Spring 2017

Soxhlet Extraction Of Avocado Endocarp and Trituration Of Avocado Mesocarp For Biodiesel Production.

Ifeoma Oraemesi
Fort Hays State University, isoraemesi@mail.fhsu.edu

SOXHLET EXTRACTION OF AVOCADO ENDOCARP

AND TRITURATION OF AVOCADO MESOCARP

FOR BIODIESEL PRODUCTION.

being

A Thesis Presented to the Graduate Faculty

of the Fort Hays State University in

Partial Fulfillment of the Requirements for

the Degree of Master of Science

by

Oraemesi, Ifeoma Stella

B.S., Anambra State University

Date_____ Approved _____
Major Professor

Approved _____
Chair, Graduate Council

This thesis for

The Master of Science Degree

by

Ifeoma Stella Oraemesi

has been approved by

_____
Chair, Supervisory Committee

_____
Supervisory Committee

_____
Supervisory Committee

_____
Supervisory Committee

_____
Supervisory Committee

_____
Chair, Department of Biological Sciences

ABSTRACT

Finding alternative sources of renewable energy is on the rise globally. Renewable sources of energy are advantageous because they are biodegradable, less toxic, and combust efficiently. More importantly, raw materials for these sources can be replenished. One alternative source of energy is biodiesel. Biodiesel is a fuel which consists of mono-alkyl esters of long-chained fatty acids obtained from vegetable oil or animal fats. They serve as efficient fuels to run diesel engines. Biodiesel is produced via transesterification of oils wherein glycerine is a by-product. Avocado (*Persea americana*) is a fleshy fruit with high lipid content, mostly monounsaturated fats, which amounts to 70% of its lipid content. These fruits serve as viable sources of biodiesel. In this research, I used the soxhlet apparatus to extract oil from the stony endocarp and trituration/geometric dilution to extract oil from fleshy mesocarp to produce biodiesel. The solvent used in both methods was hexane. About 0.48 ml of oil per g tissue was obtained from the avocado mesocarp via trituration extraction technique compared to 0.025 ml of oil per g tissue from avocado endocarp via soxhlet extraction. Oils extracted were analysed using GC-MS and were composed of fatty acids like oleic acid, palmitoleic acid, stearic acid, arachidonic acid, and myristic acid. These fatty acids were transesterified to investigate potential for biodiesel production. Avocado's high lipid content can be explored in the area of renewable energy. The mixture of saturated and unsaturated fatty acids can be advantageous in its use as biodiesel.

ACKNOWLEDGEMENTS

This thesis was made possible through the help, advice and support of many individuals. A very special thanks to Dr. Brian Maricle, my advisor, for helping me through the toughest times, times when I got confused because of the difference in the educational system I am used to, you were patient enough to help me understand and you have been a source of encouragement and inspiration. A very special thanks to Dr. Arvin Cruz, my co-advisor who worked tirelessly on this research and has been of great help. Thanks also to the members of my graduate committee, Dr. Eric Gillock, Dr. Stephen Donnelly, and Dr. Richard Packauskas, for reviewing my thesis and making recommendations along the way.

I am immensely grateful to the Department of Biological Sciences and Fleharty fellowship for providing financial support. Also to Kansas IDeA Network for Biomedical Research Excellence (K-INBRE) P20GM103418 for the research grant which was used to make this research possible. I wish to give special thanks to the Chemistry Department for helping me in the areas involving chemistry during this research.

My special thanks go to the faculty and graduate students of the Department of Biological Sciences for being supportive throughout every stage of my completing this degree. I am thankful to Mr. Mehran Shahidi, Director of Intercultural Integration for being attentive and encouraging me in my lowest times. I am grateful to the members of Liberty Foursquare Church and Foursquare Gospel Church Mile 12, Nigeria for their prayers, love and care.

And finally, I wish to thank my parents, Mr. and Mrs. Chinwuba, and my sisters, Laura and Vivian Oraemesi, for always being there for me. I appreciate your love, support, and prayers. I would not be where I am today without your help. From a distance, your words find their way to me.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page.

LIST OF FIGURES

Figure Page

PREFACE

This thesis follows the style of *Journal of Environmental Science and Health*.

INTRODUCTION

Scarcity in fossil fuel sources remains an issue of concern (Shafiee and Topal, 2009). Although more studies are being conducted (Shafiee and Topal, 2009) to address the issue on how much of these scarce resources are still left, some countries are already embracing alternative sources of renewable energy (Wustenhage et al., 2007). Such countries include Canada, Brazil, Turkey, and the United States of America (Apergis and Payne, 2010). The energy crisis experienced by countries, particularly developing nations, has heightened the need to turn towards alternative sources. In Nigeria, for example, the energy crisis is caused by overconsumption of energy resources due to exponential growth in population. Other factors such as poor infrastructure, lack of knowledge about alternative sources of energy (Domac et al., 2005), energy waste, poor distribution of energy, tax hikes, strikes by energy workers, and natural calamities (Ehinomen and Adeleke, 2012) also contribute to the crisis. The economy of Nigeria is heavily dependent on oil production and export (Sanusi, 2012). Unfortunately, most people in Nigeria do not benefit from it. Electricity, fuel, diesel, and kerosene, which ought to be in excess within the country, are actually scarce commodities (Ehinomen et al., 2012) because of corruption and misappropriation of funds (Sanusi, 2012). Because the oil produced is non-renewable, it is possible it will be used up some day (Shafiee and Topal, 2009) and that is the reason for my study. To alleviate the issue, more studies need to be conducted in order to find sources of energy that are environment friendly and renewable.

The motivation of this thesis is to explore alternative sources of renewable energy that can be used worldwide. This includes my desire to help the Nigerian economy. I am confident my study is beneficial to my fellow Nigerians by improving the standard of living

of the masses. If adopted in my country, results from this study will help create more job opportunities, reduced incidence of oil spillage and other environmental hazards, with less pollution, as well as improvement in the agricultural sector (Williams et al., 2011).

Environment friendly sources that are renewable tend to be carbon neutral, biodegradable, less toxic and burns cleanly (Speight, 2014). Such fuel sources include solar, wind, hydroelectric, geothermal, ocean tides and biomass (Johannson and Burnham, 1993). Demand on the use of biomass-based fuels have increased due to several factors (Chen et al., 2012). First, they are abundant. Second, they help reduce the amount of waste generated in landfills. Third, these materials are biodegradable; therefore, environment friendly (Apergis and Payne, 2010). Also, exploitation of these alternative sources of energy opens new avenues to create new job opportunities (Wei et al., 2010) to help sustain human needs. This in turn help improve the economy (Domac et al., 2005).

Biodiesel is a renewable fuel consisting of mono-alkyl esters of long-chain fatty acids from vegetable oil (Williams et al., 2011) or animal fats (Huber et al., 2006) that have met the requirements of American Society for Testing and Materials (ASTM) D 6751 (Wu et al., 2012) for use in diesel engines. Biodiesel also refers to pure fuel before blending with diesel fuel (Giakoumis et al., 2014). Oil is converted through transesterification (Anastopoulos et al., 2009) to methyl esters, also known as biodiesel (Baohua et al., 2012), and glycerine. Biodiesel fuels are less toxic, have lower emission (Dang et al., 2012), less air pollution (Chen et al., 2011), and are more environmentally friendly compared to fossil fuels (Baohua et al., 2012).

Avocado (*Persea americana* Mill.) of the plant family Lauraceae produces a fruit with high oil content (Mooz et al., 2012). The mesocarp (fleshy part of the fruit) makes up

60 to 75% of the total weight of avocado fruit (Costagli and Betti, 2015). Mesocarp is composed of parenchyma cells that surround uniformly distributed specialized oil containing idioblast cells (Reddy et al., 2012). The endocarp (stony part of the fruit) makes up 13% of the total weight of the fruit (Avhad and Marchetti, 2015). Avocado is a tropical fruit (Ogunwusi and Ibrahim, 2016) that stands out for its high nutritional value (Mooz et al., 2012). It is also a good source of monounsaturated fatty acids, palmitic acid, and they have low amounts of polyunsaturated linoleic acid and stearic acid (Ogunwusi and Ibrahim, 2016). Avocado oil has been used for cooking, cosmetics, and treating diseases, but has not been widely studied as a good source of oil for renewable energy (Knothe, 2013).

This is focused on comparison of the oil extracted from both endocarp and mesocarp tissues of avocado fruit. The oil from endocarp was extracted using the Soxhlet extraction technique using hexane as solvent, which has been widely studied (Meyer and Terry, 2008). The mesocarp underwent trituration with hexane as solvent for the oil extraction. This trituration technique is mostly used in pharmaceutical industries (Schachter and Harden, 1997) but has not been used in extraction of oil for energy purposes (Nakamura et al., 2004 {b}). Most researchers have used Soxhlet extraction on the mesocarp (Meyer and Terry, 2008; Mooz et al., 2012). My research compared the quantity and quality of oil from both tissues, the moisture content, and the effectiveness of the technique used.

Soxhlet extraction is a process used for liquid-solid extractions, especially for compounds with limited solubility in the solvent (Meyer and Terry, 2008). Because avocado endocarp has low solubility in the solvent (hexane), the Soxhlet was the most suitable extraction technique. By contrast, mesocarp tissue was pasty after milling, where

using Soxhlet extraction technique would have been inappropriate. In Soxhlet method, the sample is separated from the solvent based on different volatilities (Reddy et al., 2012). Specifically, the sample is dried and milled into small particles and placed in a porous thimble (Chemat et al., 2008). The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent, and a condenser is placed on top of the extraction chamber (Chemat et al., 2008). As the flask gets heated, the solvent evaporates and moves up into the condenser, where it is converted into a liquid that trickles into the extraction chamber containing the sample. Eventually, the solvent builds up in the extraction chamber and completely surrounds the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level, it overflows and trickles back down into the boiling flask (Chemat et al., 2008). As the solvent passes through the sample it extracts the oil and carries them into the flask. The oil then remains in the flask because of its low volatility. At the end of the extraction process, which typically lasts a few hours, the flask containing the solvent and lipid is removed, the solvent is evaporated and the oil is recovered (Chemat et al., 2008).

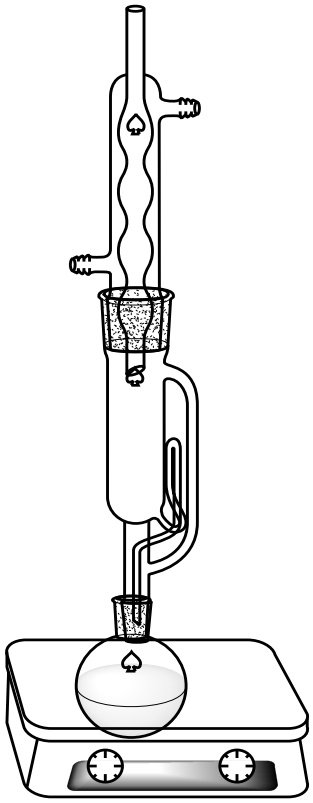


Figure 1. A typical Soxhlet extraction set-up.

Trituration technique is a solid – liquid extraction that involves the purification of an impure compound by taking advantage of the solubility differences of the compound and solvent mixture (Schachter and Harden, 1997). This extraction method is mostly used in pharmaceutical industries (Nakamura et al., 2004a). Hexane was used as the solvent since the mesocarp that was milled was sparingly soluble in it and impurities were highly soluble. The mesocarp was suspended in the solvent so that all impurities were exposed to solvent and had the opportunity to dissolve (Nakamura et al., 2004b). Then the liquid portion was decanted for further extraction process (Schachter and Harden, 1997). Trituration seemed appropriate for the mesocarp because of the nature of the mesocarp before extraction.

The oils obtained from both tissues were transesterified. Transesterification is a chemical process that involves reaction of one (1) equivalent of triglyceride with three (3) equivalents of an alcohol. The products of this reaction are one (1) equivalent of glycerol and three (3) equivalents of the methyl esters (Lin and Lin, 2012). The alkyl chains of the methyl esters will vary depending on the nature of the triglyceride present in the sample. This reaction is accompanied by a base catalyst, [B], typically sodium hydroxide, NaOH. Due to the solubility of glycerol in water, it can be easily removed by extraction. The fatty acid methyl ester mixture obtained from transesterification is referred to as biodiesel (Rachimoellah et al., 2009). Figure 2 shows the chemical equation that represents transesterification.

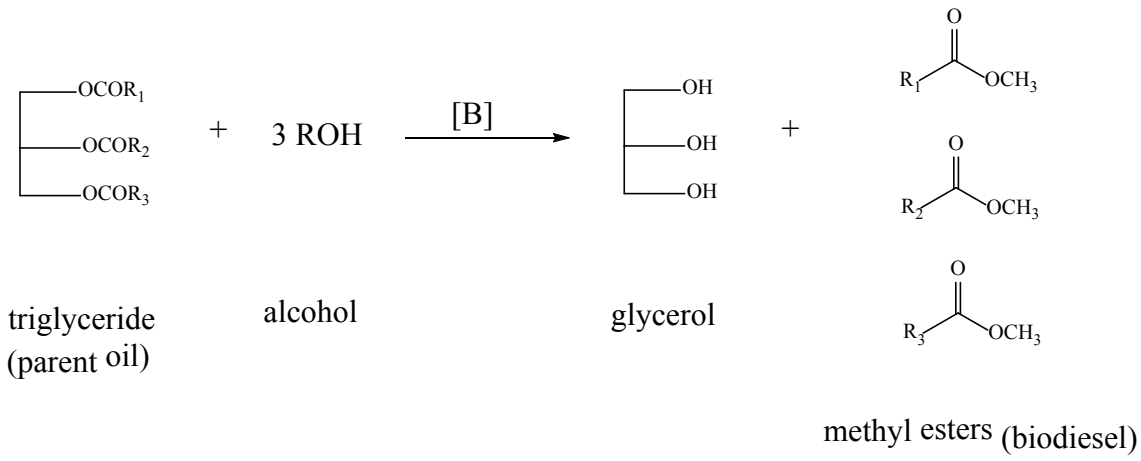


Figure 2. Transesterification reaction to produce biodiesel.

Figure 3 summarizes all the steps involved with the isolation of biodiesel from avocado fruit. The first step involves peeling and destoning of the avocado fruit. Destoning is the process of separating the endocarp from the rest of the fruit. In this project, only the mesocarp and endocarp were used to isolate biodiesel. The exocarp was discarded due to its known low lipid content. Drying process is important in removing water contents of the avocado. Lipids are impervious to water; therefore, any water in the sample will hinder the isolation of triglycerides, which are the precursors needed to generate biodiesel.

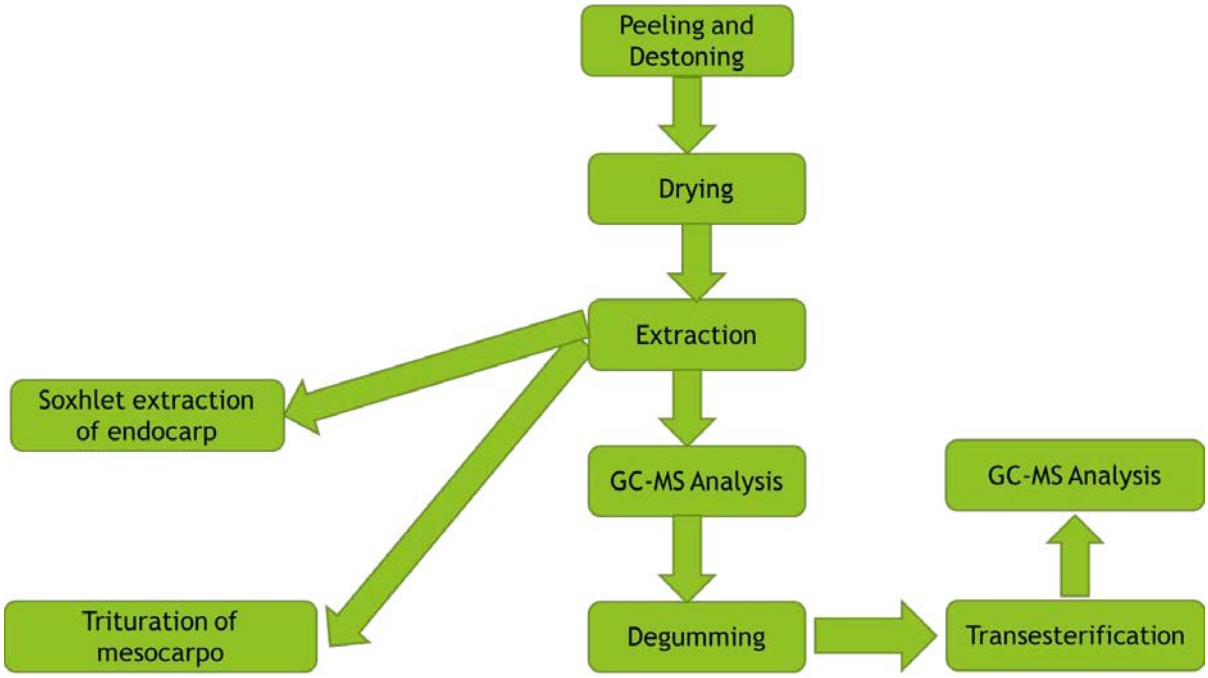


Figure 3. The overall process of isolation, degumming, purification and analysis of biodiesel.

Biodiesel consists of a mixture of methyl esters produced from transesterification of triglycerides from the fruit sample (Figure 2). Triglycerides can be taken out of the sample using various methods such as extraction with an organic solvent, supercritical fluid extraction using CO_2 and cold-press methods. In this project, the author uses Soxhlet extraction for the isolation of triglycerides present in the endocarp. Due to the pasty consistency of the mesocarp of the avocado, traditional methods of extraction may not be feasible. Here, the author used trituration methods as a novel method of extracting the triglyceride content of the mesocarp.

Degumming is the process of removing water soluble impurities from a crude sample of oily triglycerides. Impurities include pigments, metal complexes, peroxides, proteins and other water soluble vitamins and minerals (Patel et al., 2016). After degumming, the refined oil (triglycerides) was ready for transesterification to produce biodiesel (Figure 2). Various methods of chemical analysis can be employed to analyze biodiesel content, such as ^{1}H-NMR (Proton Nuclear Magnetic Resonance) spectroscopy and gas chromatograph coupled to mass spectrometry (GC-MS). In this research, GC-MS was employed due to its high sensitivity and selectivity, low-cost and relative ease of operation.

The aim of this study was to identify the difference in the oil content and moisture content of oil from the mesocarp and endocarp tissues of avocado. Another objective was to make a decision on which tissue produces suitable oil for biodiesel production. The hypothesis tested was that the oil in both tissues would differ in lipid content and moisture content. The mesocarp was hypothesized to have significantly more oil and moisture than the endocarp, but the endocarp was hypothesized to have oil of better quality for production of biodiesel.

MATERIALS AND METHODS

Sample preparation

Thirteen avocado fruits were bought from a local grocery store (Dillon's, Kroger Company) with a total mass of 2,180.22 g. The avocados were peeled (the exocarp was removed), followed by a separation of the endocarp (stony portion) from the mesocarp (fleshy portion) (Costalgi and Betti, 2015). Endocarps were cut into smaller sizes ranging from 0.60 g to 3.41 g to aid drying. The cut endocarp and mesocarp were portioned into aluminum foil weighing boats for drying.

Endocarp and mesocarp were dried initially at 25°C for two weeks, after which the temperature was increased to 45°C for one week (Reddy et al., 2012). Dried endocarp and mesocarp were milled (Reddy et al., 2012) in a Wiley mill (model 3383-L10; Thomas Scientific; Swedesboro, NJ, USA), and then stored in a refrigerator until extraction (Reddy et al., 2012). Total weight of endocarp after milling was 136.90 g, and total weight of mesocarp was 438.69 g.

Soxhlet extraction of endocarp

Extraction of oil from endocarp tissue was carried out using the Soxhlet extractor (Fig. 1) (Reddy et al., 2012). Milled endocarp tissue was divided into four batches and subjected to the Soxhlet extraction process (Table 1). These batches had the endocarp sample in the thimble ranging from 31.00 to 37.00 g and extraction solvent (hexane)

ranging from 100.0 to 150.0 ml. The extraction was carried out at 65°C for 72 hours, with constant running water throughout the process. The extract obtained from each batch ranged from 52.0 to 110.0 ml. Each batch was left to undergo Soxhlet extraction for 72 hours. After 72 hours, the extraction was stopped, and the extract was transferred to an Erlenmeyer flask sealed with parafilm.

To remove the sediments still present, the extract was vacuum filtered with a Buchner funnel and filter paper that was wet with hexane. The filtrate was transferred to a 500 ml round bottom flask for rotovap using Rotavapor – R, Büchi. In rotovap, the oil was separated from the solvent (Chen et al., 2012). The solvent evaporates but is not lost. It is instead collected in the receiving flask (Fig. 9). The round bottom flask is half immersed in a bath containing ethylene glycol and the rotovap is set to approximately 70 rpm. Evaporation was done under reduced pressure, and heat was applied at 45^{o}C. Filtrate was rotovapped until the solvent distilled into the receiving flask, typically requiring 1 hour.

Trituration of mesocarp

Dried and milled mesocarp tissue was divided into small portions and hexane was used as the solvent (Bora et al., 2001). The mesocarp was dissolved in hexane and left to stand for 48 hours at room temperature while sealed with parafilm (Fig. 10). After 48 hours, a heterogeneous mixture was obtained. A pipette was used to carefully transfer the liquid hexane (with oil extract) without disturbing the mixture. After the first trituration on all five beakers, a second trituration was carried out on the same tissue. The beakers had the mesocarp samples unequally distributed among them. The mesocarp in the beakers were

dissolved in hexane ranging from 80.0 to 155.0 ml (Table 2). Extract volumes within the range 20 to 80 ml were obtained in both trituration processes (Bora et al., 2001).

Crude extracts were subjected to analysis using a Shimadzu GCMS-QP2010SE Gas Chromatography Mass Spectrometer (GC-MS) (Shimadzu Corp., Kyoto, Japan). The crude extracts from the endocarp and mesocarp were analyzed using ether and methanol as solvents that in dissolving the samples. At the start of the analysis, The parameters included ion source temperature 200°C, interface temperature 280°C, solvent cut time of 3, 5 minutes, column oven temperature 50°C, injection time 270°C, injection mode: split, number of rinses: 1, gas: helium, pressure 26.7 kPa, total flow 22.7 ml/min, column flow 0.68 ml/min, purge flow 1.5 ml/min, linear velocity 30 cm/sec., retention time 27 min, thickness 0.25 μm, length 30 m, diameter 0.25 mm, microscan width 0.Oven temperature increased to 200°C and the pressure increased to 64 kPa at 16 min retention time to the end of the analysis. The retention time for each analysis was 27 min. The time during the analysis when peaks were seen for each of these fatty acids is the retention time. This was recorded for the fatty acids identified.

Water degumming

The soluble impurities in crude avocado oil were identified as gums which consist of phospholipids and metal complexes, free fatty acids, peroxides with their breakdown products, and pigments (Marenchino et al., 2006). Gums and metal complexes were removed by degumming (Aly, 1992) or chemical refining. The mesocarp crude extract was divided into three portions and degummed with 3.6 to 4 ml of water, stirred,

and refluxed for 30 min at a temperature of 75.0 to 85.0°C (Zufarov et al., 2008). The degummed crude extract was centrifuged at a speed of 3200 rpm for 30 min using Clay Adams Compact II centrifuge (Becton Dickinson and Company; Sparks, MD). After centrifugation, the sludge was separated from the centrifugate (supernatant) by decantation. The oil was in the supernatant.

Transesterification

0.50 g of sodium hydroxide pellets were crushed in a mortar. The crushed solid was transferred to an Erlenmeyer flask containing 14 ml methanol. This was stirred until sodium hydroxide dissolved. A 60.0 ml of oil was added to the solution and heated between 45.0 to 50.0^{o}C and refluxed for 1 hour. The mixture was allowed to cool and transferred to separatory funnel where it was left to separate into two distinct layers. The bottom layer was collected in a beaker. 10 ml of water was added to the top layer and left to stand to remove unreacted methanol and glycerine that might be present. 3 ml hexane was also added to aid separation. The mixture was left to stand until distinct layers were seen. The bottom layer was discarded while the top layer contained biodiesel.

GC-MS Analysis on Transesterified Oil

Transesterified oil from the endocarp and mesocarp were subjected to analysis using a Shimadzu GCMS-QP2010SE Gas Chromatography Mass Spectrometer (GC-MS). The parameters included ion source temperature 200.0°C, interface temperature 270.0°C,

solvent cut time of 3, 5, 10 minutes, threshold 300, column oven temperature 100.0°C, injection time 270.0°C, injection mode: split, number of rinses: 1, gas: helium, pressure 48.7 kPa, total flow 28.1 ml/min, column flow 0.74 ml/min, purge flow 1.5 ml/min, linear velocity 32 cm/sec., retention time 60 min, thickness 0.25 μm, length 30 m, diameter 0.25 mm, microscan width 0.

RESULTS

Effect of Drying

Mass of the endocarp and mesocarp were reduced after drying at 25.0°C for two weeks and 45.0°C for one week ($P < 0.001$). Avocado mesocarp replicates that were dried had masses ranging from 85.00 to 162.00 g with an average mass of 148.4 g prior to drying. Mesocarp tissue had an average moisture content of 75.1% (Fig. 4). Mass of the mesocarp replicates were significantly reduced after drying and had an average mass of 36.2 g. Avocado endocarp replicates had masses ranging from 73 to 140 g with an average of 106.9 g before drying. Endocarp tissue had an average moisture content of 37.8%. After drying, there was an average mass of 60.6 g. Mesocarp tissue had significantly higher water content compared to endocarp (T-test, $P < 0.001$; Fig. 4).

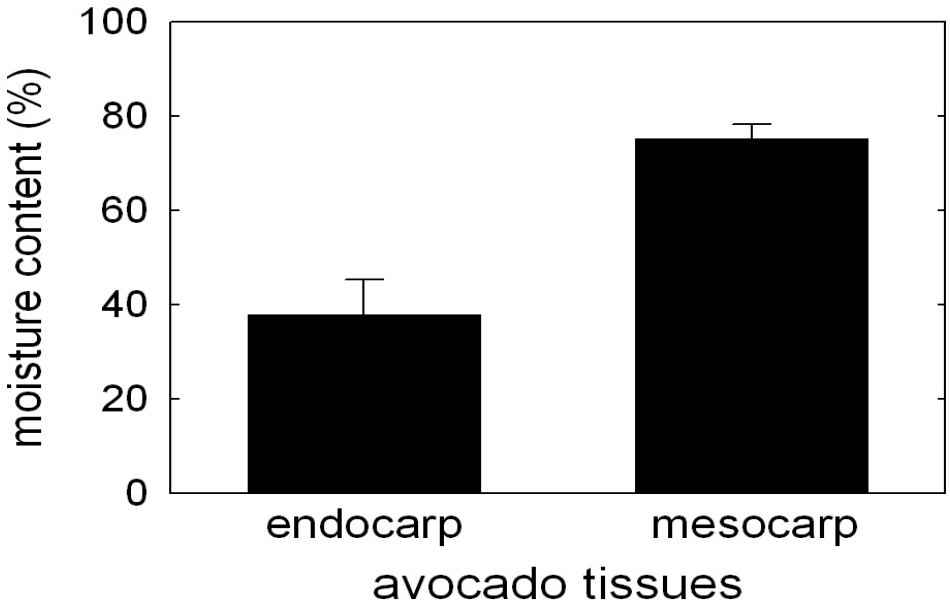


Figure 4. Moisture content of endocarp and mesocarp tissues of avocado, measured by drying tissues in the oven at 45.0°C.

Oil extracted from mesocarp tissue produced a total of 225.0 ml of oil from 13 fruits, significantly more than the endocarp, which produced a total of 3.0 ml of oil from the same 13 fruits (T-test, $P < 0.001$). Different techniques were used for different tissues of avocado (mesocarp and endocarp), where these represent the crude extract from both tissues (Fig. 5). On a per-mass basis, mesocarp produced 0.478 ml oil/g tissue whereas endocarp produced 0.0248 ml oil/g tissue (Fig. 5).

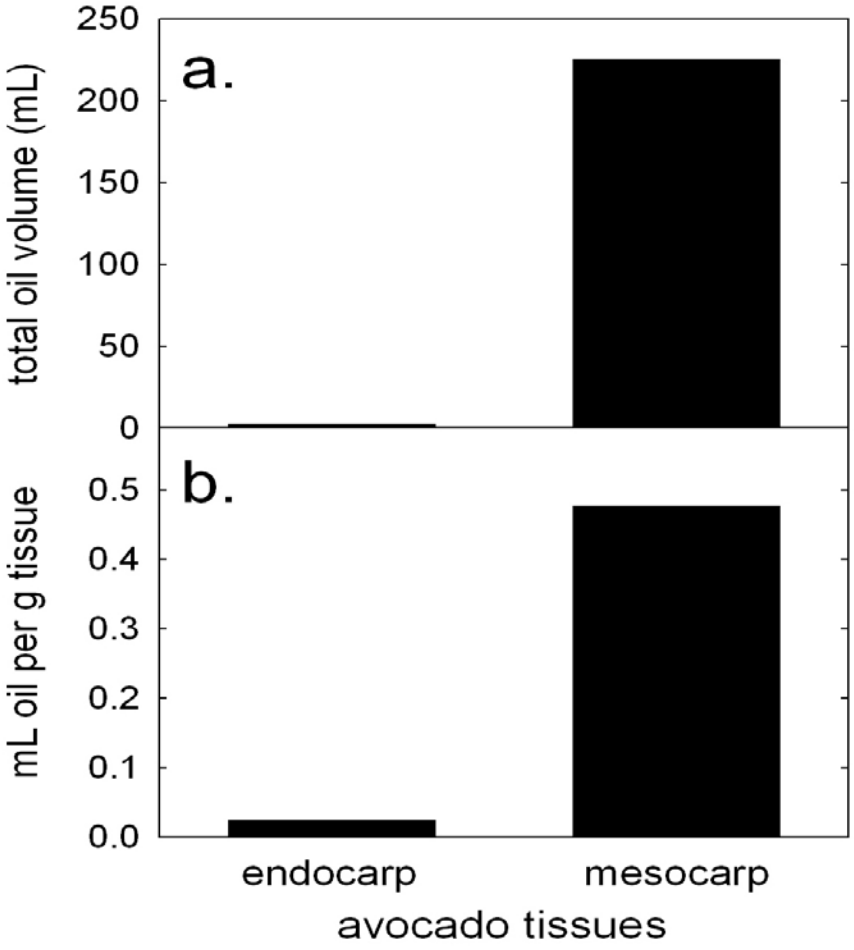


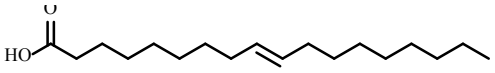
Figure 5. Volume of crude oil extracted from the endocarp and mesocarp tissues of avocado after the extraction and rotovap process.

GC-MS Analysis of crude substrate

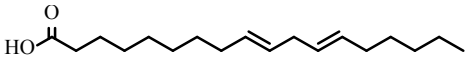
For the oil analysed from the mesocarp tissue, fatty acids like Δ^9-oleic acid, Δ^9-palmitoleic acid, $\Delta^{9,12}$-linoleic acid, $\Delta^{5,8,11,14}$-arachidonic acid, myristic acid, $\Delta^{8,11,14}$-linolenic acid, $\Delta^{9,12,15}$- linolenic acid, palmitic acid, and stearic acid were identified (Table 3). $\Delta^{9,12,15}$- linolenic acid had the highest concentration. Myristic acid and $\Delta^{8,11,14}$- linolenic acid were present in amounts greater than the other fatty acids identified. For the oil analyzed from the endocarp tissue, fatty acids like Δ^9-palmitoleic acid, $\Delta^{9,12}$-linoleic acid, $\Delta^{5,8,11,14}$-arachidonic acid, myristic acid, $\Delta^{8,11,14}$- linolenic acid, $\Delta^{9,12,15}$- linolenic acid, palmitic acid and stearic acid were identified (Table 3). Endocarp had lauric acid, Δ^6-oleic acid, $\Delta^{5,8,11,14}$-arachidonic acid, $\Delta^{9,12,15}$- linolenic acid, $\Delta^{9,12}$- linoleic acid, and Δ^9-oleic acid (Table 3). Arachidonic acid had the highest concentration. $\Delta^{9,12}$- linoleic acid and Δ^9-oleic acid were present in greater amounts when compared to the remaining fatty acids identified. Structures of the fatty acids identified in endocarp can be seen in figure 6. Both the endocarp and mesocarp tissues of avocado had oleic acid, $\Delta^{9,12,15}$- linolenic acid and arachidonic acid present, but in varying amounts. Mesocarp had 26.8% saturated fatty acids and 73.4% unsaturated fatty acids. Endocarp had 9% saturated fatty acids and 91% unsaturated fatty acids. The fatty acid methyl esters from the mesocarp tissue included Δ^9-oleic acid methyl ester, Δ^9-palmitoleic acid methyl ester, $\Delta^{9,12}$-linoleic acid methyl ester, $\Delta^{5,8,11,14}$-arachidonic acid methyl ester, myristic acid methyl ester, $\Delta^{8,11,14}$- linolenic acid methyl ester, $\Delta^{9,12,15}$- linolenic acid methyl ester, palmitic acid methyl ester, and stearic acid methyl ester were identified (Table 3). Fatty acid methyl esters from the endocarp had lauric acid methyl ester, Δ^6-oleic acid methyl ester, $\Delta^{5,8,11,14}$-arachidonic acid methyl ester, $\Delta^{9,12,15}$- linolenic acid methyl ester, $\Delta^{9,12}$- linoleic acid methyl ester, and Δ^9-oleic acid

methyl ester (Table 3). These fatty acid methyl esters had retention times ranging from 4.5 to 46.7 minutes. The mass to charge ratio (m/z) of these fatty acid methyl esters are found in Table 5.

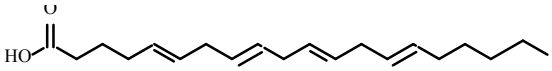
Numerous other compounds were present in the oil, including ascorbic acid, ethylisoallocholate, 7- hexadecadienol, cyclopropaneoctanoic acid, and others (Table 6). Among these compounds are other fatty acids that are not common esters (Zhao, 2012), like saturated branched chain fatty acid methyl esters, monoenoic, dienoic and trienoic fatty acid methyl esters, halogenated fatty acids, methyl esters, and others (Christie, 2017). These can be converted to methyl esters during the transesterification process. The methyl esters that were identified included hexanoic acid methyl esters, nonanoic acid methyl esters, hexanedioic acid methyl esters, and others (Table 7), their retention times were recorded (Table 7).



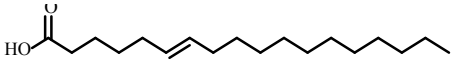
Δ^{9} – oleic acid



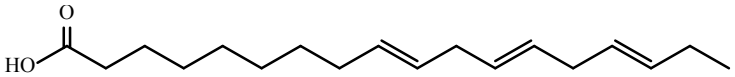
$\Delta^{9,12}$ – linoleic acid



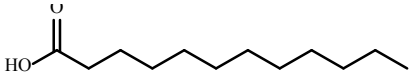
$\Delta^{5,8,11,14}$ – arachidonic acid



Δ^{6} – oleic acid

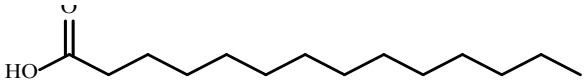


$\Delta^{9,12,15}$ – linolenic acid

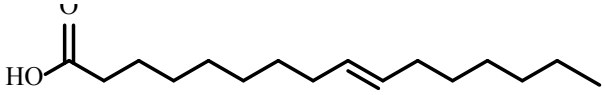


lauric acid

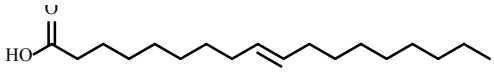
Figure 6. Structures of the fatty acids found in the avocado endocarp oil.



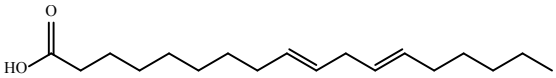
myristic acid



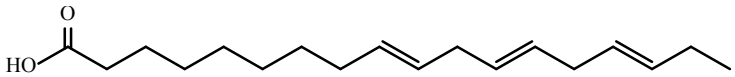
Δ^{9} – palmitoleic acid



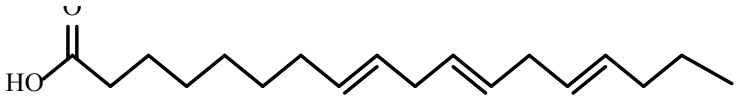
Δ^{9} – oleic acid



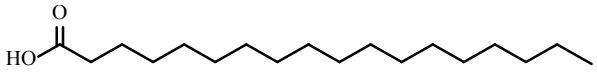
$\Delta^{9,12}$ – linoleic acid



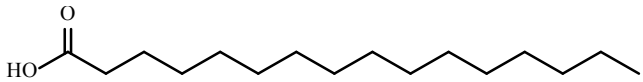
$\Delta^{9,12,15}$ – linolenic acid



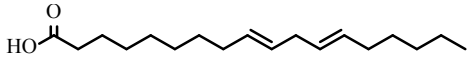
$\Delta^{8,11,14}$ – linolenic acid



stearic acid



palmitic acid



$\Delta^{9,12}$ – linoleic acid

Figure 7. Structures of fatty acids found in the avocado mesocarp oil.

DISCUSSION

Oil was extracted from avocado endocarp and mesocarp tissues. Soxhlet extraction was done on the avocado endocarp whereas trituration extraction was done on the avocado mesocarp. Significantly more oil was extracted from mesocarp than from endocarp. Oil from both tissues was degummed (Halder et al., 2009) and transesterified (Joshi and Pegg, 2007). At the end of transesterification, GC-MS was used in the analysis of the oil to determine the fatty acid methyl esters present. A mixture of saturated and unsaturated fatty acids made up the oil that was transesterified. The oil from avocado endocarp is better suited because of its low moisture content, and better oxidative stability. The endocarp is usually thrown away, so using it for a source of oil would be cost saving.

Oil Extraction Techniques

The techniques used for extraction of oil from avocado mesocarp and endocarp tissues were suitable based on the state of the tissues after they were milled (Qin and Zhong, 2016). Endocarp was a coarse solid. The Soxhlet extraction technique was best for the endocarp because oil can be extracted without direct contact of the sample and solvent (Laurens et al., 2012). In the Soxhlet extraction, the sample is separated from the solvent based on different volatilities (Laurens et al., 2012). Specifically, the sample is dried and milled into small particles and placed in a porous thimble (Chemat et al., 2008). The solvent is in a flask below the Soxhlet extractor and condenser. As the solvent passes through the sample it extracts the oil and carries them into the flask (Laurens et al., 2012). The oil then remains in the flask because of their low volatility (Mooz et al., 2012). At the end of the

extraction process, which typically lasts a few hours, the flask containing the solvent and lipid is removed, the solvent is evaporated and the oil is recovered (Chemat et al., 2008). This method was suitable for endocarp because the sample in the thimble does not dissolve when subjected to the solvent. Only the needed oil and solvent gets carried to the flask containing the solvent. The thimble serves as a filter to make sure only extract mixed in solvent was condensed to the receiving flask (Luque de Castro and Garcia Ayuso, 2000).

Trituration is a new technique for the extraction of oil from avocado mesocarp. Trituration involves the purification of an impure compound (Shachter and Harden, 1997) by taking advantage of the solubility differences of the compound and solvent mixture (Nakamura et al., 2004a). The mesocarp was suspended in the solvent (hexane) so that all impurities were exposed to solvent and had the opportunity to dissolve (Topare et al., 2011). Then the liquid portion was decanted for further extraction process. Most previous studies used the Soxhlet for both the endocarp and mesocarp tissues of avocado (Mooz et al., 2012). Trituration technique was much more suitable for the mesocarp tissue because mesocarp tissue was pasty after milling. Using the Soxhlet extraction for mesocarp would have given a yield that would not be pure oil extract. This is true because milled mesocarp tissue was sparingly soluble in hexane. Since the reaction would have been run for 72 hours for Soxhlet extraction, the whole mesocarp would have dissolved in the solvent, especially since heat was involved.

An additional benefit of both extraction techniques is that the solvent used can be fully recovered at the end of the rotovap process. The solvent recovered was pure and could be reused. The Soxhlet extraction took 72 hours in this research. An area for improving this technique might be shortening the time to find the optimum time it takes to run the

Soxhlet extraction fully. The reaction duration in my process ensured that the extraction was completely done.

Avocado Oil

Both extraction techniques worked well for the tissues. According to Kaiser et al. (1992), avocado mesocarp has 25% total lipids and avocado endocarp has 1% lipid on a fresh mass basis. This supports my results, which show significantly more oil extraction from avocado mesocarp than avocado endocarp.

There has been little work on avocado oil as a source of oil for biodiesel production (Rachimoellah et al., 2009). Both endocarp and mesocarp tissues are made of a mixture of saturated and unsaturated fatty acids (Ma and Hanna, 1999). Saturated fatty acids have a high melting and freezing point. This makes them stable at high temperature, but they have better oxidative stability Bowen, 2010). Unsaturated fatty acids have lower gel point (freezing point) and this makes them excellent for cold weather conditions (Gopinath et al., 2010). Yet, unsaturated fatty acids are prone to oxidation (Gopinath et al., 2010). Based on their composition, endocarp had 9% saturated fatty acids, and 91% unsaturated fatty acids while mesocarp had 26.8% saturated fatty acids and 73.4% unsaturated fatty acid (Table 3). Methyl esters identified in the mesocarp tissue included Δ^{9}-oleic acid methyl ester, Δ^{9}-palmitoleic acid methyl ester, $\Delta^{9,12}$-linoleic acid methyl ester, $\Delta^{5,8,11,14}$-arachidonic acid methyl ester, myristic acid methyl ester, $\Delta^{8,11,14}$- linolenic acid methyl ester, $\Delta^{9,12,15}$-linolenic acid methyl ester, palmitic acid methyl ester, and stearic acid methyl ester were identified. Fatty acid methyl esters identified in the endocarp had lauric acid methyl ester,

Δ^{6}-oleic acid methyl ester, $\Delta^{5,8,11,14}$-arachidonic acid methyl ester, $\Delta^{9,12,15}$- linolenic acid methyl ester, $\Delta^{9,12}$- linoleic acid methyl ester, and Δ^{9}-oleic acid methyl ester. Mesocarp had 26.8% saturated fatty acid methyl esters and 73.4% unsaturated fatty acid methyl esters. Endocarp had 9% saturated fatty acid methyl esters and 91% unsaturated fatty acid methyl esters. Having a mixture of both fatty acids might be complementary. Biodiesel fuel with more unsaturated fatty acids has more density but less viscosity, lower cetane number (Bamgboye and Hansen, 2008) and heating value (Berasategi et al., 2012), lower thermal efficiency, lower hydrocarbon and carbon monoxide emission, and maximum gas pressure (Gopinath et al., 2010). Considering their relative fatty acids, oil from mesocarp tissue might have a higher chance of oxidative stability when compared with endocarp, and oil from endocarp might be preferable in cold weather when compared to oil from mesocarp (Gopinath et al., 2010). Most of the fatty acids in the oil from both tissues were similar to studies conducted by Knothe (2013) and Rachimoellah et al. (2009) on avocado.

Oil from both tissues had other fatty acids that were different from the common fatty acids. These included nonanoic acid methyl esters, hexanedioic acid methyl esters, heptanoic acid methyl ester, and others (Table 7), their retention times were included in Table 7. Fatty acids on triglycerides are converted to methyl esters through transesterification (Bowen, 2010). The primary factor of oil conversion to biodiesel is triglyceride (Bowen, 2010).

CONCLUSIONS AND FUTURE DIRECTIONS

Renewable energy is an area that is becoming desirable because of its numerous benefits. Avocado as a source of biodiesel should be explored. Although avocado mesocarp produces lots of oil, the endocarp oil seemed more suitable because the oil does not need much processing for color or odor. Endocarp tissue has a lot of fatty compounds apart from the known fatty acids that can be converted to esters. In addition, endocarp tissue is typically considered as waste because it is not eaten. Mesocarp tissue contains lots of water and it is not certain that all the water content can be removed, therefore to avoid emulsion formation during the extraction process, endocarp tissue is preferred. Due to the chance of withstanding cold weather conditions, oil from endocarp is suitable for biodiesel production. Since endocarp is considered as waste, it is preferred for conversion to biodiesel.

An area for future research will be working on how to increase the amount of oil extracted from endocarp tissue. If there is enough oil extractable from endocarp tissue, there would be no need to use edible portions of the fruit for oil production for renewable energy. Another area to explore is using rotten avocados for biodiesel production. When rotten, fruits cannot be sold, therefore, turning waste into energy would be beneficial. The oil composition of a rotten avocado and fresh avocado is expected to remain the same (Qin and Zhong, 2016), so this oil can be put into good use in renewable energy. The exocarp can also be studied to determine if oil can be gotten from it. It will be another way of converting waste to fuel.

Avocado as a source of renewable energy could be beneficial to a developing country like Nigeria, who depend solely on fossil fuels (Oyejide and Adewuyi, 2011). Energy produced by photosynthesis carried out by plants years ago is responsible for fossil fuels (Bassham et al., 1950). Unfortunately, modern civilization is using up in a few centuries the excess of photosynthetic production accumulated over millions of years (Bassham et al., 1950). When the fossil fuel is used up, the only oil that will be available in Nigeria will be from contemporary sources like plants or algae (Bassham et al., 1950). Therefore, the need to look into alternative source of energy is essential. Since 2015, Nigeria has been in economic recession due to declines in the oil market. This has affected the economy of the country (Sanusi, 2012). Nigeria and other countries whose economy is dependent on oil (Ehinomen and Adeleke, 2012) should look into adopting renewable energy. Countries that have adopted renewable energy have experienced economic growth (Wei et al., 2010). The need for using biocomponents in our environment for the production of alternative sources of renewable energy and a cleaner environment is increasing globally because of its renewable tendencies (Apergis and Payne, 2010). Using avocado as a source of renewable energy will be beneficial. Renewable energy helps to create job opportunities (Wei et al., 2010) and can help improve a country's economy (Domac et al., 2005). Biodiesel is totally biodegradable, non-toxic, relatively low flammability and has a higher flash point than fossil diesel, and reduced harmful emissions (Berggmann et al., 2006).

In conclusion, avocado as a source of renewable energy has lots of potential. Among its many applications, avocado has been used for food, in skin care, and it also has great potential in oil production. It is necessary to explore further of biodiesel production from avocado.

REFERENCES

Anastopoulos, G., Zannikou, Y., Stournas, S., and Kalligeros, S. Transesterification of vegetable oils with ethanol and characterization of the key fuel properties of ethyl esters. Energies 2009, 2, 362 – 376.

Aly, S. M. Degumming of soybean oil. Grasas y Aceites 1992, 43.

Apergis, N., and Payne, J. E. Renewable energy consumption and economic growth: Evidence from a panel of OECD countries. Energy Policy 2010, 38, 656 – 660.

Avhad, M. R., and Marchetti, J. M. Temperature and pretreatment effects on the drying of Hass avocado seeds. Biomass and Bioenergy 2015, 83, 467 – 473.

Bamgboye, A. I., and Hansen, A. C. Prediction of cetane number of biodiesel fuel from the fatty acid methyl ester (FAME) composition. International Agrophysics 2008, 22, 21 -29.

Baohua,Z., Yanqing, W., Hong, X., and Zhiping, M. Enzyme immobilization for biodiesel production. Applied Microbiology and Biotechnology 2012, 93, 61 – 70.

Bassham, J. A., Benson, A. A., and Calvin, M. Path of carbon in photosynthesis. Journal of Biological Chemistry 1950, 185, 781 – 787.

Berasategi, I., Barriuso, B. Ansorena, D., and Astiasaran, I. Stability of avocado oil during heating: Comparative study to olive oil. Food Chemistry 2012, 132, 439-446.

Berggmann, A., Hanley, N., and Wright, R. Valuing the attributes of renewable energy investments. Energy Policy 2006, 34, 1004 – 1014.

Bora, P. S., Narain, N., Rocha, R. V. M., and Paulo, M. Q. Grasas y Aceites 2001, 52, 171 – 174.

Bowen, D. Effect of fatty acid structure on biodiesel. Biodiesel is good. October 18, 2010.

Chen, L., Liu, T., Zhang, W., Chen, X., and Wang, J. Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresource Technology 2012, 111, 208 – 214.

Chen, C. Y., Yeh, K. L., Aisyah, R., Lee, D. J., and Chang, J. S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. Bioresource Technology 2011, 102, 71 – 81.

Chemat, F., Tomao, V, and Virot, M. Ultrasound – assisted extraction in food analysis. Handbook of Analytical Instruments 2008, 201, 96 -99

Christie, W. W. Methyl esters of fatty acids. AOCS Annual Meeting and Industry Showcases, Orlando, Florida, USA, April 30 – May 3, 2017: The American Oil Chemists' society, AOCS foundation, Illinois, USA.

Costagli, G., and Betti, Matteo. Avocado oil extraction processes: method for cold-pressed high-quality edible oil production versus traditional production. Journal of Agricultural Engineering 2015, Vol. 46: 467, 115 – 122.

Dang, T. H., Chen, B. H., and Lee, D. J. application of kaolin based catalyst in biodiesel production via transesterification of vegetable oils in excess methanol. Bioresource Technology 2012, 145, 175 – 181.

Domac, J., Richards, K., and Risovic, S. Socio-economic drivers in implementing bioenergy projects. Biomass and Bioenergy 2005, 28, 97 – 106.

Ehinomen, C., and Adeleke, A. An assessment of the distribution of petroleum products in Nigeria. E3 Journal of Business Management and Economics 2012, 3, 232 – 241.

Giakoumis, E. G., Rakopoulos, C. D., and Rakopoulos, D. C. Assessment of NOx emissions during transient diesel engine operation with biodiesel blends. Journal of Energy Engineering 2014, 140. http://dx.doi.org/10.1061/(ASCE)EY.1943-7897.0000136

Gopinath, A., Puhan, S., and Nagarajan, G. Effect of unsaturated fatty acid esters of biodiesel fuels on combustion, performance and emission characteristics of a DI diesel engine. International Journal of Energy and Environment 2010, 1, 411 – 430.

Halder, S. K., Gosh, B. B., and Nag, A. Studies on the comparison of performance and emission characteristics of a diesel engine using three degummed non-edible vegetable oils. Biomass and Bioenergy 2009, 33, 1013 – 1018.

Huber, G. W., Iborra, S., and Corma, A. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. Chemistry Review 2006, 106, 4044 – 4098.

Johannson, T. B., and Burnham, L. Renewable energy: Sources for fuels and electricity. Island Press 1993, 1993, 4 – 44.

Joshi, R. M., and Pegg, M. J. Flow properties of biodiesel fuel blends at low temperatures. Fuel 2007, 86, 143-151.

Kaiser, C., Smith, M. T., and Wolstenholme, B. N. Overview of lipids in the avocado fruit, with particular reference to the Natal Midlands. SouthAfrican Avocado Growers' Association Yearbook 1992, 15, 78 -82.

Knothe, G. Avocado and olive oil methyl esters. Biomass and Bioenergy 2013, 58, 143-148.

Laurens, L. M. L., Quinn, M., Wychen, S. V., Templeton, D. W., and Wolfrum, E. J. Accurate and reliable quantification of total microalgal fuel potential as fatty acid methyl esters by in situ transesterification. Analytical Bioanalytical Chemistry 2012, 403, 167 -178.

Leray, C. Fatty Acid Structure. Cyberlipid center 2017, 1 – 20. http://www.cyberlipid.org/cyberlip/home0001.htm

Lin, C. Y., and Lin, Y. W. Fuel characteristics of biodiesel produced from a high-acid oil from soybean soapstock by supercritical-methanol transesterification. Energies 2012, 5, 2370 – 2380.

Luque de Castro, M. D., and Garcia Ayuso, L. E. Soxhlet Extraction. Environmental Applications 2000, 3, 2701 – 2709.

Ma, F., amd Hanna, M. A. Biodiesel production: a review. Bioresource Technology 1999, 70, 1 – 15.

Marenchino, R., Pagliero, C., and Mattea M. Vegetable oil degumming using inorganic membranes. Desalination 2006, 200, 562 – 564.

Meyer, M., D., and Terry, L.A. Development of a rapid method for the sequential extraction and subsequent quantification of fatty acids and sugars from avocado mesocarp tissue. Journal of Agriculture and Food Chemistry 2008, 58, 7439-7445.

Mooz, E. D., Gaiano, N. M., Shimano, M. Y. H., Amancio, R. D., and Spoto, M. H. F. Physical and chemical characterization of the pulp of different varieties of avocado targeting oil extraction potential. Food Science and Technology 2012, 32, 1 – 9.

Nakamura, H., Yanagihara, Y., Sekiguchi, H., Kamada, Ohtani, Y., Kariya, S., Uchino, K., Suzuki, H., and Iga, T. Effect particle size on mixing degree and dispensation. The Pharmaceutical Society of Japan 2004a, 124, 135 – 139.

Nakamura, H., Yanagihara, Y., Sekiguchi, H., Kamada, F., Kawabata, H., Ohtani, M., Saitoh, Y., Kariya, S., Suzuki, H., Uchino, K., and Iga, T. Effect of mixing method and mixing degree during the preparation of triturations. The Pharmaceutical Society of Japan 2004b, 124, 127 – 134.

Ogunwusi, A. A., and Ibrahim, H. D. Economic significance of avocado pear in Nigeria. Developing Country Studies 2016, Vol. 6, No. 3.

Oyejide, T. A., and Adewuyi, A. O. Enhancing linkages of oil and gas industry in the Nigerian economy. Making the Most of Commodities Programme (MMCP) Discussion Paper No. 8, March 2011.

Patel VR, Dumancas GG, Kasi Viswanath LC, Maples R, Subong BJJ. Castor Oil: Properties, Uses, and Optimization of Processing Parameters in Commercial Production. Lipid insights. 2016;9:1-12. doi:10.4137/LPI.S40233.

Qin, X., and Zhong, J. A review of extraction techniques for avocado oil. Journal of Oleo Science 2016, 65, 881 -888.

Rachimoellah, H. M., Resti, D. A., Zibbeni, A., and Susila. D.W.. Production of biodiesel through transesterification of avocado (*Persea gratissima*) seed oil using base catalyst. Jurnal Teknik Mesin 2009, 11, 85 – 90.

Reddy, M., Moodley, R. and Jonnalagadda, S. B. Fatty acid profile and elemental content of avocado (*Persea americana* Mill.) oil – effect of extraction methods. Journal of Environmental Science and Health 2012, 47, 529 - 537.

Sanusi, L. M. Growth prospects for the Nigerian economy. Igbinedion University Eighth Convocation Ceremony, Okada, Edo state. November 26, 2012.

Schachter, J. B., and Harden, T. K. An examination of deoxyadenosine 5′ (α-thio) triphosphate as a ligand to define P2Y receptors and its selectivity as a low potency partial agonist of the $P2Y_1$ receptor. British Journal of Pharmacology 1997, 121, 338 – 344.

Shafiee, S., and Topal, E. When will fossil fuel reserves be diminished? Energy Policy 2009, 37, 181 – 189.

Speight, J. G. Handbook of petroleum refining. CRC Press 2014, 10 - 100

Topare, N. S., Raut, S. J., Renge, V. C., Khedkar, S. V., Chavan, Y. P., and Bhagat, S. L. Extraction of oil from algae by solvent extraction and oil expeller method. International Journal of Chemical Sciences 2011, 9, 1746 – 1750.

Wei, M., Patadia, S., and Kammen, D. M. Putting renewables and energy efficiency to work: How many jobs can the clean energy industry generate in the US? Energy Policy 2010, 38, 919 – 931.

Williams, A., Burton, J., Christensen, E., McCormick, R. L., and Tester, J. Emissions from various biodiesel sources compared to a range of diesel fuels in DPF equipped diesel engines. ASME 2011 Internal Combustion Engine Division Fall Technical Conference, Morgantown, virginia, USA, October 2 – 5, 2011. Paper No. ICEF2011-60106, 223 – 241.

Wu, H., Colson, G., Escalante, C., and Wetzstein, M. An optimal U.S. biodiesel fuel subsidy. Energy Policy 2012, 48, 601 – 610.

Wustenhage, R., Wolsink, M., and Buer, M. J. Social acceptance of renewable energy innovation: An introduction to the concept. Energy Policy 2007, 35, 2683 – 2691.

Zhao, S. Analysis of fatty acid methyl esters by Agilent 5975T LTM GCMS. Agilent Technologies 2012, 1 – 6.

Zufarov, O., Schmidt, S., and Sekretar, S. Degumming of rapeseed and sunflower oils. Acta Chimica Slovaca 2008, 1, 321 – 328.

Endocarp Batch	Volume of Hexane Solvent (ml)	Volume Extract Obtained (ml)	Mass of Endocarp Wet with Hexane (g)	Mass of Dried Endocarp (g)
1	100.00	87.00	52.23	32.38
2	150.00	52.00	43.80	35.75
3	150.00	110.00	40.72	37.48
4	150.00	88.00	37.54	31.25

Table 1. The quantities of avocado endocarp subjected to Soxhlet extraction and their products.

Beaker Number	Volume of Hexane Solvent (ml)	Volume Extract Obtained (ml)
1	100.00	30.00
2	95.00	50.00
3	155.00	80.00
4	100.00	70.00
5	115.00	65.00

(a)

Beaker Number	Volume of Hexane Solvent (ml)	Volume Extract Obtained (ml)
1	80.00	20.00
2	100.00	75.00
3	120.00	50.00
4	100.00	50.00
5	100.00	65.00

(b)

Table 2. (a) First-fold trituration and (b) Second-fold trituration of oil data from avocado mesocarp.

Mesocarp Components	**Composition (%)**	**Endocarp Components**	**Composition (%)**
Δ^{9}-oleic acid	9.3	lauric acid	9.0
Δ^{9}-palmitoleic acid	6.1	Δ^{6}-oleic acid	9.0
$\Delta^{9,12}$-linoleic acid	9.7	$\Delta^{5,8,11,14}$-arachidonic acid	46.7
$\Delta^{5,8,11,14}$-arachidonic acid	11.3	$\Delta^{9,12,15}$- linolenic acid	3.3
myristic acid	17.4	$\Delta^{9,12}$- linoleic acid	16.4
$\Delta^{8,11,14}$- linolenic acid	15.0	Δ^{9}-oleic acid	15.6
$\Delta^{9,12,15}$- linolenic acid	22.0	*NA*	*NA*
palmitic acid	4.5	*NA*	*NA*
stearic acid	4.9	*NA*	*NA*

Table 3. Fatty acid composition of oil analyzed from avocado mesocarp tissue and avocado endocarp following trituration and Soxhlet extractions. Based on weight (g) of endocarp and mesocarp from 13 replicate fruits that were 136.90g and 438.69g.

Mesocarp Components	**Retention Times (min)**	**Endocarp Components**	**Retention Times (min)**
Δ^{9}-oleic acid methyl ester	16.2	stearic acid methyl ester	16.2
Δ^{9}-palmitoleic acid methyl ester	16.3	lauric acid methyl ester	22.2
$\Delta^{9,12}$-linoleic acid methyl ester	18.4	Δ^{6}-oleic acid methyl ester	16.2
$\Delta^{5,8,11,14}$-arachidonic acid methyl ester	20.0	$\Delta^{5,8,11,14}$-arachidonic acid methyl ester	20.0
myristic acid methyl ester	17.5	$\Delta^{9,12,15}$- linolenic acid methyl ester	17.9
$\Delta^{8,11,14}$- linolenic acid methyl ester	18.8	$\Delta^{9,12}$- linoleic acid methyl ester	17.5
$\Delta^{9,12,15}$- linolenic acid methyl ester	17.9	*NA*	*NA*
palmitic acid methyl ester	22.6	*NA*	*NA*
stearic acid methyl ester	9.8	*NA*	*NA*

Table 4. Retention times of fatty acid methyl ester components of oil analyzed from avocado mesocarp tissue and avocado endocarp following trituration and Soxhlet extractions. Based on weight (g) of endocarp and mesocarp from 13 replicate fruits that were 136.90g and 438.69g.

Mesocarp Components	**m/z**	**Endocarp Components**	**m/z**
Δ^9-oleic acid methyl ester	397, 380, 369, 344, 330, 314, 282, 264, 253, 234, 222, 203, 175, 151, 137, 111, 97, 83, 55, 41	lauric acid methyl ester	399,378, 357, 346, 334, 304, 281, 270, 245, 236, 207, 191, 169, 153, 138, 122, 95, 83, 55, 44
Δ^9-palmitoleic acid methyl ester	398, 377, 357, 341, 311, 282, 264, 225, 206, 187, 167, 152, 138, 111, 97, 83, 55, 44	Δ^6-oleic acid methyl ester	282, 264, 246, 235, 222, 193, 180, 151, 137, 111, 97, 83, 55, 41

(a)

Mesocarp Components	**m/z**	**Endocarp Components**	**m/z**
$\Delta^{9,12}$-linoleic acid methyl ester	264, 235, 207, 193, 179, 151, 137, 110, 95, 81, 67, 41	$\Delta^{5,8,11,14}$-arachidonic acid methyl ester	318, 262, 249, 235, 217, 203, 175, 149, 135, 121, 95, 79, 67, 41
$\Delta^{5,8,11,14}$-arachidonic acid methyl ester	318, 247, 233, 203, 175, 150, 133, 119, 91, 79, 67, 41	$\Delta^{9,12,15}$- linolenic acid methyl ester	387, 372, 351, 319, 289, 274, 248, 234, 207, 195, 165, 151, 133, 117, 95, 81, 44, 41

(b)

Mesocarp Components	**m/z**	**Endocarp Components**	**m/z**
myristic acid methyl ester	399, 389, 358, 346, 327, 315, 281, 269, 253, 227, 213, 199, 171, 157, 143, 115, 101, 88, 55, 43	$\Delta^{9,12}$- linoleic acid methyl ester	280, 256, 222, 196, 167, 151, 137, 110, 95, 81, 67, 41
$\Delta^{8,11,14}$- linolenic acid methyl ester	306, 288, 249, 235, 208, 189, 177, 150, 135, 121, 93, 79, 67, 41	Δ^{9}-oleic acid methyl ester	395, 384, 359, 341, 331, 318, 282, 264, 247, 232, 207, 178, 152, 137, 111, 97, 83, 55, 41

(c)

Mesocarp **Components**	**m/z**	**Endocarp** **Components**	**m/z**
$\Delta^{9,12,15}$- linolenic acid methyl ester	396, 384, 361, 342, 325, 310, 295, 281, 265, 244, 225, 217, 203, 193, 174, 161, 143, 135, 117, 110, 94, 81, 69, 44, 40, 35	*NA*	*NA*
palmitic acid methyl ester	256, 227, 213, 199, 171, 157, 129, 115, 97, 73, 60, 43	*NA*	*NA*

(d)

Mesocarp Components	**m/z**	**Endocarp Components**	**m/z**
stearic acid methyl ester	400, 386, 364, 341, 307, 281, 269, 253, 238, 209, 189, 149, 135, 123, 95, 85, 57, 43	*NA*	*NA*

(e)

Table 5. Mass to charge ratio (m/z) of fatty acid methyl ester components of oil analyzed from avocado mesocarp tissue and avocado endocarp (a, b, c, d, e) following trituration and Soxhlet extractions. Measures were based on weight (g) of endocarp and mesocarp from 13 replicate fruits that were 136.90 g and 438.69 g.

Endocarp **Minor Components**	**Mesocarp** **Minor Components**
Ascorbic acid	11- Hexadecynal
Tetrapentacontane	Ethylisoallocholate
11- Hexadecynal	Dichloracetic acid
Nonadecatriene	7- Tetradecenal
7-Heptadecene	7- Hexadecenal
9-Tetradecen-1-ol acetate	8, 11, 14 Docosatrienoic acid
Cis-7,10- hexadecadienal	Bicyclo (5.2.0) nonane
Oct-5-en-2-ol	Di (1-decynyl) mercury
Dodecedienylacetate	2 -cyclohexene-1-carboxaldehyde
1- Octadecyne	1,2 15,16 diepoxyhexadecane
Beta-santalol	2,6 Octadiene-1,8-diol
10-oxocyclodec-2-enecarboxylic acid	Cyclopropaneoctanoic acid
7- hexadecadienol	*NA*
14- Octadecenal	*NA*
Cyclotetradecatriene	*NA*
Trichloroacetic acid	*NA*

Table 6. Additional components in oil analyzed from avocado mesocarp tissue following trituration, endocarp tissue following Soxhlet extraction. Note: retention times of the minor components are not listed due to their relative low concentrations.

Mesocarp Components	**Retention Times (min)**	**Endocarp Components**	**Retention Times (min)**
heptanoic acid methyl ester	4.5	cyclopentaneundecanoic acid methyl ester	3.1
octanoic acid methyl ester	6.8	hexanoic acid methyl ester	4
nonanoic acid methyl ester	11.7	*NA*	*NA*
nonanedioic acid methyl ester	10	NA	NA
4- decenoic acid methyl ester	20	*NA*	*NA*
decanoic acid methyl ester	20.7	*NA*	*NA*
heptanedioic acid methyl ester	23.6	*NA*	*NA*
octanedioic acid methyl ester	43.7	*NA*	*NA*

Table 7. Retention times of other fatty acid methyl ester components of oil analyzed from avocado mesocarp tissue and avocado endocarp following trituration and Soxhlet extractions.

Mesocarp Components	Retention Times (min)
hexanoic acid methyl ester	3.1
hexanedioic acid methyl ester	13.1
heptenoic acid methyl ester	40
8- nonenoic acid methyl ester	11.2
cyclopentaneundecanoic acid methyl ester	20
undecanoic acid methyl ester	21

Table 8. Retention times of some other fatty acid methyl ester components of oil analyzed from avocado mesocarp tissue.

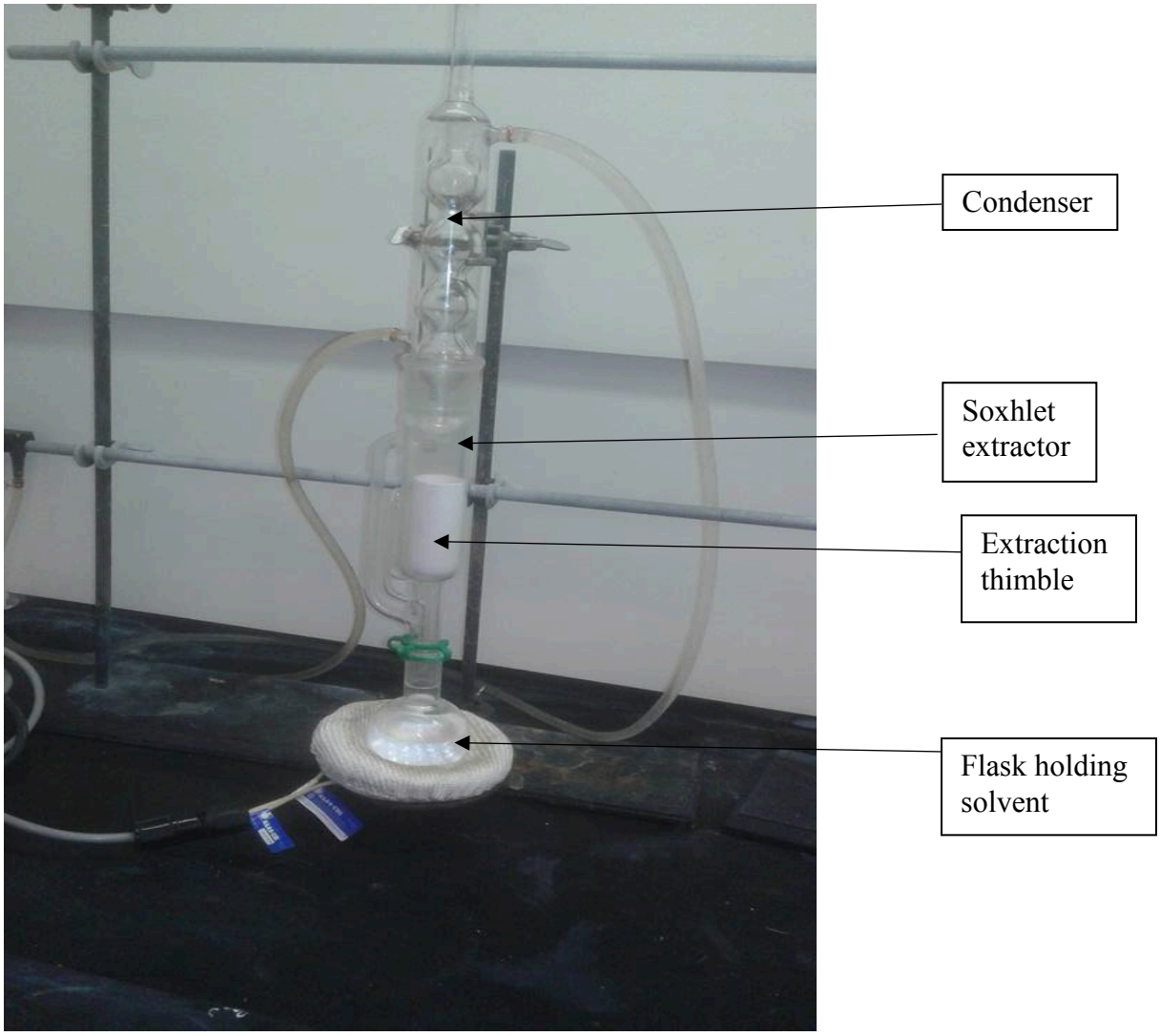


Figure 8. The Soxhlet extraction set up is comprised of a condenser (top), Soxhlet extractor (middle), an extraction thimble to hold the sample (inside the Soxhlet extractor), and a 200 ml round bottom flask (bottom) that holds the hexane extraction solvent. Lubriseal was used to grease the glassware in parts where there is a conjoining.

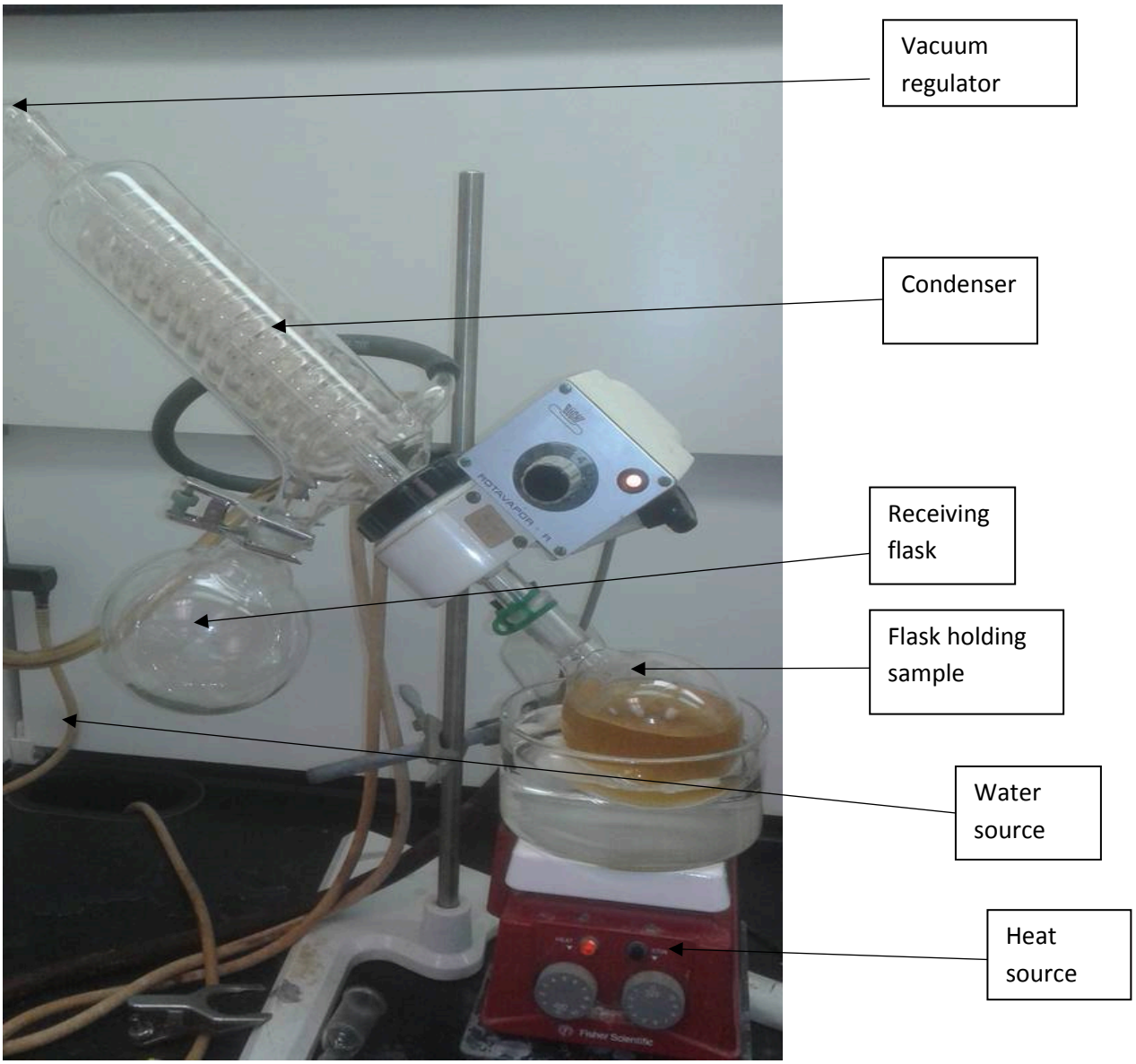


Figure 9. The rotovap set up is comprised of a condenser (top), a receiving flask (flask on the left), a round bottom flask that holds the sample (on the right), a heat source, a vacuum source, a water source, and a regulator of the rotary movement.

(a)

(b)

Figure 10. The trituration process used in the study, with avocado mesocarp dissolved in hexane (a) and divided into five portions (b).