COMPARISON STUDY ON THE EXTRACTION OF *MORINGA OLEIFERA* SEEDS OIL BY SOXHLET AND MICROWAVE METHODS

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MOHD SYAHIRAN BIN YUSUF

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (gas technology)

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JAN 2015

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (gas technology).

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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DEDICATION

To my father Yusuf bin Hasan who always support and keep praying for my success and to my beloved mother, Zaharah bt Dollah for her caring and understand me. Then, lastly for my supervisor Dr Eman N. Ali who helped me to finish my thesis.

ACKNOWLEDGEMENT

I am grateful to Almighty Allah, for his mercifulness and blessing. I would like to express sincere thanks to my supervisor, Dr Eman N. Ali, for her help, advice, and continuous support and also for her guidance through an effective well-arranged weekly meeting. Her valuable comments, feedback, interest and encouragement are indeed appreciated.

I would like to express gratitude to all the staff and colleagues of the Universiti Malaysia Pahang, Gambang campus for their friendship, help and support. Thank you to all of my friends for their constant support and friendship.

Last but surely not least, to my parents and all of my siblings for understanding, sacrifices, unwavering support and motivation to enable me to complete the thesis.

ABSTRACT

Extraction is a separation process and in this work, *Moringa oleifera* seeds oil was extracted using two methods soxhlet and microwave to find out the highest yield and best properties of oil extracted. For the soxhlet, approximately 40g of dry *Moringa oleifera* seeds are crushed and placed in a soxhlet extractor fitted with 1 L round bottom flask and a reflux condenser. Extraction took place one hour with 0.3 L n-hexane followed by removal of the solvent using rotary evaporator to get the *Moringa oleifera* oil. For the microwave, 800g of *Moringa oleifera* were placed in 1 L flat bottom flask. After adding 80 ml of solvent, the flask containing the sample was introduced to the microwave oven and adjusted with the condenser connected to a cold water recirculation system. The power, time and pressure of microwave was set as 600 W, 20 minute, and 65°C respectively. The oil collected from both methods was compared by measuring the properties such as acid no, pH, density and specific gravity (SG). For the soxhlet method, the acid no is 6.37 mgKOH, pH is 6, and density is 821.9 kg /m^3 and SG of 0.8219. For the microwave method, the acid no is 5.80 mgKOH, pH is 6, and density is 590.7 kg /m^3 and SG of 0.5907. As a conclusion, oil extracted by soxhlet method showed better yield and properties. This might be because n-haxane was used in microwave. Therefore, it is recommended to use methanol or any other polar solvent to compare between two methods.

ABSTRAK

Pengekstrakan adalah satu proses pemisahan dan dalam kerja-kerja ini, minyak biji *Moringa oleifera* telah diekstrak menggunakan dua kaedah soxhlet dan ketuhar gelombang mikro untuk mengetahui hasil tertinggi dan hartanah terbaik minyak diekstrak. Untuk soxhlet, kira-kira 40g biji *Moringa oleifera* kering dihancurkan dan diletakkan di dalam pemerah soxhlet dilengkapi dengan 1 L pusingan kelalang dasar dan kondenser refluks. Pengekstrakan berlaku satu jam dengan 0.3 L n-heksana diikuti oleh penyingkiran pelarut dengan menggunakan penyejat berputar untuk mendapatkan minyak *Moringa oleifera*. Untuk gelombang mikro, 800g daripada *Moringa oleifera* diletakkan dalam 1 L bahagian bawah rata kelalang. Selepas menambah 80 ml pelarut, kelalang yang mengandungi sampel yang telah diperkenalkan kepada ketuhar gelombang mikro dan diselaraskan dengan pemeluwap berhubung dengan sistem kitaran semula air sejuk. Kuasa, masa dan tekanan gelombang mikro telah ditetapkan sebagai 600 W, 20 minit, dan 65 ° C masing-masing. Minyak yang dikumpul daripada kedua-dua kaedah telah dibandingkan dengan mengukur sifat-sifat seperti asid tidak, pH, ketumpatan dan graviti tentu (SG). Untuk kaedah soxhlet, asid tiada 6.37 mgKOH, pH adalah 6, dan ketumpatan adalah 821,9 kg / m3 dan SG daripada 0,8219. Untuk kaedah gelombang mikro, asid tiada 5.80 mgKOH, pH adalah 6, dan ketumpatan adalah 590,7 kg / m3 dan SG daripada 0,5907. Kesimpulannya, minyak yang dikeluarkan oleh kaedah soxhlet menunjukkan hasil yang lebih baik dan sifat-sifat. Ini mungkin kerana n-haxane telah digunakan dalam ketuhar gelombang mikro. Oleh itu, adalah disyorkan untuk menggunakan metanol atau sebarang pelarut kutub lain untuk membandingkan antara dua kaedah.

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1 *INTRODUCTION*

1.1 *Motivation and problem statement*

Use of microwave energy in the chemical laboratory was described for the first time in 1986 simultaneously by Gedye and Giguerre (Gedye et al., 1986) in organic synthesis and by Ganzler and Lane and Jenkins (Lane and Jenkins, 1984) in extraction of biological samples for the analysis of organic compounds. Since then, numerous laboratories have studied the synthetic and analytical possibilities of microwaves as a non-classical energy source. Today, over 1000 and 500 articles have been published, on the subject of microwave synthesis and extraction (Pare, 1992).

Microwaves are a form of non-ionizing electromagnetic energy at frequencies ranging from 300 MHz to 300 GHz. This energy is transmitted as waves, which can penetrate in biomaterials and interact with polar molecules into materials, such as water to generate heat (Takeuchi et al., 2009). Application of microwaves in partition and extraction forms has appeared to decrease both extraction time and volume of solvet, minimizing natural affect by discharging less CO_2 in air (Lucchesi et al., 2004; Ferhat et al., 2006) and consuming only a fraction of the energy used in conventional extraction methods such as steam distillation, (Farhat et al., 2009).

Advances in microwave-assisted extraction (MAE) have led in the development of various techniques such as compressed air microwave distillation (CAMD), vacuum microwave hydro distillation (VMHD), microwave hydro distillation (MWHD), solvent-free microwave extraction (SFME), microwave accelerated steam distillation (MASD), microwave by hydro diffusion and gravity (MHG) (Farhat et al., 2009 ; Ying et al.,2013).

While a Soxhlet extractor is a laboratory apparatus invented in 1879 by Franz von Soxhlet (Jensen, 2007) It was originally designed for the extraction of a lipid from a solid material. It also has been used for a long time in leaching technique. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.

The main advantages of microwave assisted extraction over the conventional extraction techniques are reduced solvent consumption, shorter operational times, moderately high recoveries, good reproducibility and minimal sample manipulation for extraction process (Garcia-Ayuso et al., 1999)

1.2 *Problem statement*

Conventional method of oil extraction needs long time, high energy, and more solvent.

1.3 *Objective*

1. To extract oil from *Moringa oleifera* seeds using soxhlet extractor and microwave.
2. To compare the yield and properties of oil obtained.

1.4 *Scope of study*

1. This research will focus on comparison between methods of oil extraction.

1.5 *Main contribution of this work*

The main contribution of this work is to help choose the best method for oil extraction from *Moringa oleifera* seeds which can be considered a new natural product.

2.0 LITERATURE REVIEW

2.1 Moringa oleifera

Moringa oleifera (Moringaceae) known commonly as Ben oil tree or drumstick tree in English language,*'Okwe oyibo'* in Igbo, *'Gawara' or 'Habiwal'* in Hausa and*'Adagba maloye'* or '*Ewe Igbale*' in Yoruba grows rapidly in most regions and climatic conditions of Nigeria. *Moringa oleifera* is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics (Anwar et al., 2007). A number of medicinal properties have been ascribed to various parts of this tree. Most parts of this plant: root, bark, gum, leaf, fruit (pods) flowers, seeds and seeds oil have been used in folk medicine in Africa and South Asia (Fahey, 2005).

Besides that, *Moringa oleifera* also has been used for the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Morimitsu et al., 2000; Siddhuraju and Becker, 2003). The leaves, fruits, flowers and immature pods of this tree are edible and they form a part of traditional diets in many countries of the tropics and sub-tropics (Siddhuraju & Becker, 2003; Anhwange *et al.*, 2004). The leaves of *moringa oleifera* (Figure 1) are a good source of protein, vitamin A, B and C and minerals such as calcium and iron (Dahot, 1988).

Figure 1: Moringa oleifera leaves

Moringa oleifera figure 2 has been found to be a potential new source of oil especially with the advent of the need for oleo-chemicals and oils/fats derived fuels (Biodiesel) all over the world (Anwar et al., 2007). But due to lack of information on physical and chemical properties of the seed oil which has limited its applications, the need for cultivation of commercially viable crops for diversification of economy and poverty alleviation strategy of developing countries like Nigeria has made it imperative to take advantage of this economically important plant. The *Moringa oleifera* tree is shown in (Figure 3).

Figure 2: *Moringa oleifera* seeds

Figure 3: Moringa oleifera tree

2.2 Soxhlet extractor

A Soxhlet extractor is a laboratory apparatus invented in 1879 by Franz von Soxhlet (Jensen, 2007) It was originally designed for the extraction of a lipid from a solid material. It also has been used for a long time in leaching technique. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.

Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing of solid (thimble). The condenser ensures that any solvent vapour cools, and drips back down into solvent container like in (Figure 4).

Figure 4: Soxhlet extractor

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction, the solvent is removed typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

2.3 Microwave

The fundamentals of the microwave extraction (MAE) process are different from those of conventional methods (solid–liquid or simply extraction) because the extraction occurs as the result of changes in the cell structure caused by electromagnetic waves. In MAE, the process acceleration and high extraction yield may be the result of a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction (Chemat et al., 2009).

For conventional extractions the mass transfer occurs from inside to outside, although the heat transfer occurs from the outside to the inside of the substrate (Figure 5, Aguilera,2003). In addition, although in conventional extraction the heat is transferred from the heating medium to the interior of the sample, in MAE the heat is dissipated volumetrically inside the irradiated medium.

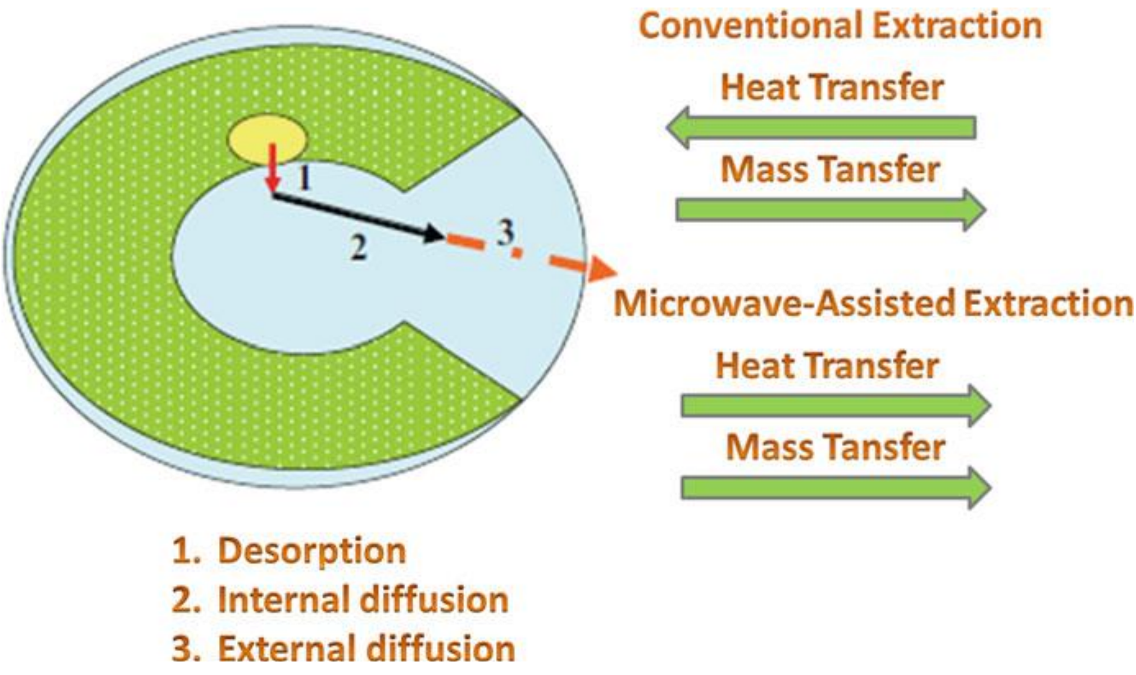

Figure 5 : Basic heat and mass transfer mechanism in microwave and conventional Extraction (Aguilera, 2003)

For the mechanism of microwave heating energy transfer occurs by two mechanisms: dipole rotation and ionic conduction through reversals of dipoles and displacement of charged ions present in the solute and the solvent (Routray and Orsat, 2011) . In many applications these two mechanisms occur simultaneously. Ionic conduction is the electrophoretic migration of ions when an electromagnetic field is applied, and the resistance of the solution to this flow of ions results in friction that heats the solution. Dipole rotation means rearrangement of dipoles with the applied field (Eskilsson and Björklund, 2000).

Energy transfer is the main characteristic of microwave heating. Traditionally, in heat transfer of the conventional process, the energy is transferred to the material by convection, conduction, and radiation phenomena through the external material surface in the presence of thermal gradients. In contrast, in MAE, the microwave energy is delivered directly to materials through molecular interactions with the electromagnetic field via conversions of electromagnetic energy into thermal energy (Thostenson and Chou, 1999).

The Microwave Assisted Extraction Process (Figure 6) is a high-speed method used to selectively extract target compounds from various raw materials. Microwave assisted extraction uses energy of microwave radiation to heat solvents quickly and efficiently. By using a closed system, extraction can be performed at higher temperatures and extraction times can be reduced drastically. It is an innovative solvent-extraction technology, offers a superior alternative to several thermal applications owing to its efficient volumetric heat production and has many advantages over conventional solid liquid extraction methods.

Figure 6: Microwave extractor

Table 1 shows the comparison of extraction using different method. Applications include the extraction of high-value compounds from natural sources including phytonutrients, nutraceutical and functional food ingredients from biomass. In the present article basis of microwave extraction technology and their advantages are discussed. The most widely used extraction processes have traditionally been based either on different liquid extraction methods or on vapor-phase extraction methods. (Starmans and Nijhuis, 1996)

Table 1: Comparison microwave extraction with other method

Parameters	Soxhlet	Sonication	Microwave	Supercritical Fluid
Sample* Weight (g)	5-10	5-30	0.5-1	1-10
Solvent	**	**	Hexane/ethanol	CO_2
Solvent volume (ml)	>300	300	10-20	5-25
Vessel volume (ml)	500-1000	500	<100	5-25
Temperature (°C)	Boiling point	Room temp	40,70,100	50,200
Time	16 hours	30 minutes	30-45 seconds	30-60 minutes
Pressure (atm)	Ambient atm	Ambient atm	1-5	150-650
Relative energy consumption	1	0.05	0.05	0.25

*Dependent on the concentration and type of sample,**Dichloromethane, acetone, hexane, cyclohexane, toluene, etc.

2.4 comparison between microwave extraction and soxhlet methods

Soxhlet is the regular method and the main reference for evaluating the performance of other solid–liquid extraction methods as it has long been one of the most used solid–liquid extraction techniques. In Soxhlet extraction the solid material containing the solutes is placed inside a thimble holder, which is connected to a flask containing the extraction solvent, and submitted to reflux. After this process, the extract is concentrated by evaporation of the solvent (Jensen, 2007)

It is a general and well-established technique, which surpasses in performance other conventional extraction techniques except, in limited field of applications, the extraction of thermolabile compounds. Furthermore, it presents other disadvantages such as poor extraction of lipids, long operation time, high solvent consumption, and operation at the solvent's boiling point (de Luque Castro and Garcia-Ayuso, 1998). The advantages of this method include no requirement of a filtration step after leaching and the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix (Wang and Welter, 2006).

While using microwave can reduce time and solvent consumption, as well as improvement in yield. For example experiment done by (Kaufmann et al.,2001) by extracting whitanolides from *Lochroma gesneroides*, showed a drastic reduction in solvent usage (5 vs. 100 ml) and in extraction time (40 s vs. 6 h). Another study concluded that the same quantity and quality of tanshiones from *Salvia miltiorrhiza* Bunge was obtained with 2 min of MAE and 90 min of Soxhlet (Pan et al., 2002). Higher yield was obtained when extracting artemisinin from *Artemisia annua* L. by MAE; in 12 min, 92.1% of artemisinin was recuperated by MAE whereas several hours were needed by Soxhlet to reach only about 60% extraction efficiency (Hao et al., 2002)

3.0 *MATERIALS* AND METHODS

3.1 Material

Moringa oleifera seeds were collected from nearby Universiti Malaysia Pahang Campus, Gambang, Kuantan Pahang. The seeds were sun-dried then grinded using domestic blender and keep at room temperature. The solvent n-hexane was supplied by Faculty of Chemical and Natural Resources Engineering /Universiti Malaysia Pahang.

3.2 Experimental procedures

3.2.1. Preparation

Preparation starts with collection of *Moringa oleifera* seeds and continues with the cleaning. Cleaning process is crucial because a clean seeds yield clean oil without any impurities. Then, seeds were undergone size reduction by grinding using domestic blender.

Figure 7: *Moringa oleifera* seeds after peel

3.2.2. Extraction of oil

3.2.2.1 Soxhlet extractor

40g of crushed *Moringa oleifera* seeds were placed into thimble of Soxhlet. Extractor fitted with 500mL round-bottom flask and condenser containing 200ml n-hexane for 1 hour (Trakampruk and Chuayplod, 2012). A heating mantle is set at 60-70^{o}C and as the heating commenced, the n-hexane was continuously heated and it started to evaporate and condense back into thimble containing ground seeds. The oil was collected and n-hexane was evaporated using rotary evaporator. The oil collected from each cycle was between 9-20 g. Therefore, the yield was calculated at an average of all runs.

3.2.2.2 Microwave

800 g of *Moringa oleifera* seeds without grinding were placed in 1000 ml flat bottom flask. After adding 80 ml volume of solvent, the flask containing the sample was introduced to the microwave oven and adjusted to a condenser connected to a cold water recirculation system; the microwave oven was turned on and the desired conditions of time (20 min) and power (600 W) and temperature of 65°C were set. After that , the hexane was evaporated in a rotary evaporator at 70°C. The essential oil was stored for futher analysis.

Procedure:

1.
   - 800 g *Moringa oleifera* was weighted.
2.
   - Put in to microwave container.
3.
   - Add 80ml of n-haxane into the microwave container.
4.
   - Put the container in microwave and set the time, power and temperature with 20 min, 600W, and 65°C respectively.
5.
   - Collect the oil from microwave container.

4.0 RESULTS

The oil obtained from both extraction methods are shown in Figure 8 and 9, for soxhlet method and microwave method, respectively. The measured properties of oil extracted by the two methods are tabulated at Table 2.

Figure 8: Oil from soxhlet

Figure 9: Oil from microwave

Table 2 : comparison of oil properties obtained from soxhlet and microwave

Moringa oleifera seeds oil properties	Soxhlet	Microwave
Density (kg/m^3)	821.9	590.7
pH	6	6
Acid no (mgKOH)	6.37	5.87
SG	0.8219	0.5907
Percentage of yield (%)	28.39	6.90

4.1 pH

For the pH, it was checked using pH meter (Figure 10). The result showed that oil obtained from soxhet and microwave has pH 6. Measure the pH important when want to do the density test because, sample must have pH between 5-8.

Figure 10: pH meter

4.2 Acid number

The total acid number (TAN) is a measurement of acidity that is determined by the amount of potassium hydroxide in milligrams that is needed to neutralize the acids in one gram of oil. In this experiment, potentiometric titrations were followed. The results showed acid no. for the oil obtained from soxhlet was 6.37 mgKOH and from microwave was 5.87 mgKOH. The acid no is important for production of biodiesel.

Figure 11: Potentiometric titirator

Procedure:-

1. 10ml sample was diluted with 50 ml ethanol (1:5) was prepared.
2. Power supply was switched on.
3. Press MODE enter and select U mode enter.
4. Press USER METH enter and select RECALL METHOD enter.
5. select method name FFA enter.
6. The diluted sample was left for 10 min. The dilution is needed to break out the molecule s of sample.
7. After 10 minutes press START and wait until being displyed EP1
8. Insert the sample value and press ENTER value.
9. wait until EP 1 shows and get the result

4.3 Density

The density, or more precisely, the volumetric mass density, of a substance is its mass per unit volume or oil concentration. To measure the density gas pycnometer was used and density for oil that obtained from soxhlet was 821.9 kg/m^3 and for microwave was 590.7 kg/m^3.

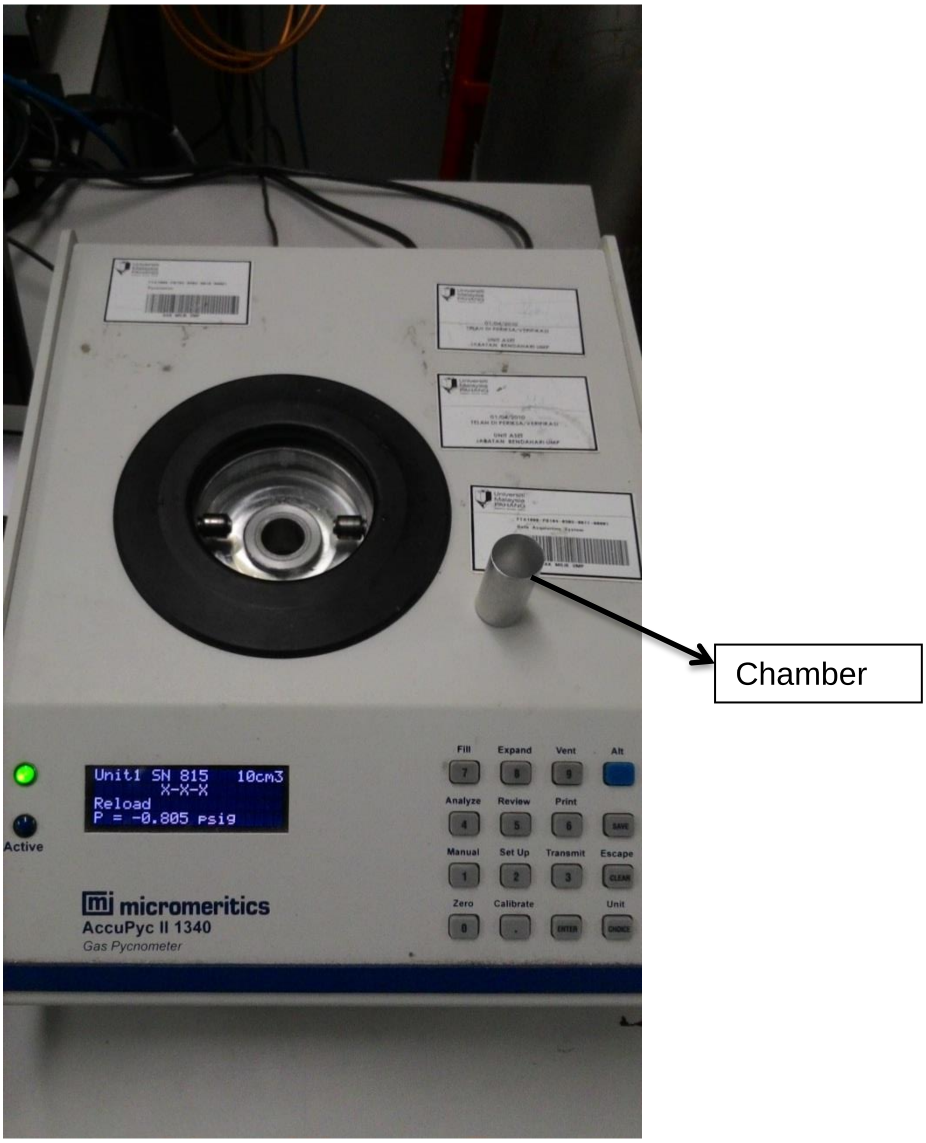

Figure 12: Chamber and AccuPyc II 1340 Gas Displacement Density Analyzer

Procedure:-

1. Put the sample into the camber and weight it.
2. Put the camber into the machine AccuPyc II 1340 Gas Displacement Density Analyzer
3. Wait and get the result.

4.4 Specific gravity (SG)

SG=$\frac{density\ of\ oil}{density\ of\ water}$

Density of water = 999.99 kg/m^3

SG of oil from soxhlet = $\frac{821.9}{999.99}$

= 0.8219

SG of oil from microwave = $\frac{590.7}{999.99}$

=0.5907

- Oil SG from soxhlet is 0.8219
- Oil SG from microwave is 0.5907

4.5 Oil yield percentage

The result of yield percentage is shown in Table 3. The percentage of oil yield was calculated using below equation:

$$\frac{Wo - W1}{Wo} \times 100\%$$

Where:

Wo- weight of sample before extraction

W_1- weight of sample after extraction

Table 3: Comparison percentage of yield obtained from both methods

Methods	weight of sample before extraction (g)	weight of sample after extraction (g)	Percentage of yield (%)
Soxhlet	40	28.64	28.40
Microwave	800	744.80	6.90

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

1. The oil from soxhlet have good properties compared to oil from microwave.

2. Oil from soxhlet also shows high percentage of yield compared to microwave.

5.2 Recommendation

Use methanol for both microwave and soxhlet for comparison because it was found that n-hexane cannot be heated by microwave as non-polar solvent.

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