J. Chem. Soc. Nigeria, Vol. 45, No.6, pp 1139 – 1146 [2020]

Comparative Physicochemical and Proximate Analyses of Different Extracts of *Persea americana*

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Received 10 September 2020; accepted 07 October 2020, published online 31 October 2020

Abstract
Avocado and Avocado oil are high in monosaturated oleic acid, a heart-healthy fatty acid that is believed to be one of the main reasons for the health benefits of olive oil. This study, was therefore carried out to determine the physicochemical and proximate composition of the seed and rind of avocado pear with the extraction and characterization of oil obtained from the seed and rind using solvent extraction. The phytochemical screening was also carried out on the seed and rind of avocado pear oil. The moisture contents were (51.05% and 71.77%) for the seed and rind respectively. The ash content of the unripe seed and rind were (0.52% and 0.55%) respectively. The fat (lipid) content in both the unripe seed and rind oil were (21.41g and 9.53 g) respectively. The unripe rind sample was richer in protein (6.4%) and unripe seed being lower (3.04%) to the rind. The crude fibre content for the seed and rind oil were (51.2 g and 2.54 g) respectively. Saponification value of the oil from unripe seed had a higher value of 258.82 mg KOH/g and for unripe rind was 203.47 mg KOH/g. The peroxide value for oil obtained from both unripe seed and rind of *Persea americana* were 0.91 mg/kg and 0.33 mg/kg respectively. Acid value was low in the unripe seed with a mean value of 0.057 mg KOH/g. It was observed that the Acid value for unripe rind was 0.058 mg KOH/g. The iodine value for rind was 182.85 (gI_2/100 g), the seed oil contains 53.78 (gI_2/100 g) of iodine. The refractive index was 1.21 and 1.35 for the unripe seed and rind oil respectively. The pH of the various avocado pear extracts also showed their slightly acidic nature.
Keywords: Avocado, Physicochemical, Extracts, Proximate, Phytochemical analyses, *Persea americana*.

Introduction

The avocado (*Persea americana*), a tree likely originating from south-central Mexico [1], is classified as a member of the flowering plant family Lauraceae [1]. The fruit of the plant, also called an avocado (or avocado pear or alligator pear), is botanically a large berry containing a single large seed [2]. Avocados are commercially valuable and are cultivated in tropical and Mediterranean climates throughout the world [3]. They have a green-skinned, fleshy body that may be pear-shaped, egg-shaped, or spherical. Commercially, they ripen after harvesting. Avocado trees are partially self-pollinating, and are often propagated through grafting to maintain predictable fruit quality and quantity [4]. Avocado is a fruit which has a high fat content of between 71 to 88% of their total calories - about 20 times the average for other fruits [5]. High avocado intake has been shown to have a beneficial effect on blood serum cholesterol levels [5]. Avocados are good source of Vitamin K, dietary fiber, Vitamin B6, Vitamin C, Folate and copper and a good source of potassium [6]; they are higher in potassium than a medium banana. Avocado is a rich source of minerals [7]. Avocado (*Persea americana lauraceae*) is an important tropical crop which is rich in unsaturated fatty acids [8].

Persea americana leaves have been reported to possess anti-inflammatory and analgesic activities [9]. Antioxidant activity and phenolic content of seeds of avocado pear was found to be greater than 70% [10]. The edible part (fruit) is very popular in vegetarian cuisine, making a substitute for meat in sandwiches and salads, because of its high fat content and high in valuable, health-promoting fats [11]. Most of the fat found in this fruit is monounsaturated, and its consumption helps reduce total cholesterol, LDL cholesterol, and triglycerides while increasing HDL cholesterol levels [12].

The fruit is not sweet but fatty, almost distinctly, yet subtly flavored, and smooth with creamy texture. Avocado fruits in many countries such as Mexico, Brazil, South Africa and India are frequently used for milkshakes and occasionally added to ice-cream. In addition to the nutritional values of its fruits, the leaves

and other parts of avocado possess medicinal properties and widely used in traditional medicines of many African countries. It is recommended for gastritis, gastroduodenal ulcer, hypercholesterolemia, hypertension, anaemia and exhaustion [13]. Various products of the plant have been effectively used for the management, control and treatment of peptic ulcers, gastritis, dysentery, diabetes mellitus, diarrhoea, insomnia, amenorrhoea, bronchitis, cough and hepatitis [13].

Therefore, in the present study, we determined the phytochemicals present in the oil of the rind and seed of avocado pear, the physicochemical and proximate composition of the unripe rind and seed oil.

MATERIALS AND METHODS

Sample collection and analysis

Ripe and unripe Avocado pears were purchased at new Benin local market, the pulp and seeds were removed to obtain the rind. The Avocado pear rinds and the seeds were thoroughly washed under tap water and then sliced into pieces with stainless steel knives on trays and sundried for 14 days. The dried rinds and seeds were then converted into powder by grinding first in an electric mill before using a grinder to obtain fine rind powder of larger surface area. The fine powder was then stored in a closed container until experiment commenced.

Data analysis of avocado pear

Phytochemical screening

Phytochemical screening was carried out using established protocols as described by Harbone [14] and Sofowora [15]. A stock solution of the Avocado pear rinds and seeds extracts obtained by solvent extraction with a concentration of 1 mg/mL of methanol was prepared and used for the screening.

Test for alkaloids

10 mL of the extract was heated and also strained. Dilute ammonia of 2 mL was mixed with 5 mL of the filtrate. To obtain the base which is alkaloidal, chloroform of 5 mL was then put and stirred gently. With acetic acid of 10 mL, the layer of chloroform was extracted. Then this was separated into dual parts. Dragendorff's reagent was put into one part and Mayer's reagent in the second. Reddish brown precipitate formation (with Dragendorff's reagent) or cream formation (with Mayer's reagent) was observed as optimistic test for the alkaloids occurrence.

Test for flavonoid

Firstly, 1 mL of NaOH was added to 3 mL of each extract. A yellow colouration indicated positive test for flavanoids. Secondly, to 1 mL of the extract, a little droplets of 1% aluminium hydroxide solution was put. The flavonoids existence was showed by a yellowish color.

Test for saponins

To 2 mL of each extract was added 5 mL of distilled water and the solution shaken vigorously for 30 seconds, stable persistent frothing indicated the presence of saponins.

Test for tannins

To 2 mL of each extract was added 1 mL of ferric chloride ($FeCl_3$). A blue-black or greenish-black precipitate indicated the presence of tannins.

Test for steroids

Five drops of concentrated $H_2SO_4$was added to 1 mL of each extract. A red colouration indicated the presence of steroids.

Test for terpenoids

2 mL of chloroform and 3 mL of concentrated H_2SO_4 were carefully added to 5 mL of each extract to form a layer. A reddish-brown color at the interface indicated the presence of terpenoids.

Test for cardiac glycosides

5 mL of each extract was treated with 2 mL of glacial acetic acid with 1 drop of ferric chloride solution; 1mL of concentrated H_2SO_4 was then added. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.

Physicochemical Properties

Acid, peroxide, iodine and saponification values were determined using the AOAC [16] method.

Determination of acid value

25 mL of diethylether and 25 mL of ethanol were mixed in a 250 mL beaker. The resulting mixture was added to 2.0 g of oil in a conical flask and few drops of phenolphthalein added to the mixture. The mixture was titrated with 0.1M NaOH to the end point with consistent shaking for which a dark pink colour was observed and the volume of 0.1M NaOH (V_o) was noted.

Free fatty acid (FFA%) (as oleic acid) = (V_o x N x 282 x 100)/ (W_o x 1000)

Where; 100 mL of NaOH = 282g of oleic acid,

N = Normality of NaOH

Wo = sample weight,

Acid value = FFA × 2.

Determination of Saponification Value

2 grams of sample were weighed into conical flask; 25 mL of 0.1N ethanolic potassium hydroxide was added. The content which was constantly stirred was allowed to boil gently for 60 minutes and flux condenser was placed on the flask containing the mixture. Few drops of phenolphthalein indicator were added to the warm solution and then titrated with 0.5M HCl to the end point until the pink colour of the indicator just disappeared. The same procedure was used for other samples and blank. The expression for saponification value (S.V) is given by:

$$V = \frac{\mathbf{56.1n(vo - vi)}}{\mathbf{m}}$$

Where; V_o= the volume of the solution -used for blank test.

V1 = the volume of the solution used for determination

n = actual normality of the HCl used.

M = weight of the sample, 56.1= constant.

0.1= concentration of alcoholic potassium hydroxide (KOH).

Determination of Iodine Value

The sample (0.4 g) was weighed into a conical flask and 20 mL of carbon tetrachloride (CCl_4) was added to dissolve the oil. Then, 25 mL of Wijs reagent was added to the flask using a safety pipette in a fume chamber.

The stopper was then inserted and the content of the flask was vigorously swirled. The flask was placed in the dark for 2 hrs 30 minutes. At the end of this period, 20 mL of aqueous potassium iodide and 125 mL of water were added. The content was titrated with 0.1M sodium thiosulphate solutions until the yellow colour almost disappeared after vigorous shaking. The same procedure was used for blank test and other samples. The iodine value (I.V) is given by the expression;

$$I.V = \frac{\mathbf{12.69 \times c\ (\ vi - v2\ )}}{\mathbf{m}}$$

Where; c = concentration of sodium thiosulphate used

V1 = volume for the blank,

V_2 = volume for the sample,

m = weight of sample.

Peroxide Value (PV)

A clean dry boiling tube was used to measure 1.0 g of oil. Powdered potassium iodide (2 g) was added and then 20 mL of solvent (2 vol glacial acetic acid + 1 vol chloroform) was added into the tube. The tube was placed in boiling water such that the mixture boiled within 30 seconds and then allowed to boil vigorously for more than 30 seconds before it was poured quickly into a flask containing 25 mL of water and the mixtures in the flask titrated against 0.002M sodium thiosulphate solution using starch as an indicator. The blank was performed at the same time. Peroxide value was calculated thus:

$$\text{Peroxide value} = \frac{\text{(S-B) X N thiosulfate X 1000}}{\text{Weight of sample}}$$

Where, S = titration of sample

B = titration of blank

pH Measurement of Solutions

A clean dried test tube was used to measure out 2.0 g of the oil and 13 mL of hot distilled water was added to the oil in the tube. The resulting mixture was stirred slowly and cooled in a water bath at 25^{o}C. A standardized pH electrode was immersed into the sample and pH value was recorded.

Measurement of Refractive Index

The refractive index of the Avocado pear oil was measured using hand-held ATAGO® master-50H rafractometer having built-in automatic temperature compensation.

Measurement of the Refractive Index of leaves

Strawberry leaf extracts were obtained after grinding the leaves by using mortar and pestle.

The Refractive Index of leaf extracts was measured using a hand-held ATAGO® master-50H

refractometer having built-in automatic temperature compensation.

Proximate composition analysis

The samples were analyzed for moisture, ash, protein, fat, fibre and carbohydrate by the method of AOAC [17].

Determination of moisture

Moisture was determined by oven drying method. A well-mixed sample (2.0 g) was accurately weighed in clean, dried crucible (w_1). The crucible was put in an oven at 100 – 105^{o}C for 2 – 4 hrs until a constant weight was obtained. The crucible was then placed in the desiccators for 30 minutes to cool. After cooling, it was weighed again (w_2). The percent moisture was calculated with following formula:

% moisture = (W1 - W2) X 100)/Initial Wt of sample
W_1 = initial weight of crucible + sample,
W_2 = final weight of crucible + sample

Determination of Ash Content

For the determination of ash, clean empty crucible was placed in a muffle furnace at 600^{o}C for an hour, cooled in desiccators.
The weight of empty crucible was noted (w_1). 1 gram of each of the samples was put in crucible (w_2). The sample was ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 550^{o}C for 2-4 hrs. The appearance of grey white ash indicated complete combustion of all organic matter in the sample. After ashing the crucible was cooled and weighed (w_3). Percent ash was calculated thus:

$$\% \text{ ash} = \frac{\boldsymbol{Difference\ in\ Wt \times 100}}{\boldsymbol{Wt.of\ sample}}$$

Difference in wt. = $w_3 - w_1$

Determination of Crude Protein

Protein in the sample was determined by kjeldahl method. The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of digestion mixture.
The mixture was then made alkaline. Ammonium sulphate thus formed, released ammonia which was collected in 2% boric acid solution and titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) and the amount of protein was calculated. 1 g of dried samples was put in a digestion flask. Concentrated H_2SO_4 (10 – 15 mL) and 8 g of digestion mixture i.e. K_2SO_4: $CuSO_4$ (8:1) were added. The flask was swirled in order to mix the contents thoroughly and then placed on heater to digest until the mixture become clear (bluish green colour). It needed 2 hrs to complete. The digest was cooled and transferred to a 100 mL volumetric flask and volume made up to mark by the addition of distilled water. Using distillation apparatus, 10 mL of digest was introduced to the distillation tube. 10 mL of 0.5N NaOH was gradually added through the same way. Distillation was continued for at least 10 minutes and NH_3 produced was collected as NH_4OH in a conical flask containing 20 mL of 4% boric acid solution with few drops of modified methyl red indicator. During distillation, yellowish colour appeared due to NH_4OH. The distillate was then titrated against standard 0.1N HCl solution until the appearance of pink color. A blank was also run through all steps as above. Percent crude protein content of the sample was calculated by using the formula:

% crude protein = 6.25* × %n

$$\%n = \frac{\boldsymbol{(S-B) \times N \times 0.014 \times D \times 100}}{\boldsymbol{Wt.\ of\ the\ sample \times V}}$$

Where; s = sample titration reading, b = blank titration reading
n = normality of HCl,
d = dilution of sample after digestion
v = volume taken for distillation,
0.014 = milli equivalent weight of nitrogen
6.25 = factor for nitrogen calculated.

Determination of Crude fat

Dry extraction method for fat determination was used. It consisted of extracting dry sample with some organic solvent, since all the fat materials e.g fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. are extracted together. 1 g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced to the extraction tube.
Weighed, cleaned and dried the receiving beaker was filled with petroleum ether and fitted into the apparatus. After 4-6 siphoning the ether was allowed to evaporate and then disconnected the beaker before the last siphoning. The extract was transferred into clean glass dish with ether washing and evaporated the ether on water bath. The dish was placed in an oven at 105^{o}C for 2 hrs and cooled in a desiccator. The percent crude fat was determined by using the formula:

$$\% \text{ Crude fat} = \frac{\textbf{Wt.of ether extract x 100}}{\textbf{Wt.of sample}}$$

Determination of Crude fibre

1 g of each deffated sample was weighed into a 250 mL beaker of 1.25% H_2SO_4 and boiled on a heating mantle for 30 minutes. The boiled samples was washed to neutralize with warm distilled water, transferred to another beaker and boiled in 250 mL of 1.25% NaOH for 30 minutes. The boiled sample was washed to neutrality with warmed distilled water, rinsed into crucibles with small quantity of ethanol and heated to evaporate the ethanol for 1hour in a hot air oven at 95^{o}C. The samples then cooled in the desiccator and weighed. The weighed samples were taken to the muffle furnace for ashing at 500^{o}C for 3hrs and reweighed again. The percent crude fibre was determined using the formular:

Where W_1= **Dry weight of the before ashing – weight after ashing x 100 / weight of sample**

Determination of Carbohydrate

Carbohydrate was calculated by difference after analysed for all the other items in the proximate analysis.

%Carbohydrate = 100 – (%moisture+ % crude protein + %crude fat + %crude fibre + %ash)

NFE represents soluble carbohydrates with other digestible and easily utilizable non-nitrogenous substances in feed**.**

Results and Discussion

There is the need to screen this fruit syrup for known bioactive secondary metabolites, to verify its folkloric use.

Table 1: Phytochemical screening of the extracts of *Persea americana*

Tests	A (fresh seed)	B (dried seed)	C (fresh rind)	D (dried rind)
Alkaloids	-	+	-	+
Flavonoids	+	-	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Steroids	+	+	+	+
Terpenoids	-	-	+	+
Cardiac glycosides	+	-	-	+

KEY: + = Present, - = Absent

Table 2: Comparison of physicochemical properties of avocado pear oil

Parameter	Unripe seed	Unripe rind
$_P$H	5.70	5.80
Refractive index	1.21	1.35
Peroxide value (mg/kg)	0.19±0.01	0.33±0.04
Saponification (mg KOH/g)	258.82±0.09	203.47±0.40
Iodine Value (gI_2/100g)	53.25±0.79	82.53±0.51
%Yield	9.27±0.02	28.51±0.00

Table 3: The proximate composition of unripe rind and seeds of avocado pear

Parameter (%)	Unripe rind	Unripe seed
Moisture	51.05±0.01	71.77±0.47
Fat	21.41±0.18	9.53±0.03
Carbohydrate	15.74±0.41	12.60±0.02
Ash	0.55±0.08	0.52±0.03
Protein	6.04±0.00	3.04±0.02
Crude fibre	5.21±0.03	2.54±0.04

Discussion

Results of phytochemical analysis showed that Avocado pear oil contained cardiac glycosides, terpenoids, tannins, flavonoids, steroids, saponins and alkaloids. Among these compounds, the tannins, flavonoids, steroids and terpenoids are already known for their antimicrobial activities [18]. Generally, the antimicrobial activity of the extracts from herb is because of the existence of several metabolites which are secondary in nature (bioactive compounds) such as saponins, tannins, flavonoids, phenolic compounds and essential oil [19]. Avocado pear oil's antimicrobial activity could also be because of the contributory effect from alkaloids, flavonoids, steroids and tannins. This proclamation is in tandem with those of earlier researchers who reported the use of alkaloids in medicine and flavonoids exhibiting cytotoxic, antifungal and anti-inflammatory activities [20].

The refractive index was determined to be (1.21 and 1.35) for the seed and rind oil respectively; the refractive index is used to follow and control hydrogenation and isomerization process [21]. Unripe rind oil of avocado pear had higher iodine value 82.85 (gI_2/100 g) than the seed oil containing 53.78 (gI_2/100 g) of iodine. Iodine value gives an indication of the degree of unsaturation of oil. The higher the iodine value, the greater the number of C=C double bonds.

Acid value is low in the unripe seed oil with mean value of 0.057 mg KOH/g. It is observed that the acid value (0.058 mgKOH/g for the unripe rind oil was close to that of the seed oil. The values were lower compared with the 8.8 mg KOH/g reported by FAO [22] for olive oil. The lower the acid value of oil, the fewer free fatty acids it contains which makes it less exposed to rancidification [23]. An increase or rise in acid value indicates rancidification of the oil [23]. Free fatty acids (FFA) are produced by the hydrolysis of oils and fats. Fat and oil are graded by their acid and free fatty acid contents, which are used as an index to determine their quality [23]. Oils processed from fresh fruits have low free fatty acids (low acid values) compared with those from many days' old fruits [23]. The level of FFA depends on time, temperature and moisture content because the oils and fats are exposed to various environments such as storage, processing, heating or frying. Since FFA are less stable than neutral oil, they are more prone to oxidation and to turning rancid. Thus, FFA is a key feature linked with the quality and commercial value of oils and fats. The acid value is used to determine the suitability of oil as edible oil [23]. Because high acid value may indicate rancidity and decrease acceptability to consumers.

Saponification value is inversely proportional to the mean molecular weight of the glycerides in the oil [24]. The oil from unripe seed had higher value (258.82 mg KOH/g) than the value for unripe rind oil was (203.47 mg KOH/g). The saponification value may serve as an important parameter in determining the suitability of oil in soap making. The higher the saponification value, the lower the fatty acids average length, the lighter the mean molecular weight of triglycerides and vice-versa [25]. Practically, fats or oils with high saponification value (such as coconut and palm oil) are more suitable for soap making [25]. Both oils could be good for soap making.

Peroxide value helps in determining the susceptibility of an oil to oxidation [26]. The peroxide value obtained from both unripe seed and rind of *Persea americana* were 0.91 mg/kg and 0.33 mg/kg respectively. Peroxides are the primary reaction products formed in the initial stages of oxidation of oil and therefore gives an indication of the process of lipid peroxidation [26]**.** The pH of fruit extract is the negative function of natural acidity in the extract, thus increase in pH is accompanied with decrease in acidity of fruit extract during storage [27]. The pH value obtained from both unripe seed and rind of *Persea americana* were 5.70 and 5.80 respectively. The pH of the various avocado pear extracts also showed their slightly acidic nature.

Tables 3, shows the proximate analysis of unripe rind and seed of avocado pear. The moisture contents were 51.05% and 71.77% respectively. The value was higher in the unripe rind than the unripe seed. This implies that they are susceptible to infection by microorganism and may have a short shelf life. The ash content was low in the unripe seed and rind (0.52% and 0.55%). Ash content is significant in foods as they account for the mineral constituents but should not be too much [28, 29]. The fat (lipid) content in both the unripe seed and rind oil were 21.41 g and 9.53 g respectively. The significance of fats in food cannot be over emphasized as they are used up by the cells of organs and glands to provide energy and in the synthesis of some of their secretions [30]. Fats are building blocks of hormones and they

insulate nervous system tissue in the body. They fuel the body and help absorb some vitamins, as such, the seed can be ground to animal feeds [30].

Proteins are complex nitrogenous substances that form an important part of living tissues. Functionally, proteins are important in food as they help in the growth and development of the body [30]. It was observed that the unripe rind sample was richer in protein (6.04%) and the unripe avocado had a lower protein content of 3.04%. The crude fibre content for the seed and rind oil were 5.12 g and 2.54 g respectively. The spongy mass of fibre helps to satisfy the appetite and also assists moving food through the alimentary canal by aiding the muscular action of the intestine thus preventing constipation [31]. Unripe avocado rind sample had the higher carbohydrate content of 15.74 g than that of the seed (12.60 g). This shows that the unripe skin of *Persea americana* is a better source of carbohydrate. Carbohydrates are ready source of energy for the body [32].

Conclusion

The study revealed that both the unripe seed and rind has high iodine value, acid value, saponification value but low level of peroxide value. The unripe *Persea americana* has high moisture, protein, fat, carbohydrate and crude fibres all necessary for the body but low ash. The therapeutic use of avocado oil can be attributed to the presence of a diverse array of bioactive compounds which are responsible for various pharmacological activities. The phytochemicals identified were; flavonoids, alkaloids, cardiac glycosides, steriods, tannins, saponins and terpenoids, though terpenoids were present in the rind oil but not detected in the seed oil. The presence of these phytochemicals in avocado oil may be the reason for their ethnobotanical uses. Also, evaluation of the proximate composition, showed that it is very rich in nutrients and therefore good for consumption by humans for the sustenance of vitality and health.

Recommendation

Since unripe *Persea americana* cannot eaten due to its hardness. It is on this basis that the following recommendations were made. The skin and seed can be used as animal feed. Public awareness should be carried out to enlighten the public on its importance, usefulness and nutritional benefits. Due to the importance of natural products in respect of their potential use for pharmaceutical and food applications, there is need to ascertain the plant chemicals that may be responsible for these health benefits.

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