STUDY ON THE EFFECT OF HYDRODISTILLATION ON YIELD AND LIMONENE COMPOSITION OF VARIOUS *CITRUS* ESSENTIAL OIL AND ITS APPLICATION AS NATURAL AROMATIC GEL

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ABSTRACT

Citrus essential oil was well known with various usages in food, beverage, medicinal and fragrance industry. The present of major compound, limonene had contributed vastly to the oil's aroma, antibacterial, antifungal and insect repellent properties. Thus, the quality of *Citrus* essential oil depends highly on the composition of limonene present in the essential oil. As such, the effect of hydrodistillation extraction process on the yield and limonene composition of essential oil extracted from peels of calamansi lime (*Citrus microcarpa*), key lime (*Citrus aurantifolia*) and kaffir lime (*Citrus hystrix*) had been studied and the potential application of *Citrus* essential oil as natural aromatic gel was examined in this research. The *Citrus* essential oil had been extracted from various *Citrus* peels by using hydrodistillation method for 3, 4, 5, 6, and 7 hours. The hydrosol collected was then separated by using dichloromethane and dried over using anhydrous sodium sulphate. The yield of essential oil collected and the composition of limonene present in the essential oil had been calculated and analysed by using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The results obtained showed that the extraction time will affect the yield and limonene composition in *Citrus* essential oil significantly ($p < 0.05$) in hydrodistillation process. Overall, the yield of *Citrus* essential oil increase with the increasing of extraction time. This is because the *Citrus* essential oil can be released from its oil sacs more easily with the aid of hot water. On the other hand, the limonene composition decreased with the increasing of extraction time after reaching the optimum point. This might due to the thermal decomposition of limonene compound when subjected to heat for long extraction period. Sensory evaluation had been carried out to evaluate on the natural aromatic gel made. Based on the results, the criteria of preciseness, intensity and persistence had been rated above moderate level while the rating for overall acceptability was above the level of slightly like. Hence, *Citrus* essential oil had the potential in making natural aromatic gel which can become a substitute for synthetic air refresher in daily life.

ABSTRAK

Minyak pati *Citrus* terkenal dengan pelbagai penggunaan seperti dalam industri makanan dan minuman, perubatan dan industri penghasilan minyak wangi. Kewujudan sebatian utama iaitu limonene dalam minyak pati *Citrus* memainkan peranan yang amat penting sebagai penyumbang kepada ciri-ciri aroma, anti-bakteria, anti-kulat dan penghalau serangga dalam tumbuhan *Citrus*. Oleh itu, kualiti minyak pati *Citrus* banyak bergantung kepada komposisi limonene dalam minyak pati. Dalam kajian ini, kesan hidrodistillasi pada hasil dan komposisi limonene dalam minyak pati *Citrus* yang diekstrak daripada kulit limau kasturi (*Citrus microcarpa*), limau nipis (*Citrus aurantifolia*) dan limau purut (*Citrus hystrix*) telah dikaji. Selain itu, potensi minyak pati *Citrus* sebagai gel aromatik semula jadi juga telah dikaji melalui penyelidikan ini. Eksperimen pengekstrakan minyak pati *Citrus* daripada pelbagai kulit *Citrus* telah dijalankan dengan menggunakan kaedah hidrodistillasi untuk selama 3, 4, 5, 6 dan 7 jam. Kemudian, hidrosol yang dikumpul telah diasingkan dengan menggunakan diklorometana dan dikeringkan menggunakan natrium sulfat. Hasil minyak pati yang telah diekstrak dan komposisi limonene yang terdapat dalam minyak pati telah dikira dan dianalisis dengan menggunakan Gas Chromatography-Mass Spektrometri (GC-MS) analisis. Keputusan yang diperoleh menunjukkan bahawa masa pengekstrakan dengan proses hidrodistillasi akan menjejaskan hasil dan komposisi limonene dalam minyak pati *Citrus* dengan ketara ($p < 0.05$). Secara keseluruhan, keputusan eksperimen menunjukkan bahawa hasil minyak pati *Citrus* akan meningkat selaras dengan peningkatan masa pengekstrakan. Hal ini kerana peningkatan masa pengekstrakan akan memudahkan pembebasan minyak pati *Citrus* daripada kantung minyak dengan bantuan air panas. Sebaliknya, komposisi limonene menurun dengan peningkatan masa pengekstakan selepas mencapai tahap optima. Hal ini mungkin disebabkan oleh penguraian terma komponen limonene apabila dikenakan haba untuk tempoh masa pengektrakan yang panjang. Berdasarkan keputusan penilaian deria, kriteria ketepatan, kekuatan dan ketahanan bau gel aromatik semula jadi telah mencapai tahap melebihi paras sederhana manakala kebolehterimaan produk tersebut berada pada tahap sedikit suka. Kesimpulannya, minyak pati *Citrus* mempunyai potensi dalam pembuatan gel aromatik sebagai pengganti kepada penyegar udara sintetik dalam kehidupan seharian.

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LIST OF SYMBOLS

^{o}C	Degree Celcius
%	Percentage
ml	Millilitre
µL	Microlitre
hrs	Hours
m	Meter
mm	Millimeter
µm	Micrometer
min^{-1}	Per minute
m/z	Mass-to-charge ratio
eV	Electron volt

LIST OF ABBREVIATIONS

CCC	Counter-current chromatography
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally recognized as safe
HD	Hydrodistillation
ISS	International Space Station
MAE	Microwave Assisted Extraction
PLE	Pressurized Liquid Extraction
SFE	Supercritical Fluid Extraction
UMP	Universiti Malaysia Pahang

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CHAPTER 1

INTRODUCTION

1.1 Background of study

An essential oil is the volatile compound that can be derived from plant material by a physical process. It is a complex mixtures whose composition may include volatile terpenic compounds with formula of $(C_5H_8)n$, where these terpenic compounds can be considered as monoterpenes when n = 2, sesquiterpenes when n = 3 and diterpenes when n = 4. Besides, it also contains terpenoid where terpenoid are oxygenated derivatives of terpenes which may contain hydroxyl or carbonyl group. Essential oil is normally originates from the plant secondary metabolism and responsible for the characteristic aroma of the plant (Chamorro et al., 2000). According to Ahmad et al. (2006), *Citrus* fruits are the most familiar and rich source of essential oil among the plants. The essential oil from the genus of *Citrus* consists of complex mixtures whose composition contain volatile terpene group where limonene is the most abundance terpene in the essential oil of *Citrus* plant. The present of terpene group in *Citrus* plant had contributed vastly to the aroma, antibacterial, antifungal and insects repellent properties of the plant.

Citrus fruits and its by-product have high economic and medicinal values because of their multiple uses such as in food industry, cosmetics and folk medicine (Silalahi, 2002; Saidani et al., 2004). Its juice had been used widely in Malaysia as flavoring in beverage and dishes. With the increasing of the demands of *Citrus* fruits juice in food and agro-food processing industry, it had produced large amount of waste or by-products such as peels which represents around 50% of the raw processed fruit after the juice extraction (Anwar et al.,

2008). The by-product from *Citrus* plant that will cause pollution and environment issues due to microbial spoilage (Sahraoui et al., 2011). Hence, the *Citrus* peels will normally be chosen for further commercialize as it exists abundantly in processing industry and contains large quantities of functional ingredients namely flavonoids, dietary fibres and essential oils (Sevevirathne et al., 2009). In this research, peels from kaffir lime (*Citrus hystrix*), key lime (*Citrus aurantifolia*) and calamansi lime (*Citrus microcarpa*) which are the most common lime used in Malaysia had been chosen for essential oil extraction because it provides a great potential for further commercial form.

An extraction method is important to isolate the essential oil from plant before further studies can be conducted on its volatile compounds (Daferera et al., 2000). The basic parameters that influence the yield of quality in essential oil extraction are the extraction time, temperature, pressure, solvent used for extraction, the process of extraction and the types of equipment used (Bjorklund & Nilsson, 2000). By choosing the appropriate extraction technology with the best parameters will eventually increase the yield and quality of essential oil that extracted from *Citrus* peels. Some recently developed extraction method such as pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) and microwave assisted extraction (MAE) had been used for essential oil extraction. However, these methods are still under research and have not used for large scale of production. Thus, hydrodistillation (HD) as one of the promising conventional extraction method had been selected for *Citrus* essential oil in this research.

1.2 Problem statement

The demand of essential oil from *Citrus* plant had been increased recently as *Citrus* essential oil can be used as the primary material in the making of perfume, soap and fragrances (Ahmad et al., 2006). However, the cost of production of *Citrus* essential oil is high due to the limited amount of supply from the plant. Thus, it is important to study on the effect of hydrostillation on *Citrus* essential oil so that a optimum yield and quality of *Citrus* essential oil can be examined in order reduce the cost of production.

Citrus fruit peels will be treated as waste materials after its juice had been squeezed out for cooking, flavouring and cleaning purpose. The fruit peels which are also the by-product may create environmental problems for the society since it contains biomaterials which may speed up the microbial spoilage. According to Kesterson & Braddok (1976), every ton of food

waste means that there will be 4.5 ton of CO_2 emissions. Thus, the *Citrus* peels will be chosen as the raw materials in *Citrus* essential oil extraction since it was the major by-product of *Citrus* after the juice had been used in cooking and cleaning purposes. Besides, fully utilizing of *Citrus* peels (by-product) has another advantage as it can increase the potential return from the *Citrus* fruit processing industry.

Many studies had revealed that synthetic air freshener contains hazardous chemicals such as phthalates and formaldehyde which may increase the risk of getting health problems. Thus, *Citrus* essential oil that had been used widely in fragrance and aromatherapy industry with a refreshing smell has a potential to be used in making natural aromatic gel which does not contain hazardous chemical that are harmful to our health (Chanthaphon et al., 2008).

1.3 Objectives

The following are the objectives of this research:

- To study on the effect of extraction time on the yield of essential oil extracted from various *Citrus* by using hydrodistillation method.
- To examine the effect of extraction time on the limonene composition in various *Citrus* essential oil extracted from hydrodistillation process.
- To study on the potential application of *Citrus* essential oil in making natural aromatic gel.

1.4 Scope

Generally, the scope of study is usually used as a guideline for conducting research. It is also very important to make sure that the objectives of the study can be achieved. In this research, peels from kaffir lime, key lime and calamansi lime were extracted by using hydrodistillation process with 3 hours, 4 hours, 5 hours, 6 hours and 7 hours of extraction time. The yield of the essential oil was calculated by using formula and the limonene composition present in *Citrus* essential oil was analysed by using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The effect of extraction time on *Citrus* essential oil was determined after the research.

The *Citrus* essential oil that extracted was then used to make natural aromatic gel. Four criteria which are preciseness, intensity, persistence and overall acceptability of aromatic gel had been tested by using sensory evaluation on 30 respondents that randomly selected from Universiti Malaysia Pahang (UMP). The respondents selected were within the age range from 20-50 years old and does not suffer from any sickness and allergy to *Citrus* product. The natural aromatic gel had been evaluated for 5 days period.

1.5 Organization of the thesis

This thesis consists of 5 chapters. Chapter 1 (Introduction) provides a description on the general idea of the research. This chapter includes the background of study, problem statements which become the guidelines to identify the objectives and scopes for this research and last but not least the organization of the thesis.

Chapter 2 (Literature review) described in details on the species of raw materials used, the properties and important of the lime essential oil extracted in industry. Besides, the principle and mechanism of extraction method, operation and advantages of gas chromatography-mass spectrometry (GC-MS) analysis and the danger of synthetic air refresher were also be reviewed in Chapter 2. Lastly, past studies on the characterization of lime essential oil had been inserted as a references and comparison for result.

The experimental procedure for lime essential oil extraction and application were illustrated in detail in Chapter 3 (Methodology). It describes clearly on sample preparation, hydrodistillation extraction, sample separation and also gas chromatography-mass spectrometry (GC-MS) analysis on the quality and yield of lime essential oil extracted. Besides, the steps of making natural aromatic gel and also sensory evaluation to determine the quality of the product had also been discussed.

Chapter 4 (Result and discussions) showed the result of experiment that had been carried out for this research. All the results obtained had been further discussed, explained and compared with the previous studies done.

Chapter 5 (Conclusions and recommendations) had conclude all the results obtained and some recommendations had been suggested for the improvement of the result in future study.

CHAPTER 2

LITERATURE REVIEW

2.1 Chapter overview

In this chapter, a detail review on the raw material used in this research had been discussed. Besides, the importance of essential oil in the industry and also the main volatile compound, limonene that contributes to quality of *Citrus* essential oil were also been discussed in this chapter. This chapter also including the detail review on the method of extraction - hydrodistillation (HD) and also the operation and advantages of gas chromatography-mass spectrometry analysis (GC-MS). Another part that of this chapter was regarding the review of the danger of the synthetic air freshener and the past study on the characterization of calamansi lime peel (*Citrus microcarpa*).

2.2 Introduction of *Citrus*

Citrus from Ructaceae family is an ancient crop which believed to have originated from Southeast Asia such as east India, north Burma and Southwest China. It is well known as one of the world's major fruit crops that comprise about 140 genera and 1300 species (Anwar et al., 2008). Several important fruits that can be classified as genus *Citrus* include oranges, mandarins, limes, lemons and grape fruits. Conventionally, *Citrus* fruits are mainly used for dessert, juice and jam production. Thus, *Citrus* varieties are frequently selected for cultivation in order to satisfy the agro-food industry and exportation demands (Hosni et al., 2010). Calamansi lime (*Citrus microcarpa*), key lime (*Citrus aurantifolia)* and kaffir lime (*Citrus hystrix*) are examples of common *Citrus* plants found in Malaysia.

2.2.1 Calamansi lime (*Citrus microcarpa*)

Figure 2.1: Calamansi lime (*Citrus microcarpa*)

Calamansi lime locally known as *limau kasturi* is belonging to the family Ructaceae. The calamansi fruit resembles a small, round shape, greenish yellow in colour and have 4-5 cm of diameter size. The outer look of calamansi lime look like a miniature of unripe lemon or orange but much smaller in size. The inner flesh of calamansi lime is juicy; taste sweet and sour, bright yellow to orange colour and has a unique and pleasant smell. Calamansi lime tree can normally grow until 3 to 5 meters (Bhat et al., 2011).

Calamansi lime is one of the common *Citrus* fruit that often used by Malaysian in seasoning dishes and as the dipping sauce for dishes. The juice from calamansi lime is extensively used in various food and beverage preparations. The fresh juice extracted is used in marinades, salad dressings, barbecue sauces, meat stews as well as in herbal tea preparations. According to Wang et al. (2008), calamansi lime is also widely produced in Taiwan.

From the research done by Lee and Najiah (2009), the extract isolated from calamansi lime showed inhibition against 7 bacterial strains such as , *Escherichia coli* (ATCC 25922), *Citrobacter freundii* (ATCC 8090), *Aeromonas hydrophila* (ATCC 49140), *Pseudomonas aeruginosa* (ATCC 35032), *Streptococcus agalatiae* (ATCC 13813), *E. tarda* (ATCC 15947), and *Yersinia enterocolitica* (ATCC 23715). Thus, the findings showed that the extract and bioactive component extracted from *Citrus microcarpa* might have potential as microbial agent for aquaculture use.

The major constituent presents in Citrus microcarpa are monoterpenes such as limonene, β-myrcene, β-pinene, α-pinene, β-phellandrene and sabinene. There are some sesquiterpenes such as elemene, farnesene and germacrene isomers had also been found in these types of *Citrus* fruits (Cheong et al., 2012).

2.2.2 Key lime (*Citrus aurantifolia*)

Figure 2.2: Key lime (*Citrus aurantifolia*)

Key lime or *limau* nipis in Malay name is other types of common *Citrus* from Ructaceae family that can be considered as a native species from Southeast Asia. It is an evergreen, spiny shrub or small tree and can grow to 6m in height. Besides, green leaves, stiff sharp spines and small white flowers are also some of the characteristics that commonly found on key lime tree.

Key lime has been used widely in traditional medicine as an antiseptic, anthelmintic, mosquito bite repellent and for stomach ailments (Apraj et al., 2011). Its juice had commonly used as flavoring agents in beverage and dishes. Furthermore, volatile compounds found in leaves of key lime showed protective effect against osteoporosis (Shalaby et al., 2011) and induced platelet aggregation (Piccinelli et al., 2008). Besides, some researches done on the key lime plant had showed that it exhibits bioactive activities for cold fevers, sore throats, sinusitis, bronchitis as well as helping asthma.

According to the research done by Pathan et al. (2012), the results showed that the extract from *Citrus aurantifolia* posses good antibacterial and antifungal activity against Mucor spp *Klebsiella pneumonia* and *Staphylococcus aureus*. This has further confirming on the great possible of the present of bioreactive compounds in *Citrus* essential oil and it will be one of the useful finding for rationalizing the use of this plant in primary health care. The main substances that contained in the essential oil of key lime are limonene, p-cymene, myrcene and b-bisabolene (Gamarra et al., 2006).

2.2.3 Kaffir lime (*Citrus hystrix*)

Figure 2.3: Kaffir lime (*Citrus hystrix)*

Kaffir lime or *limau purut* in Malay name is one of the *Citrus* plants commonly found in Southeast Asia. Kaffir lime fruits have rough and bumpy green skin. Its leaves are dark green colour with a glossy sheen and are composed of two leaflets. Its tree is a thorny bush which usually can grow until 5-10m tall. Kaffir lime originated from Thailand, Indonesia and Malaysia and it had been used widely in Asian cuisine and folk medicine.

According to Yaacob and Subhadrabandhu (1995), the leaves and fruits of kaffir lime have a pleasant lemon smell and it had been well known as medical lime. Kaffir lime is an effective cleanser and deodorizer. It had been practiced as natural bleach for many years to remove tough strain on the fabrics. The leaves of kaffir lime are popular ingredient in many Thai dishes and the fresh peels and dried fruits can be used to relieve nausea, dispel gas and control normal menstruation (Chuaeahongthong et al., 2011).

The essential oil extracted from fruit peels of kaffir lime shows an antibacterial property against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*. Due to its pleasant aroma and lower price, kaffir lime oil was usually be selected for further development as antibacterial product compare to sweet basil oil, galangal oil and lemongrass oil (Srisukh et al., 2012).

From the research done by Koh and Ong (1999), the essential oil extracted from kaffir lime can be used as an insect repellent and treatment for insect bites. Apart from that, the essential oil extracted from kaffir lime has been demonstrated to reduce blood pressure and relieve depression in human studies (Hongratanaworakit & Buchbauer, 2007). The major constituents found in the essential oil extracted from kaffir lime are limonene, a-terpineol, 2-b-pinene, terpinene-4-ol, g-terpinene, a-terpinene, and a-terpinolene (Srisukh et al., 2012).

2.3 Importance of *Citrus* essential oil in industry

Citrus essential oil can be found in *Citrus* fruits, leaves, peels and seeds. This commercial essential oil usually can be extracted by using cold pressing or distillation method. The essential oil extracted from *Citrus* plant has been classified as generally recognized as safe (GRAS) due to their wide spectrum of biological activities such as antimicrobial, antifungal, antioxidant, anti-inflammatory and anxiolytic (Pultrini, Galindo & Costa, 2006).

Although *Citrus* juice is the main commercial product, the essential oils extracted are also being exploited and subject to active trade in food industry. Due to the pleasant and widely acceptable smell of *Citrus*, the *Citrus* essential oil had been widely used as aroma flavor enhancers for soft and alcoholic beverage and also food (Sun, 2007).

Besides, *Citrus* essential oil had involved in wide range of uses in pharmaceutical industry. This is because essential oil extracted from *Citrus* plant had a sweet and pleasant flavor where it is very appropriate to be applied as a flavoring agent to mask the unpleasant taste of drugs and to cover the unpleasant smell of chemical used in hand sanitizer (Sun, 2007).

In perfumery industry, *Citrus* essential oil had formed the base of many compositions and had been added as fragrance addictive in the perfume. According to Lota et al. (2002), lemon and lime have a higher market value per pound compare to orange, grape fruit or tangerine oils in perfume industry. Thus, many of the factories had expanded its production line for extraction of lime essential oil as the demand in industry had been increased.

When *Citrus* essential oil was added to cosmetics, it can help improve the circulation and mood. *Citrus* essential oil has an astringent and toning action which able to clear oily skin and acne, helps with herpes, cuts, insect bites and also to combat congested skin (Kondo et al., 2000). Hence, *Citrus* essential oil is widely use in cosmetic industry due to its pleasant smell and well functionality in skin care and protection.

Apart from that, *Citrus* essential oil is also effective in providing calming effect and stress relieving in aromatherapy. The smell of *Citrus* essential oil can uplift and stimulate the brain cell which makes it great for brainstorming and creative thinking. Using *Citrus* essential oil in a massage of the lymph nodes will help in body detoxification and drain the lymph nodes. Besides, the essential oil can be used as an antidepressant because it promotes refreshment to tide mind (Pathan et al., 2012). Thus, *Citrus* essential oil had been applied widely in aromatherapy industry since past few decades.

Last but not least, *Citrus* essential oil has a vital usage in cleaning product. It has strong bleaching effect due to the presence of monoterpene, limonene. Thus, *Citrus* essential oil had been used widely used as an addictive in making soap and detergent to remove tough strain and oil (Chuaeahongthong et al., 2011).

2.4 Limonene

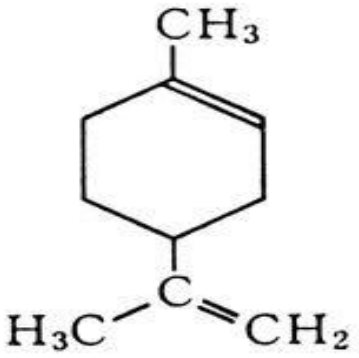

Figure 2.4: Limonene

Limonene (1-methyl-4-(1-methylethenyl)cyclohexane) is a monocyclic monoterpene with a molecular formula of $C_{10}H_{16}$. It is a major constituent present in several *Citrus* essential oil such as orange, lemon, mandarin, lime and grapefruit compare to essential oil extracted from

vegetables and plants. According to Lancas and Cavicchioli (1990), limonene is a colourless liquid with pleasant and lemon-like odour. It is practically insoluble in water but miscible in alcohol. The melting point of limonene is -74.35^{o}C and boiling point is 175.5^{o}C-176.0^{o}C. Thus, it will be in liquid state at room temperature.

Limonene had been listed as a GRAS solvent (generally recognized as safe) by the Food and Drug Admininstration (FDA). Hence, the use of limonene as a food addictive or flavoring agent in household items had been authorized by FDA. Furthermore, Environmental Protection Agency (EPA) did not require any tolerance limit for the limonene use in pesticides or insect repellent. Thus, limonene compound can be considered as one of the green compound that can be used in making natural product (Faure et al., 2013). Due to its *Citrus*-like flavor, limonene is employed as a flavoring agent in perfumes, creams, soaps, personal hygiene products, medicinal, cosmetics, household cleaning products , food and beverages such as ice-cream (Bakkali et al., 2008).

Besides, limonene had been found to possess antifungal and bactericidal properties. Therefore, the present of limonene as the major constituent in *Citrus* fruits such as lime, lemon and oranges had contributed to the antifungal and antibacterial properties of the fruits (Espina et al., 2013). According to Chanthaphon et al. (2008), the amount of limonene contained in essential oil will influence the quality of essential oil that being used for aromatherapy, antibacterial and others significantly. Thus, a higher concentration of limonene found in the essential oil will give a better quality of *Citrus* essential oil extracted (Kasuan et al., 2009).

Due to the pleasant odour, good solvent properties and relatively low toxicity, the demand of limonene as an industrial solvent to replace chlorinated hydrocarbons as a remover or stripper for wax, paints, ink, and adhesives in degreasing operations had been increased. Besides, limonene can be act as a substitute for xylene in slide preparation in many histopathologies (Lam, 2008).

Apart from that, limonene has been considered to be used on the International Space Station (ISS) as a cleansing solvent. Normally on the ISS, the low-toxicity water –soluble solvents such as ethanol and isopropanol will be used in medical applications and for hardware cleansing. However, the alcohols used are volatile, highly soluble and has a low-molecular weight. Thus, it will release into the air after used and readily to be removed together with water vapour by the humidity removal system as water condensate. Since the water

purification system in ISS has limited capacity to remove these water-borne organics, thus limonene which has low solubility in water and relatively low toxicity had been proposed as a substitute of cleansing agent to reduce the use of alcohols and other water-soluble volatile organic compounds on ISS (Lam, 2008).

According to Faure et al. (2013), limonene is a biorenewable solvent that has similar polarity and solving properties compared to heptanes. Its low toxicity properties and "green" source from *Citrus* peel make it a fine sustainable compound to replace heptanes in many chemical processes. Therefore, limonene can be evaluated as a possible substitute for heptanes in counter-current chromatography (CCC) separations, however, the density of limonene need to be concerned later in CCC purifications.

2.5 Method of extraction (hydrodistillation)

Hydrodistillation is one of method of extraction which can be traced back as early around 1000A.D., where the Arab physician and naturalist Ibn Sina using the same concept to extract the rose oil from rose petals. After that, the ancient Arabian people began to study on the chemical properties of essential oil extracted and the distillation process had started to develop. On the other hand, the Europeans only started to produce essential oils in the 12th century. In 19th century, the essential oils were widely used as medicines but it was then increasingly gaining popularity in perfumes, food and beverage industry in later time. This had lead to the large scale production of essential oil from distillation method (Pearlstine, 1998).

Essential oil can be obtained by expression, fermentation, enfleurage or extraction but distillation is the most commonly used for the production of essential oil. The process of distillation can be described as vegetal materials are mixed (or not) with water followed by heating or by introduction of water steam and the resulting vapour will then be cooled and collected in a separator. The essential oil will form two layers with water and will be drawn out. The yields of a crude essential oil after the distillation process should be further separated in order to increase the purity of essential oil (Vinatoru, 2001).

Besides, Ferhat et al. (2006) had stated that distillation is the best method to determine the essential oil content from the fruit in industry. However, the heating process should be controlled properly in order to optimized the quality and quantity of essential oils extracted.

This is because some of the compounds are very sensitive to chemical change and unstable when exposed to high temperature.

Currently in Malaysia, hydrodistillation is commonly used to extract essential oil from the plants. Hydrodistillation can be described as a technique in which the water from still flask will be boiled and distilled together with the immersing plant material for obtaining essential oil from the materials (Stanković et al., 2001). In this process, the mixture of plant material and water in a vessel is heated by the means of electrical heating elements, or by fame heating at the bottom surface of the vessel. Since the plant material has low thermal conductivity as compared to water, the heated water conducts the heat into the oil-bearing cell and released the essential oil contains inside oil sacs. This showed that the whole hydrodistillation process had been governed by convective or conduction of heat (Hanzah et al., 2011).

According to Diaz-Maroto (2005), hydrodistillation and steam distillation method are commonly used to produce essential oil industrially. Distillation had been selected for essential oil extraction due to the simple equipment used, easy operation and low cost. However, the disadvantage of distillation is long extraction time. Cold pressing method is the easiest way to extract essential oil, but it takes longer time and lower yield compare to distillation method. Solvent extraction method takes shorter time, but the separation between solvent and oil is a tough problem and it will influence the quality of essential oil extracted. Supercritical carbon dioxide extraction (SFE) and microwave assisted extraction (MAE) can extract higher yield and quality of essential oil but there are seldom used in industry because of the high cost and equipment needed (Zhou et al., 2006).