POTENTIAL APPLICATION DERIVED FROM THE ESSENTIAL OIL OF PANDAN LEAVES (*PANDANUS AMARYLLIFOLIS ROXB.)*

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POTENTIAL APPLICATION DERIVED FROM THE ESSENTIAL OIL OF PANDAN LEAVES (*PANDANUS AMARYLLIFOLIS ROXB.)*

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Thesis submitted in partial fulfillment of the requirements

for the award of the degree of

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I hereby declare that the work in this thesis entitled "Potential Application Derived from Essential Oil of Pandan Leaves (*Pandanus Amaryllifolius* Roxb.)" is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Special Dedication to my supervisor, my family members,
my friends, my fellow colleague and all faculty members
for all your care, support and believe in me.

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ABSTRACT

Pandan (*Pandanus Amaryllifolius Roxb.)* is an erect green plant with the woody aerial roots and fan-shaped sprays of long, narrow, blade-like leaves leaf. It is widely used as natural flavoring in South East Asian dishes. Pandan leaves are also used by many people as an odour repellent in their cars due to its nature as an insect repellent and its aromatic fragrance. The major compound contributing to its aromatic fragrance is 2-acetyl-1-pyrroline (2AP). The potential usage of the essential oil as a natural air freshener was studied. The effects of the extraction temperature and period on quantity and quality of extraction yield were also studied. Experimental parameters included the temperature and extraction period. In this study, essential oil was extracted from pandan leaves by using the Microwave Assisted-solvent Extraction method, followed removal of ethanol solvent by using Rotary Evaporator. The chemical compounds inside extract included 2AP were identified by Fourier Transform Infrared Spectroscopy (FTIR), while the concentration of 2AP was analyzed by Gas Chromatography-Flame Ionization Detector (GC-FID). The concentration of 2AP for each parameter was determined by comparing the internal standard graph of trimehylpyridine (TMP) using GC-FID. The results shown that yield, volume of extracted essential and concentration of 2AP in essential oil increased with the extraction temperature before reaches the optimum point. Then, they decreased for higher temperature for extraction process due to decomposition of 2AP. On the other hand, the yield, volume of extracted essential oil and concentration of 2AP in essential oil decreased with increment of extraction period and then almost constant for longer period. This is because further exposure in high temperature for long period decomposed the heat sensitive compounds inside essential oil. Therefore, the extraction process at temperature 88^{o}C and 30 minutes produced the highest yield of extract and concentration of 2AP respectively. After the optimum condition of extraction method was determined, high amount of pandan leaves extract was produced for further research. Beaker A with 25 ml sample and beaker B with 25 ml water was given to 6 rooms in Kolej Kediaman 4 for sensory evaluation. The 6 rooms were chosen random, but have almost same environment condition. There are 5 people in each room to evaluate 5 criteria which are correctness, persistence, smell, intensity and overall acceptance for one week. They also measured the volume of water and sample left in beaker in every single day. Based on the result, most panels gave high rating grade for persistence, correctness and smell. However, they gave low rating grade for overall acceptance and intensity due to low concentration of 2AP in essential oil. The recommendation is to increase the concentration of 2AP inside essential oil in order to have high performance. The essential oil of pandan leaves has high potential to be a natural air freshener in daily life.

Keywords: Pandan leaves; extraction period; 2-acetyl-1-pyrroline; temperature; yield; volume; sensory evaluation; concentration 2AP; air freshener

ABSTRAK

Pandan (*Pandanus Amaryllifolius Roxb*.) merupakan tumbuhan hijau yang tegak dengan akar berkayu lembut dan daunnya berbentuk kipas lama, sempit, seperti pisau bilah. Ia banyak digunakan sebagai perisa semulajadi dalam masakan Asia Tenggara. Daun pandan juga digunakan oleh orang ramai sebagai bau penghalau dalam kereta mereka kerana harumannya and sifatnya sebagai penghalau serangga. Sebatian utama yang menyumbang kepada haruman aromatik 2-asetil-1-pyrroline (2AP). Potensi penggunaan minyak pati sebagai penyegar udara semula jadi telah dikaji. Kesan suhu dan tempoh pengekstrakan pada kuantiti dan kualiti hasil perahan juga dikaji. Parameter uji kaji termasuklah suhu dan tempoh pengekstrakan. Dalam kajian ini, minyak pati yang diekstrak daripada daun pandan dengan menggunakan kaedah pengekstrakan Microwave, diikuti penyingkiran pelarut etanol dengan menggunakan Rotary Evaporator. Bahan kimia di dalam minyak pati termasuk 2AP telah dikenal pasti oleh Fourier Transform Infrared Spectroscopy (FTIR), manakala kepekatan 2AP dianalisis oleh Gas Chromatography-Flame Ionization Detector (GC-FID). Kepekatan 2AP bagi setiap parameter adalah ditentukan dengan membandingkan graf trimehylpyridine (TMP) menggunakan GC-FID. Keputusan menunjukkan bahawa hasil, jumlah minyak pati dan kepekatan 2AP dalam minyak pati meningkat dengan suhu pengekstrakan sebelum mencapai titik optimum. Kemudian, mereka menurun dalam suhu yang lebih tinggi untuk proses pengekstrakan disebabkan oleh penguraian 2AP. Sebaliknya, hasil, jumlah minyak pati yang diekstrak dan kepekatan 2AP dalam minyak pati yang menurun dengan kenaikan tempoh pengekstrakan dan kemudian hampir malar bagi tempoh yang lebih lama. Ini adalah kerana pendedahan pada suhu yang tinggi bagi tempoh masa yang panjang terurai sebatian yang mempunyai sifat sensitif akan haba di dalam minyak pati. Oleh itu, proses perahan pada suhu 88^{o}C dan 30 minit menghasilkan quantity minyak pati yang banyak dan kepekatan 2AP tinggi masing-masing. Selepas keadaan optimum untuk kaedah pengekstrakan ditentukan, bnayak minyak pati diekstrak daripada daun pandan dihasilkan untuk penyelidikan selanjutnya. Bikar A dengan 25 ml sampel dan bikar B dengan 25 ml air telah diberikan kepada 6 bilik di Kolej Kediaman 4 untuk penilaian deria. 6 bilik yang dipilih secara rawak, tetapi mempunyai keadaan persekitaran yang hampir sama. Terdapat 5 orang dalam setiap bilik untuk menilai 5 kriteria termasuk ketahanan, kebetulan, bau, intensiti dan penerimaan keseluruhan selama satu minggu. Mereka juga mengukur isi padu air dan sampel yang tinggal dalam bikar dalam setiap hari. Berdasarkan keputusan itu, kebanyakan panel memberi gred penarafan tinggi untuk kebetulan, ketahanan, dan bau. Walau bagaimanapun, mereka memberi gred penarafan rendah untuk penerimaan keseluruhan dan intensiti yang disebabkan oleh kepekatan rendah 2AP dalam minyak pati. Dengan meningkatkan kepekatan 2AP di dalam minyak pati, minyak pati akan mempunyai prestasi yang tinggi. Minyak pati daripada daun pandan mempunyai potensi yang tinggi untuk menjadi penyegar udara semula jadi dalam kehidupan seharian.

Kata kunci: Daun pandan; tempoh pengekstrakan; 2-asetil-1-pyrroline; suhu; isipadu; hasilan; penilaian deria; kepekatan 2AP; udara penyegar.

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LIST OF SYMBOLS

^{o}C	Degree Celcius
ε̇	Dielectric constant
ppm	Parts per million
ppb	Parts per billion
W	Walt

LIST OF ABBREVIATIONS

2AP	2-acetyl-1-pyrroline
FTIR	Fourier Transform Infrared Spectroscopy
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GC-SPME	Gas Chromatography- Solid Phase Micro Extraction
MAE	Microwave-assisted extraction
RBD	Refined, bleached and deodorized
TMP	2,4,6-trimethylpyridine
UV-Vis	Ultraviolet-visible Spectroscopy

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CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 RESEARCH BACKGROUND

Pandanus amaryllifolius leaves, generally known as pandan, is a tropical plant which belongs to the screw pine genus (Pandanaceae). Pandan is an erect green plant with the woody aerial roots and fan-shaped sprays of long, narrow, blade-like leaves (Yahya et al., 2010). The leaves of pandan are dark green in colour with a strong nutty aroma. Rutuju Jathar (2011) stated that pandan plant is native to Asia and the tropical parts of Australia. It is a sterile plant and is often propagated by people by cutting the leaves.

The pandan leaves are widely used as a natural flavoring in South-east Asia including India, Thailand, Indonesia and Malaysia. For example, people usually cook non-aromatic rice with pandan leaves to impart a resemblance of leaf aroma which is similar to aromatic rice such as basmati and jasmine rice (Bhattacharjee et al., 2004). In addition, pandan leaves are sometimes added to iced drinks prepared from fresh unripe coconuts. Many people also extract the juice from pandan leaves and use it as essence in

cake making. Mohd Nor et al. (2007) stated that it gives a fragrant flavor to the savory dishes such as jellies, puddings, custards, chicken and sweets.

Besides having aromatic properties, pandan leaves are very beneficial for various health conditions. Whole pandan plant is considered to be diuretic because it contains traces of tannin, glycosides and alkaloids (Rutuju Jathar, 2011). Therefore, it is useful for healing various wounds and diseases such as smallpox. Apart from that, pandan leaves are usually used in aromatic therapy to relieve the weak nerves, so they are considered as the pain reliever to cure chest pain, headache, reduce fever, arthritis, earache and others. Moreover, pandan leaves are used by some parents as a healthy laxative for their children. Pandan leaves are also very effective in helping women with weak body recover after give birth. Some scientists found that the pandan plant is one of the anti-carcinogenic plants and is significantly useful for curing diabetes diseases. Several types of skin disorders including leprosy can be cured by pandan leaves too.

The essential oil derived from the pandan leaves contains a number of volatile compounds from groups of alcohols, aromatics, carboxylic acids, ketones, aldehydes, esters, hydrocarbons, furans, furnanones and terpenoids. In general, these volatile compounds are ethyl formate, 3-hexanol, 4-methylpentanol, 3-hexanone, 2-hexanone, trans-2-heptenal, β-damascenone, 4–ethylguaiacol and 3-methyl-2-(5H)-furanone (Yahya et al., 2010). Since the amounts of those compounds are very minimal, this study will focus on the compound 2-acetyl-1-pyrroline (2AP). It has a popcorn odor and an appearance from colourless to yellow. It has quite a high boiling point which is 182 ^{o}C, however it is a volatile component in the natural plant. The chemical formula of the compound 2AP is C_6H_9NO. Its molecular weight is 111.142 g/mol.

The major compound contributing to the aromatic flavor of pandan is 2-acetyl-1-pyrroline (2AP). Yahya et al. (2010) reported that 2AP is a hydrophilic compound which has the odor threshold value as low as 0.1 ppb in water. Besides, 2AP is a substituted pyrroline and a cyclic imine. In a study by Buttery et al., (1983), it is

reported that the quantities of 2AP present in pandan leaves (in unit of ppm) is 10 times and 100 times more than that found in the scented milled rice and common rice respectively. Therefore, pandan leaves are one of the best sources of 2AP compound. Li, and Ho. (2003) proved that seven compounds and fractions prepared from pandan leaves have repellence against certain species of cockroaches. From the study, it is proven that it has the potential to be a natural and environmental friendly pest management tool.

Several extraction methods are used by many researchers in the past for extraction of 2AP such as simultaneous steam distillation–solvent extraction (Buttery et al. 1986; Lin et al. 1990; Mahatheeranont et al. 2001), micro steam distillation–solvent extraction (Tanchotikul and Hsieh 1991) and direct solvent extraction (Mahatheeranontet al. 2001; Bergman et al. 2000; Yoshihashi 2002) coupled with analytical methods for quantification of 2AP.

1.2 PROBLEM STATEMENT

Pandan leaves are very popular among people of South East Asia as an aromatic flavoring in their daily activities. Sometimes, people will keep pandan leaves in refrigerator for 1 day before using them for cooking. However, the storage period of pandan leaves is very short, around 2-3 days. Pandan leaves will lose its fragrance slowly and change from green color to brownish color. Lastly, people have to replace the withered leaves with fresh pandan leaves. In addition, pandan leaves are harvested in a form of bunch with their roots intact. Some farmers bring these leaves to market and sell them to people in village. Since pandan have slender leaves which is proportional to its volume and weight, therefore it is unlikely to be carried around.

Synthetic air fresheners used in cars or living spaces will increase the risk of developing a variety of health problems. For example, Natural Resources Defense Council, (2007) reported that 12 of 14 brands of common household air fresheners contain phthalates. These phthalates are hazardous chemical compounds. They are often

used to prolong the length of time the scented products retain their fragrance, however they increase the risk of experiencing endocrine, reproductive, and developmental problems. Apart of that, some artificial air fresheners contain formaldehyde which is classified as a human carcinogen by the International Agency for Research on Cancer.

According to journal of Mathure (2010), there are many factors affecting the quality and quantity of 2AP extracted with every method. Those factors are pre-treatment methods, extraction time, extraction temperature, solvent quantity, sample weight and others. It is found out that the yield of 2AP from the HS-SPME extraction methods decreases when the extraction time is too long and temperature is too high. Therefore, it shows a need for optimization of those factors before proceeding. With the optimum condition for the experiment, a large amount of high quality 2AP can be obtained in the essential oil.

1.3 OBJECTIVES

The objectives of this research are:

i. To determine the optimum extraction time and temperature in order to get highest extraction yield of essential oil and concentration of 2AP.
ii. To develop a natural air freshener derived from the essential oil of pandan leaves

1.4 SCOPE OF RESEARCH

The research scope is very important to make sure that the objectives of research are achieved. Generally, the scope is used as a guideline for conducting this research. The scope of this research can be summarized as follow:

i. In this study, the type of raw material was restricted to *Pandanus Amaryllifolius Roxb.*. The pandan leaves must be fresh and purchased from the same place.
ii. The temperature of extraction process was set at 70^{o}C, 80^{o}C, 90^{o}C, 100^{o}C and 110^{o}C.
iii. The period of microwave assisted-solvent extraction process was set at 30 minutes, 40 minutes, 50 minutes, 1 hour and 70 minutes.

iv. The powers, ratio of raw materials to solvent, as well as pressure for the microwave assisted-solvent extraction of cut pandan leaves were consistent for all experiment runs.
v. The persistence period of essential oil was tested in rooms of Kolej Kediaman 4 in University Pahang.
vi. 30 people are chosen randomly for the sensory evaluation.
vii. The sensory testing period was one week.

1.5 RATIONAL AND SIGNIFICANT

Currently, many synthetic air fresheners are proven to pose risk of developing of a variety of health problems such as asthma, breathing problem headache and Bronchial irritation. Liu et al. analysed air freshener and reported that smoke from heating contains heavy metals, allethrin and hazardous phenol O-cresol. Sharma (2001) stated that the chemical, pyrethrums in air freshener could lead to running nose and wheezing, prolonged use will damage the liver corneal and cause asthma. This study aims to develop a natural air freshener and repellent from essential oil of pandan leaves to substitute those artificial products. Some chemical compounds contained in the essential oil are used in pharmaceutical. Therefore, the essential oil form pandan leaves will help to improve the human health through the aromatherapy while reducing the risk of getting health problems (Ruratech Services, 2010). Besides, the pandan air freshener is an environmental friendly product. It only contains non-hazardous chemical compounds meaning it is safer for use in daily life. The usage of pandan leaves will also minimize the amount of hazardous chemicals in the waste water resulted from the production of air freshener.

Since essential oil is kept in small bottles, it saves space and is more convenient to carry around compared to pandan leaves. It also has a much longer persistence period which makes it more economical as the pandan leaves will wither in a few days. Besides, the aromatic properties are suitable in eliminating unpleasant odors. Furthermore, the pandan plants can be grown easily in the South-East Asia since it can adapt easily to various environments and has a short growth period. With the abundance

of pandan leaves sources, pandan leaves have great potential for commercialization in future.

CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Pandan Leaves

Pandanus amaryllifolius Roxb. plant is known as pandan leaf. It is a tropical plant originally from Vietnam. *Pandanus amaryllifolius* Roxb.is grouped in the screwpine genus belonging to family *Pandanaceae* which is a paleotropic family of 800 trees and shrubs. Among the 36 species of this family that have been found in the India, *Pandanus odoratissimus* Linn. and *Pandanus amaryllifolius* Roxb. are of commercial interest to the food industry. This plant is seldom found in the wild but it is widely cultivated by people in South-East Asia. Nowadays, it is distributed over Southern India, peninsular South East Asia, Indonesia and Western New Guinea. It is an upright green plant and has woody aerial roots. It also has the fan-shaped sprays of long, narrow, bladelike leaves. Katzer, G., (2001) reported that this plant seldom has flowers and the flowers are not big bloomer. The male flowers in this species of plant are extremely rare, and there is no scientific evidence to prove the existence of the female flower. Katzer, G., (2001) also reported that the flowering pandan plants on the Moluccas archipelago are the only known instances. This is because this species have faced the evolution there by the process of hybridisation which shares its chromosome number ($2n$=60) with most other representatives of the genus.

It usually planted by people in container or ground. It can grow well in any kind of soil. Besides, it needs a lot of water and humid environment. It prefers to semi-expose to strong sunlight on afternoon. In general, it propagates by replanting the suckers which are formed at base or plantlets with aerial roots. However, the grounded suckers are better than aerial plantlets because some plantlets may fail to propagate. It can grow till a height between 6 feet and 8 feet tall. It also has leaves around 30-50cm long when it is mature. The pandan leaves exhibit the popcorn like, pleasant aroma. This aroma is special and hard to describe but a similar scent is found in some aromatic rice varieties grown in South East Asia. For examples, Buttery, et al., (1983) reported that the Thailand jasmine rice and Basmati rice have similar aroma but less dense. However, the pandanus leaves have strong scent only during wilting. The fresh, intact pandan leaves hardly have any odour. In addition, dried pandan leaves will lost its fragrance rapidly.

2.2 APPLICATION OF PANDAN LEAVES

In general, pandanus leaves are very popular natural food flavoring in tropical Asia, from South India to New Guinea. The food which use them as flavoring such as bakery products, sweets, ice-cream, yogurt, tea drink, and coconut jam. They also are used for others purposes, but mostly related to rice (Buttery et al., 1985). For example, in India and Philipines, pandan leaves are traditionally used during cooking the non-aromatic rice in order to impart the unique fragrance (Bhattacharjee et al., 2003). Therefore, people no need to buy the expensive aromatic rice. In Sri Lanka, they are widely used together with curry leaves to cook Singhalese curries. Moreover, in Thailand, Malaysia and Indonesia, some plain rice cooked in coconut milk together with pandan leaves is a delicacy even when eaten alone (Yahya et al., 2010). The Indonesian specialty *nasi kuning* and *nasi-lemak* are made by putting entire pandan leaf with coconut water and rice. Most delicious is rice steamed in small baskets made from pandanus leaves, as often prepared in Indonesia. Sometimes, pandan leaves impart green colour to the rice too.

In addition, some food industries produce pandan cake from the essence which is juices extracted from pandan leaves. Sometimes, pandan leaves are used as the fragrant wrappers for Thailand cuisine. For example, Pandanus chicken is known as *gai hor bai toey.*It is a classical recipe and an eternal favourite in some Thai restaurants. They use pandan leaves to wrap these marinated chicken bits and then deep-fry them in a wok. Besides, the most important culinary application of pandan leaves is discovered in desserts all over the South East. For instances, young coconuts iced drinks together with pandan flavour are popular in Thailand. Some pandan leaves are made into ice cream and sold in market (Katzer, G., 2001). Pandan leaves are also frequently added in sweet puddings or custards prepared from sticky, glutinous rice. For these concoctions, water, palm sugar, pandan leaves and glutinous rice are boiled together to produce a heavy mass rice. Then, it becomes semi-solid when cooling. The thick coconut milk normally is sprinkled over it before serving.

2.3 ESSENTIAL OILS

Essential oils are natural volatile and liquid aroma compounds extracted from the various plants. They actually are not oil but have the similar properties with oil which is poor solubility in water. They also exhibit the distinctive scent and flavor which depend on the oxygenated compounds. In general, essential oils consist of terpenoids, few are benzene derivative, but their compounds mainly depend on the type of solvent and extraction methods. Table 2.1 shows the major compound inside essential oils derived from some common plants.

Table 2.1: Essential oils from some common plants

Source: Mohammad, S., Bin I., (2008)

Name	Part of plant extracted	Botanical name	Important constituent	Usages
Lemongrass and citronella	Leaf	*Cymbopogonspp*	Citral, Citronella, Terpenes	Perfumery Disinfectant
Eucalyptus	Leaf	*Eucalyptus globules Eucalyptus citriodora Eucalyptus dives*	Cineale Citronella Terpenes	Not mention
Lavender	Flower	*Lavendulaintermedia*	Linalol	Perfumery
Clove	Bud	*Eugenia caryophyllus*	Eugenol	Dentistry flavouring
Sandalwood	Wood	*Santaium album*	Sanatols	Perfumery
Nutmeg	Nut	*Myristicafragrants*	Myristicin	Not mention
Almond	Nut	*Pruniscommunts*	Benzaldeyhde	Not mention
Coriander	Seed	*Coriandrumsatiyum*	LinalolTerpenes	Not mention
Garden Angelica	Seed	*Angelica archangelia*	β-terebangeleneTerpenes	Medical Flavoring
Oregano	Leaf	*Origanumvulgare*	ThymolCarvacol	Fungicide Digestive

Essential oils can be divided into two broad categories according their volume:

I. Large volume oils are usually extracted from the leafy material. (e.g. lemongrass, cinnamon leaves, oregano and citronella)

II. Small volume oils are extracted from the fruits, seeds, buds and lesser extent, flowers. (e.g. Clover, Lavender, Coriander and Almond)

Normally, pure essential oils are very expensive because it is produce from large quantity of plant material. However, few drops of essential oil are required to achieve the desired effect on the product. They have an intermediate impact to human's sense of smell known as olfaction. When we inhale the fragrance of essential oil, olfactory receptor cells are stimulated and the impulse is transmitted to the central of the brain known as limbic system. This limbic system is linked to areas of the brain which controls the memory, breathing and blood circulation. Furthermore, the endocrine glands which regulate hormone levels in the body will be affected by limbic system. Therefore, properties and fragrance of essential oils will affect the simulation of these systems in body.

Sometimes, these oils are used in the process of massage. Human not only inhale, also absorb them through skin. They penetrate the skin and flow into bloodstream inside our body. After that, they are transported to every organs and parts of our body. Some essential oils also will be absorbed quicker into skin via the hair follicles when applied to our body. The time for body absorbs the essential oils is depending on the concentration of essential oils. High quality pure essential oils are proved to have 70 times higher concentration than plant source. As a result, our brain and body will start to react to the vapor form of essential oils in less than four seconds after they stimulate the olfactory nerves and other sensors.

In the daily life, these essential oils may be used singly or in combination to to bring the curative and restorative effect to our mind and body. It is also a gentle alternative to modern hazardous drugs. They are applied already to be an assistance material in the treatment of the physical, mental and emotional changes, skin care and therapeutic massage. They can enhance and enrich positively our daily life even used solely for sensual pleasure.

2.4 PROPERTIES OF ESSENTIAL OIL

Essential oils mainly consists of hydrocarbons derivatives, so they exhibit the properties of hydrophobic and more soluble in organic solvents. However, they also can dissolve slightly in the water to give an intense odor to the solution such as rose water and orange flower water. The chemical compounds inside essential oils are volatile enough to distill unchanged in most methods, but they will decompose when the temperature is too high. Essential oils derived from different type of plant will have different colour. They vary from the colour to yellow or brownish in colour. They also have high refractive indexes which averaging about 1.5. Apart of that, these oils show wide range of optical activity and can rotate in both directions. The chemical compounds inside the essential oils can be classified based on their functional group as in next page:

Table 2.2: Functional groups of chemical compounds

Source: Mohammad, S., Bin I., (2008)

Functional groups	Common constituents
Carboxylic acid	Benzoic, Acetic, Salicyclic and Cinnamic acids
Alcohol	Linalool, Geraniol, Terpinol, Menthol, Borneol
Aldehydes	Citral, Benzaldehyde, Cinnamaldehyde, Vanilin
Phenols	Eugenol, Thymol, Carvacrol
Terpenes	Camphene, Pinene, Limonene, Phellandrene, Cedrene
Hydrocarbons	Cymene, Styrene
Ester	Benzyl Acetate, Triglycerides, Phospholipids

In general, these constituents are mainly built from five species of atoms which are carbon, hydrogen, oxygen, nitrogen and sulphur. Nitrogen and sulphur have much lesser extent inside the essential oils. Nowadays, new analytical techniques have been created to study the chemical compounds inside these oils. As a result, the number of

chemical constituents known especially most important ones is increasing rapidly. As mentioned before, most constituents consist of class of terpenes, although others classes are found. The essential oils have a high percentage of single compound which are used for the production is called isolates. These isolates focus on extracting the more valuable and main compound rather than relatively inexpensive compounds such as hydrocarbons. They are usually used as the starting material for synthesis. However, the trace components are also important to give the oils its particular and natural odor. The composition of essential oils varies from one harvest to next and from one producer to the other.

Terpenes are the major components of resin and turpentine inside plant. They are the molecules which are built from the isoprene. Monoterpenes, sesqiterpenes, diterpenes, sisterpenes and triterpenes are family of terpenes. Besides, hydrocarbons consist of carbon and hydrogen atoms which are the most common constituents of the essential oils, but they are not the most important or valuable ones. They are often will oxidise when contact with air and produce the bad odour. They also will lower the solubility of the essential oils in the water. The oxygenated compounds such as alcohols, aldehydes, ketones, esters, carboxylic acids, acetals, oxides, lactones and phenols play the most important role to give the characteristic fragrance for the essential oils. They also represent the solubility portions inside the water and polar solvents.

2.5 BENEFITS OF ESSENTIAL OIL

Essential oils are safe, simple and natural products which used as the primary ingredients in aromatherapy. In the aromatherapy, they help individual to recover the physical and emotional ailments, give pleasure to relax. Besides, this therapy is known as a holistic, complete and natural form because the effect of the treatment on the body, mind and the emotions of person receiving it are taken in account in order to have the maximum good effect. In past, these oils of plants are used by our ancestors because of their advantages. The effectiveness of essential oils is proven by the scientific analysis in order to confirm the intuitive link and understand the relationship between nature and

human beings. Consequently, the dynamics of aromatherapy bring the essence of nature into our daily lives.

2.6 EXTRACTION OF ESSENTIAL OILS

2.6.1 Introduction

Essential oils can be extracted using a variety of methods, although some methods are not encouraged and common nowadays. Therefore, various extraction techniques are widely investigated to obtain the best or optimum method in extracting valuable natural compounds from the plants for commercialization. Traditional methods such as Soxhlet extraction have been used for many decades. There methods are time-consuming and require relative large quantities of solvents (Luque de Castro & Garcia-Ayuso, 1998). In this 21st century, there is an increasing demand for new extraction techniques with shortened extraction time, reduced solvent consumption, and increased pollution prevention (Wang, L. J. & Weller, C. L., 2006). The improved extraction methods nowadays including ultrasound-assisted extraction (Vinatoni, 2001), microwave assisted extraction (Kaufmann & Christen, 2002), supercritical fluid extraction (Marr & Gamse, 2000; Lang & wai, 2001; Meireles & Angela, 2003), improved solvent-free microwave extraction (Wang et al., 2006) and Accelerated solvent extraction (Kaufmann & Christen, 2002; Smith, 2002) are fast and efficient for extracting chemicals from solid plant materials. These techniques have the potential in working at elevated temperatures and/or pressures, greatly decreasing the time of extraction.

Recently, these techniques have become relatively mature because some potential applications for the extraction of nutraceuticals from solid plant matrices have been reported. For example, Mamidipally and Liu (2004) had used d-limonene and hexane in the Soxhlet extraction of oil from rice bran. In addition, an overview of ultrasound-assisted extraction of bioactive compounds from herbs was drafted by Vinatoru, (2001). The application of microwave-assisted leaching in process metallurgy has widely been inestigated by Ganzler, Sango, & Valko, (1986). The essential oil of *Angelica archangelica* L. Root is extracted through supercritical carbon dioxide extraction methods at 120bar and 40 ^{o}C in 2 hours. Smith, (2002) also wrote a review of

fundamentals and practical use of pressurized how water extraction for some plants. In this research, I will focus on the microwave-assisted extraction (MAE). I will discuss the information about matrix characteristics, solvent choice, liquid-solid ratio, temperature, pressure, theoretical background and extraction time

2.6.2 Microwave-assisted Extraction (MAE)

- Principles and Mechanisms

Microwave-assisted extraction (MAE) is a simple technique that provides a novel way of extraction soluble products into a fluid from a wide range of materials by using microwave energy. This technique also can be applied to both liquid phases extraction such as isolation of essential oil based on the basic physical principle. Microwaves are electromagnetic radiations with frequency from 0.3 to 300 GHz and are transmitted as waves which can penetrate biomaterials and interact with polar molecules such as water inside the biomaterials to create heat (Wang et al., 2006). For example, domestic and industrial microwave generally operate at 2.45 GHz but sometimes operate at 0.915 GHz in USA and 0.896 GHz in Europe. In conclusion, microwaves can heat a whole material via penetration and polar compounds simultaneously.

This technique offers a rapid delivery of energy to solvent and solid plant matrix with subsequent heating of the solvent and solid matrix, efficiently and homogeneously. This is because water or polar compounds within the plant matrix absorbs microwave energy and then cell disruption is promoted by internal superheating which facilitates desorption of chemicals from the matrix (Kaufmann, Christen, & Veuthey, 2001). This action will improve the recovery of nutraceuticals from the matrix. For example, microwave pretreatment of fresh orange peels led to destructive changes in the plant tissue is observed by Kratchanova, Pavlova, and Panchev, (2004) via scanning electron micrographs. These changes in plant tissue will give a considerable increase in the yield of extractable pectin. Moreover, the migration of the dissolved ions will increase penetration of solvent into the matrix and thus facilitated the release of the chemicals. From this principle, it can conclude that the effect of the microwave energy is strongly depending on the dielectric susceptibility of both the solvent and the solid plant matrix.

There are two types of commercially available MAE systems which are closed extraction vessels under controlled pressure and temperature, and focused microwaves ovens at atmospheric pressure (Kaufmann & Christen, 2002). The closed vessels for MAE system is used for extraction under drastic conditions such as high temperature. The pressure for the vessel in this system essentially depends on the volume and the boiling point of the solvents. The focused MAE system is operated at a maximum temperature which is determined by the boiling point of the solvents at atmospheric pressure. A dynamic MAE system was demonstrated by Ericsson and Colmsjo, (2000) to yield extract equivalent to yield of extract by using Soxhlet extraction, but consume a much shorter time.

- Matrix Characteristics

As mentioned before, MAE system is dependent on dielectric susceptibility of solvent and matrix, so better recoveries can be obtained by moistening samples with a substance that has a relatively high dielectric constant such as water. If a dry plant material is re-hydrated before extraction, the matrix will interact better with microwaves and hence facilitates the heating process. The microwave heating will lead to expansion and rupture of cell walls and is followed by liberation of constituents into the solvents (Spar Eskilsson & Bjorklund, 2000). In this case, the solvent can have a low dielectric constant and will remain cold during extraction. This method can be used to extract thermosensitive compounds such as essential oil from plants (Brachet, Christen, & Veuthey, 2002) However, completely dry and very wet samples is impossible to perform a good MAE when a non-polar solvent such as hexane is used as the extraction solvent (Molins, Hogendoorn, Heusinkveld, Van Zoonen, & Baumann, 1997).

Plant particle size and size distribution have a significant influence on the efficiency of the MAE system. Spar Eskilsson & Bjorklund, (2000) reported that the particle sizes of the extracted materials are usually in the range of 100 μm-2 mm. Fine powder can enhance the extraction since the limiting step of the extraction is always the diffusion of chemical out of the plant matrix. With these fine powders, they provide the larger contact surface area between the plant matrix and the solvent. For example, finely

ground cocoa powder are more easily extracted than large particles by using MAE system to get cocaine (Brachet et al., 2002)

- Solvent Choice

Solvent choice for MAE system is dependent on the solubility of the extracts of interest, the interaction between solvent and plant matrix and microwave absorbing properties of the solvent determined by its dielectric constant. For example, the efficiency and selectivity of MAE for the extraction of the color pigments from paprika powders using 30 extracting solvent (Csiktusnadi Kiss et al., 2000). Based on the results, dielectric constant of the extraction solvent mixture will influence significantly the efficacy and selectivity of MAE system. Therefore, the chosen solvent should possess a high dielectric constant to absorb strongly the microwave energy. Solvents such as ethanol, methanol and water are sufficiently polar to be a good heating medium of microwave energy (Brachet et al., 2002). Non-polar solvents with low dielectric constants such as toluene and hexane are not suitable solvent for MAE system. However, the extracting selectivity and the ability of the solvent to interact with microwave can be modulated by mixing with polar solvents (Bracht et al., 2002). One of the most common mixtures used is hexane-acetone (Spar Eskilsson & Bjorklund, 2000). For example, a small amount of water around 10% inside non-polar solvents such as hexane, xylene or toluene will improve the heating rate (Spar Eskilsson & Bjorklund, 2000).

Table 2.3: Dielectric constant for few solvents

Source: Bracht et al., (2002)

Solvents	**Dielectric constant (ε)**
Acetone	20.7
Acetonitrile	37.5
Ethanol	24.3
Hexane	1.89

Methanol	32.6
2-Propanol	19.9
Water	78.3

- Operating Conditions

During the extraction, it is important to make sure that the volume of solvent is sufficient to immerse the entire solid matrix. Generally, a higher ratio of solvent volume to solid matrix mass in conventional extraction techniques such as Soxhlet Extraction can increase the yield. However, in the MAE system, a higher ratio may be give lower recoveries. This is because of the inadequate stirring of the solvent by microwaves (Spar Eskilsson, Bjorklund, Mathiasson, Karlsson, &Torstensoon, 1999).

Besides, temperature plays an important role in affecting the recovery yield. In general, increase the temperature will improve the extraction efficiencies. However, for the extraction of thermolabile compounds, high temperatures may cause the degradation of some chemical inside the extracts. In this case, power must be chosen wisely or set correctly in order to avoid excess temperatures which leading to possible solute degradation (Font, Hernanadez, Hogendoorn, Baumann, & Van Zoonen, 1998).

- Advantages and Disadvantages of Microwave-assisted Extraction

MAE system has been considered as a potential alternative to traditional solid-liquid extraction of metabolites from plants. It also has been used to extract nutraceuticals because of several reasons. The reasons are reduced extraction time, reduced solvent usage and improved extraction yield. Furthermore, this system is comparable to other modern extraction techniques such as super fluid extraction due to its process simplicity and lower cost. By considering economical and practical aspects, MAE system has a high potential as an extraction technique for nutraceuticals.

However, an additional filtration or centrifugation is necessary to remove the solid residue during MAE compared to super fluid extraction. The efficiency of

microwaves can be very poor when the target compound or the solvent are non-polar compounds and they are volatile.

- Potential Applications of Microwave-assisted Extraction

MAE system is usually used to extract the organic compounds from Kingman, 2004; Barriada-Pereira et al., 2003; Ganzler, Salgo, &Valko, 1986; Lorenzo, Vazquez, Carro, &Cela, 1999; Spar Esklisson & Bjorklund, 2000; Tomaniova et al., 1998). However, there are few applications have been published in the nutraceutical area. Kaufmann and Christen, (2002) have publish an overview on MAE of natural products. It reported that MAE extracts nutraceutical products from plant sources in a faster manner then conventional methods. Guo et al., (2001) showed that MAE of the puerarin from the herb *Radix puerarie*could be completed within 1 min. Besides, Kwon, Belanger, Jocelyn Pare, &Yaylayan, (2003) reported that MAE could decrease the extraction time of *ginseng* saponins dramatically from 12 h to few seconds. In addition, Brachet et al., (2002) reported that the extraction of cocaine from leave with the assistance of microwave energy took only 30s, but conventional solid-liquid extraction took several hours to have same quantity of extract. For extracting the tanshinones from *Salvia miltiorrhizabunge*, MAE only needed 2 min. However, extraction at room temperature, Soxhlet extraction, ultrasonic extraction and heat reflux extraction needed 24h, 90min, 75 min and 45 min respectively to extract same amount and quality of tanshinones (Pan, Niu, & Liu, 2002). Williams, Raghavan, Orsat, and Dai, (2004) reported that MAE used to recover the 95% of the total capsaicinoid fraction from *capsicum* fruit in 15 min. This method is more efficient than the shaken flask methods and the reflux which are 24h and 2h respectively.

Besides, higher extraction yield can be achieved by using MAE system. For example, Hao, Han, Huang, Xue, & Deng, (2002) found that MAE could recover 92.1% of artemisinin from *Artemisia annual* L. within 12 min but Soxhlet extraction used several hours only have 60% recovery. Microwave-assisted extraction of glycyrrhizic acid from *licorice* root using solvent ethanol-water in 4-5min has a higher yield than solvent (ethanol-water) extraction at room temperature for 24h (Pan, Liu, Jia&Shu, 2000). Pan, Niu, & Liu, (2003) found that MAE achieved a higher yield within 4 min in

extracting polyphenols and caffeine from green tea leaves than solvent extraction at room temperature within 20h, ultrasonic extraction for 90min and heat reflux extraction for 45 min respectively. Furthermore, Shu, Ko, and Chang, (2003) also reported that MAE of ginsenosides from *ginseng* root only need 15min to extract a higher yield than conventional solvent extraction for 10h.

This extraction method can reduce solvent comsuption. Focused MAE was applied to the extraction of withanolides from air-dried leaves of *lochromagesnerioides* (Kaufmann et al., 2001a).By comparing the MAE and Soxhlet extraction, MAE has drastic reduction in organic solvent consumption (5 vs 100ml) and extraction time (40s vs 6h) (Wang, L. J. & Weller, C. L., 2006). It was found that the presence of water in the solvent of methanol will allow the faster extractions than with organic solvent alone.

2.7 2-ACETYL-1-PYRROLINE (2AP)

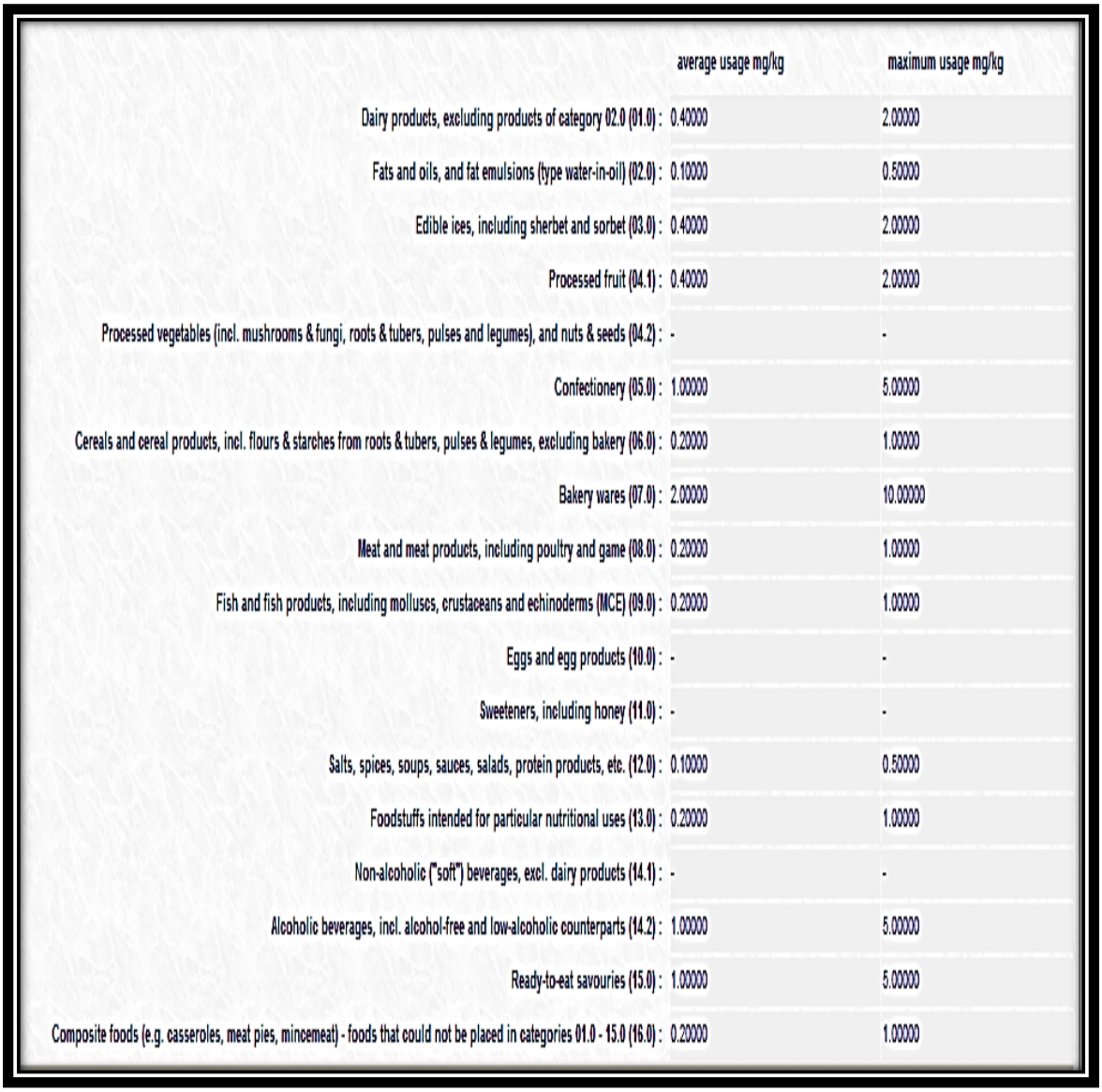
2.7.1 Introduction

In general, pandan leaves contains essential oils, carotenoids, tocopherols and tocotrienols (lee, Su, &Ong, 2004), quercetin (Miean& Mohamed, 2001), alkaloids (Busque, March, Fugueredo, Font, & Sanfeliu, 2002), fatty acids and esters (Zainuddin, 2004) and non-specific lipid transfer proteins (Ooi, Wong, Sun, &Ooi, 2006). The essential oil derived from pandan leaves contains many volatile compounds such as 2-hexanone, ethyl formate, 3-hexanol, 4-methylpentanol, 3-hexanone, trans-2-heptenal, β-damascenone, 4-ethylguaiacol and 3-methyl-2-(5H)-furanone. The major compound contributing to the aromatic fragrance of the pandan leaves is the 2-acetyl-1-pyrroline (2AP). It is a substituted pyrroline, a cyclic imine and a ketone. Its systematic name is 1-(4,5-dihydro-3H-pyrrol-2-yl)ethanone. It has high boiling point which is around 180 ^{o}C at 760mm Hg and molecular weight, 111.14176 g/g-mol. 2AP has higher solubility in polar solvent such as water than in organic solvents such hexane and the value for log P (o/w)= -1.27. Besides, the odor of 2-acetyl-1-pyrroline is similar to popcorn which is very special flavour. Its appearance normally varies from colourless to yellow solid which depending on the concentration.

A five-membered N-heterocyclic ring compound, 2-acetyl-1-pyrroline (2AP), was identified for first time as the important aroma component of cooked rice and the volatile oil of freeze-dried pandan leaves (Wongpornchai et al., 2003). For futher studies, Laohakunjit., N. and Kerdchoechuen., O. (2006) reported that this compound can be found in rice endosperm of rice varieties such as basmati and jasmine rice as a flavouring agent. Moreover, Buttery et al., (1983) had run an experiment about the concentration of 2-acetyl-1-pyrroline inside the steam volatile oil of 10 different varieties of rice. They found that the amount of 2AP present in the cooked rice varied from less than 0.006 ppm for Calrose rice to 0.09 ppm for MalagkitSunsong variety rise based on dry weight of the rice. Buttery, et al., (1988) also reported that 2AP is a hydrophilic compound which has an odor threshold value as low as 0.1 ppb in water. Therefore, this compound has gained the increasing interest among food aroma researchers. In addition, the quantities present in the pandan leaves is more than 10 times of the those found in scented milled rice and 100 times of those found in common rice after further study on this compound (Yahya, et al., 2010). Wongpornchai et al., (2003) discovered that 2AP occurs naturally in fresh *Vallarisglabra* Ktze (bread flower) leaves with a concentration of 0.53 ppm, but pandan leaves is noted as the main source 2AP by Yahya et al. (2010) with the highest concentration of 1 ppm 2AP. Based on previous studies, they discovered the lower epidermal papillae of the pandan leaves are the site of storage of 2AP by using histochemical analysis (Nadaf, Krishnan, &Wakte, 2006; Wakte, Nadaf, Krishnan, &Thengane, 2007).

After that, many experiments had run to determine the best method to extract the essential oils from pandan leaves with highest yield of 2AP. Based on the study of Bhattacharjee et al., (2003), continuous steam-distillation-extraction of freeze-dried fresh leaves of *Pandanus* yields 1 ppm of 2AP inside the steam volatile oil. Laohakunjit et al., (2004) reported that 2.77pm of 2AP has been extracted from ground fresh pandan leaves using ethanol solvent extraction. The latest method is the supercritical CO_2 extraction at 20 MPaoressure 50 ^{o}C temperature and 20 min contract yielded 0.72ppm of 2AP (Laohakunjit and Noomhorm, 2004). Bhattacharjee et al., (2005) also reported the highest yield of 2AP which is 7.16 ppm by using the supercritical extraction at 45 MPa, 60 ^{o}C and 3 h extraction time.

Apart of that, Li, J., and Ho. S. H. had run a study about the repellent activity of seven compounds and frections prepared from pandan leaves against *Blattellagermanica*(L.) using a modification of the linear tract olfactometer. In their study, pure 2-acetyl-1-pyrroline has the highest repellency (93%) among the seven compounds. In contrast, undiluted crude aqueous pandan extract displayed an attractancy of 62%. Based on the result, they concluded that the repellent activity towards cockroaches increase when concentration of 2AP in extract increase, while attractancy will decrease when the concentration of 2AP increase. They also reported that *Pandanus amaryllifolius* has the potential to be a natural and environmentally friendly pest management tool. In general, 2AP also used to flavour the meat and vegetable products or dishes or sauces (blended with other flavouring agents) such as meatloaf, gravies, soups, stews, and ketchup. It can be mixed with other known flavouring ingredients such as monosodium glutamate, onion powder, garlic powder, black pepper, and paprika. Furthermore, 2AP is used as a form a flavour enhancing composition in dried herbs such as parsley, oregano, celery, and sage. The table below shows the average of reported usages and maximum use of 2AP in industries the food categories according to Commission Regulation EC No. 1565/2000 (EC, 2000) in FGE.06 (EFSA, 2002a). The "maximum use" is defined as the 95th percentile of reported usages (EFSA, 2002i).



	average usage mg/kg	maximum usage mg/kg
Dairy products, excluding products of category 02.0 (01.0) :	0.40000	2.00000
Fats and oils, and fat emulsions (type water-in-oil) (02.0) :	0.10000	0.50000
Edible ices, including sherbet and sorbet (03.0) :	0.40000	2.00000
Processed fruit (04.1) :	0.40000	2.00000
Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds (04.2) :	-	-
Confectionery (05.0) :	1.00000	5.00000
Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery (06.0) :	0.20000	1.00000
Bakery wares (07.0) :	2.00000	10.00000
Meat and meat products, including poultry and game (08.0) :	0.20000	1.00000
Fish and fish products, including molluscs, crustaceans and echinoderms (MCE) (09.0) :	0.20000	1.00000
Eggs and egg products (10.0) :	-	-
Sweeteners, including honey (11.0) :	-	-
Salts, spices, soups, sauces, salads, protein products, etc. (12.0) :	0.10000	0.50000
Foodstuffs intended for particular nutritional uses (13.0) :	0.20000	1.00000
Non-alcoholic ("soft") beverages, excl. dairy products (14.1) :	-	-
Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts (14.2) :	1.00000	5.00000
Ready-to-eat savouries (15.0) :	1.00000	5.00000
Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0 (16.0) :	0.20000	1.00000

Figure 2.1: Average Use and Maximum Use of 2AP in Industries.

Source: Good Scent Comapny, (1980-2011)

2.7.2 Gas Chromatography Analysis of 2-acetyl-1-pyrroline (2AP)

Based the journal of Buttery, et al., (1986), collidine (2,4,6-trimethylpydrine) was a suitable internal reference standard with properties related to 2AP. He stated that it was a stable compound and reasonably close to 2AP in GC. However, the retention time for collidine was found at about 7 min under GC analysis conditions in the journal

of Laksanalamai, V., and Ilangantileke, S., (1992). The result is showed in the Figure 2.2. Apart of that, Buttery et al., (1983) compared the volatile components extracted from pandan leaves and authentic AP in Figure 2.3. They found out that the mass spectrum of the peak at 5.47 for pandan leaves while the peak at 5.49 min attained by authentic AP. Both mass spectra were observed to be almost identical.

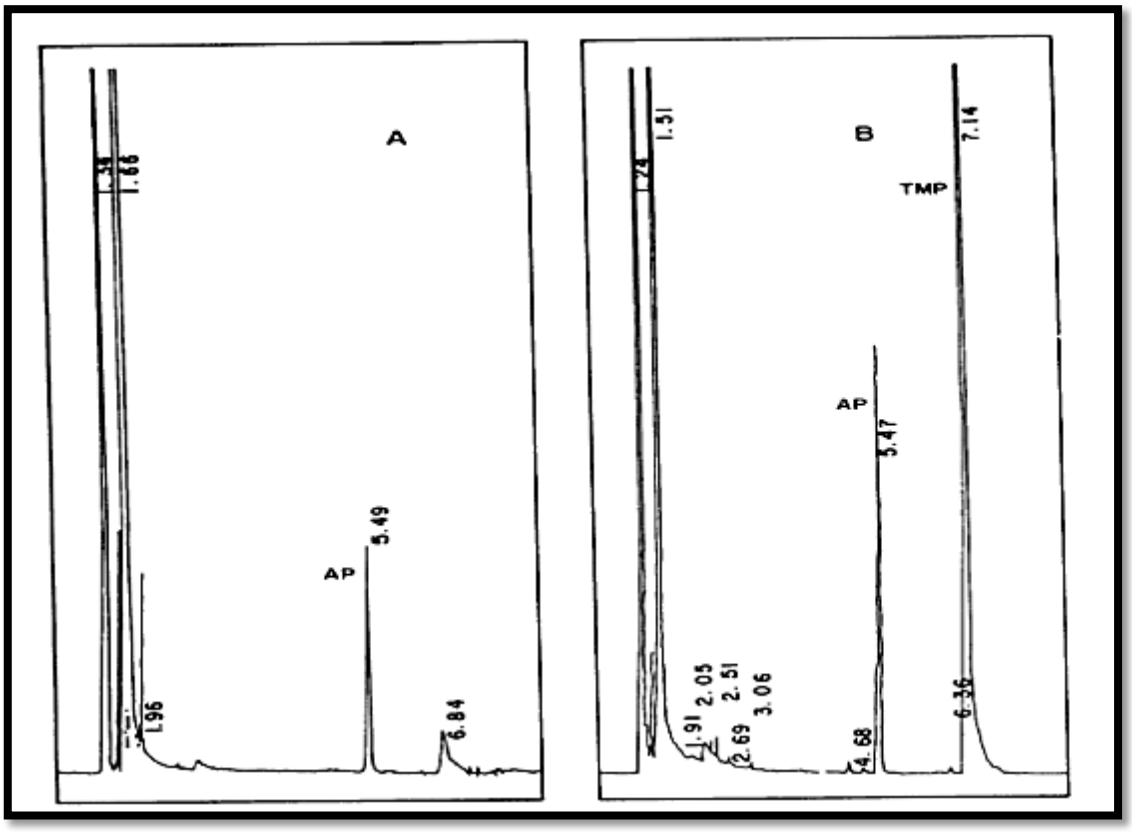


Figure 2.2: Gas Chromatograms of The Authentic 2-acetyl-1-pyrroline (A) and Volatile Oils Extracted from Pandan Leaves (B) AP=2-acetyl-1-pyrroline, TMP= collidine (2,4,6-trimethylpydrine).

Source: Laksanalamai, V., and Ilangantileke, S., (1992).

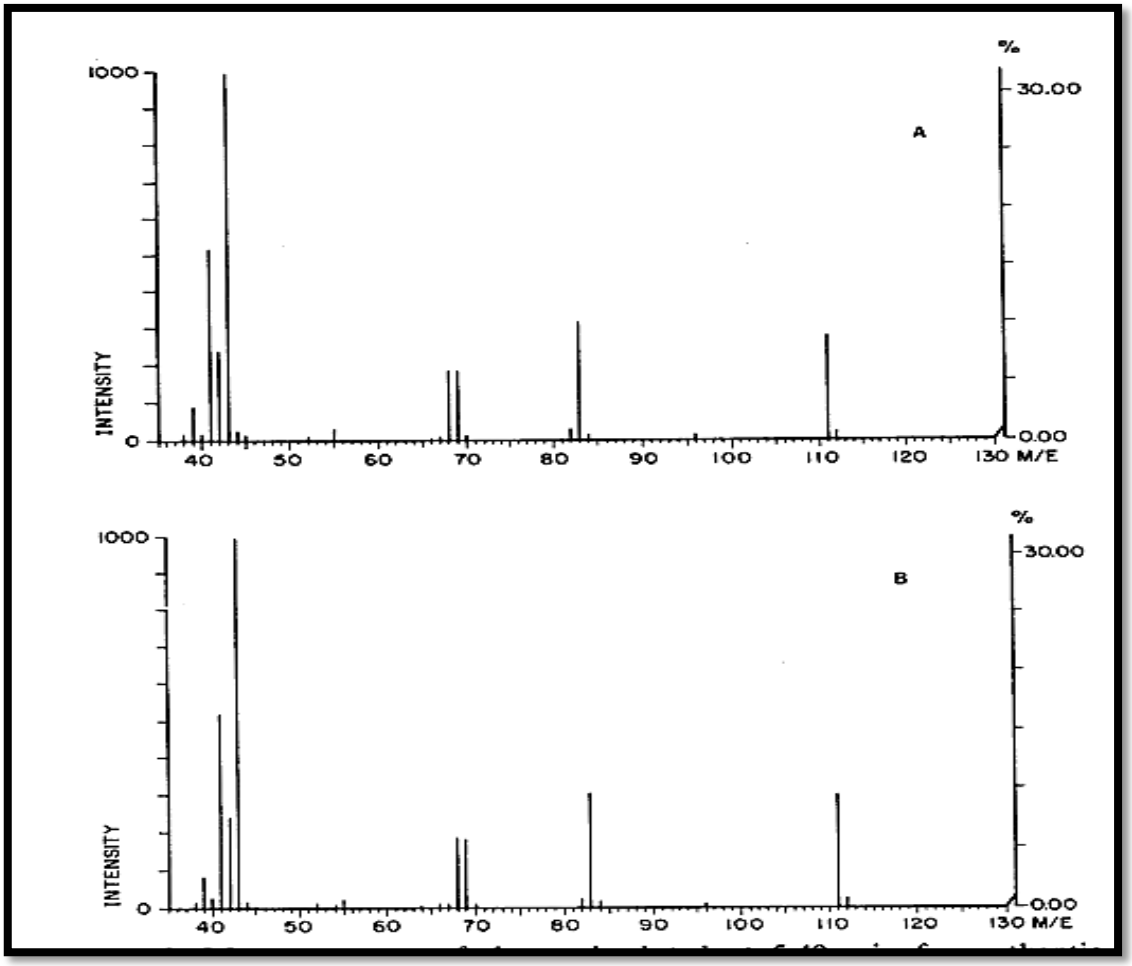


Figure 2.3: Mass Spectrum of The Peak Eluted at 5.49 min for Authentic 2-acetyl-1-pyrroline (A) Compared with That of The Peak Eluted at 5.47 min for Basic Fraction from Pandan Leaves (B).

Source: Buttery et al., (1983)

Although retention time of collidine in GC varies due to different conditions, it still is a suitable internal standard for identification of 2AP. By comparing the standard and the samples, the concentration of 2AP in rice can be estimated. Buttery, et al., (1986) use the follow below to estimate the concentration of 2AP:

$$\text{AP concentration} = \frac{\text{area of AP peak}}{\text{area of collidine peak}} \times \text{collidine concentration} \times \text{RRF}$$

Where RRF is percent recoveries of collidine and AP from the rice extract. For example, RRF was determined by adding a known quantity of the standard 2AP and collidine to

6L of water in the 12L flask and performing the extract to get the amount the recoveries of collidine and 2AP. Then, calculate the percentage of recoveries for RRF.

2.7.3 Factors Affect the Yield of 2-acetyl-1-pyrroline

For your information, slight change is sample temperature, heating time or variation of moisture can affect the amount of the extracted samples. Grimm, et al., (2001) stated that the extract for Solid Phase Micro Extraction (SPME) must reach a temperature sufficient to liberate bound volatile compounds or to thermally generate the compounds. They run experiment for the samples of rice flour in triplicate from 60 to 85 ^{o}C at intervals of 5 ^{o}C. The amount of 2AP recovered from the headspace of rice doubled as the temperature of the sample was increased from 60 to 85 ^{o}C. Apart of that, the internal standard, TMP decreased with increasing temperature. From the Figure 2.4, the recoveries of 2AP at 80 and 85 ^{o}C were not significantly different, so they choose 80 ^{o}C as the optimum temperature. They also explained that adsorption temperatures larger 85 ^{o}C resulted in the sporadic deformation and rupture of the seal of the sampling vials.

Furthermore, they did the experiment about the adsorption period of SPME. After comparing the yield of 2AP for 10, 15, 20 and 30 minutes, 15 minutes of adsorption time is chosen as optimum time because it has the highest yield. Further exposure time of SPME fibre to the headspace resulted in no significant increase in recovery of 2AP. In addition, they investigated the incubation period which is to preheat the sample prior to adsorption. They ran samples in triplicate for incubation period from 0 to 35 min at 5 min intervals. They found out that the recovery of 2AP is increasing up to 40 min when increase the incubation period (Figure 2.5). In order to minimize the time and cost spent, they chose 25 min as optimum incubation period.

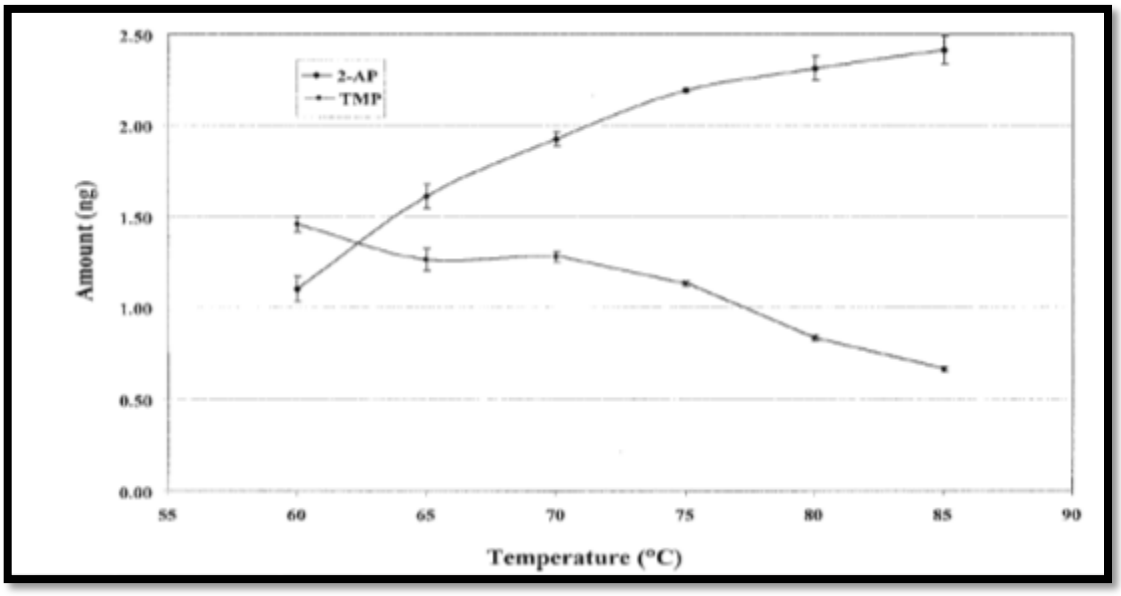


Figure 2.4: Amount of 2AP Recovered from Jasmine Rice Flour at Sampling Temperature Ranging from 60 to 85 °C. TMP is Employed as The Internal Standard.

Source: Grimm, et al., (2001)

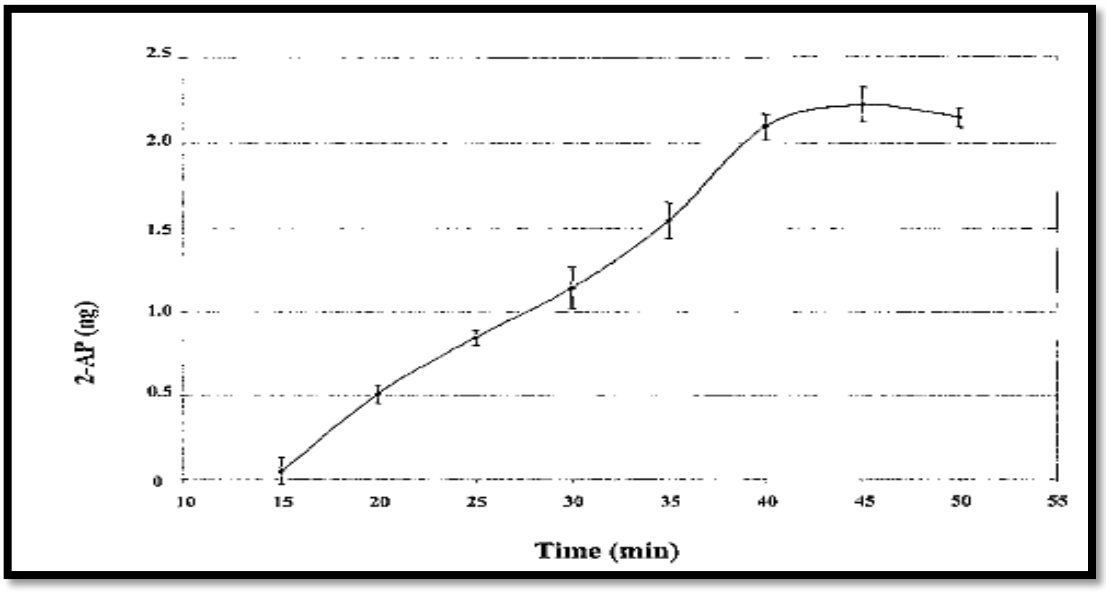
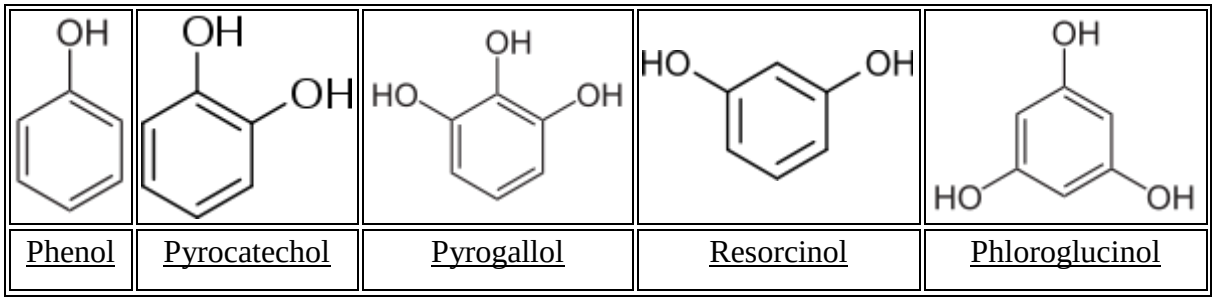


Figure 2.5: Determination of Optimum Heating Time for the Recovery of 2AP from the Headspace of Jasmine Rice.

Source: Grimm, et al., (2001)

2.8 POLYPHENOLS

Polyphenols is a very important chemical compound inside the essential oil derived from pandan leaves. They are a class of natural or synthetic or semisynthetic organic chemicals which have characterized by the presence of large multiples of phenol. The unique physical, chemical, and biologic properties of the polyphenols are dependent on the number and characteristics of the phenol. Polyphenols have the hydrophilic phenol and the hydrophobic hydrocarbons, so they are soluble moderately in the polar compounds. Besides, these polyphenols have the molecular weight from 500-4000Da and at least 13 phenolic hydroxyl groups inside. In the polyphenols, there is a limitation which 5 to 7 aromatic rings for 1000 Da molecular weight. Tannis is a subset of the polyphonels which is historically important chemical class. This is because it highlights the high density of phenolic substructures and proved polyphenols are original from plant substances (phytochemicals). In general, polyphenol compositions are normally limited to atoms carbon, hydrogen and oxygen in undefined proportion. For examples, black tea antioxidant theaflavin-3-gallate and the hydrolyzable tannin are subset of polyphenols.



Phenol	Pyrocatechol	Pyrogallol	Resorcinol	Phloroglucinol

Figure 2.6: The aflavingallate image of polyphenols

Source: Mohammad, S., Bin I., (2008)

Sometimes, the pandan leaves are put into frying oils to impart flavour to fried food. However, many people do not know that pandan leaves will help to extend the usage life of frying oil. This is because polyphenols inside the leaves will impart another special characteristic which antioxidative properties to the frying oil during cooking. The phenolic compounds in hearbs or plants are suitable to act as antioxidants

due to their redox properties. These properties also cause them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators (Fatihanim et al., 2007). Several herbs reportedly retard lipid oxidation during frying (Che Man &Jaswir, 2000; Naz, Siddiqi, Sheikh, &Sayeed, 2005), in heated oil (Khan &Shahidi, 2001; Nogala-Kalucka et al., 2005; Shyamala, Gupta, Lakshmi, &Prakash, 2005) and in food (Jaswir, CheMan, & Kitts, 2000). In addition, these natural antioxidative substances of edible herbs and plants are believed to be safer and may provide the health benefits when compare to synthetic antioxidants (Fatihanim et al., 2007). Therefore, many researchers start to investigate this area due to current consumer concerns about health.

One of the studies about these polyphenols conducted by Fatihanim et al., (2007) is the antioxidative properties of *Pandanus amaryllifolius* leaf extracts in accelerated oxidation and deep frying. The potential uses of pandan leaves extract as a natural antioxidant were evaluated in refined, bleached and deodorized (RBD) palm olein. They used the accelerated oxidation and deep frying studies at 180 ^{o}C from 0 to 40h. Based on their results, the extracts had retard significantly oil oxidation and deterioration ($P<0.05$) and comparably to 0.02% BHT in tests such as peroxide value, anisidine value and iodine value. Apart of that, they found that the oiliness, crispiness taste and overall acceptability of French fries by using the oil which is mixed with pandan leaves are not much different with oil with synthesis antioxidants. This is because pandan leaves have polyphenol with an excellent heat-stable antioxidant properties. They concluded that the pandan leaves can be a good natural alternative to existing synthetic antioxidants in the food industry.

2.9 CONCLUSION

From the previous studies, pandan leaves extract has the high potentials to be the pest management tools, natural antioxidants, natural flavouring agent and natural air-freshener with special aromatic fragrance. Since it provides many health benefits, many researchers are encourage to develop an economical, harmless, fast and high yield extraction method for the pandan leaves. Therefore, the essential oil of pandan leaves can be extracted easily in the industrial sections. Besides, I hope that there are more researches are conducted to determine the components inside pandan leaves. In future,

these natural products can replace the synthesis products as to reduce the health problems among humans.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter describes the required materials, equipment and methods to be followed for conducting the experimental work of this research. To accomplish the objectives of this research, the study is carried out in three stages. The first stage is the extraction of essential oil from pandan leaves and consists of cooling, evaporation of solvent and storage of essential oil. Subsequently, the second stage is the analysis of essential oil for the identification of 2-acetyl-1-pyrrodine (2AP), others compounds and for the measurement of 2AP concentration. The third stage is the viability study of the essential oil application as air freshener. In addition, the procedures for sample preparation and equipment set up to be conducted beforehand are also given.

3.2 MATERIAL

3.2.1 Raw Material

I. Fresh pandan leaves
II. Ethanol
III. 2,4,6-trimethylpyridine (TMP)
IV. Acetone

V. Methanol
VI. Water
VII. Gallic acid

3.2.2 Equipment

I. Fourier Transform Infrared Spectroscopy (FTIR)
II. Gas Chromatography-Flame Ionization Detector (GC-FID)
III. Ultraviolet-visible Spectroscopy (UV-Vis)
IV. Stopwatch
V. Microwave Extractor
VI. Rotary Evaporator
VII. Refrigerator
VIII. Micropipette
IX. Syringe filter
X. 100ml Beakers

3.3 METHOD OF RESEARCH

3.3.1 Sample Preparation

The fresh and mature pandan leaves were used as the sample. The pandan leaves were cut by using a pair of sharp scissors into small pieces whose sizes are around 5mm. Next, the cut pandan leaves were compressed in a 1L beaker. Then, the leaves were soaked in ethanol solvent for 5 min at room temperature to carry out pre-leaching before the extraction process is started (Munirah, 2008). Soaking the sample in solvent prior to extraction will increase the yield of extraction. Finally, the solution was filtered with filter paper to remove the pre-soaking ethanol solvent.

Figure 3.1: The Fresh and Mature Pandan Leaves

Figure 3.2: Soaked Cut Pandan Leaves in 2L Beaker

3.3.2 Microwave assisted-solvent extraction

In this experiment, the microwave extractor in the laboratory of University Malaysia Pahang was used to extract essential oil from pandan leaves. The brand of the equipment is Milestone. The extraction was performed in the closed vessel unit Ethos E. This unit is equipped with temperature sensor, vessel, rotor and automatic gas sensor. The maximum oven power for this system is 1000W. Following the procedures of Yahya et al., (2010), 50g of sample was accurately weighed and soaked in 50 ml of ethanol solvent according to the ratio of sample to solvent of 1:1. Next, this sample was placed in a sealed 2L vessel and left in microwave extractor to extract for 40 min at 80 ^{o}C. The power of microwave extractor was set at 500W. After 40 min, the extract is allowed to cool for 10 minutes before removal from the distillation collector. Then, there formed two layers of liquid in the collector of microwave due to the immiscibility of deionized water and ethanol. The bottom layer, which consists of water, was taken out. After that, the upper layer (extract) was collected in vials. Next, solvent was evaporated from the extract using rotary evaporator under reduced pressure and 78 ^{o}C bath temperature for up to 1h. Finally, the extract was stored in a refrigerator at 4 ^{o}C for further analysis. This extraction was repeated at different extraction temperatures which are 70, 90, 100 and 110 ^{o}C and different extraction periods which are 30 minutes, 50 minutes, 60 minutes and 70 minutes.

Figure 3.3: Sample in Microwave Extraction

Figure 3.4: Graph of Extraction Process in Computer

Figure 3.5: Rotary Evaporator

Figure 3.6: Essential Oil in Vials

3.3.3 Total yield of Pandan leaves extract

After collecting the extract, the yield was determined gravimetrically and measured with a measuring cylinder. From the study of Yahya et al., (2010), the extraction yield is expressed as the percentage ratio of the mass of extracted material to the mass of grinded Pandan leaves loaded in the closed vessel as follows:

$$Extractionyield(\%) = \frac{massofextractedmaterial\ (g)}{massofcutleaf\ (g)} \times 100$$

3.3.4 Gas Chromatography-Flame Ionization Detector (GC-FID)

Firstly, 0.1 ml of 2,4,6-trimethylpyridine (TMP) was taken using a micropipette. Then, the sample was mixed with 9.9 ml HPLC analytical grade acetone solvent with purity of 99.9%. Next, the solution was filtered using a 0.2 µm syringe filter. After that, it was transferred into GC vials. The aforementioned TMP is used as an internal standard. Then, quantitative analysis of extract was performed on a gas chromatography (Agilent Technologies 6890N USA) equipped with a flame ionization detector (FID) capillary column with dimension of 25.0m × 450µm (inner diameter) × 1.20µm film thickness. A 3 µl aliquot of extract containing TMP was injected into the GC-FID by using an auto sampler injection with a split ratio of 1:80. Oven temperature was set initially at 50 ^{o}C for 2 min and then increased gradually at a rate of 15 ^{o}C/min to 200 ^{o}C. Besides, the injector and detector temperatures were set at 170 and 250 ^{o}C respectively while running the GC-FID system. Carrier gas Helium was set at a flow rate of 1.3m/min. Then, the steps 1 and 2 were repeated by changing the volume of TMP and solvent which are 0.2 ml and 9.8 ml, 0.3 ml and 9.7 ml, 0.4 ml and 9.6 ml, 0.5 ml and 9.5 ml respectively. The purpose is to get the standard curve for TMP. The steps were repeated by substituting TMP with samples resulted from extraction at different temperatures and extraction periods. Finally, the concentration of 2AP was determined by comparing the peaks area in the graph.

Figure 3.7: Dilution Volumetric Flask

Figure 3.8: Gas Chromatography-Flame Ionization Detector

3.3.5 Fourier Transform Infrared Spectroscopy (FTIR)

The identification of other components inside the essential oil was carried out by using the Fourier Transform Infrared (FTIR) that can found in the lab. Although Gas Chromatography-Mass Spectrometry (GC-MS) is a more suitable for analysis, the essential oil of pandan leaves cannot be detected in this equipment due to the existence of polar chemical compounds. The essential oil was placed at the measuring platform (also known as smart performer). The spectra of oil-in-water emulsion were studied under conditions specially applied for quantitative work by using thin liquid film technique in the region from 4000 to 200 cm^{-1}. FTIR measured the change in the absorption bands and peak area of the different functional group. By comparing the absorption band of major peaks in analysis with data in the University Malaysia Pahang database, the components inside essential was identified.

Figure 3.9: Fourier Transform Infrared Spectroscopy (FTIR) and Computer Analyzer

3.3.6 Sensory Evaluation

Sensory evaluation was conducted to determine the aroma quality of essential oil extracted. It also evaluated the potential of essential oil to be natural air freshener based on the persistence of aroma. The evaluation is done by random probability. Based on the Sensory Evaluation Manual by Associate Professor Richard Mason from the

University of Queensland, the sensory evaluation criteria are determined. These criteria can be divided into four parts which are correctness, intensity, persistence and overall acceptability. After the important criteria to evaluate the essential oil are determined, the sensory evaluation form was constructed. This sensory evaluation was conducted at 6 rooms of Kolej Kediaman 4 of University Malaysia Pahang. 30 people in these 6 rooms are chosen randomly to ensure the accuracy of sensory evaluation. Firstly, 25ml essential oil was poured into a 100ml beaker. Then, it was sealed by the parafilm. Next, the 5mm diameter of rod was used to make 8 holes on the parafilm. Those steps were repeated for 4 same samples and 5 control solutions. Each room had two beakers: A is the essential oil beaker and B is control solution. They evaluated the essential oil for one week. In order to minimize the disturbance factors from environment, the size of rooms, number of people and location are same. Besides, they face to same direction to the Sun and near to each other's. There was a control solution, water to determine the evaporation rate of each room. Therefore, can determine the causes if the evaluation are not accurate.

Figure 3.10: Essential Oil and Control Solution

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 INTRODUCTION

This chapter presents all the results obtained which can be divided into three parts. First part is to analyze the results get from extraction process and GC-FID as described in the methodology of Chapter 3. Then, choose the optimum extraction period and temperature based on the screening process. The results are volume, yield of the essential oil and concentration of 2AP. Second part is to identify the components inside the essential oil which is extracted in the optimum condition. Third part is to analyze the potential of essential oil pandan leaves to be the natural air freshener by using sensory evaluation results. The evaluation is based on criteria: correctness, persistence, intensity, smell and over acceptance. The raw data for all tests are shown in appendix.

4.2 VOLUME OF ESSENTIAL BASED ON DIFFERENT EXTRACTION PERIOD AND TEMPERATURE

Table 4.1: Volume Readings of Essential oil.

sample	trial 1	trial 2	Volume
1	9.2	3.5	6.35
2	11	10.75	10.875
3	13.5	10.5	12
4	7.5	9.5	8.5
5	12.1	5	8.55
6	9.5	10.5	10
7	4.9	4.975	4.9375
8	4	9	6.5
9	4.5	10.5	7.5
10	7	7.5	7.25

where sample 1 : essential oil pandan leaves is extracted at temperature 70 ^{o}C

sample 2 : essential oil pandan leaves is extracted at temperature 80 ^{o}C

sample 3 : essential oil pandan leaves is extracted at temperature 90 ^{o}C

sample 4 : essential oil pandan leaves is extracted at temperature 100 ^{o}C

sample 5 : essential oil pandan leaves is extracted at temperature 110 ^{o}C

sample 6 : essential oil pandan leaves is extracted for 30 minutes

sample 7 : essential oil pandan leaves is extracted for 40 minutes

sample 8 : essential oil pandan leaves is extracted for 50 minutes

sample 9 : essential oil pandan leaves is extracted for 60 minutes

sample 10: essential oil pandan leaves is extracted for 70 minutes

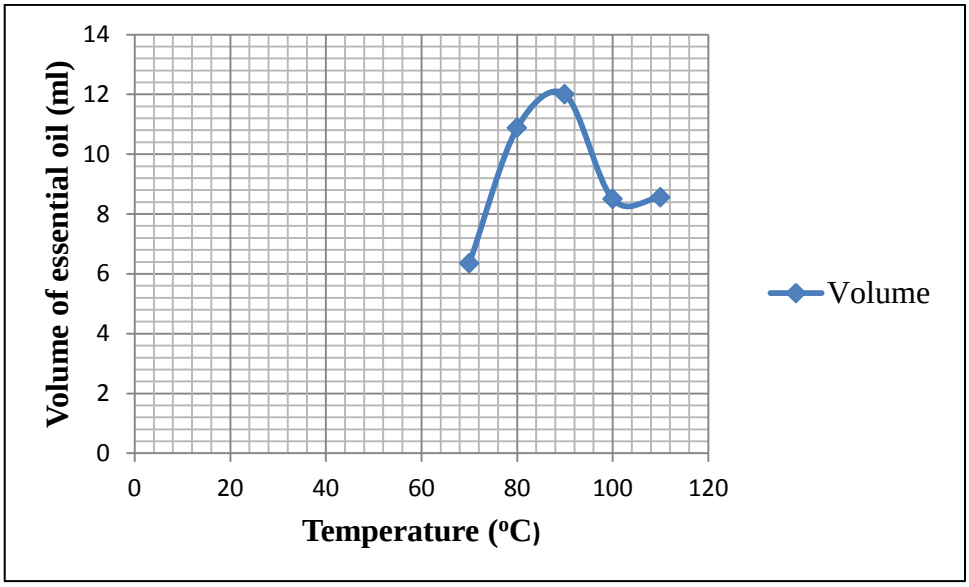


Figure 4.1: Volume of Essential oil versus Temperature

This experiment is to study the effect of varied extraction temperature on the volume of essential oil extracted. Others parameters are ensure to be constant along the experiment of different temperature. As I said in Chapter 3, the pressure of microwave extractor is 1 bar, power is 500W, mass of cut pandan leaves to be extracted is 50 g, soaked in ethanol solvent for 5 minutes prior extraction, ratio of solvent to pandan leaves is 1:1 and extraction period is 40 minutes. Based on the Table 4.1 and Figure 4.1, I found out that the volume of essential oil increases rapidly from 70 to 80 ^{o}C and then the increment of volume decreases till reach the maximum point of the graph which is 88^{o}C. This is because more heat is supplied to liberate the bound volatile compounds inside pandan leaves by having cell disruption when penetration of microwave into cell tissue of pandan leaves. This statement is supported by the study of Grimm, et al., (2001). They stated that the amount of 2AP recovered from the headspace of rice doubled as the temperature of the sample was increased from 60 to 85 ^{o}C. But, the recoveries of 2AP at 80 and 85 ^{o}C were not significantly different Next, the volume of essential oil decreases sharply till 101 ^{o}C. After that, the volume of essential oil is almost same from 101 to 110 ^{o}C. The volatile compounds which are terpenes mostly are destructed or decompose at high temperature in extraction process, so volume of essential oil is lesser. Although the readings in first trial and second trial are not in same

patterns, this maybe because the disturbance factor during experiment such as the size of cut leaves, the varied efficiency of microwave and temperature of chiller.

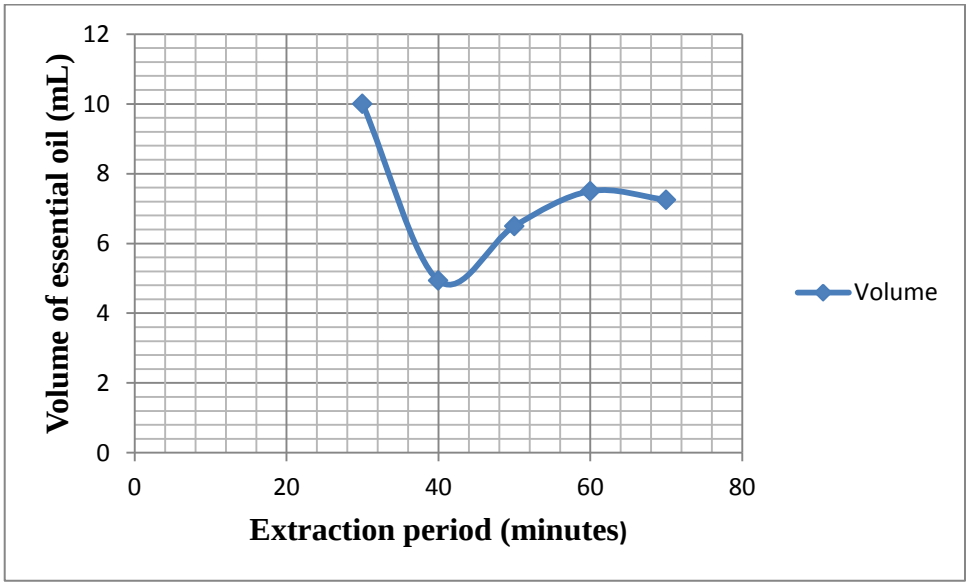


Figure 4.2: Volume of Essential Oil versus Extraction Period

This experiment is to study the effect of extraction period for pandan leaves on the volume of essential oil. Others parameters are ensure to be constant along the experiment of different extraction period. As I said in Chapter 3, the pressure of microwave extractor is 1 bar, power is 500W, mass of cut pandan leaves to be extracted is 50 g, soaked in ethanol solvent for 5 minutes prior extraction, ratio of solvent to pandan leaves is 1:1 and extraction temperature is 80 ^{o}C. Based on the Figure 4.2, extraction period extends from 30 to 41 minutes causes the volume of essential oil decreases. This is mainly because the small amount of pandan leaves needs less extraction period to complete the extraction. Before reach the suitable extraction period, the volume of essential oil should be increased because it has more time to for the process of cell disruption and more bound volatile compounds come out. However, the heat sensitive compounds maybe decompose or destructed when expose to high temperature for longer period. Then, the volume of essential oil increases from 41 to 60 minutes. Finally, it decreases again from 60 to 70 minutes. Theoretically, the volume of essential oil should do not have significant change along this extraction period. Therefore, there are some errors for my readings. The errors maybe caused by the

impurities dissolved inside the essential oil. The impurities come from the dirt on the apparatus of distillation and separate flask in microwave extraction. There are some students use the microwave extractor to extract the biodiesel and left the dirt. The dirt is hard to clean due to small diameter of apparatus.

4.3 YIELD OF ESSENTIAL BASED ON DIFFERENT EXTRACTION PERIOD AND TEMPERATURE

Table 4.2: Mass and Yield of Essential oil

sample	trial 1	trial 2	Mass (g)	Yield (%)
1	8.69	3.35	6.02	12.04
2	10.39	10.27	10.33	20.66
3	12.83	10.15	11.49	22.98
4	6.93	9.25	8.09	16.18
5	11.62	4.89	8.255	16.51
6	10.25	10.21	10.23	20.46
7	4.48	4.11	4.295	8.59
8	3.44	8.52	5.98	11.96
9	3.97	10.7	7.335	14.67
10	6.5	7.25	6.875	13.75

The yield is calculated based on the formula:

$$\text{Extraction yield (\%)} = \frac{\text{mass of extracted material (g)}}{\text{mass of cut leaf (g)}} \times 100$$

For sample 1:

$$\text{Extraction yield (\%)} = \frac{6.02\ \text{g}}{50\text{g}} \times 100\%$$

$$= 12.04\ \%$$

This calculation above is repeated to calculate for sample 2 to 10 to get the yield of essential oil.

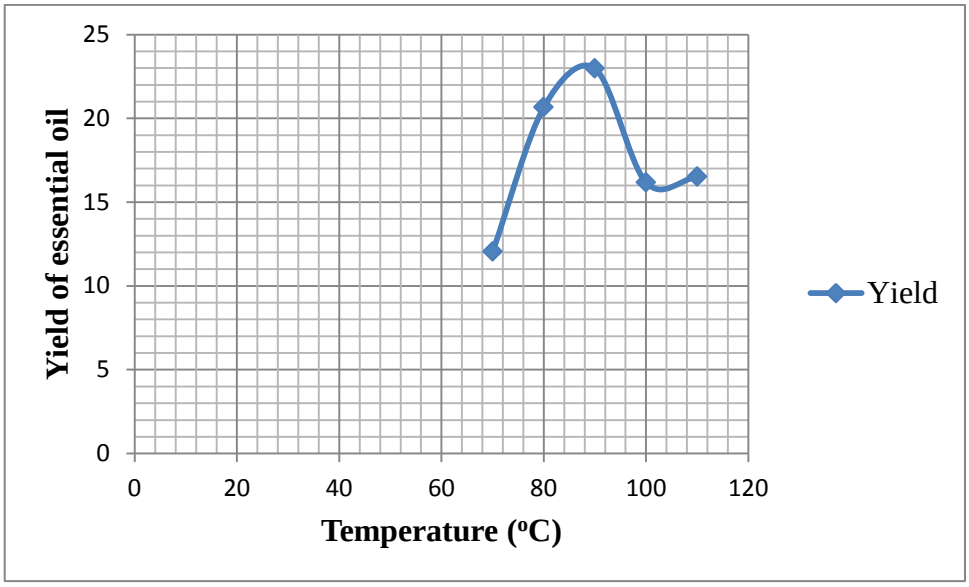


Figure 4.3: Yield of Essential Oil versus Temperature

This experiment is to study the effect of temperature on yield of essential oil. The others parameters are set to be constant along the experiment and stated in discussion for Figure 4.1. The pattern of Figure 4.3 is same with Figure 4.1. The yield of essential oil increases rapidly from 70 to 80 ^{o}C. Then, it increases slowly till reach the maximum point of the graph which is 88^{o}C. This is because more volatile compounds come out from the pandan leaves with more heat supplied during the vigorous cell disruption in higher temperature. This statement is supported by the study of Grimm, et al., (2001). The yield of essential oil decreases sharply till 101 ^{o}C and then almost constant from 101 to 110 ^{o}C. The volatile compounds which are terpenes mostly are destructed or decompose at high temperature in extraction process, so yield of essential oil is lesser. From Table 4.2, the value in trial 1 and 2 are different due to the disturbance stated in discussion for Figure 4.1.

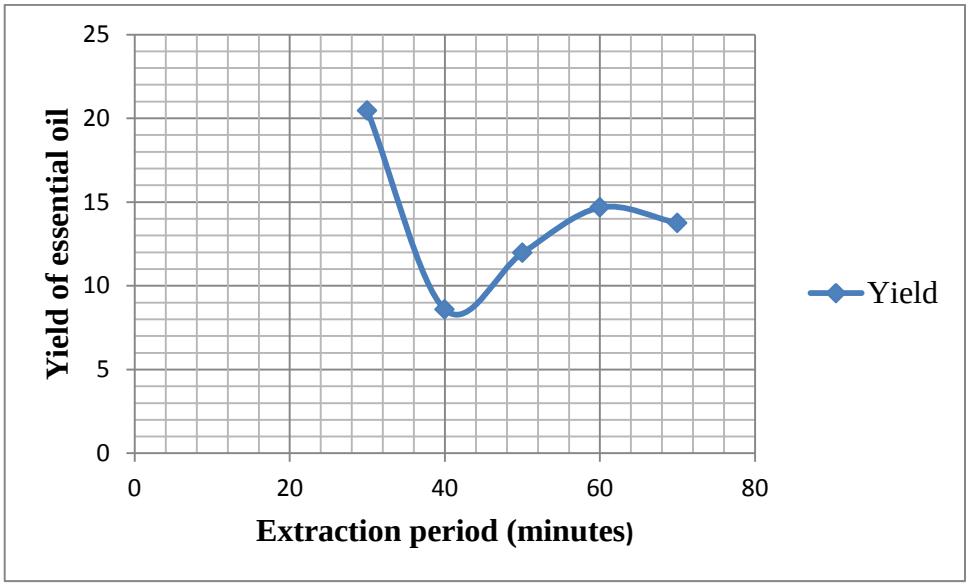


Figure 4.4: Yield of Essential Oil versus Extraction Period

This experiment is to study the effect of extraction period for pandan leaves on the yield of essential oil. The others parameters are constant along the experiment of different extraction period and stated in discussion for Figure 4.2. The pattern of Figure 4.4 is same with Figure 4.2. The yield of essential oil decreases with extraction period from 30 to 40 minutes. The change in yield is because the small amount of pandan leaves needs less extraction period to complete the extraction. Grimm, et al., (2001) stated that the recovery of 2AP is increasing up to 40 min when increase the incubation period in their journals. Therefore, the yield of essential oil increases before reach suitable extraction period and decrease for further extraction period. The explanation is stated in discussion for Figure 4.2. Then, the yield of essential oil increases from 41 to 60 minutes. Finally, it decreases again from 60 to 70 minutes. There are some errors too for the yield of essential oil because the yield should do not significant change. The explanation is same with discussion for Figure 4.2.

4.4 CONCENTRATION OF 2AP IN ESSENTIAL OIL DIFFERENT EXTRACTION PERIOD AND TEMPERATURE

Table 4.3: The Peak Area in Gas Chromatography for Internal Standard (TMP)

Concentration of Standard (µL/mL)	Peak Area (pA*S)
1	54049.4
2	55214.8
3	55549.3
4	57374.9
5	57388.5

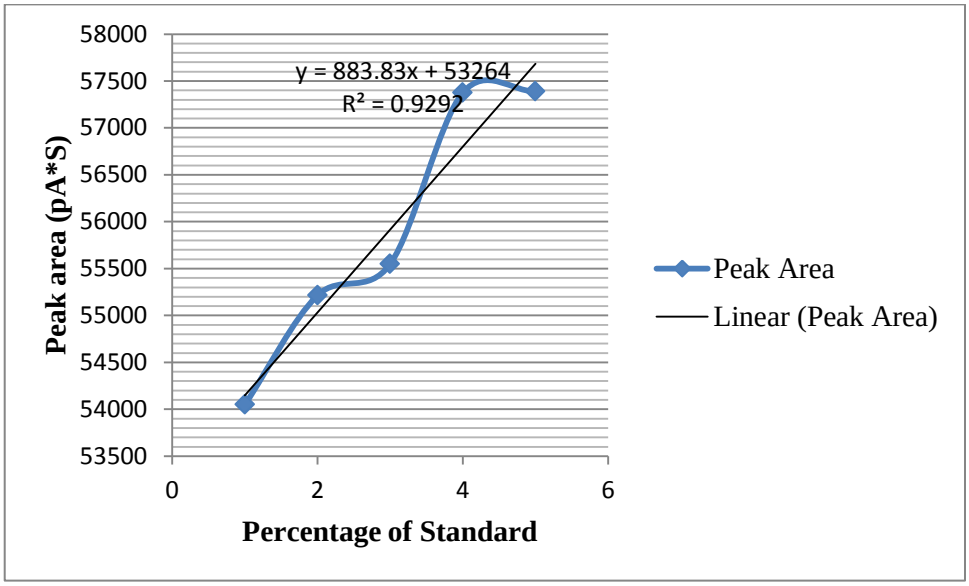


Figure 4.5: Peak Area versus Percentage of Standard

In order to get the concentration of 2AP in the sample, I did the five internal standards with different concentration for GC-FID analysis to get the standard linear. The concentration of standard is from 1 to 5 µL/mL. From the Figure 4.5, the peak area in GC-FID increases with the increment of concentration of standard. Although the values are not very accurate, it can form a good linear graph with equation:

Y= 883.8x +53264

The inaccuracy of readings maybe because amount of internal standard and ethanol solvent injected to GC vials are not very accurate during handling process of

syringe and its filter. There are some solution still left inside the syringe and causes the concentration is not exactly accurate.

Table 4.4: The Peak Area and Concentration for 2acetyl-1-pyrroline (2AP)

sample	Peak Area (pA*S)	Concentration of 2AP (µL/mL)
1	56877.8000	4.0889
2	56796.1000	3.9965
3	57223.3000	4.4799
4	57149.5000	4.3964
5	56794.8000	3.9950
6	56885.3000	4.0974
7	56284.7000	3.4179
8	55425.0000	2.4451
9	55773.8000	2.8398
10	55862.0000	2.9396

The concentration inside the solution is estimated by using the formula:

$$\text{Concentration } (\mu\text{L/mL}) = \frac{\text{Peak area} - 53264}{883.8}$$

The value is get from the linear equation of inter standard.

$$\text{Concentration } (\mu\text{L/mL}) = \frac{\text{Sample 1}}{\frac{56877.8 - 53264}{883.8}}$$

$$= 4.0889\ \mu\text{L/mL}$$

This calculation is repeated for sample 2 to 10 to estimate the concentration of 2AP in essential oil.

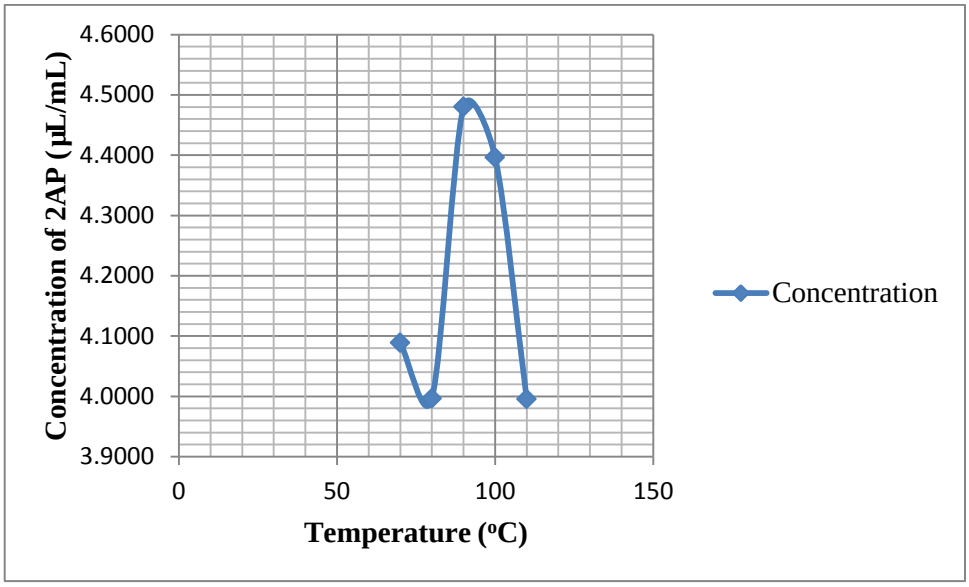


Figure 4.6: Concentration of 2AP versus Temperature

This experiment is to study the effect of extraction period on recovery concentration of 2AP in essential oil. From the Figure 4.6, the estimated concentration of 2AP decreases from 70 to 80 ^{o}C. Theoretically, the concentration of 2AP should increase with temperature before reach the optimum temperature. This statement can be proved by the result of volume and yield of essential oil in Section 4.2 and 4.3 respectively. Grimm, et al., (2001) stated the recoveries of 2AP from rice flour increased from 60 to 85 ^{o}C. Therefore, the second reading is not accurate maybe because system error in microwave extractor and temperature of chiller. Then, the concentration of 2AP increases sharply from 80 to 90 ^{o}C. This is because higher temperature in extraction process supplies more heat energy to break the chemical bound of 2AP inside the papillae, located on the lower epidermis cell of pandan leaves through the cell disruption process. In addition, high temperature causes the breakage and destruction of cell walls including papillae (Yahya, et al., 2010). The maximum concentration of 2AP in essential oil is at temperature around 90 ^{o}C under the specific condition mentioned before. After that, the concentration of 2AP also decreases sharply from 90 to 110 ^{o}C. 2AP maybe decomposed or destructed by too high temperature due to its heat sensitive properties.

By gathering the information for volume, yield of essential oil and concentration of 2AP in essential oil, I can concluded that the temperature range 88-90 ^{o}C is the optimum temperature to have highest quality and yield of essential oil. In the information of yield and concentration, temperature 88 ^{o}C has more yield of essential but a bit lower quality than 90 ^{o}C. There is bigger change in yield than concentration of 2AP across the temperature. In order to minimize the cost spent for extraction process and have maximum yield of high 2AP concentration essential oil pandan leaves, I chose the 88 ^{o}C as the optimum temperature for my experiment.

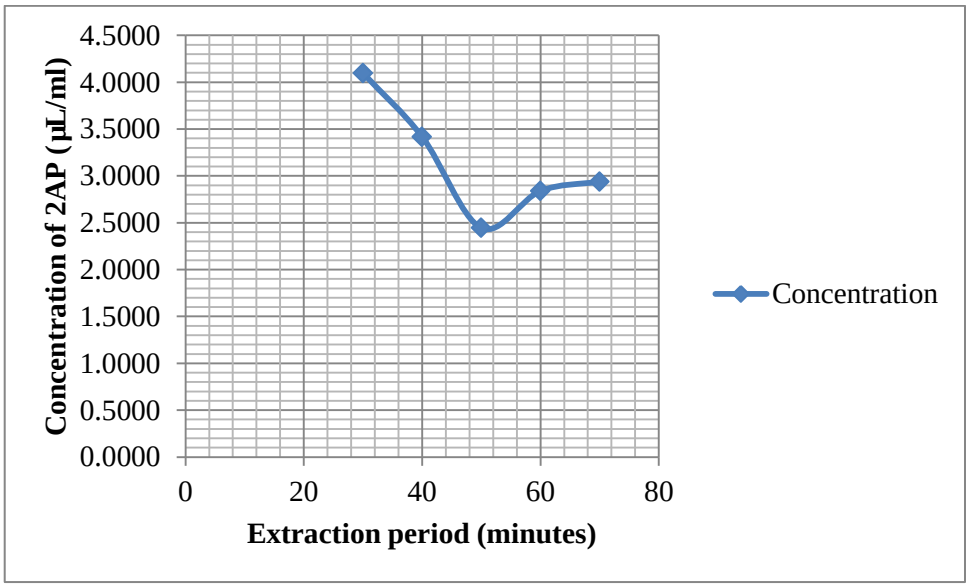


Figure 4.7: Concentration of 2AP versus extraction period

This experiment is to study the effect of extraction period on concentration of 2AP in essential oil. From Figure 4.7, the concentration of 2AP in essential oil decreases with extraction period from 30 to 50 minutes. Further exposure of 2AP for high temperature causes the decomposition possibility for heat sensitive 2AP increases. Therefore, amount of 2AP in essential oil decreases. Apart of that, the concentration of 2AP for 30 minutes extraction period is highest because small amount of pandan laves need shorter extraction period only for extraction process in high temperature condition. After that, the concentration of 2AP increases for the extraction period from 50 to 70 minutes. The concentration of 2AP in essential oil should do not have significant change across the extraction period. This is because almost all 2AP had been extracted

out and collected in the flask before extraction time finish. Therefore, extra extraction period do not extract any essential oil from dry pandan leaves. There is error in third concentration reading. It may be affected by high temperature in chiller.

By summarize the information for volume, yield of essential oil and concentration of 2AP in essential oil, I can concluded that 30 minutes is optimum extraction period to have maximum amount of highest concentration 2AP of essential oil.

4.5 ANALYSIS OF CHEMICAL COMPOUNDS INSIDE ESSENTIAL OIL BY FTIR

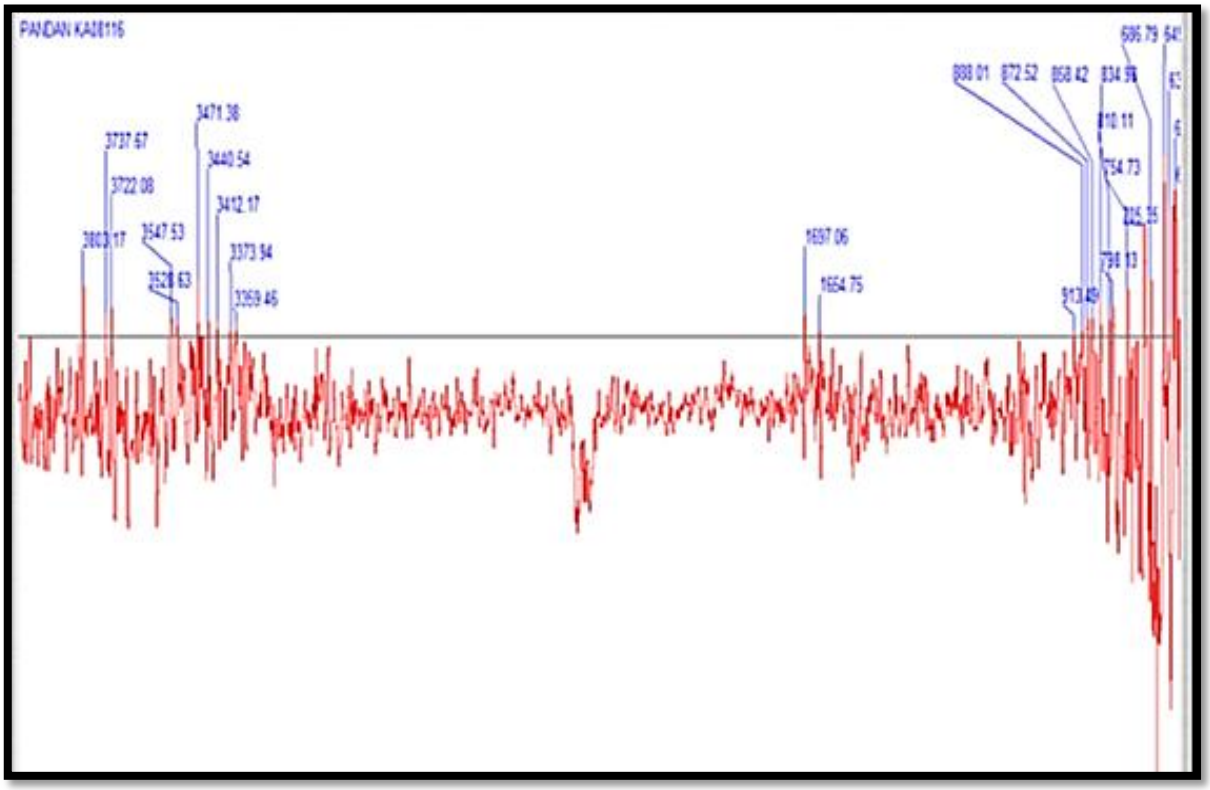


Figure 4.8: FTIR Analysis for Essential Oil

The essential oil extracted is based on the optimum conditions which are the pressure of microwave extractor is 1 bar, power is 500W, mass of cut pandan leaves to be extracted is 50 g, soaked in ethanol solvent for 5 minutes prior extraction, ratio of

solvent to pandan leaves is 1:1, extraction period is 30 minutes and extraction temperature is 88 ^{o}C. In journal of Grimm, et al., (2002), Gas Chromatography- Solid Phase Micro Extraction (GC-SPME) is best equipment to determine the chemical compounds inside essential oil of pandan leaves. Its theory is to capture aroma come out from essential oil after burning it and then identify the components. However, the laboratory does not have this equipment. Besides, Gas Chromatography-Mass Spectrometry (GC-MS) is suitable for identification of components inside essential oil although it may decompose some chemical compounds in high temperature. But, the polar components and water inside essential oil cause the identification of GC-MS fail. Therefore, FTIR is used to determine the components of essential oil pandan leaves although it is not quite suitable. The list of components is showed in the Table 4.5.

Table 4.5: List of Chemical Compounds in Essential Oil of Pandan Leaves

number	Compound name
1	Poly(styrene). Atactic
2	Triphenylphosphine
3	Poly(styrene: Vinylidene Chloride)
4	Amphetamine HCL
5	N(2-cyanoethyl)Amphetamine
6	Methyl Diphenylphosphine
7	Benzphetamine HCL
8	Phenformin HCL
9	1,3-dichlorobutane
10	3-methyl-1-cyclohexene
11	Para-toluenesulfonyl Fluoride
12	Talc
13	Salicyclic Acid
14	Phenyl Sulfoxide
15	Thiophenol
16	Chloroform
17	Sodium Thiosulfate
18	L-amphetamine
19	Nicotine
20	2-Coumaranone
21	1,6-heptadien-4-ol
22	Pyridine

24	PCP PyrrolineAnalog HCL
24	Ketamine
25	Phenol
26	Benzaldehyde
27	Formanilide
28	Phenyl Isocyanate
29	Salicylamide
30	Aniline
31	Benzonitrile

From the identification result of FTIR, the essential generally consists of functional group of pyrroline, pyridine, cyclic acid, phenols, polyphenols, aldehyde, organic amine, polystyrene, inorganic sulphate others aromatic and aliphatic hydrocarbon. However, the essential oil of pandan leaves still has some hazardous compounds such as Nicotine, Chloroform and Phenformin in trace amounts. Although they are at trace amount, need to have precaution steps before consume it.

4.6 SENSORY EVALUATION

Table 4.6: Sensory Evaluation Mean Results

Days	1	2	3	4	5	6	7
Correctness	7.26	7.04	6.30	6.57	6.04	6.18	6.07
Intensity	4.59	4.13	4.04	4.21	3.86	4.00	3.93
Persistence	6.87	6.50	6.00	5.96	5.68	5.61	5.54
Overall Acceptance	5.22	5.50	4.70	4.50	4.25	4.22	4.13
Volume Sample (mL)	26.04	26.46	26.02	25.13	23.57	23.13	22.37
Volume Water (mL)	26.07	26.67	26.69	26.20	24.98	24.70	23.98
Smell	6.53						

where for correctness, intensity, persistence and overall acceptance, the rating for catogories is from 1 to 9. This number represent:

1 = Worst

2 = Very bad

3 = moderately bad

4 = Bad

5 = Satisfactory

6 = Good

7 = Moderately Good

8 = Very good

9 = Excellent

while the rating categories for smell criteria is different than others and represent:

1 = Dislike extremely

2 = Dislike very much

3 = Dislike moderately

4 = Dislike slightly

5 = Neutral

6 = Like slightly

7 = Like moderately

8 = Like very much

9 = Like extremely

Table 4.7: Sensory Evaluation Standard Deviation Results

Days	1	2	3	4	5	6	7
Correctness	1.48	1.42	1.46	1.39	1.41	1.42	1.42
Intensity	2.30	1.10	1.38	1.62	1.31	1.25	1.30
Persistence	1.82	1.59	1.33	1.37	1.34	1.31	1.44
Overall Acceptance	1.79	0.93	0.95	0.76	0.71	0.67	0.83
Volume Sample	2.03	1.88	2.41	2.65	2.12	1.88	1.73
Volume Water	2.07	2.00	2.06	1.66	1.98	2.03	2.39
Smell	1.20						

As I stated in Chapter 3, the sensory evaluation is done by 30 people chosen randomly in 6 rooms of KolejKediaman 4. The raw data is showed in the Appendix 3. Then, these data is summarized by using the mean and standard deviation of the data for 7 days in Table 4.6 and 4.7 respectively. The formula of mean and standard deviation is showed below and next page:

Mean = Sum of X values / N

where X is raw data for rating grade in every single day

N is amount of people rate the criteria

$$Standard\ Deviation, S = \sqrt{\frac{\sum(X-M)^2}{N-1}}$$

where M is the mean of raw data for rating grade in every single day

Smell criteria in the sensory evaluation is to determine the likeness of aroma essential oil pandan leaves among the people. From the Table 4.6 and 4.7, the rating grade for the smell is 6.53, between categories of like slightly and like moderately. The standard deviation for the smell is 1.2 and is quite big because the value is more than 1. It means that many panels like the smell of essential oil moderately and some dislike the smell slightly according to their personally acceptance and likeness. This statement can be proved by raw data in Appendix 3. The essential oil releases the sweet popcorn like aroma which is pleasant aroma. The smell of essential oil is also similar to pandan leaves which its aroma liked by most people in Asia region. Therefore, this criteria show that most people can accept and like the essential oil aroma and it means that essential oil got potential to be new air freshener.

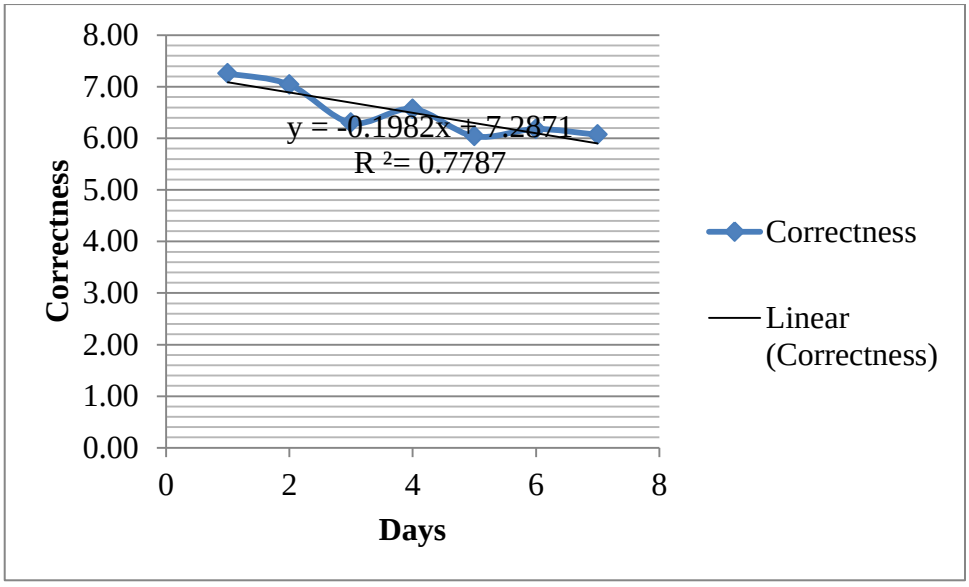


Figure 4.9: Correctness of Essential Oil versus Testing Days

Correctness means the similarity of aroma released by essential oil to aroma of pandan leaves. This evaluation is to determine the correctness of essential oil across the days. From the Figure 4.9, the initial correctness of essential oil is given high rating which is 7.26 between categories of moderately good and very good. It means that aroma of essential is quite similar to aroma of pandan leaves. Although the essential oil has some others chemical compounds inside which may affect the aroma, the amount of 2AP in essential oil is higher (at least 4 µL/mL) than other compounds. Therefore, the aroma of essential oil is mainly dependent on amount of compound 2AP. However, the rating grade of correctness decreases across the testing days. This is because mainly because some 2AP compound is evaporated by heat supplied to surrounding and affects the aroma of essential oil due to smaller concentration of 2AP. Besides, there are some pollutants in the air may dissolved into essential oil and affect the aroma. I also found that there some insects dropped into essential oil in the sample after finish the experiment. The existence of insects inside sample seriously changes the aroma of essential oil. However, the correctness decreases slowly after 7 days and still maintains in rating categories of good. It means that essential oil not easily affect by the disturbance factors from surrounding. In conclusion, the essential has the potential to substitute the pandan leaves.

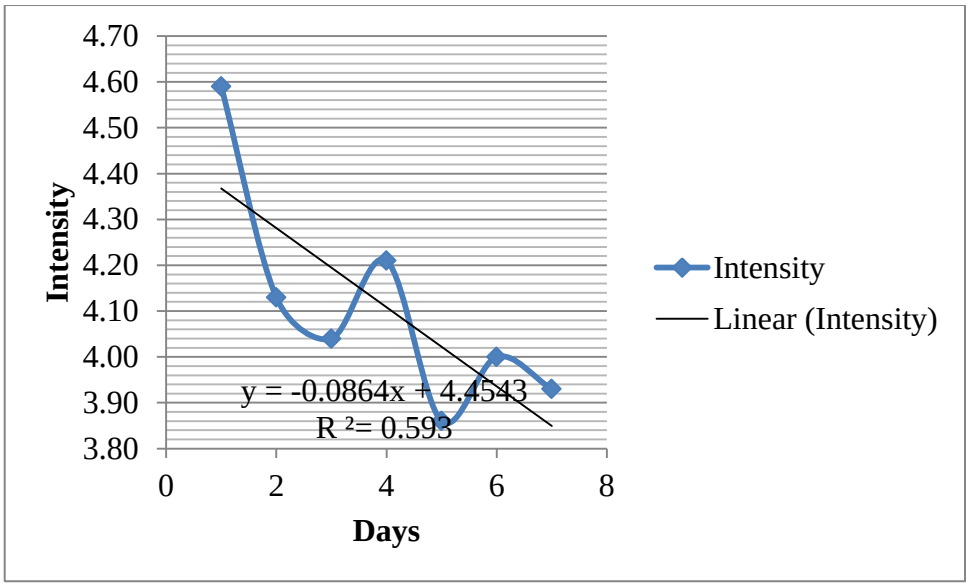


Figure 4.10: Intensity of Aroma Essential Oil versus Testing days

Intensity means the how strong the aroma of essential oil in bottle in every single days. This evaluation is to study the change of aroma intensity in essential oil across testing days. From Table 4.6, the initial mean intensity rated by 30 panels is quite low which is between categories of satisfactory and bad. The intensity of aroma is strongly dependent on the concentration of 2AP in essential oil which is the main components release the aroma. From the estimation of concentration 2AP, I found out that the highest concentration of 2AP in essential oil is very low which is 4 µL/mL only compared to normal air freshener. Therefore, it releases low intensity of aroma. From the Figure 4.10, the intensity of aroma is not in a certain pattern. The intensity aroma essential oil decreases rapidly in 7 testing days after conclude it in a linear graph. Then, the final rating for intensity is inside bad categories. This is because the volatile properties of 2AP compounds. In hot temperature environment with high wind speed, 2AP easily and faster evaporated into surrounding. In addition, there is no cover to close the hole and it causes evaporation happening every moment during testing days. Furthermore, standard deviation of aroma intensity in Table 4.7 is larger than other readings because personally sense of smell different among those panes. Some panels feel the aroma in satisfactory category and some panels have high demand in intensity. Sometimes, there are some smells mixed with aroma pandan leaves. This causes panels cannot smell the aroma when pass by the source released. The raw data for intensity is less consistent than other criteria.

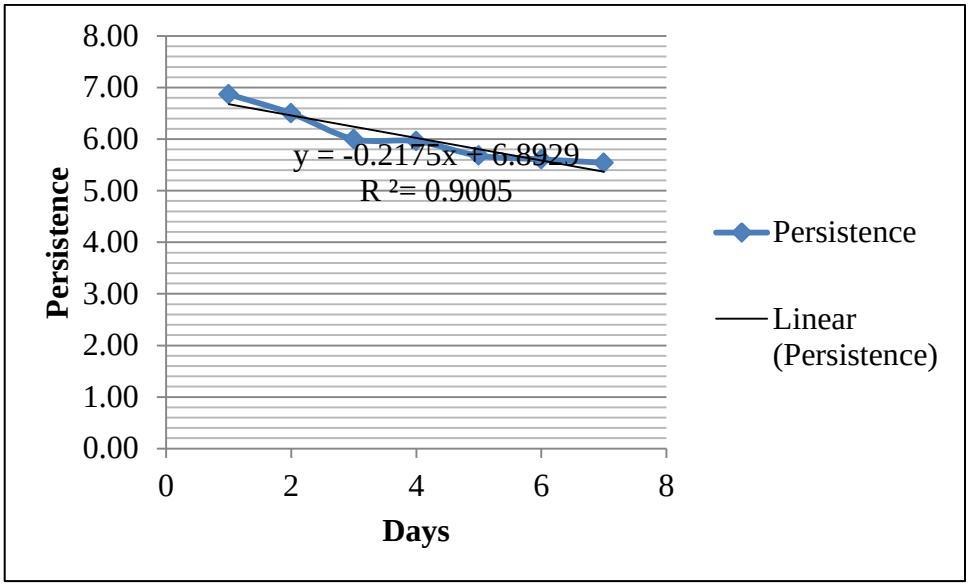


Figure 4.11: Persistence of Aroma versus Testing Days

The persistence means that how long the aroma stay in surrounding and source released. This experiment is to study whether the aroma of essential oil maintain or stay in surrounding during testing days. From Table 4.6, the initial persistence rating grade is between moderately good and good, 6.87. It means that some aroma stayed in the surrounding in first day because the intensity aroma was still high. However, the mean results show the decline pattern in the Figure 4.6 across the testing day. The reason is probably because of less concentration of 2AP from source released due to natural evaporation process in the room. As a result, the aroma of essential oil cannot maintain its performance with lower concentration of 2AP. The rating for persistence at 7^{th} day is still above the satisfactory categories means aroma essential still able to stay in surrounding. In conclusion, the potential of essential oil to be the natural air freshener is showed. Based on the comments given, they said that the aroma of essential oil did not disperse to whole room because the volume of the room is quite large, around 100 m^3. However, the aroma still stayed around the space within the source released for 7 days. Moreover, the standard deviation of persistence criteria in table 4.7 is quite large which is larger than 1. This may because the daily activities of panels inside room or room conditions affect the persistence evaluation. Sometimes, there are some smells mixed with aroma pandan leaves. This causes panels cannot smell the aroma when pass by the source released. So, some panels may rate the persistence in lower grade in those days.

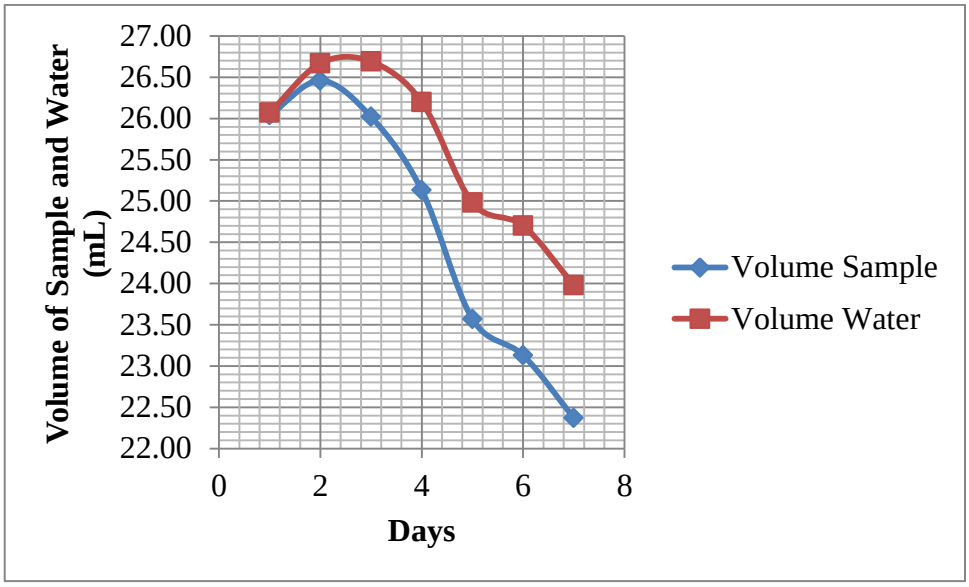


Figure 4.12: Volume of Sample and Water left in Bottle in Every Single Day.

This experiment is to determine volume of sample left in beaker due to natural evaporation with the water solution be the reference. The initial volume of sample and water I put inside the beaker is 25 ml. However, the readings given by 30 panels in first three readings have some parallax errors during taking process due to volumes of both solutions are larger than 25ml. Based on Figure 4.12, the graph for both solutions show the same pattern which declining from first to seventh day. The natural evaporation occurs slowly in those 6 rooms. These rooms usually not face the sun because the windows face inside of building. Therefore, the sunlight usually does not reach these rooms and supplies more heat energy to evaporate. Besides, the wind motion in these rooms is quite stable due to have two small fans in room and less natural flow from window. The relative humidity for these rooms is quite low. This statement is proved by readings and wind motion. Apart of that, I found out that the volume of sample left in beaker is less than water although they are put in same place and have same surface area (number and size of holes are same) are same. This is because various types of volatile compounds inside the essential oil. They have lower boiling point than water and easier to be evaporated due to low respective vapour pressure in the air. However, the difference between the final volumes of both solutions is around 1.6ml only. It means evaporation rate of essential oil relative to water is in satisfied condition. The slopes of persistence graphs for both solutions decrease from fifth to sixth day mainly because

there is raining environment is whole day. The relative humidity is high and surrounding temperature is low. Those factors slow down the evaporation process for both solutions.

Besides, the standard deviation for mean volume of both solutions are quite large which is larger than 1. It means the readings are not consistent due to large scale in the 100ml beaker and parallax error. This error affects the accuracy of estimation for change pattern of solution volume. In order to reduce this error, I should use a smaller scale for them to take volume readings.

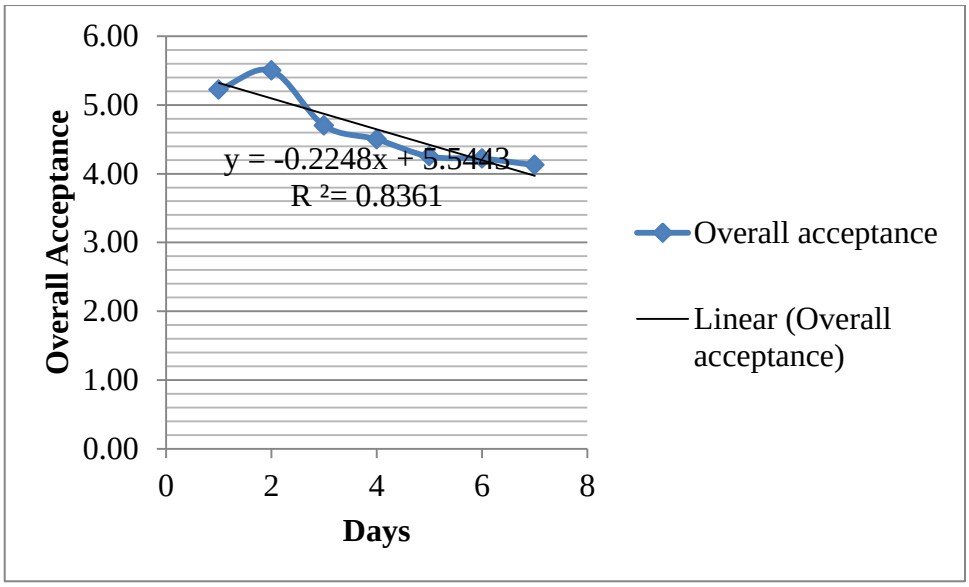


Figure 4.13: Overall Acceptance of Aroma Essential Oil versus Testing Days

Overall acceptance in the sensory evaluation represents the overall quality of essential oil to be the natural air freshener across the days. This evaluation is to study the effects of the period on the overall acceptance of essential oil. From Table 4.6, the initial overall acceptance for essential is only above the satisfactory category. This mainly because of the low intensity of aroma released from essential oil and aroma cannot disperse in to whole room. This is the main disadvantage of the essential oil due to low concentration of 2AP. In order to improve the overall quality, we increase the concentration of 2AP inside the essential oil. The overall acceptance decreases across the testing days means the overall quality are getting worse. The factor contribute most

to this phenomena is decreasing of concentration 2AP due to the natural evaporation process as mentioned before. It seriously affects the performance of essential oil pandan leaves to be a good air freshener. The final rate for overall acceptance is bad. The standard deviation for overall acceptance of essential oil is big in first day, but smaller than 1 for second to seventh day. Some essential oils have high quality in first day because it is just taken from refrigerator which maintain strong aroma of essential oil. After that, the performance of essential oil drops rapidly and many panels rate it in bad categories. For your information, the raw data for overall acceptance is much less which is around 8 to 10 panels than others. This is because they cannot see the column for rating overall acceptance in sensory evaluation form. Therefore, the analysis for overall acceptance is affected and not so accurate.

By conclude all the analysis for the sensory evaluation, the essential oil of pandan leaves has quite well performance in persistence and correctness criteria, but has lower intensity. It also has the quite low evaporate rate compare to water, so small amount of essential oil is enough to be used as air freshener for one month. The overall acceptance also rated low because of the low intensity performance of essential oil. After increasing the concentration of 2AP inside essential oil, it will have high performance in intensity criteria. ‘Thus, it also increases the grade for overall acceptance. In conclusion, the essential oil of pandan leaves is suitable to be natural air freshener with moderately high performance in almost all criteria.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

5.1.1 Optimum Extraction Temperature for Microwave Extraction

Grimm, et al., (2001) stated that slight changes in sample temperature, heating time or variation of moisture can and do affect the measured amount of analytes. One of the objectives of this project is to optimize the parameters, extraction period and temperature for microwave extraction process prior to application of essential oil. The optimization is on the basis of relative amount of essential oil and concentration of 2AP collected.

The volume and yield of essential oil for extraction temperature increase from 70 to 88 ^{o}C. This is because more heat is supplied to liberate the bound volatile compounds inside pandan leaves by having cell disruption when penetration of microwave into cell tissue of pandan leaves. Then, the temperature 88 ^{o}C is maximum point on Figure 4.1 and 4.2 in Chapter 4. The temperature has the highest yield and volume of essential oil. After that, yield and volume of essential decreased from 85 to 101 ^{o}C and almost constant for rest temperature. The volatile compounds which are

terpenes mostly are destructed or decompose at high temperature in extraction process, so volume of essential oil is lesser.

The estimated concentration of 2AP decreases initially and then increases 80 to 90 ^{o}C because supplies more heat energy to break the chemical bound of 2AP inside the papillae, located on the lower epidermis cell of pandan leaves through the cell disruption process. The temperature 90 ^{o}C has the highest concentration 2AP. The concentration of 2AP decreased from 90 to 110 ^{o}C because 2AP maybe decomposed or destructed by too high temperature due to its heat sensitive properties.

By gathering the information, the optimum temperature range is 88 to 90 ^{o}C. Since there is bigger change in yield than concentration of 2AP in temperature range, the optimum temperature is 88 ^{o}C to have maximum amount of essential oil with high concentration of 2AP.

5.1.2 Optimum Extraction Period for Microwave Extraction

The volume, yield of essential oil and concentration of 2AP have the same patterns in the initial part of Figure 4.2, 4.4 and 4.7 of Chapter 4. Before reach the suitable extraction period, the volume of essential oil should be increased because it has more time to for the process of cell disruption and more bound volatile compounds come out. However, those results decreases from 30 to 40 minutes because the heat sensitive compounds maybe decompose or destructed when expose to high temperature for longer period. Theoretically, they should do not have significant change along this extraction period because all essential oil come out from pandan leaves. Therefore, nothing happens for rest of time after 40 minutes. There are some errors for my readings caused by the impurities dissolved inside the essential oil.

By gathering the information, the optimum extraction time is 30 minutes for extraction of small amount of pandan leaves, 50 g under specific condition in this project.

5.1.3 Identification of Chemical Compound inside Essential Oil of Pandan Leaves

Table 5.1: List of Chemical Compounds in Essential Oil of Pandan Leaves

number	Compound name
1	Poly(styrene). Atactic
2	Triphenylphosphine
3	Poly(styrene: Vinylidene Chloride)
4	Amphetamine HCL
5	N(2-cyanoethyl)Amphetamine
6	Methyl Diphenylphosphine
7	Benzphetamine HCL
8	Phenformin HCL
9	1,3-dichlorobutane
10	3-methyl-1-cyclohexene
11	Para-toluenesulfonyl Fluoride
12	Talc
13	Salicyclic Acid
14	Phenyl Sulfoxide
15	Thiophenol
16	Chloroform
17	Sodium Thiosulfate
18	L-amphetamine
19	Nicotine
20	2-Coumaranone
21	1,6-heptadien-4-ol
22	Pyridine
24	PCP PyrrolineAnalog HCL
24	Ketamine
25	Phenol
26	Benzaldehyde
27	Formanilide
28	Phenyl Isocyanate
29	Salicylamide
30	Aniline
31	Benzonitrile

From the identification result of FTIR, the essential oil generally consists of functional group of pyrroline, pyridine, cyclic acid, phenols, polyphenols, aldehyde, organic amine, polystyrene, inorganic sulphate others aromatic and aliphatic hydrocarbon. However, the essential oil of pandan leaves still has some hazardous compounds such as Nicotine, Chloroform and Phenformin in trace amounts. Although they are at trace amount, need to have precaution steps before consume it.

5.1.4 Potential of Essential Oil of Pandan Leaves to be Natural Air Freshener

From the sensory evaluation result, most people chosen like the smell of essential oil in the categories between like slightly and like moderately. This criteria show that most people can accept and like the essential oil aroma and it means that essential oil got potential to be new air freshener.

Besides, the rating grade for correctness, intensity and persistence criteria show the declining patterns in Figure 4.9, 4.10 and 4.11 respectively from first day to seventh day. This main reason is natural evaporation process of 2AP inside those 6 rooms and causes the low performance of essential oil as air freshener due to low concentration of 2AP along the experiment. Apart of that, the initial persistence and correctness get quite high rating grade from panels. Their rating grade also decreases less in 7 testing days. Furthermore, the evaporate rate of essential oil in these rooms is quite slow compared to control solution, water. These criteria prove that the high potential essential oil to be air freshener with high persistence for a small amount.

. The overall acceptance also rated low because of the low intensity performance of essential oil. After increasing the concentration of 2AP inside essential oil, it will have high performance in intensity criteria. ‘Thus, it also increases the grade for overall acceptance. In conclusion, the essential oil of pandan leaves is suitable to be natural air freshener with moderately high performance in almost all criteria.

5.2 RECOMMENDATIONS

In the experiment of determining the optimum extraction temperature and period for extraction process, the apparatus of microwave extractor should wash away all the dirt left by using some chemicals. Besides, ensure that put the grease when set up the apparatus to avoid any gas come out from space between the connector. Furthermore, the temperature must be always maintained at 10 ^{o}C in order to condense all gases released from extraction of pandan leaves. Change the cutting method to grinding method in order to get equivalent size in every sample for extraction process. I should ensure the temperature for rotary evaporation is maintained at 78 ^{o}C because there are some system errors during removal solvent process. In addition, the seal for vials is made sure in good condition to avoid any gases come out during the storage period. I also should wash away all the dirt on the raw pandan leaves before extraction process. During the weighting process, calibrate the equipment in order to avoid system errors.

Apart of that, I should put my eyes same level with the readings in syringe when taking solution from volumetric flask in order to avoid the parallax errors. I also should ensure that there is not any solution left in the syringe filter by pushing syringe hardly. When doing the dilution, I should unsure the solution level reaches exactly the dilution line in volumetric flask in order to have accurate concentration of sample for GC-FID analysis.

On the other hand, I should change the large measurement beaker to smaller measurement beaker in order to have more accurate of volume sample and water left in beakers. I also should ensure two beaker put as close as possible in order to avoid any disturbance factor. The condition of each room is ensured to be almost same such as direction to sun, volume of room and amount of fan.

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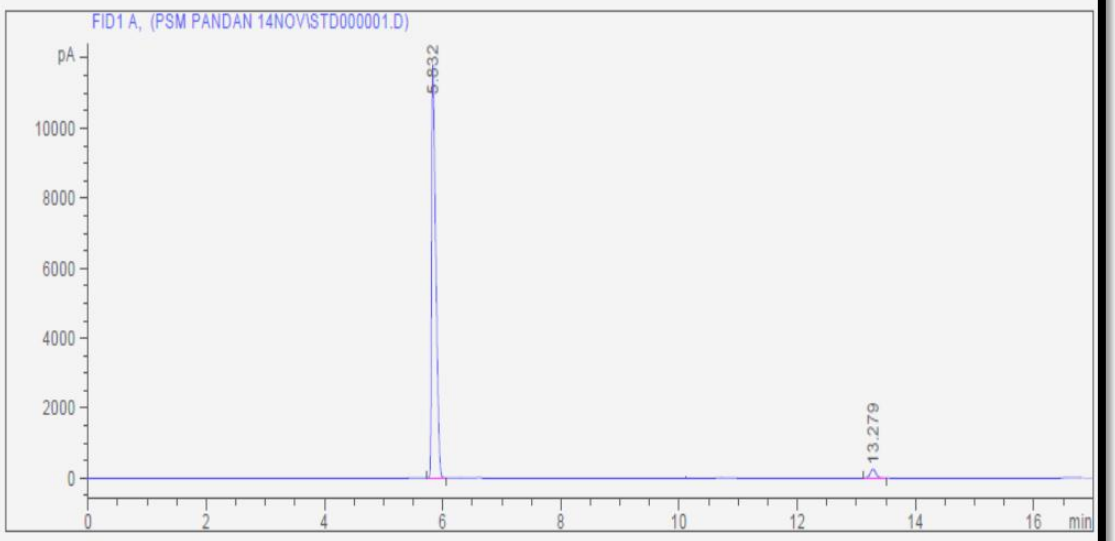
APPENDIX A

ANALYSIS DATA OF ESSENTIAL OIL FOR GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTOR

APPENDIX A.1: ANALYSIS DATA FOR INTERNAL STANDARD (TMP) 1%

Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000001.D
Sample Name: 1%

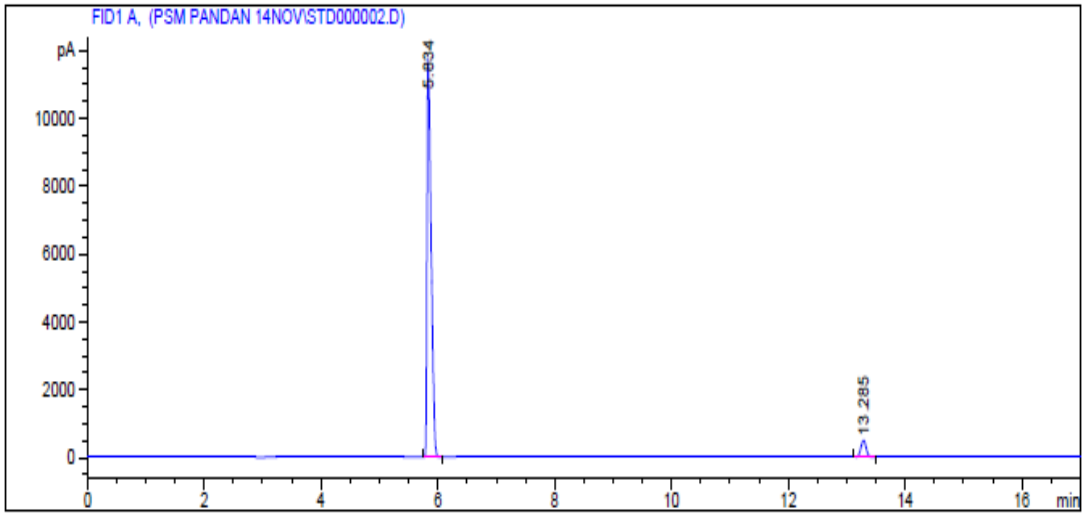
```
==========================================================================
Acq. Operator   : fiza14nov                      Seq. Line :   1
Acq. Instrument : Instrument 1                   Location : Vial 1
Injection Date  : 14/11/2011 14:21:19                 Inj :   1
                                                Inj Volume : 1 µl
Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
Last changed    : 14/11/2011 09:19:27  by fiza03nov
Analysis Method : C:\CHEM32\1\METHODS\ISOOCTANE230108.M
Last changed    : 27/12/2011 10:30:28  by wani27dec
                  (modified after loading)
Method Info     : isooctane/psm

==========================================================================
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      27 August 2009 13:05:28
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [pA*s]        %
----|-------|------|-------|----------|--------|--------------------
   1   2.185         0.0000    0.00000  0.00000
   2   2.253         0.0000    0.00000  0.00000
   3   2.308         0.0000    0.00000  0.00000
   4   2.359         0.0000    0.00000  0.00000
   5   2.762         0.0000    0.00000  0.00000
   6   4.797         0.0000    0.00000  0.00000
   7   5.832 BB S    0.0642 5.73815e4  97.39251 ?
   8  13.279 BB      0.0766 1536.27759  2.60749 ?

Totals :                   5.89178e4
```

APPENDIX A.2: ANALYSIS DATA FOR INTERNAL STANDARD (TMP) 2%

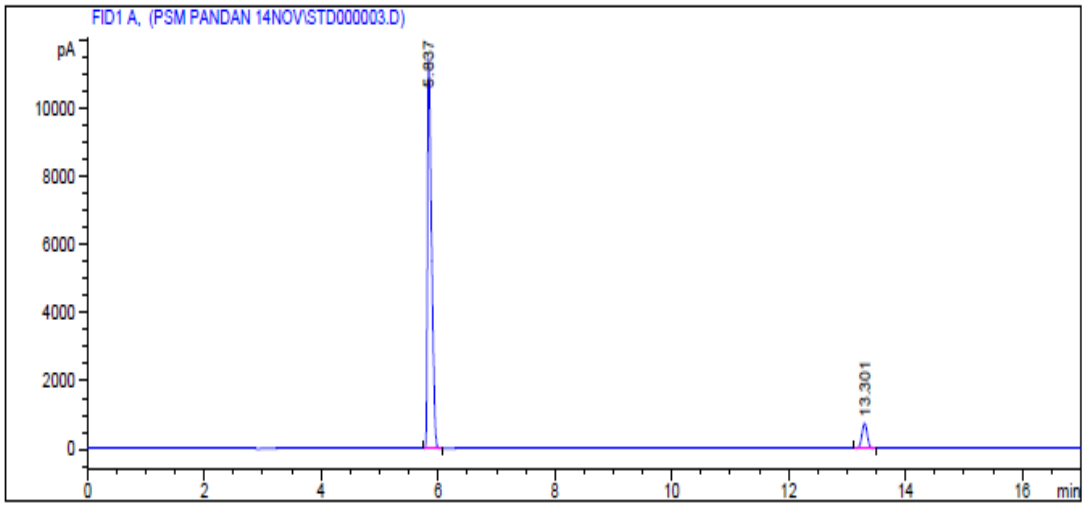
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000002.D
 Sample Name: 2%
   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :   2
   Acq. Instrument : Instrument 1                   Location : Vial 2
   Injection Date  : 14/11/2011 14:46:20                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 14:44:26  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\ISOOCTANE230108.M
   Last changed    : 27/12/2011 10:30:28  by wani27dec
                     (modified after loading)
   Method Info     : isooctane/psm

   ======================================================================
```




```
   ======================================================================
                          Area Percent Report
   ======================================================================

   Sorted By             :      Signal
   Calib. Data Modified  :      27 August 2009 13:05:28
   Multiplier            :      1.0000
   Dilution              :      1.0000
   Use Multiplier & Dilution Factor with ISTDs


   Signal 1: FID1 A,

   Peak RetTime  Type   Width     Area      Area    Name
     #   [min]          [min]   [pA*s]       %
   ----|-------|------|-------|----------|--------|--------------------
      1   2.185         0.0000    0.00000  0.00000
      2   2.253         0.0000    0.00000  0.00000
      3   2.308         0.0000    0.00000  0.00000
      4   2.359         0.0000    0.00000  0.00000
      5   2.762         0.0000    0.00000  0.00000
      6   4.797         0.0000    0.00000  0.00000
      7   5.834 BB S    0.0645 5.73649e4  94.81236 ?
      8  13.285 BB      0.0776 3138.70898  5.18764 ?

   Totals :                   6.05036e4
```

APPENDIX A.3: ANALYSIS DATA FOR INTERNAL STANDARD (TMP) 3%

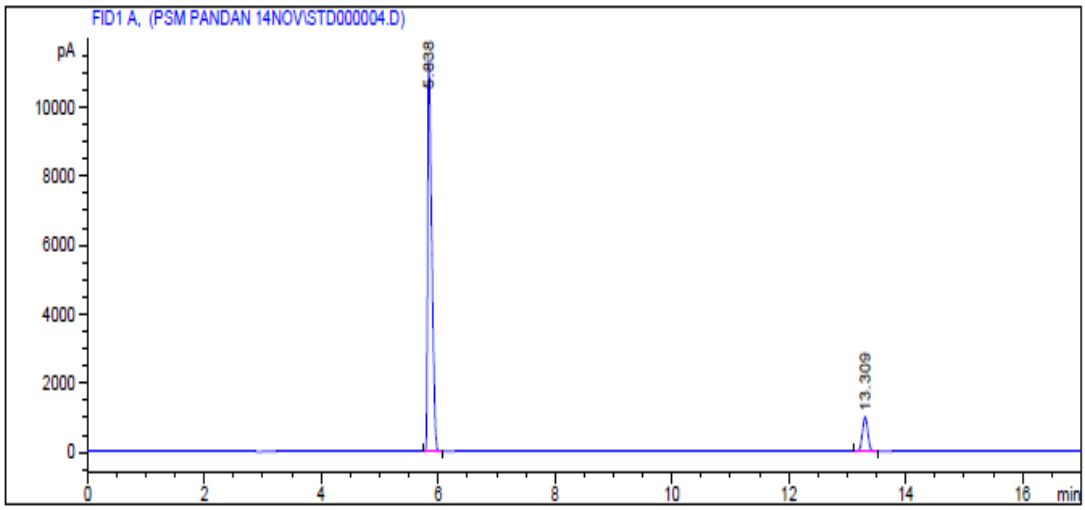
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000003.D
 Sample Name: 3%
   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :   3
   Acq. Instrument : Instrument 1                   Location : Vial 3
   Injection Date  : 14/11/2011 15:11:25                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:08:00  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\ISOOCTANE230108.M
   Last changed    : 27/12/2011 10:30:28  by wani27dec
                     (modified after loading)
   Method Info     : isooctane/psm

   ======================================================================
```




```
   ======================================================================
                            Area Percent Report
   ======================================================================

   Sorted By             :      Signal
   Calib. Data Modified  :      27 August 2009 13:05:28
   Multiplier            :      1.0000
   Dilution              :      1.0000
   Use Multiplier & Dilution Factor with ISTDs


   Signal 1: FID1 A,

   Peak RetTime  Type   Width     Area      Area    Name
     #   [min]          [min]   [pA*s]       %
   ----|-------|------|-------|----------|--------|--------------------
      1   2.185         0.0000    0.00000  0.00000
      2   2.253         0.0000    0.00000  0.00000
      3   2.308         0.0000    0.00000  0.00000
      4   2.359         0.0000    0.00000  0.00000
      5   2.762         0.0000    0.00000  0.00000
      6   4.797         0.0000    0.00000  0.00000
      7   5.837 BB S    0.0657 5.55383e4  92.13971 ?
      8  13.301 BB      0.0829 4737.88135  7.86029 ?

   Totals :                   6.02762e4
```

APPENDIX A.4: ANALYSIS DATA FOR INTERNAL STANDARD (TMP) 4%

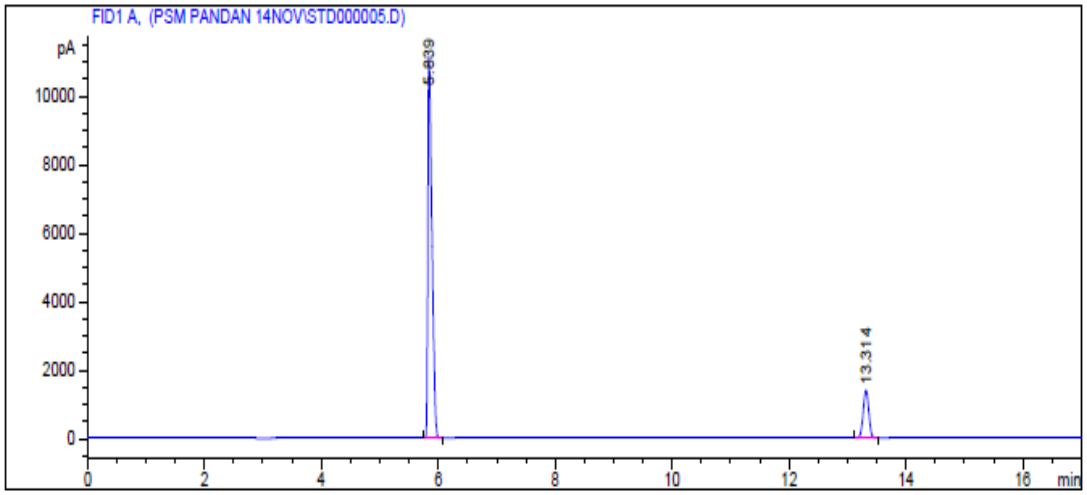
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000004.D
 Sample Name: 4%
   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :   4
   Acq. Instrument : Instrument 1                   Location : Vial 4
   Injection Date  : 14/11/2011 15:37:37                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\ISOOCTANE230108.M
   Last changed    : 27/12/2011 10:30:28  by wani27dec
                     (modified after loading)
   Method Info     : isooctane/psm

   ======================================================================
```




```
   ======================================================================
                          Area Percent Report
   ======================================================================

   Sorted By             :      Signal
   Calib. Data Modified  :      27 August 2009 13:05:28
   Multiplier            :      1.0000
   Dilution              :      1.0000
   Use Multiplier & Dilution Factor with ISTDs


   Signal 1: FID1 A,

   Peak RetTime  Type   Width     Area      Area    Name
     #   [min]          [min]   [pA*s]       %
   ----|-------|------|-------|----------|--------|--------------------
      1   2.185         0.0000    0.00000  0.00000
      2   2.253         0.0000    0.00000  0.00000
      3   2.308         0.0000    0.00000  0.00000
      4   2.359         0.0000    0.00000  0.00000
      5   2.762         0.0000    0.00000  0.00000
      6   4.797         0.0000    0.00000  0.00000
      7   5.838 BB S    0.0651 5.51816e4  89.44730 ?
      8  13.309 BB      0.0784 6510.14258 10.55270 ?

   Totals :                    6.16918e4
```

APPENDIX A.5: ANALYSIS DATA FOR INTERNAL STANDARD (TMP) 5%

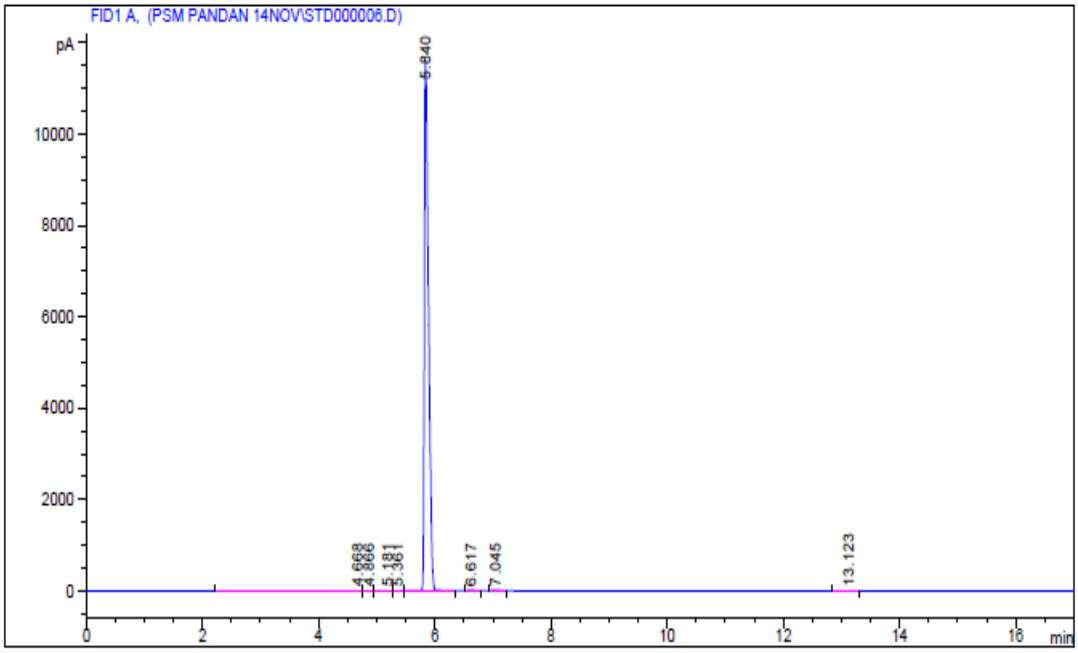
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000005.D
 Sample Name: 5%
   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :   5
   Acq. Instrument : Instrument 1                   Location : Vial 5
   Injection Date  : 14/11/2011 16:16:06                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\ISOOCTANE230108.M
   Last changed    : 27/12/2011 10:30:28  by wani27dec
                     (modified after loading)
   Method Info     : isooctane/psm

   ======================================================================
```




```
   ======================================================================
                           Area Percent Report
   ======================================================================

   Sorted By             :      Signal
   Calib. Data Modified  :      27 August 2009 13:05:28
   Multiplier            :      1.0000
   Dilution              :      1.0000
   Use Multiplier & Dilution Factor with ISTDs


   Signal 1: FID1 A,

   Peak RetTime  Type   Width     Area      Area    Name
     #   [min]          [min]   [pA*s]        %
   ----|-------|------|-------|----------|--------|--------------------
      1   2.185         0.0000    0.00000  0.00000
      2   2.253         0.0000    0.00000  0.00000
      3   2.308         0.0000    0.00000  0.00000
      4   2.359         0.0000    0.00000  0.00000
      5   2.762         0.0000    0.00000  0.00000
      6   4.797         0.0000    0.00000  0.00000
      7   5.839 BB S    0.0646 5.40135e4  85.31398 ?
      8  13.314 BB      0.0794 9297.92676 14.68602 ?

   Totals :                   6.33114e4
```

APPENDIX A.6: ANALYSIS DATA FOR SAMPLE 1

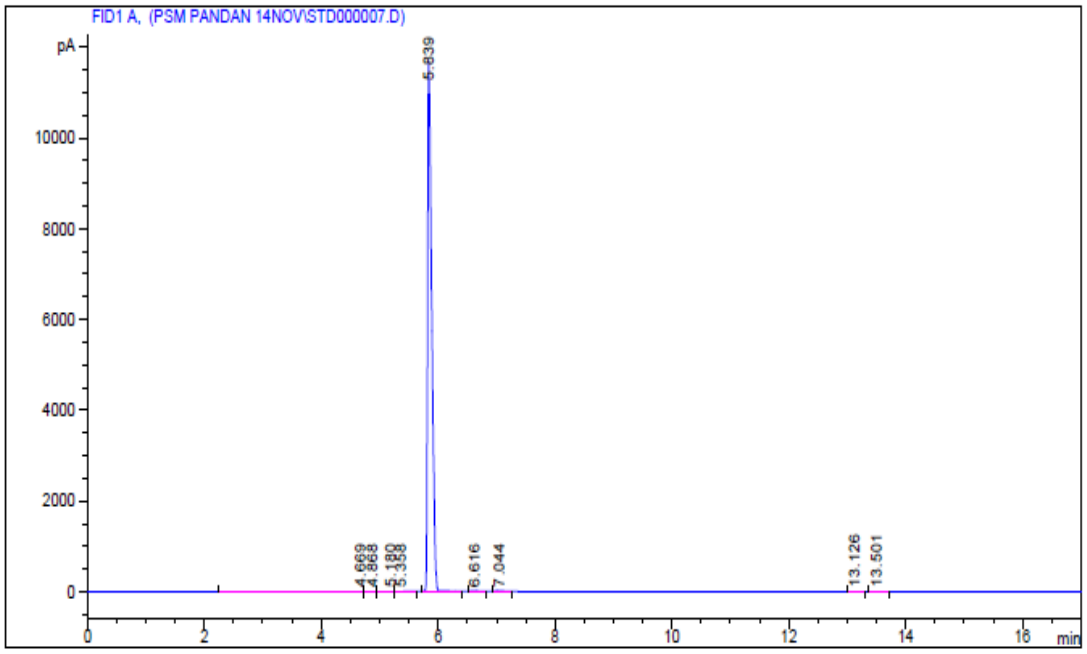
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000006.D
 Sample Name: 1

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :   6
   Acq. Instrument : Instrument 1                   Location : Vial 6
   Injection Date  : 14/11/2011 16:41:16                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ======================================================================
                            Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]       [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|----------|----------|--------|
      1   4.668 BV    0.7083   55.54321 9.34725e-1  0.09741
      2   4.866 VV    0.1205   10.27435    1.13601  0.01802
      3   5.181 VV    0.2277   13.44197 7.55168e-1  0.02357
      4   5.361 VV    0.1435    7.76343 7.04698e-1  0.01362
      5   5.840 VB S  0.0733 5.68778e4  1.15869e4  99.75168
      6   6.617 BB    0.0883    9.83624    1.73125  0.01725
      7   7.045 BB    0.0814   43.55910    8.41985  0.07639
      8  13.123 BB    0.0895    1.17236 1.60033e-1  0.00206

   Totals :                  5.70194e4  1.16007e4
```

APPENDIX A.7: ANALYSIS DATA FOR SAMPLE 2

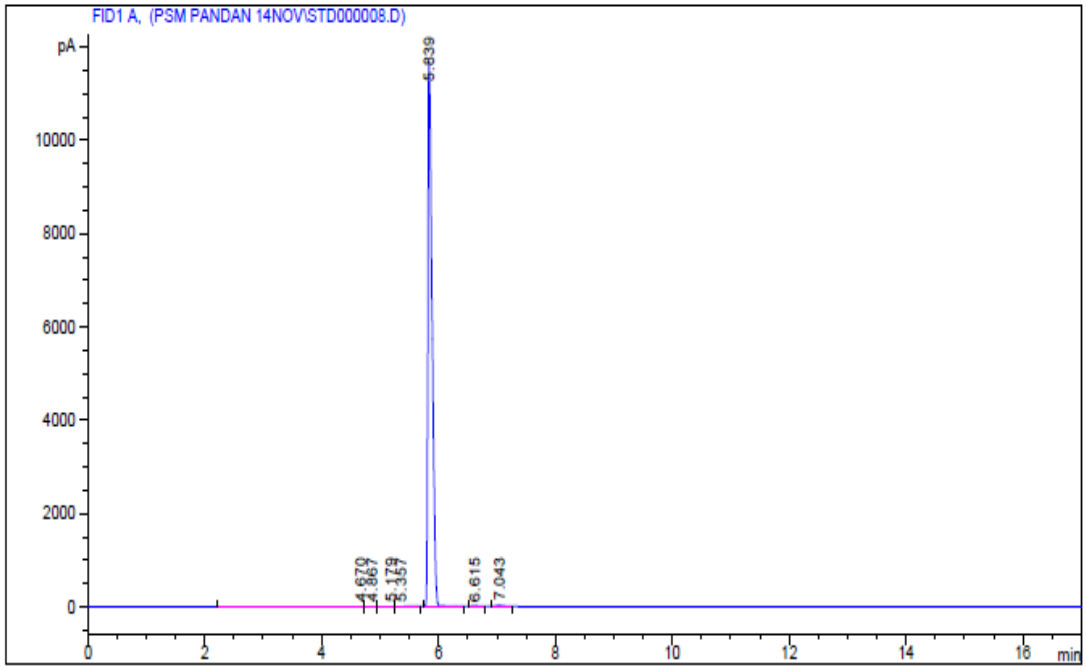
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000007.D
 Sample Name: 2

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :   7
   Acq. Instrument : Instrument 1                   Location : Vial 7
   Injection Date  : 14/11/2011 17:19:15                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ======================================================================
                              Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|----------|----------|--------|
      1   4.669 BV    0.4499   26.38673 7.07868e-1  0.04636
      2   4.868 VV    0.1187    7.24528 7.99685e-1  0.01273
      3   5.180 VV    0.2201    8.24533 4.71354e-1  0.01449
      4   5.358 VB    0.1554    4.89841 3.95822e-1  0.00861
      5   5.839 BB S  0.0759 5.67961e4  1.16404e4  99.79050
      6   6.616 BB    0.0861    9.79689    1.72988  0.01721
      7   7.044 BB    0.0804   59.84830   11.55450  0.10515
      8  13.126 BB    0.0881    1.13708 1.59675e-1  0.00200
      9  13.501 BB    0.1097    1.67832 1.98328e-1  0.00295

   Totals :                  5.69154e4  1.16564e4
```

APPENDIX A.8: ANALYSIS DATA FOR SAMPLE 3

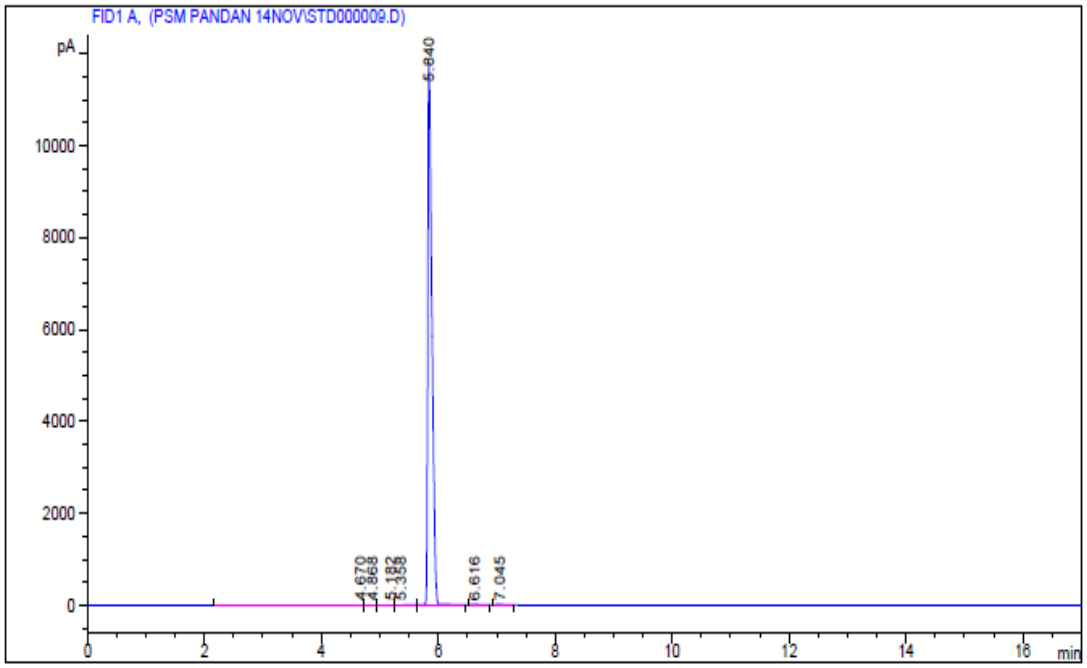
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000008.D
 Sample Name: 3

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :   8
   Acq. Instrument : Instrument 1                   Location : Vial 8
   Injection Date  : 14/11/2011 17:44:25                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ======================================================================
                            Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]       [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|----------|----------|--------|
      1   4.670 BV    0.7278   61.14646 9.97939e-1  0.10647
      2   4.867 VV    0.1239   11.14303    1.18321  0.01940
      3   5.179 VV    0.2205   15.72044 8.62886e-1  0.02737
      4   5.357 VB    0.2563   17.13249 8.31732e-1  0.02983
      5   5.839 BB S  0.0725 5.72233e4  1.16052e4  99.63718
      6   6.615 BB    0.0895    9.76966    1.74116  0.01701
      7   7.043 BB    0.0807   93.46306   17.97446  0.16274

   Totals :                  5.74317e4  1.16288e4
```

APPENDIX A.9: ANALYSIS DATA FOR SAMPLE 4

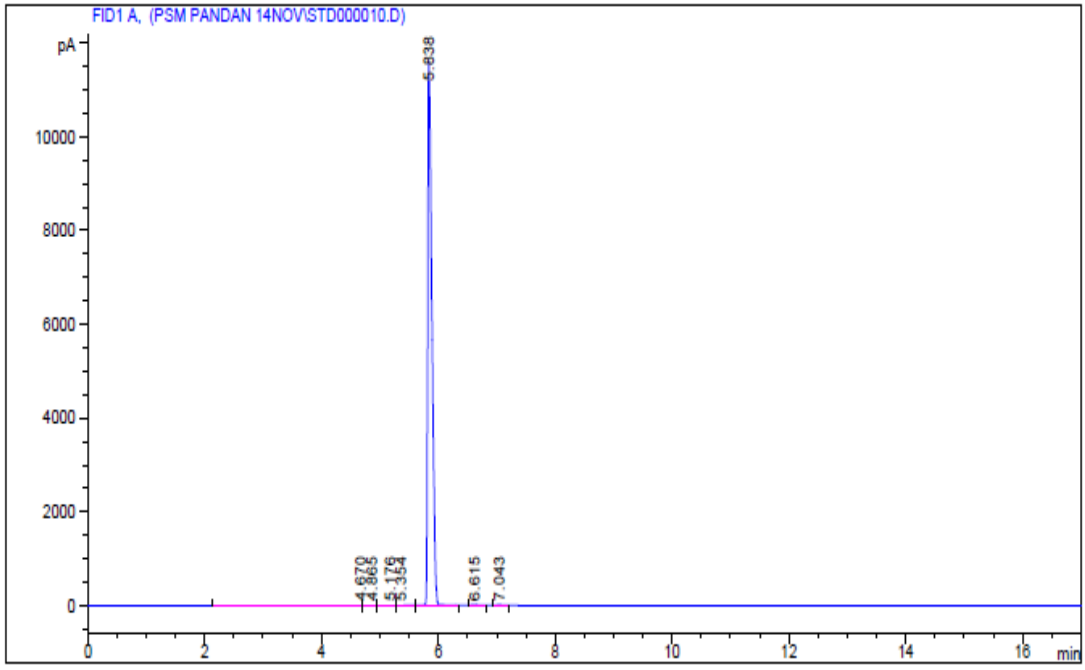
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000009.D
 Sample Name: 4

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :   9
   Acq. Instrument : Instrument 1                   Location : Vial 9
   Injection Date  : 14/11/2011 18:22:49                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ======================================================================
                            Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|----------|----------|--------|
      1   4.670 BV    0.6257   59.80402    1.14254  0.10440
      2   4.868 VV    0.1331   13.34168    1.31835  0.02329
      3   5.182 VV    0.2208   18.36036    1.03604  0.03205
      4   5.358 VB    0.2417   19.13153    1.00212  0.03340
      5   5.840 BB S  0.0752 5.71495e4  1.16575e4  99.76598
      6   6.616 BB    0.0885    9.84011    1.72704  0.01718
      7   7.045 BB    0.0818   13.57575    2.60328  0.02370

   Totals :                  5.72836e4  1.16663e4
```

APPENDIX A.10: ANALYSIS DATA FOR SAMPLE 5

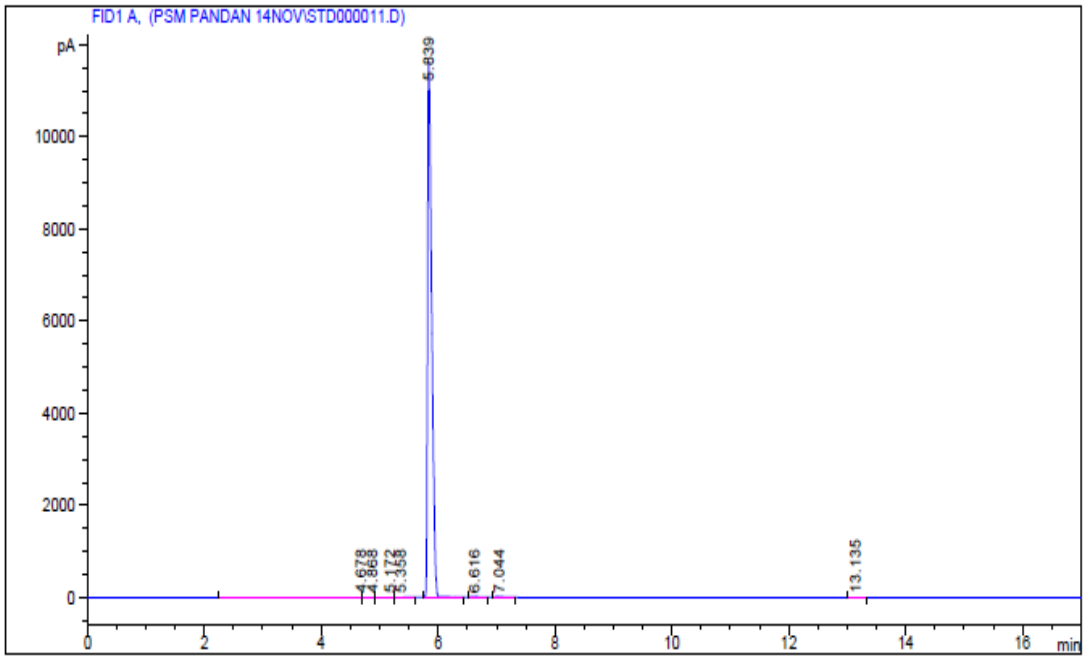
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000010.D
 Sample Name: 5

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :  10
   Acq. Instrument : Instrument 1                   Location : Vial 10
   Injection Date  : 14/11/2011 18:48:02                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ======================================================================
                            Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|----------|----------|--------|
      1   4.670 BV    0.7331   54.52075 8.82044e-1  0.09581
      2   4.865 VV    0.1425   11.93707    1.09244  0.02098
      3   5.176 VV    0.2253   14.57351 7.86020e-1  0.02561
      4   5.354 VB    0.2015   11.61219 7.30490e-1  0.02041
      5   5.838 BB S  0.0754 5.67948e4  1.15533e4  99.81040
      6   6.615 BB    0.0886    9.69517    1.72488  0.01704
      7   7.043 BB    0.0797    5.54988    1.08494  0.00975

   Totals :                  5.69027e4  1.15596e4
```

APPENDIX A.11: ANALYSIS DATA FOR SAMPLE 6

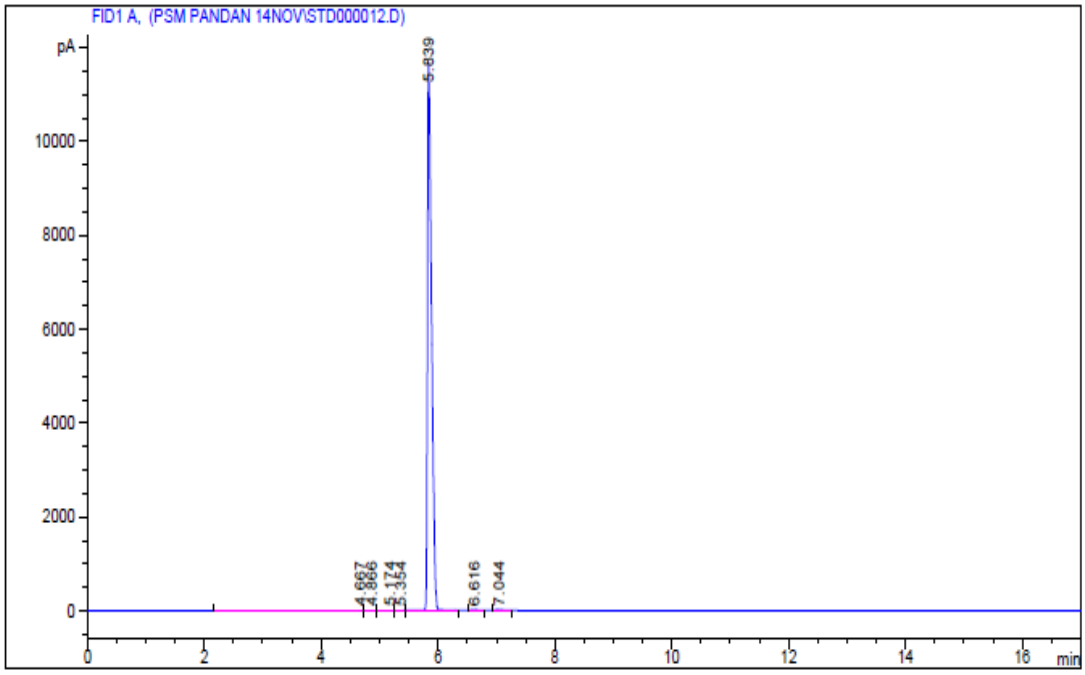
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000011.D
 Sample Name: 6

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :  11
   Acq. Instrument : Instrument 1                   Location : Vial 11
   Injection Date  : 14/11/2011 19:27:12                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ======================================================================
                          Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|----------|----------|--------|
      1   4.678 BV    0.5090   24.87917 5.80553e-1  0.04367
      2   4.868 VV    0.1277    7.44509 7.77297e-1  0.01307
      3   5.172 VV    0.2137    8.06119 4.50658e-1  0.01415
      4   5.358 VB    0.1512    4.22859 3.67599e-1  0.00742
      5   5.839 BB S  0.0702 5.68853e4  1.15953e4  99.85979
      6   6.616 BB    0.0874    9.65386    1.72285  0.01695
      7   7.044 BB    0.0805   24.48834    4.72224  0.04299
      8  13.135 BB    0.0858    1.11260 1.58623e-1  0.00195

   Totals :                  5.69652e4  1.16041e4
```

APPENDIX A.12: ANALYSIS DATA FOR SAMPLE 7

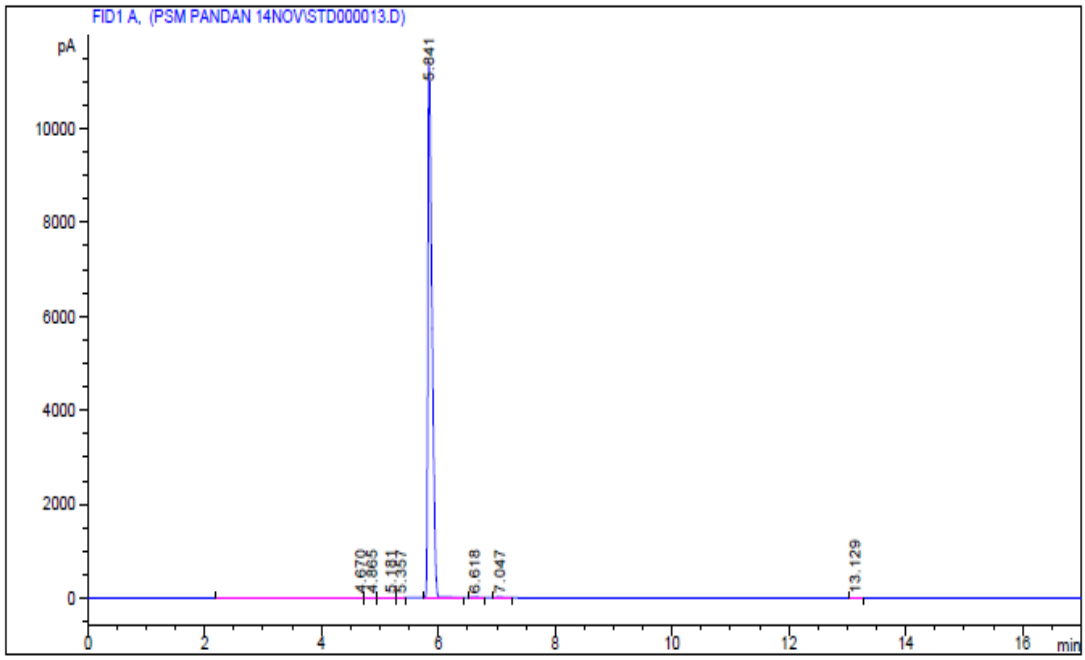
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000012.D
 Sample Name: 7

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :  12
   Acq. Instrument : Instrument 1                   Location : Vial 12
   Injection Date  : 14/11/2011 19:52:24                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ======================================================================
                          Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [pA*s]      [pA]       %
   ----|-------|----|-------|----------|----------|--------|
      1   4.667 BV    0.7185   56.97036 9.36787e-1  0.10100
      2   4.866 VV    0.1273   10.80318    1.10261  0.01915
      3   5.174 VV    0.2175   14.32592 7.97916e-1  0.02540
      4   5.354 VV    0.1288    7.18147 7.42566e-1  0.01273
      5   5.839 VB S  0.0707 5.62847e4  1.15724e4  99.78225
      6   6.616 BB    0.0885    9.79362    1.74423  0.01736
      7   7.044 BB    0.0801   23.75033    4.61162  0.04210

   Totals :                  5.64075e4  1.15823e4
```

APPENDIX A.13: ANALYSIS DATA FOR SAMPLE 8

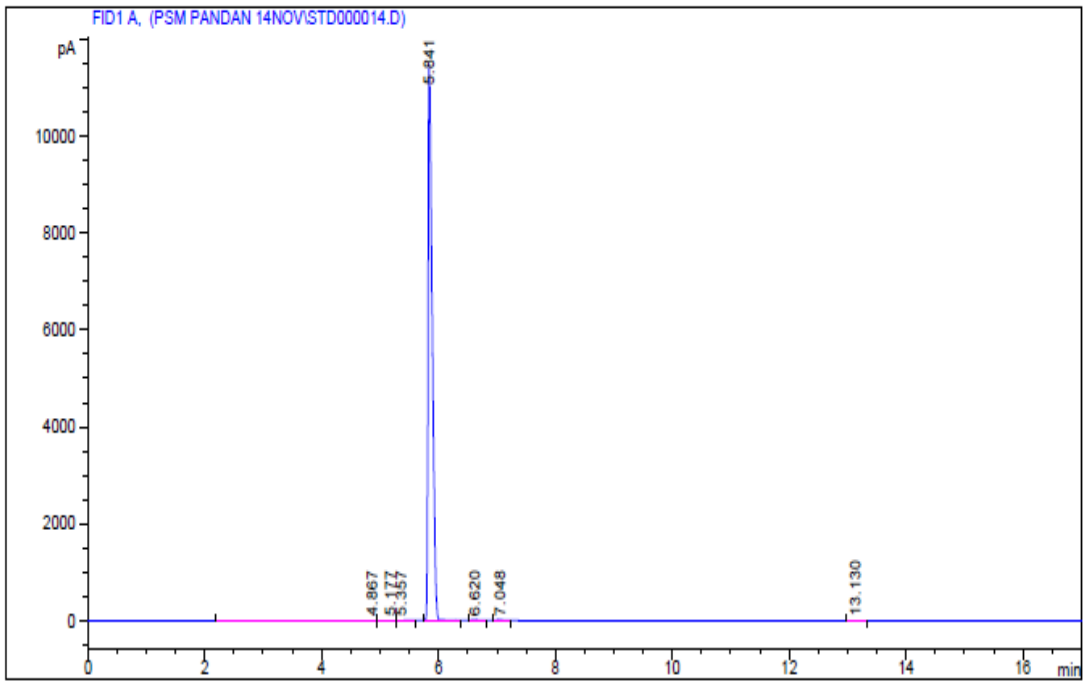
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000013.D
 Sample Name: 8

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :  13
   Acq. Instrument : Instrument 1                   Location : Vial 13
   Injection Date  : 14/11/2011 20:31:34                 Inj :  1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ======================================================================
                          Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|----------|----------|--------|
      1   4.670 BV    0.6504   62.28072    1.13109  0.11212
      2   4.865 VV    0.1346   13.76085    1.34205  0.02477
      3   5.181 VV    0.2327   20.05268    1.06481  0.03610
      4   5.357 VV    0.1199    9.31251    1.02610  0.01676
      5   5.841 BB S  0.0709 5.54250e4  1.13591e4  99.77544
      6   6.618 BB    0.0892    9.57213    1.68734  0.01723
      7   7.047 BB    0.0830    8.73686    1.67043  0.01573
      8  13.129 BB    0.0826    1.02932 1.58809e-1  0.00185

   Totals :                  5.55498e4  1.13672e4
```

APPENDIX A.14: ANALYSIS DATA FOR SAMPLE 9

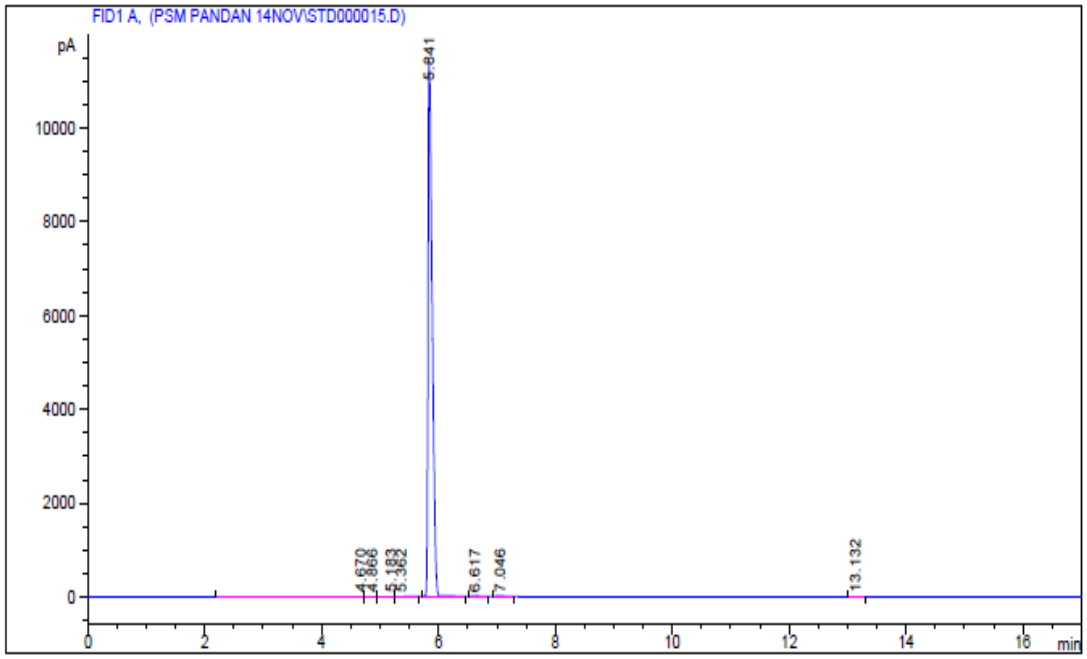
```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000014.D
 Sample Name: 9

   ========================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :  14
   Acq. Instrument : Instrument 1                   Location : Vial 14
   Injection Date  : 14/11/2011 20:56:47                 Inj :  1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```




```
   ========================================================================
                            Area Percent Report
   ========================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|---------|----------|--------|
      1   4.867 BV    0.6099   38.16051 7.45906e-1  0.06834
      2   5.177 VV    0.2294    7.25470 3.92970e-1  0.01299
      3   5.357 VB    0.1357    3.33029 3.24644e-1  0.00596
      4   5.841 BB S  0.0762 5.57738e4  1.13736e4  99.87668
      5   6.620 BB    0.0882    9.88492    1.74154  0.01770
      6   7.048 BB    0.0826    8.97109    1.72844  0.01606
      7  13.130 BB    0.1087    1.26550 1.59273e-1  0.00227

   Totals :                  5.58427e4  1.13786e4
```

APPENDIX A.15: ANALYSIS DATA FOR SAMPLE 10

```
Data File C:\CHEM32\1\DATA\PSM PANDAN 14NOV\STD000015.D
 Sample Name: 10

   ======================================================================
   Acq. Operator   : fizal4nov                      Seq. Line :  15
   Acq. Instrument : Instrument 1                   Location : Vial 15
   Injection Date  : 14/11/2011 21:36:03                 Inj :   1
                                                  Inj Volume : 1 µl
   Acq. Method     : C:\CHEM32\1\METHODS\PANDAN OIL.M
   Last changed    : 14/11/2011 15:37:06  by fizal4nov
                     (modified after loading)
   Analysis Method : C:\CHEM32\1\METHODS\B4ASHUTDOWN.M
   Last changed    : 28/12/2011 10:06:36  by wani27dec
                     (modified after loading)
   Method Info     : std testing
```

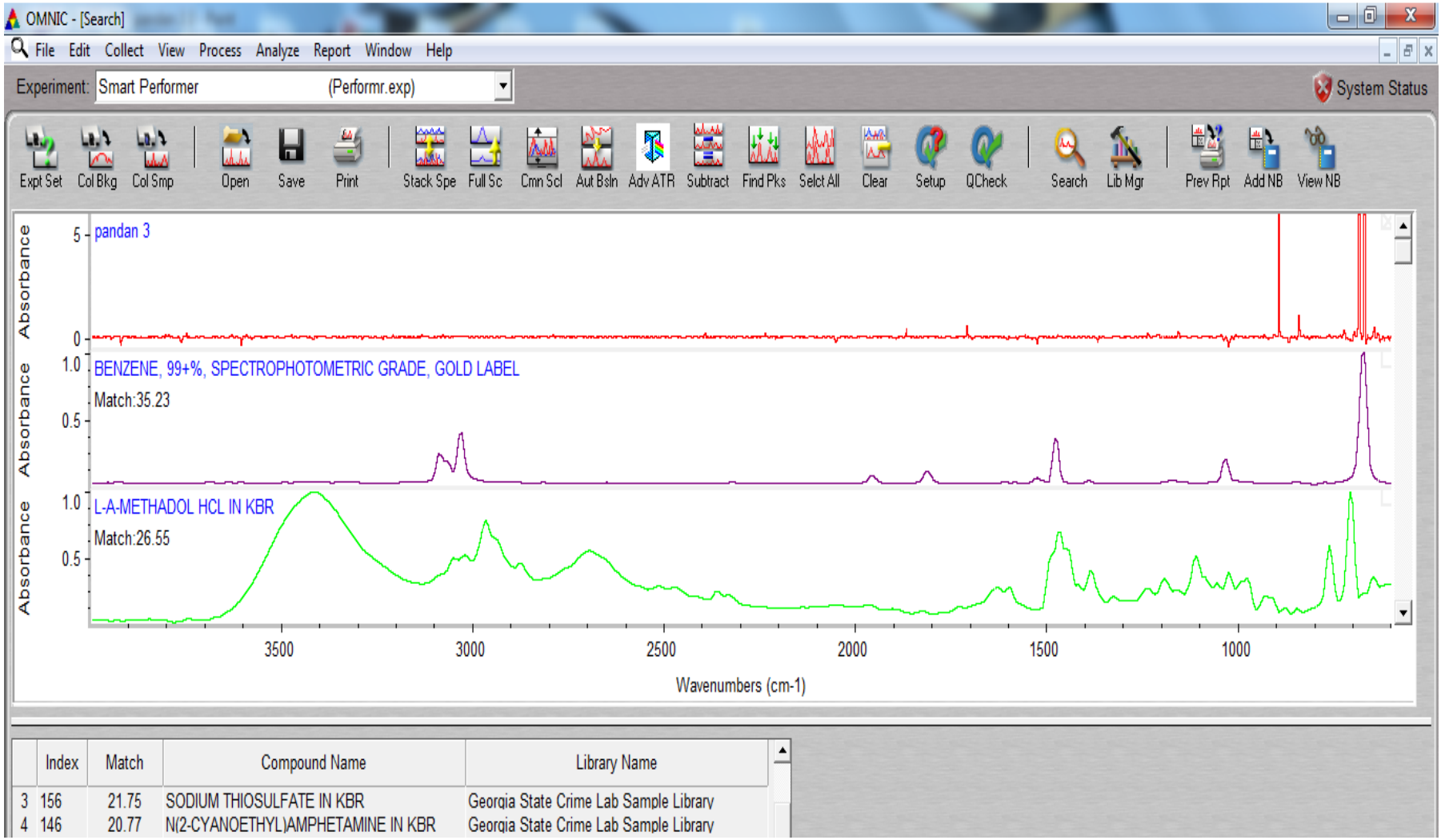



```
   ======================================================================
                          Area Percent Report
   ======================================================================

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


   Signal 1: FID1 A,

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [pA*s]      [pA]        %
   ----|-------|----|-------|----------|----------|--------|
      1   4.670 BV    0.6202   63.22636    1.21875  0.11285
      2   4.866 VV    0.1232   14.41415    1.54014  0.02573
      3   5.183 VV    0.2175   19.83776    1.10978  0.03541
      4   5.362 VB    0.2551   21.74232    1.04361  0.03881
      5   5.841 BB S  0.0715 5.58620e4  1.13414e4  99.70826
      6   6.617 BB    0.0887    9.65363    1.68879  0.01723
      7   7.046 BB    0.0805   33.53103    6.46523  0.05985
      8  13.132 BB    0.0921    1.04603 1.50461e-1  0.00187

   Totals :                  5.60254e4  1.13547e4
```

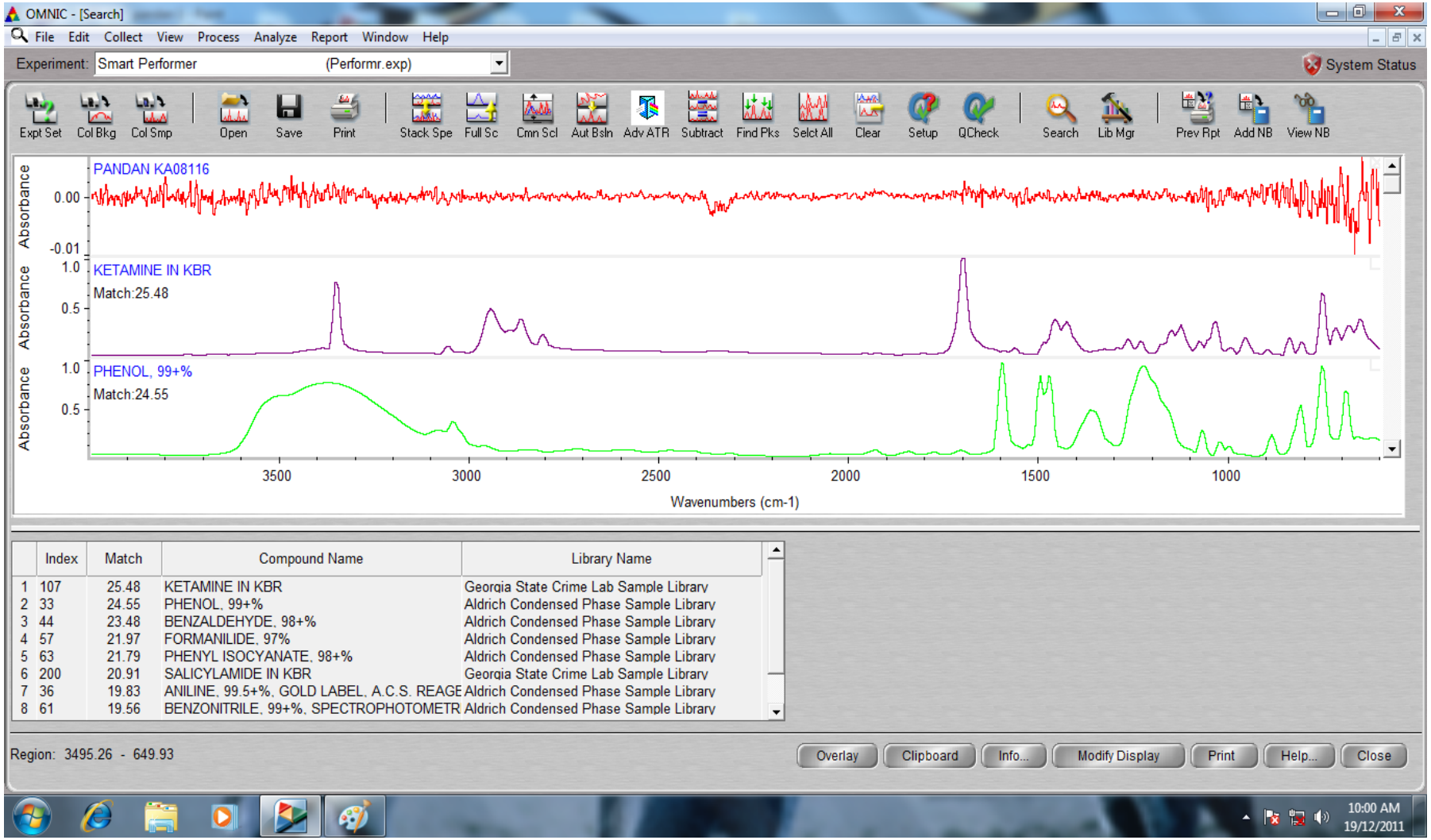
APPENDIX B

ANALYSIS DATA OF ESSENTIAL OIL FOR FOURIER TRANSFORM INFRARED SPECTROSCOPY

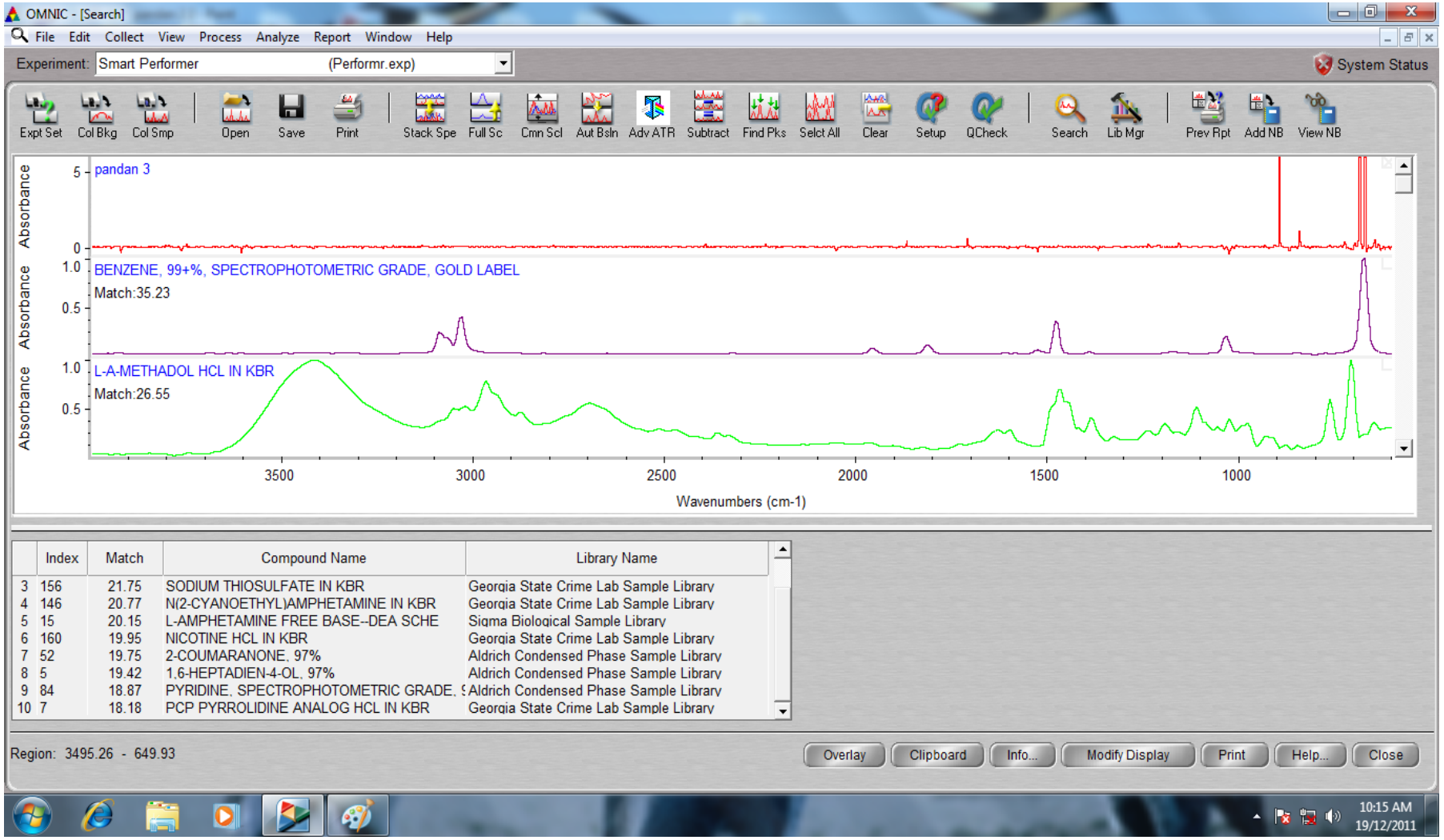
APPENDIX B.1: ANALYSIS DATA TRIAL 1 FOR ESSENTIAL OIL



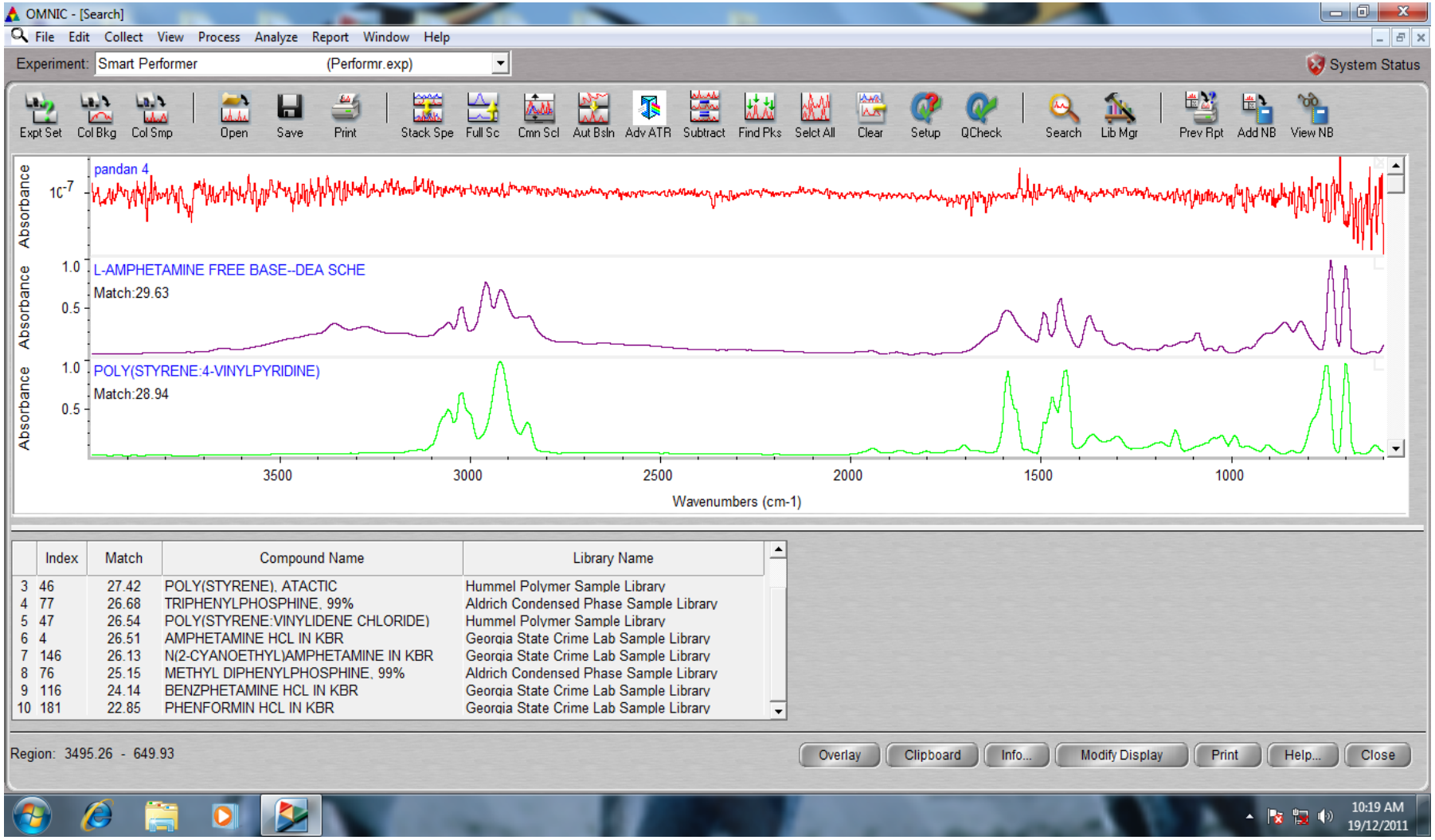
APPENDIX B.2: ANALYSIS DATA TRIAL 2 FOR ESSENTIAL OIL



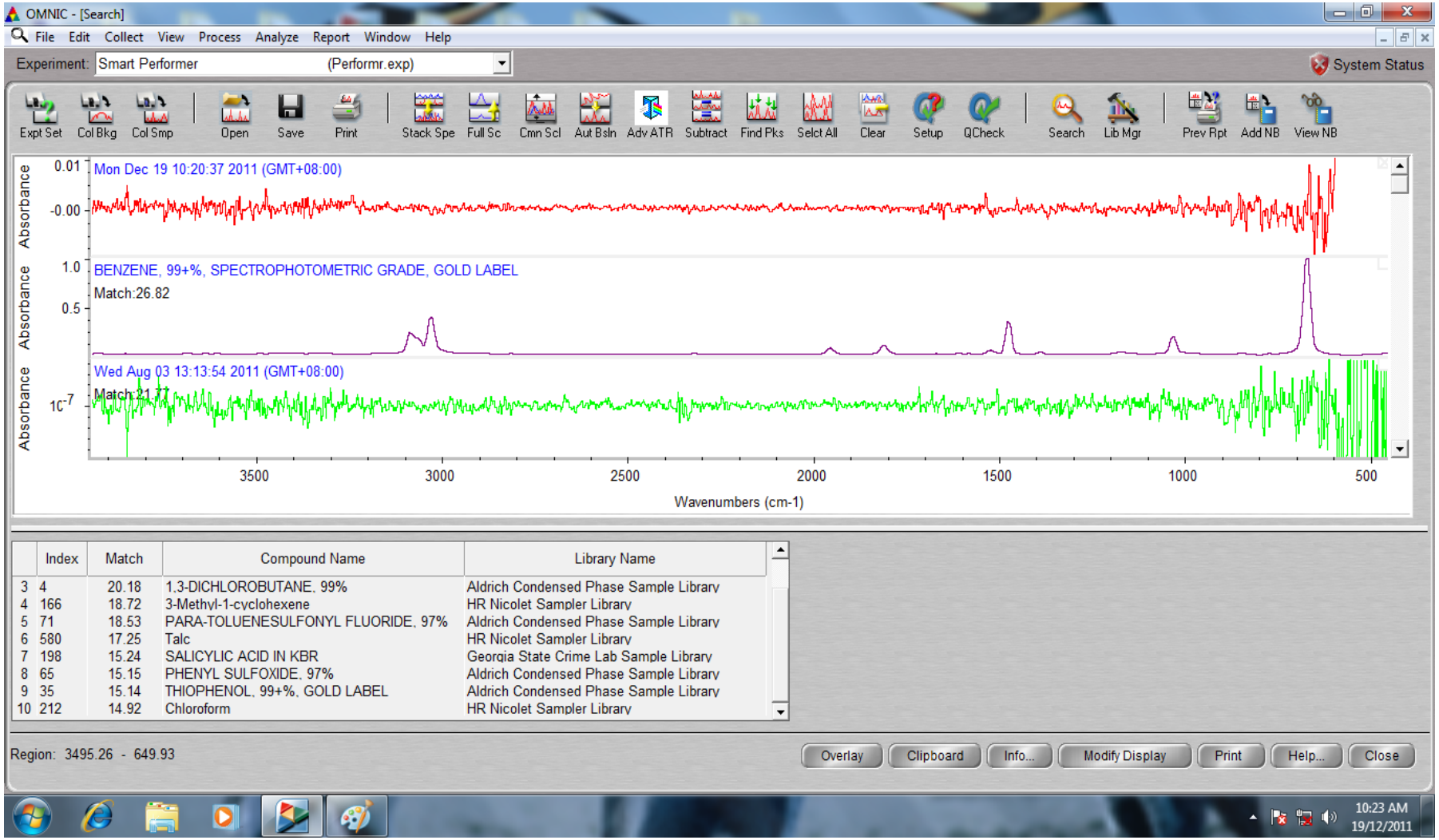
APPENDIX B.3: ANALYSIS DATA TRIAL 3 FOR ESSENTIAL OIL



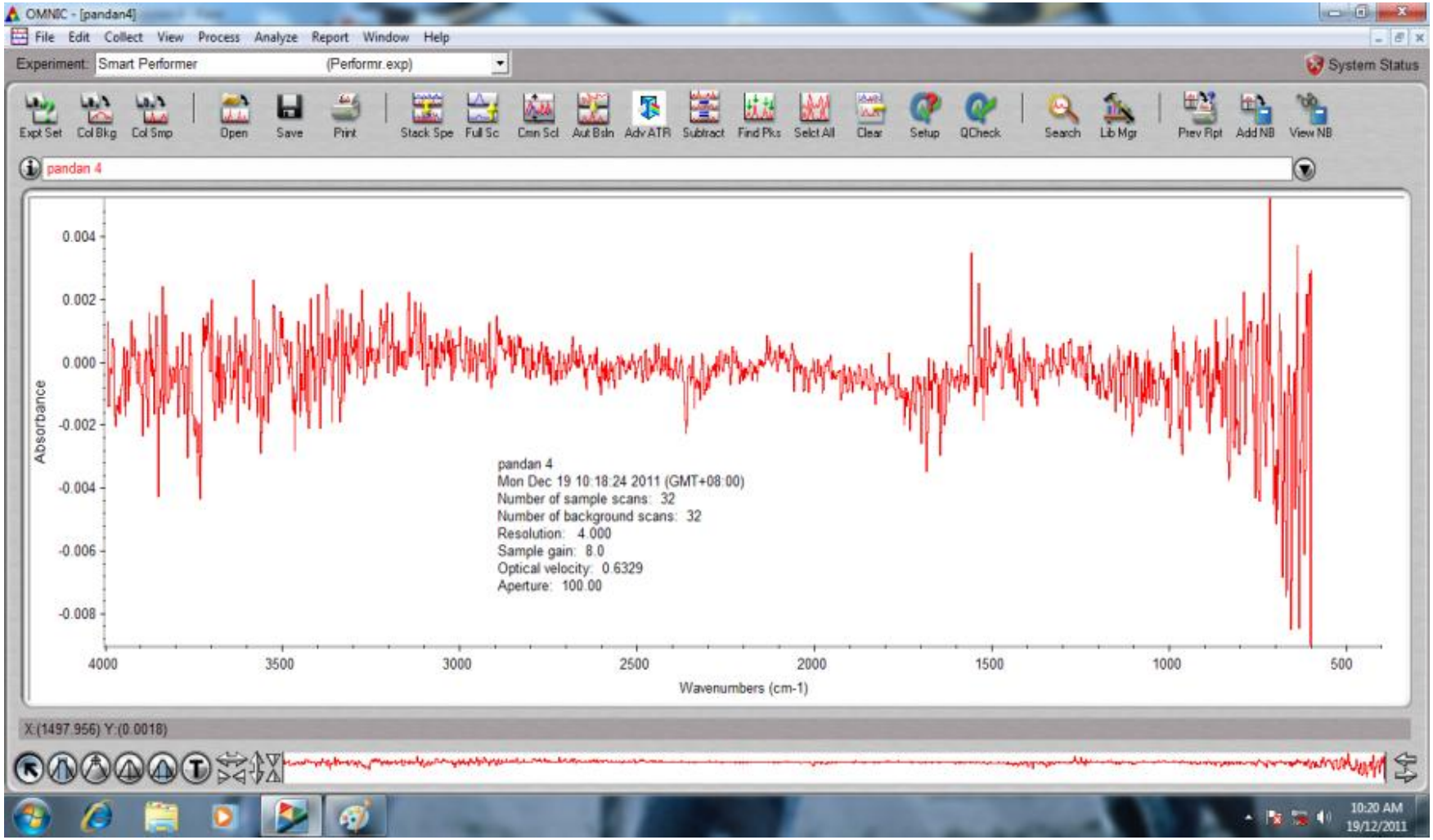
APPENDIX B.4: ANALYSIS DATA TRIAL 4 FOR ESSENTIAL OIL



APPENDIX B.5: ANALYSIS DATA TRIAL 5 FOR ESSENTIAL OIL



APPENDIX B.6: ANALYSIS GRAPH FOR ESSENTIAL OIL



APPENDIX C

RAW DATA FOR SENSORY EVALUATION FORM

Sensory Evaluation form

Item Name : Aroma of the Pandan Leaves Category: Air freshener

Direction: Check one rating for each attribute in the table. Please write your comment if there is any suggestion or advice.

Date:

Characteristics and attributes		Grading Categories								
		Excellent, (9)	Very good, (8)	Moderately good, (7)	Good, (6)	Satisfactory, (5)	Bad, (4)	Moderately bad, (3)	Very bad, (2)	Worst, (1)
Aroma	Correctness									
	Intensity									
	Persistence									
	Overall Acceptance									
		Like extremely,(9)	Like very much, (8)	Like moderately, (7)	Like slightly, (6)	Neutral, (5)	Dislike slightly, (4)	Dislike moderately, (3)	Dislike very much, (2)	Dislike extremely,(1)
	Smell									

Volume left (mL)	

Comments & Recommendations

APPENDIX C.1: RAW DATA FOR SENSORY EVALUATION IN DAY 1

Number	Characteristics and attributes					Volume Left	
	Correctness	Intensity	Persistence	Overall Acceptance	Smell	pandan	water
1	5	4	4	4	5	30	30
2	5	3	3	3	5	30	30
3	5	4	4	4	5	30	30
4	5	4	4	4	5	30	30
5	7		8		7	25	25
6	9	5	8		7	25	25
7	7	5	6	6	6	25	25
8	6	5	6	6	5	25	25
9	8	3	8		7	25	25
10							
11							
12							
13							
14	9	9	9		8	25	25
15	9	9	9	9	5	25	25
16	9	9	9		9	25	25
17	9	9	9		9	25	25
18	8	4	6		8	25	25
19	7	3	7		8	25	25
20	8	2	8		7	25	25
21	7	2	7		7	25	25
22							
23	9	4	8		7	25	25
24							
25	8	3	8		8	25	25
26	8	3	8	6	8	25	25
27	7	3	7		8	25	25
28	7	3	7		8	25	25
29	5	5	5	5	5	29	29.5

APPENDIX C.2: RAW DATA FOR SENSORY EVALUATION IN DAY 2

Number	Characteristics and attributes					Volume Left	
	Correctness	Intensity	Persistence	Overall Acceptance	Smell	panadan	water
1							
2							
3							
4							
5	7	4	7		8	25	25
6	8	5	8		7	24.5	24.5
7	6	5	6		5	24	25
8	6	5	6	6	5	24	24
9	8	3	8		7	25	25
10	7	5	4		4	28	25
11	5	5	5	5	5	28	29
12	5	5	5	5	5	28	29
13	5	4	5	5	5	28	29
14	8	4	4		8	25	25
15	4	4	4	4		24	24
16	4	4	4		5	25	25
17	5	5	5		5	25	25
18	8	3	6		7		
19	7	4	7		8	25	25
20	8	3	8		7	25	25
21	7	2	7		7	24	25
22	8	3	8	7	8	25	25
23	8	5	8		7	24	25
24	8	3	8		8	25	25
25	8	2	8	6	8	24	25
26	8	2	7	6	7	25	25
27	7	4	7		8	25	25
28	7	5	7		8	25	25
29	7	5	4		3	28	29

APPENDIX C.3: RAW DATA FOR SENSORY EVALUATION IN DAY 3

Number	Characteristics and attributes					Volume Left	
	Correctness	Intensity	Persistence	Overall Acceptance	Smell	panadan	water
1	5	5	5	4	5	29	29
2	5	5	5	4	5	29	29
3	4	4	4	4	5	28.5	28.5
4	4	4	4	4	5	29	29
5	6	3	6		7	23	25
6	8	5	5		7	24	24
7	5	5	6	6	6	24	25
8	6	5	6	6	5	23.5	23.5
9	7	2	7		7	23	25
10	5	7	6		6	28	25
11	5	5	5	5	5	27	28
12	5	4	5	4	5	27	28
13							
14	8	4	4		7	25	25
15	4	4	4	4		24	24
16	4	4	4		5	24	24
17	6	5	5		5	24	24
18	7	3	5		6		
19	6	3	6		7	23	25
20	6	3	6		7	23	25
21	7	2	7		7	24	25
22	8	2	8	6	7	24	25
23	8	4	8		8	24	25
24	8	3	8		8	25	25
25	8	2	7		8	24	25
26	8	3	8		7	24	25
27	6	3	6		7	23	25
28	6	3	6		7	23	25
29	5	7	6		6	27.5	28

APPENDIX C.4: RAW DATA FOR SENSORY EVALUATION IN DAY 4

Number	Characteristics and attributes					Volume Left	
	Correctness	Intensity	Persistence	Overall Acceptance	Smell	panadan	water
1	7	6	6	5	6	28	27
2	7	6	6	5	6	28	27
3	5	5	4	4	6	28	27
4	5	6	6	4	6	28	27
5	6	2	6		7	21	25
6	7	7	7		7	23.5	23.5
7	6	5	6		5	24	25
8	6	5	6	6	5	23	23
9	7	3	7		7	21	25
10	7	7	6		5	27	25
11	4	4	4		5	26	26.5
12	4	4	4	4	5	26	26.5
13	5	4	5	4	5	27	28
14	8	5	5		7	25	25
15	4	4	4	4		24	24
16	4	4	4		5	23	23
17	6	4	4		5	23	23
18	7	3	5		7		
19	6	3	5		7	21	25
20	6	3	5		7	21	25
21	8	2	7		7	24	25
22	8	2	7		7	24	25
23	8	4	8		7	24	25
24	8	2	8		8	24	25
25	8	2	8		8	24	25
26	8	3	8		7	22	25
27	6	3	5		7	21	25
28	6	3	5		7	21	25
29	7	7	6		5	27	27

APPENDIX C.5: RAW DATA FOR SENSORY EVALUATION IN DAY 5

Number	Characteristics and attributes					Volume Left	
	Correctness	Intensity	Persistence	Overall Acceptance	Smell	panadan	water
1	4	4	4	4	5	21	21
2	4	4	4	4	5	21	21
3	4	4	4	4	5	21	21
4	4	4	4	4	5	21	21
5	7	3	6		7	21	25
6	6	4	5		7	23	23
7	6	5	6		5	22	25
8	6	5	6	6	5	21	21
9	6	3	6		7	21	25
10	7	7	6		5	27	24
11	4	4	4	4	5	25.5	26
12	4	4	4	4	5	25.5	26
13	4	4	4	4		26	26.5
14	7	4	4		7	24	24
15	4	4	4			23	23
16	4	4	4		5	23	23
17	6	5	5		5	23	23
18	7	3	5		7		
19	6	3	6		7	21	25
20	6	3	6		7	22	25
21	8	2	7		7	22	25
22	7	2	7		7	24	25
23	7	4	7		7	22	25
24	7	2	7		7	24	25
25	8	2	8		8	22	25
26	7	2	8		7	22	25
27	6	3	6		7	21	25
28	6	3	6		6	21	25
29	7	7	6		5	26.5	26

APPENDIX C.6: RAW DATA FOR SENSORY EVALUATION IN DAY 6

Number	Characteristics and attributes					Volume Left	
	Correctness	Intensity	Persistence	Overall Acceptance	Smell	panadan	water
1	4	4	4	4	5	21	21
2	4	4	4	4	5	21	21
3	4	4	4	4	5	21	21
4	4	4	4	4	5	21	21
5	6	4	6		7	21	25
6	8	4	5		7	21	21
7	6	5	6		5	22	25
8	6	5	6	6	5	20	20
9	6	3	6		7	21	25
10	7	7	6		5	26	23
11	4	4	4	4	5	24	24.5
12	4	4	4	4	5	24	24.5
13	4	4	4	4	5	25.5	26
14	7	4	4		7	24	24
15	4	4	4	4		23	23
16	8	4	4		5	23	23
17	7	4	4		5	23	23
18	6	3	5		7		
19	6	4	6		7	21	25
20	6	4	6		7	21	25
21	7	2	7		7	22	25
22	7	2	7		7	22	25
23	7	4	7		7	22	25
24	7	2	7		7	23	25
25	8	2	8		8	22	25
26	7	2	7		7	22	25
27	6	4	6		7	21	25
28	6	4	6		7	21	25
29	7	7	6		5	26	26

APPENDIX C.7: RAW DATA FOR SENSORY EVALUATION IN DAY 7

Number	Characteristics and attributes					Volume Left	
	Correctness	Intensity	Persistence	Overall Acceptance	Smell	panadan	water
1	4	4	4	4	5	21	21
2	4	4	4	4	5	21	21
3	4	4	4	4	5	21	21
4	3	3	3	3	5	21	21
5	7	3	6		7	21	25
6	6	5	4		7	20	20
7	6	5	6		5	21	25
8	6	5	6	6	5	20	20
9	6	3	6		7	21	25
10	7	7	6		6	26	23
11	4	4	4	4	5	22.5	23
12	4	4	4	4	5	22.5	23
13	4	4	4	4	5	24	24.5
14	7	4	4		7	20	20
15	4	4	4			20	20
16	8	4	4		5	20	20
17	6	3	3		6	20	20
18	6	3	6		7		
19	6	4	6		7	21	25
20	6	4	6		7	21	25
21	7	2	7		7	22	25
22	7	2	7		7	22	25
23	7	4	7		7	22	25
24	7	2	7		7	23	25
25	8	2	8		8	22	25
26	7	2	7		7	22	25
27	6	4	6		7	21	25
28	6	4	6		7	21	25
29	7	7	6		6	25	25