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RESEARCH TITLE :

SOLVENT EXTRACTION OF OLEO-RESINS FROM VANILLA (VANILLA PLANIFOLIA ANDREWS) USING ULTRASONIC MULTIPURPOSE EXTRACTOR PILOT PLANT SYSTEM (UMEPPS)

(PENGEKSTRAKAN PELARUT OLEO RESIN DARIPADA VANILLA (VANILLA PLANIFOLIA ANDREWS) DENGAN MENGGUNAKAN PENGEKSTRAK ULTRASONIK BERSKALA LOJI PANDU PELBAGAI-GUNA)

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ABSTRACT

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is an invaluable aroma molecule, useful as food flavouring materials, fragrances, and pharmaceutical or directly in medicine. Vanilla plant (scientifically known as *Vanilla planifolia Andrews*) is the major sources of natural vanillin produced worldwide. The solvent extraction using ultrasonic multipurpose extractor pilot plant system (UMEPPS) was evaluated as a simpler and more effective alternative to conventional extraction methods for the extraction of oleo-resins from vanilla plant. The vanilla bean samples is extracted with different types of solvent (hereby referred as ethanol and hexane), varies types of vanilla bean surface area (grinded vanilla beans and chopped vanilla beans into 2 cm long) within several limits of time under direct sonication by an ultrasound probe horn located inside the ultrasonic extraction vessel. The mixture of solvent and the solute then undergo the separation process in the evaporation vessel to separate the solute from the solvent. The final separation is done in the thin film evaporator to purify the crude from the last percent of remaining solvent. The enhancement of extraction of oleo-resins from cured vanilla beans by ultrasound attribute to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave. Ethanol appears to be the best extraction solvent with the maximum extraction yield occurs when the grinded vanilla beans is used. 120 minutes seem to be the ideal extraction time for extraction using UMEPPS. The employing of ultrasound to the solvent extraction gives a significant reduction in extraction time and increasing maximum extraction yields. Vanillin was analyzed using HPLC on a Nucleosil C18 column by using water and methanol (40:60) as the mobile phase and retention time was only 2.245 min (extraction using hexane) and 2.107 min (extraction using ethanol) compared to the standard solution which is 2.159 min.

ABSTRAK

Vanilin (4-hydroxy-3-methoxybenzaldehyde) merupakan molekul aroma yang tidak terhingga nilainya, sangat berguna sebagai bahan penambah perisa makanan, bahan wangian, dan farmasi atau secara langsung dalam ubat-ubatan. Tumbuhan vanila (secara saitifiknya dikenali sebagai *Vanilla planifolia Andrews*) merupakan sumber utama bagi pengeluaran vanila asli dunia. Pengektrakan menggunakan sistem loji pandu pengekstak serbaguna ultrasonik dikatakan sebagai lebih mudah dan lebih efektif, alternatif kepada kaedah pengekstrakan konvensional untuk pengekstrakan minyak resin daripada tumbuhan vanila. Sampel kacang vanila akan diekstrak menggunakan jenis pelarut yang berlainan (dirujuk sebagai etanol dan heksana), pelbagai jenis luas permukaan kacang vanila (kacang vanila kisar dan kacang vanila yang dipotong 2cm panjang) dalam beberapa had masa di bawah sonikasi secara langsung oleh prop ultrabunyi yang terletak di dalam vesel pengekstrakan ultrasonic. Campuran bahan pelarut dan bahan terlarut akan melalui proses pengasingan di dalam vesel pengewapan untuk mengasingkan bahan terlarut daripada bahan pelarut. Pengasingan akhir akan dilakukan di dalam pengewap filem nipis untuk mengasingkan bahan kasar daripada peratusan terakhir baki bahan pelarut. Peningkatan dalam pengekstrakan minyak resin daripada kacang vanila yang dirawat menggunakan ultrabunyi dikaitkan dengan fenomena kavitasi yang terhasil di dalam bahan pelarut melalui lintasan gelombang ultrasonik. Etanol merupakan pelarut pengektrakan terbaik dengan hasil extrak maksimum terhasil apabila menggunakan kacang vanila kisar. 120 minit dilihat sebagai masa yang paling ideal bagi pengekstrakan menggunakan UMEPPS. Penggunaan ultrabunyi terhadap pengekstrakan telah memberikan pengurangan terhadap tempoh pengekstrakan dan meningkatkan hasil maksima pengekstrakan. Vanilin dianalisa menggunakan HPLC dan masa adalah 2.245 minit (heksana) dan 2.107 minit (etanol) berbanding 2.159 minit bagi larutan standard.

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LIST OF SYMBOLS

%	-	percentage
Hz	-	Herzt
kW	-	kilowatt
°C	-	degree Celcius
m	-	meter
cm	-	centimeter
mm	-	milimeter
in.	-	inches
kg	-	kilogram
µm	-	micrometer
ml	-	milimeter
µl	-	microliter
nm	-	nanometer
g	-	gram
T	-	Temperature
P	-	Pressure
L	-	liter
Min.	-	minute
i.d.	-	inside diameter

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CHAPTER 1

INTRODUCTION

1.1 The Advantages of Ultrasonic Extraction

Ultrasonic (i.e., ultrasound-assisted wave fields) extraction has been widely used for extracting non-volatile and semi volatile bioactive substances from different parts of a number of plants (Mason et al., 1999). The ultrasonic enhancement of extraction is attributed to disruption of cell walls, particle size reduction and enhanced mass transfer of the cell content via cavitations bubble collapse. Direct evidences are given, confirming that an enhanced hydration process and plant material fragmentation is the primary benefits of sonication.

The classical process of active compounds extraction from a plant material by means of a solvent is via diffusion of extractive substances (ES) through the cell walls and generally involves two main stages (Coulson and Richardson, 1991): first, dissolution of the soluble constituents on or near surfaces of solid plant particles (so called washing), and second, mass transfer of soluble constituents from the plant material into the solution by diffusion and osmotic process (so called slow extraction). Usually, the latter stage is slower than the former one and is responsible for limiting the rate of extraction process. The mechanism of ultrasonic extraction is expected to enhance the diffusivity rate, the extraction and sonication time, distribution of coefficient, selectivity and extraction rate constant.

In ultrasonic extraction method, the vanilla plants are sonicated in the extraction vessel, followed by the distillation process to separate the two phases of

mixture components. Purification is needed to remove solvent from the vanilla oleoresin. A newly designed short path distillation rig is used during this stage. The method has considerably 'speed-up' the purification process and the vanilla oleoresin was purified. This extraction plant will give extract that richer in high volatility components than that obtained by traditional methods.

1.2 The Importance of Vanillin

Vanillin (4-hydroxy-3-methoxybenzaldehyde) as shown in Figure 1.0 is the major component of natural vanilla, which is one of the most widely used and important flavoring materials worldwide. The source of vanilla is the bean, or pod, of the tropical Vanilla orchid (principally Vanilla planifolia Andrews). Although more than 12,000 tons of vanillins are produced each year, less than 1% of this is natural vanillin from Vanilla plant; the remainder is synthesized much more cheaply via chemical processes. The value of vanillin extracted from Vanilla pods is variously calculated as being between $1200 per kg and $4000 per kg, in contrast to the price of synthetic vanillin, less than $15 per kg (Lomascolo et al., 1999; Muheim and Lerch, 1999).

Vanillin is a component of vanilla oleoresin responsible for ginger's medicinal effects. Specifically, vanillin has been identified as an anti-pyretic for the pharmacologist effects, food flavor, cosmetics agent and etc. There are two main products from vanilla, vanilla oleoresin and essential oil. For the active components, there are nine main components form the vanilla's extracted; ethyl hexanoate, *p*-methoxybenzaldehyde, 5-propenyl-1,3-benzodioxole, ethyl nonanoate, one unidentified component, *p*-methoxybenzoic acid methyl ester, 3-phenyl- 2-propenoic acid methyl ester, ethyl decanoate and vanillin. Figure 1.1 shows the molecule structure and route of vanillin (4-hydroxy-3-methoxybenzaldehyde) formation provide by Zenk (1965).

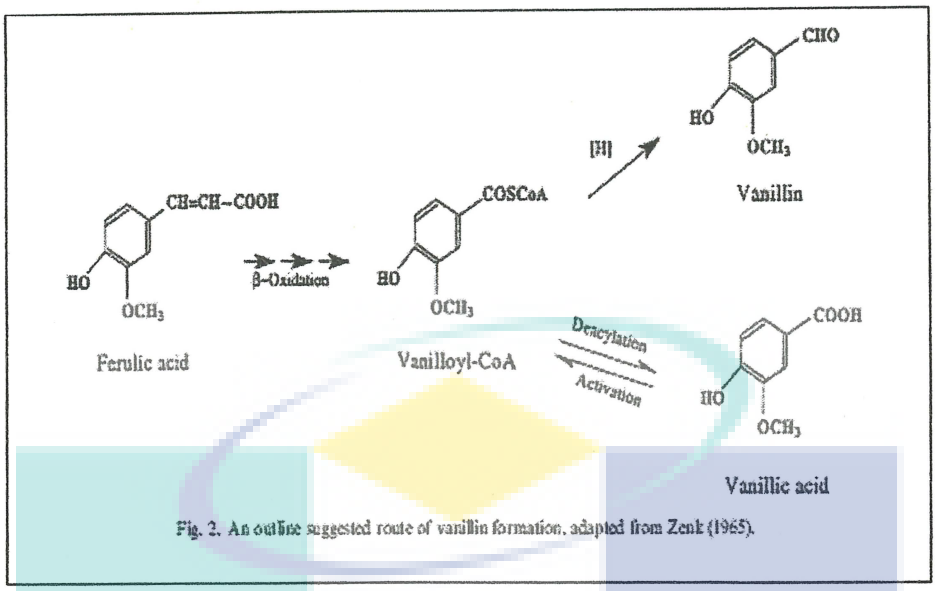


Figure 1.1 Molecule structure and route of vanillin (4-hydroxy-3-methoxybenzaldehyde) formation (Zenk, 1965)

Besides being a very popular flavor, vanillin is also used in the synthesis of drugs, and this use now surpasses its use as a flavoring agent. The largest single use for vanillin as a starting material is for the manufacture of the antihypertensive drug called Aldomet (1-3-(3,4-dihydroxyl phenyl)-2-methylalanine). L-dopa is another drug made from vanilla and is used for the treatment of several neurodegenerative diseases such as Alzeimer's and Parkinson's disease (Kumar et al., 2004). Like many polyphenols found in plants, vanillin has antioxidant and anti-tumor activity and has been reported to show antimutagen, anticlastogen and anticarcinogen (Durant et al., 2003).

1.3 Research Background

The main purpose of this study is to exploit the potential use of the vanilla be it as fragrances, cosmetics, foods and non-foods and medicinal use.

Vanilla essentials oil is obtained through steam distillation whilst the oleoresin is through solvent extraction by using organic extraction solvent and ultrasonic energy. To maximize the yield of the products from vanilla, an optimum method of sample preparation was established. This requires that the fresh vanilla undergo a series of pre-treatment. This research will focus in producing oleoresin. Oleoresin is total extracts of the natural spice or herb, representing the volatile and the non-volatile components of the spice or herb. The dried vanilla contains approximately 20 to 25% oleoresin. The appearance of vanilla oleoresin is a shinning black or very dark brown.

The current work deals with the ultrasonic extraction of vanillin from dry vanilla plant (*Vanilla planifolia*) ultrasonic multipurpose extractor pilot plant system. The main aim is to check if the ultrasound-assisted extraction method for extracting the vanillin from vanilla plant will be the best extraction method compare to the other extraction methods. Also, the work is aim to determine the different parameters such as vanilla surface area, the solvent to material ratio and sonication time (exposure time), associated with extraction process in order to obtain the maximum yield of the vanillin from vanilla plant. The other goal is to choose the best extracting solvent (from hexane and ethanol) to ensuring the highest yield of vanillin. The selectivity and the yield of the vanilla product will be analyzed using HPLC (High Performance Liquid Chromatography) and GC (Gas Chromatography).

1.4 Objective

The objective of this research is to obtain essential oil from *Vanilla Planifolia* plant source using ultrasonic extraction technique, competitive in terms of quantity and cost to essential oil produced by traditional methods, by investigating and understanding ultrasonic extraction process as an advanced high-speed extraction technique.

1.5 Research Scope

In order to achieve the objective of this research, five scopes have been identified in this research.

1) To study the influence of the variation of vanilla surface area in vanilla extraction.

   This study is conducted to discover the effect of the variation of vanilla surface area to the yield of vanilla oleo-resins using the ultrasonic multipurpose extractor pilot plant system. This done by two types of surface area which are grinding the vanilla pods into small particles and chop the vanilla beans into 2 cm pieces.

2) To study the influence of type of solvent to the yield of vanilla oleo-resins.

   The focus of this study is to observe the type of solvent that can enhance the production of yield within a specified range of time. In this study, two selected solvent was choose based on their high degree of extraction properties. There were ethanol and hexane.

3) To study the solvent to material ratio in vanilla extraction.

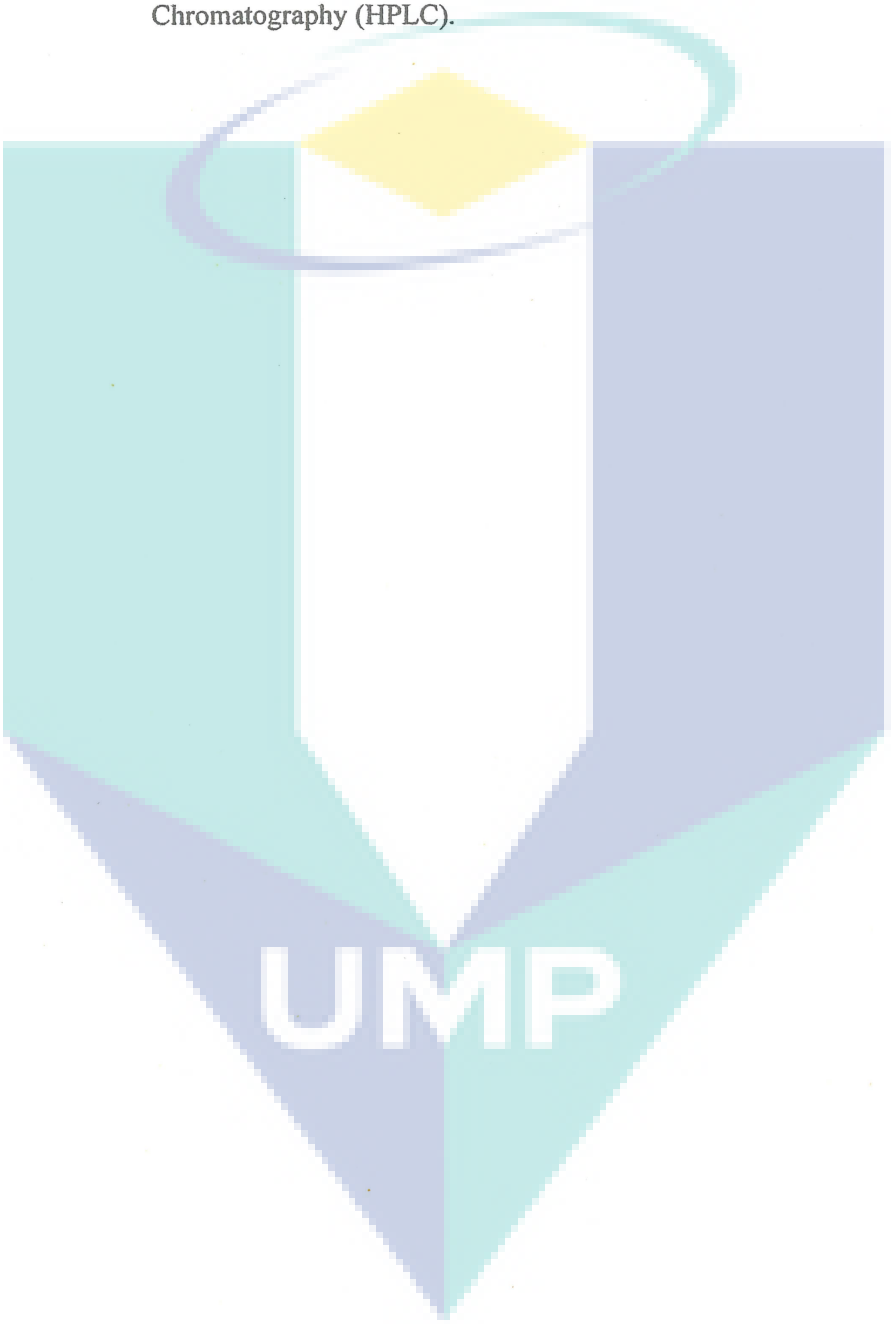
   This study is needed to identify the ideal solvent to material ratio to achieve the greatest yield of vanilla oleo-resins. The study used 1:2, 1:3 and 1:4 of the material to solvent ratio respectively.

4) To study the sonication or exposure time to the yield of vanilla.

   This study is aim to observe the optimum time needed in extracting vanilla oleoresin using the ultrasonic multipurpose extractor pilot plant system.

5) To analyze the selectivity and the yield of the vanilla product using HPLC (High Performance Liquid Chromatography) and GC (Gas Chromatography).

The selectivity and the yield of the vanilla oleo-resins has been analyzed using two analyzing equipment which are Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC).



CHAPTER 2

LITERATURE REVIEW

2.1 Separation Processes

2.1.1 Introduction of Separation Process

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. The two-phase pair can be gas-liquid, gas-solid, liquid-liquid, or liquid-solid. 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 processes is defined as any set of operations that separate solutions or mixture of two or more components into two or more products that differ in composition (Noble and Terry, 2004). Separation process is used for three primary functions of separation process which are:

a) Purification

The principal is to remove undesired components in a feed mixture from the desired species. For example, the purification of acidic gases such as sulfur dioxide which must be removed from power plant combustion gas effluents before discharging into atmosphere.

b) Concentration

The principal is to obtain higher proportion of desired components that are initially dilute in a feed stream. For example, concentration of metals present in an electroplating process by removing water. This separation allows one to recycle the metals back to the electroplating process rather than discharge into the environment.

c) Fractionation

In fractionation, a feed stream of two or more components is segregated into product streams of different components, typically relatively pure streams of each component.

Separation process is divided into two classes which are equilibrium-based process and rate-based process. These classes are designated using thermodynamic equilibrium relationships between phases and the rate of transfer of a species from one phase to another respectively.

Equilibrium-based processes are those in which the degree of separation in each stage is governed by a thermodynamic equilibrium relationship between the phases. The common processes related to this category are distillation and extraction and leaching. In distillation, 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 dissimilar

boiling temperatures. In extraction, a species is removed from a liquid in which it is dissolved by means of another liquid for which it has a higher affinity. As 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 limited 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).

2.1.2 Types of Separation Processes

1. Absorption

When the two contacting phases are a gas and a liquid, this operation is called *absorption* (Geankoplis, 2003). A solute *A* or several solutes are absorbed from the gas phase into the liquid phase in absorption. This process involves molecular and diffusion or mass transfer of solute *A* through a stagnant, nondiffusing *B* by the liquid water *C*. Usually, the exit ammonia-water solution is distilled to recover relatively pure ammonia.

2. Distillation

In the *distillation* process, a volatile vapor phase and a liquid phase that vaporizes are involved (Geankoplis, 2003). An example is distillation of an ethanol-water solution, where the vapor contains a concentration of ethanol greater than in the liquid.

3. 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 (Geankoplis, 2003). One example is extraction of acetic acid from a water solution by isopropyl ether.

4. Leaching

If a fluid is being used to extract a solute from a solid, the process is called *leaching* (Geankoplis, 2003). Sometimes this process is also called *extraction*. Examples are leaching vegetable oils from solid soybeans by organic solvents such as hexane.

5. Membrane processing

Separation of molecules by the use of membranes is a relatively new separation process. The relatively thin, solid membrane controls the rate of movement of molecules between two phases (Geankoplis, 2003). It is used to remove salt from water, to purify gases, in food processing, and so on.

6. 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 as a solid phase (Geankoplis, 2003). Examples of this separation process are crystallization of sugar from solution.

7. 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 is obtained (Geankoplis, 2003). Example includes removal of organic compounds from polluted water.

8. Ion Exchange

In an *ion-exchange* process, certain ions are removed by an ion-exchange solid (Geankoplis, 2003). This separation process closely resembles adsorption.

2.2 Extraction

In order to separate one or more of the components in a mixture, the mixture is brought into contact with another phase. The two-phase pair can be gas-liquid, vapor-liquid, liquid-liquid or liquid-solid. Extraction is the removal of 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 and Terry, 2004). Separation through extraction depends on differences in both solute solubility and density of the two phases.

There are few advantages of extraction separations. Firstly, extraction can be performed at ambient temperature. Therefore, it is relatively energy efficient and can be applied to separations involving thermally unstable molecules. Close boiling mixtures or substances that cannot withstand the temperature of distillation, even under a vacuum, usually are separated from impurities by extraction, which utilizes chemical differences instead of vapor pressure differences. Besides, 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.

Nevertheless, extraction also has its disadvantages. In extraction 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 et. al., 2001).

2.2.1 Solvent Extraction

Solvent extraction, also called as liquid-liquid extraction, is the process of transferring a substance from any matrix to an appropriate liquid phase (IUPAC Compendium of Chemical Technology, 1997). There are three major components in this extraction. There are:

1. The *solute*, which is the component or components to be removed from the feed by contacting solvent.
2. The *feed*, which is a stream containing a solvent and the solute to be removed in the extraction process. The feed is called the raffinate stream after it begins to lose the solute.
3. The *solvent*, which is the liquid that absorbs the solute. The solvent is called the extract stream when it leaves the contactor.

The feed stream (raffinate stream) and the solvent (extract stream) must be two immiscible streams. More simply, these two liquid streams must be insoluble or exhibit very low mutual solubility (Erwin, 2002).

Solvent extraction involves contacting the feed stream (raffinate stream) with an immiscible liquid solvent in which one or more of the feed stream components are soluble. Thus two different liquid phases are formed after addition or mixing of the solvent with the feed. The component that is more soluble in the solvent than in the feed will transfer to the solvent, a process known as *absorption*. Thus in solvent extraction there are two immiscible liquids of which at least one single component is soluble in both the feed and the contacting solvent. This component (the solute) transfers from one liquid phase to the other. This extraction transfer process falls into three categories:

1. The solute transfer occurs due to the solute being more soluble in the solvent liquid phase than in the feed (raffinate) liquid phase.

2. The solute transfer occurs due to the solvent phase having a greater mass flow or the solvent having a much smaller quantity of solute. Either or both of these situations may occur.

3. The solute transfer occurs because of a chemical reaction between the solute and the solvent.

When separation by distillation is ineffective or very difficult, solvent extraction is one of the main alternatives to consider. Close-boiling mixtures or substances that cannot withstand the temperature of distillation, even under a vacuum, may often the separated from impurities by extraction, which utilizes chemical differences instead of vapor pressure differences. For example, penicillin is recovered from the fermentation broth by extraction with a solvent such as butyl acetate, after lowering the pH to get a favorable partition coefficient. The solvent is then treated with a buffered phosphate solution to extract the penicillin from the solvent and give a purified aqueous solution, from which penicillin is eventually produced by drying. Extraction is also used to recover acetic acid from dilute aqueous solutions; distillation would be possible in this case, but extraction step considerably reduces the amount of water to be distilled.

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. The vapor and liquid phases are similar chemically. In solvent extraction, the two phases are chemically quite different, which leads to a separation of the components according to physical and chemical properties. Solvent extraction can sometimes be used as an alternative to separation by distillation or evaporation. For another example, acetic acid can removed from water by distillation or by solvent extraction using an organic solvent. The resulting organic solvent-acetic acid solution is then distilled.

2.2.2 Extraction Equipment

There are two main classes of solvent extraction equipment, vessels in which mechanical agitation is provided for mixing, and vessels in which the mixing is done by the flow of the fluids themselves. The extraction equipment may be operated batch wise or continuously as in absorption and in distillation. A quantity of feed liquid may be mixed with a quantity of solvent in an agitated vessel, after which the layers are settled and separated. The extract is the layer of solvent plus extracted solute, and the raffinate is the layer from which solute has been removed.

In solvent extraction, as in the separation processes of absorption and distillation, two phases in liquid-liquid extraction must be brought into intimate contact with a high degree of turbulence in order to obtain high mass-transfer rates of a material. After this contact of the two phases, they must be separated. In both absorption and distillation, this separation is rapid and easy because of the large difference in density between the gas or vapor phase and the liquid phase. In solvent extraction, the density difference between the two phases is not large, so that the energy available for mixing and separation - if gravity flow is used - is small, much smaller than when one phase is a liquid and the other is a gas. The two phases are often hard to mix and harder to separate. The viscosities of both phases also are relatively high, and linear velocities through most extraction equipment are low. In some types of extractors, therefore, energy for mixing and separation is supplied mechanically (McCabe et. al., 2001). Extraction equipment may be operated batch wise or continuously.

2.3 Ultrasonically Assisted Extraction of Bioactive Substances from Plant Materials

2.3.1 Introduction

Vegetal materials are invaluable resources, useful in daily life as food, food additives, flavors, fragrances, pharmaceuticals, colors or directly in medicine. This use of plants has a long history all over the world and, over the centuries, humanity has developed better methods for the preparation of such materials. Nowadays, there is a renaissance of interest in natural remedies, in part due to some disillusionment with modern medicine and drugs that either do not perform entirely to expectation or are accompanied by unwanted side effects. Natural remedies have the advantage that they have passed the proof of the time. On the other hand some synthetic drugs even though they have been used for over 100 years, may still need more time to proven to be absolutely harmless.

Bioactive substances from plant materials could be defined as the compounds and/or compound mixtures obtained from fresh or dried plants, or part of plants: leaves, flowers, seeds, roots and barks, by different extraction procedures. Characteristically, the active constituents are obtained together with other materials present in the vegetal mass.

Extraction is the first important step for recovery and purification of bioactive substances from different parts of plant materials. The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of materials and the rate of mass transfer. Usually, the traditional techniques require long extraction hours and have low efficiency. The extraction of bioactive components from plant materials is part of phytopharmaceutical and food technology.

Medicinal and aromatic plants provide an inexhaustible resource of raw materials for the pharmaceuticals, cosmetics and food industries and more recently in agriculture for pest control (Rice, 1975). People have learned to increase the power or usefulness of herbs by preparing medicinal compounds from them, by preserving them so that they are always available, and by finding new ways to release their active constituents. Among modern methods used to release the bioactive constituents from plant materials is ultrasonically enhanced solvent extraction. The

use of ultrasound to enhance the extraction yield is a technique that started in the 1950s with laboratory scale experiments (Schmall et. al., 1954).

2.3.2 Extraction Procedures

To obtain extract from plant materials, several methods are available. Among them:-

1. Distillation:

   (a) Direct essential oil distillation;
   (b) Water steam distillation;
   (c) Water and steam distillation.

2. Solvent Extraction:

   (a) Solvent extraction (percolation);
   (b) Maceration with solvent;
   (c) Boiling with water (infusion);
   (d) Extraction with cold fat (effleurage);
   (e) Extraction with hot fat.

3. Cold compression, which is the usual method for the natural oil industry.

4. Non-conventional extraction techniques:

   (a) Supercritical fluid extraction;
   (b) Vortical (turbo) extraction;
   (c) Extraction by electrical energy;
   (d) Ultrasonic extraction.

2.3.2.1 Distillation

Distillation means that plant materials are mixed (or not) with water followed by heating or by the introduction of water steam. The resulting vapors are cooled and collected in a separator and the essential oil or oleo-resin separates from water. This yields a crude essential oil or oleo-resin that should be further distilled. This is the main procedure for obtaining essential oil or oleo-resins, but some other extraction techniques such as extraction with light solvents can offer similar or better extracts (Vinatoru et. al., 1997).

During distillation it is obvious that the use of ultrasonic energy is futile. A distillation unit provided with an ultrasonic source will produce more rapidly boiling centers, but no collapsing bubbles. Therefore this kind of unit will be useful only to enhance boiling with little, if any, improvement in yield.

However, ultrasound can be successfully employed to enhanced extraction when low boiling point solvents are used, and the temperature of the extraction mixture is kept below it boiling point. An example showing how ultrasound can help solvent extraction of essential oils from different vegetal materials is given in Table 2.1.

It is interesting to note that at longer extraction times, [7 day maceration (M) + 30 min ultrasound extraction (US) + 240 min reflux (R)] the concentration of limonene decreases even when ultrasound is used. This is quite strange since the amount of oil is greater than that obtained via classical or ultrasound extraction. It seems obtained via classical or ultrasound extraction. It seems that maceration prevents more limonene being extracted but the mechanism not yet understood. One of the most important observations in this case is that ultrasonically assisted extraction leads to non-detectable amounts of heavy components.

Table 2.1 : Comparison between different extraction methods of 100 g of dill seeds. (M. Vinatoru, 2001)

Method used	Oil amount (g)	Extraction time	Components		
			Limonene %	Carvone %	Heavy comp. %
CE	3.00	240 min	40.79	47.29	0.09
US	3.40	30 min	49.63	48.15	-
US	3.40	60 min	51.22	45.84	-
M + US	3.50	7 days M + 30 min US	25.08	66.84	0.05
M + US	3.50	7 days M + 60 min US	20.77	65.40	0.34
M + US + R	3.55	7 days M + 30 min US + 240 min R	20.72	65.69	0.10
M + US + R	3.55	7 days M + 60 min US + 240 min R	20.28	65.92	0.34

2.3.2.2 Solvent Extraction

Solvent extraction procedures, excluding fat extraction, are more amenable to ultrasonic treatment. This can be achieved simply by the introduction of an ultrasonic transducer into the extraction unit. This is possible because in almost all cases solvent extraction uses cold solvent (percolation can be done with cold as well as with hot solvent).

2.3.2.3 Non-Conventional Extraction Techniques

Extraction with supercritical fluid is one of the newer extraction techniques that can offer very good yields. For this procedure the most often used supercritical fluid is carbon dioxide. A typical scheme for a supercritical extraction unit is given in Figure 2.1. From the bottle 8, the carbon dioxide gas after passage through a heat exchanger and filter (4 and 7) is compressed in the compressor 3, and cooled again until reaching the liquid state when it is introduced into the extraction unit 1. After completing the extraction, the liquid is transferred though a throttle 5 into the separation unit 2. Here the liquid carbon dioxide is heated to reach the gaseous state, removed, and from the bottom of the vessel the extract is drawn off through the valve 6a.

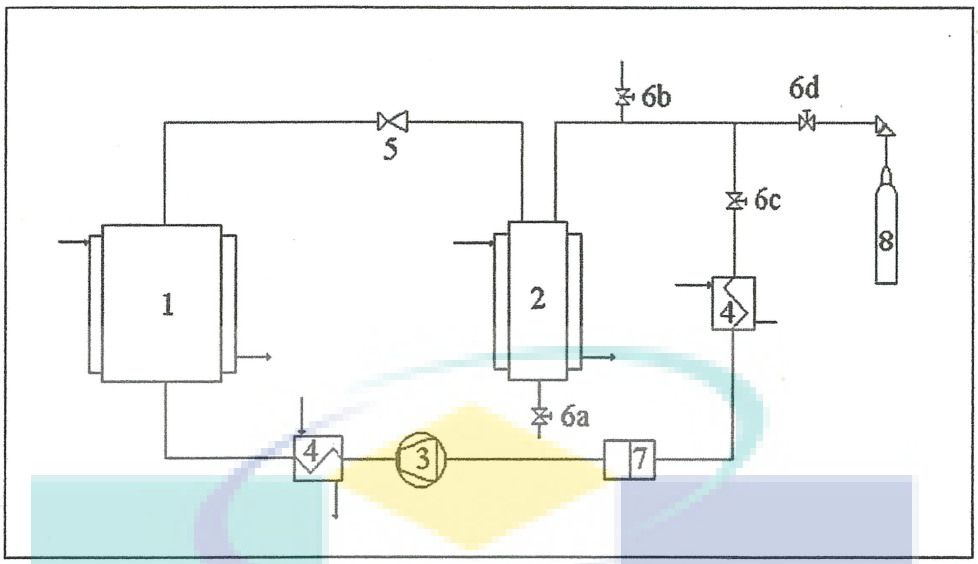


Figure 2.1 Schematic diagram of supercritical fluid extraction unit. (M. Vinatoru, 2001)

The vortical (turbo) extraction procedure uses as high speed stirrer that induces hydrodynamic cavitation, enhancing thereby the extraction yield. It is obvious that using a high speed stirrer, the contact between vegetal material and solvent is improved and therefore the diffusion process through the cell walls is increased (M. Vinatoru, 2001). Moreover, during vortical extraction hydrodynamic cavitation bubbles are produced and their collapse acts in a similar way to effect of ultrasonic devices. If the vegetal material is well milled, the differences between classical and vortical extraction diminish.

Electrical discharges within the extraction mixture were also claimed to increase the extraction yield, as suggested for the first time by Issaev and Mitev (1968) and perform by the same authors (1972) for *Cytisus laburnum* (broom). This extraction technique is represented in Figure 2.2. The alkaloid yield during the extraction of *Rauwolfia* by electrical discharge was increased by 25%, according to Boiko and Mizineko (1970). All authors working with this type of apparatus noted that during electrical discharges, cavitation bubbles are produced and that this technique therefore has similarities to ultrasonic extraction.

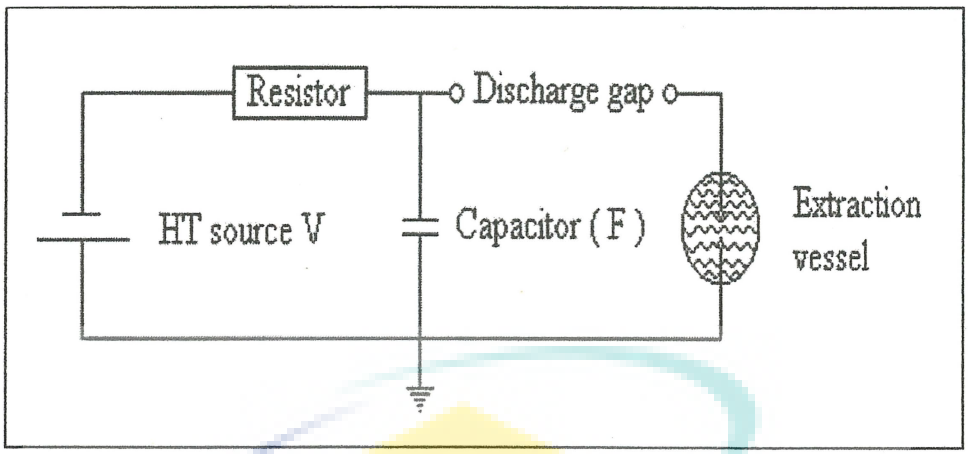


Figure 2.2 Schematic diagram of an electrical discharge extraction unit. (M. Vinatoru, 2001)

2.3.2.4 Ultrasonic Extraction

A number of papers have been published dealing with the ultrasonically assisted extraction of different vegetal materials. One of the first citations concerning ultrasonic extraction (1952) was related to hop extraction in an aqueous medium and showed that ultrasonic extraction was comparable with the boiling extraction process (Specht et. at., 1952). It was shown that during ultrasonic extraction it was possible to save some 30 to 40% of hops in the production of beer (Schmall et. al., 1953). Several references concerning ultrasonically assisted extraction are summarized in Table 2.2. It is worth noting that when high frequency ultrasound is employed, the extraction yield did not increase significantly however the degradation of the herb constituents was diminished. In the case of low frequency sonication degradation becomes more important, especially when alkaloids are being extracted. This effect could be employed as a tool to help in the extraction of medicinal compounds by using lower frequencies to assist in the degradation of toxic alkaloids during the process.

The use of ultrasound in ambient fluids is well known to cause a number of physical effects (turbulence, particle agglomeration, microstreaming and biological

cell rupture) as well as chemical effect (free radical formation). These effects arise principally from the phenomenon know as cavitation. Cavitation refers to the formation, growth and violent collapse of microbubbles in a sonication liquid due to pressure fluctuations (Leighton, 1994).

Table 2.2 : Examples of ultrasonically assisted extraction. (M. Vinatoru, 2001)

Ultrasonic frequency (kHz)	Extracted drugs	Remarks
2400	Cinchona bark	No yield improvement (too high frequency US)
400	Peanuts (oil)	Ultrasonic yield increased when hexane is used as solvent
500	Belladonna leaves	Similar yield as maceration, no decomposition of alkaloids, for short time sonication
25	Rauwolfia roots	US time 15 min; maceration 8 h for the same yield
20–40	*Datura stramonium* (thorn apple)	US offer 9% greater alkaloids in 1 h, but 40 kHz are more effective
20	Cinchona bark	US extraction gives 15% more alkaloids in 1.5 h comparing with 7 h Soxhlet.
1000	Nux vomica seeds	1.2% and 0.95% US alkaloids extraction yield in 20 min, compared with 0.64% and 0.94% in 8 h[a]
800	Digitalis leaves	US lead to similar or better yield, but decrease the glycoside yield due to H_2O_2 formation
20	Berberine	US extraction 50% greater in 0.5 h, compared with alkaline extraction for 24 h
1000	*Urtica dioica*	US extraction gives better results after 5 min, using 1 W/cm^2
800	*Inula helenium* and *Telekia speciosa*	No degradation of inulin, good yield for shorter time (10–40 min)
1000	*Amarantus retroflexus*	5 min sonication do not affect the extracted amino acids composition

Ultrasound has been shown to aid extraction in a number of plant materials by significantly reducing extraction times and increasing maximum extraction yields. An example is the extraction of helicid, a Chinese medicine used in the treatment of fatigue and listlessness, from dried seed of *Helicid erraticum* using aqueous ethanol (Zhao et. al., 1991). Conventional extraction is usually performed at reflux temperature of 80°C for 2 hours. Ultrasonic at 40°C resulted in a yield increase of 50% in the shorter time of 1 hour. Studies on the effect of ultrasound on the extraction of the main components of sage (*Salvia officinalis*) showed that cineole, thujone and boneol could be extracted better when sonicated (Salisova et. al., 1997).

The observed enhancement of extraction of organic compounds by ultrasound is attributed to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave as illustrated in Figure 2.3. During the rarefaction cycle of the sound wave cavitation bubbles are produced which fill with water solvent vapour.

During the compression cycle the bubbles and the gas within them are also compressed resulting in a resultant 'shock wave' passing through the solvent and enhanced mixing occurring. Ultrasound also exerts a mechanical effect, allowing greater penetration of solvent into the plant body. This coupled with enhanced mass transfer and significant disruption of cells, via cavitation bubble collapse, has the effect of releasing cell contents into the bulk medium.

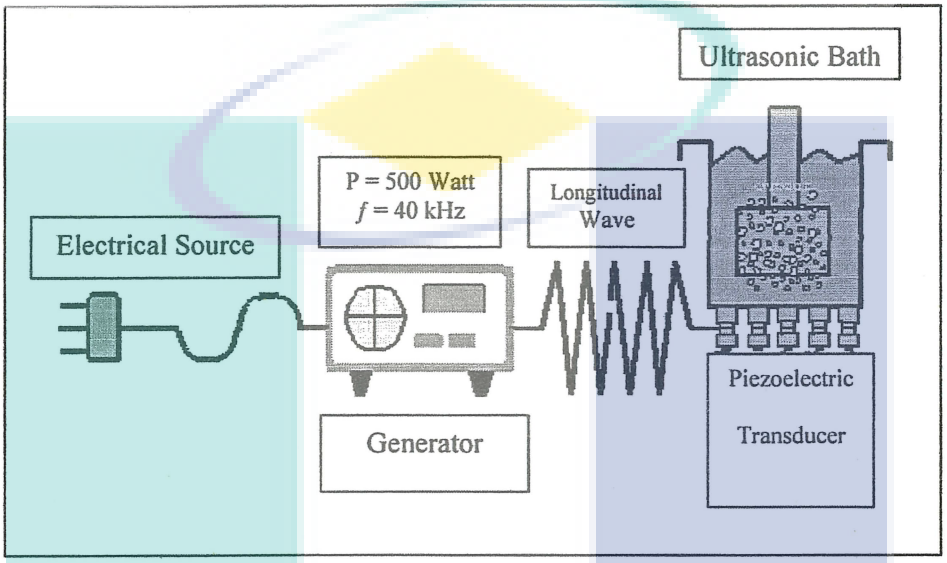


Figure 2.3 Schematic diagram of on how cavitational activity is working. (after Ziad, 2004)

Ultrasound may also produce some chemical effect due to the production of free radicals within the cavitation bubbles. Sonication of water results in the formation of highly reactive hydroxyl radicals which can combine to form hydrogen peroxide which may or may not be beneficial to the extraction process itself.

2.3.3 Extraction Mechanism

Vegetal tissue consists of cells surrounded by walls (Figure 2.4). The extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out (rinsing) the cell contents once the walls are broken.

Both phenomena are significantly affected by ultrasonic irradiation. Some cells exist in the form of glands (external or internal) that are filled with essential oil. A characteristic of such glands (when external) is that their skin is very thin and can be very easily destroyed by sonication. This explains why the extraction of essential oil, as well as fat oil, is facilitated by sonication.

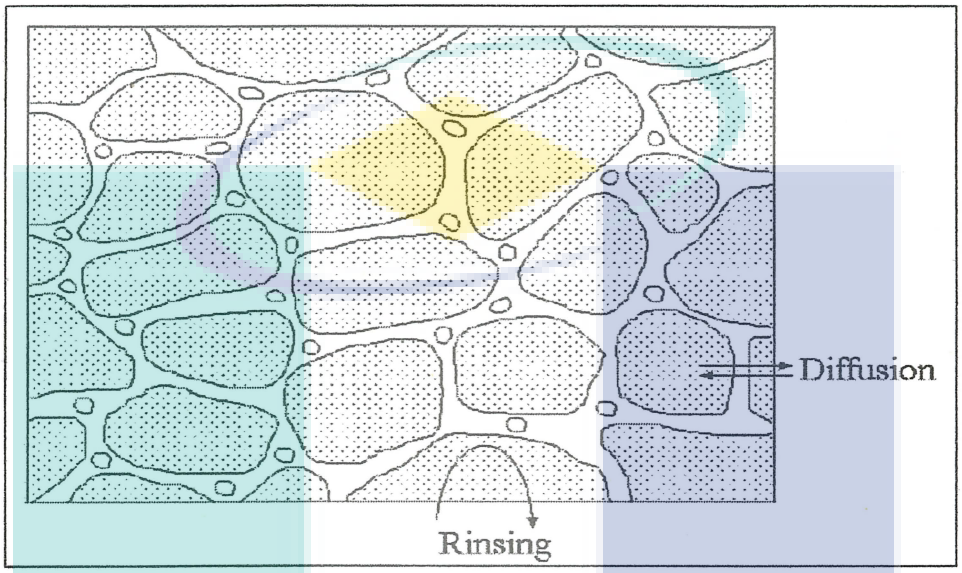


Figure 2.4 Schematic diagrams of vegetal cell structures. (M. Vinatoru, 2001)

For internal glands, it is the milling degree of the vegetal material which plays an important role and this is illustrated in Table 2.3. It is obvious that reducing the size of the vegetal material particles will increased the number of cells directly exposed to extraction by solvent and thus exposed to ultrasonically induced cavitation. This effect can be utilized by milling the material before extraction. It should be borne in mind however that powerful sonication can itself serve to mill the vegetal material. External essential oil glands are already exposed directly to the cavitating solvent and consequently are readily disrupted.

The ultrasonic breakdown of vegetal cells using normal ultrasonic extraction devices such as a cleaning bath or probe system, may not be the only mechanistic hypothesis for extraction improvement, especially when dried materials is used. This

is because solvent extraction from dried material is a two stage process involving which are:

a) Steeping vegetal materials in solvent to facilitate swelling and hydration processes;

b) The mass transfer of soluble constituents from the material to solvent via diffusion and osmotic processes.

Table 2.3 : Influenced of milling degree on the extraction of clove flowers (M. Vinatoru, 2001)

Extraction time (min)	Extraction technique	Milling degree	Eugenol extracted (g/100g)
30	Silent	not milled	4.10
30	Silent	0.1–0.5 mm	25.20
30	US	not milled	4.22
30	US	0.1–0.5 mm	32.66

Ultrasound can facilitate swelling and hydration and so cause an enlargement in the pores of the cell wall. This will improve the diffusion process and therefore enhancing mass transfer. Both steps have been shown to be sensitive to sonication as can be seen in Figure 2.5. Ultrasound increases the swelling index i.e. the water uptake by the vegetal material during sonication. The extractive value is much greater under sonication, compared with mechanical stirring. An increase in the swelling of vegetal tissue can, in some cases, break the cell walls which favor the washing out process.

The mechanical effect of ultrasound during extraction can be demonstrated in the case of ginger extraction (Balachandran et. al.,2005). The ginger particles was been analyzed by field emission scanning electron microscopy (SEM). Figure 2.6 shows that ultrasonic vibration does indeed disturb the cell walls and thereby facilitates removal of the cell contents.

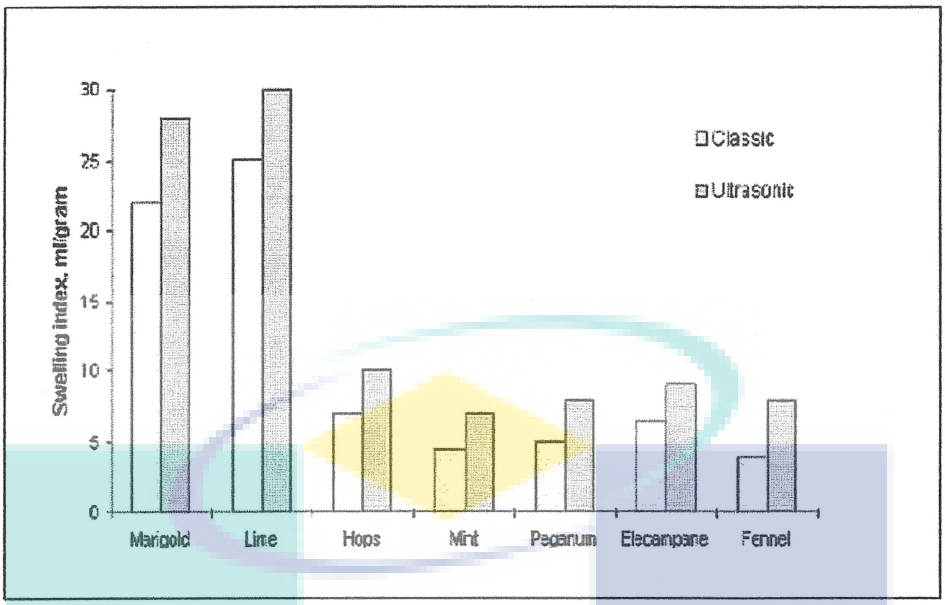


Figure 2.5 The enhancement of vegetal material swelling induced by ultrasonic treatment (M. Vinatoru, 2001)

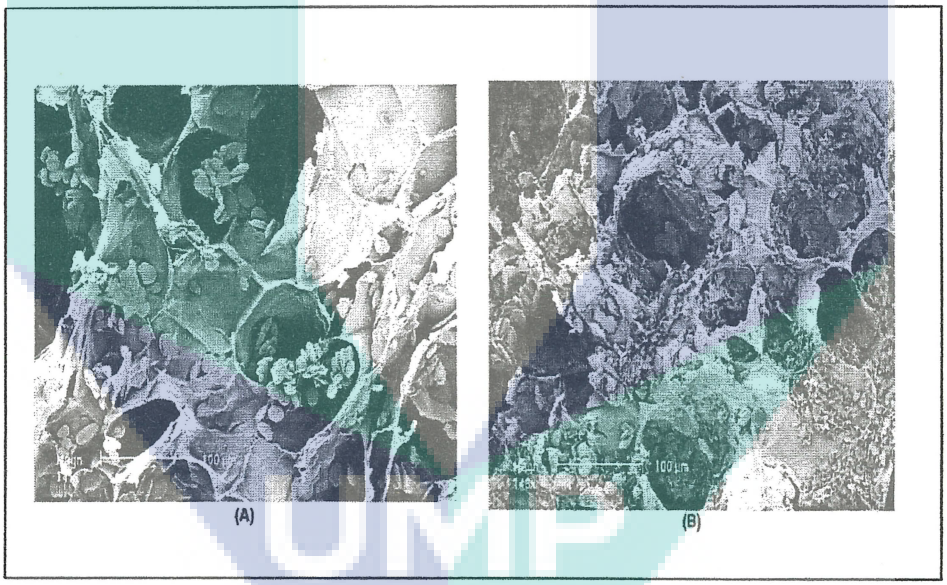


Figure 2.6 SEM pictures of ginger particles. (A) Experiments without the influence of ultrasound, (B) experiments with ultrasound. (S. Balachandran et al., 2006)

2.4 Vanilla Plant

2.4.1 Introduction

Vanilla is the name given to a genus of orchids that grow in tropical climates and to the flavor extract obtained from the fruit pods or beans of several orchid species. It is the only edible fruit of the orchid family, the largest family of flowering plants in the world. Porteres in Bouriquet (1954) describes 110 species of vanilla, distributed in the tropics of both the world and the New World. Of the 110 varieties of vanilla only two types are used commercially – Bourbon Vanilla and Tahitian Vanilla.

1. Tahitian vanilla (*Vanilla Tahitensis*)

   Tahitian vanilla is the generic name for *Vanilla Tahitensis*. This variety originates from plant stock taken to Tahiti, which probably mutated in the wild. Now regarded as a different species, the appearance and flavour is considerably different to Bourbon vanilla. Tahitian vanilla is sweet and fruity and contains less natural vanillin. It has a floral fragrance and the bean is fatter and moister than Bourbon vanilla.

2. Bourbon vanilla (*Vanilla Planifolia*)

   Bourbon vanilla is the generic name for *Vanilla Planifolia.* Originating in Mexico (vanilla's birthplace) Bourbon vanilla cuttings were taken in the 1800s and grown by the French in large plantations in Reunion then known as the Ile de Bourbon thus explaining the origins of the name. Bourbon vanilla has the familiar vanilla flavour we have come to know and love, such as that in ice cream, flavoured desserts and drinks.

Vanilla grows within the 20°C band either side of the Equator and is native to the Americas. Vanilla planifolia (also known as fragrans) grows on the Atlantic Gulf side of Mexico from Tampico around to the northeast tip of South America, and from Colima, Mexico to Ecuador on the Pacific side. It also grows throughout the Caribbean. They exhibit a wide range of life form and have terrestrial, climbing, epiphytic and saprophytic species. Apart from the large number of ornamental species which are grown for the flowers, vanilla is the only genus which has species of economic importance. Figure 2.7 shows the tabulated of world's vanilla crops.

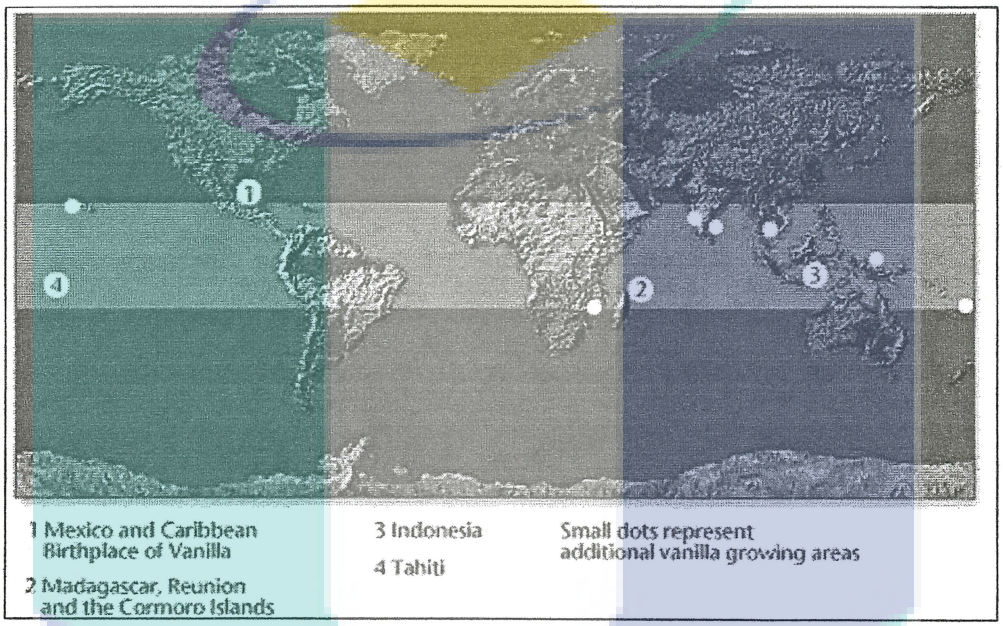


Figure 2.7 The tabulated of world's vanilla crops. (FAO, 2002)

Vanilla is the world's most labour-intensive agricultural crop, which explains why vanilla is so expensive. Tahitian vanilla has always been more expensive than Bourbon vanilla, especially now, as it is less readily available.

2.4.2 History of Vanilla Plant

Vanilla planifolia is indigenous to Mexico and may have been used up to 1000 years ago by the Totonac tribe as a flavoring. The Totonacas still grow vines with almost religious devotion because to them it was the gift of the Gods. It is not uncommon to have a few vines growing around their houses. These are watered every day as if they were the Totonacas most valuable possession. The vanilla beans were used as a tribute to the Emperor of the Aztecs.

In 1518 the Spanish Conquistador, Herman Cortez, met with Emperor Moctezuma while seeking treasure of the New World. He observed that the Emperor enjoyed a royal beverage of vanilla scented chocolate, Chocolatl. Cortez was so impressed by this regal drink that when he return to Europe, he took bags of cocoa and vanilla along with the gold, silver and jewels of Montezuma's fallen empire. Within half a century, Spanish factories were preparing vanilla-flavoured chocolate. For a quite some time the Europeans continued to use vanilla only in combination with the cocoa bean.

By 1602 vanilla began to be used as a flavouring on its own – the suggestion of Queen Elizabeth's apothecary, Hugh Morgan. From then vanilla soared in popularity and became more famous than chocolate or any other flavour known before or since. For more than 300 years after its discovery by Cortez, vanilla was produced only in its native Mexico.

Plants were tried in many countries but the orchids never bore fruit. The mystery was not solved until 1836 when a Belgian named Cherles Morren found that common insects could not pollinate the orchid. He observed that a tiny bee, the Melipone, which is found only in the Vanilla districts of Mexico, is uniquely equipped to pollinate the flowers. The bee did not survive outside Mexico and so Morren developed a method of hald-pollinating the vanilla blossoms.

Soon after this discovery, the French started to cultivate vanilla on many of their islands in the Indian Ocean, East and West Indies and Oceania; the Dutch planted it in Indonesia; and the British took it to the southern India. Eventually the

French took vanilla to Reunion, an island off Madagascar. There a former salve named Edmond Albius perfected a quick and simple method of hand-pollination which is still used to this day. This was the impetus of major cultivation in the Indian Ocean area. Vanilla is grown commercially even further a field now and the most recent venture was to go ahead Papua New Guinea. 75% of today's production is from Madagasca, Cormoro and Reunion Islands. Scientists are working to improve the vanilla flavour and use tissue culture to propagate plants.

2.4.3 Vanilla Botany

Vanilla bean is a fleshy, herbaceous vine that is perennial and climbing. It grows to a height of 10 to 15 m, supporting itself on the host plant with aerial roots. Roots are produced all along the stem, opposite to leaves. Under cultivation conditions, vanilla is trained and pruned to a height that will allow hand pollination of the flowers and subsequent harvest of the beans.

The stem is cylindrical in shape and monopodial in growth pattern; with means the central stem produces secondary branches that always remain subsidiary to the main stem. Leaves are flat and fleshy and have a short stem. They are bright green and vary between elliptical and lanceolate in shape, with an acute, rounded tip.

Arranged along the stem in an alternate pattern, vanilla leaves vary a great deal in length and width; between eight and 25cm in length and two and eight centimetres in width. In the forest it grows from the floor into the treetops –leaves are larger and healthier, the more sunlight they receive.

Vanilla flowers are fragrant, waxy and large. They are pale green- yellow in colour with a short broad labellum and the upper petals are slightly smaller than the sepals. Flowers are held on long, thick rachis in groups of 20 to 30cm. Each inflorescence measures approximately eight centimeters and usually displays three or four open flowers at a time. If flowers remain un-pollinated, they last only a day.

The fruit is a capsule, but in the trade of vanilla it is referred to as a "bean" or "pod". On the plant, before harvesting, the bean is pendulous, and cylindrical but three-angled in shape. It reaches 10 to 25cm in length and about 1.5cm in diameter, at harvest size. After the beans are harvested and cured they develop their aromatic fragrance.

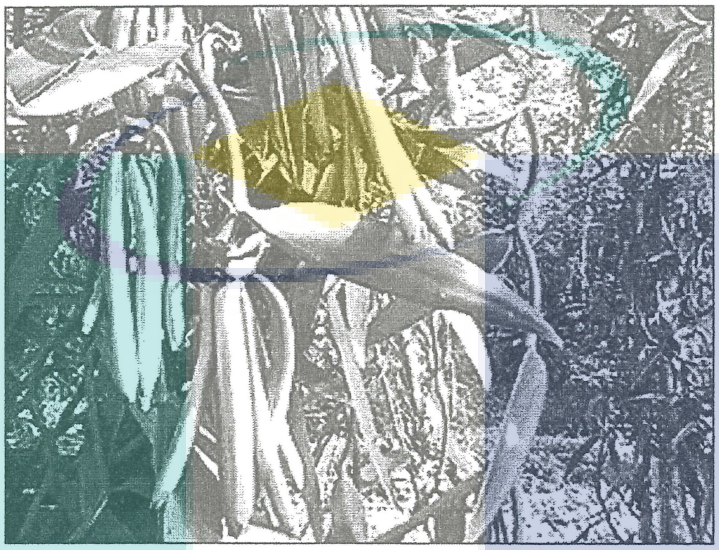


Figure 2.8 Vanilla pods

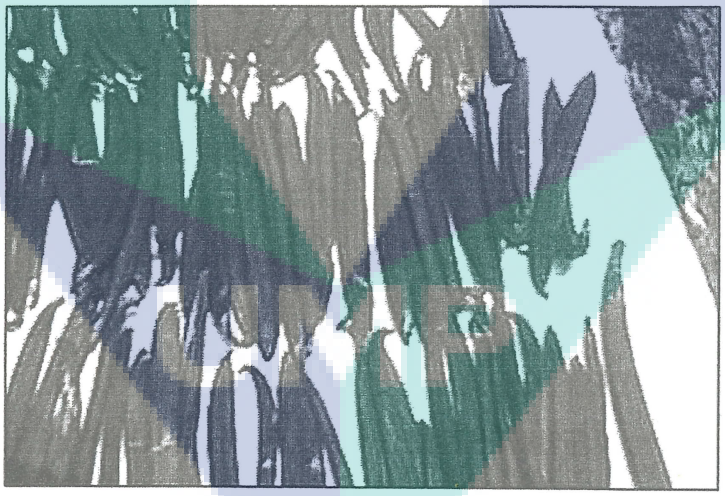


Figure 2.9 Wet vanilla beans

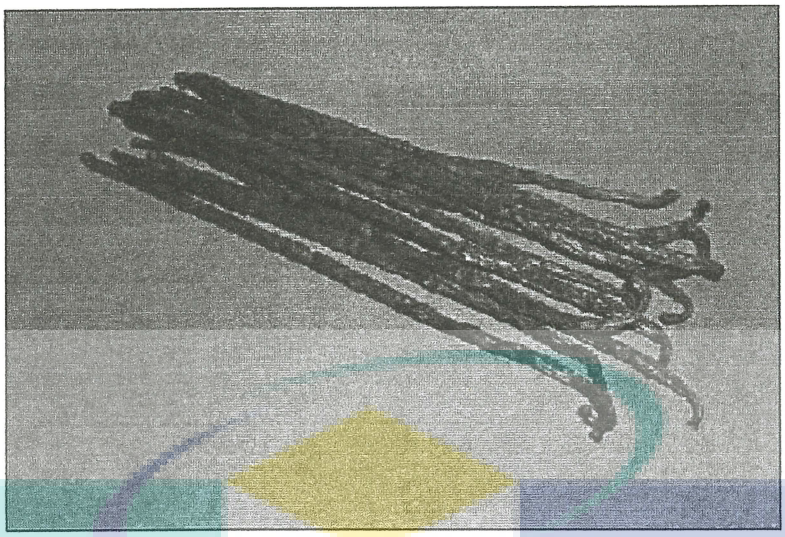


Figure 2.10 Vanilla beans after curing

In Mexico and Central America bees and hummingbirds pollinate Vanilla flowers, but self-pollination is impossible in other parts of the tropical world. Due to the structure and position of the stamen and the stigma and a lack of natural pollinators, hand pollination is necessary in most places where vanilla is farmed. The most effect method used, to hand pollinate vanilla flowers was discovered in 1841 and is still in use today. Individual flowers are pollinated in the early morning, directly after opening. A small stick of bamboo about the size of a toothpick is used to pollinate. The rostellum is pushed aside and pollen is spread from stamen to stigma by causing contact between the two.

Vanilla flowers once a year over a period of about two months. Flowers open from the base of the raceme upward, with only two or three flowers open at once. Commonly, flowers open in the early morning and remain receptive to pollination for eight hours. If fertilization has been successful, the flowers remain on the rachis for two or three days. If fertilization has not occurred, the flowers will wither and die after one day. From the state of the flowers, cultivators can judge the number of fruits that have set and control the number of beans to a plant.

In the wild as a native plant, all vanilla species grow by climbing on trees in wet tropical jungles from sea level to about 600m. Vanilla thrives in a humid, hot

climate with consistent rainfall. The best average temperature for vanilla production is 28°C, but it will tolerate a range between 21°C and 30°C. Average rainfall required is about 2000mm (80in.) spread over ten months, with two months of dry weather for flowering.

Vanilla is the world's most labor-intensive agricultural crop, which is why it's so expensive. It will take up to three years after the vines are planted before the first flowers appear. The fruits, which resemble big green beans, must remain on the vine for nine months in order to completely develop their signature aroma. However, when the beans are harvested, they have neither flavor nor fragrance. They develop these distinctive properties during the curing process.

When the beans are harvested, they are treated with hot water or heat and are then placed in the sun every day for weeks-to-months until they have shrunk to 20% of their original size. After this process is complete, the beans are sorted for size and quality. Then they will rest for a month or two to finish developing their full flavor and fragrance. By the time they are shipped around the world, their aroma is quite remarkable.

Because vanilla has always been so valuable, it has a long history of robbery and intrigue. In Madagascar, vanilla rustling was a major problem for many years. Growers branded the individual beans when they were green and the markings remained after they were dried. Whenever someone suspected their beans were stolen, they could determine by their distinctive tattoo whether or not the beans were theirs.

2.4.4 Vanilla Extract

Vanilla extract is the solution in aqueous ethyl alcohol of the sapid and odorous principles extractable from vanilla beans. In vanilla extract the content of ethyl alcohol is not less than 35% by volume and the content of vanilla constituent.

The vanilla constituent may be extracted directly from vanilla beans or it may be added in the form of concentrated vanilla extract or concentrated vanilla flavoring of vanilla flavoring concentrated to the semisolid form called vanilla oleo-resin.

Originally, everyone used vanilla beans. Vanilla extract has been commercially available for a little more than a hundred years. The first extracts were made at apothecary shops (the first pharmacies and drug stores) and were more like a tincture or syrup. They were strong and very sweet and were often used to calm upset stomachs.

Pure vanilla gives us one of the most complex tastes in the world, having well over 250 organic components creating its unique flavor and aroma. Even the same species of vanilla beans grown in different parts of the world will vary in flavor and aroma due to climate and soil differences. While some beans are higher in natural vanillin content than others, this isn't the only indicator of flavor or quality.

Vanilla extract is made by percolating or macerating chopped vanilla beans with ethyl alcohol and water. The process is usually kept as cool as possible to keep flavor loss to a minimum, though some manufacturers feel that there must be heat to create the best extraction. Most companies use a consistent blend of beans, sometimes from several regions, to create their signature flavor. The extraction process takes about 48 hours after which the extracts will mellow in the tanks with the beans from days to weeks, depending on the processor, before being filtered into a holding tank where the amber-colored liquid extract remains until being bottled.

The United States is the world's largest consumer of vanilla, followed by Europe - especially France. About 1400 tons of dried vanilla is produced worldwide each year. Our worldwide interest in natural vanilla has grown considerably in the past several years, however, and the current annual demand is for 2200 tons of vanilla.

Vanilla is not only used as a flavor in foods and beverages, but also in perfumes. It's also used in many industrial applications such as a flavoring for medicines and as a fragrance to conceal the strong smell of rubber tires, paint, and cleaning products. The dairy industry uses a large percentage of the world's vanilla in ice creams, yogurt (fresh and frozen), and other flavored dairy products. Because vanilla is so much in demand, and because it's so expensive, synthetics are often used instead of natural vanilla. In fact, 97% of vanilla used as a flavor and fragrance is synthetic.

Figure 2.11 Synthetic vanilla products. (The Vanilla.COMpany, 2006)

Synthetic vanilla is widely used and competition on markets is longstanding and becomes more fierce when the price of vanilla rockets. The price ratio between natural and synthetic vanilla products is 1:10 or even 1:15. The consumption of synthetic vanilla totals 12000 to 15000 tons per year whereas world trade in vanilla (2300 tons) represents less than 50 tons of natural vanillin. There is a clear difference between the two products. Vanilla does not consist of vanillin alone but contains several tens of aromatic compounds that give it all its value.

The aroma industry develops synthetic products held to be increasingly close to the natural model. However, the demand for food products with a 'natural vanilla' or 'vanilla' label is still as strong. It remains to be seen whether the inordinately high world prices put off industrial users. Some importers already deplored a strong

decrease in sales in early 2002. The demand for synthetic vanilla is increasing. A study by the journal The Public Ledger reported that world imports of synthetic vanilla had increased strongly since 2000. However, some observers consider that the markets for the two products - natural vanilla and synthetic vanillin - are separate.

2.4.5 Vanillin

Vanillin (4-hydroxy-3-methoxybenzaldehyde; Figure 2.12) is the major component of natural, which is one of the most widely used and important flavoring materials worldwide. Vanillin in fact occurs in trace amounts in other plants, including commercial products such as tobacco (Makkar and Beeker, 1994); however, the pods of the vanilla orchid still remain the only commercial source of natural vanillin. Although more than 12000 tons of vanillin is produced each year, less than 1% of this is natural vanillin from vanilla; the remainder is synthesized much more cheaply via chemical process. Synthetic vanillin is used in both food and non-food applications, in fragrances and as a flavoring in pharmaceutical preparations. Currently, approximately 50% of the worldwide production of synthetic vanillin is used as an intermediate in the chemical and pharmaceutical industries for the production of herbicides, antifoaming agents or drugs such as papaverine, L-dopa, L-methyldopa and the antimicrobial agent, trimethoprim (Hocking, 1997). Synthetic vanillin is also used in household products, such as air-fresheners and floor polishes.

In common with many other low-molecular weight phenolic compounds, vanillin displays antioxidant and antimicrobial properties and hence has the potential for use as a food preservative (Burri et. al., 1989; Davidson and Naidu, 2000). It is active against both Gram-positive and Gram-negative food-spoilage bacteria and has been shown to be effective against both yeasts and moulds in fruit purees and laboratory growth media (Cerruti et. al., 1997; López-Malo et. al., 1998; Fitzgerald et. al., 2003). One limitation is the strong flavor of vanillin at the minimal inhibitory concentrations required, but this may be partially overcome by using it in

combination with other, synergistic antimicrobials thus lowering the effective concentrations that are necessary (Gould, 1996). There is some evidence for antimutagenic effects of vanillin, for example in suppressing chromosomal damage induced by methotrexate in the Chinese hamster cell line (Keshava et. al., 1998).

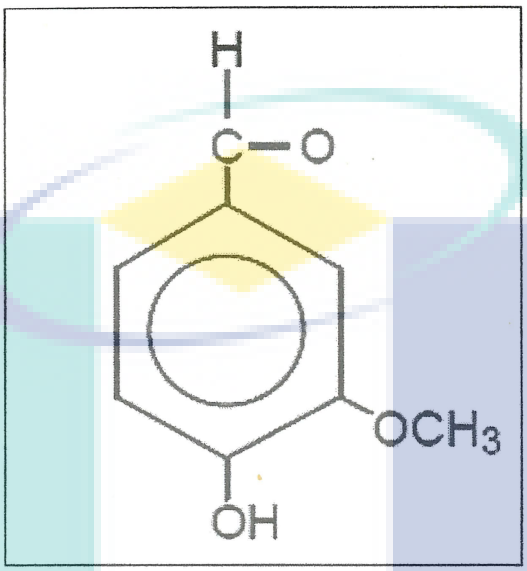


Figure 2.12 Structure of vanillin (N.J. Walton, 2003)

Vanillin is of interest to plant scientists for two main reasons. The first concerns the relationship of vanillin with the phenylpropanoid pathway and with the mechanisms of formation of benzoic acids, including 4-hydroxybenzoic acid and the signaling compound, salicylic acid (2-hydroxybenzoic acid). In particular, the mechanisms of chain shortening of putative phenylpropanoid precursors to benzoic acids in plants have remained elusive for decades.

The second relates to the commercial importance of vanillin and to the possibilities of producing the compound by biotechnology. On account of the limited supply and high price of natural vanilla and the predominance of chemically synthesized vanillin, there arose in the 1980's and 1990's an incentive to explore and develop biological sources of ''natural'' vanillin and vanilla-type flavouring that could be marketed as a realistic alternative to the chemically-synthesized substance.

CHAPTER 3

METHODOLOGY

3.1 The Overall Methodology

The overall methodology involved all the steps in achieving vanilla oleo-resins. The whole study is divided into five major sections:

a) Sample preparation of vanilla beans

b) Extraction of vanilla oleo-resins using Soxhlet Extractor (Lab scale)

c) Separation of vanilla oleo-resins and the extraction solvent using Rotary Evaporator (Lab scale)

d) Extraction of vanilla oleo-resins using UMEPPS (Pilot plant scale)

e) Analysis using GC and HPLC

3.2 Sample Preparation of Vanilla Beans

The sample of the vanilla beans will be divided into two types of vanilla surface area to study the effects of the variation of vanilla surface area to the yield of

vanilla oleo-resins. This will be done by grinding the cured vanilla pods by means of a blender to a small particles and chopping the vanilla beans into 2cm pieces as shown in Figure 3.1.

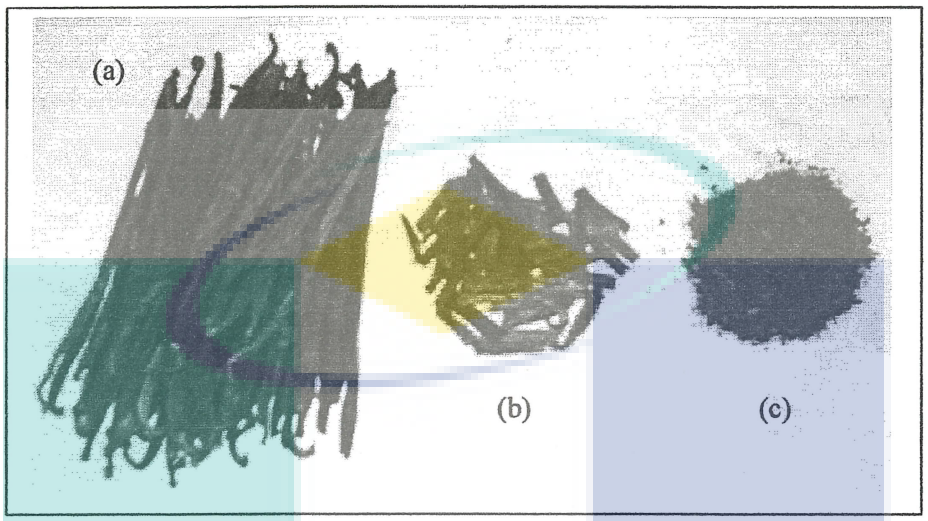


Figure 3.1 Various type of vanilla surface area. (a) Cured vanilla beans, (b) 2cm pieces chopped vanilla beans, (c) Grinded vanilla beans

3.3 Extraction of Vanilla Oleo-resins Using Soxhlet Extractor

Before running the pilot plant scale extraction process using UMEPPS, a lab scale extraction process must be conduct to compare and verify the suitable extraction solvent (in term of giving the high yield of vanilla oleo-resins) between hexane and ethanol. Besides, the purpose to conduct a lab scale extraction process before running the pilot plant scale is to choose the best vanilla surface area (between grinded and chopped vanilla beans). The lab scale experiment will be done by using Soxhlet extractor (Figure 3.2).

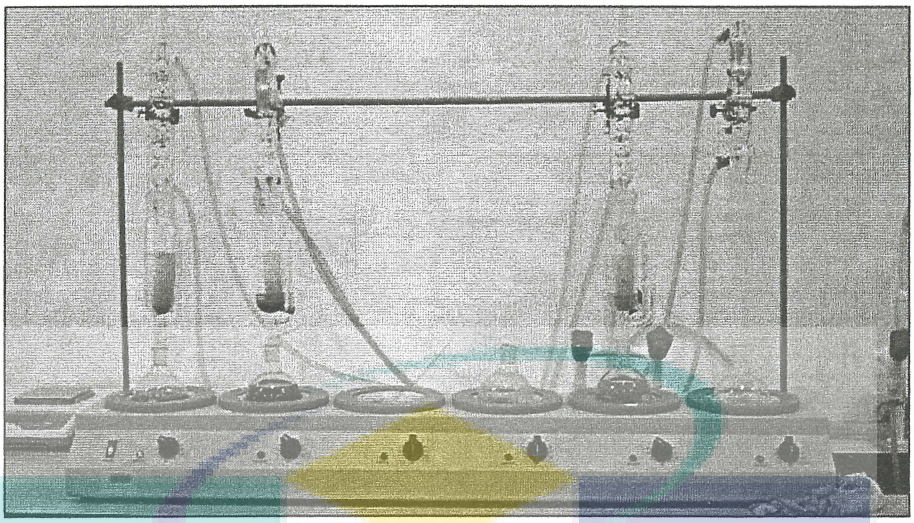


Figure 3.2 Lab scale Soxhlet extractor

3.4 Separation Using Rotary Evaporator

After the extraction is done using the Soxhlet extraction, the extract (referred as vanilla oleo-resins) and the solvent (hexane or ethanol) has to be separate to purify the extract for the further process in analysis. The process will be done by using the lab scale rotary evaporator (Figure 3.3).

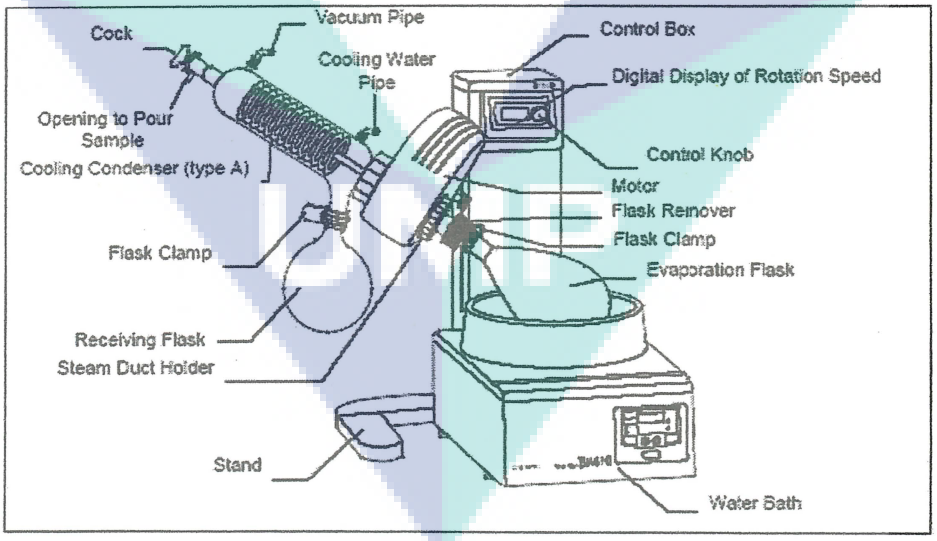


Figure 3.3 Lab scale rotary evaporator

3.5 Vanilla Oleo-resins Extraction Using UMEPPS

There are three major processes in extracting the oleo-resins from vanilla beans using ultrasonic multipurpose extractor pilot plant system. Among them are as listed below:

i. Extraction process

ii. Evaporation or Separation process

iii. Final separation process

Figure 3.4 shows the 3D technical drawing of ultrasonic multipurpose extractor pilot plant system (UMEPPS)

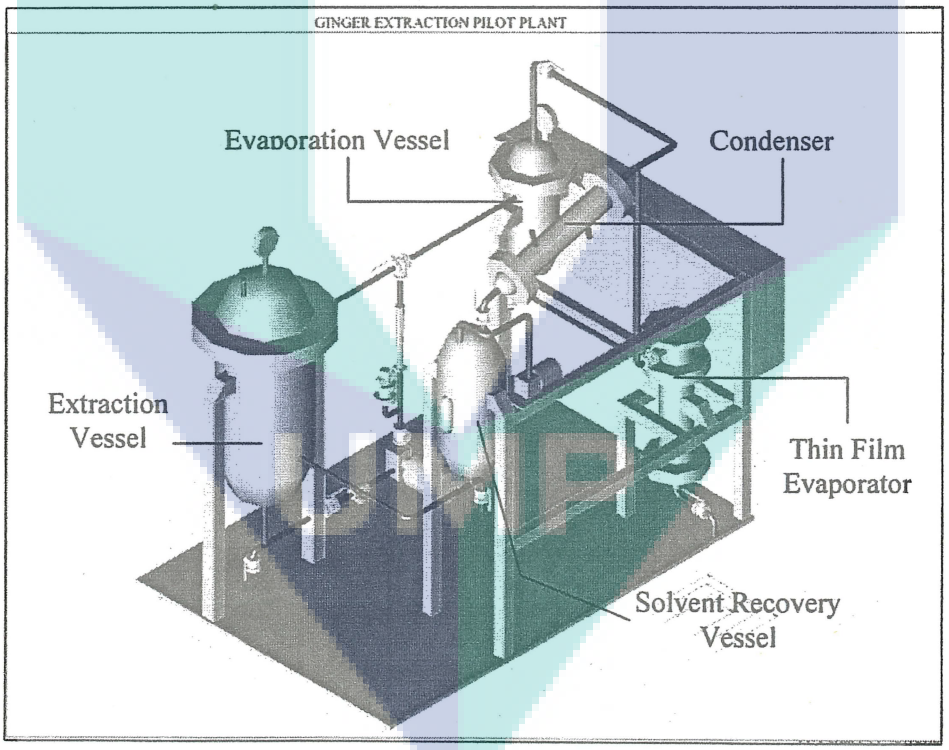


Figure 3.4 The 3D technical drawing of Ultrasonic Multipurpose Extractor Pilot Plant System (UMEPPS). (after Ziad, 2004)

3.5.1 Extraction Process

Referring to the Figure 3.5, the sample of vanilla beans will be immersed in a solvent using stainless steel bucket in the ultrasonic extraction vessel. The ratio for material to solvent are varies because this study is needed to identify the ideal solvent to material ratio to achieve the greatest yield of vanilla oleo-resins. The ratios that will be used are 1:2, 1:3 and 1:4 of the material to solvent ratio respectively.

The extraction process starts immediately after the ultrasonic energy, tangential air blower and heat energy is applied. The extraction process will be carrying out at atmospheric pressure. A process for extracting oleoresin from vanilla beans comprises the steps of supplying a solvent into the extraction chamber containing the vanilla beans. Due to the concentration gradient between the inner vanilla beans and the solvent near the vanilla beans surface, and most importantly the presence of cavitational activity generated by the probe sonicators at the solid-liquid interface and especially within the pores of the vanilla beans, solutes will diffuse from the solids into the solvent.

Figure 3.5 Ultrasonic extraction vessel.

3.5.2 Separation Process

In the evaporation vessel as Figure 3.6, the solution of solute-in-solvent is heated at a temperature in which the solvent is converted into a vapor phase. The vapor passes through a packing above the evaporation vessel. This ensures that only vapor of solvent passes through while the carried over solute can be condensed into distillate and return to the evaporation vessel. The vapor phase of the solvent is then condensed in a condenser (Figure 3.7) to form a condensate. The condensate is returned to the solvent recovery tank (Figure 3.7) for continuous extraction of the solute from the solids.

Figure 3.6 Evaporation vessel.

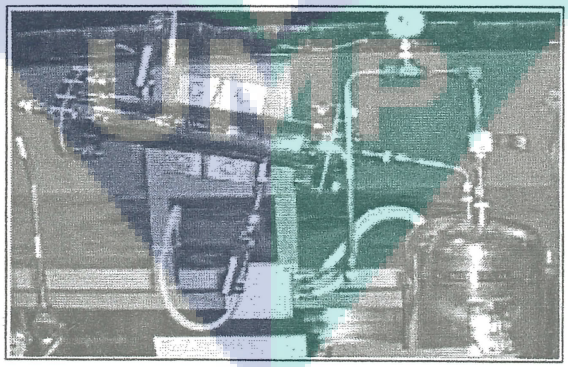


Figure 3.7 Condenser and solvent recovery vessel.

3.5.3 Final Separation Process

After evaporation process, the concentrated crude will be pumping into the thin film evaporator (Figure 3.8) for short path distillation to purify the concentrated crude from the last percent of remaining solvent.

Purification is needed to remove solvent from the vanilla oleoresin. A newly designed short path distillation rig is used during this stage. The method has considerably 'speed-up' the purification process and the vanilla oleoresin was purified. This extraction plant will give extract that richer in high volatility components than that obtained by traditional methods.

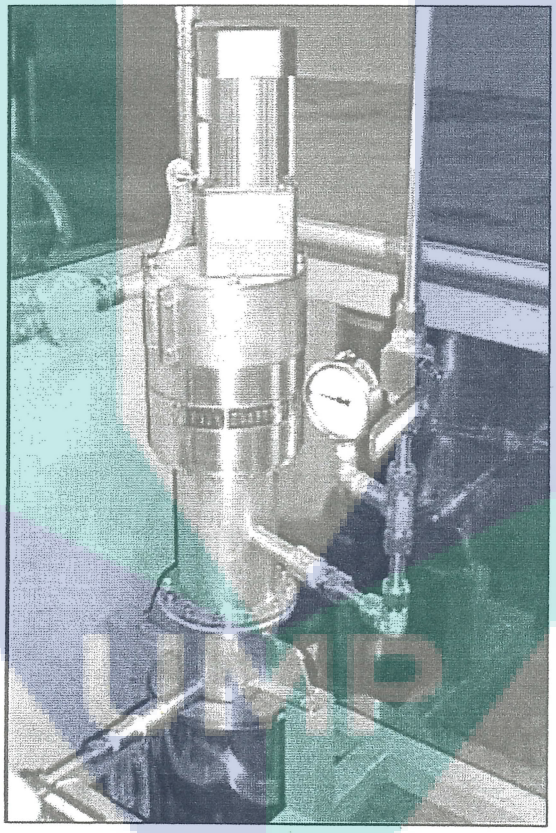


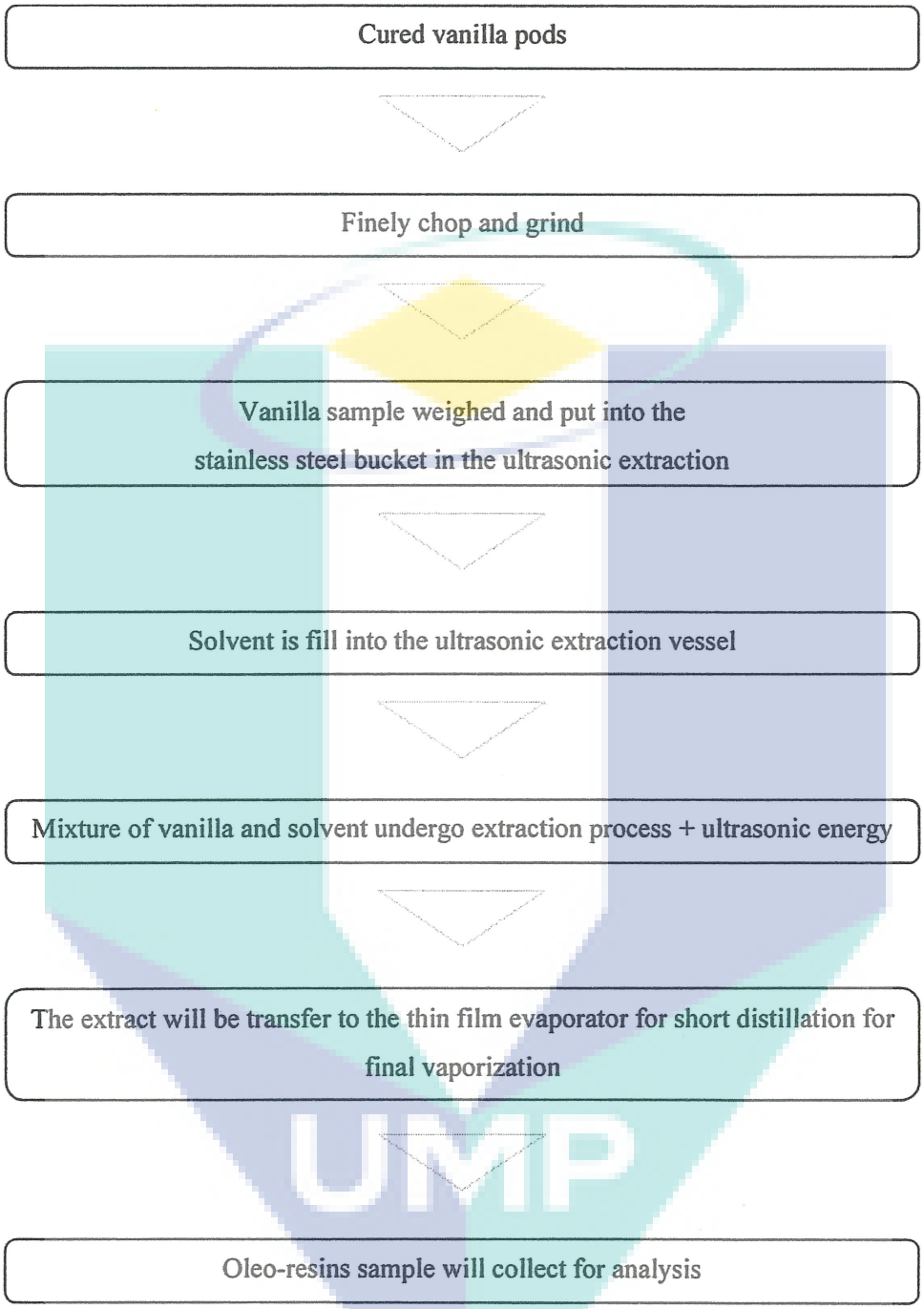
Figure 3.8 Thin film evaporator.

3.5.4 Operating Condition

Table 3.1 : Operating conditions for each equipment of the UMEPPS

Equipment	Condition
Extraction Vessel	Capacity: 25L max. Electrical capacity for clamp heater: 8 kW Setting temperature: 65°C max
Evaporation Vessel	Capacity: 5L Electrical capacity for clamp heater: 3.5 kW Setting temperature: 150°C max
Thin Film Evaporator	Electrical capacity for clamp heater: 4 kW Setting temperature: 100°C max Rotor: Graphite
Condenser	Cooling medium: cooling water Temperature: 32°C

3.5.5 Flow Diagram for Vanilla Extraction Procedure



3.6 Analysis

3.6.1 Gas Chromatography Analysis

The stationary phase is a high-boiling liquid which is usually a viscous oil or waxy substance. This high-boiling liquid is then pack into a long, narrow glass or metal column. Next, the mixture will be analyzed is load by syringe into the beginning of this column. Meanwhile, the mobile phase is an inert gas which continuously flows through the column. The components of the mixture will be distributed between the stationary high-boiling liquid (these components are either condensed or absorbed on the high-boiling liquid) and mobile gas (vapor) phase moving through the column. The gaseous mixture will flow through a detector at the end of the column and finally, if it has been successfully separate, the components will be showing as different peaks on a recorder.

A Varian 3380 gas chromatography equipped with the conditions as listed below will use to analyze the vanilla extracts for quantification.

i. Gas Chromatograph 6890N (Agilent Technology)

ii. Column: DB-Wax (J&W Scientific, Folsom, CA, USA) fused silica capillary column, (30m length, 0.32mm i.d., 0.25μm film thickness), preceded by 2m x 0.32mm uncoated precolumn.

iii. Detector: FID

iv. Carrier gas: Hydrogen (2ml/min)

v. Oven temperature: Set at 40°C for 3 min then raised to 245°C at 3°C/min for 20 min.

vi. Injection volume: 2µl

vii. On-column injector: Heated from 20 to 245°C at 180°C/min for 90 min.

viii. Detector temperature: 250°C

ix. Injection temperature: 250°C

Figure 3.9 Gas chromatography

3.6.2 High Performance Liquid Chromatography Analysis

In this study, the following condition is applied for the vanillin quantification. A Nucleosil C18 (Meta Chem) (Figure 3.10) 150 x 4.6mm with Meta guard column was selected. The mobile phase consist of 40% ultra pure water and 60% HPLC-grade methanol as the most adequate. Standard solution of vanillin is dissolved in

the mobile phase. The measurement is made by using a photodiode array detector of the most adequate maximum wavelength absorbance at 231nm. This method has been successfully applied for the determination of vanillin in some commercial extracts (Waliszewki et. al., 2006).

The other available HPLC methods for vanillin quantification are summarized in Table 3.2.

Table 3.2 : HPLC condition for vanillin quantification (K.N. Waliszewski, 2006)

Name of column	Mobile phase		Detector (nm)	Flow rate (mL/min)	Vanillin elution time (min)	Reference
	Aqueous phase moditier	Organic phase				
Reverse phase C 18	Acetic acid 0.2 M	Water–methanol (20:80)	275	1	16	Herrmann and Stockli (1982)
LiChrosorb C 8	Diluted acetic acid (10:800)	Water–methanol (90:10)	254	2.5	18	Guarino and Brown (1985)
Microsorb C18	Diluted acetic acid (10:990)	Water–methanol–acetonitrile (100:5:10)	275	1	13	Archer (1989)
LiChrospher RP18	Diluted hydrochloric acid	Water–methanol (70:30)	340	2.7	17	Lamprecht et al. (1994)
Spherisorb C 18	Acetic acid 1.25%	Water–methanol (65:35)	270	1	36	Voisine et al. (1995)
LiChrospher RP	Phosphoric acid (1:10000)	Water–acetonitrile (14:86)	278	1	7	Ehlers (1999)
Nova Pack C 18	Acetic acid 0.05 %	Water–methanol–tetrahydrofuran (70:30:0.2)	275	1	31	Jagerdeo et al. (2000)
LiChrospher 60	Phosphoric acid 1%	Acetonitril–methanol–water (2:3:95)	275	1	22	Scharrer and Mosandi (2001)

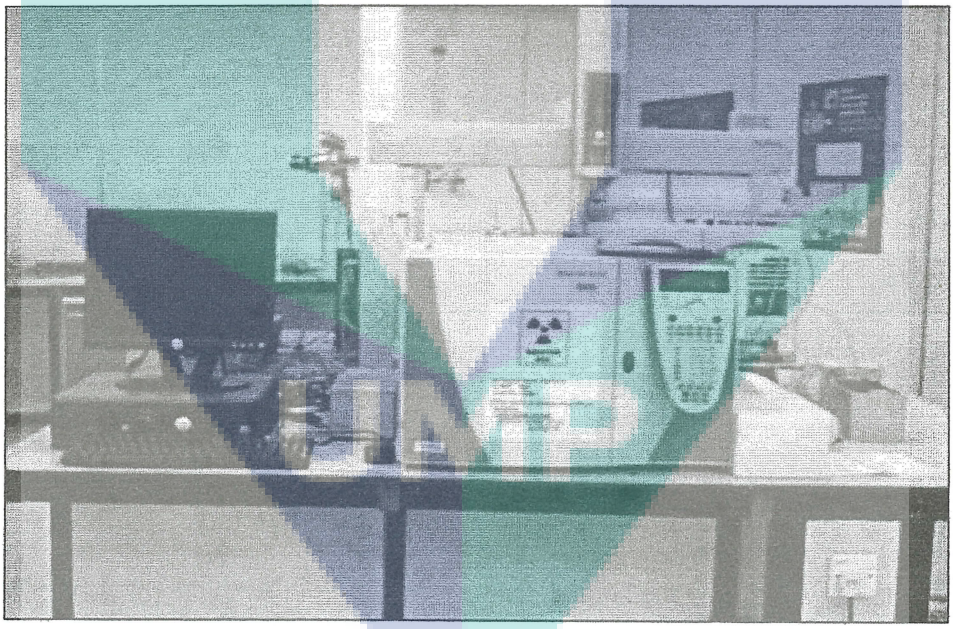


Figure 3.10 High performance liquid chromatography (HPLC)

CHAPTER 4

RESULTS AND DISCUSSIONS

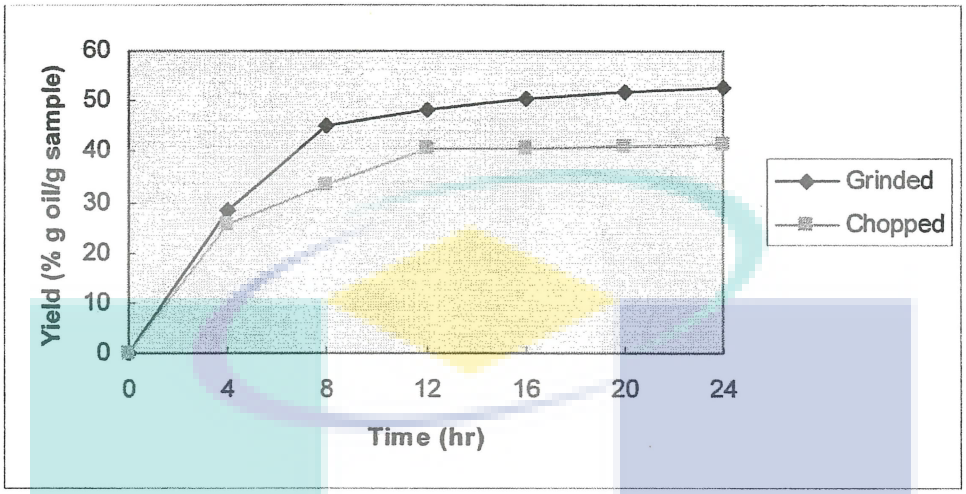
4.1 Optimization of the Experimental Condition

This research mainly concerned of the obtaining oleo-resins from vanilla plant source using ultrasonic extraction technique using UMEPPS, competitive in terms of quality and cost to oleo-resins obtained by traditional methods, by investigating and understanding ultrasonic extraction process as an advanced high-speed extraction technique. Several parameters have to be optimized in order to get the higher yield of vanilla oleo-resins. Therefore, before operating the UMEPPS, several experiments were done to discover the appropriate conditions needed for vanilla extraction. These experiments were conducted using the same method of extraction which was solvent extraction, only in lab scale using Soxhlet extractor. The ratio of vanilla plant to solvent was 1g of vanilla bean to 30ml of extraction solvent while the temperature was fixed at 80°C. The following are result and discussion pertaining to the study.

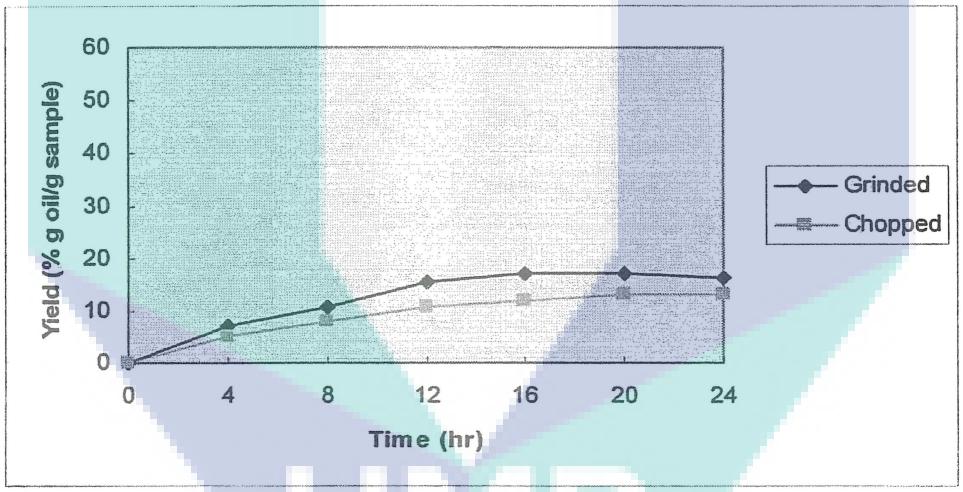
4.2 Effect of Vanilla Surface Area on Extraction of Vanilla Oleo-resins

Figure 4.1 concerned with the yield percentage of vanilla oleo-resins as a function of time and particle size (or vanilla surface area, referred as grinded and chopped) when extracted using ethanol (Figure 4.1a) and hexane (Figure 4.1b) as the extracting solvents. Based on the line graph, the rate of extraction generally

increased with a decrease in particle size because of shorter diffusion paths, consistent with other researchers (Goto et. al., 1996).



(a)



(b)

Figure 4.1 Variation of the vanilla surface area in the vanilla extraction with different types of solvent; (a) ethanol, (b) hexane

Experiments with ethanol showed a dramatic increase in yield for grinded vanilla beans. As been shown in Figure 4.1a, the extraction yields increased significantly with the extraction period extended from 4 to 8 hours for both types of

surface area, but increased slightly or leveled off from 8 to 24 hours. If compared to the extraction using hexane as the extracting solvent (Figure 4.1b), the vanilla oleo-resins yield only slightly increase from 4 to 16 hours and decrease after 16 hours of extraction. This enhancement is more pronounced for the smaller particle sizes, where the surface area to volume ratio is highest. This suggests that the particle surface area plays a major role in the effects of solvent extraction.

4.3 Effect of Type of Solvent on Extraction of Vanilla Oleo-resins

Experiments were also conducted to compare the type of extracting solvent to the yield of oleo-resins from vanilla beans. By comparing the yields of vanilla oleo-resins from two different extracting solvent, which are ethanol and hexane, one can notice that a higher yield can be achieved using ethanol as the extracting solvent than extraction using hexane as can be seen from Figure 4.2.

This phenomenon can be related with the rule of thumb for predicting solubilities which is "like dissolves like". Polar compounds tend to dissolve in polar solvents. Polar liquids are generally miscible with each other. Nonpolar solids are usually soluble in nonpolar solvents. On the other hand, nonpolar solids are insoluble in polar solvents. Nonpolar liquids are usually soluble mutually miscible, but nonpolar liquids and polar liquids "like oil and water" do not mix. As mentioned by Pérez-Silva et. al. (2006) in their food chemistry journal, volatiles from vanilla beans extract represent a wide range of functional groups. Acids and phenolic compounds which are polar compounds were predominant in vanilla extracts. These polar compounds tend to dissolved in ethanol which is a polar solvent compared to hexane which is nonpolar solvent. These reasons enough to prove on why the extraction of vanilla beans using ethanol as the extracting solvent give a higher yield compared to hexane. This suggested that the increasing of the extraction yield from the plant material depends on extracting solvent employed and increases with increasing it polarity (Veličkovič et. al., 2006).

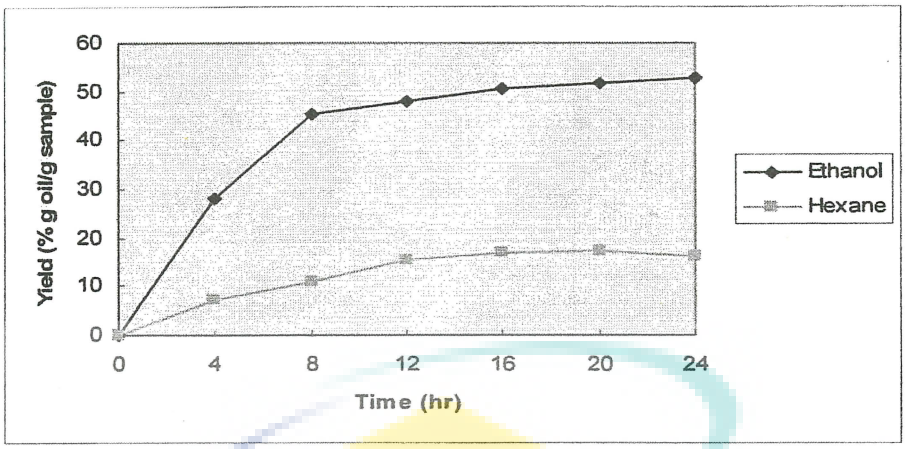
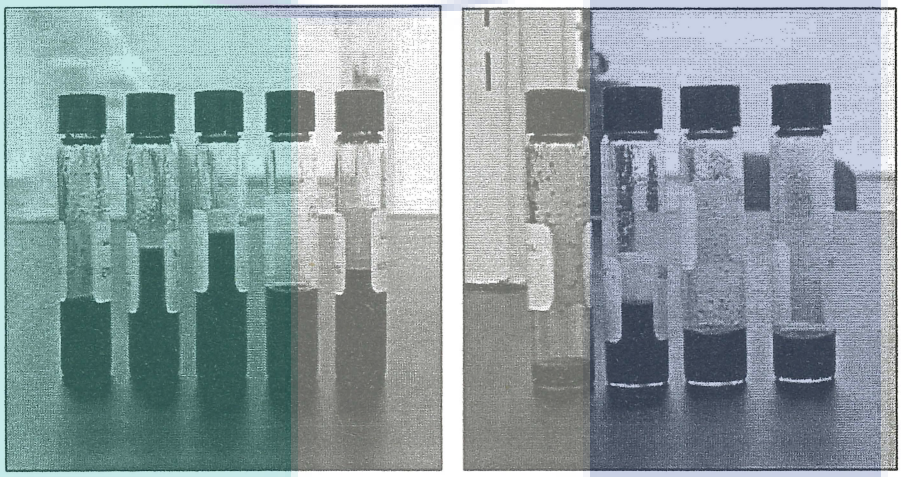


Figure 4.2 Effect of type of solvent on extraction of vanilla oleo-resins



(a) (b)

Figure 4.3 Vanilla oleo-resins using different types of extracting solvent (a) ethanol, (b) hexane

Figure 4.3 shows the yield of several experiments done by using ethanol (Figure 4.3a) and hexane (Figure 2.3b) as the extracting solvent. As can be seen from that figures, it is clearly showed that the yield of vanilla oleo-resins which extracted using ethanol as the extracting solvent give the higher yield compared to those which extracted using hexane.

4.4 The Influence of Material to Solvent Ratio on Extraction Yield

Table 4.1 illustrates the change of yield percentage due to the material to solvent ratio, where the volume of the extracting solvent was fixed for 500ml of ethanol, while the weight of the vanilla beans has been increasing linearly from 10g to 30g. As can be observed from the table, the maximum yield of vanilla oleo-resins achieved in the ratio of 3g sample to 50ml solvent which proved the higher material to solvent ratio, the higher the yields can be achieved.

Table 4.1 : Effect of material to solvent ratio on vanilla extraction yield

Weight of Vanilla (g)	Yield (% g oil/g sample)
0	0
10	30.15
20	38.02
30	47.17

4.5 Influence of Sonication Time on Extraction Yield

The extractions were carried out using UMEPPS following the experimental condition below. Experiments were performed at room temperature without allowing the temperature to rise since cavitational effects are reduced as temperature is increased (Paniwnyk et. al., 2001).

Experimental conditions:

i.	Weigh of vanilla beans	:	600g
ii.	Type of vanilla surface area	:	Chopped
iii.	Volume of extracting solvent	:	20 L
iv.	Type of extracting solvent	:	Ethanol
v.	Temperature and pressure	:	Ambient T and P

As can be seen in Figure 4.4, the extraction rate were increases dramatically when ultrasound is applied in the first 30 min. When ultrasound is applied only after 60 min of extraction time, the 'jump' in extraction rate is only slight, suggesting that the extraction had almost reached the maximum yields of oleoresin where the oleo-resins present in the vanilla beans will have already been extracted. The further increase in yield when ultrasound is applied is believed to be due to the disruption of cell walls by cavitational effects.

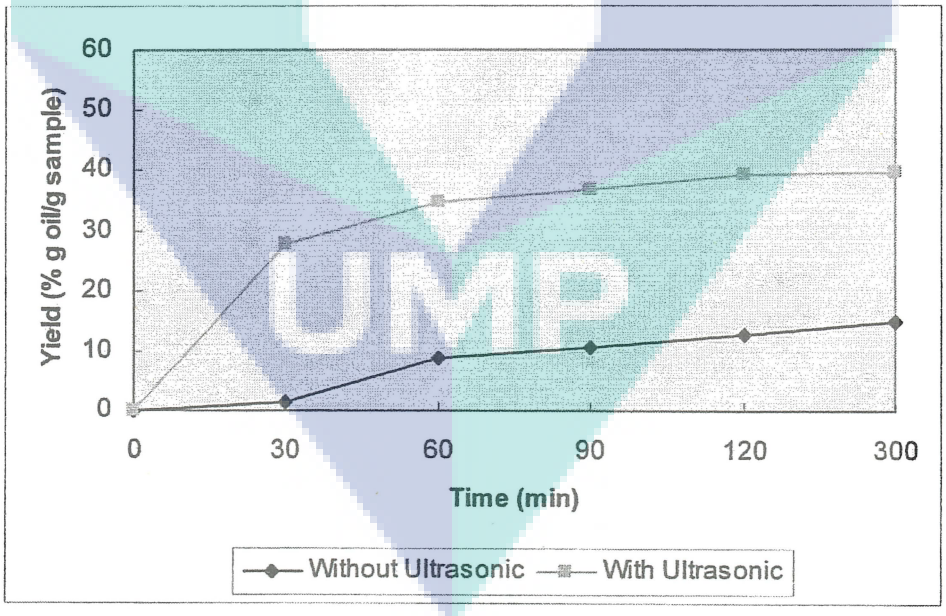


Figure 4.4 Influence of sonication time on extraction yield

4.6 Analysis

4.6.1 Analysis Using HPLC

A Nucleosil C18 column was used in the quantification of vanillin in vanilla extract. The mobile phase consisted of 40% ultra pure water and 60% HPLC-grade methanol as the most adequate. Standard solution of vanillin was dissolved in the mobile phase. The mobile phase flow rate was 1 ml/min and injection volume was 20µl. Each run lasted 5 min. The retention time for the standard solution of vanillin was only 2.159 min (Figure 4.5) at 231 nm of wavelength absorbance.

The same condition was applied to analyzed vanilla extract which was extracted using hexane as the extracting solvent. The determination of vanillin was illustrated in Figure 4.6 where the peak of vanillin occurred in the retention time of 2.122 min. While Figure 4.7 shown the chromatogram of vanillin in vanilla extract using ethanol as the extracting solvent. The peak of vanillin detected on 2.107 min of retention time. More information about the analysis is attached in Appendix A.

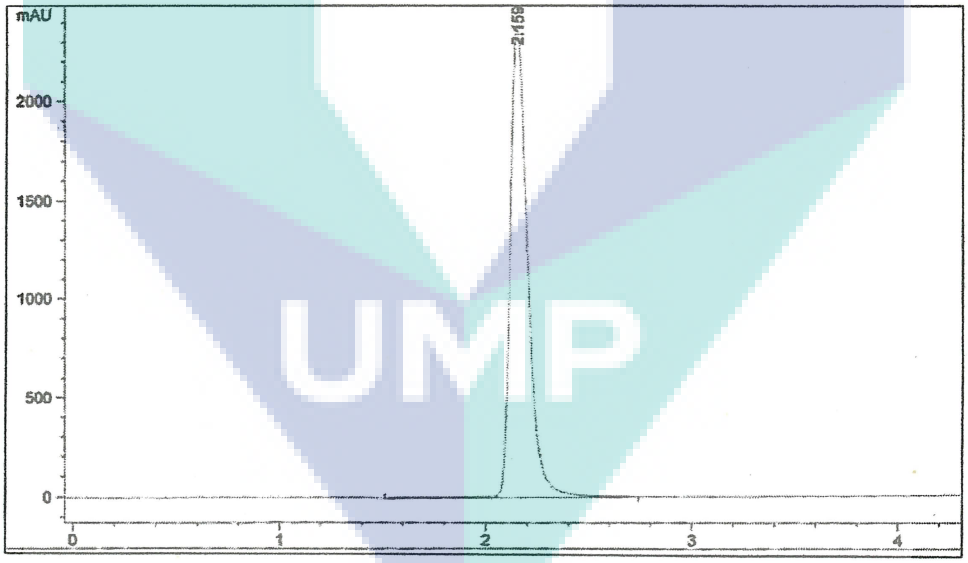


Figure 4.5 A chromatogram of vanillin standard solution using HPLC

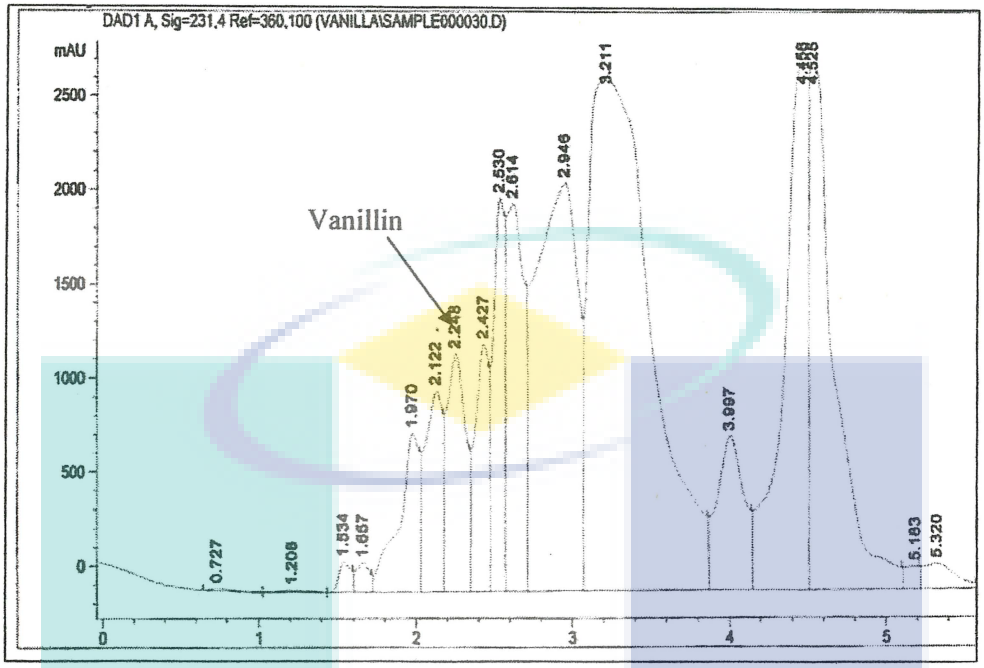


Figure 4.6 Vanillin determination in vanilla extract using hexane

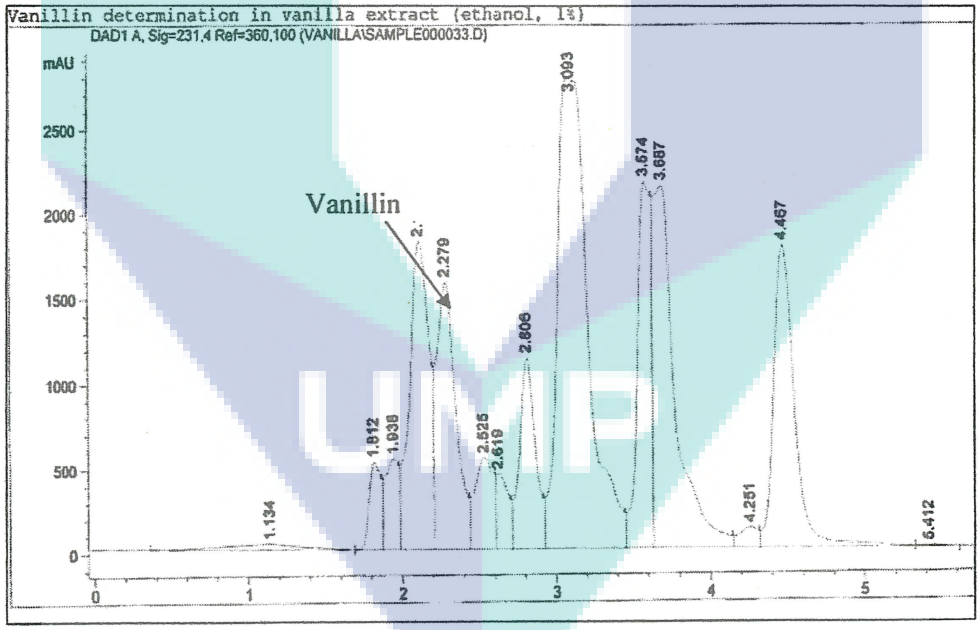
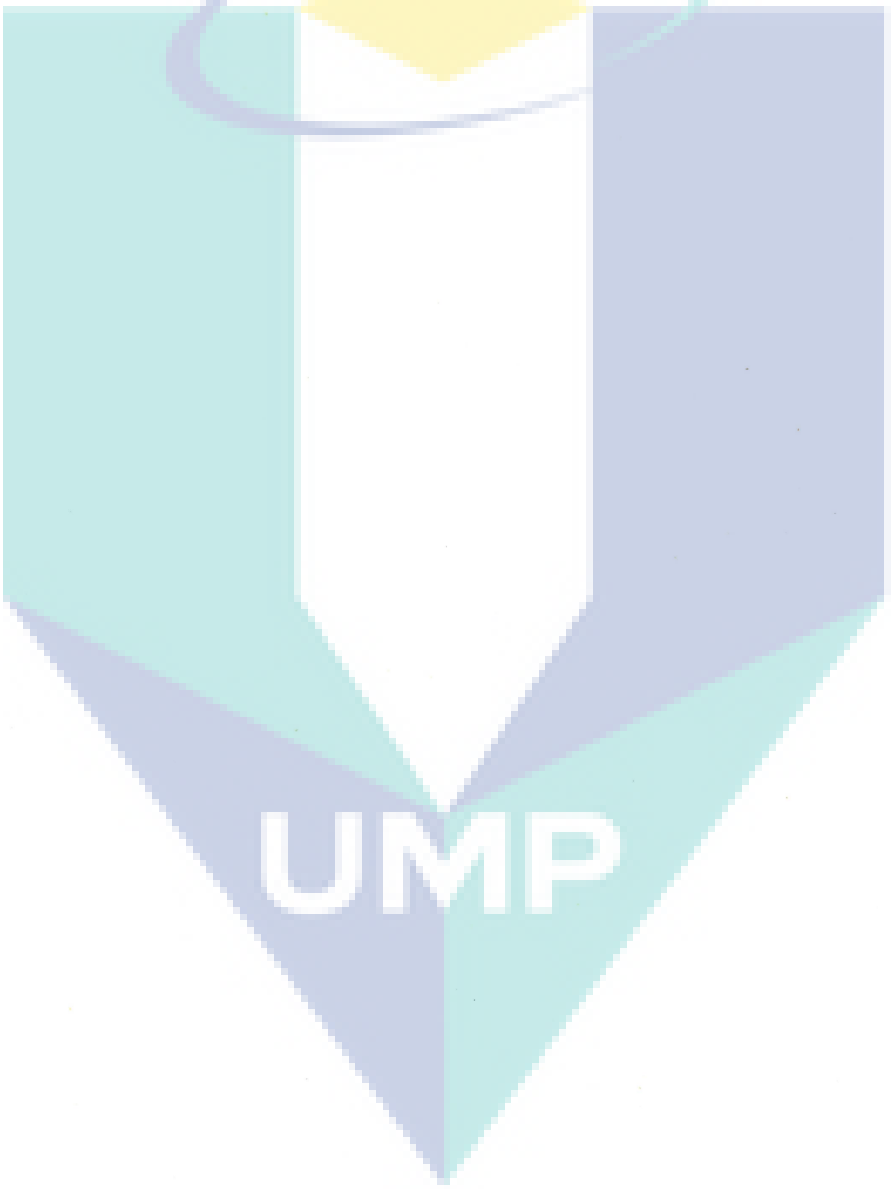


Figure 4.7 Vanillin determination in vanilla extract using ethanol

4.6.2 Analysis Using GC

According to Pèrez-Silva et. al. (2006), some of the major volatile compounds in vanilla beans including vanillin, vanillic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid could not be detected under GC-FID analysis conditions because of the saturation of the detector, but could be detected under GC-MS and GS-O condition. Therefore, the analysis of vanilla extract using gas chromatography could not be performed since GC-MS nor GC-O was not available in our laboratory.



CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In any extraction process consideration must be given to the solubility of the compound being extracted in the chosen solvent at the extraction temperature employed. In the cases of compounds with antioxidant activity, such as vanillin, ethanol appears to be suitable for solvent extractions since it give the higher yields of vanilla oleo-resins if compared to hexane.

The used of grinded vanilla beans gave a significant increase in maximum extraction of vanilla oleo-resins compared to chopped vanilla beans. In the term of analysis of vanilla extract using two different types of analysis equipment (GC and HPLC), the used of HPLC for the qualitative analysis vanillin in vanilla extract considered as the best analysis method since the peak of vanillin can be detected using a HPLC in a shorter retention time.

The other conclusion that can be drawn from this study is, the application of ultrasound to the solvent extraction of oleo-resins from vanilla beans has been proved to assist the solvent extraction towards a higher extraction yields. Ultrasound also has proven to be a much simpler and more effective means than the traditional extraction method of refluxing boiling solvents for the extraction of vanilla oleo-resin. In addition, ultrasound-assisted extraction can be carried out at lower temperature which can avoid the degradation of thermally unstable ingredients in plant materials.

5.2 Recommendations

With all the merits of ultrasonic extraction using UMEPPS, the equipment should be considered for wider application in the isolation and purification of phytochemicals from plants. But there are still some recommendations to improve the system of the multi-purpose extractor. Firstly are the scales; proper scales need to be done on each vessel to ensure accurate measurements of the fluid involved. Level indicators should be placed on the front of the vessel to make it easier to take readings during experiments. Besides that, the equipment should be designed with an effective cooling system to avoid overheating of the vessels, especially in the extraction vessel. A heat exchanger could be attached to the equipment to reduce the heat accumulated along the extraction process. Next is the condition of the pressure and temperature indicators. These indicators should be checked from time to time to ensure they are fully functional. Error on the indicators could cause unwanted mishaps such as overpressure which could lead to explosions. Automatic relief valves need to be constructed to release pressure when there is pressure build up in the vessels such as at the extraction vessel and at the evaporator. Every vessel, pipe line, pump and valve should be checked before the pilot plant is operated. Any damages such as leaks have to be corrected as soon as possible to avoid chemical and product loss. Sufficient Personal Protective Equipment (PPE) should be provided and used by the experimenter at all times during operating the pilot plant. This is to avoid from being overexposed to toxic chemicals, specifically solvents like *n*-hexane. More experiments and researches involving various parameters such as pressure, temperature and concentration need to be conducted in order to discover the optimum conditions for producing a high quality and quantity yield.

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APPENDIX A1

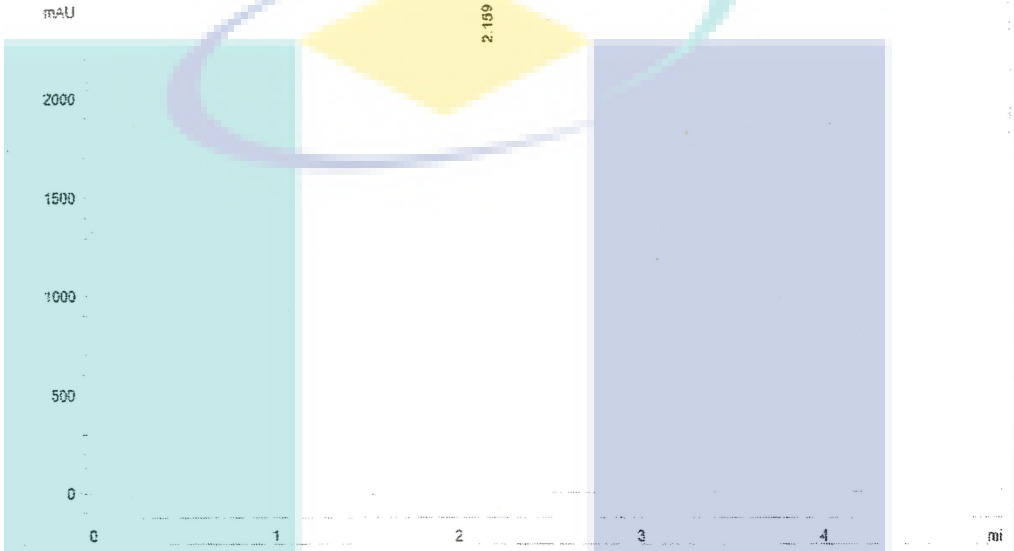
HPLC Analysis of Vanillin Standard Solution

Data File C:\Chem32\1\DATA\VANILLIA\SAMPLE\0012.D
Sample Name: vanillin

Vanillin standard method testing

Injection Date : 8/22/2006 1:06:34 PM
Sample Name : vanillin Location : Vial 2
Acq. Operator : Idayu & ruzinah
Acq. Instrument : Instrument 1 Inj Volume : 20 µl
Method : C:\CHEM32\1\METHODS\VANILLIN.M
Last changed : 8/22/2006 11:14:44 AM by Idayu & ruzinah
Tapping Coefficient

DAD1 A, Sig=231,4 Ref=360,100 \VANILLIA\SAMPLE\0012.D



External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.000e3
Use Multiplier & Dilution Factor with ISTDs

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.000e3
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=231,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.159	BB	0.0910	1.40276e4	2373.33179	1.000e5
Totals :				1.40276e4	2373.33179	

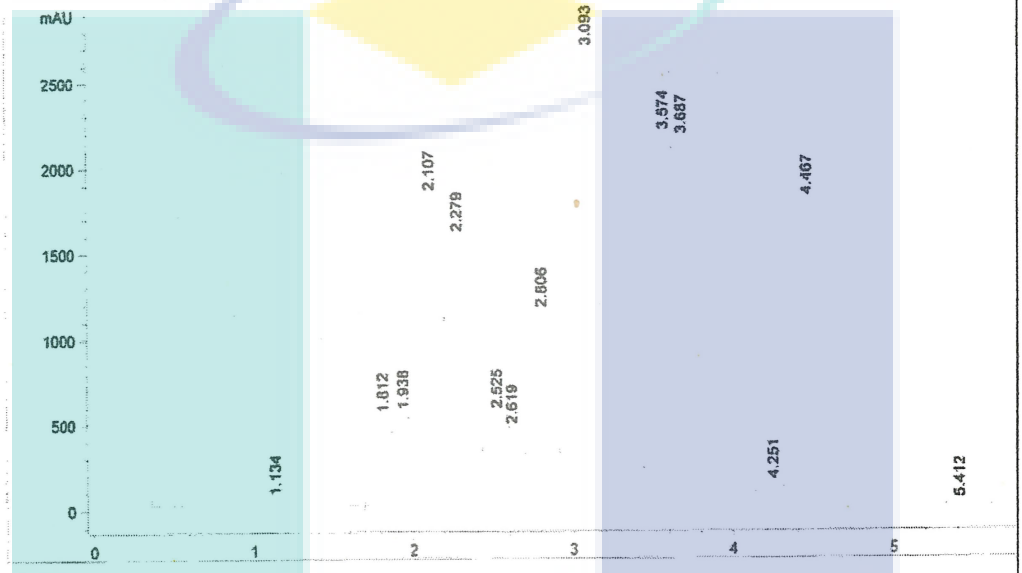
APPENDIX A2

HPLC Analysis of Vanilla Extract in Ethanol

Data File C:\Chem32\1\DATA\VANILLA\SAMPLE000033.D
Sample Name: Vanila Oleoresin

Vanillin determination in vanilla extract.

Injection Date : 9/26/2006 3:08:05 PM
Sample Name : Vanila Oleoresin Location : Vial 1
Acq. Operator : Hernawati
Acq. Instrument : Instrument 1 Inj Volume : 20 ul
Method : C:\CHEM32\1\METHODS\VANILLIN.M
Last changed : 9/26/2006 3:06:40 PM by Hernawati
(modified after loading)
Vanillin determination in vanilla extract (ethanol, 1%)



External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.000e3
Sample Amount : 1.00000 [ppm] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.000e3
Sample Amount : 1.00000 [ppm] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Data File C:\Chem32\1\DATA\VANILLA\SAMPLE000033.D
Sample Name: Vanila Oleoresin

Signal 1: DAD1 A, Sig=231,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.134	BV	0.4995	1217.01990	34.00447	848.7161
2	1.812	VV	0.0769	2699.22339	517.40576	1.882e3
3	1.938	VV	0.0869	3254.84912	535.62500	2.270e3
4	2.107	VV	0.1344	1.62448e4	1816.11206	1.133e4
5	2.279	VV	0.1239	1.34881e4	1575.02686	9.406e3
6	2.525	VV	0.1175	4499.59375	538.66785	3.138e3
7	2.619	VV	0.0816	2507.66479	445.84036	1.749e3
8	2.806	VV	0.1058	8110.52197	1129.69189	5.656e3
9	3.093	VV	0.2149	3.89830e4	2791.60767	2.719e4
10	3.574	VV	0.0885	1.34792e4	2167.69165	9.400e3
11	3.687	VV	0.1323	2.09803e4	2142.12183	1.463e4
12	4.251	VV	0.1221	1014.01251	120.58949	707.1444
13	4.467	VV	0.1440	1.68430e4	1786.26538	1.175e4
14	5.412	VV	0.1601	74.18380	7.08868	51.7337
Totals :				1.43395e5	1.56077e4	

Calibration Curves

*** End of Report ***

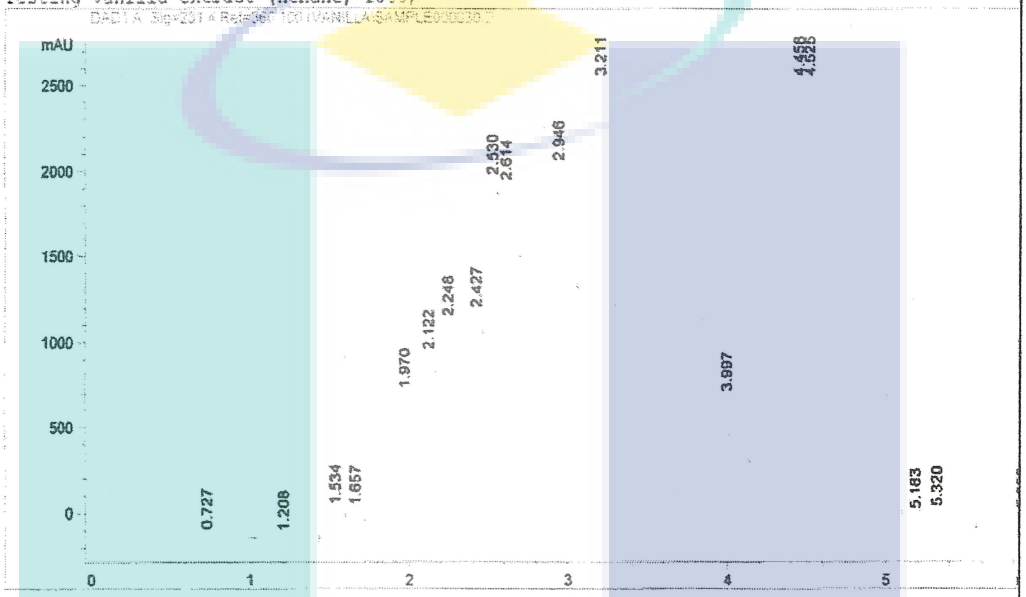
APPENDIX A3

HPLC Analysis of Vanilla Extract in Hexane

Data File C:\Chem32\1\DATA\VANILLA\SAMPLE000030.D
Sample Name: Vanila Oleoresin

Sample of Vanilla Extract using Hexane (100%)

Injection Date : 9/26/2006 2:45:53 PM
Sample Name : Vanila Oleoresin Location : Vial 1
Acq. Operator : Hernawati
Acq. Instrument : Instrument 1 Inj Volume : 20 ul
Method : C:\CHEM32\1\METHODS\VANILLIN.M
Last changed : 9/26/2006 2:50:55 PM by Hernawati
(modified after loading)
Testing vanilla extract (hexane, 100%)



External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.000e3
Sample Amount : 1.00000 [ppm] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.000e3
Sample Amount : 1.00000 [ppm] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Data File C:\Chem32\1\DATA\VANILLA\SAMPLE000030.D
Sample Name: Vanila Oleoresin

Signal 1: DAD1 A, Sig=231,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.727	BV	0.1305	94.46624	11.66290	37.0827
2	1.208	VV	0.1809	115.05742	9.52853	45.1658
3	1.534	VV	0.0752	845.14197	161.11824	331.7602
4	1.657	VV	0.0826	909.88293	154.73566	357.1743
5	1.970	VV	0.1252	7747.13818	843.04938	3.041e3
6	2.122	VV	0.1087	8298.14258	1066.89819	3.257e3
7	2.248	VV	0.1170	1.05022e4	1263.21143	4.123e3
8	2.427	VV	0.0932	8233.70117	1313.12756	3.232e3
9	2.530	VV	0.0711	1.05985e4	2090.57153	4.160e3
10	2.614	VV	0.1054	1.57886e4	2059.75293	6.198e3
11	2.946	VV	0.2709	3.99725e4	2170.15454	1.569e4
12	3.211	VV	0.3857	7.58198e4	2715.85400	2.976e4
13	3.997	VV	0.1717	9789.85254	817.54773	3.843e3
14	4.458	VV	0.1517	3.00445e4	2787.89111	1.179e4
15	4.525	VV	0.1593	3.13503e4	2786.59204	1.231e4
16	5.183	VV	0.0981	806.88556	120.47657	316.7427
17	5.320	VV	0.2041	1923.35107	135.06624	755.0109
18	5.855	VBA	0.2452	1904.82458	117.44880	747.7383
Totals :				2.54745e5	2.06247e4	

Calibration Curves

*** End of Report ***