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JUDUL : **EXTRACTION OF ESSENTIAL OILS FROM GINGER RHIZOME USING STEAM DISTILLATION METHOD**

SESI PENGAJIAN: **2006/2007**

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EXTRACTION OF ESSENTIAL OILS FROM GINGER RHIZOME USING STEAM DISTILLATION METHOD

KHAIRU AIZAM BIN IBRAHIM

A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
University College of Engineering & Technology Malaysia

NOVEMBER 2006

I declare that this thesis entitled "*Extraction of Essential Oils from Ginger Rhizome Using Steam Distillation Method*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ....

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Date : 27 NOVEMBER 2006

DEDICATION

Special dedication to my family members that always inspire, love and stand besides me, my supervisor, my beloved friends especially the one who always help me, my fellow colleagues,
and all faculty members

For all your love, care, support, and believe in me. Thank you so much.

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Praise is to God for His help and guidance that finally I'll able to complete this final year project as one of my requirement to complete my study.

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ABSTRACT

Essential oils are highly concentrated essences of aromatic plants. It can be extracted using a variety of methods such as steam distillation and solvent extraction. Essential oils have a very high commercial value due to its therapeutic properties. It is widely used in aromatherapy, medicine and as well as flavoring food and drink industries. To get the approximately pure essential oil from raw material, conventional extraction technique like steam distillation is used. Steam distillation is unlikely solvent extraction. This is because steam distillation is to produce essential oils but solvent extraction will produce oleoresin. Pure essential oil can be derived from a part of ginger plant that is the ginger rhizome by using steam distillation method. The extraction of the ginger essential oils began when steam contact to the ginger in the extraction tank. The steam carried out the essential oils from the ginger out of the rhizome and go through the condenser. Then, the steam with the essential oils will be condensed into liquid phase and will be collected in the beaker. Lastly, the two liquids will be separated. To get high quality and quality of essential oils, the fire from burner that burned the tank and produce steam in the tank must be well controlled. Apart from being effective, this study might as well discover potential savings in its operational cost and also environmental friendly.

ABSTRAK

Pati minyak adalah sangat berkepekatan tinggi daripada tumbuh-tumbuhan aromatik. Ia boleh diekstrak dengan menggunakan pelbagai kaedah seperti penyulingan wap air dan pengekstrakan dengan bahan pelarut. Pati minyak mempunyai suatu nilai komersial yang tinggi berdasarkan sifat-sifatnya yang berunsurkan nilai pengubatan. Ia digunakan dengan meluas dalam aromaterapi, perubatan dan termasuk juga industri memperisakan makanan dan minuman. Untuk mendapatkan pati minyak yang hampir-hampir tulen daripada bahan mentah, teknik yang lazim digunakan adalah seperti penyulingan wap air. Penyulingan wap air tidak seperti pengekstrakan dengan bahan pelarut. Ini disebabkan penyulingan wap air adalah untuk menghasilkan pati minyak tetapi pengekstrakan dengan bahan pelarut akan menghasilkan oleoresin. Pati minyak tulen boleh didapati daripada sebahagian daripada tumbuh-tumbuhan halia iaitu akar halia dengan menggunakan kaedah penyulingan wap air. Pengekstrakan pati minyak halia bermula apabila wap air menyentuh kepada halia di dalam tangki pengekstrakan. Wap air membawa keluar pati minyak daripada akar dan pergi melalui kondenser. Selepas itu, wap air dengan pati minyak akan diwap cairkan ke fasa cecair dan akan dikumpul di dalam bikar. Akhir sekali, kedua-dua cecair itu akan dipisahkan. Untuk mendapatkan pati minyak yang berkualiti dan berkuantiti tinggi, api daripada dapur gas yang menghasilkan wap air mesti dikawal dengan baik. Selain efektif, kajian ini juga ekonomikal melalui penjimatan kos operasinya dan ia juga adalah mesra alam.

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LIST OF ABBREVIATIONS

KUKTEM	=	Kolej Universiti Kejuruteraan dan Teknologi Malaysia
FID	=	Flame Ionization Detector
GC	=	Gas Chromatography
HPLC	=	High Performance Liquid Chromatography
WCOT	=	Wall-coated open tubular
SCOT	=	Support-coated open tubular
ML	=	Moisture lost

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CHAPTER 1

INTRODUCTION

1.1 Overview of Ginger

Ginger, a very useful herb plant, is said to be originated from India, China and Java, yet is also native to Africa and the West Indies. It is grown throughout the tropical areas of the world and also commonly found in South East Asia especially in Indo-Malaysia. The main producer of ginger is Jamaica. Ginger is scientifically named as *Zingiber officinale Roscoe*. On 1807, an English botanist, William Roscoe (1753-1831) named the plant as *Zingiber officinale* in his publication.

1.2 Physical Properties of Ginger

The name *Zingiber* is consequent from the Sanskrit word for "horn- shaped" and refers to the protuberances on the rhizome. *Zingiber officinale* belongs to the botanical family of the *Zingiberaceae.* Ginger is a perennial plant with upright reddish stem, looking like leaves, and grows from one to three or four feet in height. The stem is surrounded by the leaves. It shoots up a stem with narrow spear-shaped leaves, as well as white or yellow flowers growing directly from the root.

1.3 Usage of Ginger

Ginger has been used for a few purposes since very early times. It is used as a medicine since many years ago. It is also widely used as a cooking herb, condiment, spice and home remedy for a long time ago.

In medicinal uses, the ginger root is an effective treatment for nausea caused by motion sickness or other sickness. This kind of medical usage was found by earlier researchers, D.B. Mourey and D.E. Clayson. For morning sickness, it is not recommended to take the ginger root because morning sickness commonly associated with pregnancy. Ginger extract also has long been used in traditional medical practices to decrease inflammation.

Today, many herbalists use ginger to help treat health problems associated with inflammation, such as arthritis, bronchitis, and ulcerative colitis. To shorten the story, ginger oil is used in the treatment of fractures, rheumatism, arthritis, bruising, carbuncles, nausea, hangovers, travel and sea sickness, colds and flu, catarrh, congestion, coughs, sinusitis, sores on the skin, sore throat, diarrhea, colic, cramps, chills and fever. Beside that, ginger oil is used for cooking, as a flavoring for cookies, biscuits and cake, and it is the main flavor in ginger ale, a sweet, carbonated, non-alcoholic beverage.

1.4 Usual Methods of Obtaining Ginger Essential Oil

In *Zingiber officinale Roscoe*, there are many constituents such as acids, shoagaols, gingerol, essential oils, fiber, amino acids and minerals. There are two ways of extraction, that is using steam distillation and solvent extraction. In order to get oleoresin, solvent extraction technique is used but to obtain essential oil, steam distillation technique is used.

Steam distillation method is used for temperature sensitive material like natural aromatic compounds. For this method, there is no solvent is used to extract the material but pure water is the main component to do it.

1.5 Steam Distillation

In this research, the separation process that has been chosen is steam distillation. Steam distillation is one of the separation processes that used solid-liquid extraction theory. Liquid will be used to extract the solid. It means the essential oil will be removed from its raw material.

The extractor for this process will have three main parts. First, the steam will be supplied into the vessel. The steam will contact to the raw material and force the essential oils out of its raw material. Second, a condenser will be used to change the mixture of vapors to be two separated layer of water and essential oil. This two separated mixture occurs because of the different in density. Lastly, the mixture of water and essential oil will be collected in a vessel.

Steam distillation is most used to produce many types of essential oil such as from ginger. The process is cheaper than other extraction processes. It will not use any solvent and can make it safer than other processes.

1.6 Problem Statement

Generally, there are a few problems that arise in ginger extraction. There are many types of extraction. The extraction can be conducted with or without solvent. But, to get the essential oil, extraction through steam distillation is the most used method.

Without any solvent, pure water is used at its boiling point as steam to extract the essential oil from ginger. The steam is forced over the ginger. The steam will help to release the aromatic molecules from the ginger. The molecules of these volatile oils then escape from the ginger plant and evaporate into the hot steam. The temperature of the steam must be carefully controlled. It is because to control the ginger from burning and lost its purity.

Most of the essential oils have medicinal properties and it had been used since thousand years ago. Today, the essential oil from the ginger is widely used and the most important is that the ginger oil is used in medical field for a few sicknesses.

Nowadays, essential oil of ginger is highly needed because of the usage for medical field. The pungent components in ginger are proven beneficial in treating health problems. Many researches have been performed to discover the usage of ginger in various fields, especially in the medicinal field.

In other hand, the ginger flavor is containing aromatic and pungent component which is important in the flavor industries but recovery of both components at the same time has not been possible by conventional separation processes. To recover both components, steam distillation unit must be designed.

This equipment will be very useful for KUKTEM. KUKTEM will be one of the institutions that can produce essential oil using steam distillation method. The highly demand of the essential oil make KUKTEM take the chance to develop the technology.

1.7 Objective

The main objective of this study is to produce essential oils from the ginger rhizome using steam distillation method.

1.8 Research Scope

This research is an experimental study of steam distillation method using ginger as raw material. In order to realize the objective, three scopes have been identified. The scopes are:

i. To know the effect of extraction time to the yield of ginger essential oils.
   The experiment will be done for eight hours. After every one hour, the ginger essential oils will be collected.
ii. To study the effect of surface area of the ginger to get higher yield.
   Two different size of ginger rhizome will be prepared which are sliced and grinded to use for the experiments.
iii. To analyze the product using GC.
   This study is focus on using the gas chromatography (GC) to analyze the essential oil from raw material.

1.9 Contribution of The Study

The steam distillation equipment is expected to produce the best quality of essential oil from the ginger. There are some expected results from this research:

i. The equipment for steam distillation will be one of the most efficient and effective to produce essential oil.

ii. Application of advanced technology in ginger extracting process.
iii. Potential savings in the operational cost.
iv. The environmental friendly experiment will be conducted.

CHAPTER 2

LITERATURE REVIEW

2.1 Separation Processes

Many chemical process materials and biological substances occur as mixtures of different components in the gas, liquid, or solid phase. In order to separate or remove one or more of the components from its original mixture, it must be contacted with another phase. The two phases are brought into more or less intimate contact with each other so that a solute or solutes can diffuse from one to the other. The two bulk phases are usually only somewhat miscible in each other. During the contact of the two phases the components of the original mixture redistribute themselves between the two phases. The phases are then separated by simple physical methods. By choosing the proper conditions and phases, one phase is enriched while the other is depleted in one or more components.

Separation process is defined as a process that transforms a mixture of substances into two or more compositionally-distinct products. It is also defined as any set of operations that separate of two or more components into two or more products that differ in composition (Noble & Terry, 2004). Separation is attained by exploiting the differences between chemical and physical properties of the substances through the use of a separating agent (mass or energy). There are a few examples of separation process:

i. Absorption

When the two contacting phases are a gas and liquid, this operation is called absorption. A solute or several solutes are absorbed from the gas into the liquid phase in absorption.

ii. Distillation

In the distillation process, a volatile vapor phase and a liquid phase that vaporizes are involved.

iii. Liquid-liquid extraction

When the two phases are liquids, where a solute or solutes are removed from one liquid phase to another liquid phase, the process is called liquid-liquid extraction.

iv. Leaching

If a fluid is being used to extract a solute from a solid, the process is called leaching. Sometimes this process is also called extraction.

v. Membrane processing

Separation of molecules by the use of membranes is a relatively new separation process and is becoming more important. The relatively thin, solid membrane controls the rate of movement of molecules between two phases.

vi. Crystallization

Solute components soluble in a solution can be removed from a solution by adjusting the conditions, such as temperature or concentration, so that the solubility of one or more of the components is exceeded and they crystallize out a solid phase.

vii. Adsorption

In an adsorption process, one or more components of a liquid or gas stream are adsorbed on the surface or in the pores of a solid adsorbent and a separation are obtained.

viii. Ion exchange

In an ion exchange process, certain ions are removed by an ion-exchange solid. This separation process closely resembles adsorption.

Separation process is done for its own function. There are three primary functions of separation processes:

i. Purification

   It is used to remove undesired components in a feed mixture from the desired species.

ii. Concentration

   It is used to obtain a higher proportion of desired components that are initially dilute in a feed stream.

iii. Fractionation

   Fractionation is a separation process in which a certain quantity of a mixture (solid, liquid, solute or suspension) is divided up in a large number of smaller quantities (fractions) in which the changes according to a gradient.

The analysis of separation processes are divided into two fundamental categories:

i. Equilibrium-based processes
ii. Rate-based processes

For equilibrium-based processes, the degree of separation process in each stage is governed by a thermodynamic equilibrium relationship between the phases. Examples of separation processes in this category are:

i. Distillation
ii. Extraction and leaching

In distillation, the liquid is partially vaporized to create another phase, which is a vapor. The separation of the components depends on the relative vapor pressures of the substances. In distillation also, a different temperature at each stage alters the vapor phase equilibrium between typically binary mixtures.

The desire of a new equilibrium between the two phases at the temperature of each stage is the driving force for separation. The end result is the separation of two liquids with different boiling temperatures.

Extraction is a process where a species is removed from a liquid in which it is dissolved by means of another liquid for which it has higher affinity. While for leaching, a species is removed from a solid phase by means of another liquid for which it has a stronger affinity.

Rate-based processes are mainly about the limited of the processes by the rate of mass transfer of individual components from one phase into another under the influence of physical stimuli (such as concentration, temperature, pressure, external force). Under this category, there are a few types of processes:

i. Gas absorption
ii. Desorption or stripping
iii. Adsorption
iv. Ion exchange
v. Membrane separations

2.2 Extraction

Extraction is the process to remove one or more solutes from a liquid by transferring the solute into a second liquid phase, for which the solute has a higher affinity (Noble & Terry, 2004). This type of separation process depends on the differences in both solute solubility and density of the two phases.

In this process, there will be the advantages and disadvantages. One of the advantages is extraction can be performed at ambient temperature.

Thus, it is relatively energy efficient and can be applied to separations involving thermally unstable molecules.

2.3 Distillation

Distillation is one of the separation processes. Distillation is defined as a process in which a liquid or vapor mixture of two or more substances is separated into its component fractions of desired purity, by the application and removal of heat. Besides that, extraction processes can accommodate changes in flow rates and the solvent can be recovered and recycled for reuse. It offers greater flexibility in terms of operating conditions too, since the type, amount of solvent and operating temperature can be varied.

On the other hand, one of the disadvantages is, in this process, the solvent must be recovered for reuse (usually by distillation), and the combined operation is more complicated and often more expensive than ordinary distillation without extraction (McCabe, Smith & Harriott, 2001).

2.4 Ginger Oils Overview

The word Ginger is comes from the ancient Sanskrit word”*Singabera*” meaning shaped like a horn and the plant originates from India and being commonly found in South East Asia. The English botanists William Roscoe (1753-1831) give the plant name *Zingiber Officinale Roscoe* in an 1807 publication. Ginger oleoresin and ginger oil is derived from the fleshy part of the mesocarp of the herbs species. Ginger is a tropical herbaceous perennial with underground rhizomes from which stalks arise three feet tall. The leave is lancelote.

An example of the rhizome is shown in figure 2.0. The inflorescence comes directly from the roots (rhizomes) and ends in a spilled. The flower has an aromatic smell. The example of ginger plant is shown in figure 2.1.

Ginger has been used as a spice, condiment and flavoring agent. For nearly 2500 years, ginger has played an important role in Asian medicine as a folk remedy to promote cleansing of the body through perspiration, to calm nausea and to stimulate the appetite. Nowadays ginger is commercialize cultivated in nearly every tropical and subtropical country in the world with arable land to produce this valuable herbs. The scientific classification of ginger is further detailed in Table 2.0.

Figure 2.0: *Zingiber Officinale Roscoe* (Wikipedia, 2006)

Figure 2.1: *Zingiber Officinale Roscoe* Plant (S. Foster, 2000)

Table 2.0: Taxonomy of ginger (National Plant Database, 2004)

Scientific Classification	
Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Zingiberales
Family	Zingiberaceae
Genus	Zingiber
Species	Officinale

2.4.1 History of Ginger (*Zingiber Officinale*)

No one is sure how old Ginger is or where it come from since it has never been found growing wild. More than 5000 years ago the ancient Chinese and Indians looked upon Ginger as the 'universal medicine'. It has received praise from Confucius and Pliny. Nostradamus wrote recipes for ginger preserved in honey and Al-Quran mentions a fountain of Ginger flavored water.

Ginger reached the West at least 2000 years ago, recorded as a subject of a Roman tax in the 2^{nd} century after being imported due the Red Sea to Alexandria. Ginger is known in England before the Norman Conquest as it is commonly found in the 11^{th} century Anglo-Saxon leach books. By the 13^{th} and 14^{th} centuries it was familiar to English palates and next to pepper, was the most popular spice. In that time a pound of ginger are equal to a price of one sheep.

Ginger as a product of the Far East, was indelibly imprinted on the taste buds of Westerners before potatoes, tomatoes and corn were even known to exist by Europeans.

2.4.4 Chemical Composition of Ginger

Ginger contains ginger oil, ginger oleoresin (combination of volatile oils and resin) that account for the characteristics aroma of ginger. Volatile oils in ginger have bisabolene, cineol, phellandrene, citral, borneol, citronellol, geranial, linalool, limonene, zingiberol, zingiberene and camphene**.** 1,8-cineole, also called eucalyptol, is a major component of camphor-scented essential oils found in eucalyptus leaves, bay leaves, and other aromatic plant foliage. In Southeast Asia, cineole-rich cajuput oil is a well-known remedy for the discomfort of bruises, sprains, and pulled muscles because it stimulates blood circulation near the point of application. Recent clinical research has demonstrated 1, 8-cineole's effectiveness in reducing inflammation and pain, and in promoting leukemia cell death. Ginger also contains oleoresin (gingerol,shogoal) , phenol (gingeol, zingerone), proteolytic enzyme (zingibain), Vitamin B6,Vitamin C, Calcium, Magnesium, Phosphorus, Potassium, linoleic acid, gum, starch, lignin, acetic acid, Sulphur and asmazone. Figure 2.3, 2.4 and 2.5 below shows the structure of shogoal, gingerol and zingiberene. The pungency of ginger is due to Gingerol which is the alcohol group of the oleoresin. Ginger aroma is about 1 to 3% of volatile oils, which are bisabolene, zingiberene and zingiberol. Ginger also has primary nutrient which is Calcium, Iron, Magnesium, Phosphorus, Potassium, Protein, Sodium, Vitamin A, Vitamin B-complex and Vitamin C.

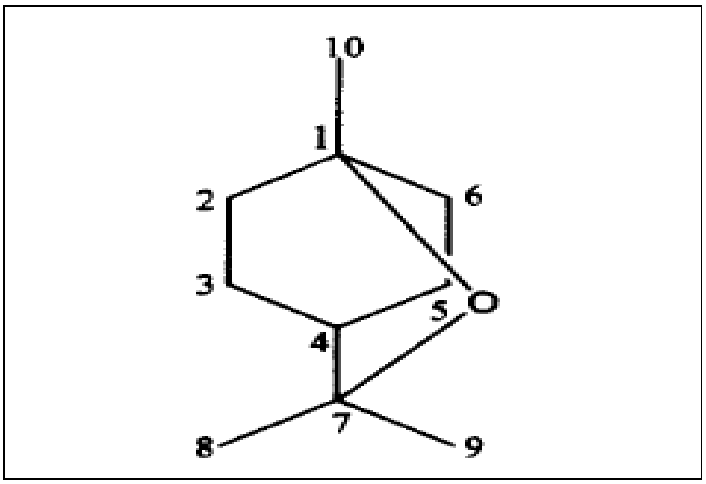

Figure 2.2: Structure of 1, 8-cineole (T. Acree, 2004)

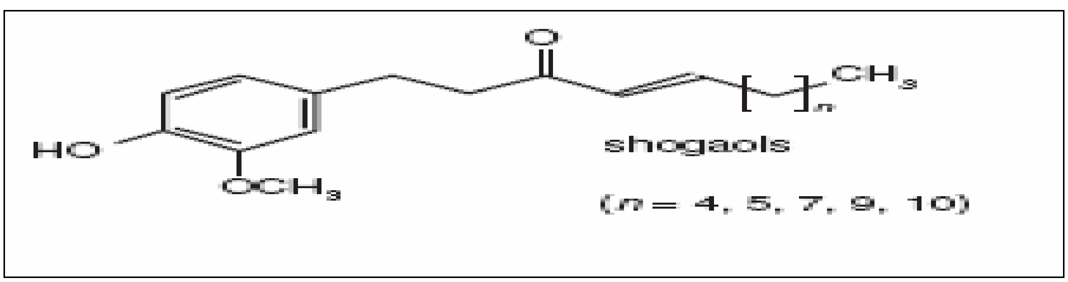

Figure 2.3: Structure of Shogoal (A. Heinrich, 2004)

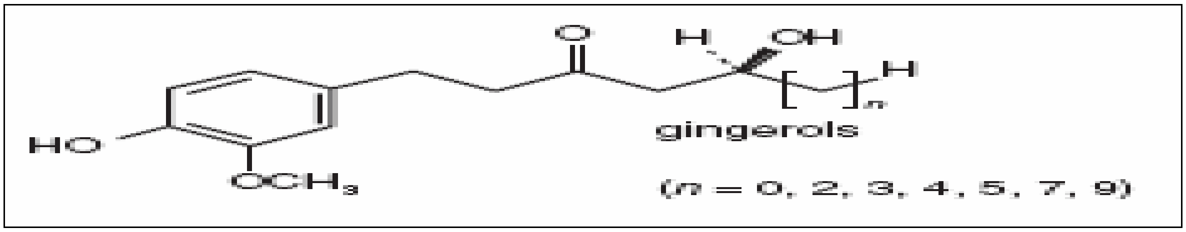

Figure 2.4: Structure of Gingerol (A. Heinrich, 2004)

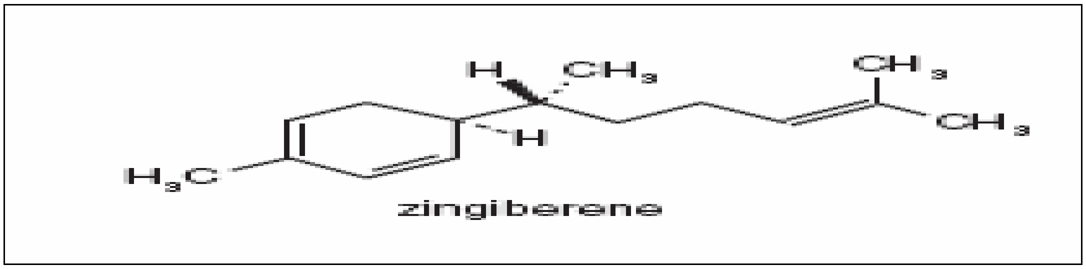

Figure 2.5: Structure of Zingiberene (A. Heinrich, 2004)

2.4.3 Ginger Oil: The Constituents

The volatile oil of ginger, which is generally prepared by steam distillation of dried comminuted rhizomes, is undoubtedly an important raw material of the food, cosmetics and pharmaceutical industries. The ginger oil consists of alpha-pinene, camphene, linalool, zingiberene, borneol, citral and many more as illustrated in Figure 2.6.

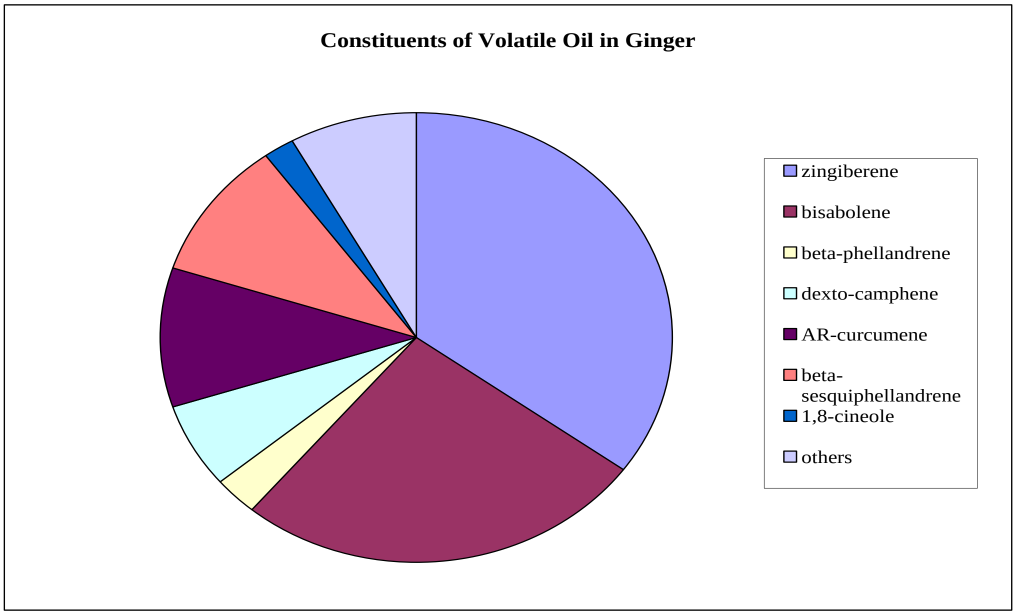

Figure 2.6: The Constituents of volatile oil in ginger (S. Foster, 200)

2.4.4 Uses and Benefit of Ginger

Ginger is mostly uses as a food flavoring. It also may be taken orally in higher amounts as an herbal remedy to prevent or relieve nausea resulting from chemotherapy, motion sickness, pregnancy and surgery. The early Greeks and Romans made extensive use of ginger as spice and as medicine.

During the third century it was apparently a very costly spice, but during the eleventh century it became cheaper, owing to extensive cultivation, and was quite generally used in Europe. Since its introduction to Western Europe in the Dark Ages, ginger has been used as a remedy for nausea and as carminative. Reports appearing in the English medical Journal Lancet in 1982 conclude that powdered ginger helped with motion sickness. Researcher conducted a double blind study on 36 college students with a high susceptibility to motion sickness.

They concluded that 940mg of powdered ginger was superior to 100mg of dimenhydrinate in reducing symptoms when consumed 25 minutes prior to test in a title rotating chair.

Figure 2.7 shows some of the product from ginger that used in medicine and cooking.

Figure 2.7: Ginger Products (S. Foster, 2000)

Gingerol in ginger is a powerful antioxidant which is to clear up the free radical that can harm within the body. Ginger is also shown to be effective against tumor growth, migraines and rheumatism.

In Chinese medicine, Ginger is widely used as a “guide drug” to “mediate” the effects of potentially toxic ingredients. Like an ancient Chinese, in India the fresh and dried roots were considered distinct medical products. Fresh ginger has been used for cold-induced disease, nausea, asthma, cough, colic, heart palpitation, swellings, dyspepsia, loss of appetite and rheumatism.

Ginger is truly a world domestic remedy. It has been well known in European homes for almost 1000 years and Asian cultures have used it for centuries.

2.8 Essential Oil

Historically, the first essential oil encountered was the oil of rose. It was discovered by the Chinese prior to the Christian era. A layer of this oil was found on a pool that was filled with rose water. Essential oils contain DNA of the plant of herbs they are extracted from. Essential oils or sometimes called volatile oils are believed to be that small portion of the plant material, which imparts the characteristics odors and flavor most closely associated with the vegetative matter which they are obtained. Most of the essential oils are used at about a level of 0.01-0.1 percent in the finished product. They are often slightly colored and have a specific gravity of about 1.

The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majority of them is fairly stable and contains a few natural antioxidants. Although most are soluble in high strength alcohol (more than 90 percent), they have poor water solubility and most contain terpenes that contribute to their poor water solubility. Some essential oils are adaptogenic.
This implies that the essential oil increase resistance and resilience to stress, enabling the body to avoid reaching collapse. Adaptogenic essential oils aid the body in maintaining homeostasis throughout stressful periods.

Essential oil is a volatile oil that is produced by steam, steam and water, or water distillation of vegetable plant matter. The vapors are condensed to yield a water condensate and an essential oil that can be separated off (usually gravity). During the distillation process, the essential oil can be continually separated off in a purpose-built separating vessel.

Essential oils may be present in many different types of plant materials (wood, bark, leaves, stems, flowers, stigmas, reproductive parts etc.) at concentrations ranging from thousandths of a percent to one or several percent.

The essential oil is not a solvent extracted material where solvents might include carbon dioxide, benzene, acetone, ethanol, or hexane. It is also not a molecular distilled product. The term essential oil is therefore not applied to carbon dioxide extracted products.

2.6 Availability of Extraction Methods

2.6.1 Steam Distillation

Steam distillation is a special type of distillation process (separation process) for temperature sensitive materials like natural aromatic compounds. Through this process the botanical material is placed in a still and steam is forced over the material. The hot steam will help to release the aromatic molecules from the plant material. The molecules of these volatile oils are then escape from the plant material and evaporate into the steam. The temperature of the steam therefore needs to be carefully controlled.
The temperature should be just high enough to force the plant material to release the essential oils, yet not too hot as it can degrade the plant material or the essential oils.

The steam containing the essential oil is passed through a cooling system to condense the steam, which then form a liquid from which the water and the essential oils is then separated. The steam is produced at greater pressure than the atmosphere and therefore it boils at above $100^{O}C$ which facilitates the removal of the essential oil at a faster rate. By doing so, it could prevent damage to the oil as well.

2.6.2 Supercritical Fluid Extraction

When CO_2 is subjected to high pressure, the gas turns into liquid. This liquid is an inert and safe solvent which can be used to extract the aromatic molecules in a process similar to that used to extract absolutes. The chief advantage, of this technique is that no solvent residue remains. This is because at normal pressure and temperature, the carbon dioxide can simply slip back to gas phase and evaporates.

2.6.3 Solvent Extraction

Another method of extraction used on delicate plants is solvent extraction, which yields a higher amount of essential oils at a lower cost. A hydrocarbon solvent is added to the plant material to help dissolve the essential oil. When the solutions are filtered and concentrated by distillation, a substance containing resin, or a combination of wax and essential oil (concrete) remains. From the concentrate, pure alcohol is used to extract the oil and when the alcohol evaporates, the oils are left behind. This is not considered the best method for extraction as the solvents can leave small amount of residue behind which could cause allergies and affect the immune system.

2.7 Steam Distillation Pilot Plant and Operation

Steam distillation pilot plant as illustrated in figure 2.8 is designed for extraction various types of essential oils. The steam distillation consists of:

i. Extraction tank
ii. Condenser
iii. Burner
iv. Oxygen tank
v. Thermometer

Figure 2.8: Steam Distillation Pilot Plant

Most essential oils are obtained from the plant material by a process known as steam distillation. Steam distillation is the most common of distillation methods today with most of our essential oils being produced via steam distillation.

Steam distillation is used in the manufacture and extraction of essential oils where the material is placed in a still and steam is forced over the material. Steam distillation brings steam in direct contact with the plant material in the vessel. The hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam.

The temperature of the steam needs to be carefully controlled. The temperature must be just enough to force the plant material to let go of the essential oil, yet not too hot as to burn the plant material or the essential oil.

The steam which then contains the essential oil is passed through a cooling system to condense the steam, which form a liquid from which the essential oil and water is then separated. The steam is produced at greater pressure than the atmosphere and therefore boils at above 100 degrees Celsius which facilitates the removal of the essential oil from the plant material at a faster rate and in so doing prevents damage to the oil. Figure 2.0 shows the basic principle of steam distillation operation.

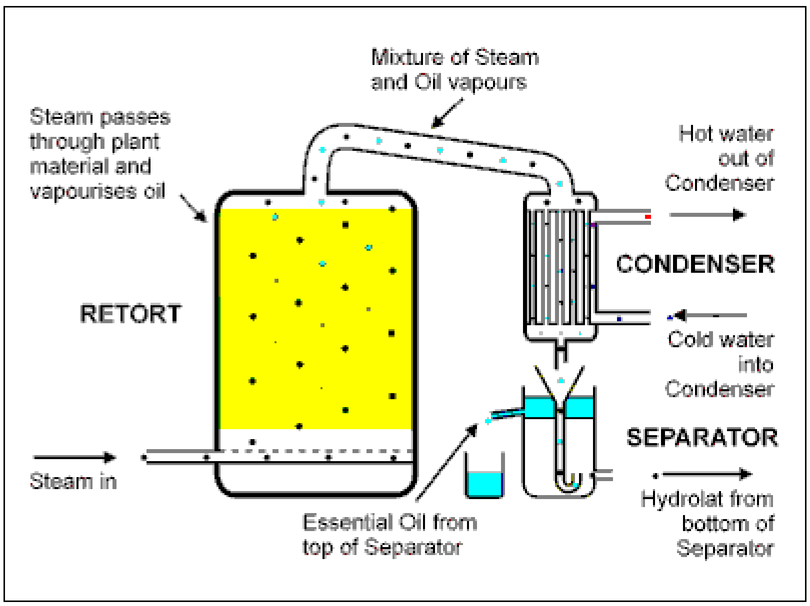

Figure 2.9: Steam Distillation Operation (Coconut Coast Natural Products, 1996)

2.8 Analysis

Essential oils can be analyzed by a few methods. There are a few different methods in analyzing volatile components in essential oils. The two most famous methods used are:

i. Gas Chromatographic (GC) analysis
ii. High Performance Liquid Chromatography (HPLC) analysis

2.8.1 Gas Chromatography Analysis

Gas Chromatography (GC) is used for analytical equipment to analyze the components in essential oils. Other than that, it can be used for separating small amounts of material and to determine whether a desired component is present.

The GC consists of an injection block, a column, and a detector. For GC analysis, a sample of essential oils needs to be vaporized and will be injected onto the head of the chromatographic column. The sample of essential oils will transport through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. Figure 2.8 has shown the schematic diagram of GC.

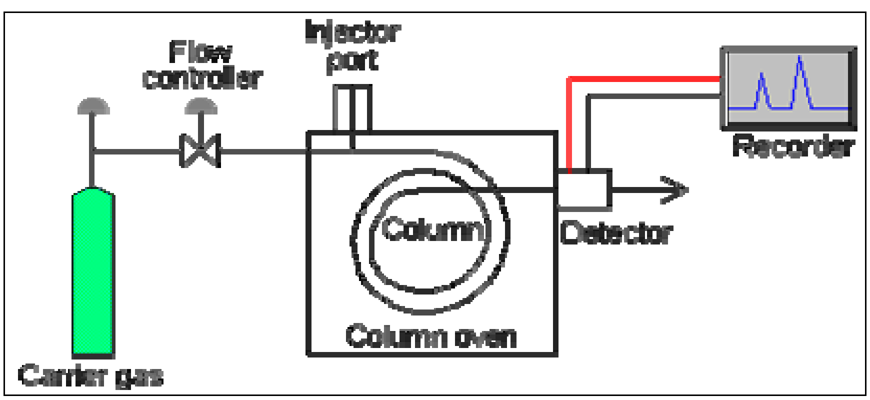

Figure 2.10: GC schematic diagram (Sheffield Hallam University, 2006)

Efficient separation of compounds in GC is dependent on the compounds traveling through the column at different rates (Feist, 2000). The rate at which a compound travels through a particular GC system depends on the factors listed below:

a. **Volatility of compound**: Low boiling (volatile) components will travel faster through the column than will high boiling components
b. **Polarity of compounds**: Polar compounds will move more slowly, especially if the column is polar.
c. **Column temperature**: Raising the column temperature speeds up all the compounds in a mixture.
d. C**olumn packing polarity**: Usually, all compounds will move slower on polar columns, but polar compounds will show a larger effect.
e. **Flow rate of the gas** through the column: Speeding up the carrier gas flow increases the speed with which all compounds move through the column.
f. **Length of the column**: The longer the column, the longer it will take all compounds to elute. Longer columns are employed to obtain better separation.

2.8.1.1 Carrier Gas

The carrier gas that will be used must be chemically inert. A few gases that commonly used are nitrogen, helium, argon, and carbon dioxide. The carrier gas is chosen depend to the type of detector that will be used. In the carrier gas system, there is also contains a molecular sieve to remove water and other impurities.

2.8.1.2 Injection System of a Gas Chromatography

The sample of any chemical compound has to be vaporized prior to analysis by GC; this is a limiting factor for many inlets. The first and most common method is to introduce a small volume of sample with a syringe. This injection may be done by hand or automatic sampler.

After that, proceed to Headspace sampling which involves taking sample of the gas above a liquid sample (headspace) and injecting it into the chromatograph.

Lastly, purge and trap which is variation on headspace analysis. A gas is bubbled through the sample and the analyte is trapped on a special kind of filter (or in a cold trap), this concentrates the analyte, the trap is then heated to desorb the analyte off of the trap and into the column (Bramer, 1996).

2.8.1.3 Columns

There are two general types of column for GC analysis. The two different columns are:

i. Packed columns
ii. Capillary columns

The packed columns contain a finely divided, inert, solid support material coated with liquid stationary phase. The columns are most packed at 1.5 - 10m in length and have an internal diameter of 2 - 4mm.

The capillary columns also known as open tubular. For these columns, the internal diameter is a few tenths of a millimeter. There are two different types of capillary columns:

i. Wall-coated open tubular (WCOT)
ii. Support-coated open tubular (SCOT)

Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase.

In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

2.8.1.4 Column Selection

Capillary columns are capable of more efficient separation. They enable more complex mixtures to be separated or resolved.
As for packed columns, they have extremely high surface area, which is an advantage for large amounts of analyte like when separating gases. Capillary columns, however, have much greater resolution so they are more widely used for analysis.

The most important consideration is the stationary phase which is the liquid that is coated onto the inside of a capillary column or on the packing material of a packed column. The stationary phase is selected to separate the compounds of interest. For instance, a polar column will retain polar molecules longer; therefore it is better for separating polar compounds. Likewise, a non-polar column is used for non-polar analytes. Other stationary phases are designed to interact with different types of functional groups (Bramer, 1996).

2.8.1.5 Detectors

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A non-selective detector responds to all compounds except the carrier gas, a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound.

Detectors can also be grouped into concentration dependant detectors and mass flow dependant detectors. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas. The table 2.1 has shown the summary for a few types of detectors.

Table 2.1: Types of detectors (Sheffield Hallam University, 2006)

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds.	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10^7
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 fg	10^5
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	10^6
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	10^3
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2 pg	10^7
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur		

Figure 2.8 shows the Flame Ionisation Detector. For this detector, the effluent from the column is mixed with hydrogen and air, and ignited. Ions and electrons will produce by the organic compound which is burned in the flame. The ions and electrons can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. FIDs are mass sensitive rather than concentration sensitive. This gives the advantage that changes in mobile phase flow rate do not affect the detector's response.

The FID is a useful general detector for the analysis of organic compounds. It has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample.

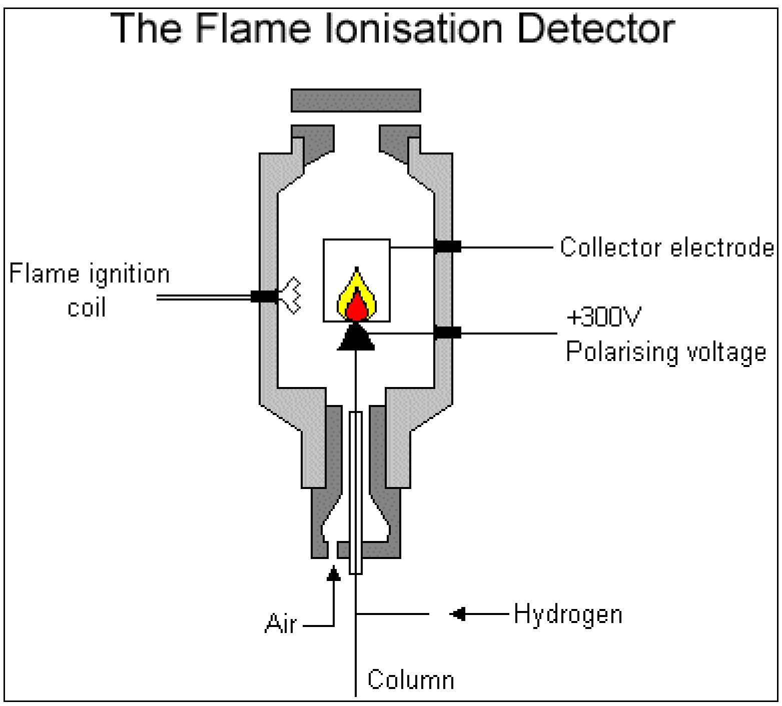

Figure 2.11: Flame Ionization Detector (Sheffield Hallam University, 2006)

CHAPTER 3

METHODOLOGY

3.1 Overview of Methodology

In producing the essential oil of ginger, there are a few steps that must be done. The steam distillation pilot plant is used to produce ginger essential oils. Steam distillation pilot plant is one of the new equipment in our lab. This experiment takes time to settle. There are a few steps to complete the experiment. There are:

i.) Sample preparation of dried ginger
ii.) Ginger oil extraction
iii.) Analysis

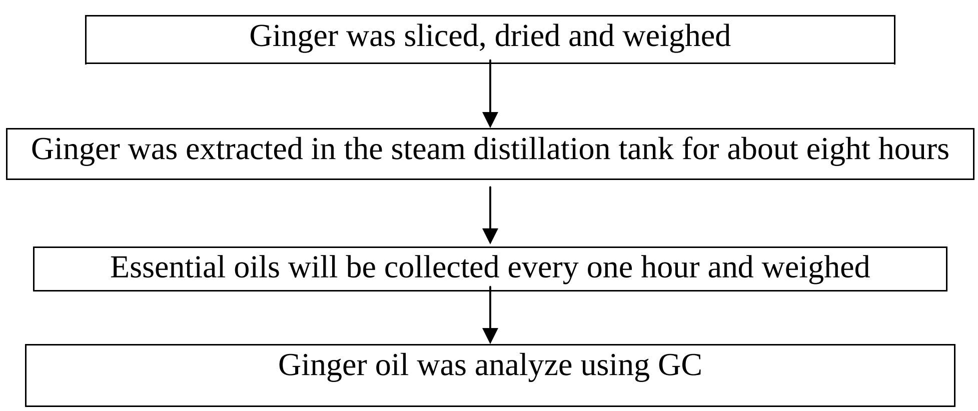

Figure 3.0: Flow Diagram for Ginger Extraction Procedure

Ginger was sliced, dried and weighed

↓

Ginger essential oils was extracted with different size
(sliced and grinded) at specified time (8 hours)
Temperature = 100^{o}C
Pressure = 1 atm

↓

Ginger oil was analyze using GC

Figure 3.1: Flow Diagram for Ginger Extraction Procedure with Different Surface Area

3.2 Sample Preparation of Dried Ginger

Fresh ginger was sliced into small shape. This is because it will easier to the slice to dry than the original shape of ginger. The slice was dried under the sunlight. The purpose for drying the ginger is to remove the moisture from ginger. Then, the dried ginger will reach at the stage which its weight is constant. The constant weight is the residual moisture in the ginger.

The percent of moisture lost (ML) of ginger is determined by the following formula:

$$ML\ (\%) = \frac{\text{Current weight of sample (g)} - \text{Initial weight of sample (g)}}{\text{Initial weight (g)}} \times 100\%$$

3.3 Ginger Oil Extraction

Fill the extraction tank with water. Ginger rhizome is placed on a platform above the water. Turn on the burner to supply heat to the tank and water. The heat will change the water into steam. In the extraction tank, steam is produced to crash the plants cells and free the oil, which will continue threw tube to the condenser to condense oil by cooling the vapor. Water at room temperature is used as cooling agent. Figure 3.2 and 3.3 shows the overall steps to obtain the ginger essential oils.

Figure 3.2: The Steam Distillation Equipment

Figure 3.3: The Ginger Essential Oil Is Collected and Separated

3.3.1 Experiment 1: Extraction of Ginger Oil in 8 Hours

Ginger is extracted using steam distillation. First, the ginger is placed on the platform in the tank. Burner is on to vaporize the water into steam. The steam will contact to the ginger. Then, the oil in the ginger will go together with the steam to the condenser. The mixture of the vapors will condense into liquid phase. Two layers of liquid are produced. Figure 3.2 shows the experiment for this research.

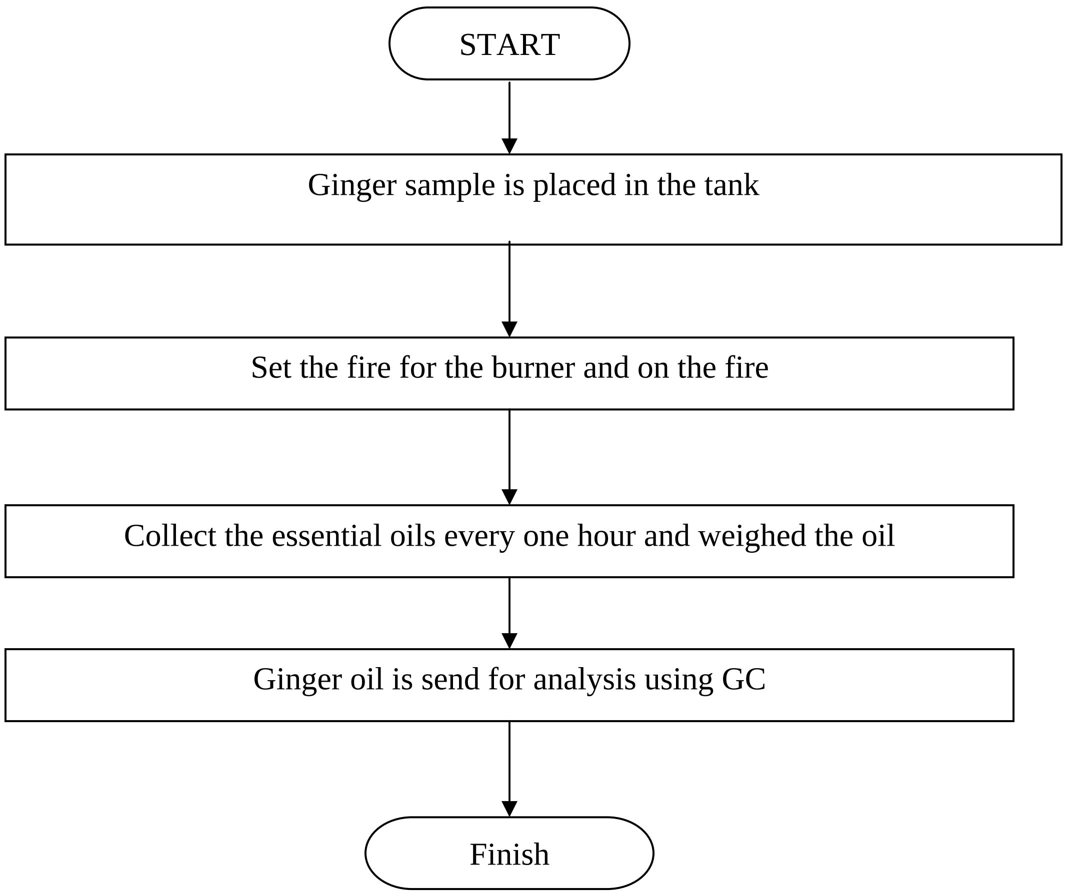

Figure 3.4: Extraction of ginger oil for sample 1

The experiment was done for about 8 hours. In about 4 kg of dried, sliced ginger was used for this experiment. Every hour, the ginger oils were weighed and the yield was calculated.

3.3.2 Experiment 2: Extraction of Ginger Oil with Different Surface Area

Ginger is extracted using steam distillation. First, the sliced ginger is placed on the platform in the vessel. Heater is on to vaporize the water into steam. The steam will contact to the ginger. Then, the oil in the ginger will go together with the steam to the condenser. The mixture of the vapors will condense into liquid phase. Two layers of liquid are produced. Figure 3.3 shows the experiment for this research.

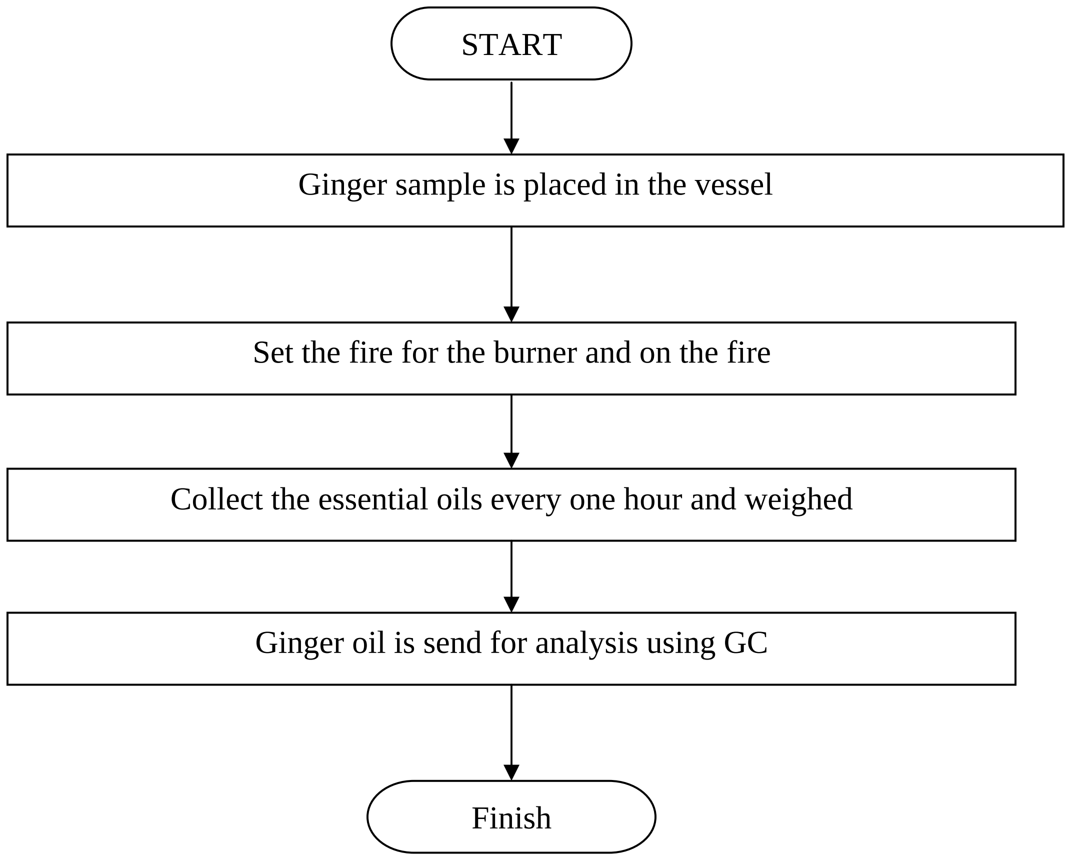

Figure 3.5: Extraction of ginger oil for sample 2

The experiment was repeated for different size of ginger rhizome. Another size of ginger is grinded ginger. Repeat the same procedure and the yields will be compared.

3.4 Analysis Using Gas Chromatography (GC)

The essential oils were analyzed using an Agilent 6890 Gas Chromatograph equipped with Flame Ionization Detector (FID) and HP-5 (polydimethyl-siloxane) MS capillary column (30m x 0.32mm and film thickness 0.25 µm. the injector and detector temperature were set at 220°C and 300°C respectively. Oven temperature was kept at 40°C for 3 min, then gradually raised to 160°C at 3°C/min, held for 10 minutes and finally raised to 280°C at 3°C/min. helium was the carrier gas and the flow rate was set at 1 ml/min. The example of standard GC result for ginger oil based on previous experiment is shown in figure 3.6.

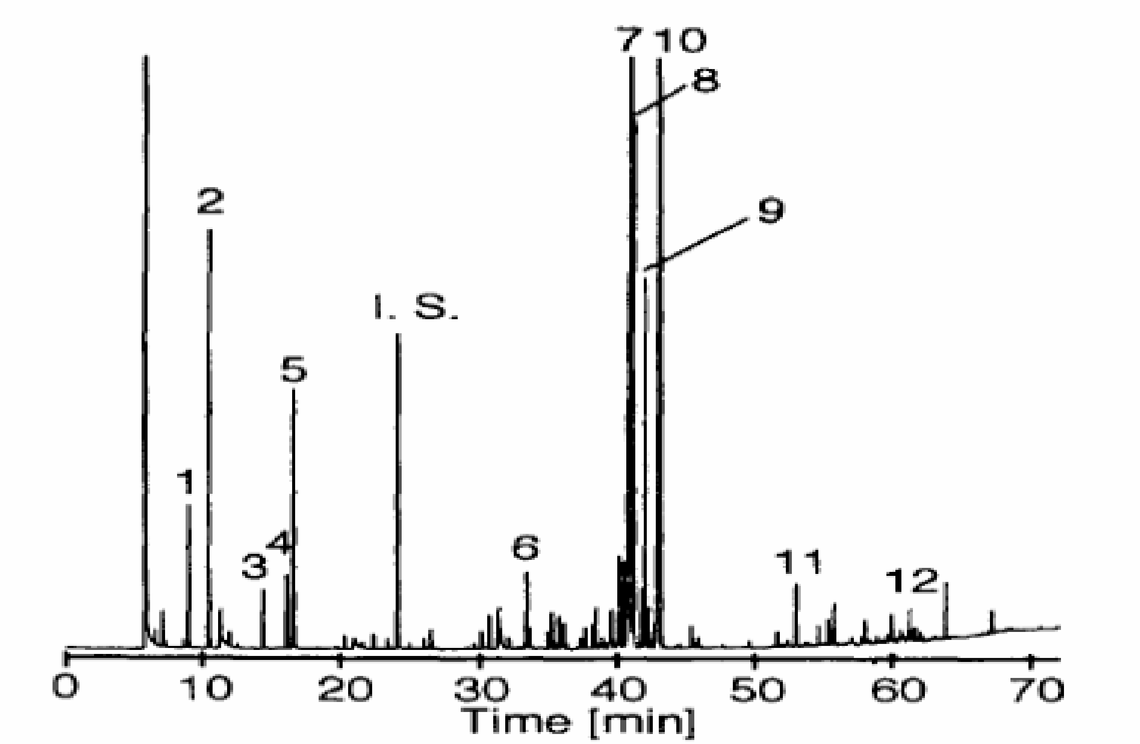

Figure 3.6: Example of gas chromatogram of ginger extraction (Yonei Y, 1995)

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

It was observed that ginger essential oil yield was yellow in color and has light smell and a bit greasy. Ginger oil that extracted using sliced gingers have light color of solution compare to oil extracted using grinded ginger. Figure 4.0 and 4.1 shows the pictures of the products. It means that there is a different color of ginger oils using different size of surface area. The color is not so different but maybe certain composition of constituents will missing or less in one of the ginger oil.

Figure 4.0: Product extracted using sliced ginger

Figure 4.1: Product extracted using grinded ginger

4.2 Quantitative Analysis

4.2.1 Amount of Ginger Essential Oil

In this experiment, the product which is ginger essential oil was collected and separated with water every one hour. The approximately pure ginger essential oil was weighed and the data was collected. There were two set of experiments had been done successfully. For the first experiment, using 4 kilograms of sliced ginger and second using 4 kilograms of grinded ginger.

All the data for the first experiment was collected and it is illustrated by table 4.0 while table 4.1 is the data for the experiment using grinded ginger.

Table 4.0: Amount of ginger oil for slice ginger rhizome

Extraction Time (hours)	Amount of Ginger Oils (grams)
0	0
1	4.1275
2	12.5479
3	17.4581
4	21.0948
5	27.6548
6	31.2187
7	33.5434
8	34.2389

In the table, it shows that the amount of ginger oil will increase every hour for the extraction time. The highest increment of the oil can be seen very clear at the one to two hours interval. At that time, the increasing of the oil is more than others interval of time. It is increasing for about 8.4204 grams from the first hour to the second hour.

After eight hours extraction, the amount of ginger oil extracted is about 34.2389 grams. It is increasing amount of 0.6955 grams from seven hours of the operation. It means that, maybe after 8 hours, there will be a little essential oil remains in the ginger.

Table 4.1: Amount of ginger oil for grinded ginger rhizome

Time (hours)	Amount of Ginger Oils (grams)
0	0
1	6.7670
2	16.1032
3	19.6542
4	27.3454
5	31.2095
6	33.3570
7	38.5439
8	40.6898

Table 4.1 shows that total amount of ginger oil after 8 hours experiment is 40.6898 grams. The total amount of ginger oil for this experiment is more than the first one. Total amount of 6.4509 grams of ginger oil for this experiment is more than experiment one.

4.2.2 Yield of Ginger Essential Oil

Figure 4.3 concerned with the change of yield percentage along with time when ginger sliced was extracted for eight hours operation. Based on the line graph, it clearly showed that the yield constantly increased with time.

The lowest yield was at the starting time while the highest yield was at the ending time of extraction. Besides that, the highest increment occurred within the range one to two hours. The difference was 0.2105% extract in one hour. Apart from that, there was only a little change that happened on the yield in the other ranges of time.

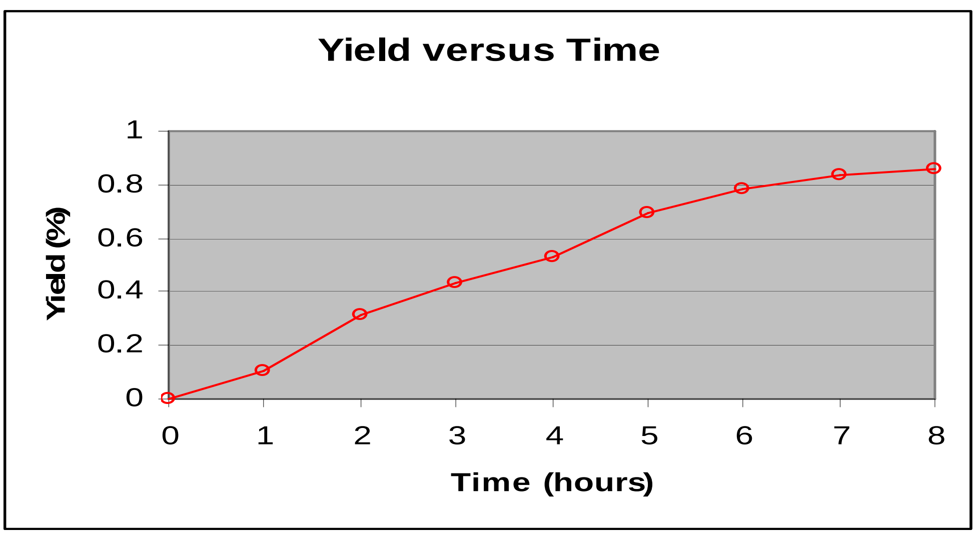

Figure 4.2: Graph yield versus time for extraction using sliced ginger

Figure 4.4 demonstrated the change of yield percentage along with time when the extraction using grinded ginger for about eight hours also. It indicated that the yield increased as the time lengthened.

The lowest yield was at the initial and at the ending of the experiment the yield was highest. The highest yield is at the ending of the extraction which was 1.0172% extract while the lowest was 0.1692% extract. Apart from that, there was a little change that occurred on the yield in the other ranges of time. The yield increase was not constant, sometimes the oil increases more but sometimes, it increases less.

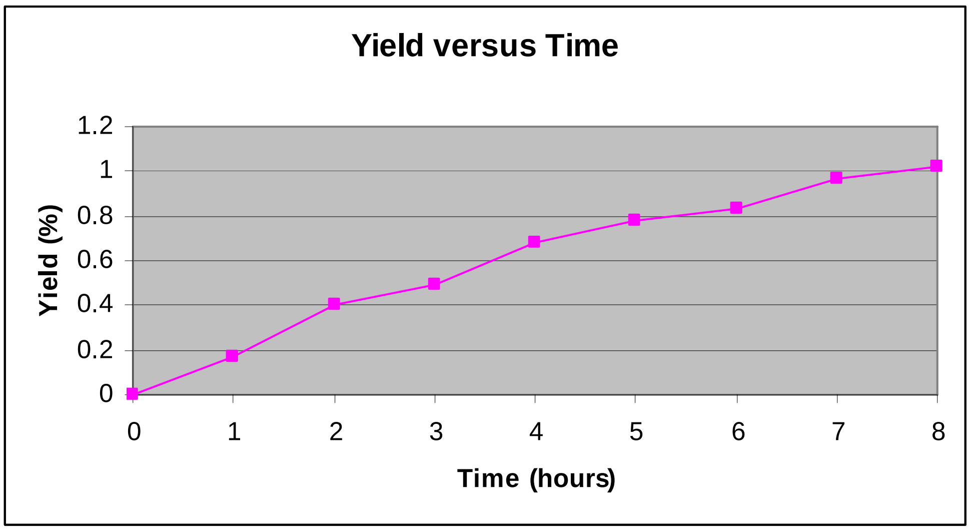

Figure 4.3: Graph yield versus time for extraction using grinded ginger

4.3 Qualitative Analysis

This chapter discusses based on the data from the experiment that had been carried out. The results describe on the analysis of the component of ginger essential oils based on the literature. The samples of ginger essential oils were compared with the standard component of ginger essential oils which is 1, 8 cineol as a valuable component. The qualitative analysis has been done using gas chromatography with a suitable method.

4.3.1 GC Analysis of Ginger Oil Constituents

Gas Chromatography analysis was done to detect the constituents of ginger oil. The chromatogram demonstrated peaks for ginger oil obtained. There are a few evident peaks along the operation time. The highest peaks for sample one (see figure 4.4) was at 41.322 minutes of retention time. The height was 7319.99512 pA and the area was 1.65296e5 pA.s. This sample was from sliced ginger extraction.

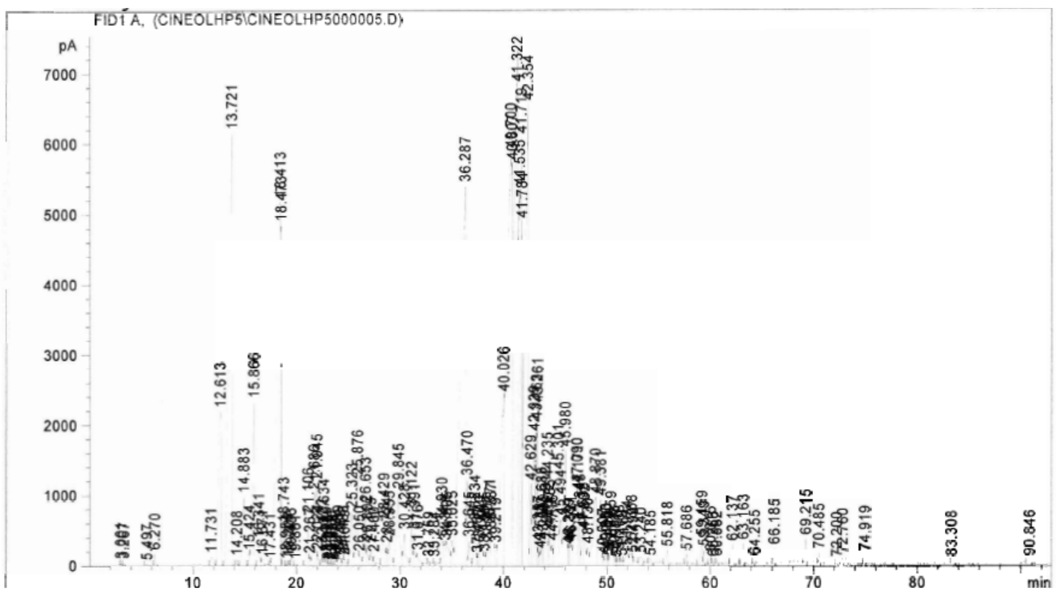

Figure 4.4: GC analysis for sample 1

Another gas chromatogram shows in figure 4.5 is for sample two. Sample two was from grinded ginger extraction. All the peaks show that the evident for ginger oil obtaining in the sample two. The highest peak was at 41.674 of retention time. At this time, the height was 8547.51758 pA.

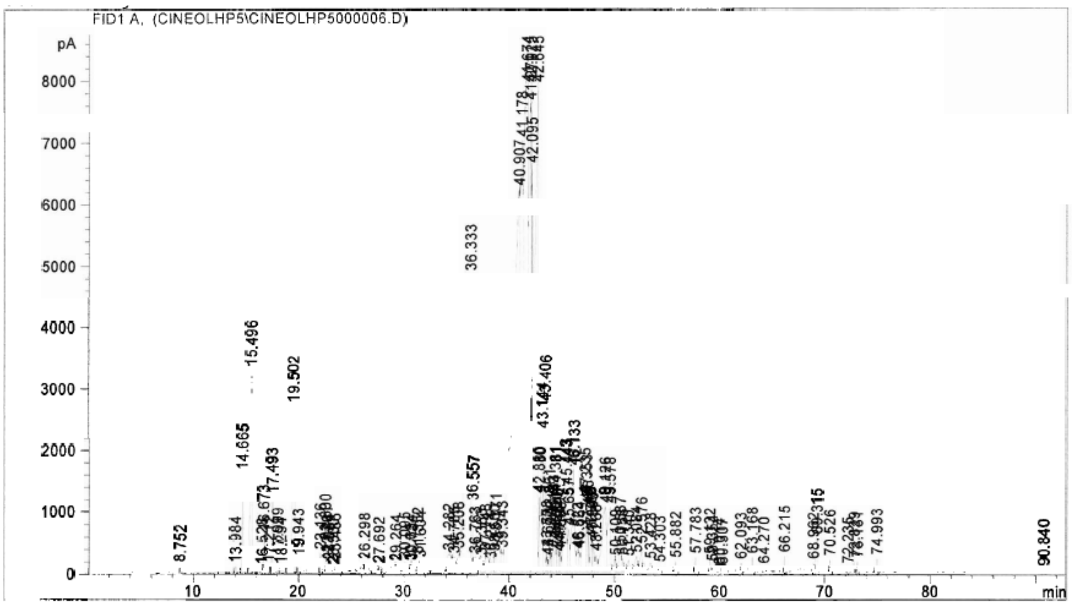

Figure 4.5: GC analysis for sample 2

4.4 Discussion

4.4.1 Ginger Extraction Using Steam Distillation Pilot Plant

Ginger oil was highly achieved using the grinded ginger rhizome compared to only slice it. Apart from that, for both experiments, the ginger oils obtained had quite high viscosity and they were yellow in color. The ginger aromas in both yields were also present indicating that the yield consisted pungent and aromatic components such as 1, 8 cineol shogaols and zingiberene. These descriptions were justified by literature review as aroma/essential ginger oil.

The amount of yield for the two experiments increased gradually as the steam supplied continuously. The heat was supplied by the burner under the tank and change water into steam. The steam was produced in the extraction tank until it contact to the raw material. The heat made the ginger pore opened and ginger oil was released. The steam carried the oil through the condenser and the two mixtures condensed in the condenser. Then, two layers of liquids were collected.

The yield for the extraction using grinded ginger was more than the other extraction. From the first hour until the ending of the operation, grinded ginger yields always more than sliced ginger yield. It was because the surface area contacted to the steam was different. For grinded ginger, the size was smaller but the surface area contacted to the steam was bigger than other prepared raw material.

For both experiments, the temperature used was approximately the same. The temperature was at around 100^0C. But, the fire controlled was not the same. The fire from the burner could not be set at the same level. Boiling point for ginger oil is lower than 100^0C. So, it could be extracted from ginger rhizome. The rapid heating of the water was influenced by the level of the fire from the burner. High level of fire from the burner produced more steam and also increased the pressure inside the tank. The fire must be well controlled to keep the vapor remains in the tank. If not, the vapor will come out from the tank through the gap between tank stopper and the tank.

For the conclusion, there is better used the grinded ginger than only sliced ginger. It is because the yield for extracting grinded ginger is more than sliced ginger yield since the yield quality resulted is almost same for both.

4.4.2 Gas Chromatography Analysis

There are a number of components in the extract but could not clearly be established in both figure 4.4 and 4.5. The sample might contain ginger oil constituents but could not be identified one by one.

The two chromatograms (See Figure 4.4 and Figure 4.5) were being compared to get the best quality between slice and grind ginger as the raw material. Between both graphs, the highest peak for sample two was superior than sample one. It shows that, sample one had more quality than sample one.

According to literature review (Yonei and Ohinata, 1995), the principal constituents of volatile oil which was zingiberene, a kind of sesquiterpene hydrocarbon was discovered highest at range of 40 to 50 minutes of retention time. The result obtained in this study (Figure 4.4 and 4.5) showed readings along 40-50 minutes (Refer Appendix A and B) appear clearly. Both samples showed that there were zingiberene in them.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The steam distillation pilot plant is effective to be used. Based on the study done, it was discovered that this technology enables to produce ginger in 8 hours extraction time. Both experiments were successfully done because the yields were quite high. This method of obtaining essential oil is more economical compared to solvent extraction. Apart from that, it is safer and environmental friendly than solvent extraction. It is because water can not cause any damage to human being and environment but solvent can damage surrounding.

Besides that, the best raw material size is that one which has bigger surface area is better to be used. It is because the bigger the surface area expose to the steam, the more the yield will be obtained. The yield appeared to be clear yellow to orange in color, similar descriptions in the literature review. The aroma is present in the yield too, indicating the existence of volatile constituents such as 1, 8 cineole. These constituents are especially valuable in the medicinal field as they could be the antidote for several diseases. From the reading, 1, 8 cineole

Lastly, the yield also depends on the fire level from the burner. If high level of fire is used, pressure is increased and the vapor contains steam and oil will come out of the extraction tank. It will come out between the tank stopper and the tank itself.
In obtaining the good quality and quantity of yield, the method that has been done is using grinded ginger with good control of fire level.

5.2 Recommendations

There are a few recommendations provided to improve the system of the steam distillation pilot plant. First is the steam supplied; proper steam supplied is needed because the fire can not be easily controlled. If big fire is used, the pressure in the tank will increase and the vapor will come out of the tank. The essential oil will discharge from the extraction tank through the hole at the tank. So, the steam supplied must be electronically controlled in order to get a constant flow rate of steam supplied to the tank. Apart from that, burner is used and is connected to methane gas tank. It is quite danger to use methane gas tank in the lab without teaching engineer as observer. If leaking occur when fire is on, there will be very dangerous because explode will take place. Other than that, the stopper at the top also must be surrounding by a thicker rubber to prevent the vapor come out from the tank. If this leakage is not to prevent, there is losing of essential oil at the top of the tank. There will be losing of steam with essential oils through this leakage so the yield of the product will be affected. Apart from that, the drain pipe and valve are suggested to be bigger in order to make the cleaning method easier. Sometimes, the raw material will fall down to the bottom of the extraction tank. The raw material will block the water in the drain pipe. It will be difficult to drain the water out of the tank after the experiment. So, to make the cleaning process easier, adjust the size of the drain pipe and valve. Lastly, more experiments and researches involving various parameters such as pressure, steam flow rate and fire level need to be conducted in order to discover the optimum conditions for producing a high quality and quantity yield of essential oil.

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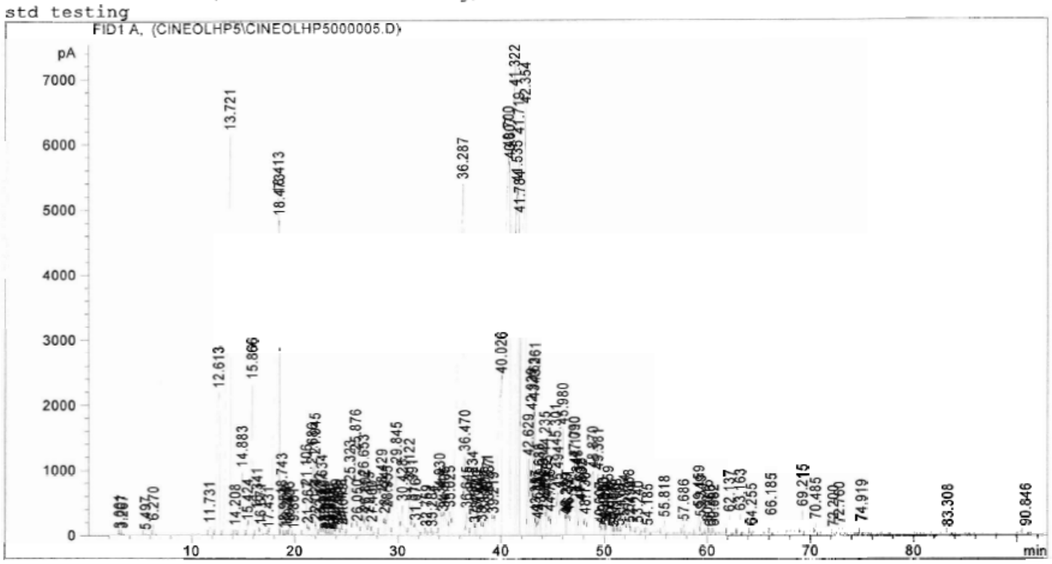
APPENDIX A

Data File C:\CHEM32\1\DATA\CINEOLHP5\CINEOLHP5000005.D
Sample Name: A1

```
Injection Date   : 17/10/2006 22:56:34            Seq. Line :    5
Sample Name      : A1                              Location : Vial 6
Acq. Operator    : Miza                                 Inj :    1
Acq. Instrument  : Instrument 1                  Inj Volume : 1 µl
Acq. Method      : C:\CHEM32\1\METHODS\CINEOL.M
Last changed     : 17/10/2006 14:15:17  by Faisal
Analysis Method  : C:\CHEM32\1\METHODS\CINEOL.M
Last changed     : 18/10/2006 11:23:14  by Miza
                   (modified after loading)
```

std testing



External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	3.001	VV	0.1093	265.42377	33.18534	0.01486
2	3.267	VV	0.0837	185.60422	31.07530	0.01039
3	5.497	BV	0.1416	145.21207	12.13608	0.00813
4	6.270	VV	0.1066	1382.80701	160.37862	0.07744
5	11.731	VV	0.2254	2042.77039	119.49220	0.11439
6	12.613	VV	0.2249	3.84297e4	2188.60864	2.15202
7	13.721	VV	0.2371	1.21680e5	6141.82227	6.81395

Data File C:\CHEM32\1\DATA\CINEOLHP5\CINEOLHP5000005.D
Sample Name: A1

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
8	14.208	VV	0.0923	474.19131	77.57983	0.02655
9	14.883	VV	0.1550	1.16524e4	970.89227	0.65252
10	15.424	VV	0.1063	1366.16211	155.85783	0.07650
11	15.866	VV	0.1443	2.73322e4	2322.23633	1.53057
12	16.341	VV	0.1571	3895.84009	326.69296	0.21816
13	16.671	VV	0.1712	1204.74011	92.31900	0.06746
14	17.431	VV	0.1074	326.47211	41.69004	0.01828
15	18.413	VV	0.3166	1.37351e5	5175.36035	7.69149
16	18.473	VV	0.0273	9236.82227	4832.15527	0.51725
17	18.743	VV	0.0857	3530.72949	551.15143	0.19772
18	18.954	VV	0.0573	113.62622	30.59627	0.00636
19	19.207	VV	0.0741	315.16702	63.33803	0.01765
20	19.436	VV	0.0859	638.80280	114.91101	0.03577
21	19.861	VV	0.1036	156.00404	21.77595	0.00874
22	21.106	VV	0.1238	5679.83691	691.36719	0.31806
23	21.267	VV	0.1259	1030.59338	112.44909	0.05771
24	21.686	VV	0.1413	1.07595e4	1018.40356	0.60252
25	21.945	VV	0.1334	1.19389e4	1166.81384	0.66856
26	22.024	VV	0.0719	828.65198	161.47311	0.04640
27	22.432	VV	0.0770	1299.28467	248.42163	0.07276
28	22.634	VV	0.0856	3017.37036	528.64612	0.16897
29	22.830	VV	0.1144	1036.52576	135.32292	0.05804
30	23.026	VV	0.1004	167.11104	23.37276	0.00936
31	23.211	VV	0.1043	242.76559	33.58570	0.01359
32	23.338	VV	0.1055	168.76268	22.76165	0.00945
33	23.508	VV	0.1157	146.77733	16.83435	0.00822
34	23.777	VV	0.1372	841.65033	89.13928	0.04713
35	24.009	VV	0.1552	1595.89917	153.62288	0.08937
36	24.182	VV	0.1366	989.75916	99.90760	0.05543
37	24.483	VV	0.1260	1251.40002	147.52220	0.07008
38	24.619	VV	0.1581	1103.01843	85.17915	0.06177
39	25.323	VV	0.2011	1.19626e4	749.93707	0.66989
40	25.876	VV	0.1594	1.51469e4	1223.99011	0.84821
41	26.050	VV	0.1546	1721.68274	142.88446	0.09641
42	26.653	VV	0.1481	9126.92383	838.00836	0.51110
43	26.802	VV	0.1468	3153.13403	292.46304	0.17657
44	27.175	VV	0.1234	2373.55640	262.76331	0.13292
45	27.460	VV	0.1184	1148.82800	130.85388	0.06433
46	28.429	VV	0.1895	8921.28809	610.59253	0.49958
47	28.724	VV	0.1104	2157.49902	260.98499	0.12082
48	28.955	VV	0.1737	4698.19678	354.23773	0.26309
49	29.845	VV	0.2496	2.10702e4	1030.30200	1.17990
50	30.428	VV	0.1799	6344.47461	451.48474	0.35528
51	31.122	VV	0.1121	6328.01367	776.48083	0.35436
52	31.391	VV	0.1296	4413.43164	457.43301	0.24715
53	31.676	VV	0.2223	2695.56909	157.80450	0.15095
54	32.759	VV	0.1400	623.04279	62.14361	0.03489
55	33.284	VV	0.1131	486.52423	58.47468	0.02724
56	34.030	VV	0.1524	6357.49023	547.78864	0.35601
57	34.341	VV	0.1457	3486.38184	293.35168	0.19523
58	34.505	VV	0.2434	6976.01855	342.89676	0.39065
59	35.025	VV	0.1611	4170.45410	354.77557	0.23354
60	36.287	VV	0.3996	1.78400e5	5383.95459	9.99016
61	36.470	VV	0.0836	7643.84375	1212.19751	0.42805
62	36.645	VV	0.1169	3064.65649	344.16125	0.17162
63	37.234	VV	0.1597	7090.64502	579.68823	0.39707
64	37.467	VV	0.0629	527.85028	118.61224	0.02956
65	37.622	VV	0.1099	2802.31665	333.83475	0.15693
66	37.740	VV	0.2146	5236.41162	290.11377	0.29323
67	38.156	VV	0.1491	1628.20288	141.61482	0.09118
68	38.407	VV	0.1203	2579.54395	299.96365	0.14445
69	38.671	VV	0.1509	5862.56152	526.54944	0.32830
70	38.787	VV	0.2245	8811.03320	468.38159	0.49341
71	39.219	VV	0.1467	3143.51685	260.65259	0.17603
72	40.026	VV	0.3014	6.11107e4	2405.02979	3.42212
73	40.700	VV	0.3839	1.90809e5	5859.89258	10.68506
74	40.807	VV	0.0930	4.09968e4	5694.99414	2.29577
75	41.322	VV	0.2674	1.65296e5	7319.99512	9.25636
76	41.535	VV	0.1311	5.62796e4	5334.58545	3.15159
77	41.719	VV	0.1235	5.95526e4	6067.82275	3.33488

Data File C:\CHEM32\1\DATA\CINEOLHP5\CINEOLHP5000005.D
Sample Name: A1

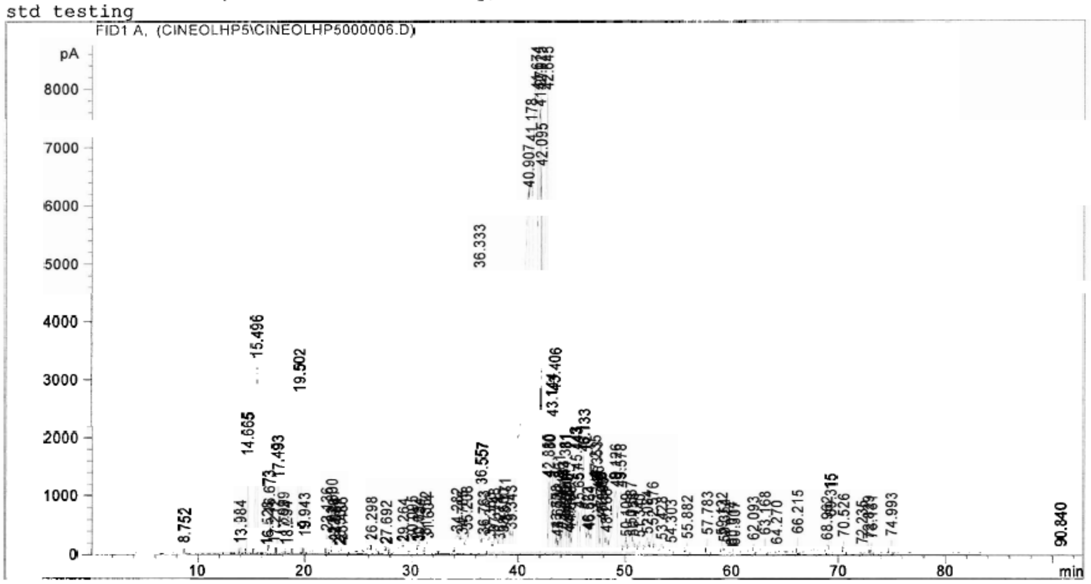
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
78	41.784	VV	0.0420	1.45979e4	4871.36475	0.81747
79	42.354	VV	0.2606	1.42194e5	6540.55225	7.96267
80	42.629	VV	0.1030	8843.94727	1146.12878	0.49525
81	42.939	VV	0.1702	2.57245e4	1850.81531	1.44054
82	43.163	VV	0.1083	1.57214e4	2009.01257	0.88038
83	43.261	VV	0.0653	1.06545e4	2247.19409	0.59664
84	43.357	VV	0.0767	1482.88989	285.19138	0.08304
85	43.533	VV	0.1050	1487.11450	186.60471	0.08328
86	43.688	VV	0.1032	4515.06250	691.25061	0.25284
87	43.830	VV	0.0781	1674.10425	304.57141	0.09375
88	44.012	VV	0.1015	5347.35156	696.77698	0.29945
89	44.235	VV	0.1263	1.16464e4	1188.90259	0.65218
90	44.372	VV	0.0882	3104.34741	481.73044	0.17384
91	44.485	VV	0.1078	4031.88696	512.36133	0.22578
92	44.716	VV	0.1255	2711.41870	294.14920	0.15184
93	45.301	VV	0.2242	2.39255e4	1302.90381	1.33980
94	45.494	VV	0.1770	9140.59766	650.10077	0.51186
95	45.980	VV	0.2003	2.54708e4	1621.39429	1.42633
96	46.289	VV	0.0667	1271.25525	244.44876	0.07119
97	46.397	VV	0.0829	1804.21704	266.64920	0.10103
98	46.521	VV	0.1445	3102.05981	286.05481	0.17371
99	47.090	VV	0.2297	2.06731e4	1108.10510	1.15767
100	47.171	VV	0.1091	7871.87549	1008.42175	0.44082
101	47.348	VV	0.1106	4128.83594	590.30994	0.23121
102	47.638	VV	0.1447	5102.92432	469.95651	0.28576
103	47.802	VV	0.1802	5816.73682	445.85724	0.32573
104	48.130	VV	0.1579	2939.20898	248.57687	0.16459
105	48.870	VV	0.2970	2.35640e4	954.69128	1.31956
106	49.381	VV	0.2155	1.62390e4	926.20636	0.90936
107	49.607	VV	0.1075	774.62250	105.74301	0.04338
108	49.833	VV	0.1238	1499.40430	171.88106	0.08396
109	49.950	VV	0.1089	1330.08997	178.57939	0.07448
110	50.129	VV	0.0788	476.12335	76.29545	0.02666
111	50.359	VV	0.2323	6319.92822	347.26126	0.35391
112	50.718	VV	0.1415	1372.97437	142.39146	0.07688
113	50.907	VV	0.0985	356.72919	51.72325	0.01998
114	51.168	VV	0.1462	1694.68616	154.17131	0.09490
115	51.419	VV	0.1729	990.07172	71.34169	0.05544
116	51.934	VV	0.2433	3970.74048	216.15469	0.22236
117	52.368	VV	0.1554	3605.75928	297.57782	0.20192
118	52.499	VV	0.0812	686.21442	115.70921	0.03843
119	53.240	VV	0.2530	2359.34399	115.63884	0.13212
120	54.185	VV	0.2409	1416.42517	76.19032	0.07932
121	55.818	VV	0.1527	2297.17676	200.42761	0.12864
122	57.686	VV	0.1272	1317.44580	144.74123	0.07378
123	59.159	VV	0.1397	3645.92285	352.52182	0.20417
124	59.349	VV	0.0989	1320.40552	205.57188	0.07394
125	60.015	VV	0.1048	989.67596	132.96681	0.05542
126	60.263	VV	0.1295	607.87152	62.47809	0.03404
127	60.582	VV	0.1400	671.01599	65.78906	0.03758
128	62.137	VV	0.1334	2967.26782	287.62823	0.16616
129	63.163	VV	0.1067	2321.69482	302.07269	0.13001
130	64.255	VV	0.1133	682.96686	79.47610	0.03825
131	66.185	VV	0.0989	1535.83289	229.92661	0.08600
132	69.215	VV	0.1148	3233.30859	366.76776	0.18106
133	70.485	VV	0.1037	1198.09509	166.97870	0.06709
134	72.200	VV	0.1069	386.65872	51.31313	0.02165
135	72.760	VV	0.1124	795.66815	99.39427	0.04456
136	74.919	VV	0.0904	844.75549	134.34923	0.04731
137	83.308	VV	0.1207	492.90530	55.44448	0.02760
138	90.846	VV	0.1823	784.24194	54.67965	0.04392
Totals :				1.78575e6	1.18783e5	

==

APPENDIX B

```
Data File C:\CHEM32\1\DATA\CINEOLHP5\CINEOLHP5000006.D
Sample Name: A2

    =================
    Injection Date  : 18/10/2006 00:41:31          Seq. Line :   6
    Sample Name     : A2                            Location : Vial 7
    Acq. Operator   : Miza                               Inj :   1
    Acq. Instrument : Instrument 1                Inj Volume : 1 µl
    Acq. Method     : C:\CHEM32\1\METHODS\CINEOL.M
    Last changed    : 17/10/2006 14:15:17  by Faisal
    Analysis Method : C:\CHEM32\1\METHODS\CINEOL.M
    Last changed    : 18/10/2006 11:23:14  by Miza
                      (modified after loading)
    std testing
```




```
    ==================================================================
                          External Standard Report
    ==================================================================

    Sorted By              :      Signal
    Multiplier             :      1.0000
    Dilution               :      1.0000
    Use Multiplier & Dilution Factor with ISTDs
    ==================================================================
    ==================================================================
                             Area Percent Report
    ==================================================================

    Sorted By              :      Signal
    Multiplier             :      1.0000
    Dilution               :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A,
```

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	8.752	BB	0.0645	463.25223	109.28921	0.02855
2	13.984	VB	0.0801	477.18564	95.82558	0.02941
3	14.665	VB	0.0886	9991.32031	1604.86670	0.61573
4	15.496	BV	0.1246	3.10954e4	3281.09961	1.91630
5	16.528	VV	0.0723	347.08374	74.59274	0.02139
6	16.673	VV	0.0689	2722.27051	611.48492	0.16776
7	17.212	VV	0.0879	601.26611	106.46113	0.03705

Data File C:\CHEM32\1\DATA\CINEOLHP5\CINEOLHP5000006.D
Sample Name: A2

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
8	17.493	VV	0.0866	7164.55273	1219.14819	0.44153
9	17.999	VV	0.0980	1421.21094	233.59753	0.08758
10	18.294	VV	0.0696	362.35962	80.41840	0.02233
11	19.502	VV	0.1739	3.54036e4	2701.20264	2.18180
12	19.943	VV	0.0638	945.54596	221.42012	0.05827
13	22.136	VV	0.0801	1701.15857	324.86667	0.10484
14	22.600	VV	0.0871	2772.81860	474.98175	0.17088
15	22.761	VV	0.1241	2555.25830	275.88968	0.15747
16	22.884	VV	0.1551	1758.51843	150.76408	0.10837
17	23.330	VV	0.0796	419.37122	76.86871	0.02584
18	23.486	VV	0.1675	2006.04004	156.51010	0.12363
19	26.298	VV	0.2868	3505.69800	155.66043	0.21604
20	27.692	VV	0.1835	1451.16931	94.34206	0.08943
21	29.264	VV	0.2467	2571.39844	127.25582	0.15847
22	30.095	VV	0.3578	5129.17090	170.59309	0.31609
23	30.654	VV	0.1268	1378.02039	131.95184	0.08492
24	30.734	VV	0.1989	2147.56226	129.18472	0.13235
25	31.352	VV	0.2023	3724.69458	249.34007	0.22954
26	31.604	VV	0.3015	4721.86719	191.64485	0.29099
27	34.282	VV	0.3587	9280.20313	306.12198	0.57191
28	34.785	VV	0.2633	5730.39697	257.81198	0.35314
29	35.208	VV	0.1689	4340.21631	335.52353	0.26747
30	36.333	VV	0.3657	1.48961e5	4830.85742	9.17992
31	36.557	VV	0.1372	1.18553e4	1095.96472	0.73060
32	36.763	VV	0.2020	3987.71460	259.90710	0.24575
33	37.363	VV	0.1589	2964.93945	227.69521	0.18272
34	37.748	VV	0.1223	2923.63086	301.17896	0.18017
35	38.274	VV	0.1335	1790.25037	163.83006	0.11033
36	38.600	VV	0.0329	680.86487	295.22513	0.04196
37	38.771	VV	0.0966	3676.18945	478.43781	0.22655
38	39.343	VV	0.1544	4431.08594	348.28967	0.27307
39	40.907	VV	0.5405	2.84889e5	6229.83691	17.55671
40	41.178	VV	0.2094	1.22990e5	7019.15625	7.57945
41	41.674	VV	0.2926	2.10727e5	8547.51758	12.98638
42	41.971	VV	0.1746	1.11516e5	7625.52100	6.87233
43	42.025	VV	0.0505	2.77119e4	7981.09277	1.70779
44	42.095	VV	0.0505	2.29097e4	6595.62451	1.41184
45	42.645	VV	0.2665	1.78091e5	8037.06152	10.97510
46	42.880	VV	0.0931	7992.91650	1225.71558	0.49258
47	43.144	VV	0.1677	2.89931e4	2274.73462	1.78674
48	43.406	VV	0.1358	2.95744e4	2720.85425	1.82257
49	43.578	VV	0.0766	1802.47778	352.79764	0.11108
50	43.673	VV	0.0693	1029.52686	217.16457	0.06345
51	43.861	VV	0.0866	5044.12012	870.96710	0.31085
52	43.973	VV	0.0787	1829.37244	346.19003	0.11274
53	44.183	VV	0.1014	5656.13965	763.33264	0.34857
54	44.381	VV	0.1266	1.21869e4	1240.57544	0.75104
55	44.520	VV	0.0873	3117.01172	496.28149	0.19209
56	44.646	VV	0.1032	3948.69849	547.34656	0.24334
57	44.744	VV	0.0589	1321.24792	280.92010	0.08142
58	44.868	VV	0.1017	2281.12134	329.82184	0.14058
59	45.443	VV	0.2358	2.63066e4	1353.48694	1.62118
60	45.657	VV	0.1855	1.00623e4	700.97491	0.62010
61	46.133	VV	0.2142	2.87370e4	1666.18848	1.77096
62	46.523	VV	0.1450	3859.50977	323.86728	0.23785
63	46.664	VV	0.1315	3538.93188	337.09689	0.21809
64	47.235	VV	0.2312	2.31143e4	1208.34216	1.42445
65	47.353	VV	0.1210	9343.80469	1100.67065	0.57583
66	47.486	VV	0.0970	4129.99805	678.26300	0.25452
67	47.785	VV	0.1618	7044.36768	575.65985	0.43412
68	47.895	VV	0.0311	1137.36438	489.04700	0.07009
69	47.940	VV	0.1313	4793.10791	485.09613	0.29538
70	48.268	VV	0.1607	3517.68384	285.54807	0.21678
71	49.126	VV	0.3267	2.87795e4	1060.18970	1.77358
72	49.578	VV	0.2338	1.99494e4	1053.94971	1.22941
73	50.109	VV	0.0972	1444.60132	207.62732	0.08903
74	50.537	VV	0.2247	6896.29736	415.38141	0.42499
75	50.753	VV	0.0981	1467.52087	222.08269	0.09044
76	51.340	VV	0.1623	2468.05420	202.39185	0.15210
77	52.084	VV	0.2388	4805.58301	259.74261	0.29615

Data File C:\CHEM32\1\DATA\CINEOLHP5\CINEOLHP5000005.D
Sample Name: A2

Peak #	RetTime [min]	Type	Width [min]	Area	Height [pA]	Area %
78	52.576	VV	0.1791	5706.71045	410.84891	0.35168
79	53.428	VV	0.1793	2250.28684	155.98051	0.13868
80	54.303	VV	0.1677	1302.71472	104.28282	0.08028
81	55.882	VV	0.1641	2188.87256	175.99930	0.13489
82	57.783	VV	0.1379	2609.49585	247.80611	0.16081
83	59.132	VV	0.1168	1975.88660	235.82469	0.12177
84	59.354	VV	0.1235	1014.49982	118.96925	0.06252
85	60.014	VV	0.1215	639.88232	70.73090	0.03943
86	60.307	VV	0.1608	915.21832	72.71173	0.05640
87	62.093	VV	0.1188	1444.39307	160.71815	0.08901
88	63.168	VV	0.1178	2203.44800	249.97174	0.13579
89	64.270	VV	0.1201	742.71960	88.33508	0.04577
90	66.215	VV	0.1042	2004.15906	271.27066	0.12351
91	68.962	VV	0.1210	1453.18457	164.65703	0.08955
92	69.315	VV	0.1277	5605.20361	560.29395	0.34543
93	70.526	VV	0.1072	1598.62231	213.85455	0.09852
94	72.235	VV	0.1171	732.38672	88.93322	0.04513
95	72.829	VV	0.1270	1622.28967	175.27205	0.09998
96	73.131	VV	0.1033	1290.05273	187.54303	0.07950
97	74.993	VV	0.1099	1843.73877	229.11691	0.11362
98	90.840	VV	0.2674	1068.43030	50.79280	0.06584

Totals : 1.62268e6 1.02312e5

Calibration Curves

*** End of Report ***

APPENDIX C

Month/ Activity	August	September	October	November
Install the equipment				
Sample preparation				
Extraction process				
Collect & record result				
Data analysis				
Preparing for final report				
Final presentation				
Submission of final report &Correction				