \frac{100}{x} \times 0.04y \text{ respectively}$$

where: x = weight of avocado pulp and seed sample taken

y = weight of BaSO4 precipitate formed.

oxygen was deduced indirectly as follows:

$$\% \text{ oxygen in avocado pulp and seed } = 100 - (\% \, C + H + N + S + Ash) \text{ -- } \quad (6)$$

Nitrogen was determined using the Micro Kjeldahl method as adopted by Udeme, [24] along with $10cm^3$ of concentrated H_2SO_4 and pipetted with $5cm^3$ of 2% boric acid.

Mineral composition

In chemical composition Mineral analyzed, Sodium and potassium were determined using flame photometer. Phosphorus was determined by vanado-molybdate yellow method. Calcium and magnesium was carried out using Versanate EDTA complexometric titration method while iron, zinc, copper were estimated using a unicam atomic absorption spectrophotometer based on the procedures followed Onwuka [20]**.** All the analysis was carried out at the Department of Animal Production and Health Laboratory, Federal University of Technology, Akure, Nigeria.

Phytochemical analysis assayed

Alkaloids

Harborne [13] method as adopted by Gbadamosi [30] was used for the analysis of alkaloids in samples. 200ml of 10 % acetic acid in ethanol was added to 5.0g of powdered sample in 250ml beaker. The mixture was covered and allowed to stand for 4hour. The mixture was sieved and the filtrate was concentrated on a water bath to one-fourth of its original volume, concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until precipitation was completed and the solution was allowed to settle. The precipitate collected was washed with dilute NH_4OH and then filtered. The residue was dried and weighed. The alkaloid content was calculated using the formula:

$$\% \text{ alkaloid } = \frac{\text{Final weight of the sample}}{\text{Initial weight of the extract}} \times 100\text{- - - -} \quad (7)$$

The experiment was replicated three times. The result was expressed as mg/100g.

Flavonoids

Five millilitres (5 ml) of the extract was mixed 5 ml of dilute hydrochloric acid (HCl) and boiled for 30 minutes. The boiled extract was allowed to cool and then filtered. One millilitre (1 ml) of the filtrate was then added to 5 ml of ethyl acetate and 5 ml 1% ammonia solution. The absorbance was taken at 420nm

Tannins

Ten millilitres (10 ml) of the sample was pipette into 50 ml plastic bottle containing 50 ml of distilled water. This is shaked 1 hour on a mechanical shaker. The solution was filtered and 5 ml of the filtrate was mixed with 2 ml FeCl3in 0.1 NH4Cl. The absorbance was measured at 120 nm [31]**.**

Phytates

One gram (1 g) of the sample was extracted with 0.2 M HCl. Fe^{3+} solution was added to 0.5 ml of the extract. The mixture was heated in a water bath for 30 mins, cooled and then centrifuged. 1.5 ml of 2, 2-bipyridine solution was then added to 1 ml of the supernatant. Absorbance was read at 519 nm using distilled water as blank [13]. The result was expressed as mg/100g [31].

Cyanogenic Glycoside

This was carried out using Buljet's reagent one gram (1 g) of the fine powder of the sample was soaked in 70% ethanol for 2 hours and then filtered. The extract was then purified using lead acetate and disodium hydrogen tetraoxosulphate (vi) (Na2HPO4) solution before addition of freshly prepared Buljet's reagent. Theabsorbance was taken at 550 nm [31]**.**

Saponins

Five millilitres (5ml) of the sample was dissolved in aqueous methanol. Then 0.25 ml of aliguot was taken for spectrophotometric determination for total saponins at 544nm [31].

Steroids

The extract was diluted with normal NH_4OH solution. Two millilitres (2ml) of the dilute was mixed with 2 ml of chloroform in a test tube. Three millilitres (3ml) of ice-cold acetic only dried was added to the mixture and two drops of concentrated H_2SO4 was continually added to the mixture and allowed to cool. The absorbance was taken at 420nm [31].

Phenols

Ten milliliters (10 ml) of the sample was boiled with 50ml acetone for 15 minutes. Five milliliters (5 ml) of the solution was pipette into a 50 ml flask. Then 10 ml of distilled water was added. This was followed by the addition of 2MnNH$_4$OH and 5ml concentrated amyl alcohol. The mixture was left for 30 minutes and the absorbance was taken at 505nm [31]

Result and Discussion

Table 1: Effect of sample treatments on physical properties of avocado pulp and seed

Parameters	Unit	Pulp	Seed
Moisture content	(% d.b)	78.39±0.17	57.65±0.27
Major Diameter	(mm)	11.46 ± 0.08	12.76 ± 0.08
Intermediate Diameter	(mm)	9.11 ± 0.41	6.78 ± 2.00
Minor Diameter	(mm)	9.54 ± 3.01	5.41 ± 1.06
Sphericity	%	0.387 ± 0.13	0.274 ± 1.03
Roundness	%	4.04	15.72
True density	(kg/m^{-3})	1078.82 Brownish black	1097.34
Colour		Purplish-black	
Mass	(g)	383.5	421.94
Bulk density	(g cm^{-3})	10.61	15
Coefficient of static friction Glass Plywood Galvanized iron steel		0.27 ± 0.04 0.10 0.12	0.111 ± 0.11 0.86 3.02
Surface area	(mm^2)	1700.04	1010.11
Repose Angle	(˚)	9.53	59.47

Table 1 shows the effect of sample treatments on physical properties of avocado pulp and seed. The moisture content of the pulp (78.39±0.17 % d b) was found to be higher than that of the seed (57.65±0.27% d b). It shows the shape of avocado pear as oblong; a shape in which the diameter of the vertical axis is greater than the diameter of the horizontal axis. The average weight of avocado pulp and seed was determined to be 383.5g and 421.94g using four replicates; this is close to the value of 374g of Hass variety of avocado as reported by Nwaokobia [14]. The value of density obtained for the pulp and seed is 1078.82 and 1097.34kg/m3 respectively, when compared with the value of 987kg/m3 reported by FAO, it can be stated that the value is greater due to different species and environmental factors. The static coefficient of friction on various surfaces, namely, glass, plywood, galvanized iron steel also increased linearly with increase in moisture content. The seed has the highest static coefficient of friction and Angle of repose on all the surfaces.

Table 2: Proximate composition (%) of the samples.

Constituents	Pulp	Seed
Ash	1.89±0.01	1.00±0.01
Moisture content	78.39±0.17	57.65±0.27
Volatile matter	26.97±0.01	1.02±0.01
Fixed Carbon	58.35±0.04	17.07±0.04
Carbohydrate	8.45±4.12	12.71±3.41
Fibre	5.09±0.16	3.01±0.43
Protein	19.34±1.52	2.50±0.43
Fat	15.73±1.19	11.64±1.41

Values are in means ± standard deviations of triplicate determinations. ND= Not Detected.

Table 3: Ultimate analysis % of the samples.

Constituents	Pulp	Seed
Total carbon	83.20±0.014	16.11±0.03
Hydrogen	1.05±0.07	1.95±0.07
Nitrogen	4.17±0.01	6.43±0.01
Oxygen	50.29±0.07	86.78±0.09
Sulphur	0.02±0.01	0.01±0.01

Values are in means ± standard deviations of triplicate determinations. ND= Not Detected.

Table 2 and 3 represents the proximate and Ultimate analysis of the sample pulp and seed. In the proximate result, it shows that the pulp has the highest fat of 31.64±1.41 than the seed. This implies that it is an oil fruit and the dietary fat quality can exert a protective effect on cardiovascular diseases [3]. The consumption of foods rich in monounsaturated fatty acids (MUFA), dietary fibers, and antioxidants has been associated with lipid profile improvement and body weight loss as reported by Silva Caldas [22]. It also shows that it has the highest amount in all the proximate and Ultimate analysis studied except in oxygen and sulphur where the seed has the highest. In the investigated samples, the little quantity of ash possessed by the samples shows that the pulp and seed can hinders the growth of micro-organism. This was also shown by Fahimdanesh [12] who had similar results in the Ash content of Iranian Mango Seed. The pulp has a Fibre content of 5.09±0.16 which is higher than 1.08±0.43 and 2.87 ± 0.00 reported by Nwaokobia and Egbuonu [14] and [11] respectively. Thus, can protects the intestine from packing and increases faecal bulk. It also reduces the risk of colon cancer by diluting the augmented concentrations of colonic bile acid that arises due to high fat diet [9].

Table 4: Phytochemical constituents of *P. Americana* leaf, fruit and seed (mg/100g)

Constituents	Pulp	Seed
Saponins	0.15±0.02	18.91±2.01
Tannins	0.13±0.02	0.26±0.22
Flavonoids	4.05±0.12	5.00±0.11
Cyanogenic glycosides	0.01±0.00	0.04±0.02
Alkaloids	0.15±0.00	0.70±0.12
Phenols	3.04±0.11	7.00±1.18
Steroids	2.08±0.19	0.08±0.00
Phytates	5.11±0.11	3.31±0.19

Values are in means ± standard deviations of triplicate determinations.

Table 4 shows the phytochemical compounds present in the extract. The following were found to be present in the seed; steroids, Cyanogenic glycosides, phenolics, flavonoids, saponins, phytates, tannins and alkaloids. These phytochemicals are responsible for the antimicrobial activities of the seed as stated by Umamaheswari [25]. The results show that the avocado seed has highest levels of total phenols and flavonoids which also agrees with the results reported by Wang [26]**.** It is well known that alkaloids are used to cure of malaria, diabetes, hypertension and also used as tranquilizer. Flavonoids is also well known to be manufactured by plants in response to microbial attack. Therefore, they are effective antimicrobial substances against a lot of microorganism. Saponins are also used as anti-inflammatory, tranquilizer, anti-lipidemic, anti-carcinogenic and agents. Hence they have the ability to stimulate the heart muscle [7] and [21]**.** They are anti-allergic, anti-cancer, anti-inflammatory and so on. Plants that contains tannin are used to treat non-specific diarrhoea, swellings of mouth, throat and slightly injured skin. This explains the rich content of antimicrobial activities displayed by the seed extracts and shows the potential of avocado as source of therapeutic agents.

Table 5. Mineral composition of the P. americana seed and pulp samples (%).

Constituents	Pulp	Seed
Total carbon	83.20±0.014	16.11±0.03
Hydrogen	1.05±0.07	1.95±0.07
Nitrogen	4.17±0.01	6.43±0.01
Oxygen	50.29±0.07	86.78±0.09
Sulphur	4.85±1.03	0.10±0.01
Sodium	50.04±1.01	58.07±0.02
Potassium	60.35±0.93	25.02±0.01
Calcium	40.41±0.06	30.63±0.00
Magnesium	48.49±1.07	38.57±0.09
Iron	29.56±9.01	13.11±0.00
Zinc	7.12±1.02	9.29±0.01
Phosphorus	18.22±1.22	34.63±0.02
Copper	1.12±0.12	1.01±0.09

Table 4 shows the Mineral analysis of avocado pulp and seed in mg/100g. Result showed the presence of Na, K, Mg, Ca, Fe, P, Cu and Zn in pulp and seed examined. Sodium (58.07±0.02%) for seed and the lower amount of it in the pulp (50.04±1.01%) which was quite higher than 12.61±1.19 as reported by Arukwe [4]**.** High content of sodium in the body has been linked with hypertension [19], but this may not be possible if there is a higher content of potassium. Potassium is essential for balancing electrolyte and the control of high pressure and can help to prevent kidney stones, osteoporosis, stroke and high blood pressure and also helps to strengthen the bones [19]**.** The high magnesium composition of the pulp indicate that avocado can be used to cure diabetes, hypertension, depress and osteoporosis. Iron composition of avocado indicates that the fruit can be used to synthesize red blood cells and help prevent iron-deficiency anemia [4]**.** The report also shows that the seed and the pulp contains good amount of phosphorus. This helps in the formation of bones and teeth. These variations of the report with other results perhaps may be due to seed varieties, agricultural conditions, conditions of the climatic and methods used for determination. The pulp and seed studied contained appreciable amounts of magnesium, phosphorus, Oxygen, Sulphur, Iron, Zinc, Copper and calcium. These are very important in human health. They are required for formation of teeth and bones, blood clotting and other function of the body [19]**.**

Conclusion

Persea americana pulp and seed is a fruit with excellent physical and chemical characteristics, with moisture, protein, fat, ash and other comparable elements. Its levels of bioactive compounds that enhances physiological and cellular activities in the body are also comparable in the different constituent fractions of the fruit. Thus, for every 100g of avocado, its edible portion (pulp) has, an average, 3.04±0.11mgof total phenolics, 4.05±0.12mg of flavonoids, 5.11±0.11mg of phytates, 0.01±0.00mg of Cyanogenic glycosides, 0.13±0.02mg of Tannins, 0.15±0.02mg of Saponins and 0.15±0.00 mg of Alkaloids. The non-edible parts (seeds) were found to have an average of 7.00±1.18 mgof total phenolics, 5.00±0.11mg of flavonoids, 3.31±0.19mg of phytates, 0.04±0.02mg of Cyanogenic glycosides, 0.26±0.22mg of Tannins, 18.91±2.01mg of Saponins and 0.70±0.12mg of Alkaloids. From the results obtained in this research, P. americana can be an outstanding substitute for fat in baking industry, considering its chemical components and the benefits of its compounds [28]**.** In addition, taken into consideration the specie and diversity of the plant varieties, cultivation is easy and the fruit is readily availability, any time of the year. The crop can be used as raw material in the cosmetic and pharmaceutical industries, and also acts as a medicinal value in herbal preparation [20]**.** The pulp residue can also be used in manufacturing food products for man and animal consumption. Several studies have proven the health benefits of P. americana intake, especially in reducing saturated fat and prevention of other related heart diseases. With a lot of researches still going on about P. americana and its nutritional characteristics and benefits, there should be an increase in P. americana production and utilization in Nigeria, and in other countries. From the experiment carried out on physical properties and results obtained, the test shows that moisture content plays an important role in determining mechanical properties of avocado pulp and seed.

Conflict of Interests

The Authors declare that they have no conflict of interest.

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