CHEMICAL VARIABILITY IN VOLATILE OILS OF BENTONG GINGER: A CHEMOMETRIC STUDY

FARAH SYUHAIDAH BINTI AHMAD JAHIDDIN

FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR

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FARAH SYUHAIDAH BINTI AHMAD JAHIDDIN

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Name of Candidate: **FARAH SYUHAIDAH AHMAD JAHIDDIN**

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CHEMICAL VARIABILITY OF VOLATILE OILS OF BENTONG GINGER: A CHEMOMETRIC STUDY

ABSTRACT

The volatile oil of ginger (*Zingiber officinale*) possesses various pharmacological properties corresponding to its chemical compositions, which in turn, depends on its source material and preparation method. In this study, preliminary experiments were carried out to investigate the variations in volatile oil of ginger prepared based on different method (dry/fresh samples), extraction duration and size reduction process (grate/slice samples). The ginger volatile oil yield can be quantitatively improved by using a fresh sample with grated form and a longer extraction cycle. Gas Chromatography-Mass Spectrometry (GC-MS) was conducted and the chromatogram profile was subjected to Principal Component Analysis (PCA). Results show that extract of fresh samples was predominated by the presence of monoterpenes while sesquiterpenes were more profound in the grated samples; meanwhile, the extraction duration demonstrated a direct and inverse relationship on monoterpenes and sesquiterpenes. As the optimum extraction procedure has been determined, the variability in the volatile components was further explored using pattern recognition tools. The results revealed a tendency for the volatile oil components to cluster, probably due to the geographic origin of the ginger material, therefore highlighting that the terpene components in Bentong ginger could be used to differentiate it from other commercial gingers. The overall findings demonstrate that different sample preparation methods and geographical origin will affect the quantity and/or quality of ginger extract obtained.

Keywords: volatile oil, ginger, principal component analysis, hydrodistillation.

KEBOLEHUBAHAN KIMIA DALAM MINYAK MUDAH MERUAP HALIA BENTONG: KAJIAN KIMOMETRIK

ABSTRAK

Minyak mudah meruap halia (*Zingiber officinale*) mempunyai pelbagai sifat farmakologi berdasarkan komposisi terpena yang bergantung kepada varieti sampel tersebut dan kaedah penyediaannya. Dalam kajian ini, ujian awal telah dijalankan bagi menyiasat variasi yang terdapat dalam minyak pati halia yang diperolehi melalui kaedah tetapan penyulingan hidro berdasarkan parameter yang khusus seperti kesegaran/kekeringan sampel, ketebalan bahan dan tempoh pengekstrakan. Berdasarkan data eksperimen, penghasilan minyak boleh diperbaiki dari segi kuantitatif dengan menggunakan sampel segar, mengurangkan ketebalan partikel dan tempoh pengekstrakan yang panjang. Sementara itu, kromatografi gas-spektrometri jisim-analisis komponen utama telah dilakukan yang mana keputusan tersebut memperlihatkan dominasi monoterpena di dalam ekstrak sampel segar dan sesquiterpena di dalam sampel yang diparut; sebaliknya, bagi tempoh pengekstrakan menunjukkan kesan antagonistik diantara monoterpena dan sesquiterpena. Dengan mengurangkan kesan berkaitan kaedah pengekstrakan, kajian lebih mendalam tentang perbezaan yang ditunjukkan komponen mudah meruap tersebut telah dilakukan melalui kaedah Analisis Komponen Utama (PCA). Keputusan yang diperolehi telah memperlihatkan kecenderungan pengelompokan bagi komponen-komponen tersebut berkemungkinan disebabkan tempat asal geografi halia. Hal ini menunjukkan keupayaan komponen terpena dalam halia Bentong yang berkemungkinan boleh digunakan untuk membezakannya dengan halia komersial yang lain. Keseluruhan penyelidikan ini telah memperlihatkan kepelbagaian kaedah penyediaan dan sumber bahan akan memberikan kesan kepada kuantiti dan/atau kualiti ekstrak halia yang diperolehi.

Kata Kunci: Minyak mudah meruap, halia, analisis komponen utama, penyulingan hidro

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LIST OF SYMBOLS AND ABBREVIATIONS

ºC	:	Degree Celsius
%	:	Percent
~	:	Approximate
$\sum$	:	Sum
$\hat{y}$	:	Predicted value of y
cm	:	Centimeter
cm^{-1}	:	Reciprocal centimeters
g	:	Gram
h	:	Hour
ha	:	Hectare
kg	:	Kilogram
kg/h	:	Kilogram per hour
L	:	Liter
min	:	Minute
ml	:	Milliliter
ml/min	:	Milliliter per minute
ml/g	:	Milliliter per gram
µm	:	Micrometer
mm	:	Millimeter
R^2	:	Coefficient of determination
v/w	:	Volume by weight
W	:	Watt
w/w	:	Weight by weight

LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	:	Analysis of variance
e.g.	:	for example
etc.	:	Etcetera
CO_2	:	Carbon dioxide
DF	:	Degree of Freedom
FT-IR	:	Fourier-transform infrared spectroscopy
GC-MS	:	Gas chromatography–mass spectrometry
HCA	:	Hierarchical Cluster Analysis
i.e.	:	That is
n.d.	:	No date
NIST	:	National Institute of Standard and Technology
PCA	:	Principal component analysis
RM	:	Ringgit Malaysia

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Appendix A: Images of ginger plants, ginger rhizomes and collection site in Janda Baik………………………………………………………. 108

CHAPTER 1: INTRODUCTION

1.1 Background of ginger

Zingiber officinale, commonly known as ginger, is an herbaceous perennial plant belonging to the family *Zingiberaceae* of the genus *Zingiber*. This herbal plant is native to warm tropical climates, particularly India and the Southeast Asia, and was introduced to European countries by Arab Traders. Today, gingers are extensively cultivated in China, Africa, Nepal, Australia, Nigeria, and most Southeast Asian countries including Thailand, Indonesia, and Malaysia. Asians are known for using ginger in their cooking (Chan et al., 2011). People tend to value the underground stems of the plant (or the ginger rhizome) in contrast to the ginger leaves since it is usually used as a cooking spice, food condiment, or herbal remedy (Shahrajabian et al., 2019). Hence, this research is confined to the ginger rhizome because of its multitudinous applications.

1.1.1 Production of ginger

According to the data in 2019 by Food and Agriculture Organization of the United Nations (FAO), the global ginger production in the past several years has mainly been attributed to Asian regions (81.8%) followed by Africa (17.6%), Americas (0.4%), and Oceania (0.2%). In 2017, the global ginger production reached 300 kilotons with India being the largest producing country and contributing to approximately 35% of the world's total production (1000 kilotons) followed by China (580 kilotons), Nigeria (349 kilotons), Nepal (279 kilotons), Indonesia (216 kilotons), and Thailand (167 kilotons).

Malaysia ranks 13th on this list, with an overall production reported to be about 14 kilotons from an area of 974 ha, considerably lower compared to its neighboring countries; Thailand and Indonesia (Food and Agriculture Organization of the United Nations, 2019). In fact, Malaysia imported about 30 kilotons of ginger from other countries such as India, Thailand, and China to fulfill domestic needs (Gan, 2017). Numerous strategies and agricultural programs have been introduced by the local government including better incentives and agriculture schemes, and land preservation initiatives. The government has also provided sufficient infrastructure to boost crop production and to reduce dependency on imported products. These relentless endeavors have borne fruit, as the ginger production in the country has improved by a small increment over the years, as shown in Figure 1.1.

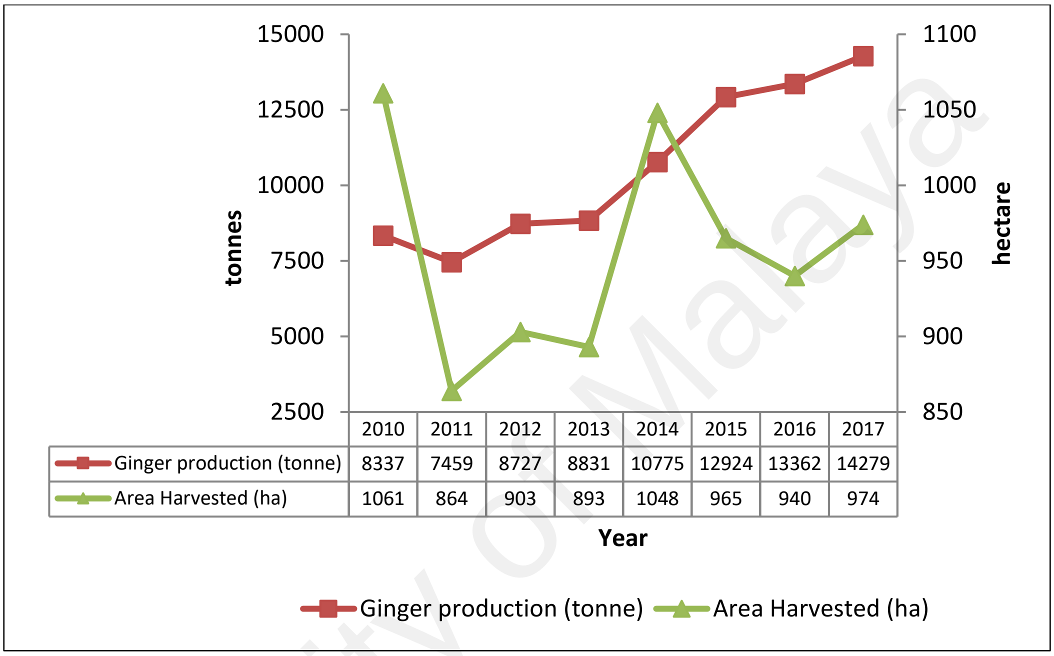

Figure 1.1: Total ginger production and total area harvested in Malaysia from 2010-2017 (source: http://www.fao.org/faostat/en/#data/QC)

The ginger crop is well suited to cultivation in a tropical climate, which has a total annual rainfall of 2,000–2,600 mm (Rashid et al., 2013). In Malaysia, the main ginger-cultivating sites are situated in Sabah (Keningau and Tambunan), Pahang (Bentong), Selangor, Sarawak, and Johor, as shown in Figure 1.2 (Suhaimi et al., 2016). This plant species has adapted in well-drained soil such as lateritic loam, sandy loam, red loam, and clay loam. A friable loam with pH 6-6.5 that is also rich in humus is optimal for growing ginger (Sharifi-Rad et al., 2017). Ginger is propagated using mature rhizomes that are more than 9-month old (or above), also called seed rhizomes. The preserved seed rhizomes are cut into smaller pieces of 2.5–5.0 cm length with each piece normally containing at least two buds (Suhaimi et al., 2012). Young ginger can be harvested after

3–6 months while mature ginger can be harvested 8–10 months after planting. Throughout the years, many ginger-based products have been introduced in the form of powder, instant drinks and personal care products.

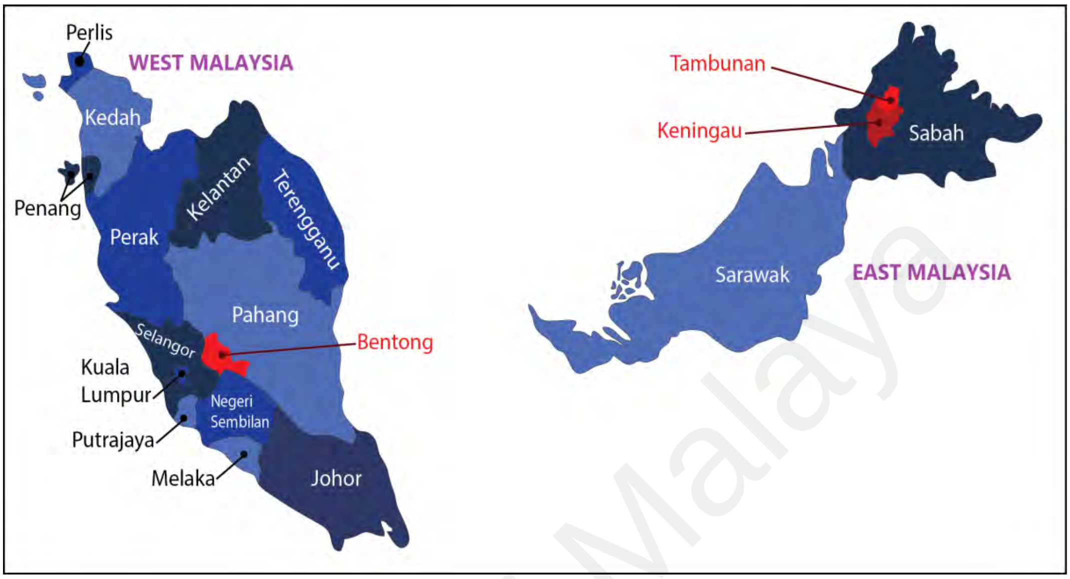

Figure 1.2: Ginger producing sites in Malaysia

1.1.2 Indicators of ginger quality

The term quality is often subjected to a high standard/value of product, and “degree of excellence or its suitability for a particular purpose” (Abbott, 1999). Each individual usually has different perceptions of product quality, as decisions are made solely based on personal preference and need. It is commonly viewed that a good quality product will attract more consumers; hence, industries, as well as the scientific community, should work intensively to improve existing product quality assessments.

Recent studies have merely focused on assessing the external and internal qualities of plant materials, as stipulated by Titova et al. (2015) and Alam et al. (2018). This approach is in agreement with the guidelines by the World Health Organization (2011) for the quality control of medicinal plants. Similarly, a report by Barrett et al. (2010) also regarded these elements as critical factors for defining product quality and consumer acceptability. External quality denotes the physical and morphological properties of the plant material, which includes shape, size, gloss, and color, while internal quality is typically interpreted using chemical means that are not easily perceived by the naked eye such as flavor (taste and aroma) and nutritional value (Camelo, 2004). Therefore, the quality of ginger could be determined based on its morphological characteristics, taste attributes (oleoresin) or aroma attributes (volatile oil).

1.1.2.1 Morphological characteristics

Brito et al. (2016) stated that visual appearances could provide a reasonable indication of the quality of plant material to a certain extent. Based on selected morphological characteristics, Dharmadasa et al. (2014) managed to differentiate *Pogostemon heyneanus* varieties. Based on the evaluation of both macro- and micro-morphological properties, the *Medicago sativa* L. plant was discriminated according to its respective cultivars (Moawed, 2016). Similarly, the same approach was demonstrated in Ahmed et al. (2016) when characterizing the species of several medicinal plants. However, morphological indications become complicated and unfeasible in the absence of appropriate quality describers particularly for dried, comminuted, and processed plant materials (Kumar et al., 2016; Yu et al., 2018). For example, such limitations have caused much concern over the improper sorting and grading of Nepalese gingers (Asia Network for Sustainable Agriculture and Bioresources, n.d.). Table 1.1 and Figure 1.3 show the typical morphological characteristics of the ginger rhizome. Due to a major morphological resemblance, the evaluation of internal attributes such as oleoresins or volatile profiles may overcome the above-mentioned limitations.

Table 1.1: Descriptions of a typical ginger rhizome

Morphology	Descriptions	References
Size	5–15 cm length: 3–6 cm width; 0.5–1.5 cm thickness	Sharma, 2017
Shape	Laterally compressed, short flattened ovate with oblique fingers or branches on the top-side. The fingers are 1 cm to 3 cm long and terminate in a depressed scar	Eltahir et al., 2018; Ghosh et al., 2011
Surface	Longitudinally striated or fibrous	Ghosh et al., 2011
Color	Yellowish-brown	Rashid et al., 2013
Odor	Aromatic	

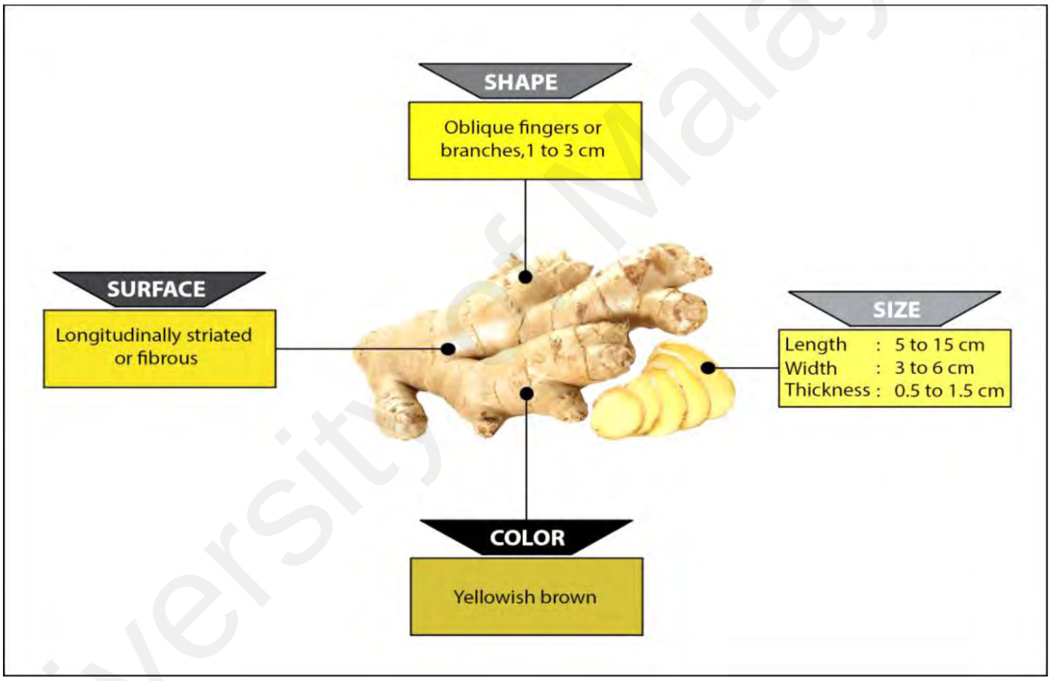

Figure 1.3: The morphological structure of ginger rhizome

1.1.2.2 Oleoresins

It is widely known that the distinctive taste of ginger is mostly attributed to its oleoresin components (Eleazu & Eleazu, 2012). In terms of quality, the superiority of ginger is reportedly associated with high oleoresin content (Goudar et al., 2017). Such non-volatile extracts comprise major bioactive constituents that lend ginger its pungent principles (Pradhan, 2013). Based on the findings of Jayashree et al. (2014) and Yudthavorasit et al. (2014) it is suggested that the relative content of these pungent compounds could plausibly serve as a quality indicator. 6-gingerol is the key pungent principle alongside its dehydrated-forms (shogoal and zingerone) where the latter compounds are promoted through the conversion of thermo-labile gingerol, as illustrated in Figure 1.4.

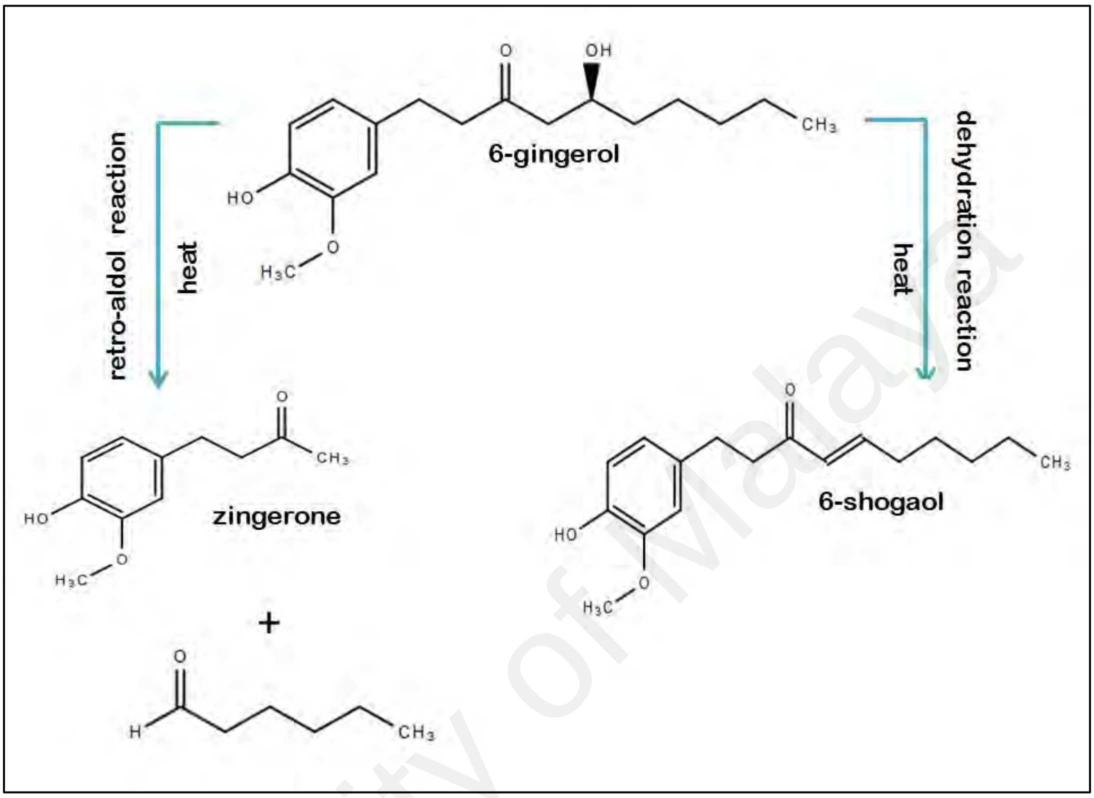

Figure 1.4: Degradation reaction scheme from gingerol to zingerone and shogaol (adapted from Gupta et al., 2016)

1.1.2.3 Volatile oils

Besides the oleoresins, the quality of ginger, namely aroma, is characterized by the chemical mixture present in its volatile oils. As described by Yadav et al. (2004), the content of the volatile oil is deemed as one of the most common and important measures for specifying the quality of ginger and ginger products for international trade (export and import). This stipulation is in accordance that of Nampoothiri et al. (2012) and Sukari et al. (2008), who recommended volatile profiling as indispensable for the quality control and authentication of ginger.

Figure 1.5 lists the common volatiles in ginger, of which many are responsible for its distinctive odor. For instance, the citral (geranial and neral) and α-terpineol in the ginger produce a citrus-like aroma while oxygenated sesquiterpene such as nerolidol is responsible for its woody aroma. Meanwhile, citronellyl acetate and citral also contribute to the fragrance of ginger oil (Morsy, 2017).

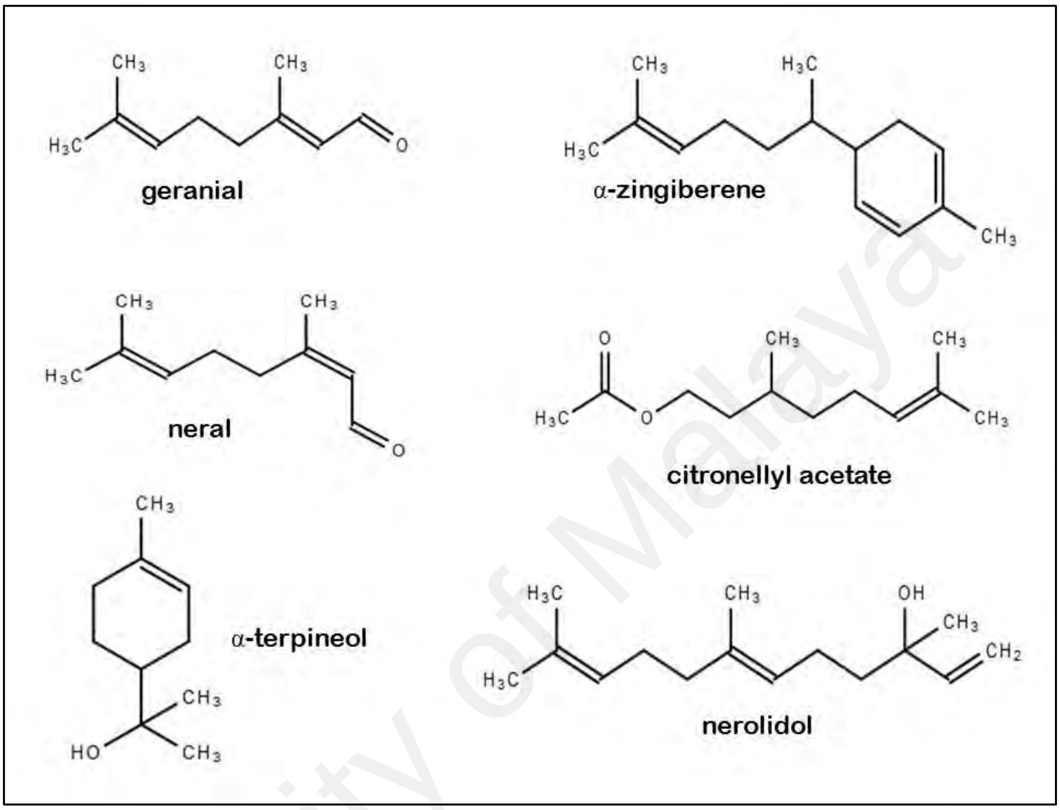

Figure 1.5: Common volatile components reported in ginger oil

1.1.3 Variability of ginger oils

Many factors could contribute directly or indirectly towards the compositional variations in ginger oil. These factors boil down to two main variations i.e. the plant materials themselves and the oil preparation method (Amechrouq et al., 2015; Benomari et al., 2016; Buang et al., 2014; Hasmita et al., 2015).

1.1.3.1 Geographic origin of plant material

It is generally agreed that the geographic origins i.e. the growing/cultivation regions of a plant somehow reflect the quality of the plant and could influence the chemical distributions including the volatile oils in the plant material. Various volatile components have been found to vary according to geographic origins, as reported for a wide spectrum of plant species such as rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita*), etc. (Hannour et al., 2017; Marwa et al., 2017; Meziane-Assami et al., 2013; Satyal et al., 2016). It is believed that the deviation in cultivation practice, soil, and climate conditions across geographical regions induce the variations in the ginger oil produced (Hasani et al., 2017; Jamshidi et al., 2009).

1.1.3.2 Preparation methods

Under normal practice, plant samples are subjected to pre-treatment such as washing, peeling, drying, and comminution before extraction. Conventional washing involves immersing the ginger rhizome in boiling water followed by drying under the sun; but pressure washing is preferred in modern-day, as it is more efficient for reducing microbial load (Plotto, 2002). Peeling or scrapping facilitates the drying process as well as minimizes mold growth and fermentation; however, these steps might also degrade the volatile constituents and some pungency characteristics of the plant (Yiljep et al., 2005). Dehydration or drying is arguably the most fundamental preservation practice, as it can conserve several quality aspects of ginger. The process helps to reduce the moisture content to 8%–10%, correlating to about 60%–70% weight loss (Plotto, 2002). The significant removal of moisture content delays deteriorative biochemical changes and controls microbial spoilage. Nevertheless, these processes may alter the physical, nutritional, anti-oxidant, and organoleptic properties of the sample material (Ding et al., 2012; Zhang et al., 2017b).

Larger plant materials are normally mechanically reduced in size into smaller solid masses, or coarse or fine particles via cutting, chopping, crushing, grinding, pulverizing, or a combination of all the above (Tischer et al., 2017). This step aims to increase the surface area of the sample materials, thus, facilitating the mass transfer of the volatile oil during extraction. This process not only affects the yield but could also drive up the quality of the extracts (Jalgaonkar et al., 2013; Rao et al., 1999; Tischer et al., 2017).

Other than those described above, other aspects related to the extraction process such as extraction techniques and duration are also crucial factors that affect the quality and quantity of volatile oils (Gavahian et al., 2015; Hasmita et al., 2015). In sum, a reasonable mix of all the above factors would result in the production of ginger oil with the desirable yield and quality.

1.2 Bentong Ginger

The Bentong region of Pahang covers 183,112 hectares of area that is mostly hilly and suitable for agricultural development. This region is synonymous with its premier agriculture produce known as the "Bentong ginger", which is mostly cultivated nomadically in the highland slopes, particularly in Kampung Bukit Tinggi and Kampung Janda Baik of Bentong district. It is believed that the geography in which the gingers grow, the climatic conditions, as well as agricultural practice, are crucial factors that determine its superior quality.

Bentong gingers have bigger rhizomes than normal ginger. This variety is pale in color externally, light yellow internally, and has low fiber content and unique sensory attributes (Suhaimi & Mohamad, 2012). Possibly due to consumer preferences and market demand, its price tag is relatively higher (~RM 24 per kilogram) compared to common ginger, which is sold at a rate between RM 5 and RM 8 per kilogram (Tho, 2018). Because of its renowned specialty, premium gingers are vulnerable to the risk of fraudulence such as substitution with cheaper gingers (usually of lower quality) but retailed at the same prices. Such unscrupulous acts could be motivated by the desire to gain quick profits, a lack of moral and ethical values, as well as inadequate enforcement of the law.

One of the preventive measures that have been enforced is the introduction of a quality label, namely a geographical indication (GI) by the Malaysia government agency, the

Intellectual Property Corporation of Malaysia (MyIPO). At present, Bentong ginger is registered under the GI scheme by the Agriculture Bureau of Pahang to sustain market recognition as well as to ensure its quality and reputation remain intact. Other scientific control measures could also be derived through an exploratory investigation of the candidate quality describers of Bentong ginger.

As previously mentioned, the volatile profile of ginger oil could provide a reasonable "fingerprint" that could reveal the identity of the plant materials. The variation in these patterns could help the research community to gain insight into the marked attributes or superiority of Bentong ginger. In this context, the distinctive characteristics and patterns of ginger can be summarized with the aid of chemometrics (Blank et al., 2015; Jayashree et al., 2014; Liu et al., 2014; Satyal et al., 2016).

1.3 Objective of Research

This study aims to explore the chemical variability in the volatile oil extracted from Bentong ginger. To achieve this aim, the below research objectives were developed:

- To investigate the variations in the volatile oils of Bentong ginger related to the sample preparation methods, sample size and extraction duration using Factorial Design
- To evaluate the chemical variability in the volatile oils derived from Bentong ginger via Exploratory Analysis

CHAPTER 2: LITERATURE REVIEW

2.1 Terpenes/Terpenoids

Volatile oils consist of a multitude of organic components, mainly from the family of terpenes. The basic structure of terpenes is made up of 2-methyl-1,3-butadiene or isoprene units. Terpene group compounds are usually fairly hydrophobic with molecular weights below 300 Daltons and they can be classified into two categories: hydrocarbons and oxygenated compounds. The first category of compounds is hydrocarbons (commonly refer to terpenes) which only consist of hydrogen and carbon molecules, whereas the oxygenated forms, also known as terpenoids, are the second category of components that contain hydrogen, carbon and oxygen that belongs to various chemical classes such as alcohols, ketones, aldehydes, esters, ethers or oxides and phenol. The biosynthesis of terpenes (the term terpene is used to indicate both terpenes and terpenoids) involves two primary metabolic processes (a) mevalonate (b) mevalonate-independent pathway (Mahato et al., 2017). The classification of terpenes can also be subjected to the number of isoprene units in the structure: hemiterpenes (1 unit), monoterpenes (2 units), sesquiterpenes (3 units), diterpenes (4 units), sesterterpenes (5 units) and poly-terpenes (many units). Generally, monoterpenes and sesquiterpenes are the most common and abundant terpenes present in the plant volatile oils.

2.1.1 Monoterpenes

Monoterpenes are derived by the attachment of two isoprene units, each of which is formed by a 5-carbon base. These compounds are synthesized in plastids and are relatively volatile, due to their lower boiling points (140-180 °C) (Gutensohn et al., 2012; Moghaddam & Mehdizadeh, 2016). They are naturally present as hydrocarbon or as the oxygenated forms and are often thought to have essential pharmacological uses. Pinenes exhibit fungicidal activity (Silva et al., 2012). A review by Koziol et al. (2014) suggested that β-mycrene is associated to estrogenic activity whereas the oxygenated monoterpenes such as linalool, citral and citronellal pose antimicrobial properties. Other than medicinal uses, some monoterpenes especially the oxygenated derivatives are highly odoriferous and have been useful as fragrance in beverages, cosmetics and perfume industries. L-borneol and eucalyptol are found in *Rosmarinus officinalis* (rosemary) oil and are said to has camphor-like odor while L-carvone in volatile oil of *Mentha spicata* (spearmint) has spicy-minty and sweetish medium-strength odor (Morsy, 2017).

2.1.2 Sesquiterpenes

Sesquiterpenes are made up from the combination of three isoprene units and are the second most abundant compounds present in volatile oil of plant after the monoterpenes. The production of these metabolites is said to occur in the cytosol (Gutensohn et al., 2012). Volatile oils containing sesquiterpenes are less volatile compared to the volatile oil consisting monoterpenes as the constituents have lower vapor pressure and their average boiling points are 100 °C higher than monoterpenes (>200 °C) (Espinoza et al., 2016). Similar to the monoterpenes, sesquiterpenes and their oxygenated forms pose some medicinal values. Hydrocarbon sesquiterpenes such as germacrene-D and β-caryophyllene have been reported to exert antibacterial effects (Vieira et al., 2009). Zhao et al. (2010) have discovered a strong antioxidant activity in volatile oil of *Curcuma* due to the presence of certain sesquiterpenes and their oxygenated derivatives. Some authors have reviewed the pharmacological activities and mode of actions of the sesquiterpenes and their oxygenated forms from several medical plants (Awouafack et al., 2013; Dosoky & Setzer, 2018). Besides the biological activities, the sesquiterpenes could also be used as flavoring agents as some of these metabolites such as ar-curcumene and β-sesquiphellandrene are said to be partly associated to the distinctive flavor of ginger. Similarly, the cis- and trans-sesquiphellandrol are regarded as the essential contributors to the ginger flavor (Zachariah, 2008).

2.2 Volatile oil Extraction Techniques

Apart from the origin of the plant material, the preparation process also affects the variability in volatile components in an extract. Therefore, the choice of extraction technique, its settings, and its constraints should be properly controlled to preserve the quality of the extracted oil. In brief, most of the extraction processes will progress through the following steps: (1) the solvent permeates into the solid matrix (plant material); (2) the solutes dissolve in the solvent; (3) the solute-water solution diffuses out of the solid matrix; (4) the extracted solutes collected (Zhang et al., 2018).

2.2.1 Conventional extraction methods

Water is normally preferred over organic solvents as the extraction medium because of its cost and green advantages. Moreover, it is also immiscible in oil and therefore facilitates the purification process of the volatile oils. Because of these reasons, conventional hydrodistillation and steam distillation are widely used in the industry.

2.2.1.1 Hydrodistillation

Hydrodistillation is the oldest and simplest technique for the separation of volatile oils from plant materials. This method uses boiling water. The hydrodistillation process involves direct contact between the samples and the heat transfer medium i.e. water. At the same time, the surrounding water molecules also act as a barrier that protects the derived volatiles from overheating (Tongnuanchan & Benjakul, 2014). The volatile components and the water form an azeotropic mixture, which evaporates together at similar pressure, subsequently condensing and separating because of their densities and immiscibility (Golmohammadi et al., 2018). Under normal circumstances, extraction is carried at atmospheric pressure but it could also be performed in a vacuum at a lower temperature to reduce the impact of heat exposure (Milojevic et al., 2013). Table 2.1 several hydrodistillation conditions and the volatile recoveries of various plant species.

Table 2.1: Hydrodistillation of volatile oils from various plant materials

Material	Operating Conditions	Major Components	Reference
Zingiber officinale	2 kg/5 L of water, 24 h	Camphene, 1,8-cineol, α-pinene,	Sa-Nguanpuag et al., 2011
Thymus daenensis	100 g of dried plant material, 3 h	Carvacrol, thymol, p-cymene, γ-terpinene	Rowshan et al., 2013
Amorpha canescens	50 g of plant material, 3 h	Germacrene D and germacrene D-4-ol	Lis et al., 2014
Mentha piperita L.	1000 mL of water, 3h	Menthol, menthone, menthofuran	Beigi et al., 2018
Rosmarinus officinalis L.	100 g/800 mL of water, 3 h	Cineole, camphor	Elyemni et al., 2019

Gavahian et al. (2015) evaluated the structural changes in *Mentha piperita* L. (peppermint) when subjected to hydrodistillation and steam distillation. They reported that steam distillation caused the glands of the plant to erupt while hydrodistillation caused the glands to wrinkle and break down to some extent, resulting in a higher yield. According to Mahfud et al. (2017) hydrodistillation techniques are also practically useful for source materials with smaller particle size. The immersion of a comminuted sample into boiling water was found to enhance the heat transfer area per unit mass; as opposed to steam distillation, where the sample materials became relatively packed and would more likely agglomerate, thus hampering the extraction process.

2.2.1.2 Steam distillation

As its name suggests, the oil extraction process in steam distillation is carried out via a current of steam. By such extraction means, there are risks that the oil components could degrade due to hydrolysis, but these risks can be controlled better than hydrodistillation and it is more suitable for thermo-labile aromatics. The steam distillation technique can be classified into two general categories i.e. "wet" and "dry" (Axtell & Fairman, 1992; Tandon, 2008). "Wet" steam distillation (commonly known as water and steam distillation) is a combination of conventional hydrodistillation and is the preferred steam distillation method for lab-scale extraction. During the extraction, the sample materials are placed above a water reservoir in an alembic or on a perforated grid. The steam produced from the reservoir helps release the aromatic components by piercing and breaking down the cell structure of the sample materials. The volatile oils are passed through a cooling system, condensed, and collected. One of the major drawbacks of this practice is that the condensate formed on the sample would slow down the extraction process. To control such waterlogging, baffle and a properly controlled heating process must be used. “Dry” steam distillation is based on the same extraction principle, but the samples are supported on a perforated grid and are extracted with a saturated steam flow directed from an external boiler instead.

According to literature reports, steam distillation has been widely used in oils and fragrance research. Furtado et al. (2018) reported that this technique managed to yield 0.86% (w/w) of volatile oil from *Blepharocalyx salicifolius* with the major components being bicyclogermacrene, globulol, viridiflorol, γ-eudesmol, and α-eudesmol. Elsewhere, this step was used to derive volatile oils for another study on the antibacterial properties of *Boesenbergia rotunda*, *Curcuma mangga*, and *Kaempferia galangal* (Baharudin et al., 2015). In comparison to the Soxhlet extraction technique, Wong et al. (2014) found that steam distillation produced a lesser oil yield for cinnamon (*Cinnamomum zeylanicum*). Despite this result, the latter is still more preferred, as it resulted in greater recovery of cinnamaldehyde (the characteristic component of cinnamon oil). Charles & Simon (1990) reported that the amount of oil distilled from different parts of basil species was generally higher using this technique compared to hydrodistillation, but no significant difference was observed in its volatile composition. It is believed that the variations in results are mainly associated with the quality of the plant material and could be attributed to differences in operating conditions during the extraction process.

2.2.2 Modern extraction techniques

Conventional techniques are widely used in commercial-scale volatile oil production. With the advent of technological advancements, many extraction techniques have been developed, but some might not necessarily fit the purpose of commercial oil production. These techniques include Microwave-Assisted Extraction and Supercritical Fluid Extraction.

2.2.2.1 Microwave-Assisted Extraction

Microwave-Assisted Extraction has gained increasing importance when it comes to volatile oil isolation, as it offers some advantages over conventional techniques. For instance, this method can improve extraction yield. It also has high extraction efficiency and is suitable for the extraction of thermo-sensitive materials because of its rapid processing time, low energy requirement, and less solvent consumption, as listed in Table 2.2.

Table 2.2: Extraction of volatile oils via Microwave-Assisted means

Technique	Material	Remarks	Reference
Microwave-assisted hydrodistillation	*Thymus vulgaris* L.	Saves energy and extraction time	Golmakani & Rezaei, 2008
	Pogostemon cablin	Better quality, environmentally friendly, less extraction time	Kusuma & Mahfud, 2017b
	Rosmarinus officinalis L.	Minimizes extraction time, increases oxygenated compounds	Elyemni et al., 2019

Table 2.2, continued

Technique	Material	Remarks	Reference
Microwave accelerated steam distillation	*Lavandula angustifolia*	Better yield than steam distillation, accelerated extraction process	Sahraoui et al., 2008
	Citrus auranticum L	High extraction efficiency and less extraction time	Kusuma et al., 2016
Microwave-assisted simultaneous distillation extraction	*Taxus chinensis* var. *mairei*	High yield, effective for the simultaneous separation of volatile oil and polysaccharides, less energy and time consumption	Zhao et al., 2016
Microwave-assisted Soxhlet extraction	*Illicium verum* Hook. f.	No major change in quality	Nam et al., 2017

The working mechanisms of microwave assisted extraction are based on interaction between microwave irradiation and the polar molecules presents in the sample material/solvent medium that lead to migrations of ions and rotation of dipole simultaneously. As the consequence, heat is generated (Tatke & Jaiswal, 2011). In the case of volatile oils extraction using microwave assisted extraction, microwave radiation selectively interacts with the polar molecules i.e. the water present in the vascular tissue, the trichomes, and the glands of the plant samples (Destandau et al., 2013). The generation of heat (by dipole rotation and ionic conduction) leads to liquid vaporization within the cell and internal superheating, which generates tremendous pressure on the cell wall due to the swelling of the plant cell. This pressure pushes the cell wall from inside, lowering the mechanical strength of the wall to the extent that it ultimately ruptures, thus allowing the release of the oil from the sample materials into the surrounding medium

(Destandau et al., 2013; Kusuma & Mahfud, 2017b; Nitthiyah et al., 2017; Veggi et al., 2013).

It is known that extraction is often associated with the process of heat-mass transfer. Similar to conventional extraction, the mechanism of mass transfer in microwave assisted extraction happens from the inside of a substrate to the outside (surrounding). On the other hand, the heat transfer mechanism of microwave assisted extraction is different from those of conventional methods. In the case of conventional extraction such as hydrodistillation, heat transfer takes place from the outside to the inside of the substrate, notably due to convection and conduction occurring via the surrounding water. On the contrarily, the heat transfer in microwave assisted extraction occurs partly from the inside of plant matrix to the water surrounding (heat liberated from the internal heating of in situ water within the cell) and that it was also mostly from the outside water surrounding to the interior of the plant matrix, similar to the conventional process. The synergistic effect of the two transport phenomena, mass, and heat, which are working in the same direction (i.e. from inside to outside), may, therefore, result in a rapid increase in oil yield during the microwave assisted extraction process (Kusuma & Mahfud, 2017b; Sadeghi et al., 2017).

In general, the efficiency of microwave assisted extraction is highly dependent on the property of both solvent and sample matrix i.e the ability to absorb microwave and converting it to heat energy. This property is partly depending on the dissipation factor, $(\tan \delta)$ that can be expressed in the equation below:

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'} \quad (2.1)$$

where ε' is the dielectric constant that represents the ability of the solvent molecules to absorb microwave energy while ε'' stands for dielectric loss, stands for the dielectric loss, which is the efficiency by which microwave energy is converted into heat (Armstrong, 1999; Destandau et al., 2013). Water has the lowest dissipation factor amongst other most commonly used solvent in microwave assisted extraction, due to its high value of dielectric constant. This means that the rate of microwave absorption by the solvent is higher than the rate of heat dissipation (Eskilsson & Björklund, 2000; Veggi et al., 2013). As a result, a rapid increment in the extraction temperature would most likely to occur as shown in the study by Golmakani & Moayyedi (2015). They found that the temperature recorded in microwave assisted hydrodistillation was seven times higher compared to the conventional method and such trend was likely related to the high dielectric constant of the water. In some cases, this phenomenon can facilitate the diffusivity of the compounds in the matrix. Yet, in other cases, it can negatively affect the extraction process such as promoting degradation of the analyte and/or "explosion" of the solvent. Hence, for this extraction process, it is highly advisable to select a solvent with high dielectric constant and dissipation factor to ensure more homogenous heat distribution in the sample as well as prevent from overheating of the samples (Al Jitan et al., 2018).

2.2.2.2 Supercritical Fluid Extraction

Supercritical Fluid Extraction (SFE) is an environmentally friendly and innovative extraction technique that uses supercritical fluid to recover volatile oils. Supercritical fluid refers to any solvent at a supercritical state (above or near its critical temperature and pressure), which normally possesses intermediary properties between liquid and gas. Supercritical CO_2 (supercritical carbon dioxide) is the most commonly used supercritical fluid due to its good extractive capacity and less toxicity. Moreover, its critical point at 30.9 °C and 73.8 bar is easily accessible. This advantage enables the extraction process to be performed at mild operating conditions (40–60 °C), which benefits the recovery of thermo-labile compounds. It is generally agreed that CO_2 is an excellent solvent for extracting non-polar species such as alkenes and terpenes, so it is widely used to extract volatile oils (Table 2.3).

Table 2.3: Application of supercritical CO_2 for the extraction of volatile oils

Material	Application Purpose	Reference
Thymus vulgaris	A study on supercritical CO_2 extraction of volatile oil based on a mathematical model	Melo et al., 2000
Marchantia convoluta	An investigation into the effect of extraction parameters on oil yield	Xiao et al., 2007
Schinus molle L.	Extraction of volatile and non-volatile compounds via supercritical CO_2	Barroso et al., 2011
Tetraclinis articulata	A study on the effect of extraction conditions and extraction capacity on volatile and non-volatile content	Herzi et al., 2013
Citrus sphaerocarpa Tanaka	A comparison between supercritical CO_2 and conventional methods and an optimization study	Suetsugu et al., 2013
Zingiber officinale Roscoe	A study on the effect of extraction parameters on oil yield	Mesomo et al., 2012
Rosmarinus officinalis	A comparison of the performance between hydrodistillation and supercritical CO_2	Ouzzar et al., 2015

In addition to CO_2, alternative solvents such as water, nitrous oxide (N_2O), and ethane can also be used for SFE, particularly for the selective extraction of either polar or non-polar constituents of plant metabolites. The application of supercritical water for extraction is limited due to its inaccessible critical point and corrosive nature (Capuzzo et al., 2013). Although N_2O is an explosion risk, it could still benefit the extraction of organic bases. Supercritical ethane has the same capability to carry out extraction tasks as that of supercritical CO_2 albeit at a considerably lower pressure and using a lower solvent-to-feed ratio. Due to potential explosion and health risks associated with its use, however, supercritical ethane is not favorable (Raeissi et al., 2008).

Other than the type of solvent used, the efficiency of SFE is also influenced by its operational settings such as extraction pressure, CO_2 flow rate, and particle size. Generally, most works agree that increasing extraction pressure and flow rate will enhance extraction yield, with similar results also being observed for samples with reduced particle size (Melo et al., 2000; Norodin et al., 2016; Suetsugu et al., 2013).

According to Hu et al. (2013), the extraction of *Cuminum cyminum* oil yields was most affected by the following factors (in ascending order): pressure > CO_2 flux > particle size > temperature. Also, the use of a modifier and fluid flow rate adjustment could affect extraction efficiency (Khaw et al., 2017; Xiao et al., 2007; Xu et al., 2011).

It is worth noting that the use of conventional techniques is still preferred over modern extraction techniques, as they involve simple operational processes that produce high quality volatile oil and more importantly, they are generally cost-effective. Based on the above literature review, it can be noted that the variability in the quantity and the quality of volatile oils greatly depends on the extraction technique as well as the origin of the sample materials. Even with the same technique, a deviation in operational settings could also contribute to some variations to a certain extent (Hasmita et al., 2015).

2.3 Chemometrics

Chemometrics refer to the use of mathematical and statistical approaches to manipulate and analyze chemical data for diverse objectives—an application commonly driven by the complexity of the multivariate nature of experimental data (Roussel, 2014). Chemometrics applies across several main areas including design of experiment and pattern recognition, further discussed in sections below (O'Donnell et al., 2014; Olsson, 2006).

2.3.1 Design of Experiment

A Design of Experiment (DOE) refers to the systematic planning of an experiment for efficient data collection and effective analysis via mathematically modeling the effects and interactions between control factors and responses related to the process under investigation (Durakovic, 2017). The application of DOE would permit a simultaneous study of the controllable factors based on the mathematical relationship established between the factors and the responses being studied (Tarley et al., 2009). Using DOE, the operational cost and the time consumed could be strategized. A study should be conducted with a screening design as the initial step, followed by optimization design (Lawson, 2003). However, in many cases, the experimental objectives, the number of factors and the study constraints also dictate the choice of research design. DOE has been widely applied in the agriculture industry especially, to improve product quality and to develop new products. In most cases, classical design such as Factorial Design is preferred due to its simplicity.

2.3.1.1 Screening study

The main function of a screening study is to shortlist the key factors that affect the response that is defined according to the objective of the experiment, e.g. the yield of extract in this case. This is driven by the assumption that only a few factors may greatly impact the process while others are relatively insignificant. Of the available design options, classical two-level designs e.g. Factorial Designs and Placket-Burman designs are most commonly adopted, where the postulated model for the response could be generalized as below:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \cdots \tag{2.2}$$

where X_i and X_j represent the levels of factor i^{th} and j^{th}; and β_0, β_i, and β_{ij} are the coefficients of the polynomial (Fadil et al., 2015; Setyawan et al., 2018; Tarley et al., 2009). The adequacy of a model can be evaluated statistically i.e. using Analysis of Variance (ANOVA), a lack-of-fit test, and the coefficient of determination (Aydar et al., 2017). Table 2.4 lists several past screening studies that have focused on optimizing the yield of volatile oils/components from various sources.

Table 2.4: Past screening studies based on experimental design

Design	Material	Factor	Response	Remarks	Reference
Full Factorial Design	*Cymbopogon citratus*	Evaporator temperature, volumetric feed flow rate	Citral content	Both factors were significant; higher content with temperature increase	Tovar et al., 2010
	Zingiber officinale	Extraction time, microwave extracting power, sample type	Oil yield	Effect of extraction time > microwave power > sample type	Shah & Garg, 2014
	Zingiber officinale	Sample freshness, size reduction, extraction time	Oil yield	All factors were significant	Ahmad Jahiddin & Low, 2019
Plackett-Burman design	*Rosmarinus officinalis*	Extraction time, individual effect, harvest period, drying, material-t-plant ratio, drying temperature, cutting of the plant	Oil yield	All factors were significant except the cutting of the plant	Fadil et al., 2014
	Pistacia lentiscus	Processing time, individuality, moisture, division of plant material, material-to-plant ratio	Oil yield	All factors were significant except the division of plant material	Haloui et al., 2015
	Mentha piperita	Harvest period, individual effect, cutting of plant, material-to-plant ratio, extraction time, drying temperature, drying process	Oil yield	All factors were significant except the drying process and the cutting of the plant	Fadil et al., 2016

(a) *Factorial design*

Factorial Designs refer to either Full Factorial Designs or Fractional Factorial Design. Full Factorial Designs consist of all the possible combinations of factorial settings while Fractional Factorial Design only includes a select subset of combinations (O'Donnell et al., 2014). A Full Factorial Design is practical for experiments that involve fewer factors—typically fewer than five (Fukuda et al., 2018). The minimum number of runs required is a function of the number of factors (Setyawan et al., 2018):

$$n = l^k \tag{2.3}$$

where n is the number of runs, l is the factor level, and k is the number of factors. Therefore, the number of runs increases drastically as the number of factors increases. In this regard, the two-level Full Factorial Design has been widely applied, where $n = 2^k$ (Braimah et al., 2017; Elazazy, 2017). Such designs are useful for screening the main effects and interactions related to the postulated model (O'Donnell et al., 2014; Wass, 2010).

2.3.1.2 Optimization Study

Optimization designs are practically useful when a linear model is unable to explain experimental data adequately. In such cases, higher-order models must be used to provide the curvature details related to the chosen factors (Fukuda et al., 2018). Based on Response Surface Methodology (RSM), second- and higher-order models supported by optimization designs could reasonably enable the estimation of the optimal region(s) that correspond to the desired response (Aydar, 2018). Of the available response surface designs, Central Composite design (CCD) and the Box-Behnken design (BBD) have been widely used to optimize the yields of volatile oils based on various extraction techniques as shown in Table 2.5. RSM has also been employed in optimization studies focusing on a particular bioactive compound rather than the extracts as a whole. Wang & Liu (2009) adopted the optimization methodology to investigate the solvent extraction conditions for recovering carotenoids from *Brassica napus* L. (rapeseed). Meanwhile, Jang et al. (2017) also used optimization methods to study the ultra-assisted extraction of glycyrrhizic acid from licorice.

Table 2.5: Past optimization studies on volatile oil extracts using RSM

Design	Extraction Technique	Material	Factor	Reference
BBD	Steam distillation	*Eucalyptus tereticonis*	The solid-to-solvent ratio was insignificant; 105 minutes of extraction time and 0.032 kg/h steam rate	Galadima et al., 2012
	Steam distillation	*Zingiber collinsii*	570 mL water to 100 g of material with 1.09 mm particle thickness at 290 min of extraction time,	Chau et al., 2017
CCD	Hydrodistillation	*Zingiber officinale*	2660 mL of water volume at 23.15 hours with medium-size particles	Buang et al., 2014
	Pressurized hot water extraction	*Lavandula angusifolia*	Extraction temperature at 162.76 °C, pressure of 29.84 bar, static time of 10.45 min, dynamic time of 18.12 min, flow rate of 0.69 mL/min	Kamali et al., 2014
	Microwave-assisted extraction	*Rosmarinus officinalis*	85 min extraction time with microwave power of 888 W, and 0.5 mL/g water-to-sample ratio	Akhbari et al., 2018

The integration of experimental designs can facilitate systematic data collection using a reduced number of experimental runs while providing a relevant and reasonable amount of information concerning the system under investigation. The results of this study will serve as a basis for controlling the variability introduced by the extraction settings; thus, enabling a subsequent investigation into the variability due to the samples.

2.3.2 Exploratory Analysis

Exploratory Analysis or unsupervised pattern recognition is the work-horse of Multivariate Analysis. It provides the first insight into the structure of variables and between samples without prior knowledge of the nature or classification of the samples (no predetermined classes) (Yudthavorasit et al., 2014). This technique helps the researcher explore the underlying relationships between the samples, and highlights the relationships between the variables to uncover possible outliers (Biancolillo & Marini, 2018). The most commonly-used techniques include Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

2.3.2.1 Principal Component Analysis

Principal Component Analysis (PCA) converts the original data matrix ($\mathbf{X}_{nm}$) consisting of n samples and m variables into two matrices, namely scores (**T**) and loadings (**P**) while retaining about the same information with a reduced dimension (Yudthavorasit et al., 2014). In simpler words, it involves a mathematical transformation that reduces the number of variables into a manageable number of variables (Jolliffe & Cadima, 2016). This new dataset comprises uncorrelated variables known as principal components (PCs). The first PC (PC1) accounts for the highest variance, and the succeeding PCs denote the remaining variance in the data. All this information allows the detection of potential anomalous or outlying observations, groupings, and trends in the data. It even helps predict the positions of the new samples in the hyperplane (Biancolillo and Marini, 2018). Therefore, PCA has been widely applied for the exploration and authentication of plant materials (Elaissi et al., 2012; Liu et al., 2014; Satyal et al., 2016).

2.3.2.2 Hierarchical Component Analysis

Hierarchical Cluster Analysis (HCA) transforms the observed dataset into a statistical graphical representation by assigning the samples into clusters. The samples are presented in a one-dimensional sequence known as a dendrogram or a hierarchical tree diagram. The degree of the association between the samples is represented in terms of distance, where the samples with a small distance reflect a high degree of similarity and are grouped into the same cluster whereas distant samples are partitioned. As a result, clusters are formed during the mathematical process while the distance pattern makes it simpler to interpret the data. Several studies have demonstrated the feasibility of HCA in discriminating plant materials according to their species and geographical origin (Elaissi et al., 2012; Satyal et al., 2016).

2.4 Authentication of geographical origin

In addition to species authentication, the authentication of geographic origin is a crucial aspect of quality control of raw materials before production (Wang & Yu, 2015). This is because the quality of the products derived from even the same species is somewhat different if the growing environment, namely the geographic origin of the plant, is different. The quality indicators associated with measurable variations provide insightful information about the geographic origin of a product. Therefore, many studies have investigated the effect of geographic origin authentication and traceability via fingerprinting/profiling (Ruiz-Samblás et al., 2013; Sattler et al., 2015; Xu et al., 2019).

2.4.1 Elemental fingerprinting

Elemental fingerprinting/profiling is one of the major approaches for authenticating the geographic origin of plant material. Because the natural diffusional movement of elemental traces follows a common process—transferring from rocks to soil followed by absorption by plants—the plant materials can be discriminated through their elemental compositions. Aside from naturally-occurring processes, accumulation due to other factors such as agricultural practices (e.g. the use of fertilizer and pesticides) and environmental contamination may further alter elemental patterns, which, in turn, enhance the discrimination power of geographic origin.

Atomic spectrometry is the most prominent technique for sampling elemental data for fingerprinting and profiling with the aid of chemometric tools (Luykx & van Ruth, 2008). Geana et al. (2013) showed that such combinations proved practically useful to discriminate Romanian wine according to their provenance. Based on a multi-elemental data matrix derived from combining inductively-coupled plasma (ICP) and multivariate statistics, Kwon et al. (2014) managed to distinguish the geographic origins (Korea and China) of both *Paeonia albiflora* and *Astragalus membranaceus*. The research by Li et al. (2015) was able to trace the geographic origin of *Marsdenia tenacissima* to four provinces in China using pattern recognition techniques i.e. PCA, Linear Discriminant Analysis (LDA), and k-Nearest Neighbor analysis (KNN). LDA has also been used to discriminate between Asian and African teas using a similar approach (Fernández-Cáceres et al., 2001). In terms of non-destructive techniques, Neutron Activation Analysis (NAA) has been conducted to discriminate between phellinus mushrooms from Cambodia and those from Korea and Vietnam (Lim et al., 2012).

2.4.2 Nutritional profiling

Nutritional profiling is another possible means for authenticating plant geographic origins. Besides its importance as dietary information, nutritional profiles have been employed to standardize and authenticate plant materials (Chanda, 2014). Parameters such as carbohydrates, proteins, fats, and fiber content are commonly studied, as various works have proven the considerable impact of geographic factors on the proximate nutrient levels of plants. For instance, significant differences were found in the protein and the crude fiber content of gingers from China and Thailand; this discrepancy could help characterize these gingers (Sultan et al., 2005). The analysis conducted on India and Sri Lanka turmeric also observed differences in the fiber and protein contents (Madhusankha et al., 2019). Haron et al. (2014) also demonstrated variations with respect to concentrations of crude protein and total carbohydrate in seeds of *Nigella sativa* from different geographical origins; Yemen Iran and Malaysia. Hence, the nutritional profile could serve as a diagnostic tool for verifying the quality and regional identity of plant materials.

2.4.3 Metabolite fingerprinting

Metabolite fingerprinting is widely adopted for authentication purposes, including for authenticating the geographic origins of plant materials. The metabolomic variations in sample materials could selectively be captured using appropriate chromatographic and spectrometric factors, for instance, gas Chromatography-Flame Ionization Detector (GC-FID), Gas Chromatography-Mass Spectrometry (GC-MS), Ultra/High-Performance Liquid Chromatography (U/HPLC), Near-Infrared (NIR) Spectroscopy, Nuclear Magnetic Resonance (NMR) Spectroscopy, etc. In the case of this study, GC-MS is considered one of the best options for providing the required information about the volatile metabolites related to the quality of flavor and the fragrance of Bentong ginger. Table 2.6 displays a wide spectrum of metabolite fingerprinting techniques that have been used to authenticate plant geographic origins.

In most cases, the incorporation of chemometrics is extremely helpful to reveal the underlying metabolomic variations and patterns from collected data. If the selected metabolic compositions have sufficient discriminatory power, together, they will form a characteristic "fingerprint" of the plant for screening and authentication purposes. For instance, Yudthavorasit et al. (2014) demonstrated the potential of gingerols and their derivatives in the regional discrimination of ginger samples from Thailand, China, India, Vietnam, and Malaysia using HPLC and chemometrics; where multiple metabolic fingerprints proved necessary to enhance the discriminatory power in certain cases. Similarly, the concentrations, distributions, and proportions of certain terpenes from *Zingiber officinale* were also found to vary with respect to country of origin, therefore suggesting the potential of using terpene markers for regional discrimination (Nampoothiri et al., 2012; Raina et al., 2005; Sultan et al., 2005).

Table 2.6: Geographic origin authentication using various analytical techniques

Extract	Technique	Material	Metabolites	Origin	Reference
Volatile oils	GC-MS	*Rosmarinus officinalis*	Terpene composition (α-pinene and camphor)	Multiple regions in Algeria	Meziane-Assami et al., 2013
	GC-MS	*Thymus vulgaris*	Terpenes composition	France and Serbia	Satyal et al., 2016
	GC-MS	*Ajuga laxmannii*	Terpene composition	Multiple regions in Turkey	Köse et al., 2015
	GC-MS	*Salvia ceratophylla*	Terpenes composition	Multiple regions in Turkey	Başer et al., 2015
Solvent extract	HPLC-DAD	*Olea europaea*	Phenolic composition	Multiple regions in Portugal	Vinha et al., 2005
	UPLC	*Allium sativum*	Allicin content	Egypt and China	Mostafa et al., 2015
	NMR	*Astragalus* roots	Saponins and isoflavonoids content	Multiple regions in China	Zheng et al., 2015
Powdered raw plant	NIR	*Ganoderma lucidum*	Polysaccharides and triterpenoid saponins content	Multiple regions in China	Chen et al., 2008

CHAPTER 3: METHODLOGY

3.1 Overview of research methodology

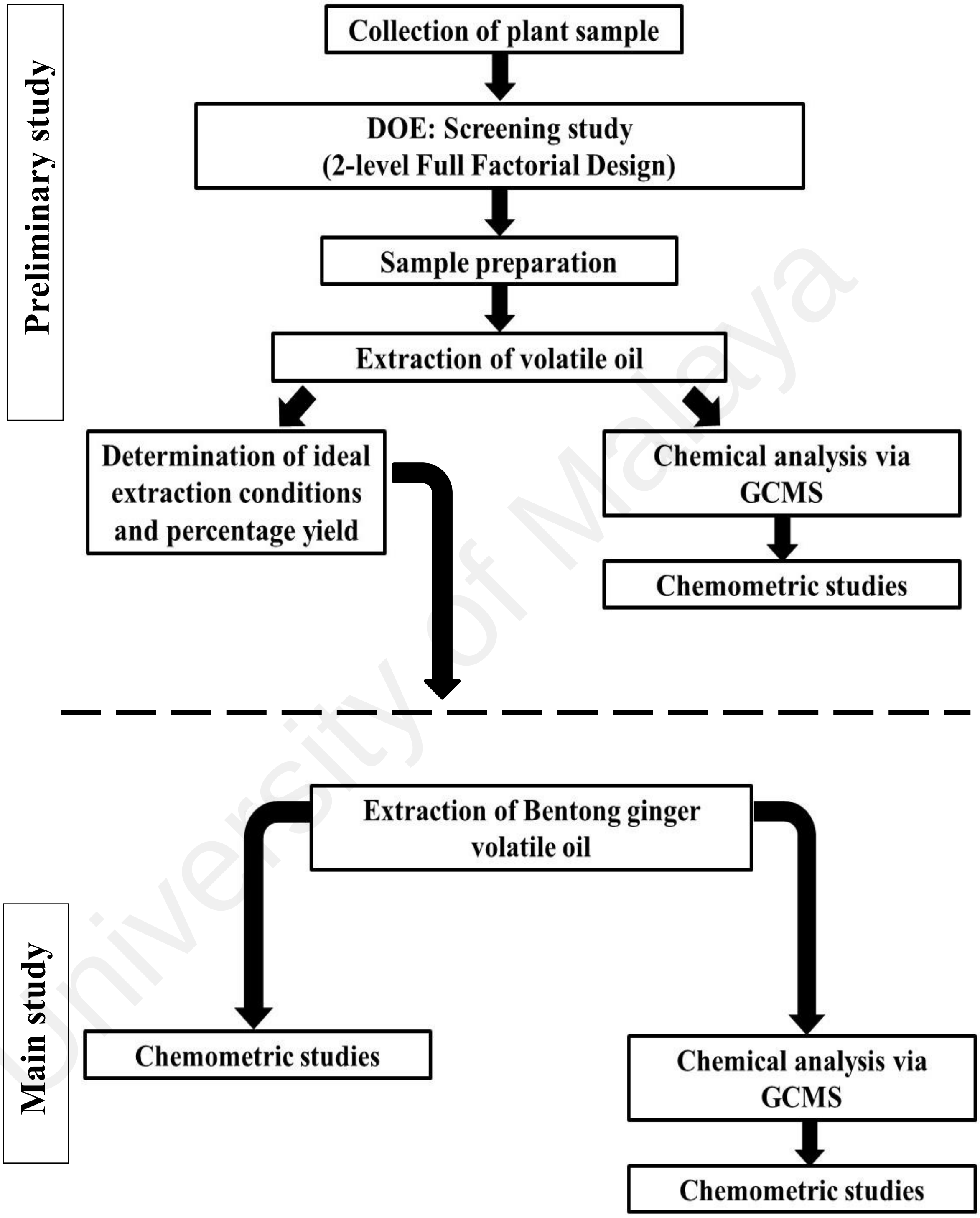

Figure 3.1: Overview of the research methodology

3.2 Sampling of plant materials

Figure 3.1 shows the flow of the study that can be divided into two parts; the preliminary study and the main study. In the preliminary study, 4 kg of fresh Bentong gingers were sampled from a ginger plantation located at Janda Baik, Pahang, to model the relationship between various factors and the volatile oil yield of ginger. The study also aims to explore the effects of these factors on the variability of terpene content in the ginger. For metabolomic fingerprinting, fresh Bentong gingers (BTG) were sampled from different plantation sites in Janda Baik, Pahang, between May 2017 and January 2018, whereas non-Bentong gingers (NG) were purchased from local markets around Kuala Lumpur and Selangor for comparison purposes.

3.3 Experimental design

Table 3.1 indicates the domain of the factors under investigation while Table 3.2 shows a two-level Full Factorial Design matrix with duplicate runs for each treatment that was constructed using JMP Pro 12 Software (SAS, USA) with the runs set in random order. A full factorial of three factors requires $2^3 = 8$ experiments and when it is replicated two times, it will generate 16 experiments. In this study, the experimental yield of volatile oil is defined as:

$$y = \frac{M_0}{M_1} \times 100\% \tag{3.1}$$

where M_0 is the mass of the oil (g) and M_1 is the mass of the feed (g), which can be analyzed using statistical methods such as regression models (Linear, Interaction or Quadratic) (Aydar, 2018). A quadratic model is typically used for optimization study (response surface design) instead of screening study. As suggested by Tarley et al. (2009), a quadratic model requires all the factors to be at least three level and that the projection of two-level factorial design does not fit a quadratic model. The most common designs for this model are the three-level factorial, Box-Behnken design (BBD) and Central

Composite design (CCD). Thus, in this screening study, the output response (percentage yield) is postulated as:

$$\hat{y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_{12} + \beta_{13} X_{13} + \beta_{23} X_{23} + \beta_{123} X_{123} + \varepsilon \tag{3.2}$$

The mathematical model was evaluated with Analysis of Variance (ANOVA) (Fukuda et al., 2018).

Table 3.1: Study domain of the design

Factor	Low Level (-1)	High Level (+1)
Sample freshness, X_1	Not treated	Microwave-treated
Size reduction, X_2	Grating	Slicing
Extraction duration, X_3	3 h	6 h

Table 3.2: Two-level factorial design

Run number	X_1	X_2	X_3
1	+1	+1	-1
2	+1	+1	+1
3	+1	+1	-1
4	-1	+1	+1
5	-1	-1	+1
6	+1	-1	-1
7	-1	-1	+1
8	-1	-1	-1

3.4 Laboratory preparation

Fresh ginger rhizome samples were washed to remove soil and dirt before subjecting them to different treatments according to the design matrix (Table 3.2).

3.4.1 Size reduction

Based on the design, the sample was cut into two nominal sizes by either grating or slicing. The grating was carried out using a Kenwood Food Processor FP120 (400W motor), which gave a thickness of ~4 mm whereas slicing was done with a knife, which provided a thickness of ~10 mm.

3.4.2 Microwave treatment

For the preparation of the dried ginger samples, ~500 g of freshly grated/sliced rhizomes was heated in a household microwave oven (Samsung, ME711K) equipped with a swirl tray at 800 W for 21 min.

3.4.3 Hydrodistillation

The extraction of volatile oils was performed via laboratory-scale hydrodistillation using a Clevenger-type apparatus as in Figure 3.2. For the preliminary study, a series of extraction was carried out per the sequence designed in Table 3.2. Briefly, 500 g of the pre-treated sample materials were immersed in 1000 mL of ultrapure water (UPW) and boiled for 3 hours or 6 hours as per the run. The distilled oil was subsequently collected and dried over anhydrous sodium sulfate to remove traces of water. The recovery of the oil was kept in an amber vial at ~4 °C until ready for the GC-MS analysis. For metabolomic fingerprinting, hydrodistillation was performed according to the optimum conditions derived from the preliminary study.

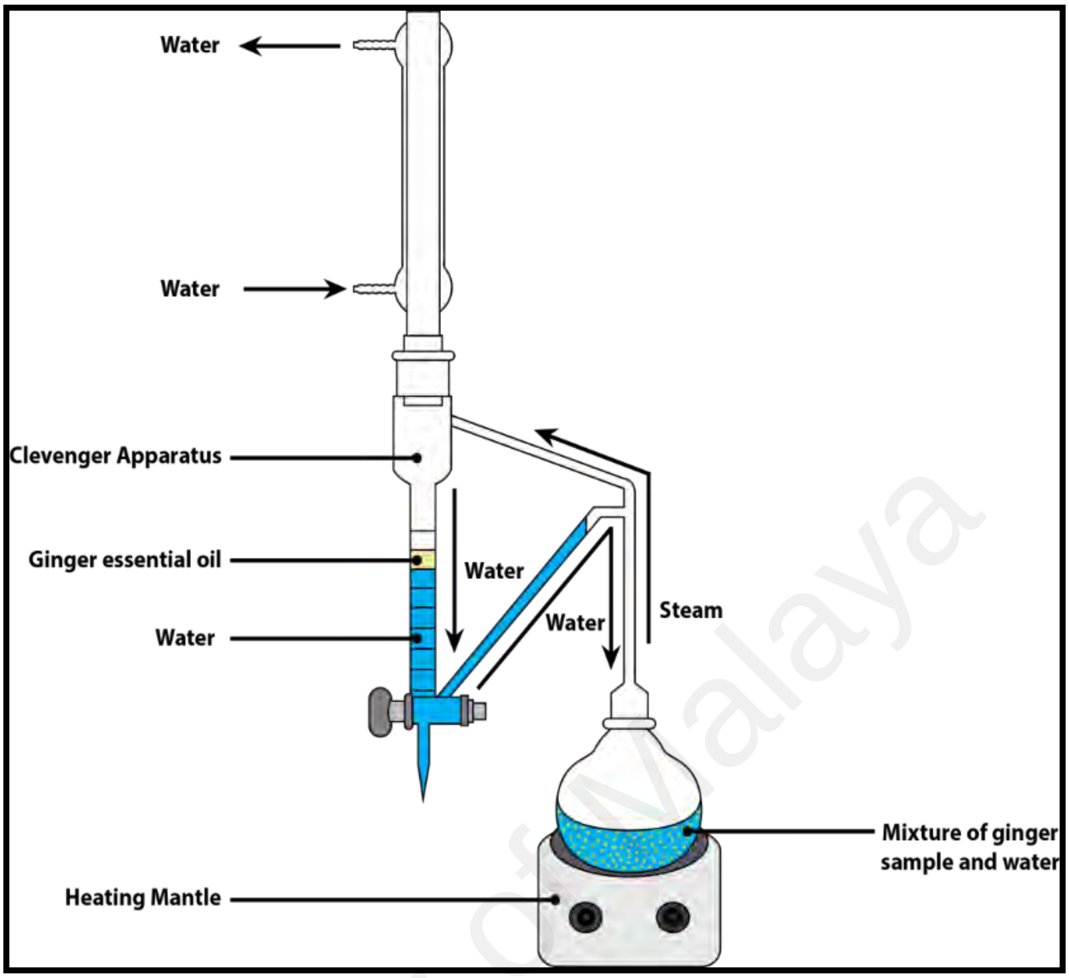

Figure 3.2: Setup of hydrodistillation

3.5 Fourier Transform-Infrared Spectroscopy (FT-IR)

The FTIR spectra of the samples were determined using a Perkin-Elmer attenuated total reflectance (ATR)-FTIR Spectrometer Spectrum 4000. Briefly, a small quantity of the oil sample was placed onto the plate and the ATR crystal area, which was first cleaned. A pressure arm was positioned over the sample area and would then scan in a single-bounce ATR mode. The spectrum was recorded with 32 scans at 4 cm^{-1} resolution at a range between 400 cm^{-1} and 4000 cm^{-1}.

3.6 Gas Chromatography-Mass Spectrometry (GC-MS)

The ginger oil was diluted with hexane up to a ratio of 1:10 (v/v) before being analyzed using a Shimadzu QP2010 Gas Chromatograph equipped with a DB-5 (30 m × 0.25 mm i.d. × 0.25 µm) column based on the method of An et al. (2016) with modifications.

Briefly, a split injection mode (with a split ratio of 50) was used, where the oven temperature was held initially for 3 minutes at 50 °C and increased to 120 °C at a rate of 4 °C/min. Then, the temperature was again held for 8 minutes, heated to 200 °C at 4 °C/min, and held for 3 minutes. Following that, the temperature was increased to 250 °C at 10 °C/min and held for 5 minutes. The helium carrier gas was introduced at a flow rate of 1.22 mL/min. The MS fragmentation was performed in electron impact (EI) mode at 70eV with a source temperature of 200 °C, and a mass acquisition range between 50 m/z and 700 m/z. Each sample was analyzed in triplicates.

The volatile components were identified by mapping their mass fragmentation patterns with the data from the NIST library and based on the mean of retention index (RI). The RIs of the target compounds were derived from the homologous series of n-alkanes (C_8-C_{20}) and compared with the reference values reported in several works (Akarchariya et al., 2017; Azar et al., 2013; Baharum et al., 2010; Camara et al., 2018; Formisano et al., 2010; Judzentiene et al., 2010; Khan et al., 2016; Liu et al., 2010; Luo et al., 2017; Pang et al., 2017; Petrović et al., 2018; Sivasothy et al., 2011; Yang et al., 2018). The relative proportion of volatile constituents was denoted as the percentage obtained from peak area normalization. The volatiles that contributed more than 0.3% of the total area was retained as the variables and their relative peak area (RPA) were calculated for fingerprinting using the pattern recognition technique.

3.7 Pattern Recognition

The relative peak area (RPA) was evaluated using pattern recognition tools such as HCA and PCA with JMP Pro version 12 software (SAS, USA). A two-way HCA dendrogram was derived based on Ward's algorithm, while PCA was conducted on the covariance matrix.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Preliminary study

The preliminary study applied a full factorial two-level experimental design and exploratory analysis to study the quantitative and qualitative variations in ginger volatile oil with respect to extraction conditions. A further investigation of hydrodistillation time was also done to identify the optimal extraction conditions that would quantitatively improve ginger oil yield.

4.1.1 The percentage yield of *Zingiber officinale* volatile oil

The percentage yields of *Zingiber officinale* (ginger) oil were in the range of 0.04%–0.17% w/w (on a wet weight basis) depending on the treatment received. The results were comparable to the report by López et al. (2017), which recorded the ginger oil yield of 0.06% w/w. In contrast, such recovery range was relatively lower than those reported in previous studies (0.10%–0.46%) regarding similar species (El-Ghorab et al., 2010; Nandi et al., 2013; Shah & Garg, 2014; Toure & Zhang, 2007). Despite the differences, all the results were somehow in agreement with Dinde et al. (2017) and Djilani & Dicko (2012), which suggested the total content of plant volatile oils is very low, generally less than 1%. For instance, the oil yield of *Zingiber officinale* var. *rubrum Theilade* (halia bara) rhizomes were recorded at 0.02% w/w (Sivasothy et al., 2011). Similarly, Ocheng et al. (2015) recorded 0.05%–0.39% w/w of the yield of volatile oil from 16 aromatic plants from Uganda. The oil yield of *Blepharocalyx salicifolius* and *Myracrodruon urundeuva* was in the range of 0.045%–0.069% w/w and 0.08%–0.13% w/w, respectively (Costa et al., 2014). Also, the average oil yield of *Rosmarinus officinalis* L. (rosemary), *Ocimum basilicum* L. (basil), and *Lavandula dentata* L. (lavender) after 30 minutes of steam distillation was 0.0051%, 0.0038%, and 0.0032% w/w, respectively, whereas the oil of *Anethum sowa* L. recorded a yield of 0.30% w/w (Cassel et al., 2009; Saleh-e-In et al., 2010). In contrast, the oil of the *Cinnamomum altissimum* Kosterm (cinnamon) and the fruit peels of *Citrus hystrix* (kaffir lime) produced much higher yields (more than 1%) (Abdelwahab et al., 2017; Yusoff et al., 2013). Furthermore, in some cases, the yield of volatile oils could reach more than 10%, as per a study on *Syzygium aromaticum* (clove) (Safrudin et al., 2015). The above findings show that the variation in the yield of volatile oil is influenced by extraction conditions as well as other factors including plant species and geographic origin.

4.1.2 Statistical evaluation of *Zingiber officinale* oil yield

The results from the statistical analysis showed that an interaction model best represents the relationship between the output response (percentage yield) and its variables. A three-factor two-level Full Factorial Design was used to cover all the effects and interactions in this study as shown in Table 4.1 and the model can be expressed in terms of the effect's coefficients:

$$\hat{y} = 0.091 + 0.032X_1 + 0.012X_2 + 0.016X_3 - 0.002X_{12} + 0.001X_{13} + 0.006X_{23} - 0.001X_{123} \tag{4.1}$$

Table 4.1: Parameter coefficient estimates

Name of parameters	Coefficient	Effect	Standard error	ratio t	p-value
Intercept	b_0	0.0914688	0.002739	33.40	<.0001
Sample freshness	b_1	0.0315188	0.002739	11.51	<.0001
Size reduction	b_2	0.0123063	0.002739	4.49	0.0020
Extraction duration	b_3	0.0156813	0.002739	5.73	0.0004
Sample freshness × size reduction	b_{12}	-0.001844	0.002739	-0.67	0.5197
Sample freshness × extraction duration	b_{13}	0.0007062	0.002739	0.26	0.8030
Size reduction × extraction duration	b_{23}	0.0055688	0.002739	2.03	0.0764
Sample freshness × size reduction × extraction duration	b_{123}	-0.001156	0.002739	-0.42	0.6840

In this study, the threshold of the $p-value$ was set to 0.05. The exclusion and inclusion of each coefficient of the effect/interaction is decided based on the $p-value$. Based on Table 4.1, the coefficient of effect/interaction is associated with a standard error, a $t-ratio$ and a $p-value$, where, generally, the smaller the magnitude of $p-value$ ($p-value < 0.05$), the more significant the corresponding coefficient terms and these terms should be included in the regression model (Ravikumar et al., 2007). Contrarily, the coefficient terms that were not statistically significant ($p-value > 0.05$) indicate that the output responses are not affected by varying the input factors level, thus these terms could be excluded from the regression model (Hribernik et al., 2009; Fukuda et al., 2018). According to the above arguments, the estimated percentage yield can be expressed per the following equation:

$$\hat{y} = 0.091 + 0.032X_1 + 0.012X_2 + 0.015X_3 \tag{4.2}$$

where the factors, b_1, b_2, and b_3 are related to the sample freshness, the particle thickness, and the extraction duration, respectively, which, in turn, significantly influence the yield of the oil; those interactions (b_{12}, b_{13} , b_{23} and b_{123}) that were not significant were also accounted for. This equation denotes that using the fresh sample in grated form and extending the extraction duration to 6 hours led to an increment in the yield of oil. Among the three factors, sample freshness was deemed the most influential, as reflected by the magnitude of coefficient b_1.

As mentioned, the adequacy of the postulated model based on the factorial design was subsequently evaluated via Analysis of Variance (ANOVA) and a lack-of-fit test. Based on the summary of ANOVA results (Table 4.2), where $p < 0.001$, $R^2 = 0.960$, and adjusted $R^2 = 0.924$, the experimental data fit the empirical model adequately while the lack-of-fit test was tested with $p = 0.379$. The results of the Shapiro-Wilks test demonstrated that the residuals were normally distributed, where N(0,0.008) and $p =$

0.6165. The ANOVA, the lack-of-fit test, and the residual analysis results demonstrate that the model is a good simulation of the extraction experiment.

Table 4.2: Analysis of Variance (ANOVA) results

Source of variance	DF	Sum of squares	Mean squared	F-ratio	p-value
Model	7	0.02283237	0.003262	27.1835	< 0.0001
Error	8	0.00095993	0.000120		
Total	15	0.02379229			
R^2-value	0.959654				
Adjusted R^2-value	0.924351				

4.1.2.1 Effect of freshness/drying process

Drying is a complex procedure involving simultaneous mass transfer and heat, so it needs proper and precise process controls. In general, this process entails the vaporization of both loosely and bound water within the solid material into the atmosphere, which leads to the reduction of volume and weight of aromatic plants (Karam et al., 2016). Besides that, drying is also identified as the simplest method to preserve plant materials and can prolong its storage life by reducing the occurrence of enzymatic or biochemical reactions, as well the growth of microorganisms that could alter the characteristics of the plant product. Based on the current experimental results, the dehydration process resulted in a low recovery of the volatile oil per the finding of (Sasidharan & Menon, 2010). Similar results were also found for *Artemisia afra* Jacq. Ex Willd. and *Cymbopogon citratus* (Ashafa & Pitso, 2014; Hanaa et al., 2012). El-Gohary et al. (2018) found changes in the yield of *Mentha* sp. volatile oil after subjected to different drying methods. Besides, a lesser amount of oil was recovered from the dried leaves of *Rosmarinus officinalis* (rosemary), with microwave-dried samples showing the most significant loss in yield (Verma & Chauhan, 2011).

The above cases of lower volatile oil yield could be due to some volatile components which are more sensitive to the water portion being vaporized along with the water in the evaporation process (Sellami et al., 2011). In this study, the ginger samples were dried via microwave-drying. This method is highly preferred, as it can significantly reduce drying time and it rapidly dehydrates the plant. Nevertheless, some authors have shown that the use of a microwave for drying may cause a low recovery of volatile oil compared to other drying methods (Ashafa & Pitso, 2014; Beigi et al., 2018). During microwave drying, polar molecules such as water absorb microwave energy; thus causing interactions known as ionic migration and dipole rotation, resulting in heat generation that promotes the evaporation of water from the surface of plant materials (Destandau et al., 2013; Orsat et al., 2006). The improper control of microwave power may cause an undesirable rise in temperature, therefore severely rupturing the plant materials, and hence accelerating not only the evaporation of moisture but also the plant's volatile oil. This result is per those of Ghasemi et al. (2013) and Raghavan et al. (1997) on *Oreganum majorana* L. and *Melissa officinalis* L, respectively.

Despite the above arguments, many reports have also shown that certain drying methods have had positive effects on the yield. These works posit that the process resulted in an increment in the oil of some plants, including *Origanum* sp., *Mentha piperita* and *Laurus nobilis* (Beigi et al., 2018; Ozdemir et al., 2018; Sellami et al., 2011). A similar result was also found for dried *Zingiber officinale* (ginger) samples (El-Ghorab et al., 2010; Mahboubi, 2019). In these cases, the damages to the cell structure caused by the drying process could promote the release of oil, thus resulting in higher yield. The varied results could be due to the method used for the drying process. It is known that the effect of drying methods on the quantity and the quality of the products is dependent on the mechanisms involved in each process, which tend to vary from one process to another (An et al., 2016; Beigi et al., 2018; Ozdemir et al., 2018). The difference in operational conditions (temperature and time) of the drying processes could also result in these differences (Hasmita et al. 2015; Sellami et al., 2011). Volatile oils stored in particular secretory structures such as trichomes, oil cells, and ducts depend on the plant species. In the case of ginger, the oil globules accumulate within the secretory cell that resides at the cell parenchymal wall of the rhizome (An et al., 2016; Karam et al., 2016). Some studies have suggested that a high drying temperature would cause severe damage to these storage cells; thus, accelerating the evaporation of the oil. Furthermore, it has been found that the low recovery of oil could also be due to the decomposition of some heat-sensitive components via autoxidation and hydro peroxidation at high operational temperature (Mashkani et al., 2018). Besides that, the longer the plant material is exposed to heat (generated during the drying process), the lower the recovery of the volatile oil, which is very volatile.

Raw ginger is highly perishable (due to a high moisture content of about 80%–90%). Therefore, the plant can deteriorate quickly, making the transportation and marketing of these products expensive. Besides, dry ginger normally contains less than 10% moisture content and is a value-added commodity in trade. It is also widely used for the manufacturing of ginger-based products such as ginger essence, ginger powder, ginger oleoresins, ginger oil, and soft drinks. All these products are also served as flavoring agents in various foods. Hence, ginger must be subjected to post-harvest procedures such as the drying procedure to ensure favorable product quality with minimal changes to its chemical, organoleptic, and physical properties.

4.1.2.2 The effect of size reduction

This study found that size reduction had a significant effect on the yield of oil. A higher amount of volatile oil was recovered from grated ginger (~4 mm thickness) compared to sliced ginger (~10 mm thickness). Hence, using plant material with a small particle size could lead to high recovery of volatile oil, with such a trend also shown for a few other plants including *Marrubium vulgare* L., *Illicium verum* Hook. f., and *Citrus* spp. (Jalgaonkar et al., 2013; Nam et al., 2017; Yahya et al., 2018). Similarly, the oil yield of lemongrass decreased with increasing particle size (1–3 cm) while whole *Piper nigrum* L. berries (black pepper) produced the lowest oil yield in comparison to ground-size form: 0.50 mm and 0.25 mm, respectively (Kumoro et al., 2010; Singh et al., 2014). One study on the hydrodistillation of *Rosmarinus officinalis* L. (rosemary) oil suggested that a better extraction yield could be achieved using a sample with (Ouzzar et al., 2015). The mechanical impact applied during the comminution process could cause the secretory cell (containing the oil) to rupture and subsequently enhance the release of the oil. The high oil recovery of the reduced sample particle size can also be attributed to the increased surface area, thereby enhancing the mass transfer of the oil to the surrounding water (Jalgaonkar et al., 2013).

However, a reverse trend was observed in the leaves of *Pogostemon cablin* (patchouli), where a larger particle size produced higher oil yield compared to smaller particle size (Shah et al., 2017). This result may be attributed to a rise in temperature during the comminution process, which could have led to the loss of volatile oils from the plant material. It is said that the large-scale grinding of materials is commonly associated with temperature rise problems in the processed materials by up to 90 °C, resulting in vaporization and a loss of volatile oil (Karam et al., 2016). In fact, a similar result was reported for *Cuminum cyminum* (cumin) (Sowbhagya et al., 2008). The large-scale grinding of cumin into cumin powder produced lesser oil recovery due to increased temperature during the milling of the powder (70 °C –80 °C) compared to the yield obtained from cumin flakes (the temperature attained was 27 °C). Besides that, fine particle size is susceptible to agglomeration, which, in turn, obstructs the interaction of plant particles with solvent molecules and lowers the percolation of the solvent; therefore, resulting in inefficient volatile oil extraction (Sayyar et al., 2009; Yunus et al., 2013).

4.1.2.3 Effect of extraction duration

The result indicates that a better recovery of oil could be expected in the case of longer hydrodistillation times. For example, Hasmita et al. (2015) found that the yield of ginger volatile oil increased with increasing hydrodistillation time. Extraction time also had a significant effect on the oil yield of *Rosmarinus officinalis* (rosemary) and *Pistacia Lentiscus*, where the oil yields of both species were increased with a longer extraction time (Fadil et al., 2014; Haloui et al., 2015). The same trend has also been observed in other plant species such as *Citrus aurantium* and *Schinus terebinthifolius* (Barbosa et al., 2007; Ellouze & Abderrabba, 2014). Plus, other similar results were observed with different extraction methods such as microwave-assisted hydrodistillation (Jeyaratnam et al., 2016). Prolonging the extraction time by up to 90 min caused an increment in the oil yield of *Cinnamomum cassia* (cinnamon). However, the yield tended to drop beyond 90 minutes. A study on the structural change of peppermint leaves indicated that boiled water caused the plant glands to wrinkle and erupt causing an enhanced outpouring of stored volatile oil during the hydrodistillation process. This result suggests that prolonged extraction time could probably maximize the contact time between the medium and the plant material, and thus increase the mass transfer process of the volatile oil (Gavahian et al. 2015).

Most studies have suggested that that the yield can be increased with increased extraction time until saturation is reached (maximum yield) (Phuc et al., 2019). In general, the common extraction time for hydrodistillation is between 3 hours to 6 hours, but in some cases, the extraction process can be extended by up to 8 hours (Rusdi et al., 2016). Therefore, the extraction time must be optimized to avoid overheating the samples, as this will result in the potential loss of thermolabile substances due to the continuous exposure to heat and high temperature. In this study, the hydrodistillation procedure was carried out by prolonging the extraction process until saturation was reached (no change in the yield). The optimal extraction time was determined by plotting an extraction curve of oil yield against time.

4.1.3 Hydrodistillation extraction curve

The hydrodistillation extraction curve of oil yield against time was plotted as shown in Figure 4.1. The curve was used to study and determine the optimal extraction time needed to achieve the maximum yield of volatile oil in this study. Based on Figure 4.1, it can be seen that the yield of the volatile oil obtained increased rapidly with increasing hydrodistillation time until 5 hours of extraction and then remained constant as the extraction duration was prolonged past the 5-hour mark. This observation is attributed to the basic mechanisms of hydrodistillation known as the washing stage and the diffusion stage, as stipulated by (Milojević et al., 2008).

As mentioned previously, the comminution of the samples before extraction enhanced the release of volatile oils at the surface of the plant particles. During the early stage of the extraction process (also known as the washing stage), the solvent starts to wash out this volatile oil from the external surface of the plant material, therefore resulting in a rapid increment in the oil yield (fast oil distillation). The latter process (or diffusion stage) involves the diffusion of volatile oils from the interior of the plant particles (undestroyed reservoirs) to their external surfaces followed by the oil distillation. At this stage, the rate of distillation tends to decrease until a nearly constant oil yield is reached because of the slow process of oil diffusion (Pornpunyapat et al., 2011).

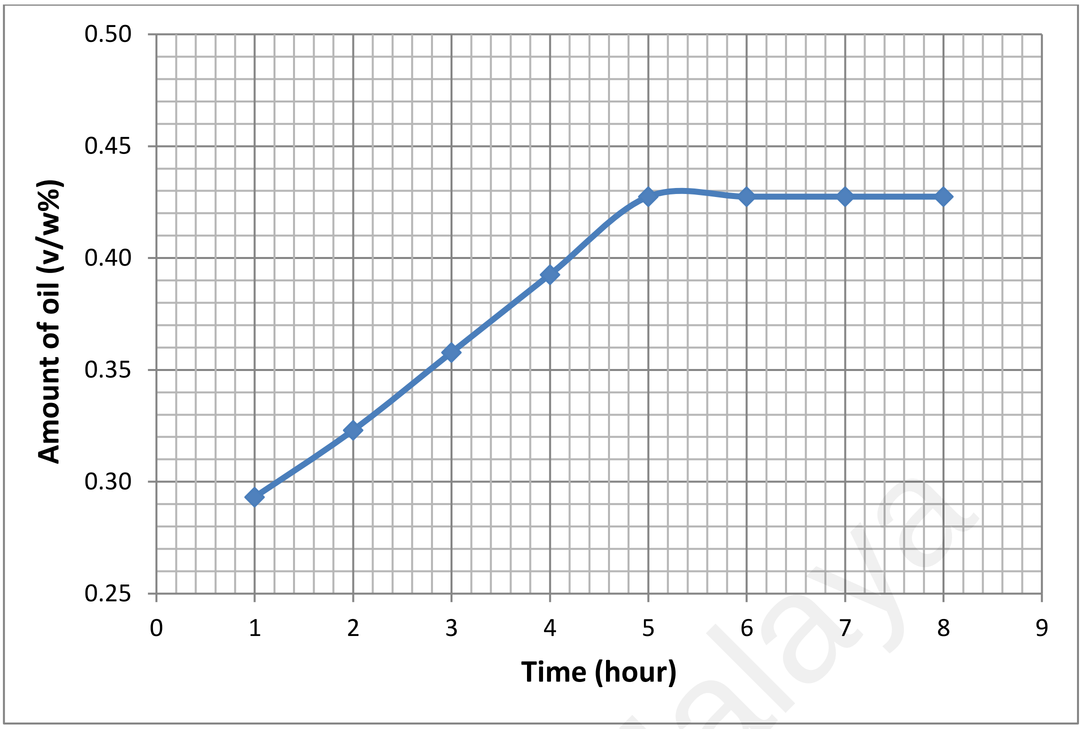

Figure 4.1: Extraction curve of ginger volatile oil

Figure 4.1 shows a similar extraction curve to that of Hasmita et al. (2015), who reported an increase in the ginger oil yield from 30 minutes to 240 minutes after which it remained constant after 240 minutes. This finding is per previous works as well, namely Shah et al. (2017), Teoh et al. (2012) and Phuc et al. (2019), which investigated *Zingiber cassumunar*, *Pogostemon cablin* and *Jasminum sambac* (jasmine), respectively. Studies suggest that upon reaching a certain time, the oil yield will remain constant, as the oil content in the plant materials keeps decreasing and then drops to its minimum level (Kusuma & Mahfud, 2017a). These results can be explained using Fick's Second Law of Diffusion, which states that a final equilibrium between the solute concentrations in the plant matrix and the solvent might be reached after a certain time, after which no change in the oil yield will be observed (Ranitha et al., 2014).

Nevertheless, the above findings were slightly different from other analyses on *Zingiber zerumbet* and *Rosmarinus officinalis* L. (rosemary) by Azelan et al. (2018) and Boutekedjiret et al. (2003), in which the oils were observed to gradually increase up to a certain amount of time and then decrease as the extraction time further increased. The reduction of oil upon extending the duration of extraction might be due to long exposures to undesirable conditions such as light, oxygen, and temperature, which induce the decomposition and oxidation of the oil components (Tan et al., 20113). Some studies have found that increasing the extraction duration did not improve the oil yield, and in fact, increased power consumption and wastage of biomass due to charring. Therefore, based on the above results, the highest oil yield could be achieved by using grated fresh ginger with a 5-hour extraction time, so this same experimental condition was used for the subsequent study (the main study).

4.1.4 Chemical characteristic of the volatile oil

The chemical characteristics of the volatile oil were determined using Fourier Transform Infrared Spectroscopy (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS).

4.1.4.1 Determination of functional groups of volatile oil using FT-IR

Fourier Transform Infrared Spectroscopy can be employed to interpret the functional groups of volatile oil components. The mechanism of infrared spectroscopy is based on the molecules absorbing specific frequencies of infrared radiation (IR). When a sample is irradiated with infrared radiation, some frequency of the radiation is absorbed, which usually induces the molecules (such as functional groups) to vibrate. These molecules are a unique reflection of the molecular structure. The IR region can be subdivided into three regions—near-IR, mid-IR, and far-IR. Absorption bands in the near-infrared are approximately 14000–4000 cm^{-1} and can excite harmonic and overtone vibrations. The mid-infrared is in the range 4000–400 cm^{-1} and is attributed to the fundamental vibrations and associated rotational-vibrational structure, while the far-infrared is used in rotational spectroscopy and is approximately within the range of 400–10 cm^{-1}.

Table 4.3: Band intensity and functional groups from FT-IR spectrum of ginger volatile oil

No.	Wavenumber (cm^{-1})	Intense	Functional group
1	3444	Weak, Broad	O-H stretching
2	2963-2861	Strong, sharp	C-H stretching
3	1676	Sharp	C=O stretching
4	1482-1340	Sharp	C–H bending, O-H bending
5	1195-950	Medium, Sharp	C-O stretching, C-O-C bend, C-H
6	900-800	Sharp	C=C aromatic
7	730	Sharp	C-H bending

Figure 4.2 represents the IR spectrum of the extracted ginger volatile oil and each of the characteristic bands were identified with respect to the functional groups as shown in Table 4.3 (Adinew, 2014; Berechet et al., 2015; Shahzadi, 2017). The characteristic bands observed in the volatile oil were in the range of 4000–450 cm^{-1}. The broad band at 3444 cm^{-1} corresponded to the oxygen-hydrogen (O-H) stretching of alcohols in the plant. The strong band of carbon-hydrogen (C-H) bond stretching was also observed in the range of 2963–2861 cm^{-1}. The band located at 1676 cm^{-1} represented the carbon-oxygen double bond (C=O) stretch in non-conjugated carbonyls, ketone, ester groups and aldehyde. Besides, the band at 1482-1340 cm^{-1} could be attributed to the bending of the O-H bond (phenol or tertiary alcohol) or C-H bond. The spectral region of 1195–950 cm^{-1} corresponds to the carbon-oxygen (C-O) stretching of primary, secondary, and tertiary alcohols, the ether linkage (C-O-C) stretching bond for alkyl-substituted ether or C-H of aromatic ring. The bands in the range of 900–800 cm^{-1} refer to presence of aromatic carbon-carbon double bond (C=C) bond, while the sharp band at 730 cm^{-1} represents the out-of-plane bending of the C-H bond, skeletal carbon-carbon (C-C) bond vibrations or methylene $(CH_2)_n$ rocking. The result above shows that ginger oil contains a mixture of compounds from different chemical classes such as alkenes, aldehydes, ketones, esters, and alcohols.

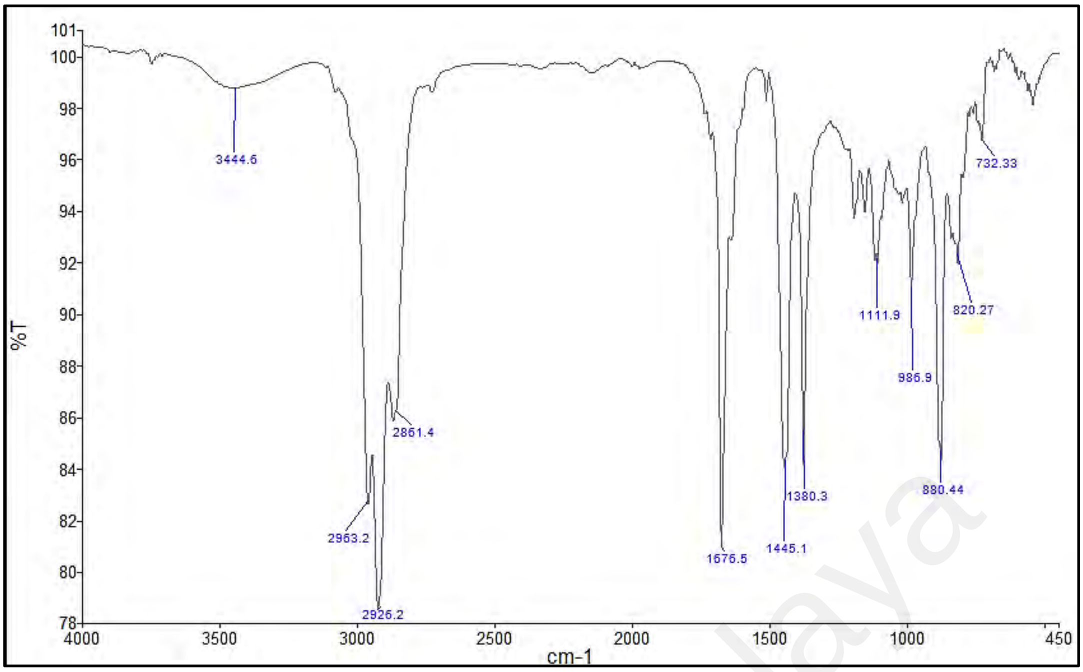

Figure 4.2: FT-IR spectrum of ginger volatile oil

4.1.4.2 Identification of volatile oil components via GC-MS

Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical technique that is commonly used for the separation and identification of mixtures of volatile organic compounds. The procedure starts with the injection of the sample into the GC inlet for heating and vaporization. The vapor is then transported by the carrier gas onto the chromatographic column where the compounds (comprising the mixture of interest) are separated. The latter part includes the compounds exiting the end of the chromatographic GC column where it is fragmented via ionization before analysis in the mass spectrometer. The fragmented compounds are separated based on mass-to-charge and then transformed into a usable signal known as shown in Figure 4.3. Based on Table 4.4, the ginger volatile oil is made up of a mixture of compounds that could be categorized into four main chemical groups, which are monoterpene hydrocarbons (17.96%), oxygenated monoterpenes (42.17%), sesquiterpenes hydrocarbons (30.17%), oxygenated sesquiterpenes (3.55%), and non-terpenes (1.57%). In general, the separation principle of the volatile oil using a less polar stationary phase (such as DB-5) is primarily dependent on boiling point differences/volatility which can be related to the chemical structure. (Chamorro et al., 2012; Najdoska-Bogdanov et al., 2015). That is, compounds elute in the order of their increasing boiling points. As discussed in section 2.1, monoterpenes have a smaller number of carbon (lower molecular weight) in comparison to sesquiterpenes, making it has lower boiling point and evaporates easily. Theoretically, typical elution pattern of the volatile oil components can be presented with monoterpenes (the most volatile), followed by sesquiterpenes and diterpenes (the least volatile), respectively (Attokaran, 2017). Most of the terpenes and their oxygenated derivatives have similar molecular weight, thus, similar in boiling point in which they frequently co-elute or appear overlapped in the chromatogram. This problem can be solved with the use of polar stationary phase as more polar compounds (oxygenated derivatives) are more retained in the column or applying a slower oven temperature rate to widen the elution range of the components.

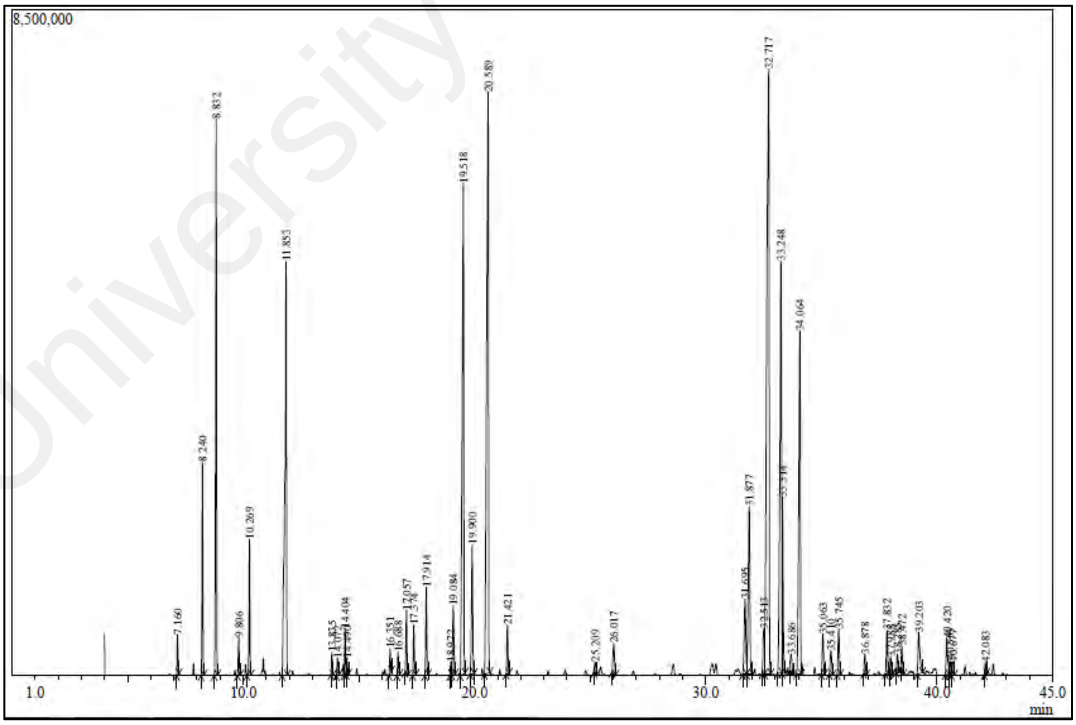

Figure 4.3: GC-MS spectrum of ginger volatile oil

Table 4.4: Volatile composition (%) from volatile oil obtained from ginger rhizome

No.	Compound	Composition (%)	Mass Fragment ions*	Ri^a	Ri^b
1	2-heptanol	0.24 ± 0.07	45,55,83	903	902
2	tricyclene	0.04 ± 0.02	77,93,105, 121	922	922
3	α-pinene	3.00 ± 0.12	77,93,105, 121	932	934
4	camphene	8.49 ± 0.35	67,79,93,107, 121	949	949
5	Sabinene	0.07 ± 0.01	77,93	971	974
6	β-pinene	0.76 ± 0.05	69,79,93	977	977
7	methyl heptenone	0.21 ± 0.06	55,69,93,108	984	988
8	β-myrcene	1.94 ± 0.09	69,79,93	989	988
9	octanal	0.01 ± 0.01	56,84,100	1003	1002
10	α-phellandrene	0.23 ± 0.03	77,93	1006	1004
11	3-carene	0.08 ± 0.01	79,93,105, 121	1008	1010
12	p-cymene	0.07 ± 0.01	91,119	1023	1025
13	β-Phellandrene	2.93 ± 0.98	77,93	1030	1030
14	eucalyptol	7.84 ± 1.64	43,81,108, 139	1031	1032
15	terpinolene	0.35 ± 0.02	79,93,105, 121	1084	1086
16	2-nonanone	0.26 ± 0.05	43,58,71	1089	1093
17	linalool	0.57 ± 0.11	55,71,93,121	1099	1101
18	2-nonanol	0.15 ± 0.03	55,69,97,126	1101	1108
19	camphor	0.01 ± 0.01	55,83,93,108	1144	1147
20	citronellal	0.47 ± 0.09	41,55,69,95	1151	1148
21	camphene hydrate	0.10 ± 0.01	43,71,86,96, 111	1153	1152
22	cis-verbenol	0.24 ± 0.05	55,67,81,94, 109,119	1160	1159
23	endo-borneol	0.93 ± 0.16	95,110,121,139	1170	1168
24	α-terpineol	1.36 ± 0.20	59,81,93,121,136	1193	1186
25	nerol	0.13 ± 0.04	41,69,93,121	1223	1227
26	β-citronellol	1.36 ± 0.19	41,55,81,94	1226	1231
27	neral	11.56 ± 1.36	41,69,84,94, 109	1238	1235
28	geraniol	1.63 ± 0.20	41,69,93,123	1248	1249
29	trans-2-decenal	0.08 ± 0.01	55,70,83	1258	1259
30	geranial	15.57 ± 1.83	69,84,93	1268	1273
31	bornyl acetate	0.11 ± 0.01	95,121	1279	1289
32	2-undecanone	0.62 ± 0.07	43,58,71	1288	1289
33	citronellyl acetate	0.25 ± 0.02	69,81,95,109,123	1340	1353
34	α-copaene	0.08 ± 0.03	105,119,161	1365	1380
35	geranyl acetate	0.04 ± 0.03	69,93	1370	1384
36	β-elemene	0.39 ± 0.08	67,81,93,107,147	1382	1389

Table 4.4, continued

No.	Compound	Composition (%)	Mass Fragment ions*	Ri^a	Ri^b
37	γ-elemene	0.02 ± 0.02	96,107,121	1426	1424
38	allo-aromadendrene	0.02 ± 0.02	91,105,161	1456	1458
39	germacrene D	1.11 ± 0.19	41,81,91,105,161	1477	1484
40	α-curcumene	2.50 ± 0.36	55,91,105, 119, 132	1480	1483
41	β-selinene	0.69 ± 0.10	55,67,105, 161, 189	1490	1489
42	α-zingiberene	12.86 ± 2.11	69,77,93,119	1493	1495
43	α-farnesene	5.65 ± 1.00	55,93,107, 119	1503	1507
44	β-bisabolene	1.73 ± 0.23	69,93,161	1504	1510
45	γ-cadinene	0.25 ± 0.08	83,91,105, 119,134	1513	1518
46	β-sesquiphellandrene	4.87 ± 0.81	69,93,161	1521	1524
47	elemol	0.99 ± 0.09	59,81,93,107,161	1545	1548
48	nerolidol	0.91 ± 0.09	55,69,93, 107,161	1560	1557
49	β-eudesmol	1.39 ± 0.14	59,149	1648	1652
50	cis,cis-farnesol	0.10 ± 0.04	41,69,81	1705	1717
51	trans, trans-farnesal	0.16 ± 0.04	41,69,84	1733	1730

A total of 51 components, including geranial (15.57%), α-zingiberene (12.86%), neral (11.56%), eucalyptol (7.84%), camphene (8.49%), α-farnesene (5.65%), β-sesquiphellandrene (4.87%), α-pinene (3.00%), ar-curcumene (2.50%), and β-mycrene (1.94%), were the major components of the extracted volatile oil. The identified compounds are almost similar to those of previous reports. Sukari et al. (2008) found that ginger volatile oil comprised geranial (21%) as a major component together with β-citronellol (17.4%), trans-geraniol (6.9%) and borneol (2.8%). Singh et al. (2008) revealed geranial (25.9%) as the predominant component of ginger oil, followed by α-

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[a] Calculated retention index with respect to a homologous series of n-alkanes (C$_8$-C$_{20}$)

[b] Retention index from published data

* Mass Fragment ions of compound obtained from mass spectrometry

zingiberene (9.5%), (E, E) α-farnesene (7.6%), neral (7.6%), and ar-cucurmene (6.6%). Nour et al. (2017) and Sharma et al. (2016) found zingiberene to be the principal component of ginger volatile oil. This result is in contrast with the study conducted by El-Ghorab et al. (2010), who found that the major components of fresh and dried ginger oil comprised camphene and α-terpineol. Sa-Nguanpuag et al. (2011) found that the oil obtained from hydrodistilled ginger samples contained camphene, eucalyptol, and α-pinene as major compounds while β-phellandrene and eucalyptol were present predominantly in the solvent extraction oil. Such variations in the composition of ginger volatile oil might be attributed to the different sample preparation, extraction settings, and methods used, as well as the geographic origin of the samples.

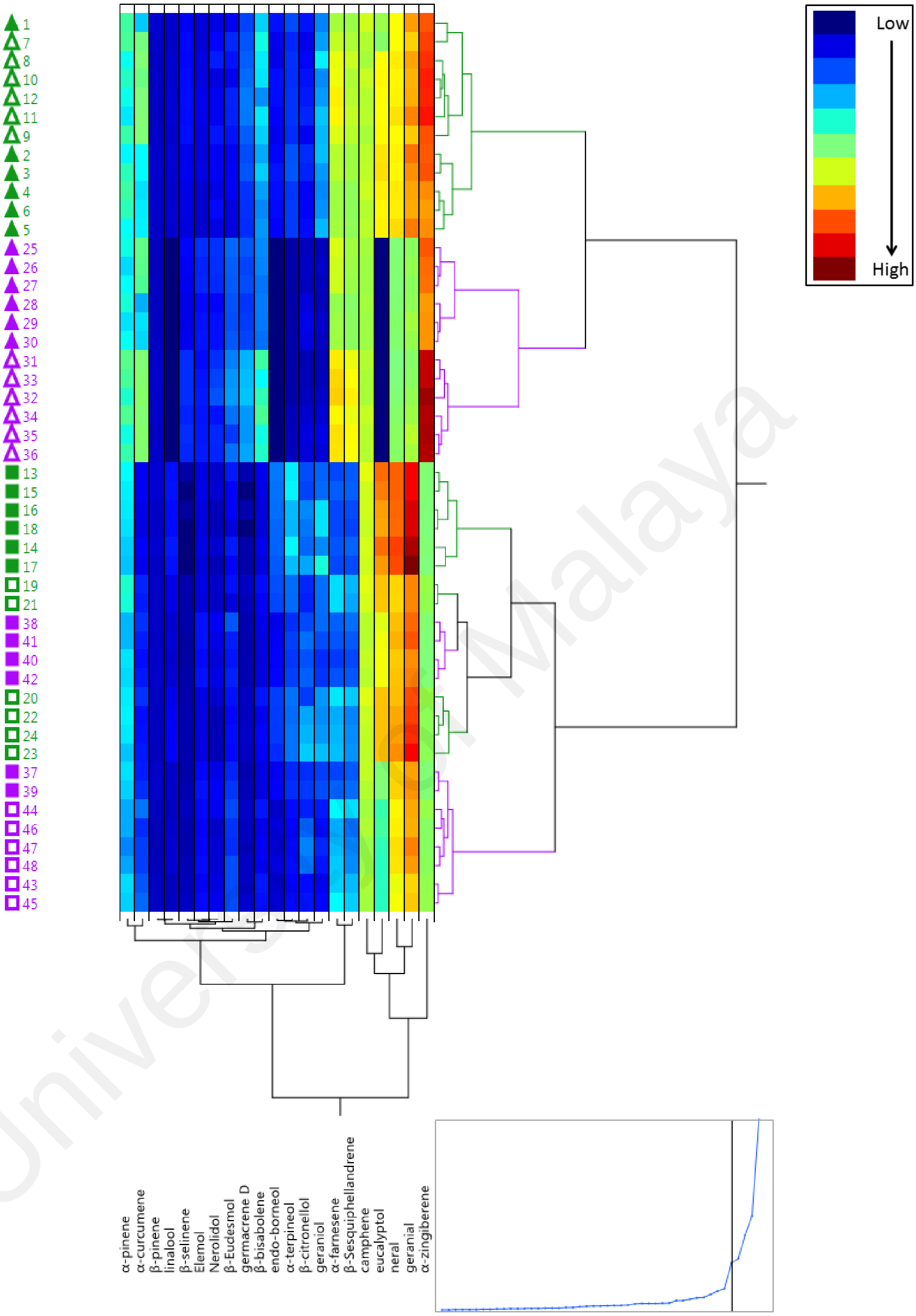
Ginger volatile oil is widely used in some industries including pharmaceutical, cosmetics, and even fragrance industries. Most of the ginger's volatile components greatly contribute to its characteristic flavor and aroma. As mentioned in previous studies, the extraction conditions do not only affect the oil yield but also the quality attributes of the volatile oil. The effect of extraction settings on the composition of ginger volatile oil can be further studied with the help of Exploratory Analysis.

4.1.5 Exploratory Analysis of ginger oil

The correlation between the volatile oil composition and extraction settings was studied using Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA).

4.1.5.1 Hierarchical Cluster Analysis (HCA)

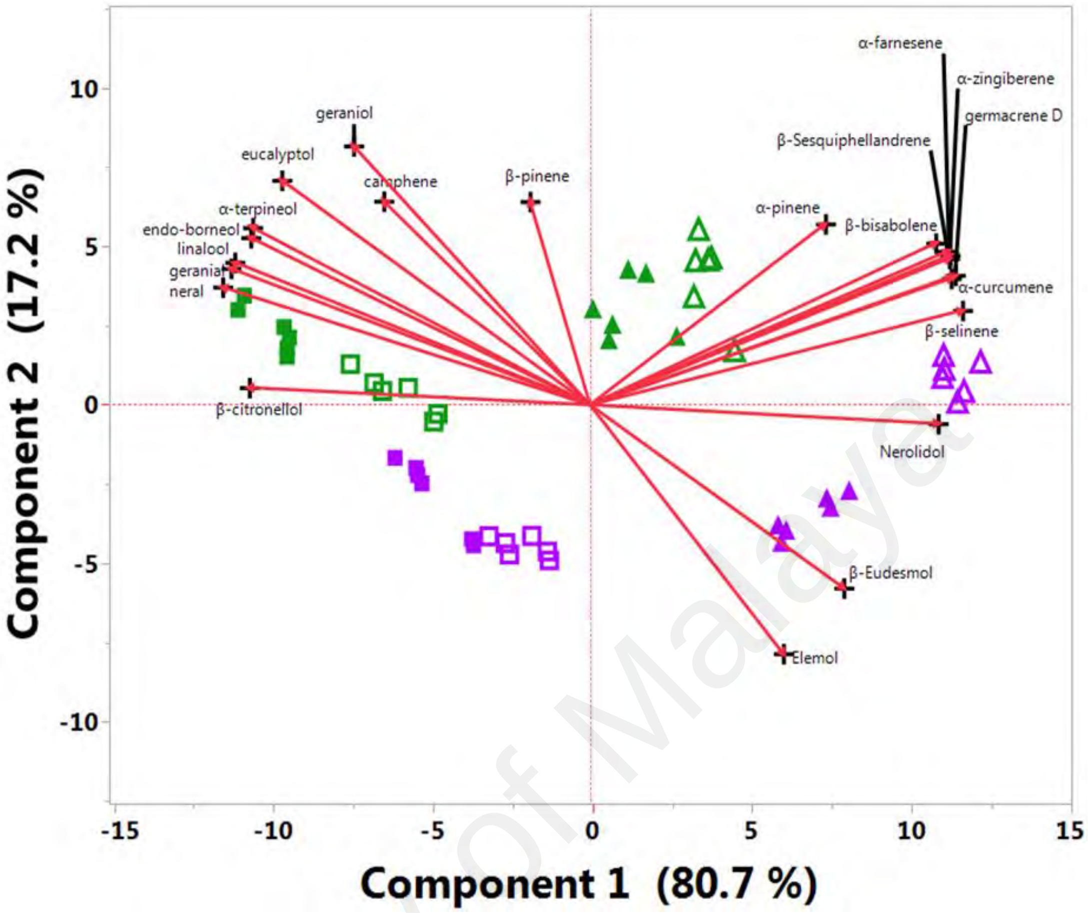
The oil samples (as displayed in the first column on the left) were labeled accordingly with respect to the respective extraction settings. The dendrogram in Figure 4.4 showed that the grated and sliced samples were separated into different cluster, showing that the volatile profiles between the two preparations were greatly different. In terms of sample freshness, some of the sliced dried samples had similar profiles with those of the fresh samples (four of them were grouped into the fresh cluster). Despite these results, the majority of the dried samples were well segregated from the fresh samples. Based on these results, the different particle sizes and sample freshness have resulted in a significant variation in the terpene composition. The affected terpenes can be evaluated based on the HCA color map. The color map provides visual information on the relative abundance of respective terpene compounds using a whole range of color codes (as shown in the legend to the right of the color map). Based on the HCA color map in Figure 4.4, grated samples displayed different color codes (ranging from green-to-yellow-to-red) from those of the sliced samples (ranging from blue-to-green) with respect to the sesquiterpene components. From these results, it can be deduced that the sesquiterpene compounds were more prevalent in the grated samples compared to the sliced samples. Besides that, the color intensity between the cells of the dried gingers and the non-dried gingers was slightly different because of the content of some monoterpene compounds including linalool, endo-borneol, neral, geranial, and eucalyptol; suggesting that the drying process could affect the level of most monoterpene components. The justification for these HCA results was then compared with the results of Principal Component Analysis (PCA).



fresh = green, dried = purple; grated = triangle, sliced = square; 3 hours = solid, 6 hours = hollow

Figure 4.4: Dendogram representing the variability in ginger volatile oil subjected to different treatments

4.1.5.2 Principal Component Analysis (PCA)



fresh = green, dried = purple; grated = triangle, sliced = square; 3 hours = solid, 6 hours = hollow

Figure 4.5: PCA scores and loading plot of ginger volatile oil

The PCA is a multivariate technique that helps the researcher identifies inherent patterns in an unbiased manner, as well as to help indicate similarities and differences in the samples. The analysis explores the underlying relationships between samples and their chemical compositions based on the treatment rendered. Figure 4.5 demonstrates the scores and loadings onto a reduced dimension where PC1 and PC2 accounted for ~97.9% of the total variance. For the clustering pattern on Figure 4.5, a high degree of clustering with a minimal overlap was observed between the grated (~4 mm) samples and the sliced (~10 mm) samples, clearly separated by PC1; while PC2 mainly corresponded to the effect of drying on the samples. Hence, the result shows that the pretreatment process such as the drying of ginger indeed influenced a loss of some terpene compounds, especially monoterpenes, while the size reduction process caused the sesquiterpenes to dominate the sample. In addition to the above, the result of the PCA also indicates the antagonistic effects of heating duration. These results are per those of HCA.

In general, the results of HCA and PCA are comparable to that of a previous report by Nour et al. (2017), who stipulated that volatile oil extracted from dried ginger produced a lower content of monoterpenes than distilled fresh ginger oil. Similarly, Sasidharan et al. (2010) found a decrease in monoterpene content in dry ginger oil. The geranial content was also found to reduce from 8% to 4.4% due to the dehydration of ginger. The same trends were reported in Ding et al. (2012) and An et al. (2016) for similar species. Monoterpenes are retained better in fresh samples in comparison to dried ones, as these compounds have low stability against heat, and so have a higher tendency to undergo chemical changes and degradation upon drying. According to Beigi et al. (2018), the dehydration of *Mentha piperita* L. (peppermint) leaves demonstrated a similar result in which the microwave-dried leaves (200 W and 400 W) showed a significant loss in the oxygenated monoterpenes compared to other drying methods; while Mashkani et al. (2018) and Pirbalouti et al. (2013) and deduced that microwave drying caused a decrement to some monoterpene compounds of basil landraces and *Thymus daenensis* (thyme) volatile oils, respectively. Besides, microwave-dried *Laurus nobilis* L. leaves also lost major compounds (3-carene, α-camphenal and myrtenal) in its oil compared to its fresh and air-dried samples (Dahak et al., 2014).

Alcohol-based compounds (borneol and heptanol) and most oxygenated monoterpenes (geranial, 2-undecanone, and neral) are commonly affected by the drying process, as these compounds seem to have more affinity for the water fraction contained in the plant samples and are therefore removed with the water fraction during the drying process. Besides, the polarity of these classes of compounds also allows them to interact with microwave energy. When the polar molecules absorb the microwave energy, ions will migrate and dipoles will rotate, therefore possibly resulting in the generation of heat within the material, eventually promoting evaporation and compound loss. It has been found that compounds with high polarity, a high dielectric constant, and a short-chain with low molecular weight are much more likely to degrade upon microwave drying (Heindl & Muller, 2007). Nevertheless, microwave drying could also produce a contrasting result, as per the volatile oils of *Laurus nobilis* L. and *Rosmarinus officinali*s Linn. (Sellami et al., 2011; Verma & Chauhan, 2011). In general, most studies agree that the dehydration processes significantly affect the relative percentage of volatile components in plants and may cause the total content of the main chemical groups in the volatile oil to decrease or increase. The influence of the dehydration process on the retention or release of volatile components is highly dependent on drying methods, drying conditions, as well as the characteristics of individual compounds and herb plants such as plant species, secretory structures, and their localization in the plants (Ashafa et al., 2008; El-Gohary et al., 2018).

The volatile oil profiles of grated and sliced gingers can be distinguished based on sesquiterpene content. From the PCA result, it can be concluded that reducing the size of the plant material may result in a loss of monoterpenes, and thus causing sesquiterpenes to dominate the content of grated ginger. This result may be attributed to the synthesis of short alkenes as well as the isomerization of similar compounds. In principle, the large surface area of the grated sample (~4 mm) would enhance the release of volatile oils during extraction. However, this characteristic may also cause some drawbacks due to the highly volatile nature of monoterpenes. During the mechanical grating process, a force is applied by the mechanism of cutting, abrasion, impact or a combination of several of them in which 99% of the input energy is dissipated as heat while only 1% is used to reduce the size of the plant material. Exposure to heat generated during the process would lead to a rapid loss of most monoterpenes, as they have a high vapor pressure and a low boiling point, as shown in the analyses of *Baccharis articulata* (Lam) Pers and leaves of *Juniperus monosperma* and *J. osteosperma* (juniper) (Adams et al., 2014; Tischer et al., 2017).

In terms of heating duration, generally, the individual compounds of the volatile oil had a distinctive kinetic behavior with respect to distillation time. Also, most monoterpenes could be recovered with a shorter distillation time whereas longer exposure caused the total content of sesquiterpenes to increase as mentioned in previous studies (Barbosa et al., 2007; Cavalcanti et al., 2015; Zheljazkov et al., 2013). The antagonism between them justifies the effect of heating duration within the experimental boundary. Overall, the two proposed pattern recognition analyses (HCA and PCA) could be applied in combination to guarantee a quality evaluation of ginger volatile oil.

4.2 Main study: Analysis of Bentong ginger oil

The second part of the research study or the main study was focusing on determining the distinctive attributes of the ginger based on its oil profile. The oil profile of the Bentong ginger was compared with other gingers/non-Bentong ginger that were obtained from local markets. Similar to the preliminary study, the oil percentage yield and the chemical characteristics of the oil were determined, followed by Exploratory Analysis (HCA and PCA). As expected, the result of the analyses showed quantitative and qualitative variations between the two samples. The variation was most likely associated to different geographical origin and the justification on this presumption was discussed further in the following section.

4.2.1 The percentage yield of Bentong ginger volatile oil

The extraction of Bentong ginger volatile oil was carried out and the yield was compared to that of non-Bentong ginger. Based on the results, the non-Bentong ginger had a much lower oil yield (0.13% ± 0.03) compared to Bentong ginger (0.20% ± 0.03) per wet weight. Previous study has compared three domestic gingers (Keningau, Bentong, and China) and noted a variation in their yield of ginger oil with the highest yield was recorded in China (5.67%) followed by Bentong (5.00%) and Keningau (4.33%) respectively (Vairappan et al., 2012). The oil content of fresh ginger collected from Ondo region, Nigeria, was recorded as 0.17%, as shown by Famurewa et al. (2011). This yield is somehow lower than ginger obtained from China (0.21%), Bangladesh (0.23%), and India (0.20%) (Gupta et al., 2011; Nandi et al., 2013). Meanwhile, fresh ginger rhizomes from the Philippines produced 0.2%–1.0% of ginger oil yield whereas the extraction of oil from Korean ginger via liquid CO_2 produced a higher yield, at 0.33% (Vernin & Cyril, 2016). Toure & Zhang (2007) found that Guinean ginger produced a higher amount of volatile oil compared to Chinese ginger at 0.44% and 0.22%, respectively. Similar results have been shown in other studies by Sultan et al. (2005), Buang et al. (2014) and Nampoothiri et al. (2012). Variation in the oil yield was also observed in other plant species such as *Salvia fruticosa* Mill. (Greek sage), which produced an oil yield ranging from 0.25%–4.00% with respect to different sources (Cvetkovikj et al., 2015). The oil recovery of the dried fruits of *Xylopia aethiopica* from Sudan was considered higher in contrast to samples from other African countries (Elhassan & Ayoub, 2014). Meziane-Assami et al. (2013) found the oil yield of *Rosmarinus officinalis* (rosemary) from Algeria to range from 0.33%–1.47% while Louasté et al. (2019) and Hcini et al. (2013) observed the yield of volatile oil of similar species plants originating from Moroccan regions and Tunisia of about 1.57%–2.90% and 1.40%–1.80%, respectively. In fact, similar trends have also been observed for other medicinal plants such as *Lippia alba* (Teles et al., 2012). Despite the differences in oil yield, the data alone was not sufficient enough to be used in differentiating Bentong ginger from other gingers. Therefore, a chromatographic analysis must be done to determine the chemical profile of plant metabolites that form the characteristic pattern representing the authenticity of the samples.

4.2.2 Chemical analysis of Bentong ginger volatile oil

Bentong ginger oil exhibited a total of 58 volatile constituents, compared to 56 constituents identified in the non-Bentong ginger volatile oil, as presented in Table 4.5. These volatile compounds can be categorized into 4 main chemical groups: monoterpene hydrocarbons (14.32% and 16.50%), oxygenated monoterpenes (32.37% and 20.99%), sesquiterpene hydrocarbons (34.73% and 40.32%), oxygenated sesquiterpenes (6.97% and 7.58%), and non-terpenes (2.97% and 0.96%). It is important to note that some components were only found in the Bentong ginger samples such as 2-heptanol, 2-heptyl acetate, 2-nonanone, 2-nonanol, cis-verbenol, and citronellyl acetate while isoborneol, 4-terpineol, α-Bergamotene, and valencene were only observed in the non-Bentong ginger samples. However, these components were only found in a few of the Bentong samples and the non-Bentong samples, so these samples may not practically represent the quality attributes of both samples. The volatile phytochemical composition of Bentong ginger samples were characterized by α-zingiberene (9.14%), geranial (7.27%), α-farnese (6.91%), neral (5.87%), eucalyptol (5.40%), and β-sesquiphellandrene (5.03%); while the composition of non-Bentong ginger samples consisted of α-zingiberene (12.93%), β-sesquiphellandrene (7.23%), β-phellandrene (5.75%), α-curcumene (4.57%), camphene (4.54%), and geranial (4.29%).

Table 4.5: Chemical composition of the volatile oil of Bentong ginger (BTG) and non-Bentong ginger (NG)

No	Ri^a	Ri^b	Composition (%)	Mass Fragment ions*	Composition (%)	
					BTG	NG
1	903	902	2-heptanol	45, 55,83	0.50 ± 0.04	n.d
2	922	922	tricyclene	77,93,105,121	0.26 ± 0.02	0.37 ± 0.02
3	932	934	α-pinene	77,93,105,121	2.07 ± 0.07	2.20 ± 0.06
4	949	949	camphene	67,79,93,107, 121	3.59 ± 0.12	4.54 ± 0.14
5	971	974	sabinene	77,93	0.15 ± 0.03	0.19 ± 0.01
6	977	977	β-pinene	69,79,93	0.75 ± 0.03	0.67 ± 0.03
7	984	988	methyl heptenone	55,69,93,108	0.29 ± 0.04	0.58 ± 0.09
8	989	988	β-mycrene	69,79,93	1.57 ± 0.03	1.30 ± 0.03
9	1006	1004	α-phellandrene	77,93	0.50 ± 0.03	0.63 ± 0.03
10	1008	1010	3-carene	79,93,105,121	0.13 ± 0.04	0.07 ± 0.03
11	1023	1025	p-cymene	91,119	0.14 ± 0.05	0.15 ± 0.03
12	1030	1030	β-Phellandrene	77,93	4.53 ± 0.20	5.75 ± 0.17
13	1031	1032	eucalyptol	43,81,108,139	5.40 ± 0.83	3.19 ± 1.22
14	1038	1044	2-heptyl acetate	87,98	0.35 ± 0.15	n.d
15	1057	1060	γ-terpinene	93,121,136	0.08 ± 0.04	0.09 ± 0.03
16	1084	1086	terpinolene	79,93,105,121	0.55 ± 0.03	0.54 ± 0.03
17	1089	1093	2-nonanone	43,58,71	0.46 ± 0.21	n.d
18	1099	1101	linalool	55,71,93,121	0.85 ± 0.03	0.56 ± 0.02
19	1101	1108	2-nonanol	55,69,97,126	0.38 ± 0.03	n.d
20	1144	1147	camphor	55,83,93,108	0.15 ± 0.04	0.24 ± 0.02
21	1151	1148	citronellal	41,55,69,95	0.79 ± 0.05	0.31 ± 0.03
22	1160	1159	cis-verbenol	55,67,81,94, 109,119	0.53 ± 0.07	n.d
23	1162	1160	isoborneol	96,110,121	n.d	0.16 ± 0.03
24	1170	1168	endo-borneol	95,110,121, 139	1.40 ± 0.04	2.13 ± 0.05
25	1179	1180	4-terpinenol	71,93,111	n.d	0.48 ± 0.03
26	1193	1186	α-terpineol	59,81,93,121, 136	1.75 ± 0.03	1.53 ± 0.04
27	1223	1227	nerol	41,69,93,121	1.40 ± 0.32	2.13 ± 0.68
28	1226	1231	β-citronellol	41,55,81,94	1.72 ± 0.17	0.64 ± 0.02
29	1238	1235	neral	41,69,84,94, 109	5.87 ± 0.17	3.38 ± 0.09
30	1248	1249	geraniol	41,69,93,123	3.30 ± 0.45	1.40 ± 0.12

Table 4.5, continued

No	Ri[a]	Ri[b]	Composition (%)	Mass Fragment ions*	Composition (%)	
					BTG	NG
31	1258	1259	trans-2-decenal	55,70,83	0.12 ± 0.04	0.08 ± 0.03
32	1268	1273	geranial	69,84,93	7.27 ± 0.25	4.29 ± 0.13
33	1279	1289	bornyl acetate	95,121	0.19 ± 0.05	0.27 ± 0.02
34	1288	1289	2-undecanone	43,58,71	0.87 ± 0.06	0.30 ± 0.03
35	1327	1328	δ-elemene	93,121,136	0.18 ± 0.03	0.23 ± 0.01
36	1340	1353	citronellyl acetate	69,81,95,109,123	0.35 ± 0.15	n.d
37	1359	1363	cyclosativene	94,105,107,119,161	0.30 ± 0.10	0.36 ± 0.06
38	1365	1380	α-copaene	105,119,161	0.60 ± 0.03	0.57 ± 0.02
39	1370	1384	geranyl acetate	69,93	1.40 ± 0.66	0.28 ± 0.09
40	1382	1389	β-elemene	67,81,93,107,147	1.34 ± 0.04	1.44 ± 0.05
41	1414	1420	β-ylangene	105,120,161	0.28 ± 0.11	0.29 ± 0.09
42	1426	1424	γ-elemene	96,107,121	1.21 ± 0.04	1.27 ± 0.03
43	1431	1432	α-Bergamotene	93,107,119	n.d	0.16 ± 0.03
44	1454	1458	trans-β-farnesene	69,93,120,133	0.68 ± 0.03	1.01 ± 0.03
45	1456	1458	allo-aromadendrene	91,105,161	0.57 ± 0.02	0.42 ± 0.02
46	1472	1479	y-muurolene	105,161	0.48 ± 0.20	0.49 ± 0.09
47	1477	1484	germacrene D	41,81,91,105,161	2.22 ± 0.05	2.25 ± 0.05
48	1480	1483	α-curcumene	55,91,105,119,132	3.80± 0.31	4.57 ± 0.14
48	1490	1489	β-selinene	55,67,105,161,189	0.31 ± 0.02	0.28 ± 0.01
50	1492	1489	valencene	93,105,119, 161	n.d	0.12 ± 0.05
51	1493	1495	α-zingiberene	69,77,93,119	9.14±0.55	12.93±0.34
52	1503	1507	α-farnesene	55,93,107,119	6.91±0.44	4.13±0.09
53	1504	1510	β-bisabolene	69,93,161	1.67±0.37	2.57±0.08
54	1521	1524	β-sesquiphellandrene	69,93,161	5.03±0.19	7.23±0.15
55	1545	1548	elemol	59,81,93,107,161	1.40±0.06	1.53±0.03
56	1560	1557	nerolidol	55,69,93,107,161	1.34 ± 0.04	1.28 ± 0.04

Table 4.5, continued

No	Ri[a]	Ri[b]	Composition (%)	Mass Fragment ions*	Composition (%)	
					BTG	NG
57	1571	1576	germacrene D-4-ol	81,105,123	0.10 ± 0.05	0.12 ± 0.04
58	1586	1586	7-epi-cis-sesquisabinene hydrate	69,82,93,119	0.72 ± 0.02	1.20 ± 0.03
59	1625	1621	γ-eudesmol	119,133,161, 189,204	0.31 ± 0.03	0.36 ± 0.12
60	1648	1652	β-eudesmol	59,149	2.19 ± 0.07	2.19 ± 0.05
61	1705	1717	cis,cis-farnesol	41,69,81	0.42 ± 0.03	0.47 ± 0.03
62	1733	1730	trans, trans-farnesal	41,69,84	0.49 ± 0.04	0.43 ± 0.04

The result shows that α-zingiberene was the most prominent compound in the Bentong ginger and non-Bentong ginger oil samples, accounting for 9.14% and 12.93% of the total composition of the Bentong ginger oil and non-Bentong ginger oil, respectively. This result is in line with most previous studies, which reported zingiberene as the main compound of ginger oil (Sharifi-Rad et al., 2017; Shirooye et al., 2016; Toure & Zhang, 2007; Yamamoto-Ribeiro et al., 2013). In contrast, Vairappan et al. (2012) found neral as the major metabolite in ginger oil from Bentong and China and geranial as the major component in a specimen from Keningau. The report also showed that the presence of α-zingiberene was only found in the specimen from China. Hirmizi et al. (2015) found eugenol and eugenol acetate as the main components of ginger oil collected from Janda Baik, Pahang. Singh et al. (2008) and Sukari et al. (2008) also identified geranial as the

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[a] Calculated retention index with respect to a homologous series of n-alkanes (C_8-C_{20})

[b] Retention index from published data

* Mass Fragment ions of compound obtained from mass spectrometry

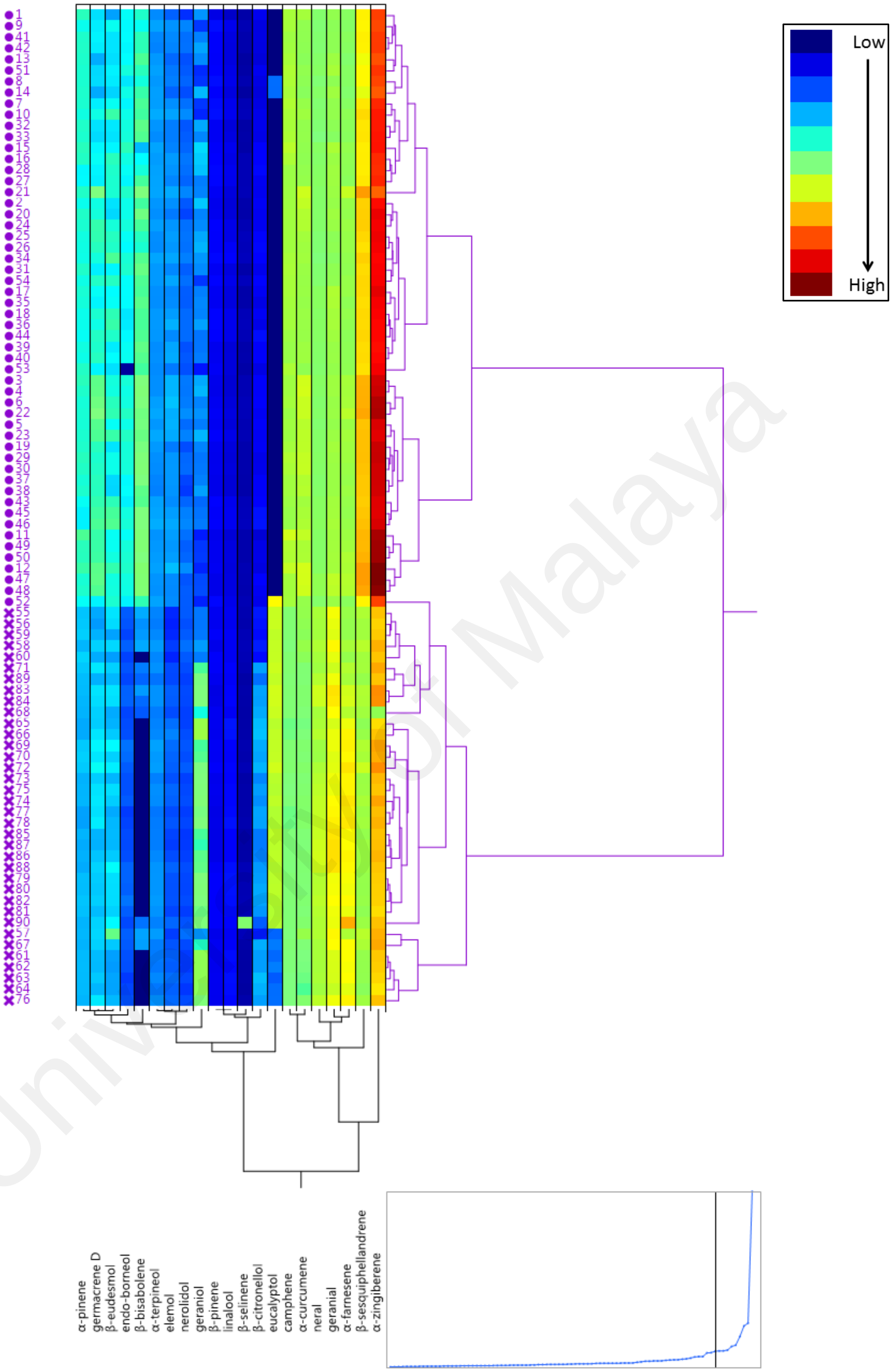
n.d : not detected

major component of ginger oil whereas El-Baroty et al. (2010) and López et al. (2017) described β-sesquiphellandrene and eudesmol respectively as the major compounds of ginger volatile oil.

Zingiberene is a monocyclic sesquiterpene, which is the principal aroma of ginger that is responsible for its distinctive flavor. This compound has also been used as a remedy for the treatment of diseases. It is known to have anti-inflammatory, antioxidant, and anti-proliferative effects on cancer cells (Gupta et al., 2016). Malek et al. (2005) identified zingiberene as the most common component of *Zingiber officinale* Rosc. var. *officinale* (common ginger), as well as other *Zingiber* species including *Zingiber officinale* var. *rubrum* (jahe merah), *Zingiber officinale* var. *rubrum Theilade* (halia bara), and *Zingiber officinale* var. *rubrum* Theilade (halia padi), with content ranging from 3%–17%. In fact, this compound could make up to 38% of ginger volatile oil (Aziz et al., 2012). Zingiberene is formed in the isoprenoid pathway via the catalytic activity of the enzyme zingiberene synthase (Gupta et al., 2016). Despite a majority of the compounds being present in both samples, the differences in the concentration of the main components, as well as the other compounds, were very pronounced. The result was further analyzed via Exploratory Analysis i.e. HCA and PCA.

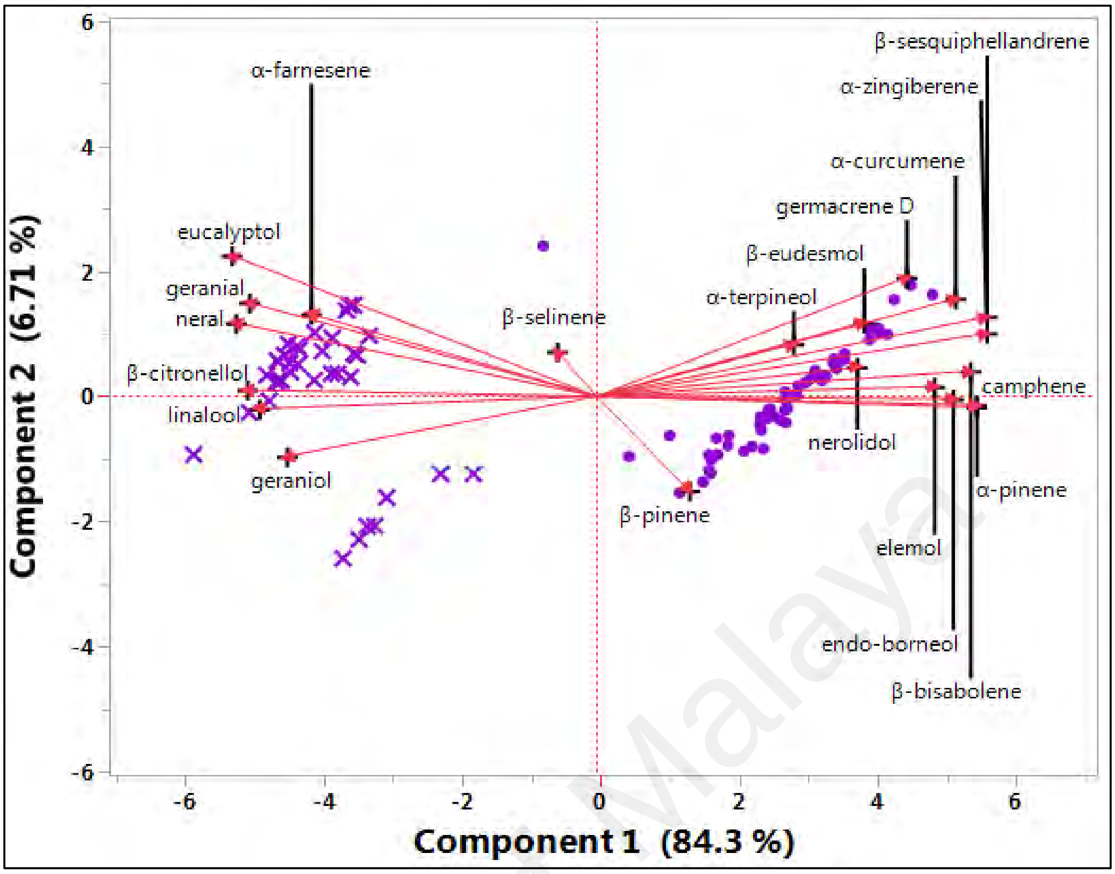
4.2.3 Exploratory Analysis of Bentong ginger volatile oil

HCA and PCA were performed and the results are shown in Figure 4.6 and Figure 4.7, respectively. The general structure of the dendrogram generated by HCA displays two main clusters, in which, the first cluster (labeled as ●) includes the oils from non-Bentong ginger while the second cluster (labeled as **X**) comprised the oil from Bentong ginger. The clear separation (formation of two clusters) between Bentong and non-Bentong samples indicated that the oil profiles of the two samples were significantly different from one another. Similar to the preliminary analysis, the HCA dendrogram color map was used to determine the differences in the two samples in relation to their relative abundance of terpene/terpenoid content. The color map showed that the Bentong gingers were mainly comprised of oxygenated monoterpenes (or monoterpenoids). As per the geranial cell, the color codes of the Bentong gingers were yellow (indicating a fairly high level of components) whereas the non-Bentong ginger samples demonstrated a green color (indicating a medium-low level of components). Besides that, the color differences between the Bentong samples (green color code) and the non-Bentong samples (blue color code) were also observed with respect to eucalyptol and geraniol. Plus, the low intensity of the blue color representing linalool and β-citronellol in the Bentong samples indicated a higher composition of these compounds than non-Bentong ginger samples. The HCA also revealed that sesquiterpenes/sesquiterpenoids were less prevalent in the samples of Bentong ginger. For instance, the color of the cell representing α-zingiberene indicated much lesser components in Bentong ginger oil (yellow-to-orange) compared to those of non-Bentong ginger (red-to-dark red). Similarly, the cell of β-sesquiphellandrene in the Bentong ginger samples and the non-Bentong ginger samples displayed a green color code and orange color code, respectively. The same trend was also observed in other sesquiterpene compounds such as germacrene D, β-eudesmol, elemol, and β-bisabolene.



Non-Bentong ginger = ● ; Bentong ginger = X

Figure 4.6: Dendrogram representing the variability in non-Bentong ginger volatile oil and Bentong ginger volatile oil



Non-Bentong ginger = ● ; Bentong ginger = ×

Figure 4.7: PCA scores and loading plot of non-Bentong ginger volatile oil and Bentong ginger volatile oil

The PCA data in Figure 4.7 showed that the first two principal components represented ~91.0% of the total variance, in which the first axes contributed ~84.3% of the total variance while the second axes accounted for ~6.7% of the total variance. PC1 enabled the separation of a majority of the samples of Bentong ginger from the non-Bentong ginger. These differences were mainly attributed to the variability in terpene composition, as the oils extracted from the Bentong samples were predominantly characterized by oxygenated forms of monoterpenene whereas sesquiterpenes/sesquiterpenoids were prevalent in the volatile oil extracted from the non-Bentong gingers. It is thus important to note that the PCA result is in line with the report generated from the HCA. A possible explanation for the inverse relationship observed between the monoterpenes and sesquitepenes could be due to the pathways to monoterpenes and sesquiterpenes that compete for common precursors (Wohlmuth et al., 2006). Monoterpenoid content is synonymous with the quality attributes of Bentong ginger volatile oil.

As expected, the above analyses showed quantitative and qualitative variations in volatile oil of Bentong ginger and non Bentong ginger. The variation was certainly not related to the extraction conditions as the extraction procedure has been standardized for both samples. Besides the sample preparation, factor such as geographical origin may also influence the variability in volatile oil composition. It has been suggested that plant oil profile is particularly representative and unique to each geographic location. This is accordance to previous study by Vairappan et al. (2012). The study revealed that Bentong ginger volatile oil recorded the highest level of oxygenated monoterpenes compared to ginger from China and Keningau. The ginger obtained from the Temerloh region of Pahang was extracted under optimized conditions with the oil constituents predominantly comprising sesquiterpenes (Buang et al., 2014). The ginger volatile oil from other ginger-producing countries also expressed some variability in oil compositions (Gupta et al., 2011; Mahboubi, 2019). For instance, the relative content of monoterpenes and sesquiterpenes were found to vary in ginger oils from several regions of North-East India (Kiran et al., 2013). Previous studies have also reported that ginger oil from Northern India showed a fairly high concentration of monoterpenes (mainly oxygenated monoterpenes) whereas the ginger oil from Southern India was oxygenated and rich in sesquiterpenes (Gupta et al., 2011; Koch et al., 2017; Raina et al., 2005). Ginger oil from Mauritius and Madagascar predominantly contained monoterpenes (Gupta et al., 2011; Gurib-Fakim et al., 2002). On the other hand, the oils of ginger from Japan, Vietnam, and Nigeria have been described to have high sources of sesquiterpenes; and yet, there were differences in the relative percentage of several major components in the three ginger specimens (Koch et al., 2017; Mahboubi, 2019; Onyenekwe & Hashimoto, 1999). Australian gingers are known to be a good source of oxygenated monoterpenes, particularly neral and geranial, with relatively low levels of sesquiterpene hydrocarbons (Wohlmuth et al., 2006). Toure & Zhang (2007) found more sesquiterpene components in samples from China while samples from Guinea consisted of mostly monoterpene fractions. Ginger oil from China and Bangladesh were compared and the results revealed a high amount of sesquiterpenes in the China samples while the Bangladesh ginger oil had more monoterpene compounds (Nandi et al., 2013). In contrast, Aziz et al. (2012) found that the oil of gingers from China contained a high content of monoterpenes while the oil of Bangladesh gingers predominantly comprised sesquiterpenes. Other variations in oil composition were also observed in other plant species such as *Lippia alba*, *Zataria multiflora* and *Hyptis suaveolens* (Azevedo et al., 2002; Mesa-Arango et al., 2009; Sadeghi et al., 2015). Considering those findings, it can be concluded that the formation of two clusters in HCA and PCA could possibly related to difference in geographical origin of the two samples.

4.2.4 The effect of geographical origin on Bentong ginger oil profile

It is known that Bentong gingers were cultivated in Janda Baik of Pahang whereas for the non-Bentong ginger, all of the samples were presumed to have different origin, such as China (as per stated on their labels). The result from exploratory analyses showed that the samples of Bentong ginger were clearly differentiated from those of non-Bentong ginger. As for the non-Bentong samples, despite they were collected from various local markets in Klang Valley, all of the samples clustered together and can be classified as a single group. The clustering of these samples implied that all the non-Bentong samples have similar chemical profile and were closely related than to those from Bentong origin. Therefore, it is worth noting that the variation pattern observed in the volatile oil is consistent with the geographical label that could serve the authentication of Bentong ginger, which is the main subject of this study.

It is suspected that the Bentong regions in which the gingers are grown could have influenced the quality composition of their volatile oils. Şanli & Karadoğan (2016) and Sardrodi et al. (2017) suggested a strong association between the effect of geographic origin on the composition of plant volatile oil based on the differences in environmental conditions of the cultivation regions such as altitude, temperature, sunlight exposure, rainfall or a combination thereof, all of which may alter many eco-physiological reactions in plant bodies. Most plants would normally alter their metabolim, growth and reproduction processes correspond to their surroundings for survival. In this regard, the biosynthesis of plant metabolites could as well be affacted which result in variation in the volatile oil composition.

4.2.4.1 Altitude effect

The variation in the major constituents of plant volatile oils is sometimes linked to the so-called altitude effect. As described previously, Bentong ginger is mainly cultivated in the highlands of Bentong region. Altitude was found to affect the chemical composition of *Achillea aucheri* Boiss volatile oil (Sardrodi et al., 2017). The study indicated an increment in total monoterpenes and a decrease in sesquiterpenes with elevated altitude, similar to Azevedo et al. (2002), who investigated *Hyptis suaveolens* volatile oil and found that samples from a low altitude mainly comprised sesquiterpenes, whereas monoterpenes dominated the samples from higher altitudes. These results, however, did not agree with the findings of Pavela et al. (2019), who showed a reduced monoterpene content in *Thymus zygiformis* var. *majellensis* oil with increased elevation. Also, the study found no correlation between volatile oil composition and altitude for *Thymus longicaulis* and *Thymus spinulosus*. These contradictions suggest that the altitude effect could be too general to explain the variations in the findings. The most recent study on this subject found a link between the physiological and biochemical characteristics of the plants with the climate changes at higher altitudes, stating that these plants experienced different climate conditions; often with lower temperature, strong sunlight radiation, and fairly high rainfall (Cui et al., 2018; Li et al., 2014).

4.2.4.2 Temperature effect

The Bentong ginger cultivation site has temperature between 24 °C and 28 °C that could drop to ~22 °C (especially at night). The moderately cold temperature of the region could influence the concentration level of certain terpene compounds in the ginger. *Aloysia triphylla* and *Pelargonium graveolens* volatile oils contained the highest amount of citral and geraniol, respectively, during winter season, which typically has a low temperature (Sgarbossa et al., 2019; Soni et al., 2015). Similarly, the oil of *Ocimum basilicum* was found to have a high oil yield that was richer in oxygenated monoterpenes during winter while the samples taken in summer predominantly comprised sesquiterpene hydrocarbon with a lower oil yield (Hussain et al., 2008). The differences in yield might be associated with the high temperature during summer, leading to the partial evaporation of some constituents of the oil. High temperatures induce a rapid change in plant physiological status (e.g. stomatal function), which subsequently affects the plant production of terpenes. Plus, some terpenes such as monoterpenes and its oxygenated form are highly volatile at high temperatures, thus affecting emission rates and the compound concentration in tissue. A study on the emission rates of biogenic volatile organic compounds (BVOCs) showed an increment in isoprene and monoterpene emissions at high temperature, resulting from more active synthesis in the plants, but a partly higher release rate from storage, which lowered the concentration of such compounds in the tissue (Guenther et al., 1993; Holopainen et al., 2013). Despite the above findings, Ramak et al. (2014) review of past related studies showed that the effect of high temperature on the monoterpene pool size was unclear due to an inconsistency in the results such as those published by Constable et al. (1999) and Snow et al. (2003) on *Pseudotsuga menziesii*.

4.2.4.3 Sunlight exposure

Besides the above factors, sun exposure is also one of the important factors that stimulate the biosynthesis of most secondary metabolites. Plants growing at high elevations have a relative advantage due to sufficient light exposure for growth and development (Gale, 2004). Anasori & Asghari (2008) found that the production of zingiberene and gingerol in the callus culture of *Zingiber officinale* Rosc. was prompted by light. Barra (2009) review study described light as being responsible for the increment in the monoterpene content of *Ocimum basilicum* L. and *Satureja douglasii* Benth. Such trend was justified by Ghaffari et al. (2019) who reviewed the finding by Zhang et al. (2017a) in regards to the distinctive responses of some volatile oil components towards sunlight. The group stipulated that the biosynthesis of linalool and ocimene was hindered to a great extent in the absence of sunlight. Yet, the re-exposure of the compounds to the light has resulted in increment of their contents. Sunlight is a key factor in plant growth and development, as it provides energy for photosynthesis. Secondary metabolites such as terpenes are formed from photosynthetic carbon obtained from photosynthesis. It is said that light intensity could induce or halt the activity of certain enzymes (involved in the mevalonic acid pathway, the precursors of terpenes), leading to the predominance of a particular biosynthetic process (Şanli & Karadoğan, 2016). Despite multiple reports stating the influence of light on terpenoid content, no prevalent behavior has been observed and it is believed that the content could also be highly dependent on other factors including the plant species and type of metabolites (Singh & Sharma, 2015).

4.2.4.4 Rainfall

Environmental conditions such as rainfall could also have influenced the concentration of monoterpenes in Bentong ginger. Water availability is very important for the growth of many plants including ginger. A sufficient amount of water is required in the early rapid growth phase of ginger, followed by a fairly high water supply during the growing period and dry weather (less water) during the maturity phase (before harvesting). Bentong ginger grows in a region where rainfall is well distributed over the year (average annual rainfall of 2200 millimeters) accompanied by the dry season, making the area conducive for the cultivation of the crop. Increased water availability was found to induce the production of monoterpenes and its oxygenated forms in *Mentha spicata* (Marino et al., 2019). A similar result was found for *Lippia thymoides* oil where high amounts of oxygenated monoterpenes were observed in the samples during the rainy season (Silva et al., 2018). However, the published reports on a possible relationship between the variability in plant volaitle oil and water availability are inconsistent. In contrast, the oil of *Achillea millefolium* predominantly comprised oxygenated monoterpenes with lower water availability (Alvarenga et al., 2018). Based on the above discussion, possible outcomes could arise as a result of the combined synergistic effect of abiotic factors such as temperature, light, and rainfall, which consequently influence the enzymatic activity in plants and interfere with the biosynthesis of certain secondary metabolites.

4.2.4.5 The nature of cultivation soil

In addition to the factors mentioned above, the nature of the soil in which the plant is cultivated is sometimes responsible for the chemical variability in plant volatile oil. The local farmers in Janda Baik often practice the nomadic cultivation of Bentong ginger to overcome soil infertility, as well as to replenish the nutrient level in the soil, where replanting on the same land can only be done at least after 6 years (Suhaimi et al., 2014). According to the local farmers, Bentong ginger is said to face difficulty thriving in other regions outside Janda Baik and the characteristics of those gingers are different from those cultivated in Janda Baik, suggesting that the region has a unique soil for cultivating the plant. However, such a claim could not be proven due to a lack of scientific data regarding the soil nutrient content in the region. Previous studies have demonstrated a correlation between the content of volatile oil metabolites with the soil characteristics of the collection sites of *Thymus migricus* (Yavari et al., 2010). This result is also per that of Şanli & Karadoğan (2016). The differences in soil textures and climates between the low altitude and the high altitude Lake region of Turkey have been found to affect the concentration of some monoterpene constituents in the volatile oil of *Kundmannia anatolica*. The emission of terpenoids could be induced by nutrients in the soil such as nitrogen, which promotes the electron transport rate and leaf photosynthesis, which provide ATP requirements and carbon substrate availability for the synthesis of isoprene. Besides that, phosphorus is also identified as a key component of NADPH and ATP for the biosynthesis of terpenoids. The precursors of terpenoid (isopentenyl diphosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) contain high-energy phosphate bonds, suggesting that phosphorus could indeed influence terpenoid production (Ormeño & Fernandez, 2012).

4.2.4.6 Biotic stress

Another possible factor that can induce the domination of certain metabolites is biotic stress. As discussed earlier, a plant produces different oil constituents in response to their local surroundings; temperature, light, water availability, as well as the nature of the soil in which it is planted, and, recent evidence has implied that biotic stresses such as pathogen attack (e.g. fungal, bacterial, and viral) could also lead to the same result (Mazid et al., 2011; Sánchez-Sánchez & Morquecho-Contreras, 2017; Sharifi-Rad et al., 2017). The cultivation of highland crops is more susceptible to waterlogging caused by excessive rainfall and an ineffective irrigation system, both of which could result in soil-borne diseases (caused by fungal and bacterial) and nematode infestation (Suhaimi et al., 2016; Zuraida et al., 2016). It is suggested that excessive exposure to this condition could trigger the biosynthesis of certain terpenes/terpenoids such as oxygenated monoterpenes as part of the plant's defense mechanism since this class of compounds is known to exhibit a broad spectrum of antibacterial activity that is better than hydrocarbon terpenes

(Guimarães et al., 2019; Li et al., 2019). The cytotoxicity of terpenes against microbes is mostly associated with the compound's hydroxyl, aldehyde, and ketone groups; in which terpenoids with hydroxyl groups (alcohol) possess higher antimicrobial activity than those with carbonyl groups (İşcan, 2017; Zengin & Baysal, 2014). Previous studies have suggested that most plants respond to pathogen attacks via intricate and dynamic defense systems that include structural barriers and the production of a wide spectrum of defensive secondary metabolites (War et al., 2012). Plant roots have been found to release diverse volatile constituents that act as antimicrobial agents that protect the plant from underground insect pests, and by attracting the natural enemies of the root-eating insect pests. This result is per the findings of Montanari et al. (2011), who found that vegetative parts release volatiles to protect the plant. These volatiles deter herbivores from attacking, whereas floral volatiles could be emitted to attract specific pollinators. For instance, conifer releases a complex mixture of terpenoids upon tissue disruption as a first-line direct defense against bark beetles and their associated fungal pathogens (Fantaye, 2014). Ebadollahi (2011) review study revealed that volatile monoterpenes or volatile oil are important components in protecting plants from pathogenic fungi as well as insect pests. For instance, pine produces α-pinene, which is described as a defense mechanism against *Ophiostoma minus and* ectosymbiont fungi *Entomocorticium* sp. (Garcia et al., 2008).

Based on the above discussion, the environmental conditions of the Bentong region have been identified as a potential factor influencing the chemical variability in the Bentong ginger volatile oil. The domination of monoterpenoids in the volatile oil is not only functionally important to the plant itself but is also beneficial for humans. Sasidharan and Menon (2010) identified citral and geraniol as the acrylic monoterpenoids in ginger oil that demonstrate a strong repellent property against cockroaches. Besides, this oil could potentially be used as an antioxidant agent in the agrifood industry due to the strong antioxidant properties of monoterpenoids compared to other terpenes (Bhavaniramya et al., 2019; Zengin & Baysal, 2014). Other than that, this oil could also be used as a natural fragrance since monoterpenoids such as neral, eucalyptol, and geranial are highly odoriferous (Morsy, 2017).

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study aimed to investigate the variation in ginger volatile oil caused by different extraction treatments and to explore the chemical variability in Bentong ginger via Exploratory Analysis.

The preliminary study revealed that the yield of ginger volatile oil could vary between 0.04% and 0.17% depending on the administered treatment. Based on the outcomes of this study, extraction settings such as sample freshness, size reduction, and extraction duration could significantly affect the oil yield. Plus, extending the extraction process to 5 hours using a fresh ginger sample with reduced particle thickness could quantitatively improve the yield. The quality attributes of ginger based on terpene content varied according to the extraction settings; monoterpenes dominated the fresh samples, while sesquiterpenes were prevalent in the grated samples and showed antagonistic effects due to the extraction time.

The Bentong ginger had a higher oil yield compared to that of non-Bentong ginger, at 0.20% ± 0.03 w/w and 0.13% ± 0.03 w/w, respectively, but both samples had α-zingiberene as the main component. The chemical variability of the volatile oil from Bentong ginger was strongly attributed to the domination of monoterpenes, which could be related to the environmental factors in the cultivation site. Overall, this study suggests that the proper manipulation of extraction conditions could increase the yield and quality of ginger oil to meet industry standards. Furthermore, the result also showed that multivariate analyses combined with the volatile oil profile of aromatic plants could be used to determine the quality of the plant based on its origin.

5.2 Significance of the study

The rationale of this study is to highlight the quality attributes of Bentong ginger with respect to its volatile oil using the chemometric approaches. The application of metabolic profiling combined with exploratory analyses such as PCA and HCA has successfully discriminated the volatile oil based on extraction settings and geographical origin. More importantly, this approach has provided the ability to discover and recognize a characteristic pattern and chemical components (domination of monoterpenes) that associated to the Bentong ginger oil, which to the knowledge of the author have not been previously reported. This in turn would serve as an important data especially for the purposes of quality control of Bentong ginger in overcoming food frauds and problem of adulteration.

5.3 Recommendations

Based on this study, it is recommended that future work include a soil analysis of the ginger cultivation site to better understand the effect of this variable on the variability of ginger volatile oil. Additionally, more financial support is needed to encourage more studies to investigate the mineral content and element isotope ratios in Bentong ginger and its potential as a medicinal herb, as it has not been extensively studied and there is limited information regarding this area.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

PUBLICATION

1. **Ahmad Jahiddin, F. S.** & Low, K. H. (2019). Evaluation of terpene variability in the volatile oils from *Zingiber officinale* using chemometrics. *Current Analytical Chemistry, 15*(1). doi:10.2174/1573411015666190710221141