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RESEARCH ARTICLE

Analysis of white pepper essential oil components using gas chromatography-mass spectroscopy

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Abstract

Introduction: White pepper is an important commodity used to produce essential oils. Differences in the oil components are determined by which region the peppers used were sourced from. **Objectives:** The aim of this study was to analyse the components of the essential oils produced by white peppers from different regions and to examine the specificity of these peppers. **Methods:** This analysis was carried out using the chromatography gas – mass spectroscopy (GC-MS) method on essential oil samples made from peppers obtained from the Java, Sumatera, Kalimantan, and Sulawesi Islands. **Results:** The results showed that white pepper essential oil contains 22 components, and that *α-pinene, β–pinene, Δ-carene, sabinene, dl-limonene*, and *caryophyllene* were major compounds within the oils. Furthermore, it showed that *α-pinene, sabinene*, and *caryophyllene* compounds were most found in samples from the Kalimantan, Sumatra, and Sulawesi Islands respectively. Meanwhile, those from Java Island contained *β– pinene*, Δ-*carene*, and *caryophyllene* in similar quantities.

Introduction

Pepper crops form some of the most widely cultivated plantations in Indonesia. According to the 2017 statistics on the county's plantation commodities, this spice was also widely grown on the Java, Sumatra, Kalimantan, and Sulawesi Islands. Pepper plants produce black and white fruits, which can only be distinguished as a result of differences in post-harvest handling procedures (Zhao *et al*., 2005). White pepper can be used as a cooking spice, flavouring, meat preservative and traditional medicinal ingredient (Direktorat Jendral Perkebunan, 2019).

Essential oils are a mixture of low molecular weight molecules that are responsible for producing distinct aromas. The quality, freshness and uniqueness of the oils make them very valuable (Calo *et al*., 2015). Nonetheless, heat, humidity and oxygen greatly affect their stability, thus decreasing their quality. Essential oils are volatile and can be produced from the roots, leaves, stems, fruits, and seeds of various plants. They can also be obtained through distilling water vapour and from some animals, such as musk, sperm whales, and even microorganisms (Li *et al*., 2019).

The use of essential oils in the industry is quite extensive. In the food industry, they are widely used as flavouring ingredients or flavour enhancers (Calo *et al.*, 2015; Z. Liu *et al*., 2019). Meanwhile, in the pharmaceutical industry, they are widely used as antibacterial agents (Calo *et al*., 2015; Tamokou *et al*., 2017). These oils dissolve in fat underneath the skin because they are biodegradable and can be absorbed into blood vessels. In research carried out by Hu and the authors (2019) and Demirok Soncu and the authors (2018), it was shown that they act as very effective antifungal agents.

In the cosmetics industry, essential oils such as Patchouli oil, fragrant root and sandalwood are widely used as perfumes, while those made from pepper, ginger, cinnamon, cloves and coriander are widely used as cooking spices (Calo *et al*., 2015).

Essential oils are insoluble in water but are soluble in alcohol, ether, and fixed oils. Generally, they are colourless and liquid at room temperature. The composition of these oils is very dependent on the species of plant that they were extracted from, their growth location, the time of harvest, and the extraction techniques used. Based on their chemical structure, the compounds within these oils can be classified as terpenes, straight-chain compounds without side chains, phenylpropanoids (benzene derivatives), and more (Moghaddam & Mehdizadeh, 2017).

Various factors can qualitatively and quantitatively affect the chemical composition of essential oils. One of which is the environment, as it can greatly affect the metabolic pathways of plants, which in turn leads to variations within their chemical components. In addition, plant age, rainfall, luminosity, soil composition, environmental pollution and microorganisms in the soil greatly affect the chemical components produced by plants (Fokom *et al*., 2019).

Essential oil analysis is generally carried out using gas chromatography (GC) or gas chromatography-mass spectroscopy (GC-MS) methods. GC is a chromatographic method that uses gas as a mobile phase (L. Liu *et al*., 2007).

Method

This study was carried out using experimental methods, starting with sample preparation, distilling the essential oils, determining the optimum conditions needed for GC-MS, and analysing the oils using GC-MS at optimum conditions.

Materials and tools

Materials: toluene, aqua, n-hexane and white pepper seeds samples from the Java, Kalimantan, Sumatera, and Sulawesi Islands. Tools: GC-MS Agilent Technologies 7890A, a steam distillation apparatus, and glassware.

Sample preparation

Raw white peppers were obtained from the Java, Kalimantan, Sumatera and Sulawesi Islands. These were purchased directly from the farmers on these islands. In order to further ensure the authenticity of the materials, a determination process was carried out in the Institute Technology of Bandung (ITB) Bioengineering Science School laboratory.

Essential oil distillation

The steam distillation method was used to distil these oils. This method resembled the boiling method; however, in this case, the water did not come into direct contact with the distilled materials.

Essential oils analysis with GC-MS

After determining the conditions needed for the GC-MS analysis, the essential oils were then analysed under the optimum conditions.

Result and discussion

Sample preparation

Based on the plant determination results, the sample proved to be white pepper.

The plant's taxonomy was as follows:

Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Magnoliidae
Order: Piperales
Family: Piperaceae
Species: *Piper nigrum L*.
Synonym: *Muldera multinervis Miq*.

Distillation of the essential oils

The distillation process for the essential oils lasted 6 hours and was declared complete when the produced condensate no longer contained oil.

Analysis of essential oil components

The optimum conditions needed for the GC-MS oil analysis can be seen in Table I. Analysis of the essential oils using the GC-MS showed that the essential oils made from white peppers from the Java, Kalimantan, Sumatra and Sulawesi Islands contained almost the same components. Moreover, it showed that there were 22 component compounds within the oils. The essential oil components can be seen in Table II. White pepper chromatogram samples from the Java, Kalimantan, Sumatra and Sulawesi Islands can be seen in Figure 1.

Of the 22 compounds identified, the 6 with the largest compositions differentiated the essential oils based on which region they originated from. These six compounds were *alpha-pinene, beta-pinene, delta-3-carene, sabinene, dl-limonene*, and *caryophillene*. The white pepper from Java Island was more dominant in *beta-pinene, dl-limonene*, and *delta-3-carene* compared to those from the other islands. *Sabinene* was most commonly found in the essential oils from Sumatra Island, *alpha-pinene was most commonly found in the essentials oils from* Kalimantan Island, and *caryophyllene was most commonly found in the essentials oils from* on Sulawesi Island. This study showed that the differences in cultivation location affected the relative composition of the essential oils.

Table I: The optimum GC-MS conditions

Condition	Description
Gas chromatography	*Agilent Technologies* 7890A
The brand	HP-5MS, length 30m, diameter
Coulomb	0.25 mm
Carrier gas	Helium
Detector	Mass spectroscopy
Injection volume	1 mL
Injection technique	Split
Split ratio	25:1
Temperature programme:	
Injector temperature	250°C
Initial temperature	40°C
Temperature rate	10°C/minute
Final temperature	40°C
Intercept temperature	280°C
Detector temperature	250°C
Mass spectroscopy:	
Merck	*Agilent Technologies* 5975C
Mass range	40-550
Resolution	1188

Table II: Essential oil components present

Retention time (minutes)	Compound names
6.006	1-isopropyl-4-metthylbicyclo[3.1.0]hex-2-ene
6.130	Alfa pinene
6.401	Camphene
6.817	Sabinene
6.881	2-beta-pinen
7.079	Beta-Myrcene
7.338	I-Phellandrene
7.435	Delta.3 Carene
7.544	alfa-Humulene (CAS)
7.678	Benzene, 1-methyl-3-(1-methylethyl)-
7.744	dl-Limonene
8.242	y-Terpinene
8.731	Cyclohexene, 1-methyl-4-(methylethylidene)-
8.879	Linalool L
12.492	q-Elemen (CAS)
13.146	Copaene
13.388	Beta Elemenen
13.998	Caryophyllene
14.277	Guaia-1(5),11-diene
14.676	Alfa-Humulene
15.426	Beta-Seline (CAS)
15.630	Alfa-Selinene

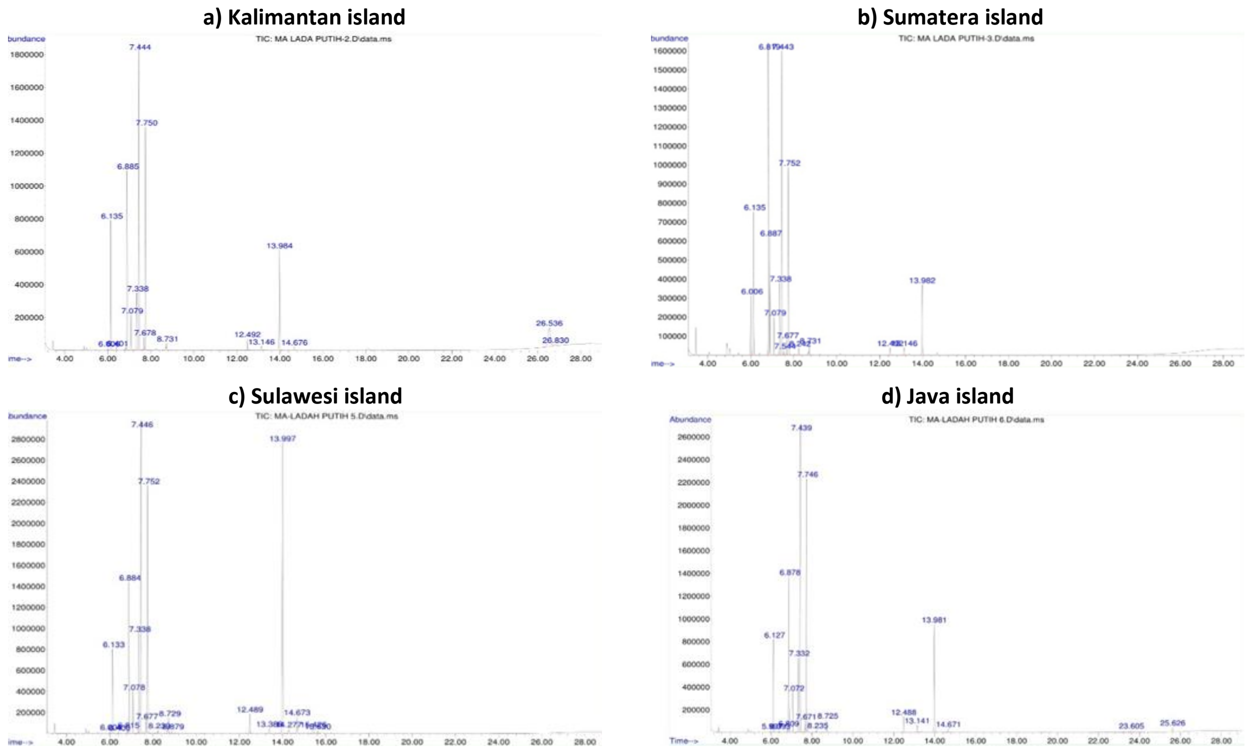

Figure 1: Chromatogram of white pepper's essential oils sample

The spectrum of the six main molecules can be seen in Figure 2. The identity of each molecule was determined based on the value of the spectrum match factor (MF) of the tested molecules against the data library. Match factor data can be seen in Table III. MF values that are close to 999 or greater than 700, indicate that the molecular identities are very compatible (Reichenbach *et al*., 2019). The data library used was the NIST11. The results were similar to those obtained by Liu in 2007. Liu, Song and Hu analysed the white pepper essential oil components using the GC-MS method without mentioning the location from which the pepper was obtained. They also showed that the main components of this pepper were *caryophillene, 3-carene, d-limonene, beta-pinene*, and *alpha-pinene*.

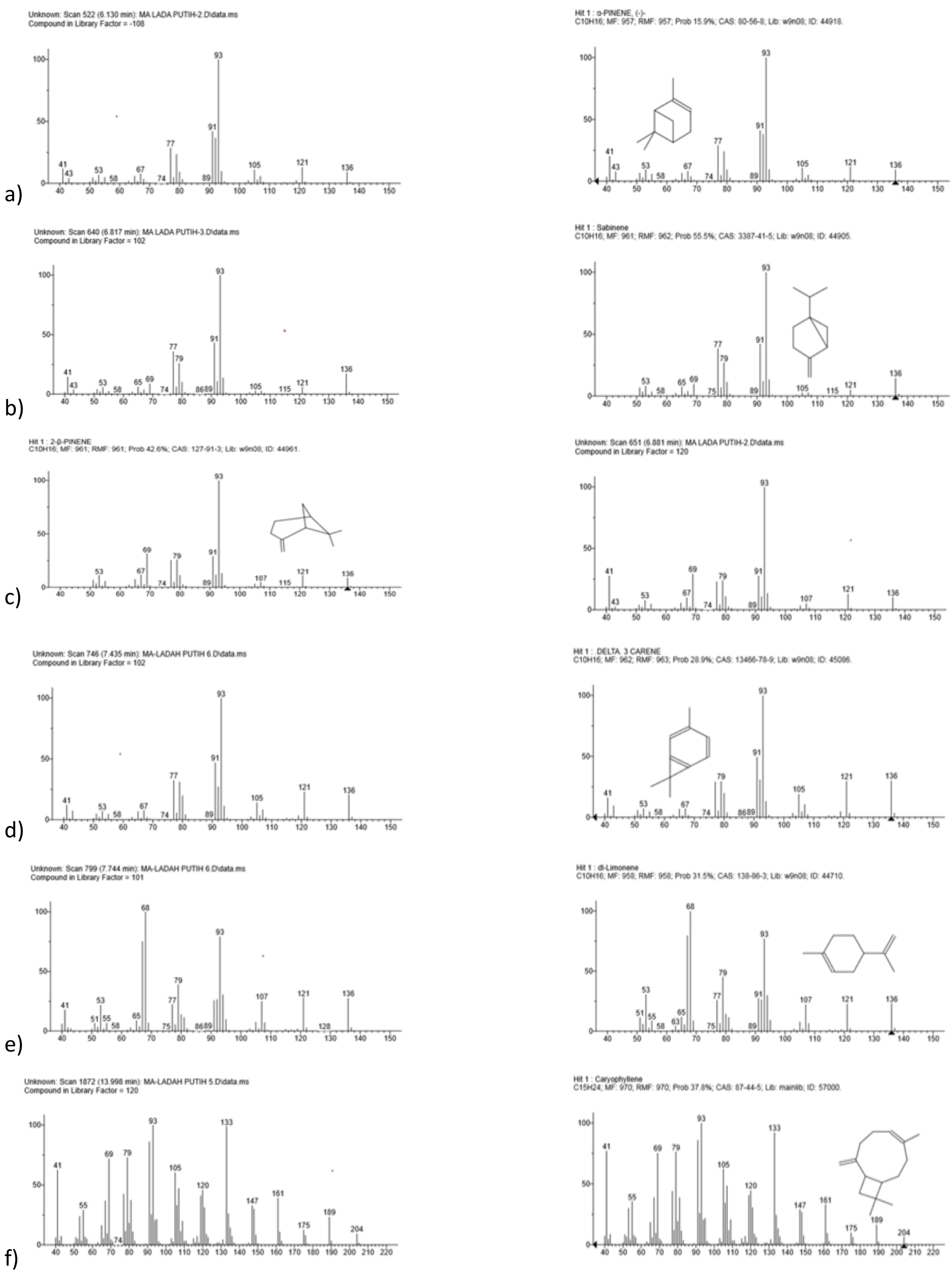

Figure 2: Spectrum of the six main molecules

Table III. Match Factor (MF) values of main molecules in the white pepper samples

No	Compound	Retention time	MF	%Area			
				Java	Sumatera	Kalimantan	Sulawesi
1	*α-pinene*	6,130	957	7,83	9,92	10,65	5,20
2	*Sabinene*	6,817	961	0,41	21,58	0,14	0,24
3	*Beta-Pinene*	6,881	961	13,03	8,18	14,82	9,48
4	*Δ-3-carene*	7,435	962	27,83	21,39	24,98	21,37
5	*dl-Limonene*	7,744	958	21,68	15,41	17,86	16,08
6	*Caryophyllene*	13,998	970	13,18	6,99	11,68	30,90

Conclusion

The white pepper essential oil from Java, Sumatra, and Kalimantan Islands contained 22 constituent compounds. In addition, those from different islands have specific compounds which relate to their differences in composition. *Alfa-pinene, Sabinene, and caryophillene* were dominantly found in white pepper from the Kalimantan, Sumatra, and Sulawesi Islands, respectively. Meanwhile, those from Java Island contained *beta-pinene*, *delta-carene* and *caryophillene* in similar quantities.

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