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Effects of Extraction Solvents on Oil Yield from *Persea Americana* Seed and Its Characterization

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Abstract: This study focused on the extraction yield of oil from *Persea Americana* seed using three different extraction solvents of hexane, diethylether and chloroform respectively. The *Persea Americana* seeds were prepared prior to extraction process thereby removing dirts and the seeds were washed, dried, crushed and ground to 600μm particle size thereby increasing surface area for oil extraction operational rate of *Persea Americana* seeds. The ground *Persea Americana* seeds were soaked for a period of 14 hours to 70 hours under ambient operating conditions. Soxhlet extractor was used in the extraction process and extracted oil was separated from solvent via distillation process. The results showed that the optimum conditions for the yield of oil was obtained at 70 hours for hexane, diethylether and chloroform extraction solvents respectively but diethylether extraction solvent showed higher percentage yield of oil in comparison with other extraction solvents. Characterization of the extracted oil from *Persea Americana* seeds were performed and the results depicted extracted oil physiochemical properties such as refractive index, P^H value, density, specific gravity, iodine value, viscosity, saponification value etc and the results of these parameters are in tandem with other previous studies of *Persea Americana* seeds and within the range of acceptable value of European and United States of America standards.

Keywords: Soxhlet Extraction, Persea Americana Seed, Hexane, Diethyl-Ether, Chloroform, Characterization

1. Introduction

Avocado seed or *Persea Americana* is by product of fruit processing industry and have a potential novel oil seed crop that represents a considerable amount (up to 16%) of the total fruit, has a rich phytochemical profile and a long history of ethnobotanical use. Modern research into the bioactivities of *Persea Americana* remains in its nascent stages. Currently, the seed is an under-utilized resource and a waste issue for avocado processors. *Persea americana* seeds is often discarded after taking the pulp of the fruit. However, research has shown that the avocado seed is a good source of carbohydrate, protein, fat and some mineral elements such as calcium, phosphorus, potassium and magnesium as well as high concentration of anti-nutritional factors such as phytate, oxalate and cyanogenic glycosides making the seed to appear potentially toxic [1]. This high anti-nutritional factors present in the raw seeds of *Persea americana* could be recognized as a potential threat in the use of these seeds in animal and human nutrition, in spite of its nutritional composition, although proper processing methods, such as soaking and boiling, can reduced the levels of these anti-nutrients present in the raw seeds to a great extent. There is ethno-pharmacological information on the use of seeds for the treatment of health-related conditions [2], especially in South American countries where avocados are endemic and currently grown on a large scale. Current research has shown that avocado seeds may improve hypercholesterolemia, and be useful in the treatment of hypertension, inflammatory conditions and diabetes. Seeds have also been found to possess insecticidal, fungicidal, and anti-microbial activities. The avocado seeds are rich in phenolic compounds, and these may play a role in the putative health effects [3, 4].

However, the seed of the avocado is quite bitter, so you may not want to use it in your food. Just keep it handy for your cosmetic needs. First of all, the fruit solid wastes should be characterized so that they can be reused. In this study, avocado seed wastes from different fruit juice industry have been analyzed with various chemical and instrumental analysis methods, and their characteristics have been defined. This data

is thought to be useful in terms of preventing both environmental pollution and waste of resources by putting solid wastes into good use as secondary raw material in different industries rather than transferring them to disposal areas local fruit juice processing avocado seed as an alternative solution to current leather chemicals, the rise in price of which has had an adverse effect on the economy of the country [5].

Avocado seed oil is a vegetable oil obtained from the seed of avocado plant, also known as *Persea americana*. It is a drying, amber coloured liquid with an irritating-like odour. The oil can be extracted from the seed by expression, solvent extraction or combination of both, but because avocado seed contains relatively small amount of oil, solvent extraction seems the most viable method. Avocado seed oil also has many benefits, such as producing ecofriendly, biodiesel, paint and Studies in rats have shown that the oil from the avocado seed helps to increase the soluble collagen in the skin [6]. As you age, your body naturally loses its ability to rebuild the collagen, but avocado seed oil helps to naturally restore it. Collagen helps to improve the overall tone of the skin by getting rid of wrinkles, dry flaky skin, cellulite and sclerosis of the skin. It also contains many antioxidants which help you to feel great. By massaging avocado seed oil into your scalp, you will not only increase the growth of your hair, but it will come back to life with a new shine. Therefore, the seed oil is so popular in hand and body lotions, shampoos and other cosmetics; it simply helps you to look your very best from top to bottom. Avocado seed oils have been reported to be used in healing skin eruptions. Avocado oil is mainly sold for direct consumption due to its interesting contribution of fatty acids, vitamins, antioxidants, among other compounds [7]. Historically, extracts of avocado seeds have also been used as ink for writing and research in laboratories and this has explored the potential colorant properties of a polyphenol oxidase-produced colored avocado seed extract. Hence, great importance are attached to oils from fruits and seeds due to their numerous applications, functions and uses to mankind [8, 9]. This research study will focus on oil yield from *Persea Americana seeds v*ia the applications of three different solvents, thereby studying the effect of extraction solvents and extraction time on the *Persea Americana* seed oil yield. This shall be achieved through seed preparation, solvents extraction process using soxhlet method, oil yield variation with different solvents and extraction time and characterization of extracted oil to test for its quality and purity.

2. Materials

Solvent extraction also known as liquid-liquid extraction is a method of separating compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase [10]. Studies have shown that when comparing solvent extraction such as soxhlet extraction and mechanical extraction using centrifugal force, solvent extraction was probably, until recently the most common method of extracting oil from seeds such as avocado fruit [11]. Thus, Soxhlet extraction is the most widely utilized solid–liquid extraction technique in research, and it is one of the most traditional techniques still being used. It is a popular method and used as a reference for several existing modern extraction techniques [12]. The soxhlet extraction process usually separates a soluble compound from an insoluble compound. Hexane has become the solvent of choice for solvent extraction because of high stability of the solvent, low evaporation loss, low corrosiveness, little greasy residue and better odour and flavour of the extracted products [13]. Although, solvent extraction method can be used for extraction and pre-concentration of a wide range of nonvolatile or semi volatile species from water using only routine laboratory equipment, its use is decreasing in most situations because solvents of the required purity tend to be expensive, and can also cause problems with proper disposal after use [14]. Three extraction solvents are applied in this research study for removal of oil from Avocado seed and the choice of a particular solvent for the extraction of essential oil is generally influenced by the sensitivity of the essential oil to the action of heat and water, volatility of the essential oil and water solubility of the essential oil.

2.1. Hexane

Hexane is a very volatile aliphatic hydrocarbon, and a constituent in the paraffin fraction of crude oil and natural gas applied industrially as chemical and laboratory reagent. Laboratory grade *n*-hexane contains approximately 99% *n*-hexane. Laboratory and industrial solvents such as *"*hexane*"* and petroleum ether contain *n*-hexane from <0.1% to as much as 33%. Physical and chemical properties of hexane extractive solvent are shown in Table 1 [15].

2.2. Diethyl Ether

Diethyl ether is a mobile, very volatile, highly flammable liquid used as an inhalation anesthetic and as a solvent for waxes, fats, oils, perfumes, alkaloids and gums. It is mildly irritating to skin and mucous membranes. Diethyl ether is a clear colourless liquid with an anesthetic odour and some of its properties are shown in Table 2. [15]

Table 1. *Physical and Chemical Properties of Hexane [15].*

Property	Data
Molecular Weight	86.18
Color	Colourless
Physical State	Liquid
Melting Point	-95°C
Boiling Point	69°C
Density	0.6603 at 20°C
Odour	Faint, perculiar odour
Odour Threshold:	
Water	0.0064mg/L
Air	130ppm
Solubility:	Insoluble
Water	9.5mg/L
Organic Solvent(s)	Miscible with alcohol, chloroform, ether
Vapour Pressure	150mmHg at 25°C 138mmHg at 24°C
Flash Point	-22°C
Autoignition Temperature	225°C

Table 2. *Physical and Chemical Properties of Diethyl ether [15].*

Property	Data
Molecular Weight	74.12
Color	Colourless
Physical State	Liquid
Melting state	-116°C
Boiling Point	34.6°C
Density	0.71g/cm^3
Odour	A characteristic odour
Solubility: Water	Partially soluble in water
Organic Solvent(s)	Soluble in organic solvents
Vapour pressure	422mmHg at 20°C
Flashpoint	-40°C

2.3. Chloroform

Chloroform is a clear, colourless, volatile liquid with a pleasant etheric odour that volatilizes readily from soil and surface water and undergoes degradation in air to produce phosgene, dichloromethane, formyl chloride, carbonmonoxide, carbon dioxide, and hydrogen chloride Chloroform is indirectly produced when chlorine reacts with organic compounds [16].

Table 3. *Physical and Chemical Properties of Chloroform [16].*

Property	Value
Molecular Weight	119.38
Color	Colourless
Physical State	Liquid
Melting state	-63.2°C
Boiling Point	61.3°C
Density	1.485g/cm^3 at 20°C
Odour	Pleasant, ethereal, nonirritating pleasant, sweet
Odour Threshold:	
Water	2.4ppm (w/v)
Air	85ppm (v/v)
Solubility:	Insoluble
Water	7.22x10^3 mg/L
Organic Solvent(s)	Miscible with principal organic solvent, alcohol, benzene
Vapour pressure	159mmHg at 20°C 160mmHg at 20°C
Flashpoint	None
Autoignition temperature	>1000°C

3. Methods

Persea Americana was sourced from a farm in Port Harcourt, its preparation and processing at the laboratory of the department of Chemical/Petrochemical Engineering, Rivers State University, Nkpolu-Oroworukwo, Port Harcourrt prior to extraction analysis. Analytical grade reagents were applied in this research study.

3.1. Preparation of Sample

The sourced avocado fruits (*Persea Americana)* were washed with clean water to remove dirt, its outer layer peeled-off and the edible part of the ripe avocado fruits were consumed thereby removing the inner seeds as depicted in Figure 1.

Figure 1. *Dirt Free Persea Americana seeds after separation from its fruits.*

Size reduction or crushing of the *Persea Americana* seeds were carried out and the reduced seed size shown in Figure 2 were sun dried to reduce or remove moisture contents, and further dried in an oven at 85°C for a period of six (6) hours for effective and efficient drying of crushed seeds. The dried crushed *Persea Americana* seeds were ground mechanically in a grinder to a particulate size of 600μm sieve as highlighted in Figure 3 and the resultant products stored for oil extraction operation. It should be emphasized that grinding weakens or ruptures the *Persea Americana* seeds cell walls and enhances the release or liberation of *Persea Americana* seed oils or fats via extraction process [17]. Hence, the ground *Persea Americana* seeds sample were inserted into a bag and weighed at 40g to improve extraction process.

Figure 2. *Persea Americana Seeds Reduced Size after Drying.*

Figure 3. *600μm Size of Persea Americana Seeds after Drying.*

3.2. Sample Extraction Operation

40g of blended *Persea Americana* (Avocado fruit) seeds was added separately into fifteen (15) different sample bottles of known weights, and 200ml of the three extractive solvents namely hexane, diethyl-ether and chloroform were added into five (5) different bottles containing the 40g of the blended *Persea Americana* seed for specified period. The

mixtures were shaken vigorously with the flasks covered or sealed and allowed to stand for effective extraction process between fourteen (14) and seventy-two (72) hours respectively at ambient conditions. The samples were decanted and filtered at the end of the extraction period (time) and the filtrates in each case were distilled to recover the oil and its volume and percentage oil yield determined thus [1, 8].

$$Oil\ Yield\ (\%) = \frac{Weight\ of\ Oil}{Weight\ of\ Sample\ on\ a\ Dry\ Matter\ Basis} \times 100$$

3.3. Soxhlet Extraction Process

The Soxhlet extractor was fitted to a round bottom flask that contained the mixture of solvent and oil from each of the extractive solvents. 40g of the ground *Persea Americana* seed was weighed, put into a thimble (semi permeable membrane) and placed into the soxhlet tube. A constant volume of 200ml of different extractive solvent namely hexame, diethylether and chloroform of 95% purity respectively were poured into the round bottom flask for different operational process to the boiling point of the solvent. The vapour passed up through the tube, cooled in the condenser and the condensed solvent slowly fills the Soxhlet tube. This operational process is continued until large amount of solvent is evaporated from the oil. The extracted oil was then recovered into crucibles of different known weights, dried in an electric oven and weighed to determine the mass of oil. This operational procedure was repeated or carried out for all samples at different extraction time and respective extraction solvent.

3.4. Characterization of Extracted Persea Americana Seed Oil

Characterization of the extracted oil from *Persea Americana* seeds refers to its identification and purity using different characterizing properties such as refractive index, free fatty acids, saponification value etc.

3.4.1. Determination of Oil Refractive Index

Abbe refractometer was applied in determining the refractive index of the extracted oil from Persea Americana seeds in line with other studies of Joshua *et al*. [1] and Gidigbi *et al*. [18] respectively. Thus, the mean value of quadricate analysis was evaluated.

3.4.2. Determination of Oil Free Fatty Acid

The value is defined as the amount of milligram of alkali solution (sodium hydroxide) required to neutralize the free fatty acids present in one gram of oil. The value is a measure of the amount of fatty acids that has been freed by hydrolysis from their triglycerides combination via the action of water, temperature and lypoltic enzymes. Thus, 2g of the oil was weighed into a round bottom flask and 30ml of 95% hexane was added. The mixture was then stirred with a magnetic stirrer for homogeneous mixture of sample, which was warmed and allowed to cool and a drop of phenolpthalein indicator was added. The mixture was titrated with molar solution of sodium hydroxide (NaOH), shakening vigorously until it gives a faint permanent pink colour.

$$\%\ Free\ Fatty\ Acid = \frac{28.2 \times V \times N \times 100}{W}$$

3.4.3. Determination of Oil Soponification Value

The value is defined as the milligram of potassium hydroxide required to sponify one gram (1g) of fat or oil. The saponification value is an index of the mean molecular weight of the glycericides comprising fat. The procedure involved weighing of 0.2g of the oil into a bottom flask and 10ml of potassium hydroxide (KOH) was added. The flask was connected to a reflux condenser and heated in a water bath for one hour with occasional mixing or shaking. The flask and condenser were removed to cool, two drops of phenolpthalein indicator was added to the content of the flask and titrated against 0.5M of hydrochloric acid (HCl) with a blank test carried out simultaneously.

3.4.4. Determination of Oil Relative Density

The density of the extracted *Persea Americana* oil depends on the mass, volume and the extractive solvent applied in the extraction operational process. The relative density of the extracted oil from *Persea Americana* seeds was deduced via the technique applied by Joshua *et al.* [1], which involved washing, drying and weighing of a specific density bottle (W_A). The specific density bottle was filled with distilled water and weighed (W_B). The water was poured off and the bottle was dried to its initial constant weight and then filled with the extracted oil sample and weighed (W_C).

$$Relative\ Density = \frac{W_C - W_A}{W_B - W_A}$$

3.4.5. Determination of Oil Iodine Value

The iodine value of an oil or fat is the percentage weight of halogen calculated as iodine absorbed by the oil under the condition of the test. It is a measure of the degree of unsaturated or the number of double bonds present in it. The glycerides of the unsaturated acids do unite with a certain amount of halogen in order to break the double bonds and saturate the acid glycerides. The iodine value is often constant or within a certain range for a particular oil and is therefore more useful for identification of oils. The experimental procedure involve weighing of 0.2g of the oil and its dissolution in 10ml of carbon tetrachloride. The mixture is transferred into a clean dried bottle and sealed for adequate shaking to achieve uniform mixture content. 20ml of Wij's reagent is added to the mixture via a pipette. The stopper moistened with potassium iodine solution used to cork the bottle that was allowed to stand in dark for 30minutes. A blank test was also carried out simultaneously. After removing it from the dark, 15ml of ten percent (10%) potassium iodine solution was added followed by addition of 200ml of distilled water. The mixture is poured into a conical flask and with 0.1M $Na_2S_2O_3$ solution using starch solution as indicator towards the end of the titration continuously until the blue colour disappeared. Thus, the disappearance of the deep blue colour indicates the end point.

$$Iodine\ Value = 12.6C(V_1 - V_2)m$$

3.4.6. Determination of Oil Viscosity

The extracted *Persea Americana* oil viscosity was determined via the experimental procedure. 0.2g of the extracted *Persea Americana* oil was measured and added into a capillary viscometer tube that was partially dipped into an oil bath with magnetic stirrer with hot plate applied as the heating apparatus or vessel. At an oil temperature of 40°C, the extracted *Persea Americana* oil in the viscometer tube was pumped to the upper mark of the tube and the operational period monitored. The monitoring period began as the extracted *Persea Americana* oil started falling down the tube and this operation terminated as the oil fell to the bottom mark and the operational period or time noted.

4. Results

The result analysis of the solvent extraction operation using three different extractive solvents (hexane, diethylether and chloroform) from *Persea Americana* seed and its characterization are discussed thus.

4.1. Effect of Extraction Solvents

The investigation of the effect of three different extraction solvent such as hexane, diethylehter and chloroform on the extraction of oil from *Persea Americana* seeds at different extraction operational time is shown in Figure 4.

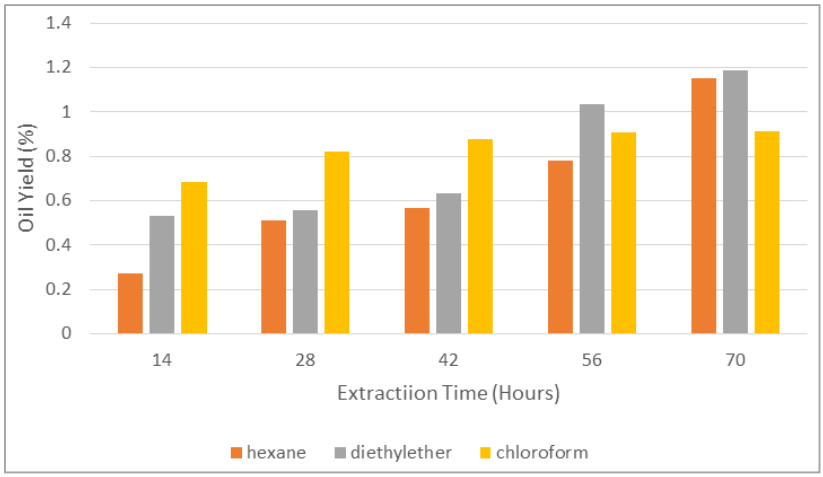

Figure 4. *Oil Yield against Time of Hexane, DiethylEther and Chloroform Extraction Solvents.*

It can be deduced therefore that the yield of extracted oil from *Persea Americana* seeds increases with increase in soaking or extraction time for hexane, diethylehter and chloroform solvents respectively. Thus, maximum extracted oil yield was achieved at maximum extraction time of seventy (70) hours with diethylether extraction solvent yielding the maximum extracted oil among the three applied extraction solvents.

4.2. Effect of Extraction Time

The yield of extracted oil from *Persea Americana* seeds using hexane, diethylether and chloroform as extraction solvents were compared with extraction time as shown in Figure 5.

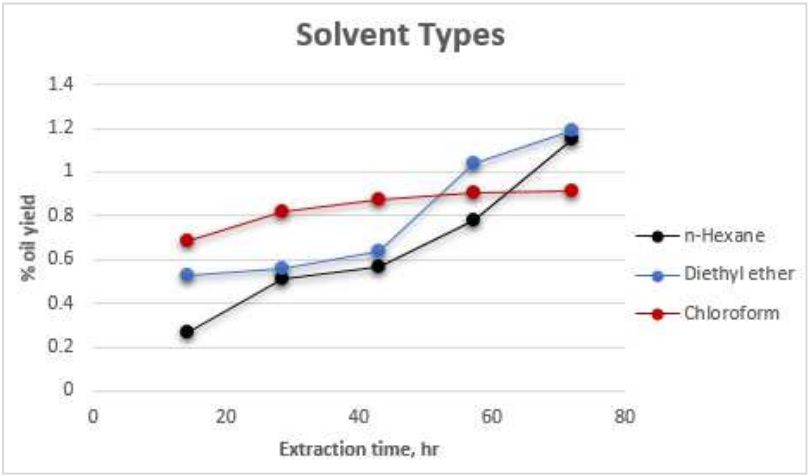

Figure 5. *Plots of Extracted Oil Yield against Time.*

There is gradual increase yield as the extraction operational time increases due to more available soaking period for oil extraction from *Persea Americana* seeds. As the extraction process progressed, there is gradual increase of extracted oil with chloroform as solvent until 42 hours followed by a relatively or steady yield of extracted oil as extraction process continued. Also, the extracted oil yield using diethylether as solvent is relatively slow or gradual until 42 hours when there is sharp increase in the yield of the extracted oil. Thus, extraction solvent diethyether showed maximum yield of extracted oil from *Persea Americana* seeds among the three extraction solvents (hexane, diethylether and chloroform) applied in this research study.

4.3. Extracted Oil Characterization Analysis

The extracted oil from *Persea Americana* seeds using three different extractive solvents were characterized to check its quality and purity and the characterization results compared with standard values of the United States of America and Europe as shown in Table 4. The extracted oil showed refractive index of 1.45 and this is in tandem with previous studies of Joshua *et al.* [1] and Dagde [6] respectively. Also, the P^H value of the extracted oil (6.0) is within the acceptable P^H value standard of United States and Europe and conforms with previous study by Tafere [19].

Table 4. *Comparison of Extracted Oil with the United States of America and Europe.*

Properties	Extracted Oil	EN-14214	ASTMD-6751
Density (Kg/m^3)	870	860-900	
P^H	6.0	5.0-6.7	7.0-9.0
Viscosity (cst)	4.8	3.5-5.0	1.9-6.0
Iodine Value ($gI_2/100g$)	48	<120	
Free Fatty Acid	1.66		
Saponification Value (mgKOH/g)	186.47		
Refractive Index	1.45		

Furthermore, density, viscosity and iodine values of the extracted oil are within the European and United States standard and are in agreement with other studies of Joshua *et al* [1], Dagde [6] and Tafere [19] respectively. In addition, the free fatty acid and saponification values of the extracted oil yielded closed value with the previous study of Dagde [6] and are within the range of values of other studies of Joshua *et al.* [1], Shiferaw [5] and Tafere [19] respectively.

5. Conclusion

This study highlighted the extraction of oil from *Persea Americana* seed via the application of three different extraction solvents (hexane, diethylether and chloroform). The percentage yield of the extracted oil was more with diethylether as extraction solvent in comparison with hexane and chloroform as extraction solvents respectively. Also, the extracted oil was characterized using standard analysis procedures and the characterization results compared with other previous extraction studies of *Persea Americana* seed with high degree of conformation and values are within the range of European and United States standard. Thus, the extracted oil from *Persea Americana* seed can be used as an alternative to energy oil and its physicochemical characteristics showed that it has some industrial potential and utilization.

Nomenclatures

C: Concentration of Sodium thiosulphate used
V_1: Volume of Sodium thiosulphate used for the blank
V_2: Volume of Sodium thiosoulphate used for determination
M: Mass of the sample
V: Volume in ml of standard Potassium hydroxide
N: Normality of the Potassium hydroxide solution
W: Weight in gram of the sample
W_A: Weight of Dried Specific Bottle
W_B: Weight of Specific Bottle with Distilled Water
W_C: Weight of Density Bottle with Extracted Oil

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