SOXHLET EXTRACTION OF PHILIPPINE AVOCADO FRUIT PULP VARIETY 240

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Abstract: Avocado *(Persea Americana)* is a fruit that contains a high value of oil and chlorophyll concentration. There had been many studies regarding the extraction and optimization of the avocado pulp oil yield. Those studies showed that Soxhlet Extraction proved to be the most practical process in extracting avocado oil. This study investigated the oil yield and oleic acid content of the avocado oil extracted using Soxhlet extraction with hexane and ethanol as solvents at different extraction times and amounts of solvents. The physical and chemical properties of the oil were determined too. The variety 240 of the Philippine avocado pulp was peeled, destoned and underwent freeze drying. After which it was dried, ground and accurately measured to 30 grams per experimental run. The constant sample mass was extracted using n-Hexane and 96% ethanol as solvents at varying amount, specifically 250ml and 350ml. The extracted oil was then separated using a rotary evaporator. Oil yield was computed and then analyzed for its oleic acid content using gas chromatography. Best conditions were concluded from the preliminary experiments and were replicated to further stress the effects of the varying conditions. The highest oil content obtained using ethanol was 73.56% (w/w) while 40.33% (w/w) was obtained using hexane. The total moisture content was 89.387%. Oleic acid was found to be the most dominant fatty acid in both solvents. With the use of ethanol, oleic acid had a maximum percentage of 41.91% while 43.37% for hexane.

Key Words: Avocado Fruit Pulp; Soxhlet Extraction; Oil Yield; Oleic Acid

1. INTRODUCTION

Persea Americana, commonly known as Avocado, is a fruit that contains a high value of oil and chlorophyll concentration which when extracted results to an oil of emerald green color (JAOCS, 1988). There had been many studies regarding the extraction of avocado pulp. Most of these have indicated the high amount of oleic acid present in avocado. Oleic acid is said to be an effective aid to lower serum cholesterol levels and low-density lipoproteins in the human body (Alvarado et al., 2003). Studies have been tested on the extraction and optimization of oleic acid, such as Microwave

Assisted Solvent Extraction, Supercritical Carbon Dioxide Extraction and Centrifugal Extraction. Among all of these, based on De Castro (2010) and Wang (2006) studies, it is said that Soxhlet Extraction is the simplest, cheapest and most practical process in extracting avocado oil. Using the other studies obtained as basis, there were certain generalizations that were observed. In the study of Mostert (2007), she concluded that ripe fruit yields higher oil compared to unripe fruit. The study of Werman (1987) supplemented the observation made by Mostert for the same reasons as they mentioned that the last few months of fruit ripening (peak ripening period), maximum capacity of the oil content of avocado as well as its oleic acid content can be obtained. Additionally it was supported that freeze drying is the best method for drying due to its superior benefits (Mostert, 2007 & Wang, et al., 2010).

The solvent extraction using n-hexane and 96% ethanol was executed in this study primarily to determine the best conditions that would give highest oil yield with highest oleic acid content using freeze dried Philippine variety (240) avocado flesh pulps. In addition to that, there was also the investigation of the best optimization parameters needed in order to further improve the said process. The crude extracts obtained were analyzed through Gas Chromatography.

2. METHODOLOGY

The avocado fruits obtained from a farm in Batangas were left to ripen for 5-7 days at a room temperature of 25 °C (Villa-Rodriguez, 2010 and Ozdemir, 2003). The seed, seed coat and skin were removed. The pulp of the ripened fruit was cut into smaller pieces and was placed in ice bags. It was then left in the freezer for a couple of days to be frozen. The frozen avocado samples were set into the freeze dry system for a couple of days until all of its moisture content was removed. The moisture content (Werman et al, 1987) of the sample was obtained by calculating for the water lost upon the drying process.

$$\text{Moisture content (\%): } W = 100(M_o - M)/M_o \qquad \text{(Eq. 1)}$$

where:

M	=	the final weight of the dried sample
M_o	=	initial weight of the fresh sample

The freeze-dried avocados were then ground with the use of mortar and pestle, weighed, and contained in a sealed vessel to reduce unnecessary changes. Extraction (Fig. 1) was performed with the parameters used listed as controlled variables and constants shown in

Table 1. The varying conditions were determined from the preliminary runs in which further experiments were done to optimize the best conditions for each of the solvent used.

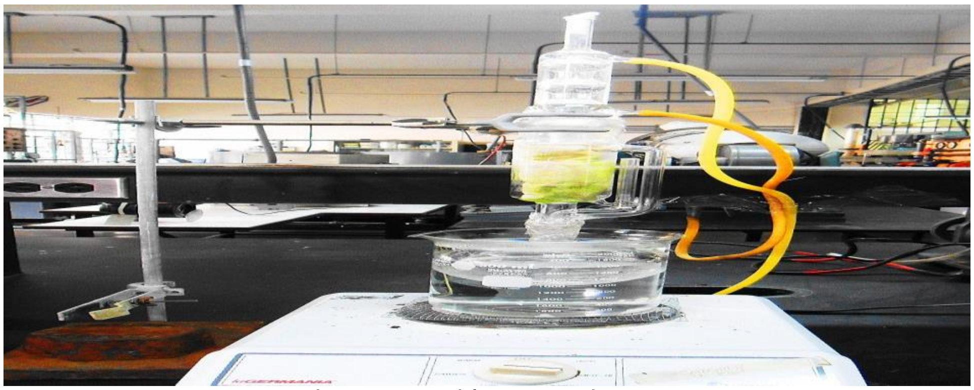

Figure 1. Soxhlet Extraction Set-up

From the preliminary runs, the response of the sample to n-hexane is faster than that of ethanol hence giving 1 and 2 hours for n-hexane's high and low parameter and 4 and 8 hours for ethanol. The oil extracted using n-hexane showed to be better at an extraction time of 2 hours. This may be due to the rapid solubility of the oil present on the facade of the powdered sample and higher mass transfer driving force since the concentration of the oil present in the fresh solvent is minimal. Accordingly, the highest oil yield using ethanol as solvent was obtained at an extraction time of 8 hours. Same analogy to that of n-hexane, exposing the sample longer to the solvent produces higher oil yield. The properties of hexane allows it to extract oil yield at a shorten time compared to ethanol thus the shortened extraction times. In contrast, due to ethanol's properties, it renders the solvent to longer extraction time but allows it to extract higher oil yield compared to Hexane. Also it was shown on the study made by Saxena, et al., (2011) that longer extraction times decrease the amount of oil yield for hexane and leaves the sample with wastes while for Ethanol longer extraction times allows it to extract higher oil yield.

Table 1. Constants and Variables

Constants For Hexane and Ethanol		**Variables**		
Freeze drying period	3 days	Solvent used	n-Hexane	96% Ethanol
Extraction Temperature	100°C	Amount of Solvent	250 ml and 350 ml	250 ml and 350 ml
Appearance of sample	Powdered	Extraction Time	1 & 2 hours	4 & 8 hours
Grams of sample (per run)	30 grams			
Drying process of the fruit	Freeze dried			
Freeze drying temperature	-40°C			
Shape of boiling flask	Round bottomed flask			
Size of the boiling flask	1000 ml			

After extraction was done, the crude oil samples were then placed to the rotary evaporator to remove the remaining solvent. The oil yield was computed as,

!"# !"#$%&#!' (Eq. 2)

where: %= !"#$!" !"#$%& ×100

gram of sample = 30 grams of freeze-dried avocado pulp

The oil samples obtained were tested for the following physical and chemical properties: pH, specific gravity, refractive index, acid value, free fatty acids and iodine value. The components of the crude avocado oil were also analyzed through Gas Chromatography (GC).

3. RESULTS AND DISCUSSION

In any extraction process, the oil yield and quality of oil is affected by the moisture content of the raw material. In this study, the moisture content of avocado pulp was measured by the amount of water evaporated from freeze dryer. The total moisture content of the sample fruits is 89.387 % (w/w). In contrast, fresh avocado pulp is not suitable to use in Soxhlet extraction because it contains a lot of moisture, thus no oil can be extracted. In choosing the best conditions, several factors were considered: the oil yield percentage, the fatty acid profile and the physical and chemical properties of the oil.

Table 2. Runs for Determination of Percentage of Oil Yield for Hexane and Ethanol

Solvent	Amount of Solvent in ml	Extraction hour	Extraction Temperature ^{o}C	Grams of dried sample	Oil Yield in %
Hexane	250	1	100	30	30
Hexane	250	2	100	30	**32**
Hexane	350	1	100	30	26.33
Hexane	350	2	100	30	29.67
Ethanol	250	4	100	30	24
Ethanol	250	8	100	30	53.33
Ethanol	350	4	100	30	36.33
Ethanol	350	8	100	30	**66.0**

Table 3. Replication of Best Conditions for Hexane and Ethanol

Solvent	Amount of Solvent in ml	Extraction hour	Extraction Temperature ^{o}C	Grams of dried sample	Oil Yield in %
Hexane	250	2	100	30	**40.33**
Hexane	350	1	100	30	39.22
Ethanol	250	8	100	30	52.11
Ethanol	350	8	100	30	**73.56**

Table 2 shows the highest oil yield using hexane as solvent, under the conditions of 250 ml solvent extracted for 2 hours at 100°C, is 32% (w/w). While for ethanol, 66% (w/w), under the conditions of 350 ml solvent extracted for 8 hours at 100°C. For hexane, it is shown that lower amount of solvent and at a longer period of time, generates higher oil yield. Hexane's properties allow it to vaporize quickly and heat up faster. Lower oil yield of hexane is due to its low boiling point (60^{o}C) and low dielectric constant (1.89) therefore it is only mildly capable to produce higher heat. On the other hand ethanol exhibited higher oil yield for higher amount of solvent and at longer period of time.

Ethanol has a strong solubilizing capability, and high electro negativity with a dielectric constant of 24.3, which make it capable to heat faster than hexane. Also, it has a hydroxyl group present and shorter carbon chain rendering oil yield to be extracted easier (“Ethanol and Hexane”, 2011). Table 3 shows the highest oil yield obtained for ethanol with 73.56% (w/w) while 40.33% (w/w) for hexane, when replicated. Table 3 supports the values obtained from Table 2 and verifying even further that the parameters chosen from Table 2 were indeed the best conditions that can give the highest oil yield content for both solvents.

These results were comparable with that of the works of Ortiz et al. (2004) who obtained a 50%; Mostert (2007), 31% and Villa-Rodriguez (2010), 21% yield with hexane as solvent with almost similar conditions and methods applied except for the variety of avocado used. The same can be said for ethanol when compared to the studies of Werman (1987), with 52.1% and Adama, K.K. & Edoga, M. (2011), 35.9%. In addition, same observations were noted too by Botha, B.M. (2004) on the importance of the extraction time and temperature used. Higher oil yield can be obtained at longer extraction time which was verified when the time was increased to 10 hours for ethanol as solvent (data not shown).

Table 4. Replication of Best Conditions of Fatty Acids for Hexane and Ethanol

Solvent	Amount of Solvent in ml	Extraction hour	Oil Yield in %	Palmitic Acid	Palmitoleic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid
Hexane	250	2	**40.33**	27	9.63	**43.37**	17.26	2.22
Hexane	350	1	39.22	26.66	10.15	44.68	16.61	1.45
Ethanol	250	8	52.11	25.54	10.95	43.18	18.21	1.6
Ethanol	350	8	**73.56**	24.42	12.58	**41.91**	19.14	1.58

During replication of these experimental runs, the physical and chemical properties were now considered. Table 4 shows the obtained fatty acid values from the best conditions chosen as given in Table 3. Oleic acid was found to be the most dominant fatty acid in both solvents. With the use of ethanol, oleic acid had a maximum value of 41.91% while for hexane, 43.37%. Although there were higher oleic acid contents generated for both hexane and ethanol in terms of the other conditions, their differences were not so significant. As seen in Table 4, in terms of oil yield, ethanol seems to be a better solvent than hexane, although hexane needs shorter time to extra the desired oil.

Compared to the study made by Adama, K.K. & Edoga, M. (2011), the % oleic acid obtained was 79%, which was higher than this study (43.37% and 41.91% for hexane and ethanol. respectively). In contrast, both the Palmitic (7%) and Linoleic acid (13%) contents were lower in their study. These differences may be due to certain factors. Firstly, the boiling point used in the study of Adama was 78 ^{o}C (for ethanol); second, oven drying was used instead of freeze-drying. Another factor was the extraction time used which was shorter than this study. This implies that the oleic acid is highly sensible to the heat that is generated at longer period of extraction.

Physical and chemical properties of avocado oil extract, such as its pH, specific gravity, refractive index, acid value and iodine value were also obtained. Based on the best conditions chosen for hexane: 5.32 (pH), 0.7563 (sp. gravity), 1.432, refractive index (RI), 4.51, acid value (AV), and 63.86 iodine value (IV). While for ethanol: 5.27 (pH), 0.8946 (sp. gravity), 1.454 (RI), 2.22(AV), and 30.98 (IV). According to the range of values given by Requijo-Tapia (1999), all the oils are acidic; however the oil with the lowest acid value (highest quality of oil) is from the 8-hour ethanol extraction. Considering all other factors, it can be concluded that 8-hour ethanol extraction is a good choice for a solvent.

Based on all the data obtained from the studies of Ozdemir & Topuz, (2003) & Mostert, M. E. (2007) and from the results of this study, it was noted that the difference in the values of oil yield content and oleic acid is due to different factors. First, total percentage of oil yield and oleic acid content from avocado pulp is influenced by the varying harvest and ripening periods used, fruit pre-treatment, and varieties of the fruit used. Secondly, the extraction method and the conditions used also prove to be a critical factor for the difference in the values, color, and quality of the oil.

4. CONCLUSIONS

This study investigated the crude oil obtained from the variety number 240 of the Philippine avocado pulp. Best conditions that resulted to high oil yield and high oleic acid content using hexane as solvent were 250 ml of solvent extracted for 2 hours with 36.17% oil yield and 43.46% oleic acid content (average values). On the other hand, for ethanol, 350 ml of solvent extracted for 8 hours resulted to 69.78% oil yield and 40.71% oleic acid content (average values). Both results showed that as the extraction time increases, the oil yield also increases as well as the oleic acid content. Purification of the oil extract is recommended so as to achieve a high quality oil and may be other new extraction method be applied in order to shorten the extraction time.

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