I INTRODUCTION

1.1 Background

Indonesia is a rich country with abundant natural resources and local endemic foods full of flavor; however, many have not been well known. One of the local endemic food is andaliman (*Zanthoxylum acanthopodium DC).* It is locally known as Batak Pepper, a traditional spice from North Sumatera with a unique citrus aroma and numbing and tingling trigeminal sensations. Three types of andaliman have been identified: Sihorbo, Simanuk, and Sitanga; however, out of all three, Simanuk andaliman is the mostly used and cultivated one because of its more robust flavor and higher yield (Wijaya *et al.* 2018). Andaliman fruits, the commonly used part of the species, change its color from green to red as it matures, however, green andaliman is commonly used because the red ones rot quickly and lose the characteristic andaliman flavor (Wijaya *et al.* 2018)

Zanthoxylum, as the genus of andaliman, has more than 500 species (Wijaya *et al.* 2018), and numerous studies have been conducted for *Zanthoxylum* chemical composition, aroma, flavor, and functional benefit. Unfortunately, the research, exploration, usage, and popularity of andaliman are behind other species such as *Z. piperitum* (Japanese pepper) and *Z. bungeanum* (Sichuan Pepper).

The main hurdles in andaliman usage are the rapid rotting and flavor degradation, mostly due to high moisture content. Good processing techniques will be crucial to expand andaliman usage by local farmers and industries. The effect of drying to the sensorial intensities (aroma and trigeminal) of andaliman has been studied previously by sensory analysis, and optimization of oven drying method to dry andaliman has also been conducted (Napitupulu et al. 2020); however, sensory analysis on andaliman tends to be difficult and inaccurate due to sensory fatigue from its numbing sensation. Furthermore, the drying process's impact on the volatiles and numbing compounds of andaliman has not been understood.

Studies on the effect of drying to volatiles and chemical composition in food commodities have been done on many types of food such as apples (Krokida and Philippopoulos 2006), bay leaves (Díaz-Maroto *et al.* 2002), grape skin (de Torres *et al.* 2010), oregano (Figiel *et al.* 2010), kaffir lime leaves (Raksakantong *et al.* 2012) thyme (Calín-Sánchez *et al.* 2013; Rahimmalek and Goli 2013; Dehghani Mashkani *et al.* 2018), ginger (Ding *et al.* 2012; An *et al.* 2016), rosemary (Szumny *et al.* 2010; Calín-Sánchez *et al.* 2011), spearmint (Antal *et al.* 2010), and even to *Z. myriacanthum* (Sriwichai *et al.* 2019) with different recommendations of drying technique were proposed to dry each commodity. However, this type of study has not been done to andaliman. Therefore, a study is needed to determine changes of volatiles and numbing compounds of andaliman by various drying techniques: oven drying, freeze drying, sun drying, sun drying with shade-cloth, and shade drying.

The numbing sensation of andaliman is due to unsaturated alkylamides, also known as sanshools. Many types and isomers of sanshool compounds have been identified; however, the main sanshool compound found in andaliman is α-sanshool (Wijaya 2000; Wijaya *et al.* 2018), whereas the main sanshool compound in Sichuan Pepper and Japanese Pepper was reported as hydroxy α-sanshool (Sugai, Morimitsu, and Kubota 2005; Yang 2008). Analysis of sanshool compounds is usually conducted using column chromatography or HPLC (High-Pressure Liquid Chromatography); however, these methods tend to be time-consuming and require large amounts of costly solvent and expensive stationary phase (Sugai, Morimitsu, and Kubota 2005; Wang *et al.* 2011).

On the other hand, aroma analysis is conducted using GC-MS (Gas Chromatography-Mass Spectrometry), which requires two analytical methods to get the complete picture of both the aroma and taste of andaliman. However, three studies managed to identify sanshool compounds by GC-MS (Adesina and Akinwusi 1986; Tirillini and Stoppini 1994; Reyes-Trejo *et al.* 2019), which will shorten the research timeline and reduce the cost to analyze the quality of dried andaliman. Previous research showed that direct solvent extraction of andaliman by diethyl ether and chloroform produced the most representative extract to fresh andaliman aroma, which resulted in 24 tentatively identified volatiles with geranyl acetate and limonene as the major compounds and 11 aroma-active compounds with citronellal and limonene as the key aroma compounds (Wijaya et al. 2001), similarly, chloroform extraction managed to extract sanshools from *Z. piperitum* (Sugai, Morimitsu, and Kubota 2005). Therefore, chloroform extraction is the preferred method to extract both aroma and sanshool compounds from andaliman.

A consumer-based sensory analysis on andaliman aroma was conducted to compare the result GC-MS analysis. Rate All That Apply (RATA)-Hedonic Test is the method of choice because it was quick and able to differentiate samples that have similar sensory characteristics from each other (Meyners et al. 2016). Conducting an affective test simultaneously with RATA analysis was also found to increase the discrimination between samples (Jaeger and Ares 2015). These tests are crucial to evaluate consumer acceptance of the aroma of andaliman.

This study aimed to confirm the GC-MS condition to detect both aroma and sanshools, analyze the impact of drying process on the volatiles, numbing compounds, physical and sensory characteristics, and determine the essential volatiles that played a substantial role in andaliman aroma quality and acceptance based on their correlation with favorable sensory attributes using PLS regression and Pearson correlation analysis.

1.2 Problem Statement

Andaliman is a highly perishable Indonesian spice with a unique yet unstable citrus-like aroma and numbing trigeminal sensation. Drying is a simple way to extend andaliman shelf life; however, it has been reported that each drying method would result in a different sensory profile. Studies on the impact of drying on andaliman profiles of volatiles, numbing compounds, and aroma are still quite limited. Moreover, different methods are typically conducted to characterize both andaliman aroma and numbing compounds, increasing research cost and complexities.

1.3 Research Objectives

The main objective is to evaluate the impact of drying on the volatiles and numbing compounds in andaliman (*Z. acanthopodium*).

1.4 Research Sub-Objectives

The sub-objectives of this research are to:

1. Identify the condition of GC-MS method to separate, detect, and identify both aroma and taste numbing components of andaliman.
2. Identify the physical and sensory profiles of variously dried andaliman
3. Determine the important volatiles to andaliman favorable sensory attributes

1.5 Hypothesis

The hypotheses of this research are:

1. Chloroform extraction and GC-MS technique can extract, identify, and quantify the aroma and taste numbing compounds of andaliman
2. There will be measurable differences in the volatiles, numbing compounds, and aroma profile of dried andaliman by different drying techniques.
3. Changes in volatiles composition will have a similar effect to the aroma perception of andaliman as detected by a consumer-based sensory panel
4. There will be certain dried andaliman that is most accepted by the panelists
5. There will be certain volatiles that could be utilized as markers of andaliman flavor quality.

1.6 Scope of the Research

The scope of this research is:

1. Green andaliman was of Simanuk variety, obtained directly from a local farmer in Gotting Raya Village, Simalungun District, North Sumatera.
2. Fresh green andaliman for GC-MS confirmation step was purchased from *Pasar Senen,* Jakarta.
3. Chloroform maceration was used as the sole extraction, and GC-MS was used to analyze the volatiles and sanshool compounds.
4. The identification of volatiles was based on Wiley WN808 Mass Spectral Library, confirmed with the literature's Linear Retention Index.
5. Identification of α-sanshool was based on comparing the mass spectra with the literature, while identification of hydroxy α-sanshool was based on commercial standard
6. Sensory aroma analysis was conducted utilizing Rate All That Apply method with 40 untrained panelists
7. Data analyses were based on One-way ANOVA, Duncan Post-Hoc, Principal Component Analysis, Partial Least Square Regression, and Pearson Correlation analysis.

1.7 Benefits of the Research

This research will illuminate the change in volatiles, numbing compounds, and sensory profiles of dried andaliman, find a better method to dry andaliman, and find a new technique in analyzing aroma and sanshool compounds, and to obtain important markers to andaliman aroma qualities.

II LITERATURE REVIEW

2.1 Zanthoxylum

Andaliman belongs to the *Zanthoxylum* genus, which is part of the *Rutaceae* family (Yang 2008), the same family of citrus fruits, with more than 500 species currently known (Wijaya *et al.* 2018). While each *Zanthoxylum* species has its own set of aroma profile and chemical composition, most of them possess this unique numbing and tingling trigeminal sensation (Yang 2008). Two of the well-known kinds are the Sichuan peppers from China, which consist of many species such as *Z. simulans, Z. bungeanum, Z. schinifolium,* and the Japanese pepper or *sansho* with the Latin name of *Z. piperitum.* Traditionally, *Zanthoxylum* is not only used as spices, but also for its health benefits as described in Table 1 as follows:

Table 1. Traditional usage of *Zanthoxylum* species

Species	Habitat	Traditional Usages
Z. acanthopodium	North Sumatera	Alleviate diarrhea, stomachache, toothache, food preservatives, spices (Suryanto *et al.* 2004; Parhusip *et al.* 2005; Yanti *et al.* 2011)
Z. alatum	The Himalayans	Cholera, diarrhea, dyspepsia, diabetes, coughing, fever, headache, infection, toothache, antiseptic (Prakash *et al.* 2012; Mukhija *et al.* 2014)
Z. americanum	North America	Pulmonary and bronchial diseases, burn, coughing, itchiness, wounds (Bafi-Yeboa *et al.* 2005)
Z. armatum	India, China, Taiwan, Nepal, Pakistan, Malaysia	Anti-flatulence, aids in digestion, antiparasitic, fever, toothache, anti-inflammation, insecticidal, antifungal, antimicrobial (Singh and Singh 2011; Kumar et al. 2014)
Z. bungeanum	China and Southeast Asia	Stomachache, toothache, ascariasis, diarrhea, dysentery, spices, stimulating saliva, dan increasing appetite (Bowers *et al.* 1993; L.-C. Yang *et al.* 2013; Tang *et al.* 2014)
Z. leprieurii	Cameroon	Indigestion, gonorrhea, anti-parasite, infertility (Misra *et al.* 2013)
Z. limonella	Thailand	Stomachache, toothache (Tangjitjaroenkun *et al.* 2012)
Z. nitidium	India, Australia	Anti-inflammation, burn, cancer, toothache, nerve pain, stomachache, throat pain, arthritis, snake bite (Chen *et al.* 2011; Hu *et al.* 2013; Liu *et al.* 2014)
Z. piperitum	Japan, Korea, China	Diuretic, stomachache, anti-parasitic, dan indigestion (Hatano *et al.* 2004; Hwang and Kim 2012)
Z. rhetsa	India	Asthma, bronchitis, heart complaint, rheumatoid, stimulant, *astringent*, improve digestion, local anesthetic (Alphonso and Saraf 2012; Ahsan *et al.* 2014)
Z. schinifolium	China, Korea, Japan	Spices and medicines (Diao *et al.* 2013)
Z .rhoifolium	Atlantic coast	Anti-inflammation, anti-infection, malaria, flatulence, colic pain, dyspepsia, ear pain, toothache, snake bite (Freitas *et al.* 2011)
Z. zanthoxyloide	Cameroon	Wound healing, anti-parasitic, alleviate edema (Misra *et al.* 2013)

2.2 Andaliman

Andaliman (*Zanthoxylum acanthopodium* DC.) is an Indonesian endemic plant from North Sumatera. It is widely used by the Batak tribe as one of their traditional cooking spices and folk medicines because of its unique citrus aroma and numbing or tingling trigeminal sensation when consumed by a human (Wijaya 2000; Wijaya et al. 2001; Wijaya et al. 2018). It is a perennial shrub plant with up to 5 m in height and thorns within the branches (Wijaya *et al.* 2018). Based on Keng (1978), andaliman is classified in the Division: *Spermatophyta,* Subdivision: Angiospermae, Class: *Dicotyledoneae*, Order: *Geraniales*, Family: *Rutaceae*, Genus: *Zanthoxylum,* Species: *acanthopodium DC.*

Figure 1. Fresh green andaliman (left) and fresh mature red andaliman (right)

The fruits, which are the commonly used part of the plant, change their color from green to red as they ripen, as shown in Figure 1. While both the green and red fruits could be used as spices, the red fruits readily rot and turn black, losing the characteristic andaliman flavor (Wijaya *et al.* 2018). Therefore, the unripe green fruits are typically used because of the longer shelf life. Each fruit contains only one hard-skinned, shiny black colored seed (Wijaya *et al.* 2018). In North Sumatera alone, there are at least three types of andaliman: Sihorbo, which has larger fruit and less aromatic flavor, Simanuk, which is commonly found in the markets because of its more robust flavor and higher yield, and Sitanga, which has a robust fruity flavor but unpleasant aroma disliked by the locals (Wijaya *et al.* 2018)

One of the main challenges in using fresh andaliman is that it is exceptionally perishable because of the high moisture content and unstable flavor compounds (Yang 2008; Wijaya *et al.* 2018). Drying of andaliman has been proposed to extend its short shelf life (Napitupulu et al. 2020). Drying managed to reduce its water content and activity; however, drying also affected its flavor profile due to the degradation of aroma and taste. While the effect of different drying methods on the andaliman overall aroma and trigeminal intensity has been reported (Napitupulu et al. 2020), little is known about the change of andaliman aroma profile, volatiles, and numbing compounds by different drying methods. A table summarizing recent studies of andaliman regarding its application in the field of food science and technology is provided in Table 2 as follow:

Table 2. Recent studies of andaliman in the field of food science

Objective	Result	Reference
Trigeminal active compound	A substituted alkylamide (α-sanshool): 2E,6Z,8E,10E-N-(2'methylpropyl)-dodecatetraenamide, MW: 247.1936, $C_{16}H_{25}ON$	(Wijaya 2000)
Aroma compound	Direct solvent extraction by diethyl ether and chloroform produced the most representative extract to fresh andaliman aroma; 24 tentatively identified volatiles with geranyl acetate and limonene as the major compounds and 11 aroma-active compounds with citronellal and limonene as the key aroma compounds	(Wijaya et al. 2001)
Antioxidant activity	Hexane-ethanol extract of andaliman had higher protection activity than BHT in an aqueous system, but not in an emulsion or fat system. The antioxidant activity was relatively stable under heat and pH, with a 13.6 % reduction after heating 175 °C for 120 min.	(Tensiska *et al.* 2003)
Antiradical activity	Ethanol andaliman extract possessed antiradical activity based on DPPH test similar to BHT and Vitamin E	(Suryanto *et al.* 2004)
Antimicrobial	Ethyl acetate extract of andaliman disrupts Bacillus cereus' growth, with MIC 0.2% and MBC 0.8%, by causing permeability and reducing hydrophobicity of the *B. cereus* cell wall.	(Parhusip *et al.* 2005)
Spray-dried andaliman powder	Ethyl acetate and ethanol andaliman extract was spray-dried with a mixture of maltodextrin and gum arabic (3:2) at an extract to carrier ratio of 1:8. The spray-dried powder gave a similar sensorial characteristic of fresh andaliman.	(Akyla 2014)
Drying method to preserve andaliman sensory characteristic	Freeze drying produced the best dried-andaliman; however, due to its high cost, convective oven drying was proposed as the ideal method, which was further optimized, resulting in 54 °C and 480 min as the ideal drying parameter	(Napitupulu et al. 2020)
Andaliman chili sauce formulation	5% of andaliman and 0.6% of citric acid was ideal for producing a product that could be accepted by sensory as well as stable during storage of 28 days	(Ephmara 2014)
Stability of andaliman antioxidant activity and sanshool content	Dried andaliman is recommended to store in the whole form, at a lower temperature, and in a packaging blocking oxygen and air, to preserve the numbing sensation and sanshool content.	(Inggita 2015)

2.3 The aroma of Andaliman and Zanthoxylum

Although each *Zanthoxylum* species varies in the sensory profiles and its aroma compounds, *Zanthoxylum* fruits generally have distinctive fresh, floral, spicy, and green aroma with unique numbing trigeminal sensations (Yang 2008). Andaliman has strong citrus notes and could even mask the fishy aroma of raw fish or meat (Wijaya et al. 2001). Such unique application was evident in traditional Bataknese food, *naniura,* a raw fish dish that has been fermented overnight using andaliman as one of the main flavor contributors causing the dish to have a unique citrus-like and peppery aroma of andaliman (Wijaya *et al.* 2018).

Previous study on andaliman odorants utilizing GC-MS has reported 24 tentatively identified compounds with the main volatiles were geranyl acetate and limonene with relative peak areas of 32.04% and 15.80% (Wijaya et al. 2001). However, even though geranyl acetate was the main aroma components, the key aroma compounds of andaliman, determined by the GC-Olfactometry AEDA method, were citronellal and limonene with flavor dilution (FD) factor of 128 and 32, respectively. High FD factor meant that those compounds had high aroma intensity and significantly contributed to the aroma of andaliman. Citronellal only had 5.63% relative peak area but had the highest FD factor, while geranyl acetate had 32.04% relative peak area but only had 4 FD factor (Wijaya et al. 2001). This phenomenon clearly showed that the most abundant compounds did not necessarily contribute significantly to the andaliman aroma. Table 3 below showcases the aroma-active compounds of andaliman, which shows that a citrus-like aroma dominates the aroma of andaliman.

Table 3. Fresh Andaliman aroma-active compounds (Wijaya et al. 2001)

Compounds	FD Factors	Relative Peak Area (%)	Aroma Description
Citronellal	128	5.63	Citrus, strong, warm
Limonene	32	15.80	Orange peel, sweet
Geranyl acetate	4	32.04	Citrus, floral, acid
Geraniol	4	3.34	Floral, lime leaf
Geranial	8	2.57	Lemon, sweet
β-myrcene	8	2.00	Citrus, sweet, cooked
β-citronellol	8	0.84	Citrus, warm
Linalool	8	0.76	Citrus, floral
(z)-β-ocimene	4	0.39	Citrus, sweet, cooked
Neral	8	0.22	Lemon, sweet
A sesquiterpene	4	0.13	Woody

Research on the key aroma compounds of Japanese pepper leaves or *Z. piperitum DC* showed that (Z)-3-hexenal, (Z)-3-hexenol, and 2-tridecanone, linalool, geraniol, citronellol, and citronellal to be the potent odorants (Kojima *et al.* 1997). Another research showed that the key aroma compounds of Japanese pepper green fruits were geraniol, linalool, citronellal, limonene, β-phellandrene, and methyl cinnamyl acetate, while of the ripe fruits were geraniol, citronellal, linalool, geranial, and geranyl acetate, and of the dried pericarps were geraniol, citronellal, and linalool geranyl acetate, methyl cinnamate (Jiang and Kubota 2004).

The main constituents in the aroma of other *Zanthoxylum* species that have been previously reported are summarized in Table 4 as follows. Limonene and geranyl acetate are the most reported aroma compounds in *Zanthoxylum* species. Interestingly, Yang (2008) did not manage to extract a significant amount of geranyl acetate from *Z. schinifolium,* whereas Chang and Kim (2008) managed to extract them. The different growing locations, China vs Korea might have caused this difference.

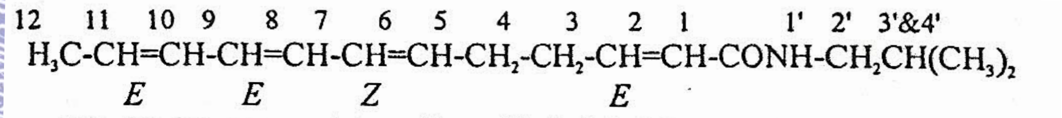
Z. schinifolium and *Z. bungeanum*, although are recognized as part of the Sichuan peppers, have significantly different properties. *Z. schinifolium* has red-colored pericarps and favored by flavorists while *Z. bungeanum* has green colored pericarps and favored by perfumer due to its strong refreshing and green aroma (Yang 2008). Andaliman, on the other hand, seems to be quite similar in aroma to Japanese pepper (sansho) *Z. piperitum* due to its limonene, citronellal and geranyl acetate content (Wijaya et al. 2001).

Table 4. Aroma constituents of other Zanthoxylum species

Species	Plant Parts	Extraction Method	Major Aroma Compounds (% Area)	Reference
Z. acanthopodium DC	Fruit	Diethyl ether extraction	Geranyl acetate (32.04%) Limonene (15.80%) Citronellal (5.63%)	(Wijaya et al. 2001)
Z. bungeanum (China)	Fruit	Hydro-distillation	Sabinene (5.55%) Limonene (12.3%) Linalool (13.2%) Linanyl acetate (15.2%)	(Yang 2008)
Z. piperitum	Fresh Green Fruit	Methanol extraction	d-Limonene (41.14%) β-Phellandrene (25.60%), Citronellal (1.82%) Geranyl acetate (10.11%)	(Jiang and Kubota 2004)
Z. piperitum	Dried Pericarps	Methanol extraction	d-Limonene (29.54%) β-Phellandrene (17.79%), Citronellal (7.26%) Geranyl acetate (17.71%)	(Jiang and Kubota 2004)
Z. piperitum	Fresh Mature Fruit	Methanol extraction	Geranyl acetate (40.04%) Citronellal (16.22%) Limonene (11.47%) Geraniol (11.02)	(Jiang and Kubota 2004)
Z. piperitum DC	Green fruits	Methanol extraction	β-phellandrene (42%), d-limonene (23%), β-pinene (11%)	(Wu *et al.* 1996)
Z. piperitum DC	Leaves	Steam distillation	2-tridecanone (25.2%), (Z)-3-hexenol (19.25%), 2-undecanone (15.18%),	(Kojima *et al.* 1997)
Z. piperitum DC AC (China)	Fruits	Steam distillation	Limonene (18.04%) Geranyl acetate (15.33%) Cryptone (8.52%)	(Chang and Kim 2008)
Z. schinifolium (China)	Fruit	Hydro-distillation	Linalool (29.3%) Sabinene (13.1%) Limonene (14.3%) Linalyl acetate (3.99%)	(Yang 2008)
Z. schinifolium DC AC (Korea)	Fruits	Steam distillation	β-Phellandrene (22.54%) Citronellal (16.48%) Geranyl acetate (11.39%)	(Chang and Kim 2008)

2.4 Taste of Andaliman

Besides its unique and pleasant aroma, andaliman also has a unique trigeminal sensation described as tingling and numbing in the mouth (Wijaya 2000). Attempt to isolate the specific compound in andaliman has been conducted, which reported that andaliman contained substituted alkylamides or also known as α-sanshool, (2E,6Z,8E,10E-N-(2'methylpropyl)-dodecatetraenamide, with a molecular mass of 247.1936, and a molecular formula of $C_{16}H_{25}ON$ as shown in Figure 2 (Wijaya 2000; Sugai, Morimitsu, Iwasaki, *et al.* 2005). This compound has two main isomers, α-sanshool and β-sanshool, that differ only cis or trans configuration of the double bond at the 6th carbon atom (Sugai, Morimitsu, Iwasaki, *et al.* 2005).



$H_3C\text{-}CH{=}CH\text{-}CH{=}CH\text{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}CH{=}CH\text{-}CONH\text{-}CH_2CH(CH_3)_2$

2*E*, 6*Z*, 8*E*, 10*E*-N-(2'-metilpropil)-dodekatetraenamida (Sanshool).

Figure 2. Structure of α-sanshool from Andaliman (Wijaya 2000)

Many researchers have attempted to find new unsaturated alkylamides in *Zanthoxylum* species, which lead to the discovery of at least 25 types of pungent compounds ranging from α-sanshool, β-sanshool, γ-sanshool, δ-sanshool, hydroxy α-sanshool, hydroxy β-sanshool, hydroxy γ-sanshool, hydroxy ε-sanshool, hydroxy-ζ-sanshool, dihydro γ-sanshool, bungeanool, isobungeanool, hydroxy-γ-isosanshool, dihydro-bungeanool, tetrahydro-bungeanol, and others (Kashidawa et al. 1997; Xiong et al. 1997; Chen et al. 1999; Sugai, Morimitsu, Iwasaki, et al. 2005; Yang 2008; Bader et al. 2014; Tao et al. 2017; Jie et al. 2019).

However, despite the similarities in their structures of the isomers and variations of sanshool compounds, each has its unique characteristics, threshold values, Scoville units, and pungency durations, as summarized in Table 5 (Sugai, Morimitsu, Iwasaki, *et al.* 2005; Yang 2008).

Table 5. Sensory qualities of sanshools (Sugai, Morimitsu, Iwasaki, et al. 2005)

Compounds	Sensory characteristics	Threshold value ($\times 10^{-5}$ g/mL)	Scoville ($\times 10^{5}$ SU, mL/g)	Pungency duration (s)
α-sanshool	Burning, numbing tingling	1.3	0.8	±580
β-sanshool	Numbing, bitter	1.4	0.7	±300
γ-sanshool	Burning, numbing, bitter, fresh,	0.9	1.1	±380
δ-sanshool	Burning, numbing, fresh	0.9	1.1	±230
hydroxy α-sanshool	Tingling, numbing	3.8	0.26	±300
hydroxy β-sanshool	Numbing, astringent, bitter	7.8	0.13	±200

Also, it is now known that not all sanshool compounds are present in each *Zanthoxylum* species and their contents vary from species to species (Yang 2008). α-sanshool was the chief sanshool compound that contributes to andaliman trigeminal sensation (Wijaya 2000; Wijaya et al. 2018), whereas hydroxy α-sanshool was the key sanshool compounds in Japanese pepper and Sichuan pepper (Sugai, Morimitsu, and Kubota 2005; Yang 2008).

The mechanism behind its pungent and numbing characteristics are still under debate; however, it can be attributed to TRPV1 channels (contribute to the burning sensation from capsaicin), TRPA1 channels (stimulated by pungent compounds from mustard or horseradish), as well as sensory neuron excitement by modulating and inhibiting potassium channels (Koo et al. 2007; Bautista et al. 2008; Jie et al. 2019; Reyes-Trejo et al. 2019).

2.5 Drying of Andaliman

Fresh andaliman is its most used form; however, due to its high moisture content, it is prone to rotting that starts after 4-5 days of room temperature storage post-harvest, which turns its color from green to black within 10-14 days and loses its original flavor (Wijaya *et al.* 2018). The flavor of andaliman and *Zanthoxylum* species is unfortunately not very stable, with a significant degradation in aroma and tingling sensation could be observed after long term storage, especially if not stored properly since the conjugated triene system in sanshool compounds are sensitive to degradation by oxygen and UV light (Yang 2008; Wijaya *et al.* 2018). On top of that, the unsaturated alkylamides are also degraded by high temperature with 30% degradation was observed when it was subjected to 160°C for 1 hour (Yang 2008).

Drying has been proposed as a simple method to extend the shelf life of andaliman by reducing the moisture and water activity so that biological and chemical degradation is inhibited (Wijaya et al. 2018; Napitupulu et al. 2020). Different drying methods affected the overall aroma and trigeminal intensities of andaliman (Napitupulu et al. 2020). Convective oven drying at 54 °C for 8 hours was deemed to be the optimum method of preserving the sensory characteristics of andaliman considering the ease and cost of application compared to other methods such as solar drying, shade drying, freeze drying, and far infra-red oven (Napitupulu et al. 2020). Freeze drying resulted in the best sensory characteristics of andaliman (Napitupulu et al. 2020) and generally preserve the aroma of the fresh food due to low temperature during the drying process; however, the high cost might hinder its application in the industry (Krokida and Philippopoulos 2006). Although the study on the effect of drying on the overall sensory intensities of andaliman has been done, studies on the impact of drying on the volatiles, numbing compounds, and aroma profiles of andaliman are still limited.

A recent study from Thailand was conducted to analyze the aroma component of dried makwhaen (*Z. myriacanthum*) using solar, shade, oven, and microwave drying. Each drying technique gave a different aromatic profile such that the citrus aroma dropped significantly compared to the fresh produce. Compounds that represent fruity aroma were affected mostly by oven drying and microwave drying. In contrast, compounds representing herbal aroma were increased by shade drying under the shade and microwave drying (Sriwichai *et al.* 2019).

The loss of volatile compounds during the drying process generally are affected by a few factors such as loss of water-soluble volatiles as the moisture is removed as well as the difference in vapor pressure between nonpolar and polar compounds (Baranauskiene *et al.* 2006; Nöfer *et al.* 2018; Sriwichai *et al.* 2019).

Although drying processes tend to lower the sensory attributes of fresh produce, specific sensory characteristics could somehow be preserved since each volatiles' loss varied from each other (Szumny *et al.* 2010).

Generally, drying processes can be grouped into two main categories: natural and artificial/mechanical drying (Schweiggert *et al.* 2007). Natural drying depends on the removal of moisture by solar energy or ambient air, while mechanical drying removes water in food by convective/oven drying, vacuum drying, freeze drying, and microwave drying (Díaz-Maroto *et al.* 2002; Dehghani Mashkani *et al.* 2018; Nöfer *et al.* 2018; Téllez *et al.* 2018). Natural drying cannot remove a large amount of moisture rapidly with a consistent standard; however, it is still widely used because of its low cost and capital requirement (Dehghani Mashkani *et al.* 2018; Sriwichai *et al.* 2019). Sun drying is typically done by directly exposing food material to sunlight or indirectly using solar panels to heat the food products (Téllez *et al.* 2018). The food quality tends to degrade tremendously by direct sun drying because it is exposed to solar radiation; therefore, it was proposed to use a shade-cloth to improve the final product quality by limiting the exposure to sun radiation (Téllez *et al.* 2018).

Convective oven drying is widely used because it manages to dry food rapidly; however, it was reported to cause a considerable loss in the quality and volatile compounds due to exposure to high heat during the process (Díaz-Maroto *et al.* 2002; Nöfer *et al.* 2018). The drying process rate is generally governed by the temperature of the air, the humidity of the air, the velocity of the air, and the dimension of the food itself (Krokida and Philippopoulos 2006; Fellows 2016). However, out of those four factors, air temperature and size were the most contributing factor of drying rates (Onwude et al. 2016)

Freeze drying tends to preserve the original texture and flavor characteristic of the food (Díaz-Maroto et al. 2002; Nöfer et al. 2018), but its high cost might limit its usage industry (Krokida and Philippopoulos 2006). Freeze drying is done by freezing the water in the food matrix into ice, which will be transformed into vapor through sublimation as the pressure is reduced and heat was provided to give the latent heat of sublimation at the chamber where the frozen food is kept (Coumans *et al.* 1994; Fellows 2016). This process tends to give a porous product with low density and rehydrate quickly (Fellows 2016). Interestingly, higher pressure (150-250 Pa) preserves the volatiles of freeze-dried spearmint better than lower pressure (10-30 Pa) despite the longer drying time required to remove moisture (Antal *et al.* 2010). The freezing rate also affects the texture as well as the volatile retention in freeze-dried food. More giant ice crystals are formed at a lower freezing rate, which could damage the texture. However, the aroma compounds tend to be preserved because they are concentrated in the food matrix and are encapsulated by the dissolved solids (Coumans *et al.* 1994).

2.6 Extraction of Aroma Compounds

Volatile compound identification begins with the extraction and concentration of aroma molecules. There are various kinds of extraction procedures. Each of them has its strengths and weaknesses; three of those extraction techniques are briefly explained as follows. Regarding andaliman aroma, direct solvent extraction was reported to produce the best representative aroma to fresh andaliman compared to other extraction techniques such as headspace, simultaneous distillation extraction, or vacuum distillation (Wijaya et al. 2001).

- Direct Solvent Extraction

Solvent extraction is done by immersing plant materials in an organic solvent with a different density than water (hexane, pentane, diethyl ether, chloroform, dichloromethane, and others) to extract volatiles in samples. The solvent, in which volatile compounds are dissolved, is further concentrated before analysis. Although this procedure can obtain a complete volatile compounds profile, non-volatile materials (waxes, pigments, lipids, plasticizers from laboratory equipment) can be extracted as well (Rowan *et al.* 2011; Z. Yang *et al.* 2013). However, this method managed to produce the most representative aroma of andaliman (Wijaya et al. 2001). Choosing the solvent used in the extraction process is the key to successful aroma extraction. It was reported that diethyl ether and chloroform could produce extracts that were representative of fresh andaliman with scores of 3.14 and 3.00 out of 4.00, respectively, whereas ethanol (2.58) and acetone (1.58) gave much lower similarity scores (Wijaya *et al.* 2001).

- Simultaneous distillation and extraction (SDE)

This procedure is widely used for extracting aroma compounds because this method combines the isolation and extraction of volatiles and manages to extract the most volatile materials compared to other methods. The samples are boiled in water to distill the volatile compounds, and at the same time, solvent vapor, distilled from the other side, extracts the volatiles from the steam and is condensed by cold water. SDE, however, is not without drawbacks since it may introduce artifacts during the process from pollution, oxidation, and thermal degradation (Chaintreau 2001). Andaliman aroma extract by SDE tends to not resemble the real aroma of fresh andaliman because of the long boiling period (Wijaya et al. 2001).

- Solid Phase Microextraction (SPME)

Since its first introduction in the 1990s, SPME has become one of the leaders in microextraction methods, especially after the commercialization of more robust fibers, higher extraction efficiencies, and selective coatings. SPME is based on reaching equilibrium between matrix and SPME coatings in the headspace area. Therefore, more prolonged exposure does not mean a higher concentration of volatile extracted by the coatings (Merkle *et al.* 2015). SPME is not only more rapid and more straightforward than other extraction methods, but it also does not need to use any solvent. It is also sensitive and able to prevent losses of volatiles during extraction, clean up, and concentration stages of traditional extraction methods (Nerín *et al.* 2009; Merkle *et al.* 2015). Choosing the correct fiber is one of the keys to successful aroma extraction by SPME. Many commercial fibers are now available, and each of them has an affinity towards a specific class of molecules. For examples: polydimethylsiloxane (PDMS) is useful for analyzing nonpolar molecules, while carboxen (CAR), divinylbenzene (DVB) and polyacylate (PA) are useful for more polar molecules, by combining two or more fibers, bipolar fiber characteristics can be achieved (Merkle *et al.* 2015).

2.7 Identification of Volatile Compounds

The most common way to identify volatile compounds after extraction is through gas chromatography-mass spectrometry (GC-MS). Gas chromatography is a column-based separation technique using gas as the mobile phase and immobilized liquid or inert solid support as the stationary phase. The analyte is injected into the column head and vaporized by an oven under a gradient of temperature to separate the volatiles by their size, boiling point, and polarity as the volatiles travels through the column (Ismail 2017).

Mass Spectrometry is a typical detector attached to GC, which can ionize molecules and separate them by their mass-to-charge ratio (m/z) using a mass analyzer and detect them with a very sensitive detector (Smith and Thakur 2017). GC-MS can separate, identify, and quantify volatile compounds. Semi quantification is done by calculating the peak area ratio between the target compound and a known quantity of internal standard. Identification is made typically through comparing with standard, searching through mass spectral libraries such as Wiley W8N08 library (John Wiley and Sons, Inc., USA), and confirmed by comparing the linear retention indices (LRI) to the literature.

Determination of LRI (linear retention index) of each component that appears in the sample is done by comparing the retention time to a series of standard alkanes C_7-C_{40}, injected to the GC-MS with similar sample injection conditions. LRI values are calculated by using the formula:

$$\mathrm{LRIx} = \left[\frac{(tx-tn)}{(tn+1-tn)} + n\right] \times 100$$

LRIx = Linear Retention Index of component x
tx = retention time of the component x (min)
tn = retention time of (n) - standard alkanes that appears before x component (min)
tn + 1 = retention time of (n+1) - standard alkanes that appears after x component (min)
n = number of C atoms from the standard alkanes that appear before the x component

2.8 Sensory Profiling Method

Sensory characterization is a powerful and frequently used sensory science tool to offer a complete picture of products' sensory characteristics. Quantitative Descriptive Analysis (QDA), one of the techniques in descriptive analysis, has been used extensively for the past 50 years; however, this method requires trained panels and tends to be time-consuming and expensive to conduct. Therefore, new methods to conduct consumer sensory profiles have been developed and can be categorized into four groups based on the approaches (Varela and Ares 2014):

1. Individual attributes evaluation: Free Choice Profiling (FCP), flash profiling, Check All That Apply (CATA), Rate All That Apply (RATA)
2. Global differences evaluation: sorting and napping
3. Reference comparison: Polarized Sensory Positioning (PSP)
4. A global description of specific products: open-ended questions

Although consumer-based sensory analysis delivers a valid and reproducible set of data, it cannot replace the classic descriptive analysis, which is highly accurate because the panels have been trained to identify and quantify each sensory attributes (Ares *et al.* 2014; Ares and Jaeger 2015). However, descriptions provided by trained panels often do not mirror consumer perceptions (Saldaña *et al.* 2019); therefore, consumer-based sensory methodologies are gaining popularity.

CATA is a rapid consumer-based sensory profiling method that presents consumers with a list of words or descriptors. Consumers are instructed to choose the applicable attributes to describe product characteristics (Ares and Jaeger 2015). CATA methodology has been extensively used because it captures consumers' perception of products while being perceived as a manageable test to the consumer. However, CATA has one critical limitation in its inability to measure each sensory attribute's intensity, which might prevent discrimination between samples with similar sensory attributes but differ in intensities (Meyners *et al.* 2016).

Therefore, a modified test was created by pairing CATA questions with rating the intensity of each attribute on a scale, which resulted in the Rate All That Apply (RATA) method (Ares *et al.* 2014). It is imperative to design the questionnaire as best as possible to get the best result from this technique by maximizing consumers' cognitive efforts by limiting the maximum amount of terms to 40 descriptors (Ares and Jaeger 2015).

III RESEARCH METHODS

3.1 Time and Place

This research was conducted from November 2019 – August 2020 at IPB University Food Science and Technology Chemistry Laboratory, IPB University Food Science and Technology Food Processing Laboratory, IPB University Food Science and Technology Sensory Evaluation Laboratory, IPB Inter-University Center Building (PAU) Chemistry and Biochemistry Laboratory, IPB Analytical Service Laboratory, *Balai Besar Pascapanen Bogor,* and *Laboratorium Kesehatan Daerah (Labkesda) Jakarta*.

3.2 Materials

The fresh material for the experiment was fresh green *Simanuk* andaliman obtained directly from a local farmer in Gotting Raya Village, Simalungun District, North Sumatera at 1437 MSL, and fresh green andaliman purchased from *Pasar Senen,* Jakarta. *Simanuk* andaliman was used during the drying, extraction, physical and volatile analysis to measure the impact of drying on andaliman aroma and numbing compounds. They are purchased directly from the grower, packed in a cardboard box lined with wrapping paper, transported by bus to Medan, North Sumatera, which was then directly shipped by plane to Jakarta where it was kept at a refrigerator for a maximum of 3 days before drying or extraction. Before drying or extraction, the green andaliman was separated from the red and senescent ones, and the berries were cut off from the twigs using scissors. The andaliman was identified by the Indonesian Institute of Science (LIPI) Research Center for Biology No. 822/IPH.1.01/If.07/VIII/2020 as *Zanthoxylum acanthopodium DC*.

Andaliman from *Pasar Senen* was transported directly to Jakarta by motorcycle courier and kept in the refrigerator constantly before further analysis. It was used as the material to confirm the GC-MS method to analyze andaliman aroma and numbing compounds.

The chemicals used in this research were chloroform (Merck, Darmstadt, Germany), alkane external standard C_7-C_{40} (Sigma Aldrich, St Louis, USA), 1,2,4 trichlorobenzene (Sigma Aldrich, St Louis, USA), hydroxy α-sanshool standard with >98% purity (Chengdu Biopurify Phytochemicals, Chengdu, China), Whatman filter paper no 1, Na_2SO_4 anhydrous (Merck, Darmstadt, Germany), and aluminum pouch and aluminum cups.

3.3 Equipment

Gas Chromatography 7890A – Mass Spectrometry 5975C (Agilent Technologies, Santa Clara, CA, USA), laboratory-scale benchtop convection oven (Thermo Fisher Scientific, Waltham, USA), Freeze drier Alpha 1-2 LDplus (Martin Christ, Osterode, Germany), rotary evaporator (Buchi, Flawil, Switzerland), food grinder, scale, Aw-meter WA-360 (Shibaura Electronics Co., Ltd, Saitama-city, Japan), Chromameter CR-400 (Konica Minolta, Tokyo, Japan), 55% black plastic sunshade cloth, digital thermometer and hygrometer were used in this study.

3.4 Research Procedures

This research began with drying fresh andaliman from Simalungun, North Sumatra, using natural and artificial drying techniques. The fresh and dried andaliman were subjected to physical analysis such as water activity, moisture content, yield, color, and bulk density to evaluate each drying process's effectiveness. The research continued by confirming the GC-MS condition to detect aroma and sanshool compounds using fresh andaliman obtained from *Pasar Senen*. The volatiles and taste numbing compounds from dried and fresh andaliman were extracted by macerating ground andaliman in chloroform overnight twice. After filtration, the filtrate was dried with Na_2SO_4 anhydrous, concentrated by rotary evaporator, volume-adjusted to 10 mL, and analyzed with GC-MS. Identification was based on the built-in WN808 mass spectrum library, LRI, hydroxy α-sanshool retention time, and comparing mass spectra of α-sanshool from the literature. The relative quantity of α-sanshool, hydroxy α-sanshool, and volatiles was analyzed by calculating the peak area ratio between each compound and the peak area of an internal standard and multiplied to the quantity of the internal standard.

The drying process was duplicated (two biological replications); the physical analysis was done in triplicate (three technical replications). In comparison, the extraction and GC-MS analysis were done in duplicate (two technical replications). Sensory analysis of the aroma of andaliman was conducted using the RATA-Hedonic test to profile the aroma qualities and the acceptance of andaliman. A Focus Group Discussion (FGD) was conducted beforehand to select the aroma attributes that will be evaluated during the RATA analysis. As a guidance during the FGD, a set of aroma attributes was first selected based on the aroma descriptors of the aroma-active compounds of andaliman and previous aroma study of other *Zanthoxylum* species using a sensory test (Wijaya et al. 2002; Jiang and Kubota 2004; Sriwichai et al. 2019; Yang 2020)

The physical, GC-MS and sensory analysis data were evaluated by one-way ANOVA and Duncan Posthoc Test. Additionally, the chemometric and sensory data were analyzed by PCA, PLS Regression, and Pearson Correlation to investigate the impact of drying on andaliman chemical and physical characteristics and to analyze the correlation between aroma qualities of andaliman and its volatiles. Each step of this research is illustrated in the flow charts in Figure 3-11 as follows.

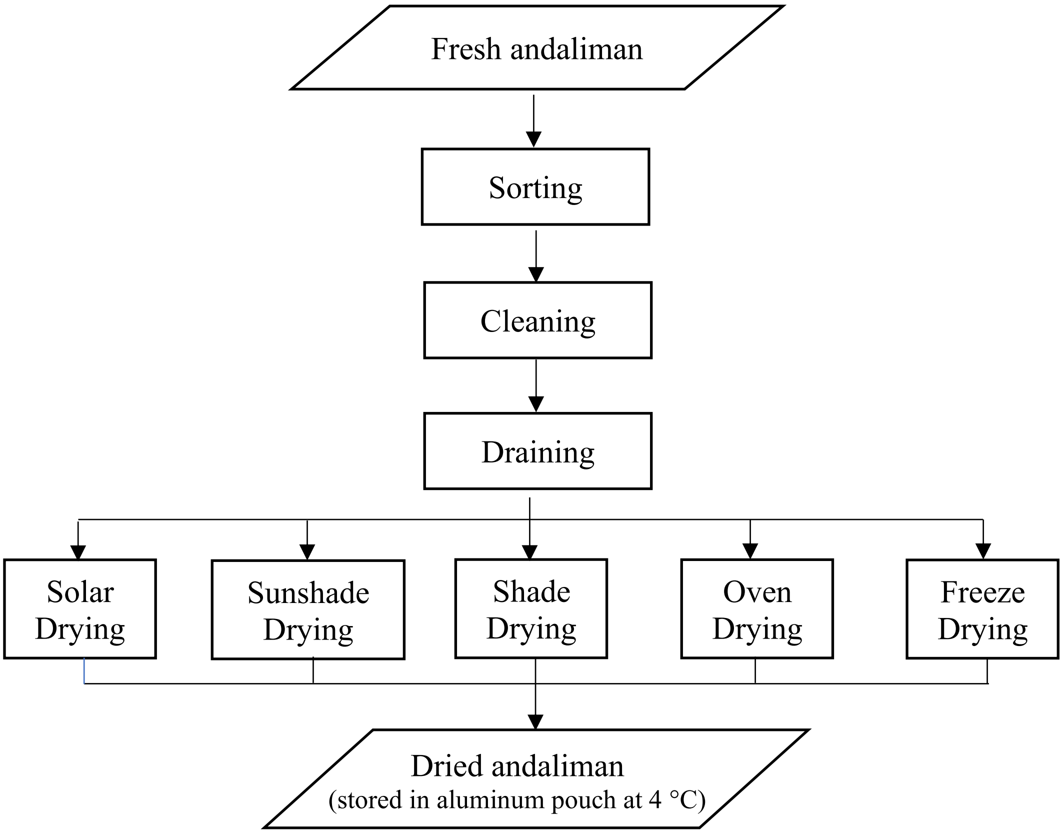


Figure 3. Research flow chart of drying of andaliman

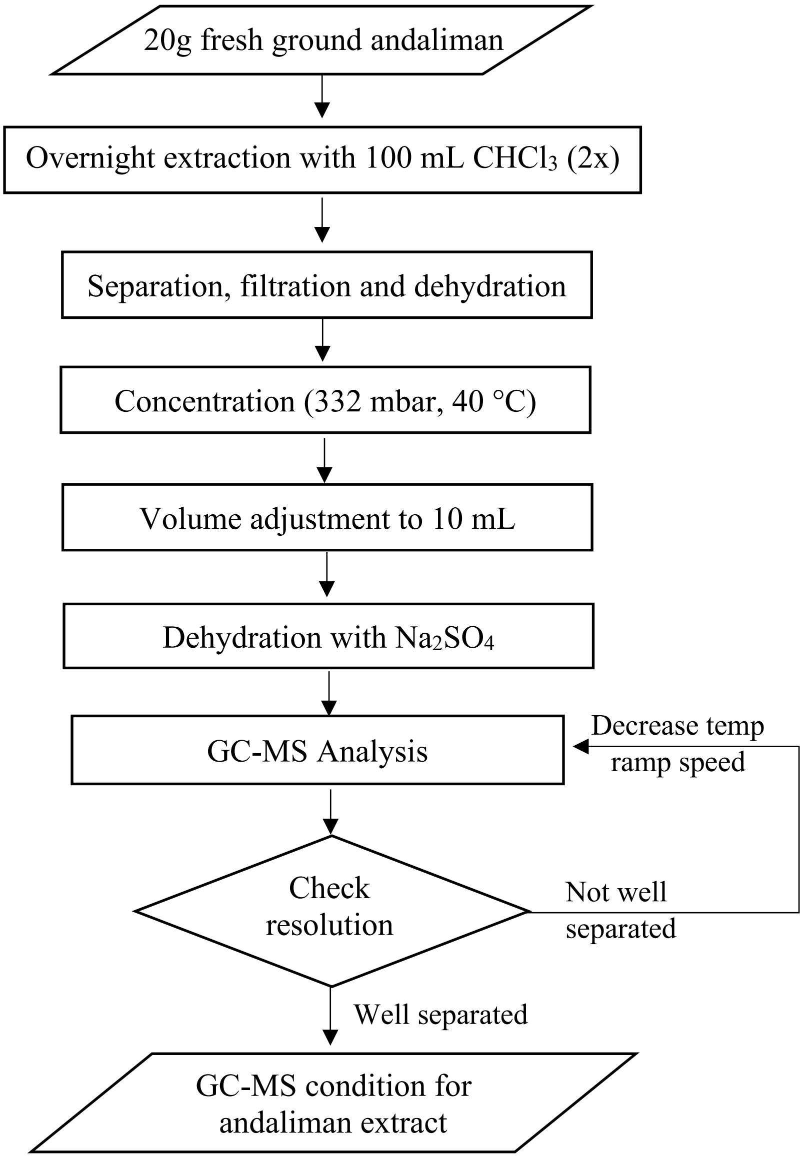


Figure 4. Research flow chart of confirmation of GC-MS condition to detect andaliman volatiles and numbing compounds

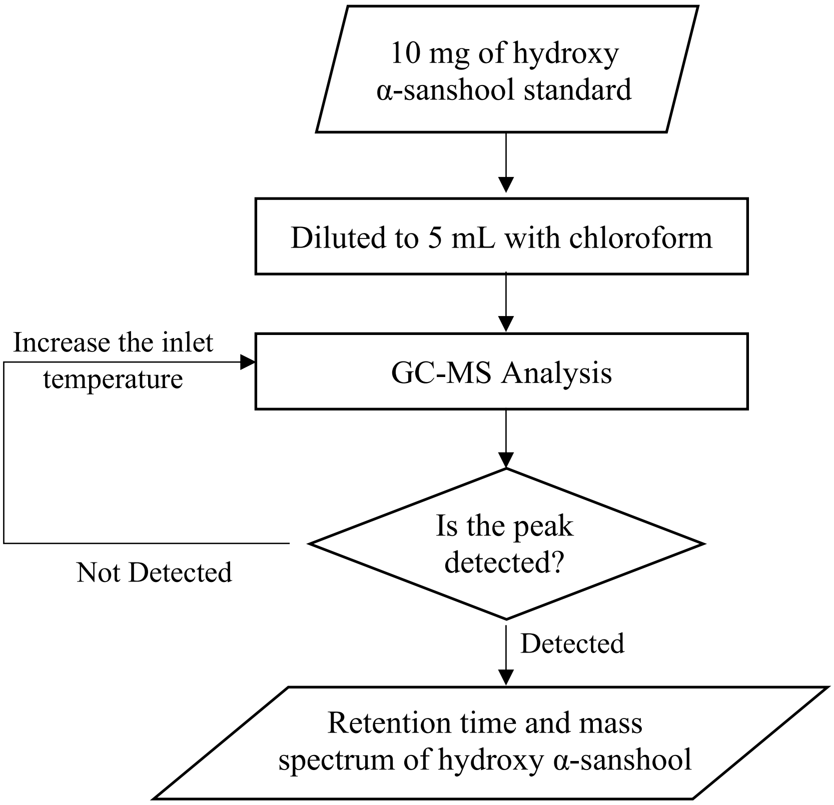


Figure 5. Research flow chart of confirmation of GC-MS condition to detect hydroxy α-sanshool

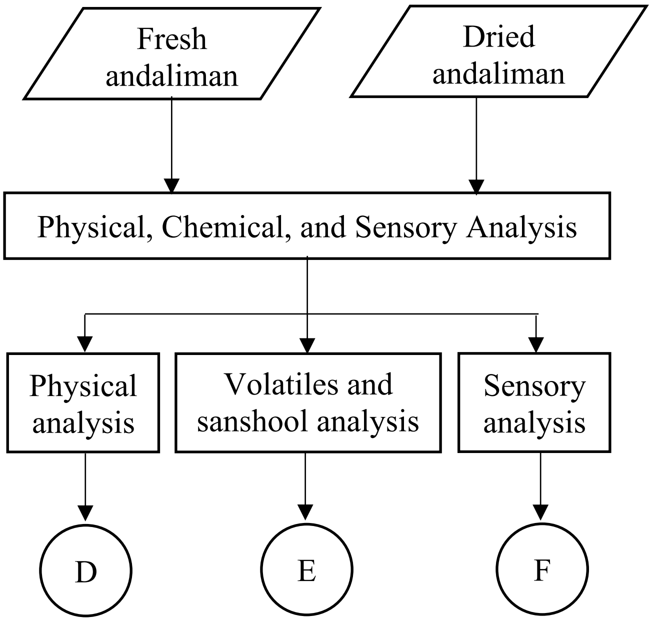


Figure 6. Flow chart of physical, volatiles, sanshool, and sensory analysis

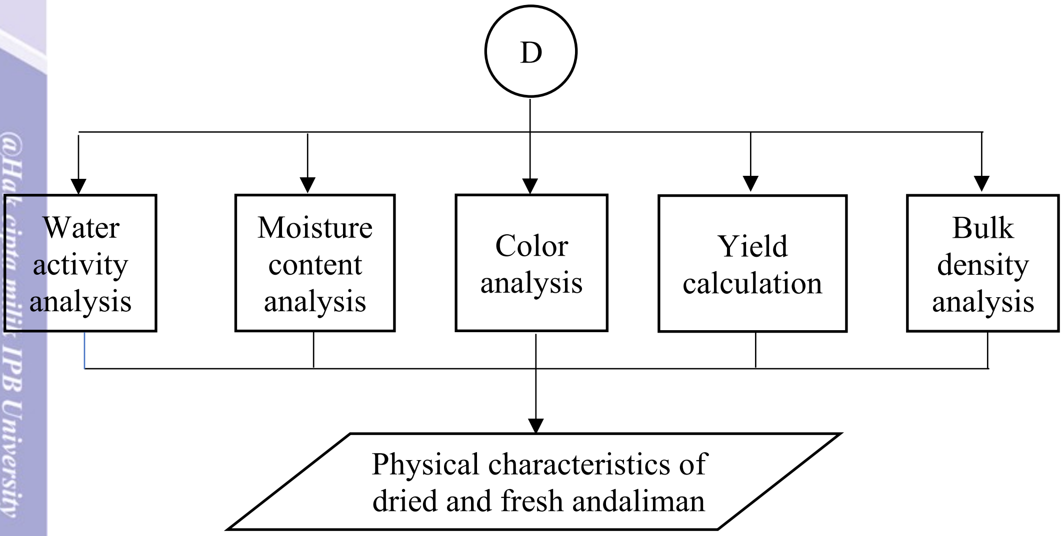


Figure 7. Research flow chart of physical characterization of andaliman

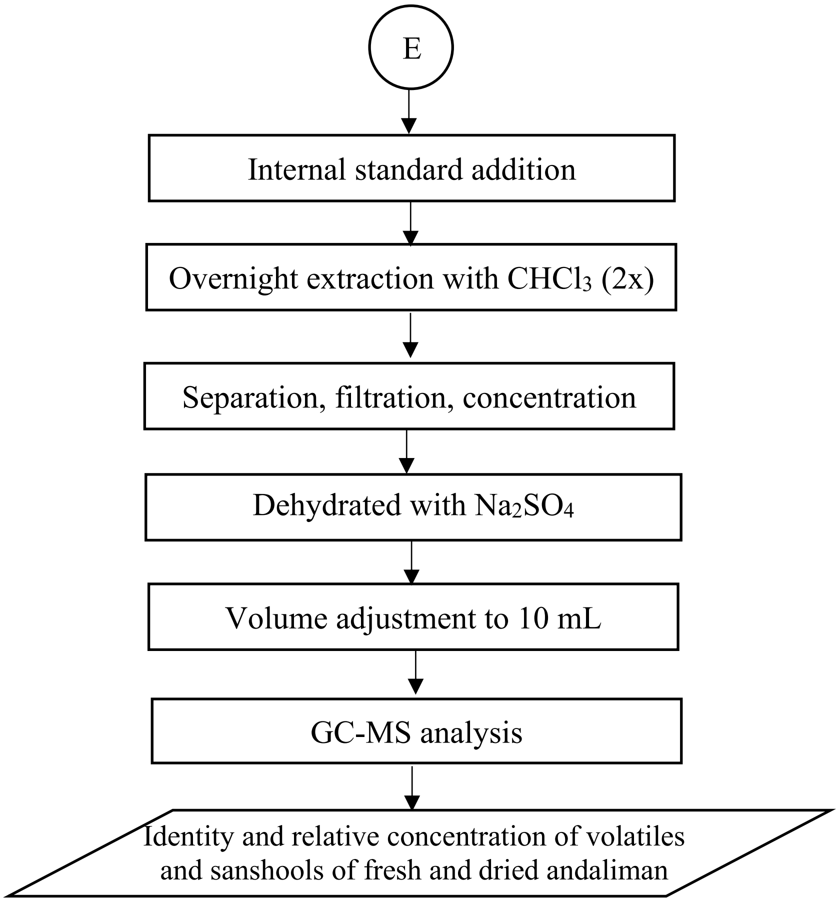


Figure 8. Research flow chart of volatiles and sanshool analysis

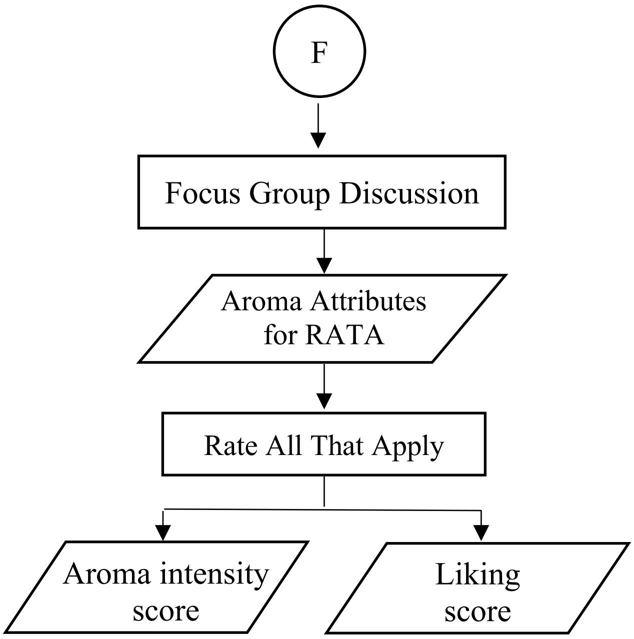


Figure 9. Research flow chart of andaliman sensory analysis

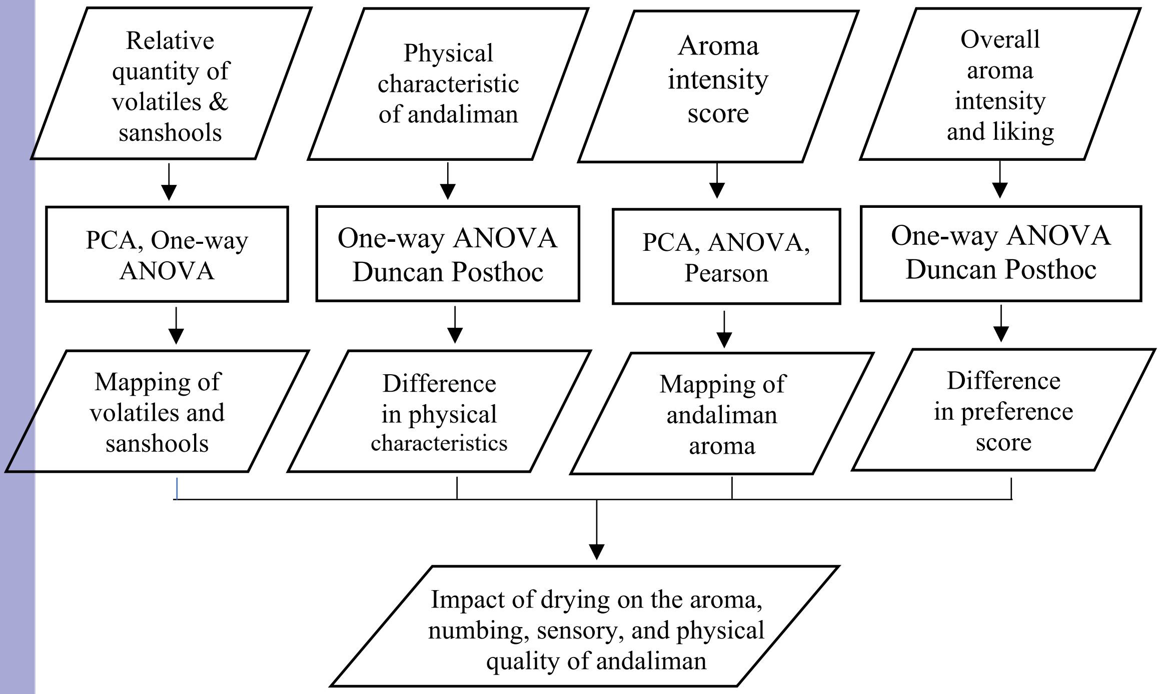


Figure 10. Research flow chart of statistical data analysis to evaluate the effect of drying to andaliman quality

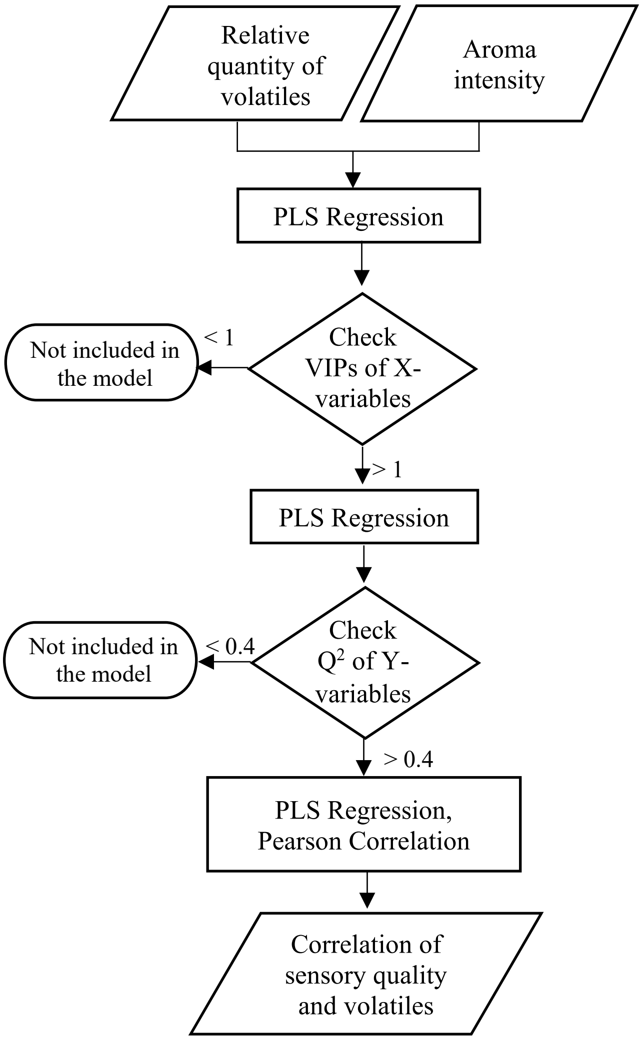


Figure 11. Research flow chart of statistical data analysis to correlated sensory intensities based on its volatiles

3.4.1 Drying of Andaliman

Drying is proposed to be one of the common solutions in extending the shelf life of many herbs and spices due to its ability to reduce the moisture level to less than 10% (Díaz-Maroto *et al.* 2002; Schweiggert *et al.* 2007). Different drying methods will result in different characteristics of the products (Díaz-Maroto *et al.* 2002; Szumny *et al.* 2010; Nöfer *et al.* 2018; Sriwichai *et al.* 2019); therefore, it will be crucial to compare the characteristics of andaliman that has been dried using various drying techniques. Andaliman grows in Indonesia, a tropical country, where one of the most popular methods to dry food products is by sun-drying (Schweiggert *et al.* 2007). Artificial drying using a heated oven is typically proposed as a cost-effective and simple method to accelerate the drying process and minimize the change in sensory characteristics (Schweiggert *et al.* 2007; Choi *et al.* 2017). Despite the high cost, freeze drying is still the top drying method to maintain the quality of dried food products (Nöfer *et al.* 2018). Therefore, in this study, andaliman will be dried using five methods: direct sun drying, sun drying with shade-cloth (sunshade), shade drying, convective oven drying, and freeze drying. The drying condition is based on previous research of drying andaliman to obtain the best sensory characteristic (Napitupulu et al. 2020).

Before drying, green andaliman fruits were separated from the red and senescent ones as well as from the stems, leaves, or other debris before being washed, drained, and air-dried in an air-conditioned room for two hours to remove excess water. The fruits were then dried using the following drying techniques. The dried andaliman was packaged in aluminum pouches and kept in a freezer before further analysis. The whole drying process will be duplicated to ensure the reproducibility of the data.

3.4.1.1 Sun Drying (SD) (Napitupulu et al. 2020 with modification)

400 g of andaliman was spread on 2 aluminum trays (25x35 cm) and exposed to direct sunlight to result in a similar yield compared to oven drying for 54 hours including the time after sunset. After each sunset, the drying process was moved indoors to protect the andaliman from harsh weather.

3.4.1.2 Sunshade Drying (SSD)

Because sun-drying exposed andaliman to damaging UV light, sun drying of andaliman with a shade-cloth was proposed to protect andaliman from direct UV that might degrade the sanshool compounds (Yang 2008). 400g of andaliman was spread on 2 aluminum trays (25x35 cm), covered with 55% shade-cloth and dried under sunlight for a total of 54 hours, including the time after sunset. To improve the airflow, the shade-cloth was placed around 20 cm above the andaliman with 10 cm ventilation space using a cage made from PVC pipes. The usage of sunshade was found to improve the quality and discoloration of dried stevia leaves (Téllez *et al.* 2018). Similar to SD, the andaliman was moved indoors after each sunset.

3.4.1.3 Shade Drying (SHD)

400 g of andaliman was spread on 2 aluminum trays (25x35 cm) and left to dry inside an air-conditioned room with dim light at 20-25 °C and relative humidity of 42-47% for 96 hours (Chen et al. 2012 with modification). This method was an improvement of the previous research showing that outdoor shade drying took almost 6 days to complete with unsatisfying sensory characteristics (Napitupulu et al. 2020). The long drying period was caused by the high relative humidity and weather condition in the Bogor area that often rained during the day.

Shade drying is commonly used to dry *Z. myriacanthum* in northern Thailand and resulted in the most preferred essential oil aroma (Sriwichai *et al.* 2019), however, this method was found to degrade the trigeminal and aroma characters of andaliman (Napitupulu et al. 2020), therefore, it will be of interest to study the chemical changes of andaliman flavor by shade-drying technique.

3.4.1.4 Convective Oven Drying (OD)

400 g of andaliman was dried in a laboratory-scale benchtop convection oven at 54°C for 480 min, based on previous research that optimized the convective oven drying to achieve the best andaliman sensorial quality (Napitupulu et al. 2020).

3.4.1.5 Freeze Drying (FD)

400 g of green andaliman fruits were frozen overnight at -20°C and lyophilized for 40-41 hours with condenser temperature of -50 °C and the pressure of 0.040 mbar. Freeze-dried andaliman was shown to have the highest trigeminal sensation of dried andaliman (Napitupulu et al. 2020), possibly due to the drying process's low temperature since sanshools are not heat-stable (Yang 2008).

3.4.2 Modeling of Drying Kinetics

During the drying process, the samples' weight was recorded periodically except during freeze drying (every 1 hour for oven drying and every 6 hours for the other drying methods). The moisture content dry basis (g water/g dry solid) from each period is calculated using the following formula (Naderinezhad et al. 2016):

$$\mathrm{Mt} = \frac{m_t - m_s}{m_s}$$

M_t = moisture content dry basis (g water / g dry solid)
m_t = the mass of the sample mass at a particular time (*t*)
m_s = the mass of the dry solid from loss on drying (LOD)

The moisture ratio (MR) could then be calculated by using the following formula (Naderinezhad et al. 2016):

$$\mathrm{MR} = \frac{M_t - M_e}{M_o - M_e}$$

MR = moisture ratio
M_t = moisture content dry basis at a particular time
M_o = initial moisture content dry basis
M_e = equilibrium moisture content dry basis

The M_e during the drying process is often exceptionally low because of the elevated temperature and low relative humidity (RH). Therefore, the previous equation could be simplified to the following formula without significant change in MR value (Naderinezhad et al. 2016; Nöfer et al. 2018). From there, the drying curve was built by plotting MR against time.

$$\mathrm{MR} = \frac{M_t}{M_o}$$

A curve fitting software, Table Curve 2D version 5.01 (Systat Software, Inc, San Jose, CA, USA) was used to fit the most used drying models to the calculated MR data against time. Several thin layer drying models were considered for the modeling: Page, Modified Page II, Henderson and Pabis, Logarithmic, Midili-Kucuk, and Weibull model (Onwude et al. 2016; Nöfer et al. 2018). The equation of each model is as follows: k is the drying constant while t is the time, and a, b, c, and n are constants.

Page	$MR = \exp(-kt^n)$
Modified Page (II)	$MR = \exp[-(kt)^n]$
Henderson and Pabis	$MR = a \exp(-kt)$
Midilli-Kucuk	$MR = a \exp(-kt) + bt$
Logarithmic	$MR = a \exp(-kt) + c$
Weibull	$MR = a - b \exp(-kt^n)$

The best model to describe the general dying kinetics of all dried andaliman were determined by the R^2 and Standard Error of Fit. The R^2 value should be as close as to 1 while the SE of Fit should be as close as to 0.

3.4.3 Confirmation of GC-MS condition

Sanshool compounds are typically analyzed by using HPLC while aroma compounds are analyzed by GC-MS, however, some studies have managed to detect sanshool compounds by GC-MS (Adesina and Akinwusi 1986; Tirillini and Stoppini 1994; Reyes-Trejo *et al.* 2019) which could speed up the analysis process.

Because this method is not common, a confirmation of GC-MS condition to detect both aroma and sanshool compounds needs to be conducted. Firstly, 20 g of fresh andaliman was twice extracted overnight with chloroform (100 mL each) and filtered with Whatman filter paper no 1 to remove the residue. The extract was dehydrated with Na_2SO_4 anhydrous, concentrated with a rotary evaporator (332 mbar, 40 °C) to reach precisely 10 mL. 1 µL of the solution was injected into the GC-MS using the following condition as a starting point (Wijaya et al. 2001; Reyes-Trejo et al. 2019) – with modification.

- GC-MS: 7890A and 5975C (Agilent Technologies, USA)
- Column: HP-5ms 325°C (30m x 250 µm x 0.25 µm)
- Ionization mode and voltage: Electron Ionization (EI) 70 eV
- Ion temperature: 200°C
- Injection: 1µL, Split 1:100
- Carrier gas: Helium (1mL/min)
- Oven: 40°C (4 min), increased to 280°C for 4 minutes (4°C/min)
- Injector and detector temperature: 250°C
- Acquisition mode: 20 scan/s – mass range 45-550 m/z

If the sanshool peak were not found in the chromatogram, then the injection temperature would be increased to ensure that sanshool compounds were volatilized entirely and entered the column. If the peaks were not well separated, then the temperature ramp would be slowed down to ensure sufficient peak separation.

After that, a confirmation was made by injecting 10 mg of hydroxy α-sanshool standard, which had been diluted to 5 mL with chloroform to obtain a 2 mg/mL concentration. 1 µL of the solution was then injected into GC-MS with the previous condition to ensure that the condition managed to analyze sanshools.

3.4.4 Characterization of fresh and dried andaliman

3.4.4.1 Physical characteristic analysis

The primary purpose of drying is to reduce the water content and water activity so that chemical and biological degradations could be inhibited. Therefore, analysis of yield, moisture content, water activity, color, and bulk density was done to confirm each drying process's result. Physical analysis of dried and fresh andaliman was conducted in triplicate

3.4.4.2 Yield Calculation

The following formula calculated the yield of each drying process:

$$yield = \frac{weight\ of\ andaliman\ after\ drying (g)}{weight\ of\ andaliman\ before\ drying\ (g)} \times 100\ \%$$

3.4.4.3 Moisture content

The moisture content of andaliman was be expressed as Loss on Drying (LOD) by oven drying at 105°C for 3 hours or until constant weight (BSN 1992).

1. Firstly, an empty aluminum dish was dried in the 105°C for 1 hour and cooled in a desiccator before being weighed.
2. Approximately 2 g of ground andaliman was weighed into the dish.
3. The dish was then oven-dried initially for 3 hours
4. After that, it was cooled in a desiccator before weighing.
5. The drying was then continued and reweighed every hour until a constant weight was achieved
6. The moisture content was be calculated by the following formula as:

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} \times 100$$

W1 = sample weight (g) before drying
W2 = sample weight (g) after drying

3.4.4.4 Water activity

The water activity of fresh and dried andaliman fruits was measured by putting 1-3 grams of ground andaliman sample into an Aw-meter WA-360 (Shibaura Electronics Co., Ltd, Saitama-city, Japan) at ambient temperature.

3.4.4.5 Bulk density (ISO 1998)

The whole andaliman were filled to a 100 mL measuring cylinder, and the weight of 100 mL andaliman was then recorded. The bulk density would then be expressed in g/mL by using the following formula:

$$bulk\ density = \frac{weight\ of\ andaliman\ (g)}{volume\ of\ andaliman\ (mL)}$$

3.4.4.6 Color analysis

The color of each dried and fresh andaliman was analyzed using chromameter CR-400 (Konica Minolta, Japan) to give the color indication of each sample (L*, a*, b*). Calibration was done by inserting the x and y values from the calibration plate's cover and measuring a white calibration plate's color values for three times. The result of the analysis will result in the L*, a*, and b* values. The L values indicate the brightness (0 = black, 100 = white), a values indicate the red-green color (-80 – 0 = green, 0 – 80 = red), while the b values indicate the blue-yellow color (-70 – 0 = blue, 0 – 70 = yellow).

Only the pericarps were used during the color analysis to prevent bias from the exposed black seeds; on the other hand, the color of fresh andaliman was measured using whole fresh andaliman fruits. Since the color of andaliman was not uniform, the measurement was conducted by firstly filling three sample cups with the andaliman. Three readings were taken from separate locations within each cup, and the average value of the three readings was calculated. The process was then repeated with the other two cups, which resulted in 3 average measurements, each from 3 readings, to obtain each batch's overall color (Whetzel 2016).

3.4.5 Characterization of volatiles, α-sanshool, and hydroxy α-sanshool

3.4.5.1 Volatiles and sanshool extraction (Sugai, Morimitsu, and Kubota 2005)

The dried andaliman (both seeds and pericarps) was ground to pass a 40-mesh screen while the fresh andaliman was ground for 10-20 seconds using a dry blender. 1,2,4-trichlorobenzene was used as the internal standard (IS) to quantify each peak area in the chromatogram by diluting 0.1043 grams of IS to 10 mL with chloroform to obtain a concentration of 1% w/v concentration of IS. 5 g of ground dried andaliman (both seeds and pericarps) and 200 µL of IS was macerated overnight twice with chloroform (100 mL each) in a refrigerator. As the control, 20g of fresh andaliman (both seeds and pericarps) and 200 µL of IS was macerated overnight twice with chloroform (100 mL each) in a refrigerator. The mixture was filtered through a Whatman filter paper no 1 to separate the extract from the residue. The residue left on the filter paper was rinsed twice with more chloroform to prevent any loss of aroma and sanshool compounds.

The filtrate was then be dehydrated over sodium sulfate anhydrous, filtered, and concentrated under reduced pressure using a rotary evaporator (332 mbar, 40 °C) to reach 10 mL. The extracts were kept in a PTFE lined screw-capped test tubes, covered in aluminum foil, and stored at -20 °C before further analysis.

3.4.5.2 Semi-quantitative analysis of aroma and sanshool compounds

1 µL of the extract was then injected to GC-MS based on the confirmed condition as follows:

- GC-MS: 7890A and 5975C (Agilent Technologies, USA)
- Column: HP-5ms 325°C (30m x 250 µm x 0.25 µm)
- Ionization mode and voltage: Electron Ionization (EI) 70 eV
- Ion temperature: 200°C
- Injection: 1µL, Split 1:10
- Carrier gas: Helium (1 mL/min)
- Oven: 40°C (4 min), increased to 280°C for 4 minutes (2°C/min)
- Injector and detector temperature: 250°C
- Acquisition mode: 20 scan/s – mass range 45-550 m/z

Identification of volatiles was made by comparing the mass spectra with the built-in W8N08 mass spectra library (John Wiley and Sons, Inc., USA) and the LRI (Linear Retention Index) of each peak with the literature. By injecting an external standard of alkane series C_7-C_{40} under the same condition, the LRI of each peak was calculated and compared with the literature. Sanshool compounds in andaliman would be represented by α-sanshool and hydroxy α-sanshool, which were the majority of sanshool compounds in *Zanthoxylum* (Sugai, Morimitsu, and Kubota 2005; Yang 2008). Because no commercial α-sanshool standard was available for purchase, its identification was made by comparing the mass spectra with that from literature (Reyes-Trejo et al. 2019) while the hydroxy α-sanshool was identified from the retention time and mass spectra of the standard that had been injected under the same condition.

After each peak had been identified, the area under each peak from the GC-MS chromatogram was integrated using Openchrom software (Lablicate GmbH, Hamburg, Germany) before converting to a concentration value relative to the internal standard using the following formula. Because each sample had different moisture content, the concentration was reported on a dry-weight basis by dividing the concentration with each sample's dry matter percentage.

$$[A] = \frac{peak\ area\ of\ B}{peak\ area\ of\ IS} \times \frac{C\ mL\ SI}{D\ g\ material} \times E\ {}^{g}/_{mL} \times 10^{6}\ {}^{\mu g}/_{g}$$

A = concentration of the component of interest (µg/g material)
B = component of interest
C = volume of the internal standard (mL)
D = weight of the material (g)
E = concentration of internal standard (g/mL)

3.4.6 Consumer-Based Sensory Analysis: RATA-Hedonic Test

Since the sensory analysis involved human subjects, it had been approved by the Human Research Ethics Committee of IPB University No. 261/IT3.KEPMSM-IPB/SK/2019. Consumer-based sensory analysis using the Rate All That Apply

(RATA)-Hedonic method was conducted to evaluate the aroma of fresh and dried andaliman. A 120-minute focus group discussion (FGD) was conducted beforehand with seven panelists who have participated in sensory analysis and could articulate aroma descriptions. This step was conducted to select the most representative aroma descriptors of andaliman. All andaliman samples were presented to the panelists, and an initial list of aroma attributes based on the aroma descriptors of andaliman aroma-active compounds (Wijaya et al. 2001) and sensory descriptive analysis of other *Zanthoxylum* species (Jiang and Kubota 2004; Sriwichai et al. 2019; Yang 2020) were used as the guideline during the discussion as could be seen in the appendix. The panelist was explained of each aroma attributes and smelled the andaliman that had been grounded to 40 mesh, and check the aroma attributes that were present in each sample utilizing a method that was similar to Check-All-That-Apply. Each used attribute was discussed with the panel to reach a consensus of all aroma attributes that could best describe the andaliman aroma.

For the RATA-Hedonic analysis, 40 untrained panelists were recruited to rate the intensity of each aroma attributes. The panelists must be in healthy condition, not pregnant, within the age of 18 – 60 years old, and had not smoked for at least two hours before the analysis. They were simultaneously presented with six 3-digit coded samples, which consisted of fresh (3.8 g) and dried (1.0 g) ground andaliman at random order. They were given a brief oral and written explanation of the sensory analysis process and each aroma attribute's definition. Then, they were instructed to smell the samples, choose the aroma descriptors they thought were present in each sample, and rate the intensity based on a 6-point scale, with 1 = slightly applicable and 6 = very applicable (Meyners *et al.* 2016). If the panelist did not feel that an aroma attribute was present in the sample, they were instructed to leave the rating blank. Simultaneously, a hedonic question was asked for all samples to evaluate each product's overall liking by the panelists. The overall liking was rated with a 9-point scale (1 = dislike extremely and 9 = like extremely) (Meilgaard et al. 2016). The template for the sensory analysis is attached in appendix 1.

3.4.7 Statistical Data Analysis

The physical analysis data were subjected to a one-way ANOVA and Duncan posthoc test to observe any significant differences. The relative quantities of volatiles and aroma attribute scores were subjected to one-way ANOVA. One-way ANOVA and Duncan’s New Multiple Range Test (DNMRT) were conducted using IBM SPSS Statistics version 25 (IBM, Armonk, NY, USA) while PLSR and PCA were conducted using XLSTAT 2014 (Addinsoft, New York, NY, USA).

The relative concentrations of each volatiles were auto-scaled and subjected to multivariate data analysis by Principal Component Analysis (PCA), which is an unsupervised multivariate analysis that could visualize the data grouping and trends as well as inspect any outliers (Yip and Yong Chan 2013; Li *et al.* 2019).

The average score of each aroma attribute from the sensory analysis was auto-scaled and subjected to PCA, which can confirm the aroma analysis done previously and see the effect of drying to the aroma of andaliman based on consumer sensory profiling. The overall liking test data were analyzed using one-way ANOVA and Duncan posthoc to check whether a sample was more accepted than the other by the panelists. Determination of favorable aroma attributes were conducted with Pearson correlation analysis between aroma intensities and overall liking score.

Correlation of chemometrics and sensory data has been done to multiple food products such as durian and lai fruit (Belgis et al. 2017), wine (González Álvarez et al. 2011; Jung et al. 2014; Nguyen et al. 2019), Sichuan Pepper (Yang 2020), Coffee (Ribeiro et al. 2009), and others. Partial Least Square Regression (PLSR) analysis is one of the most commonly used methods to find such correlations (Seisonen et al. 2016). An initial PLSR analysis was conducted against all identified compounds and all aroma attributes to identify the compounds used for the correlation analysis. Only compounds showing variable importance for the projections (VIP) greater than 1 and volatiles that had been previously identified as the aroma-active compounds in andaliman were selected for further analysis. Another PLSR was conducted with the selected volatiles and all aroma attributes. Aroma attributes showing Q^2 values of less than 0.4 were removed for further analysis. This variable reduction step was crucial to reduce the number of irrelevant variables, thus improving the prediction quality and decreasing the model complexity (Seisonen et al. 2016). Pearson correlation analysis was also conducted using XLSTAT to evaluate the significant correlations based on the PLSR results (Belgis et al. 2017).

3.4.8 Design of Experiment

This study was conducted using Completely Randomized Design or *Rancangan Acak Lengkap* with one factor, namely drying method, and six levels, fresh andaliman, sun drying, sunshade drying, shade drying, oven drying, and freeze drying.

IV RESULTS AND DISCUSSION

4.1 Drying of Andaliman

Once the andaliman had arrived from Medan, the fruits were moved in plastic boxes and kept in a refrigerator to slow down the degradation process. The green fruits were meticulously separated from the twigs and red fruits by scissors, triple washed to remove smaller debris, and air-dried in an air-conditioned room for 2 hours to remove excess water. Then, the andaliman was dried based on the previously mentioned drying methods, namely sun drying (SD), sunshade drying (SSD), shade drying (SHD), oven drying (OD), and freeze drying (FD). The results of each drying process compared to the fresh andaliman fruits could be seen in Figure 12. Once the drying processes were completed, the andaliman pericarp shrunk, exposing the shiny black seeds, and the pericarp color turned black brown. Freeze drying could visibly maintain the greenest product compared to other drying processes due to low temperatures during the drying process.

Figure 12. The results of the drying process
fresh andaliman - FA (a), sun-drying - SD (b), sunshade drying - SSD (c), shade drying - SHD (d), oven drying – OD (e), freeze drying - FD (f)

The moisture ratio (MR) was then plotted against time to observe the change in moisture during the drying process as well as to find the drying model that could best describe the drying of the fast-drying method (OD) and slow drying methods (SD, SSD, and SHD). As shown in Figure 13, OD was the fastest of all drying methods used in this study due to its higher and constant temperature. The blower's airflow also increased the evaporation rate. This method resulted in less variability during the drying process than other methods as the temperature was monitored continuously by a thermostat. However, the fruits needed to be laid in a single layer to ensure that all fruits were equally exposed to the hot air. Therefore, a large enough container should be used during the drying process.

During SD and SSD, the MR of andaliman experienced the most significant drop in the first 24 hours of the drying process because most of the free water was being evaporated. At night, the MR was relatively constant and could even increase on the second night due to moisture absorption from the ambient air. SHD was the slowest of all drying methods due to the low temperature (20-25°C) used during the process. The low relative humidity was the only main driver of water removal throughout the drying process, and after 48 hours, the decrease in MR was marginal. Based on a previous trial, there was negligible weight loss after 96 hours.

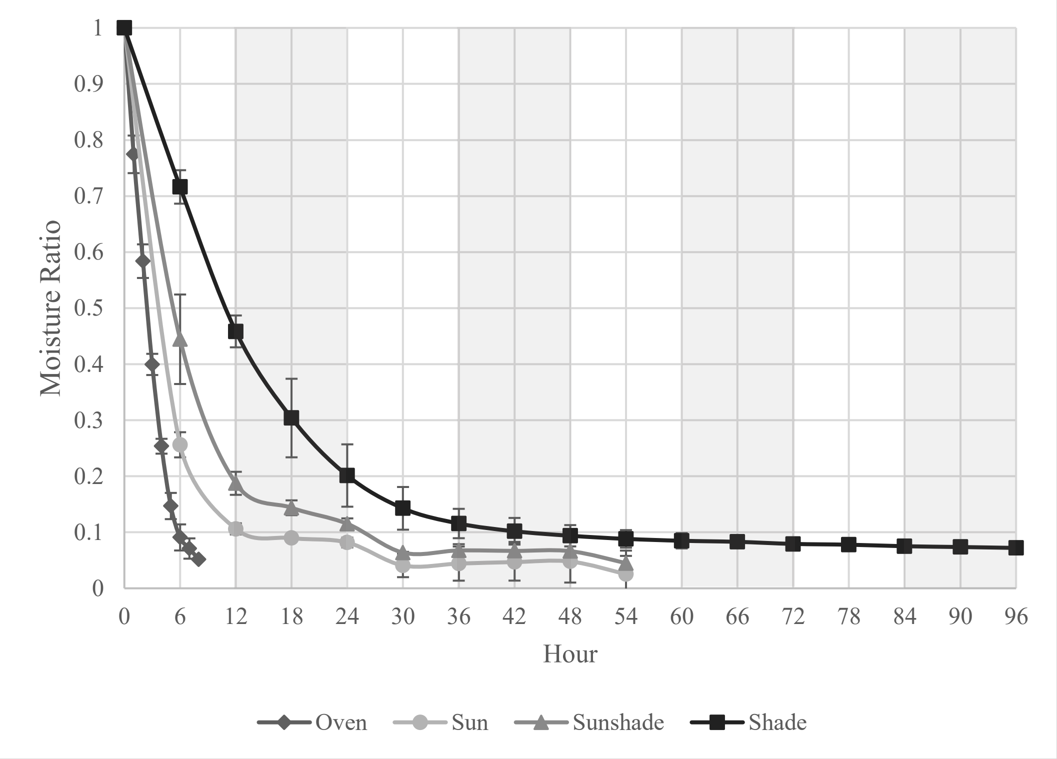


Figure 13. Moisture Ratio (MR) vs. time of 4 drying methods (shade symbolize nighttime)

SD and SSD depended on the weather condition to dry the andaliman fruits. As shown in Figure 14, the temperature fluctuated throughout the drying period, and the temperature difference could alter the drying rate between each replication. During the two replications, the weather condition was extremely hot, with sunny weather and temperature reaching 45°C during the day. When the temperature was at the peak, the relative humidity was also at the lowest. At night, the RH increased dramatically while the temperature dropped, which explained the slower drop in the MR. Since the drying was conducted during the Jakarta dry season, the drying process was completed at 54 hours. However, the drying duration could vary significantly if the weather were not favorable to efficiently dry andaliman, such as when the weather was cloudy or rainy.

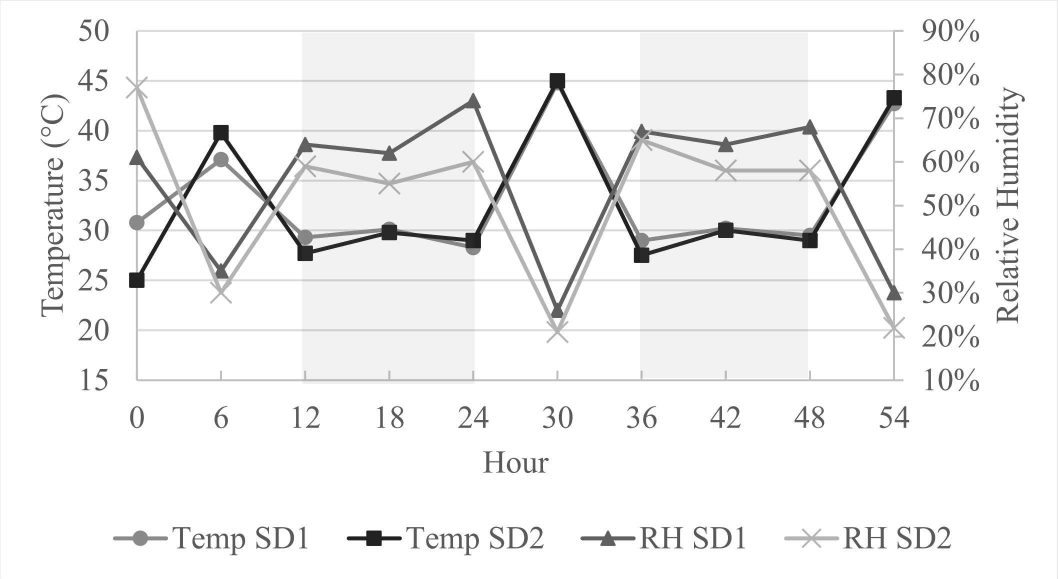


Figure 14. Temperature and relative humidity fluctuation during sun-drying (SD1: sun-drying first replication, SD2: sun-drying second replication)

The sunshade usage that blocked 55% of the sunlight managed to reduce the temperature by up to 5 °C during the day, which increased the RH, especially at the peak temperature at noon when the weather was at the sunniest time. The lower temperature and higher RH would explain the slower drop of MR during SSD compared with SD.

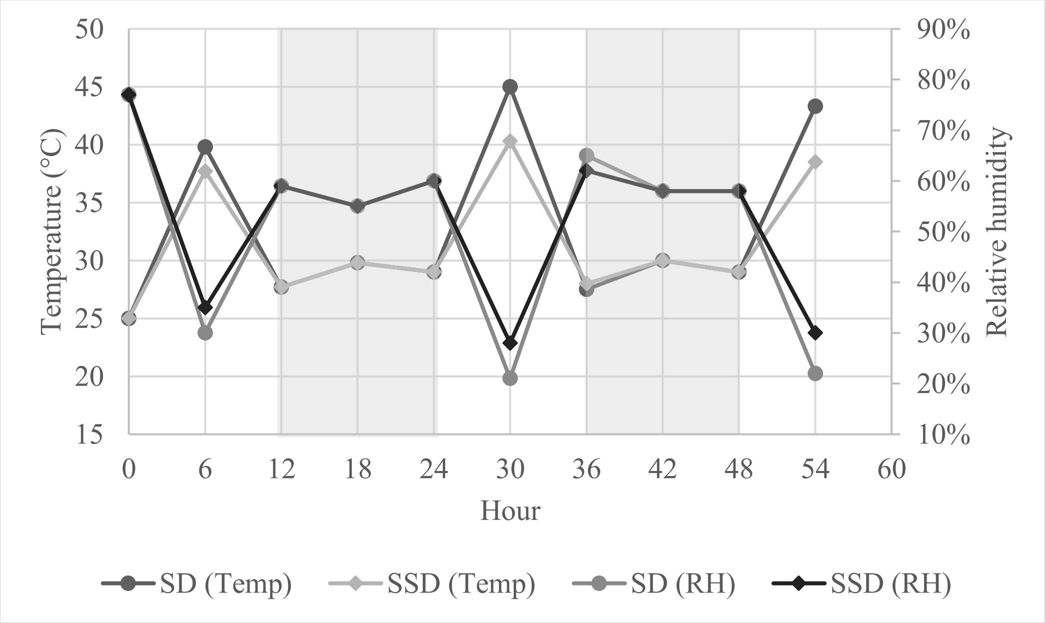


Figure 15. Effect of the sunshade on temperature and relative humidity (SD: Sun Drying, SSD: Sunshade Drying, RH: Relative Humidity)

4.2 Drying Kinetics

Six drying model was fitted into the MR vs. time data of each drying process using Table Curve 2D software (Systat Software, Inc, San Jose, CA, USA) resulting in the model parameters, as shown in Table 6. Generally, the Logarithmic model could better describe the drying kinetics of andaliman by various drying techniques, as shown by the highest R^2 value greater than 0.99 for all drying methods. In contrast, the Weibull model could not successfully describe the drying kinetics of andaliman. The logarithmic model has been known to explain the thin layer drying mechanism of several food products such as apples (Rayaguru and Routray 2012), pumpkins (Doymaz 2007), Basil leaves (Kadam et al. 2011), and others. The drying constant of OD was the highest indicating that it has the fastest drying rate compared to other drying methods.

Table 6. Drying kinetics andaliman by various drying techniques

Model	Parameter	Treatment			
		OD	SD	SSD	SHD
Page	k	0.0039	0.0109	0.0048	0.0018
	R^2	0.9965	0.9933	0.9837	0.9678
	SE Fit	0.0204	0.0245	0.0361	0.0477
Modified Page II	k	0.0053	0.0064	0.0024	0.0010
	R^2	0.9965	0.9933	0.9854	0.9678
	SE Fit	0.0204	0.0245	0.0361	0.0477
Henderson & Pabis	k	0.0057	0.0034	0.0020	0.0010
	R^2	0.9856	0.9717	0.9676	0.9595
	SE Fit	0.0411	0.0502	0.0539	0.0535
Logarithmic	k	0.0045	0.0042	0.0025	0.0013
	R^2	0.9921	0.9933	0.9919	0.9905
	SE Fit	0.0316	0.0251	0.0277	0.0263
Midilis Kucuk	k	-0.0023	0.0036	0.0022	0.0011
	R^2	0.9967	0.9877	0.9890	0.9912
	SE Fit	0.0204	0.0340	0.0323	0.0253
Weibull	k	0.0330	0.0778	0.1151	0.3011
	R^2	0.1760	0.9422	0.8225	0.5596
	SE Fit	0.3328	0.0761	0.7751	0.1822

k = drying constant (min^{-1}), R^{2,} = coefficient of determination,
SE Fit = Standard Error of Fit

4.3 Physical Characteristics of Dried Andaliman

The fresh and dried andaliman were analyzed for their physical characteristics to measure the finished product qualities and to ensure that each drying process could result in stable products throughout the shelf life.

4.3.1 Yield

Each drying process's yield was calculated by comparing the resulting weight after drying with the original weight before drying. As shown in Figure 16, shade drying resulted in a higher yield than other drying processes, whereas SD and FD resulted in a lower yield than other methods. However, based on one-way ANOVA, it was found that all drying processes did not result in significantly different yield values due to the P-value of 0.790, which was greater than the alpha of 0.05.

OD resulted in a yield of 26.4%, while a previous study by Napitupulu et al. (2020) reported that OD produced a yield of 24.04%, which was lower than other drying methods. In contrast, this study found that FD and SD resulted in yield values of 25.1% and 24.7%, respectively, while the previous study by Napitupulu et al. (2020) reported yield of 26.7% and 26.8%. SHD resulted in a similar yield to the one obtained in the previous study (Napitupulu et al. 2020) although the previous study did not use any air conditioning. It should be noted that this trial experienced quite a sizeable standard deviation between replicates from the variations in the initial moisture content of the fruits.

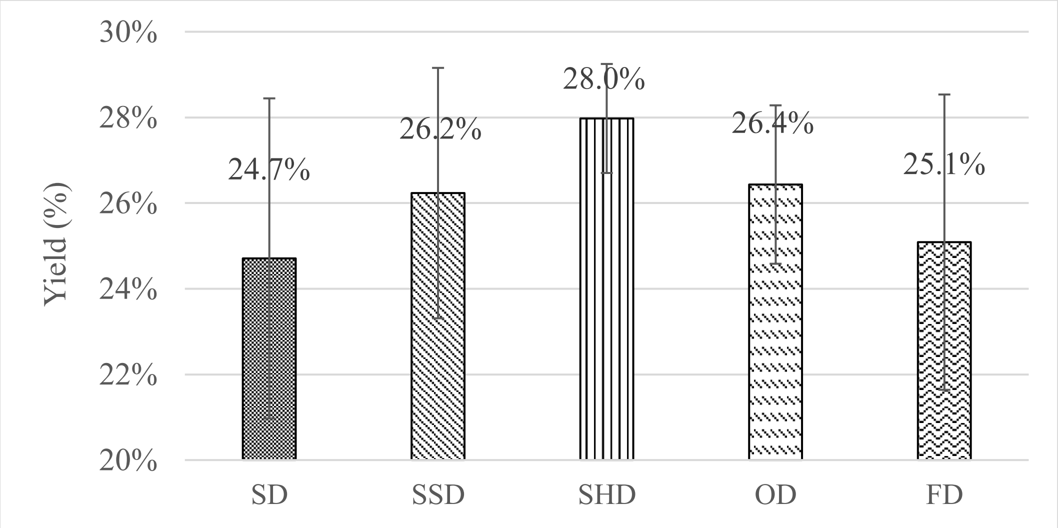


Figure 16. The yield of each drying process
sun drying – SD, sunshade drying - SSD, shade drying - SHD, oven drying – OD, freeze drying – FD. No significant differences (P-value of 0.790).

4.3.2 Moisture Content (Loss on Drying)

The moisture content of andaliman was expressed as Loss on Drying values by subjecting the samples at 105 °C for three hours or until a constant weight was achieved. This method resulted in elevated values due to the high content of volatile oils evaporated during the process (Charoensup et al. 2016). Sun-drying (SD) managed to reduce the LOD from 77.62 % down to 10.09 %, whereas shade drying (SHD) only reduced the LOD to 15.91 %, as could be seen in Figure 17. Freeze drying suffered from a significant standard deviation in the LOD values, which was probably caused by the structure's collapse during the drying process, thus hindering a complete lyophilization of the residual moisture in the fruits. Elevated room temperature could cause the structure to collapse since the machine was not placed inside an air-conditioned room.

It is worth noting that the success of SD and SSD depended very much on the weather condition during the drying process. A period of cloudy day or rain would hinder the complete drying of the fruits, thus requiring an extended period to achieve a complete drying. Despite all this, all drying methods managed to reduce the LOD to less than 17.90%, which was the maximum recommended LOD values of dried *Zanthoxylum* (Charoensup et al. 2016). Therefore, it could be concluded that drying targets have been achieved.

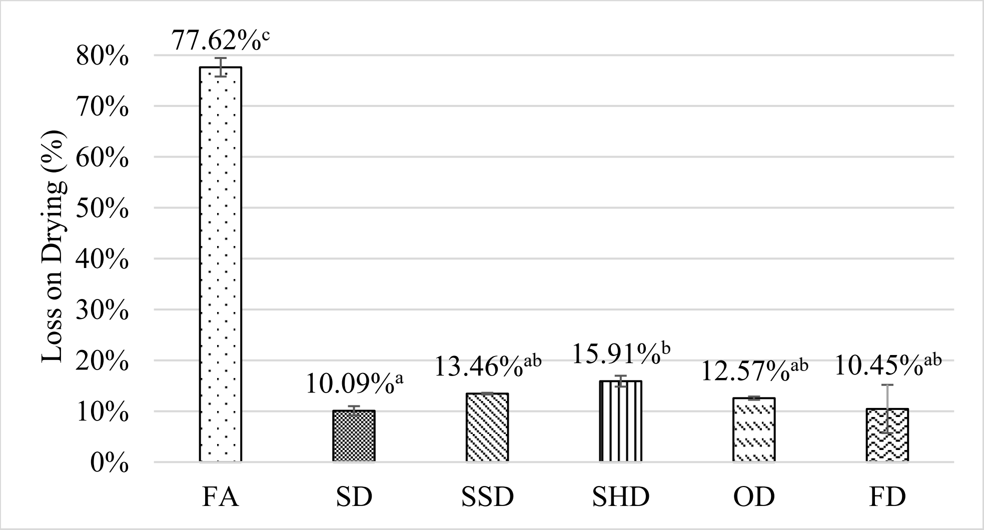


Figure 17. Loss on drying (LOD) values of fresh and dried andaliman
fresh andaliman – FA, sun-drying – SD, sunshade drying - SSD,
shade drying - SHD, oven drying – OD, freeze drying – FD
a, b, c, d – mean sharing similar letters were not significantly different at a 95% confidence interval based on the Duncan posthoc test.

4.3.3 Water Activity

As could be seen from Figure 18, SD and FD resulted in the lowest water activity amongst all drying methods, which could be caused by the hot weather and long drying duration experienced during the sun drying as well as the long duration and low pressure during the freeze drying that could evaporate most of the residual moisture present in the samples. These values were lower than the ones previously reported by Napitupulu et al. (2020). However this study showed similar water activity from OD with the previous study (Napitupulu et al. 2020).

A similar trend of the yield, water activity, and loss on drying values could be observed by comparing the graphs in Figure 16 – 18. SD and FD resulted in the lowest yield, LOD, and a_w, whereas SHD resulted in the highest yield, LOD, and a_w. On the other hand, OD and SSD resulted in similar yield, LOD, and a_w. The high yield of SHD could be attributed to the samples' high residual moisture, which could be observed by the high a_w and LOD compared with other drying methods.

Low moisture foods' water activity should be less than 0.60 to prevent molds from growing in the products (Pinkas and Keller 2014). All drying methods managed to reduce the water activity to less than 0.60; however, shade drying resulted in a water activity of 0.585, which was remarkably close to 0.60. At one replication, the water activity was even higher than 0.60. Therefore, this drying method should not be used commercially without further modifying the drying parameters to reduce the water activity to significantly less than 0.60. Reducing the relative humidity while increasing the airflow could be some of the solutions to lower the water activity during shade drying.

Storage of the finished products should also be of concern since the water activities of andaliman could increase when stored at a relative humidity > 60% since the products would reach equilibrium with the environment, thus increasing the water activity to higher than 0.60, which could allow for mold proliferation during the storage (Pinkas and Keller 2014). Bacteria could not grow at a_w of less than 0.85 (Pinkas and Keller 2014); however, drying does not mean that bacteria were not present within the samples because pathogenic bacteria such as *Salmonella* could still survive the drying process and cause outbreaks of foodborne illnesses upon consumption (Gurtler et al. 2014). Further research on ensuring microbiologically safe dried andaliman should be conducted.

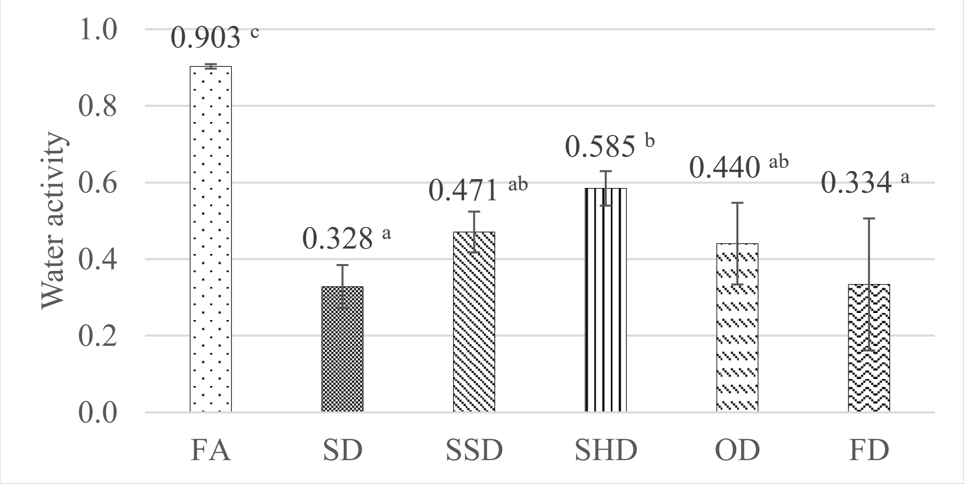


Figure 18. The water activity of fresh and dried andaliman
fresh andaliman – FA, sun-drying – SD, sunshade drying - SSD,
shade drying - SHD, oven drying – OD, freeze drying – FD
a, b, c, d – mean sharing similar letters were not significantly different at a 95% confidence interval based on the Duncan posthoc test.

4.3.4 Poured Bulk Density

Bulk density is a measure of weight per volume of an ingredient affected by numerous factors such as size, shape, moisture content, and other processing factors (Schuck 2011; López-Córdoba and Goyanes 2017). Low bulk density is generally unfavorable in the industrial application as it would increase the volume of the ingredients, thus increasing the cost to pack, store, and ship the ingredients (Schuck 2011). There are three main methods to measure bulk density, aerated, poured, and tapped (López-Córdoba and Goyanes 2017), however, poured bulk density is commonly used to measure the density of spices such as black pepper (ISO 1998).

As shown in Figure 19, the drying of andaliman decreased the bulk density of the fruits with freeze-dried andaliman possessing the lowest bulk density than other dried andaliman despite not significantly different from sun-dried, sunshade-dried andaliman. The sublimation of ice during freeze drying created pores within the structure of the andaliman, thus decreasing the bulk density; however, this porous structure could collapse if the temperature during lyophilization was elevated beyond its collapse temperature (Krokida et al. 1998). Therefore, strict control of the temperature during freeze drying must be maintained to ensure consistent results from batch to batch.

Whole dried andaliman had lower bulk density than the bulk density of whole black pepper, 0.600-0.620 g/mL (BSN 1995), which indicated that dried andaliman required larger space during shipping and storing than whole black pepper. This phenomenon could be explained by the opening of the pericarps during drying, which created space within the product.

Grinding the whole andaliman would increase the bulk density of dried andaliman (Napitupulu et al. 2020); however, upon long term storage of powdered andaliman, the numbing sensation was significantly reduced than that of whole dried andaliman (Inggita 2015).

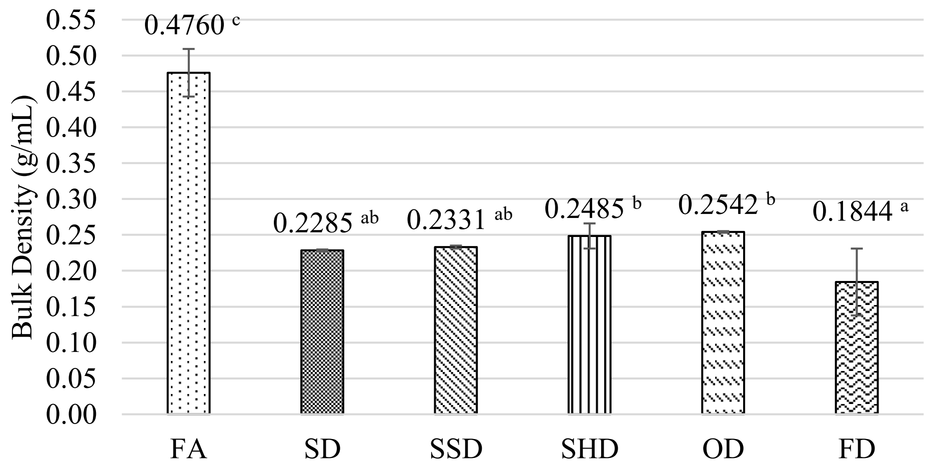


Figure 19. Bulk density of whole fresh and dried andaliman
fresh andaliman – FA, sun-drying – SD, sunshade drying - SSD,
shade drying - SHD, oven drying – OD, freeze drying – FD
a, b, c, d – mean sharing similar letters were not significantly different at a 95% confidence interval based on the Duncan posthoc test.

4.3.5 Color

The color of andaliman pericarp was not uniform; therefore, measuring the color was quite a challenge. Each sample was separated into three containers, and three measurements were taken at three separate locations from each container. Those three measurements were then averaged to obtain a single value of that container. The averaged measurements from each container were then averaged to obtain the overall color values of each batch of andaliman. This method reduced the standard deviation of non-uniform colored samples such as pretzel bites (Whetzel 2016). Grinding of andaliman would result in a more uniform sample for evaluation; however, when fresh andaliman was ground, the color immediately changed from bright green to dark green due to the release of acids from the cells, which transformed chlorophyll into pheophytin. This change of color would certainly hinder an objective comparison.

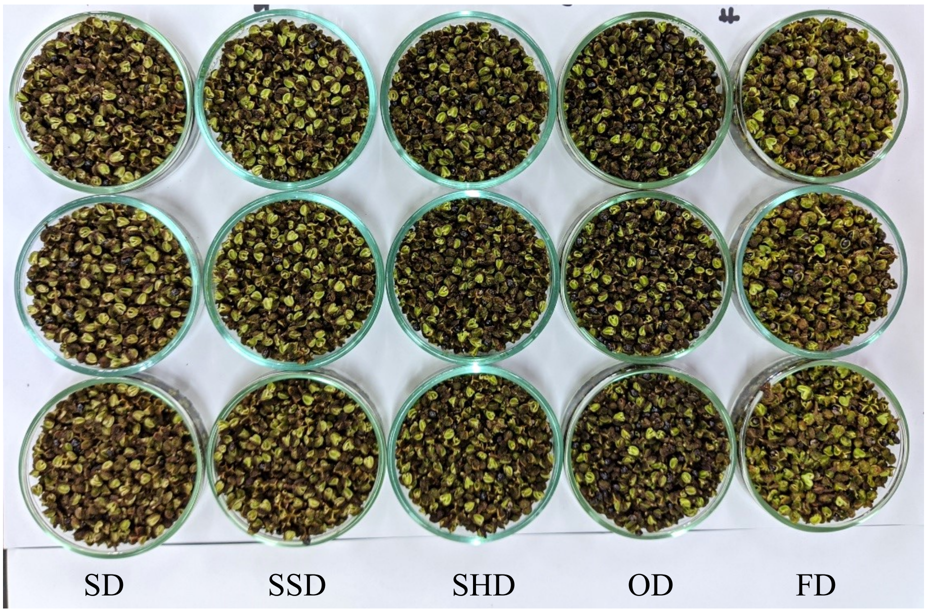


Figure 20. Color measurement of andaliman pericarp
(sun-drying – SD, sunshade drying - SSD, shade drying - SHD, oven drying – OD, freeze drying – FD)

No significant difference was found in the L* values of all andaliman even though the L* value of freeze-dried andaliman was the highest amongst all samples. The lack of significant difference might have been caused by the large color variations and the light-colored inner part of the pericarps, increasing the color's overall lightness when measured by the chromameter.

One of the most noticeable changes when drying andaliman was the degradation of andaliman green color into black-brown, which could have been caused by enzymatic browning due to polyphenol oxidase (Karnady 2015) or by chlorophyll degradation due to chlorophyllase and chlorophyll-degrading peroxidase (Chen et al. 2012).

No significant difference was found with the a* values of all dried andaliman. However, there was a significant difference when the a* values of dried andaliman were compared to the fresh andaliman, indicating a substantial green color reduction. This study found that OD resulted in a lower a* value than SHD, which agreed with Chen et al. (2012) that found OD of green Sichuan pepper resulted in a lower a* value compared to SHD. This phenomenon was caused by the increase in the activities of chlorophyll degrading peroxidase and pheophorbidase during the initial part of shade drying. On the other hand, OD reduced all chlorophyll degrading enzymes' activity immediately after the start of drying (Chen et al. 2012).

Table 7. Color analysis of fresh and dried andaliman pericarp

Treatment	L*	a*	b*
FA	24.97 ± 2.07^{a}	-3.81 ± 0.45^{a}	20.38 ± 0.43^{b}
SD	25.18 ± 2.38^{a}	1.01 ± 1.01^{b}	14.74 ± 1.38^{a}
SSD	25.71 ± 1.54^{a}	0.23 ± 0.52^{b}	15.21 ± 1.24^{a}
SHD	25.93 ± 1.64^{a}	-0.12 ± 1.25^{b}	15.32 ± 0.71^{a}
OD	24.72 ± 2.77^{a}	-0.57 ± 1.09^{b}	15.35 ± 1.54^{a}
FD	29.26 ± 2.57^{a}	-1.00 ± 0.42^{b}	19.38 ± 1.14^{b}

L*: (+) light – (-) dark; **a***: (+) red – (-) green; **b***: (+) yellow – (-) blue
Fresh andaliman - FA, sun drying – SD, sunshade drying - SSD,
shade drying - SHD, oven drying – OD, freeze drying – FD
a, b, c, d – mean sharing similar letters were not significantly different at a 95% confidence interval based on the Duncan posthoc test.

Usage of sunshade improved the color of dried andaliman by reducing the a* value compared to SD, although no significant difference was found between them. On the other hand, FD resulted in the greenest color compared to all drying methods because of its low temperature, suspending the activity of chlorophyll degrading enzymes. SD resulted in the worst color, whereas FD resulted in the best color of dried andaliman, which agrees with the similar conclusion reported by Roshanak et al. (2016).

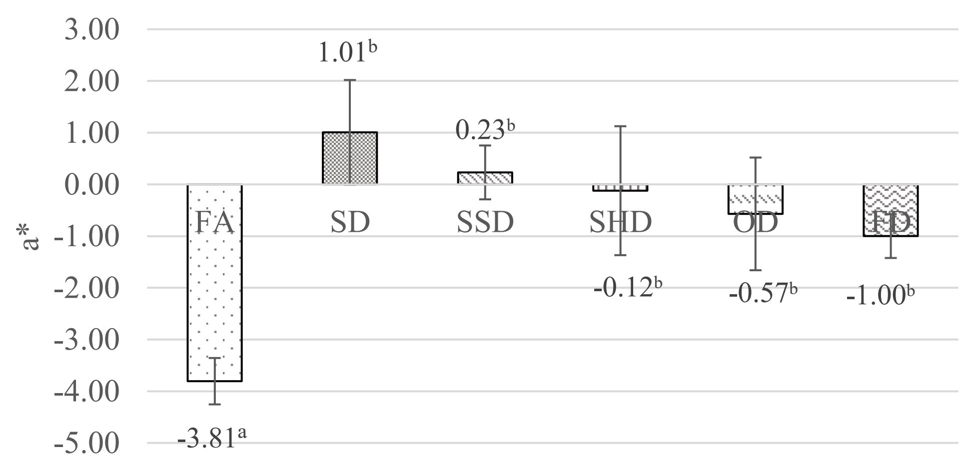


Figure 21. a* values of fresh and dried andaliman
Fresh andaliman - FA, sun drying – SD, sunshade drying - SSD,
shade drying - SHD, oven drying – OD, freeze drying – FD
a, b, c, d – mean sharing similar letters were not significantly different at a 95% confidence interval based on the Duncan posthoc test.

4.4 Confirmation of GC-MS Condition

A confirmation of the GC-MS condition that could analyze both volatiles and sanshool compounds were conducted by injecting a chloroform extract of fresh andaliman obtained from *Pasar Senen* into the GC-MS using split injection at 1:100 ratio with an initial oven temperature of 40 °C (4 minutes) ramped to 280 °C for 4 minutes with temperature ramp speed of 4°C/min.

As shown in Figure 22, the TIC from the analysis showed three prominent peaks that were later identified as limonene, geranyl acetate, and α-sanshool. However, numerous shorter peaks were challenging to identify upon closer look because the tall limonene peak overshadowed them. Therefore, we decided to further refine the method by decreasing the split ratio to 1:10 to increase the heights of the peaks of other compounds.

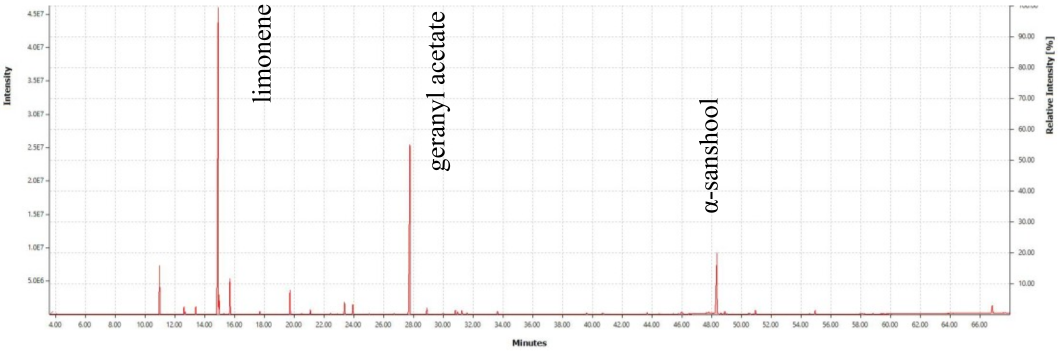


Figure 22. TIC of andaliman extract based on the initial method (split 1:100)

Once the split ratio was decreased, the peaks were more visible in the chromatogram, and further decrease would widen the peaks too much. However, after further observation, the geranyl acetate peak coeluted with another compound, as shown in Figure 23b. As geranyl acetate was a major aroma compound in andaliman, it was necessary to separate the two coeluted peaks. One of the most common ways of separating coeluted peaks was to decrease the temperature ramp speed, which in this case, the speed was reduced from 4°C/min to 2°C/min.

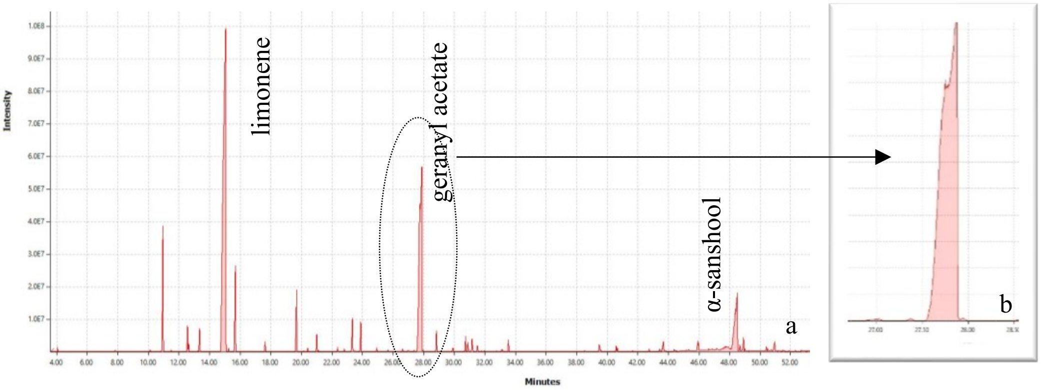


Figure 23. TIC of andaliman extract on 1:10 split (a), TIC of coeluted peak (b)

By decreasing the temperature ramp speed, the coeluted peaks were separated and further identified as geranyl acetate and methyl cinnamate (Figure 24). This slow temperature ramp speed separated smaller coeluted peaks and increased the accuracy of the compound identification. The downside was that each running took twice a longer time to complete as the total run time was increased from 67 minutes to 128 minutes. Autosampler usage improved the analysis throughput as the analysis could be conducted continuously and improve the accuracy as no human error was present during sample injection.

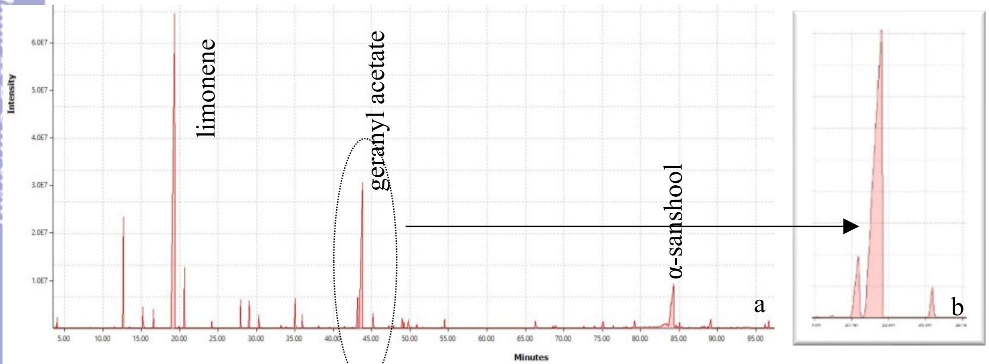


Figure 24. TIC of andaliman extract at 2°C/min (a), TIC of separated peak (b)

To further ensure that this method could analyze sanshool compounds, 1 µL of the diluted hydroxy α-sanshool standard was injected into the GC-MS using the previous method. It was found that hydroxy α-sanshool was eluted at approximately 89 minutes, as shown in Figure 25. Based on this, we could then identify the peak of hydroxy α-sanshool in andaliman extract, and we could confidently conclude that this GC-MS parameter managed to detect sanshool compounds.

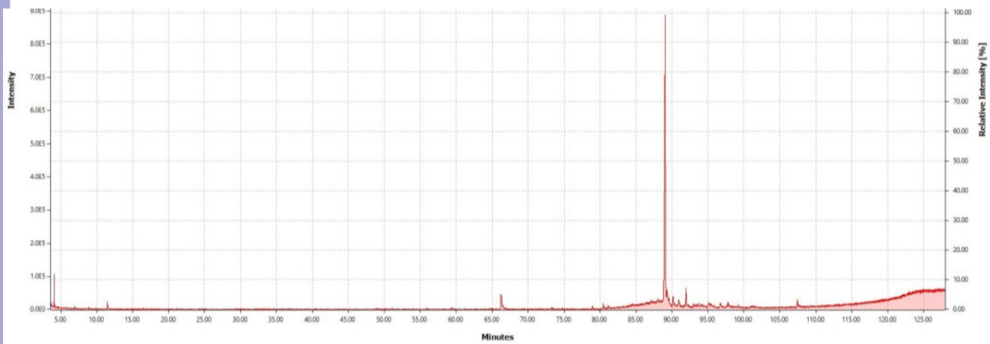


Figure 25. TIC of diluted hydroxy α-sanshool standard

The mass spectra of hydroxy α-sanshool obtained by this method are shown in Figure 26 for future reference. The spectra indicated that the molecular ion of hydroxy α-sanshool is 263, which agrees with its molecular formula of $C_{16}H_{25}NO_2$.

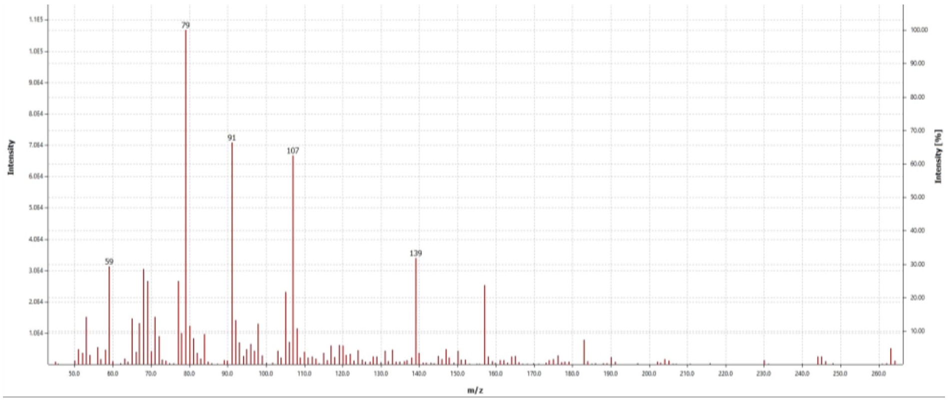


Figure 26. Mass spectra of hydroxy α-sanshool standard

On the other hand, α-sanshool was tentatively identified by comparing the resulting mass spectra with the literature. As shown in Figure 27, the mass spectra of the α-sanshool peak, eluted at around 84 minutes, showed a molecular ion of 247 m/z, which corresponded to its calculated molecular weight the formula of $C_{16}H_{25}NO$. This tentative identification should be clarified in the future using a high purity standard. Its isomers were also evident as there were other shorter unknown peaks with a molecular ion of 247. The highest peak with molecular ion 247 and similar mass spectra pattern with the literature was tentatively identified as α-sanshool since it is the main sanshool compound in andaliman.

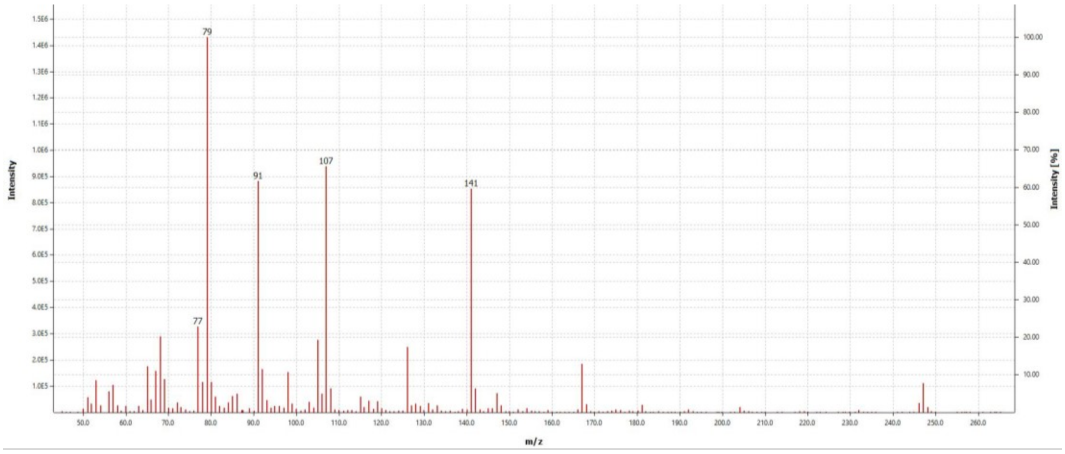


Figure 27. Mass spectra of α-sanshool peak

Out of curiosity, we tried different solvents to extract fresh andaliman, namely diethyl ether, chloroform, ethanol, and hexane, using the fast ramp GC-MS (4°C/min) to increase the analysis throughput. The TIC overlay is compiled in Figure 28 as follows. Hexane managed to extract the most compounds due to its low polarity compared to other solvents; however, it also extracted a significant amount of wax that turns the extract into a solid upon refrigeration. This high amount of wax would require extensive clean-up that could remove crucial compounds. Extraction by chloroform and diethyl ether, on the other hand, managed to result in a similar chromatogram profile.

In contrast, ethanol extraction resulted in the most different chromatogram, as shown by the missing limonene peak and lower areas of all other compounds overall. This difference was probably caused by its high polarity resulting in inefficiencies to extract non-polar volatiles as well as the lower pressure and higher temperature required to evaporate ethanol during the concentration process. Thus, we decided to use chloroform to extract the volatiles and sanshool compounds as it has been successfully used before (Sugai, Morimitsu, and Kubota 2005) and the fact that it could be condensed during the rotary evaporation process. Diethyl ether was highly volatile and could not be condensed back to liquid by the rotary evaporator, causing significant air pollution in the laboratory, since the evaporator was not placed inside a fume hood and was not equipped with a water chiller to condense the diethyl ether vapor efficiently.

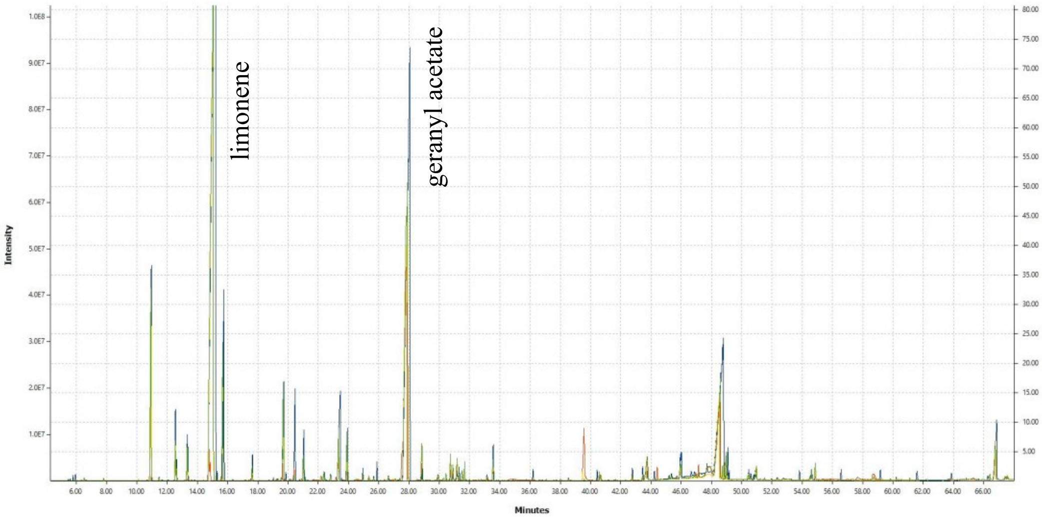


Figure 28. Overlay of TIC resulted from different solvent extraction
red – ethanol, yellow – chloroform, blue – hexane, green – diethyl ether

4.5 Volatiles Contents of Andaliman

Fresh andaliman was ground for 10-20 seconds, while dried andaliman was ground to 40 mesh to increase the surface area and produce uniform particle size amongst all dried andaliman. The ground andaliman was added with 200 µL of 1% 1,2,4 trichlorobenzene as the internal standard and macerated with 100 mL chloroform overnight twice in a refrigerator before concentrated to 10 mL. The extraction and GC-MS analysis were done in duplicate for each biological replicate of dried and fresh andaliman. The result of the extraction could be seen in Figure 29 as follows. The fresh andaliman extract was lighter in color than the dried andaliman extracts, which could be attributed to the moisture content in the fresh andaliman. The presence of water might have reduced chlorophyll extraction's effectiveness by diluting the solvent used during the extraction (Su et al. 2010).

Figure 29. Chloroform extract (in duplicate) of fresh and dried andaliman

Interestingly, the TIC of fresh andaliman was quite similar to the TICs of dried andaliman. As a comparison, TIC of fresh and oven-dried andaliman extract was shown in Figure 30. The peak pattern was almost identical, differing mostly in the peak area of each compound. To further investigate the difference between each drying process, relative quantities of each compound were calculated by comparing each compound's peak area to the peak area of the internal standard (1,2,4-trichlorobenzene).

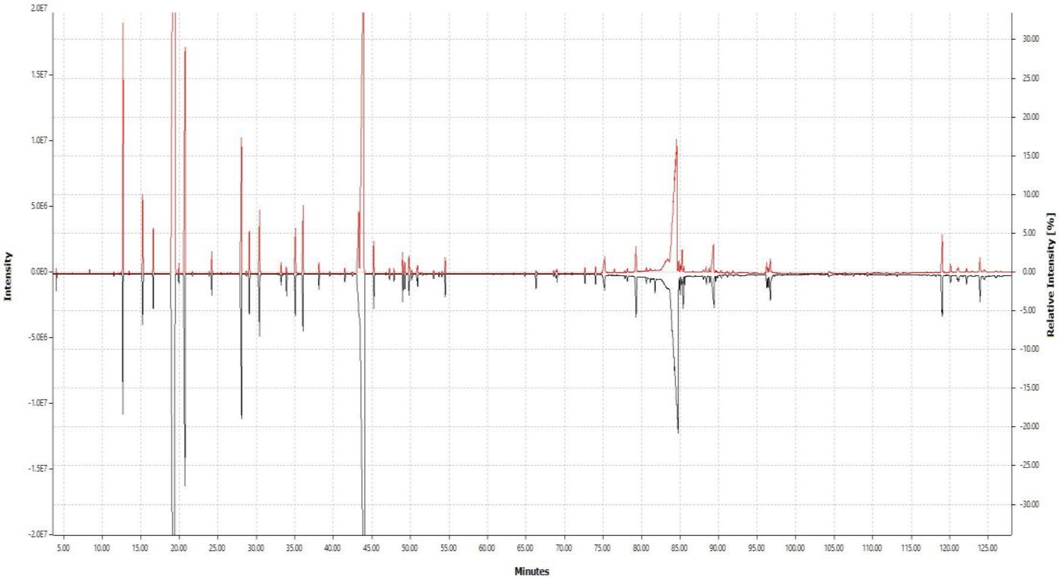
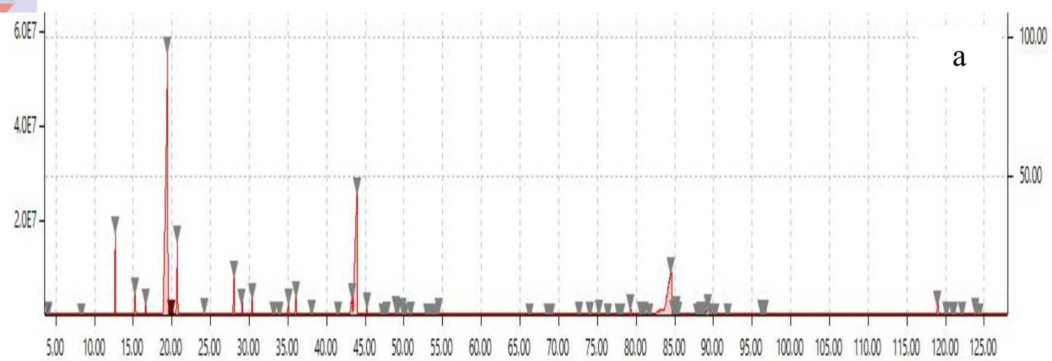
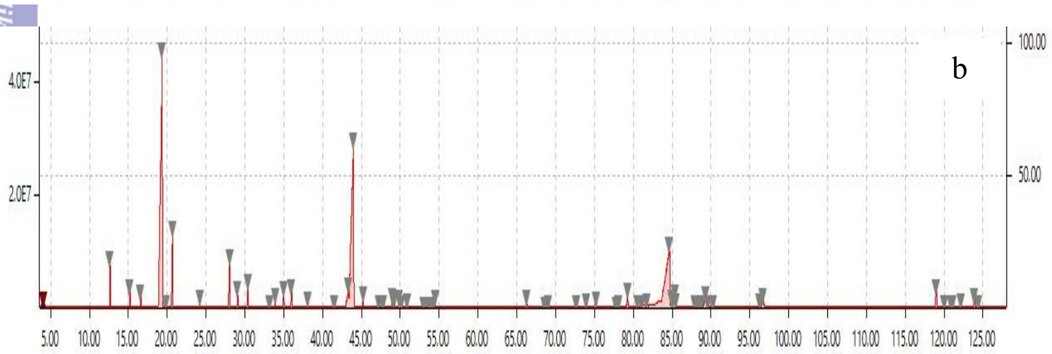
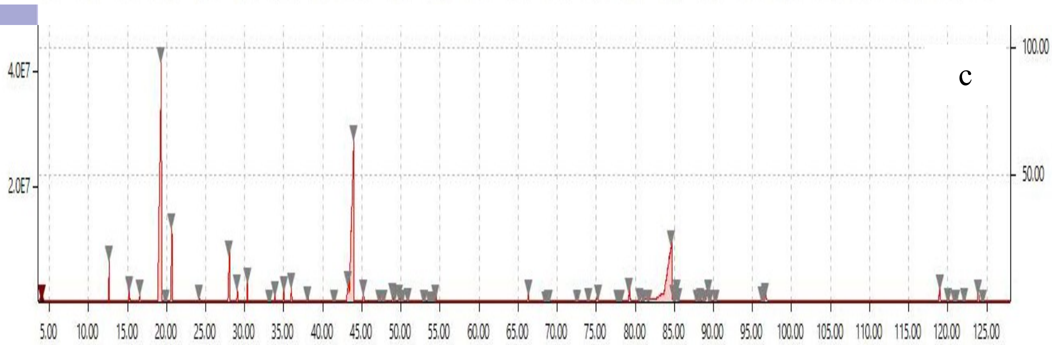


Figure 30. TIC comparison of fresh (top) vs. oven-dried andaliman (bottom)

Representative chromatograms of the extracts from each drying process could be seen in the Figure 31. All extracts resulted in a similar chromatogram profile, albeit differing in the area of each peak.


a
6.0E7
4.0E7
2.0E7
100.00
50.00
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00 75.00 80.00 85.00 90.00 95.00 100.00 105.00 110.00 115.00 120.00 125.00


b
4.0E7
2.0E7
100.00
50.00
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00 75.00 80.00 85.00 90.00 95.00 100.00 105.00 110.00 115.00 120.00 125.00


c
4.0E7
2.0E7
100.00
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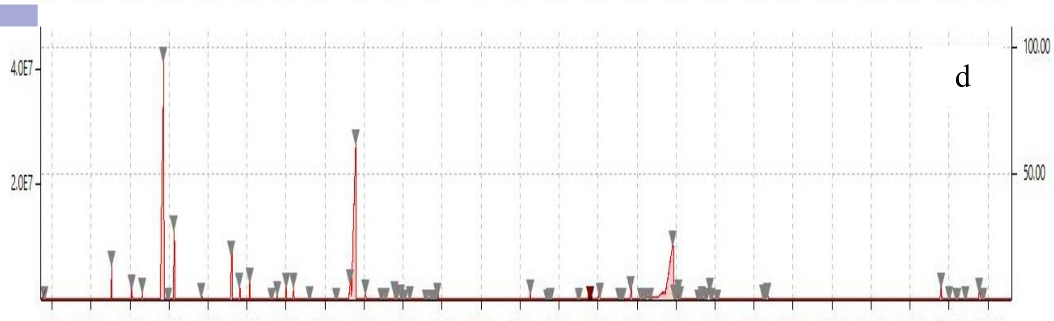
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00 75.00 80.00 85.00 90.00 95.00 100.00 105.00 110.00 115.00 120.00 125.00


d
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2.0E7
100.00
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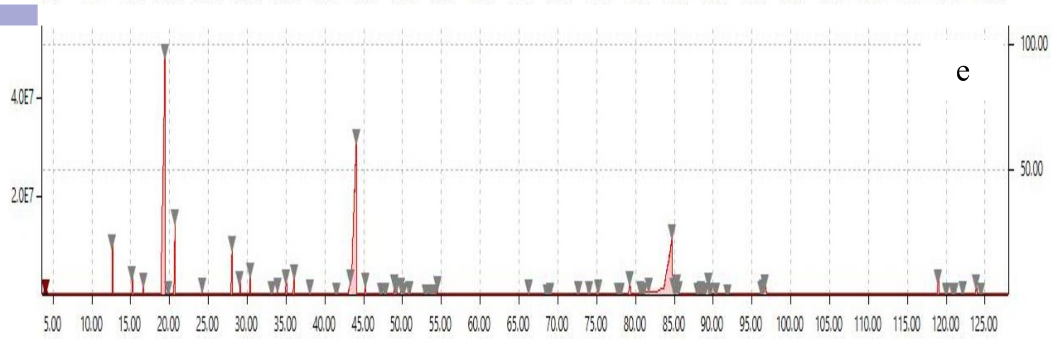
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00 75.00 80.00 85.00 90.00 95.00 100.00 105.00 110.00 115.00 120.00 125.00


e
4.0E7
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5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00 75.00 80.00 85.00 90.00 95.00 100.00 105.00 110.00 115.00 120.00 125.00

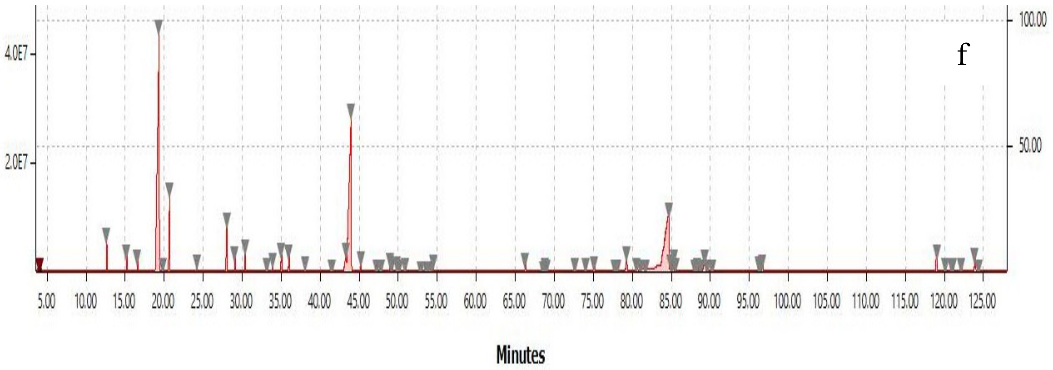

Figure 31. Representative TIC of GC-MS analysis of each andaliman samples
(a) Fresh (b) Sun Drying (c) Sunshade Drying (d) Shade Drying
(e) Oven Drying (f) Freeze Drying

Table 8. The relative content of identified compounds in andaliman samples

No	Compound	Odor[a]	ID[b]	RI[c]	CRI[d]	FA	SD	SSD	SHD	OD	FD
Total relative content of identified compounds						63969.3 ± 4694.2	56429.1 ± 12148.6	57804.7 ± 12148.8	64722.5 ± 4504.4	65872.8 ± 20160.6	61763.8 ± 11962.9
Acetal											
1	1,1-diethoxyethane	Nutty	RI, MS	725	<800	30.4 ± 4.3	38.8 ± 29.3	43.4 ± 33.4	39.7 ± 9.8	62.4 ± 6.5	41 ± 2.1
Aldehyde											
2	(E)-2-hexenal*	Green	RI, MS	846	848	31.2 ± 10	nd	nd	nd	nd	Nd
Ester											
19	(E)-methyl cinnamate	Balsamic	RI, MS	1376	1381	1055.6 ± 511.6	1087.4 ± 481.9	1087.4 ± 541.4	1293.2 ± 16.4	934.8 ± 444.2	1176.4 ± 350.5
34	Methyl ferulate		RI, MS	1854	1830	36.7 ± 2.4	31.1 ± 2.4	32.2 ± 0.2	36.2 ± 3.9	27.8 ± 9.4	32.4 ± 1.1
40	Ethyl palmitate*	Waxy	RI, MS	1993	1992	31.2 ± 10	nd	nd	nd	nd	Nd
Monoterpene alcohol											
9	Linalool	Floral	RI, MS	1095	1098	175.3 ± 18.8	157.5 ± 22.5	164.6 ± 20.8	157.4 ± 9.8	186.6 ± 40.4	163.5 ± 23.6
12	α-terpineol	Terpenic	RI, MS	1186	1186	696.6 ± 105	541.6 ± 237.1	582.5 ± 266.2	788.5 ± 139.6	740 ± 175.3	665.5 ± 97.5
13	β-citronellol	Floral	RI, MS	1223	1228	79.3 ± 23.4	44.9 ± 15.3	51.9 ± 7	53.8 ± 3.9	83.6 ± 49.1	66.9 ± 25.4
15	Geraniol	Floral	RI, MS	1249	1255	652 ± 3.2	679.9 ± 147.5	711.1 ± 179.4	685 ± 153.9	642.1 ± 67.1	695.3 ± 87.6
Monoterpene aldehyde											
10	Citronellal	Floral	RI, MS	1148	1153	1583.2 ± 602.4	1615.5 ± 451.9	1757 ± 539	1782.9 ± 210.5	2127.1 ± 760	1841.4 ± 407.8
14	Neral	Citrus	RI, MS	1235	1238	64.5 ± 5.1	195.5 ± 69	238.2 ± 67.4	293.5 ± 55.5	246.6 ± 21.9	244.7 ± 53.4
16	Geranial	Citrus	RI, MS	1264	1269	755.3 ± 235.6	452.9 ± 118.7	478.9 ± 149.7	561.9 ± 54.2	678.1 ± 312.2	508.5 ± 78.4
Monoterpene ester											
17	Geranyl formate	Floral	RI, MS	1298	1300	107.2 ± 8.2	112.6 ± 34.2	117.6 ± 35.7	145.9 ± 25.8	154.7 ± 36.4	132.2 ± 20
18	Citronellyl acetate	Floral	RI, MS	1352	1353	53.6 ± 13.5	70.1 ± 17.3	80.6 ± 12.7	86.4 ± 3.5	85.8 ± 39.1	86.7 ± 18.7
20	Geranyl acetate	Floral	RI, MS	1379	1392	13149 ± 969.2	15302 ± 3634.9	15345.9 ± 3706	17532 ± 988.4	17118 ± 4838.7	16833.9 ± 3656
Monoterpene hydrocarbon											

3	α-pinene*	Herbal	RI, MS	932	928	2826.9 ± 591.1	1194.3 ± 349.6	984.4 ± 17.7	847.3 ± 81.2	1337.7 ± 59.5	713.4 ± 112.4
4	Sabinene*	Woody	RI, MS	969	968	758.9 ± 95.8	381.6 ± 105.1	369.8 ± 101.6	474.8 ± 61.1	538.5 ± 111.4	411.3 ± 89.8
5	β-myrcene*	Spicy	RI, MS	988	989	507.5 ± 56.6	336.5 ± 30.1	321.4 ± 20.7	330.1 ± 58.1	390.8 ± 29.9	274.2 ± 78.8
6	Limonene	Citrus	RI, MS	1024	1030	28092.9 ± 29.4	19298.5 ± 3006.3	19510.5 ± 1718.7	20544.3 ± 1722.1	21857.5 ± 7024.3	19523.3 ± 3561.3
7	(Z)-β-ocimene	Floral	RI, MS	1032	1037	69.7 ± 24.6	53.9 ± 25.3	54.7 ± 28.5	61.4 ± 8.6	69.6 ± 37.9	67.7 ± 18.6
8	(E)-β-ocimene	Sweet herbal	RI, MS	1044	1049	2426.4 ± 1150.6	2069 ± 865.7	2040.6 ± 1014.2	2299.2 ± 318.1	2690 ± 1325.7	2600.1 ± 633.8
Sesquiterpene											
21	β-caryophyllene	Spicy	RI, MS	1417	1412	418.2 ± 76.1	426.3 ± 65.8	432.3 ± 20.3	387.5 ± 95.9	403.1 ± 136.1	323.3 ± 52.3
22	α-caryopyllene	Woody	RI, MS	1452	1445	59.3 ± 10.6	64.4 ± 10.6	63.7 ± 3.7	60.2 ± 14.4	69.4 ± 7.4	51 ± 7.5
23	(E)-β-farnesene	Woody	RI, MS	1454	1455	59.8 ± 6.7	72.6 ± 4.4	75 ± 4.3	74.2 ± 14.6	80 ± 12.2	68.7 ± 8.8
24	Germacrene D	Woody	RI, MS	1480	1473	263 ± 45.3	285.6 ± 43.2	294.9 ± 26.7	282.2 ± 62	318.3 ± 42.2	248.8 ± 36.4
25	β-selinene	Herbal	RI, MS	1489	1477	125.9 ± 9.8	133.8 ± 8.7	137.5 ± 3.2	130.3 ± 25.2	146.2 ± 32.7	110.4 ± 23.2
27	(Z,E)-α-farnesene	Woody	RI, MS	1491	1493	40.6 ± 3.8	57.6 ± 20.1	57.9 ± 17.6	50.7 ± 19	56.4 ± 18.7	42.9 ± 5.2
26	α-selinene	Spicy	RI, MS	1498	1487	203 ± 23.7	209.3 ± 14.9	215.9 ± 6	204.5 ± 42.7	233.7 ± 51.7	176.7 ± 36.4
28	(E,E)-α-farnesene	Woody	RI, MS	1505	1505	72.1 ± 1.5	90.8 ± 7.2	93.1 ± 9.5	103.6 ± 17.8	110.3 ± 22.8	96.8 ± 14.3
35	Neophytadiene	odorless	RI, MS	1840	1836	41.6 ± 14.1	42 ± 6.1	57.6 ± 5.8	110.2 ± 55.1	38.9 ± 6	104.2 ± 9.7
Sesquiterpene alcohol											
31	(E)-Nerolidol	Floral	RI, MS	1561	1560	25.8 ± 2.6	32.1 ± 0.1	35.2 ± 0.5	41 ± 11.6	39.3 ± 5.2	35.2 ± 2.2
32	Germacrene D-4-ol		RI, MS	1574	1567	194.7 ± 19.8	230.3 ± 8.5	234.5 ± 5.2	246.6 ± 49.3	265.6 ± 43.8	231.3 ± 26.8
Sesquiterpene ester											
36	(2E,6E)-farnesyl acetate	Oily	RI, MS	1845	1838	55 ± 5.8	74.8 ± 3.8	79.2 ± 6.9	85.3 ± 19.9	86.1 ± 11.8	78.1 ± 4.3
Diterpene alcohol											
46	(E)-phytol*	Floral	RI, MS	2116	2108	9.2 ± 3.2	81.8 ± 12.3	43.6 ± 19.7	24 ± 17.9	188.7 ± 15.8	26.1 ± 2.9
Secondary amides											

47	α-sanshool		MS		2175	6879.7 ± 1633.5	7994.9 ± 3489.5	8531 ± 3914.7	10765.2 ± 450.3	10464 ± 3925.5	10721.5 ± 2228.5
55	hydroxy α-sanshool		S, MS		2286	644.9 ± 136.3	550.2 ± 341	606.9 ± 308.5	823.2 ± 43.1	773 ± 371.4	798.2 ± 161
Alkaloids											
33	N,N-Dimethyltryptamine		RI, MS	1750	1785	78.4 ± 59.2	281.8 ± 37.4	524 ± 179.6	451.2 ± 91.5	310.8 ± 146.7	340 ± 22.8
Phytosterol											
65	Campesterol		RI, MS	3131	3189	67.2 ± 10.5	82.9 ± 21.9	116.4 ± 5.4	156.3 ± 41.9	97.8 ± 48.8	125.8 ± 18
66	Stigmasterol*		RI, MS	3170	3224	47.9 ± 4.4	98.5 ± 24.1	112.3 ± 31.3	161 ± 42.6	115 ± 13.5	143.4 ± 1.3
67	β-Sitosterol*		RI, MS	3203	3283	238.8 ± 31.9	378.5 ± 0.5	436.3 ± 22.3	585.1 ± 180.5	375.2 ± 79.9	526.8 ± 0.4
Carboxylic acid											
39	Palmitic acid	Waxy	RI, MS	1959	1966	288.7 ± 95.6	326.7 ± 98.8	417.3 ± 65.3	511.7 ± 157.6	321.6 ± 20.8	268.2 ± 15.6
Lignan											
62	Episesamin		RI, MS	3151	3121	505.3 ± 95.6	786.6 ± 8.2	785.3 ± 108.1	958.8 ± 222.8	930.7 ± 97.4	701.7 ± 8.7
Unknown											
29	unknown				1541	38.7 ± 1.9	40.2 ± 26	33.8 ± 24.4	27.5 ± 16	42.6 ± 22.6	14.8 ± 0.9
30	unknown				1553	18.2 ± 2.7	28.6 ± 19.2	30.3 ± 14.8	25 ± 12.1	26.9 ± 8.4	18.8 ± 1.6
37	unknown				1912	62.4 ± 13.3	69.5 ± 29	83.7 ± 22.8	101.6 ± 17.1	101.2 ± 37.3	102.6 ± 10.4
38	unknown				1941	64.7 ± 11.7	70.4 ± 41.5	90.3 ± 26.5	109.9 ± 15.8	108.5 ± 40.4	110.4 ± 12.6
41	unknown				2022	27.5 ± 7.1	29 ± 8.5	30.2 ± 9.1	39.2 ± 4.3	38.5 ± 10.1	35.9 ± 3.7
42	unknown				2029	39.8 ± 4.8	47.9 ± 23.7	47.4 ± 24.2	57.6 ± 21.6	67.8 ± 17.3	66.3 ± 10
43	unknown				2053	403.6 ± 10	395.3 ± 230.7	411.9 ± 256.4	625.7 ± 111.6	636.2 ± 148.9	585.2 ± 67
44	unknown				2083	44.3 ± 5	55.4 ± 2.9	53.4 ± 12.6	66.7 ± 14.5	66 ± 14.3	63.6 ± 8.5
45	unknown				2094	29.4 ± 4.4	30.9 ± 2.9	26.7 ± 9.5	38.7 ± 4.2	40.9 ± 14	39.5 ± 8.9
48	unknown				2181	174.1 ± 97.5	320.4 ± 212.8	310.7 ± 189.9	242.5 ± 135.6	250.2 ± 59.8	210.2 ± 5.5
49	unknown				2185	49.4 ± 3.9	37 ± 31.1	40.8 ± 27	65.3 ± 4.3	59.2 ± 28.9	61.1 ± 8.2
50	unknown				2191	418.4 ± 219.4	668.9 ± 347	613.8 ± 278	475 ± 231.6	516.8 ± 91.3	421.3 ± 38.3

51	unknown	2195	68.7 ± 13.3	108.6 ± 20.5	96.7 ± 5.7	91 ± 28	98.5 ± 4.8	81.7 ± 3.4
52	unknown	2254	26.1 ± 3.6	40.2 ± 6.2	40 ± 21.9	29.7 ± 10.8	39.6 ± 3.3	36.5 ± 3.6
53	unknown	2263	52.8 ± 11.1	66.6 ± 26.5	74.3 ± 34.2	93.7 ± 15	93.6 ± 28.9	90.2 ± 11.4
54	unknown	2273	39.7 ± 9.2	51.7 ± 22	55.6 ± 24.1	70.7 ± 4.1	70.2 ± 31.4	71.3 ± 12.2
56	unknown	2292	16.4 ± 7.7	34.9 ± 22.7	28.4 ± 16.9	40.5 ± 7.2	44.1 ± 28.5	35.1 ± 5.7
57	unknown	2309	59 ± 38.6	68.4 ± 37.9	77.8 ± 52.1	60.1 ± 33	55.9 ± 6.3	52.9 ± 8.2
58	unknown*	2348	33.7 ± 2.3	nd	nd	nd	20.5 ± 7.7	nd
59	unknown	2459	122 ± 10.3	155.5 ± 30.2	164.6 ± 60	211.8 ± 39.8	193 ± 65.5	213.6 ± 1.1
60	unknown	2463	32 ± 19.8	62.5 ± 42.9	51.3 ± 26.3	69.1 ± 15.8	76.3 ± 68.8	71.3 ± 11.4
61	unknown	2471	213.5 ± 27	329.5 ± 14	290.4 ± 4.5	322.2 ± 34	361.8 ± 129.6	322.2 ± 34.6
63	unknown	3156	111.1 ± 11.1	90.8 ± 12	94.9 ± 4.4	127.1 ± 36.7	102.9 ± 12.6	120.2 ± 1.3
64	unknown	3184	37.6 ± 0.4	32.1 ± 9.5	34 ± 9.7	47.7 ± 11.8	38.5 ± 11.6	44.9 ± 3
68	unknown	3302	26 ± 13.7	42.9 ± 30.7	80.9 ± 15.5	44.3 ± 14.4	92.6 ± 19.7	38.1 ± 1.1

[a] Odor quality based on literature (Won and Hyung 2005; Moon et al. 2006; Miyazawa et al. 2016; The Good Scents Company 2019)

[b] Identification based on the mass spectra of the detected compound with the built-in library or the literature (MS) and confirmed with the linear retention indices in the literature (RI) or by commercial standard (S)

[c] LRI based on the literature (Babushok et al.; Adam 2017; NIST 2018)

[d] Linear Retention Indices were calculated based on HP-5MS capillary column using C_7-C_{40} alkane standard

* Significantly different at $\alpha = 0.05$

(mean ± SD from 2 biological replicates in µg/g d.w.)

The relative contents of volatiles could be seen in Table 8 and were reported as the mean of ± SD of two biological replicates (n=2). Each biological replicate value was obtained from the average of two technical replicates. As this study utilized limited biological replications, the results suffered from a sizeable standard deviation. Out of the 67 peaks that were detected from all samples, only 42 peaks were managed to be tentatively identified. Twenty-nine compounds of which were from the terpenoids family. Previous research using the GC-O method determined that the potent odorants of andaliman were β-myrcene, limonene, (Z)-β-ocimene, linalool, citronellal, β-citronellol, neral, geraniol, geranial, geranyl acetate, with limonene and citronellal were determined to be the key odorants (Wijaya et al. 2001). All those compounds were also found in this study despite in different proportions. This study found that limonene was the major volatiles followed by geranyl acetate; however, Wijaya et al. (2001) reported that geranyl acetate was the major volatiles followed by limonene. This difference could be caused by different raw materials or longer maceration since it was reported that a longer maceration increased the limonene extracted from andaliman (Meutia et al. 2015). Although no significant difference was found in the total content of volatiles; OD resulted in the highest volatiles content probably due to the formation of a partially dried layer on andaliman pericarp during the initial part of the drying, acting as a barrier for volatiles from diffusing out of the matrix (Buchaillot et al. 2009).

Limonene has been reported as one of the major volatiles from the fruits of Japanese *Z. piperitum* (Jiang and Kubota 2004), Chinese *Z. piperitum* (Chang and Kim 2008), and Chinese *Z. bungeanum* (Yang 2008). Geranyl acetate has also been reported as one of the major aroma compounds of *Z. piperitum* (Jiang and Kubota 2004; Chang and Kim 2008), indicating that andaliman might have a similar aromatic profile with Japanese pepper/sansho. Linalool was reported as the major aroma of Chinese *Z. schinifolium* (Yang 2008); however, linalool was found to be a minor aroma compound in andaliman during this study. Sabinene was reported as the major aroma compound of *Z. myriacanthum* from Thailand (Sriwichai et al. 2019); however, it was not found as the major aroma compound in andaliman.

As shown in Table 8, SD and SSD had the lowest volatiles content, while FA, SHD, OD, and FD had similar total content of identified volatiles. The contents of certain compounds such as geranyl acetate, citronellal, and neral tended to increase upon drying. In contrast, the contents of several compounds such as limonene, (E)-2-hexenal, geranial, α-pinene, sabinene, and β-myrcene tended to decrease upon drying, resulting in a relatively similar total content of volatiles but different in its proportions. Fresh andaliman was the only one that had (E)-2-hexenal, which contributed to a fresh, green, leafy odor (Burdock 2010). All drying processes removed (E)-2-hexenal, agreeing with previous research showing its disappearance upon drying bell peppers (Luning et al. 1995).

Fresh andaliman had the highest limonene content, whereas all dried andaliman had lower limonene contents, explaining the decrease in the citrus note intensity detected during the sensory analysis. This reduction corresponded to previous research that reported a reduction of Japanese pepper (*Z. piperitum*) limonene content upon drying (Jiang and Kubota 2004).

The content of linalool, geraniol, and (Z)-β-ocimene tended to stay the same upon drying. On the other hand, β-myrcene content decreased significantly upon drying with freeze drying had the lowest content of β-myrcene. Linalool and β-myrcene content remained the same when kaffir lime leaves were dried using an oven at 50 °C but were significantly decreased when the leaves were dried at 60 and 70 °C (Jirapakkul et al. 2013).

The content of geranial tended to decrease while the content of neral tended to increase upon drying. The ratio of geranial:neral in fresh andaliman was around 9:1, while in dried andaliman, the ratio changed to approximately 7:3. Geranial is the trans isomer of citral, which isomerized to its cis-isomer (neral) when catalyzed by glycine, an amino acid, to reach an equilibrium ratio of geranial:neral of 6:4 (Wolken et al. 2000). The same isomerization was also observed when pure neral was incubated with glycine. This isomerization, catalyzed by the native amino acid content, was predicted to happen during the drying process.

Fresh and oven-dried andaliman had the highest content of β-citronellol, while the sun, sunshade, and shade dried andaliman had the lowest content of β-citronellol. Geranyl acetate and citronellal contents in dried andaliman tended to be higher than in fresh andaliman. This increase of specific volatiles has been previously reported in other dried products (de Torres et al. 2010; Jirapakkul et al. 2013). This increase was of interest as drying tended to decrease volatiles content.

Two hypotheses had been proposed to explain this increase of volatiles upon drying. The release of bound volatiles could increase certain andaliman volatiles. It was reported that some volatiles in Japanese pepper (*Z. piperitum*) leaves were bound to sugar molecules forming glycosides (Kojima et al. 1997; Jiang et al. 2001). Volatiles exist in their free forms or bound to other molecules such as sugar to form glycosides (Liu et al. 2017). Plants typically stored the volatiles in its glycoside forms because it is much more water-soluble and less reactive than in the aglycone forms (Zheng et al. 2016). When volatiles are bound to a sugar molecule, it loses the odor activities. Its aroma activity would only be present when the glycosides are hydrolyzed, either by enzymes of the acidic environment (Hjelmeland and Ebeler 2015). Enzymatic hydrolysis using the native enzyme within the leaves released volatiles of the glycosides fraction of Japanese pepper leaves (Kojima et al. 1997; Jiang et al. 2001; Jiang and Kubota 2001). This phenomenon was investigated because slapped or crushed Japanese pepper leaves were much for aromatic than the whole Japanese pepper leaves (Jiang and Kubota 2001). Therefore, it was postulated that cell destruction during drying released the native enzymes hydrolyzing the glycosidically bound volatiles. Simultaneously, the reduction of water could increase the acidity of the materials, which hydrolyzed the glycosidically bound volatiles and liberated the aglycones (de Torres et al. 2010).

Alternatively, better extraction was hypothesized to occur when dried products were used because of the destruction of cells and organelles that contain the compounds, thus increasing the amount of volatiles extracted (de Torres et al. 2010). A study on lipid extraction from biomass with different moisture content could also explain this phenomenon. It was found that the presence of moisture lowered the lipid extraction efficiency by a nonpolar solvent such as hexane. However, no difference was observed when using polar solvent such as methanol (Malekzadeh et al. 2016). Reduced contact between the hydrophobic solvent and the materials distributed in the water phase decreased the biomass's lipid extraction. When methanol and hexane were used in combination with a ratio of 1:1, the extraction efficiency was significantly increased to the similar level of dried biomass (Malekzadeh et al. 2016). Therefore, future research using a combination of chloroform and methanol should be conducted to compare the extraction efficiency of fresh and dried andaliman.

Table 9. Change in Odor Activity Values of andaliman aroma-active compounds

Compounds	Flavor Dilution Factor[a]	Threshold in Water (ppm)[b]	Odor Activity Value (OAV)					
			FA	SD	SSD	SHD	OD	FD
Citronellal	128	0.006	263,867	269,250	292,833	297,150	354,517	306,900
Limonene	32	0.2	140,465	96,493	97,553	102,722	109,288	97,617
Linalool	8	0.006	29,217	26,250	27,433	26,233	31,100	27,250
β-myrcene	8	0.014	36,250	24,036	22,957	23,579	27,914	19,586
Geranial	8	0.032	23,603	14,153	14,966	17,559	21,191	15,891
Neral	8	0.03	2,150	6,517	7,940	9,783	8,220	8,157
β-citronellol	8	0.04	1,983	1,123	1,298	1,345	2,090	1,673
Geranyl acetate	4	0.46	28,585	33,265	33,361	38,113	37,213	36,595
Geraniol	4	0.04	16,300	16,998	17,778	17,125	16,053	17,383
(z)-β-ocimene	4	0.034	2,050	1,585	1,609	1,806	2,047	1,991

[a](Wijaya et al. 2001) [b](van Gemert 2011)

The Odor Activity Values (OAV) of each andaliman aroma-active compound was shown in Table 9. These values were calculated by comparing the concentration of each aroma-active compound concentration in each andaliman samples and their threshold concentration in water (Qian and Wang 2005). Citronellal was evident as the andaliman key aroma compound indicated by the extremely high OAV, which agreed with its high flavor dilution factor (Wijaya et al. 2001). Even though citronellal was not a major volatile compound in andaliman, it had an extremely low threshold, which could explain its high intensity reported from previous study. Despite their high concentration, limonene and geranyl acetate had lower OAV compared to citronellal because of their high odor threshold values.

The changes in citronellal OAV between each drying process were not that substantial. However, we could see some decreasing trend in the OAV of limonene from fresh to dried andaliman, explaining the lower citrus intensity of dried andaliman. There are also some substantial changes in the OAV of neral upon drying; however, neral has a milder citrus-like aroma compared to its trans-isomer, geranial; therefore, the increase in neral content is predicted not to alter andaliman aroma substantially. On the other hand, geranial did not show a tremendous change in OAV between fresh and variously dried andaliman. It is predicted that as geranial was liberated from the glycosides, its isomerization into neral could also be happening simultaneously, increasing the concentration of neral in andaliman.

PCA was conducted with the means of identified compounds relative quantities from all andaliman samples. As shown in Figure 32, 72.35% variation of the data could be explained by the PCA (PC1: 47.60% and PC2: 24.74%). The FA was separated in the second quadrant from the other dried andaliman. At the same time, OD was also separated in the first quadrant from other dried andaliman. SD and SSD were grouped around the middle of the score plot while the SHD and FD were grouped at the second quadrant.

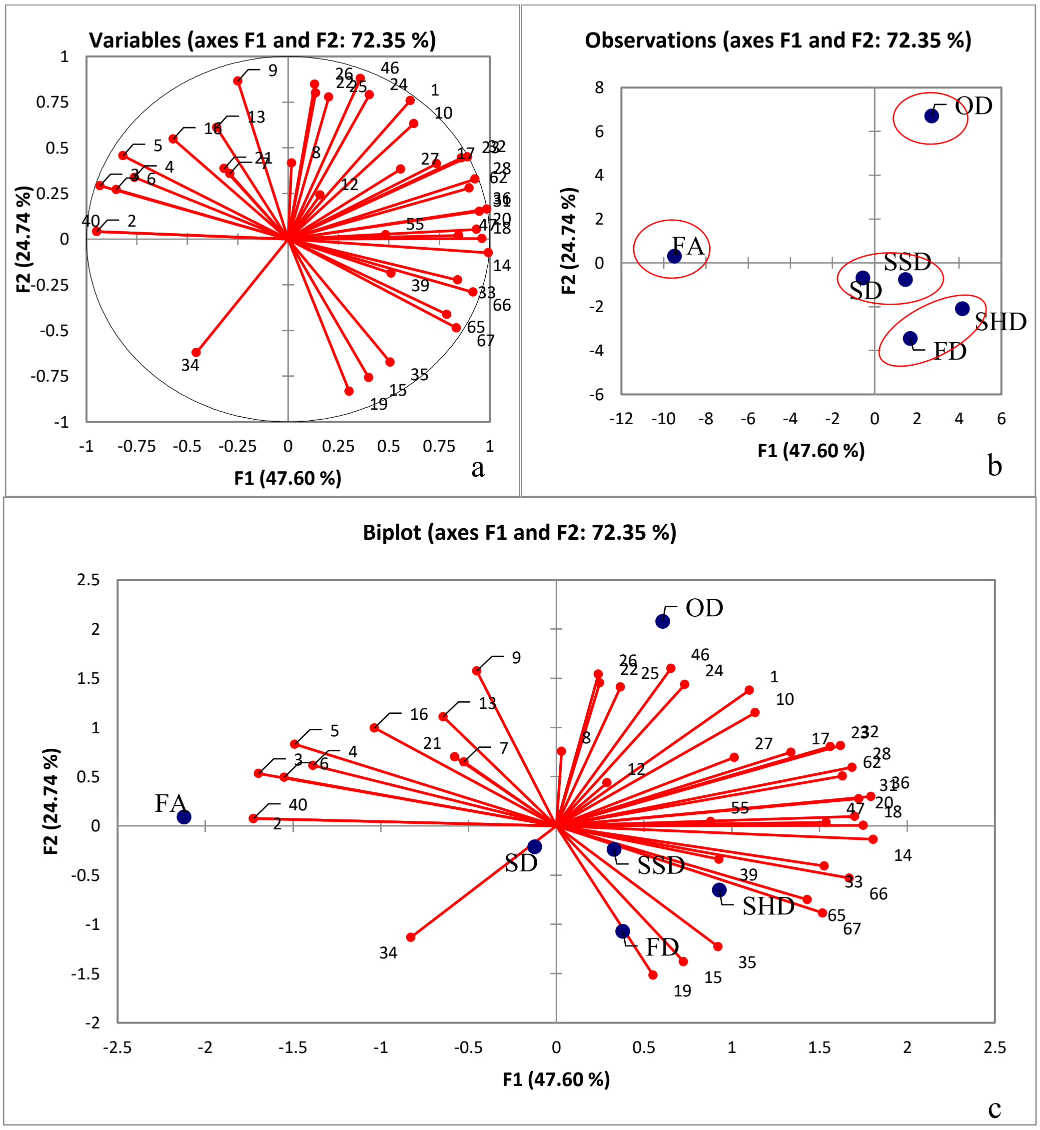

Figure 32. PCA of the average relative quantities of identified compounds Loading plot (a), score plot (b) and biplot (c)

It could be inferred that drying methods utilizing sun radiation would result in a similar volatiles profile while drying methods without heat would also result in a similar volatiles profile. Oven drying resulted in a vastly different product than other drying methods since it managed to dry the andaliman in the shortest time. Therefore, since no drying method was clustered closely with fresh andaliman, it could be concluded that no drying method managed to result in similar volatiles profiles to fresh andaliman.

By looking at the biplot (Figure 32c), it could be deduced that a lot of the volatiles were positively associated with the oven-dried andaliman. Based on the PCA biplot (Figure 32c), compound 2,3,4,5,6, and 40 ((E)-2-hexenal, α-pinene, sabinene, β-myrcene, limonene, and ethyl palmitate, respectively) were more associated with fresh andaliman. At the same time, those six compounds were also positively correlated with each other. On the other hand, limonene (compound 6) was negatively correlated with neral (compound 14). Compounds 1, 10, 22, 24, 25, 26, 46 (1,1-diethoxyethane, citronellal, α-caryopyllene, Germacrene D, β-selinene, α-selinene, and (E)-phytol, respectively) were more associated with oven-dried andaliman. Geraniol, (E)-methyl cinnamate, and neophytadiene, compound 15, 19, and 35, respectively, were associated with freeze-dried andaliman.

4.6 Sanshool Contents of Andaliman

The identity of the hydroxy α-sanshool peak was confirmed by comparing it with a commercial standard. In contrast, α-sanshool peak identity was predicted as the largest peak at around 84 minutes with a molecular weight of 247 and a mass spectral pattern like that previously reported in the literature (Reyes-Trejo et al. 2019). As shown in Table 8, many compounds, especially those with higher boiling points, could not be successfully identified. Some were predicted to be the isomers of sanshool compounds but impossible to identify without pure known standards or other analytical methods such as Nuclear Magnetic Resonance analysis.

As shown in Figure 33, α-sanshool, followed by hydroxy α-sanshool, were the two main sanshools in andaliman. The content of α-sanshool extracted in this study ranged from 0.68 - 1.07 %. This α-sanshool content was lower than the content of hydroxy α-sanshool reported in the pericarps of the green fruits of Japanese Pepper (*Z. piperitum*) that ranged from 1.4 – 3.1 % (Sugai, Morimitsu, and Kubota 2005) as well as to the content of hydroxy α-sanshool content of Sichuan Pepper pericarps that ranged from 3-6% (Yang 2008).

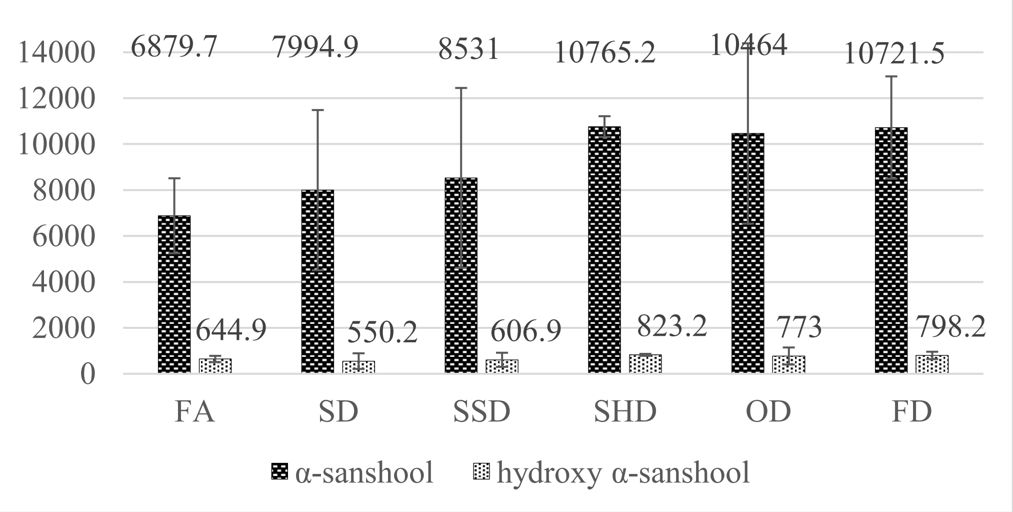

Figure 33. Relative quantities of andaliman numbing compounds
fresh andaliman - FA, sun drying – SD, sunshade drying - SSD,
shade drying - SHD, oven drying – OD, freeze drying – FD
(mean ± SD from 2 biological replicates in µg/g d.w.)
(No significant difference at α = 0.05)

However, andaliman α-sanshool content was much higher than what was reported in Japanese Pepper with a range of 0.43 – 0.67 % (Sugai, Morimitsu, and Kubota 2005). It is crucial to note that the previous analyses were conducted using only the pericarps, which contained most of the numbing compounds and volatiles in *Zanthoxylum.* In contrast, this study used the whole andaliman containing both seeds and pericarps. The seeds of *Zanthoxylum* comprised approximately 43-49% of the total fruit weight while containing barely any sanshool (Sugai, Morimitsu, and Kubota 2005). The previous studies also analyzed the sanshool content with HPLC instead of GC-MS, which might have caused some difference in the concentration of sanshool compound.

The threshold of α-sanshool was reported to be roughly a third of the threshold of hydroxy α-sanshool. Additionally, the burning duration of α-sanshool was also reported to be roughly twice the duration of hydroxy α-sanshool (Sugai, Morimitsu, Iwasaki, et al. 2005). Therefore, although the content of α-sanshool in andaliman is much lower than the concentration of hydroxy α-sanshool in Japanese and Sichuan Peppers, andaliman still could possess a similar numbing intensity because of α-sanshool low threshold and high trigeminal duration.

Like the change in quantities of specific volatiles, drying tended to increase the amount of α-sanshool and hydroxy α-sanshool extracted during the analysis. However, no significant difference was observed, probably due to the variation of sanshool content between each batch of fresh andaliman and the fact that this study only utilized two biological replications. Further research should be conducted to confirm the result of this study.

It was reported that variation in the date of harvest of Japanese Pepper resulted in variation of sanshool concentration. Sanshool compounds also are readily oxidized because of their conjugated triene system and are degraded upon prolonged exposure to heat and UV light (Yang 2008). When the sanshool contents were compared amongst all drying methods, sun and sunshade drying generally resulted in lower sanshool contents than shade and freeze drying (low temperature) as well as to oven drying (fast process). SHD, OD, and FD also protected the andaliman from UV exposure, explaining the higher content of sanshool compounds. The usage of sunshade did not increase sanshool content tremendously.

A previous study reported that SHD resulted in a lower trigeminal sensation than other drying methods such as SD, OD, and FD. On the other hand, freeze-dried, followed by oven-dried andaliman, had the highest trigeminal sensation (Napitupulu et al. 2020). The high content of sanshool compounds in the oven and freeze-dried andaliman found in this study could explain the high trigeminal sensation of OD and FD. However, this study found that shade dried andaliman had a high sanshool compound content similar to the oven and freeze-dried. This similarity showed that the usage of air conditioner managed to preserve sanshool compounds, probably due to the increased water evaporation rate.

4.7 Sensory Profile of Dried Andaliman

A Focus Group Discussion (FGD) was initially conducted with seven panelists who have done multiple sensory analysis and were articulate in describing food products aroma. A Check-All-That-Apply method was utilized during the analysis by providing the FGD panelists with 22 possible aroma attributes (Appendix 1) that could describe andaliman. All attributes that were used by the panelists were individually discussed to reach a consensus between the panelists that resulted in 15 tentative aroma attributes. The aroma attributes were then tested by having the panelists evaluate the andaliman sample using the Rate-All-That-Apply method.

Figure 34 shows that the 15 aroma attributes managed to separate the andaliman samples based on each aroma attributes' intensities as evaluated by the FGD panelist. Therefore, the attributes were decided to be used during the RATA session. An additional attribute of “overall aroma intensity” was added during the final RATA session to measure the aroma intensity of andaliman samples.

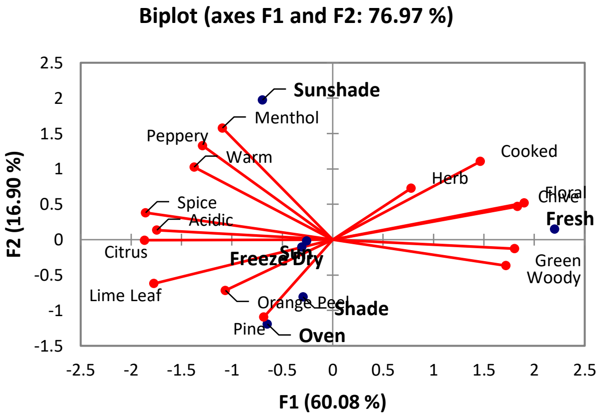

Figure 34. PCA biplot of mock RATA session by the 7 FGD panelists

Based on the 15 selected aroma attributes, sensory profiling was conducted by utilizing 40 untrained panelists from IPB University (7 male and 33 female) to evaluate the intensities of each aroma attributes and the liking score of fresh and dried andaliman. The panelists were of good health, did not smoke before the sensory analysis, were not pregnant, and were within the age range of 21-49. 92.5% of the panelists have heard of andaliman previously; however, only 35% of the panelists have consumed andaliman beforehand. One panelist admitted to smoking before the analysis; therefore, he could not participate in the session. The overall aroma of each andaliman could be described by the spider chart, as seen in Figure 35. Citrus, orange peel, and lime leaf aroma could best describe the overall aroma of andaliman, which would indicate that the citrus-like aroma dominated andaliman aroma. This strong citrus aroma was similar to the green Japanese peppers' aroma, which was dominated by citrus aroma (Jiang and Kubota 2004).

FA had the highest citrus aroma intensity. In contrast, SD had the lowest citrus intensity, which could be explained by the highest limonene content in FA and the degradation of limonene due to the UV radiation from sunlight (Mull et al. 2017). OD and FD were similar in citrus aroma, although they were lower in intensity than FA. This finding was similar to previous research that drying tended to lower Japanese pepper's citrus aroma (Jiang and Kubota 2004). Intriguingly, orange peel and lime leaf aroma were lower in fresh andaliman than oven-dried andaliman, which had the highest intensity in those two attributes.

Fresh andaliman had the lowest warm aroma than all dried andaliman; in contrast, fresh andaliman had the highest green aroma compared to all dried andaliman. The high intensity of green aroma could be attributed to the content of (E)-2-hexenal in fresh andaliman. Dried andaliman also had a lower floral aroma than fresh andaliman even though the difference was not significant. The drying process seemed to increase the spice, peppery, cooked, menthol, and chive aroma of andaliman. The sunshade usage managed to increase the citrus and orange peel aroma compared to sun-drying without a sunshade. Overall, it could be seen that oven-dried andaliman had the highest intensity in most attributes compared to other drying techniques.

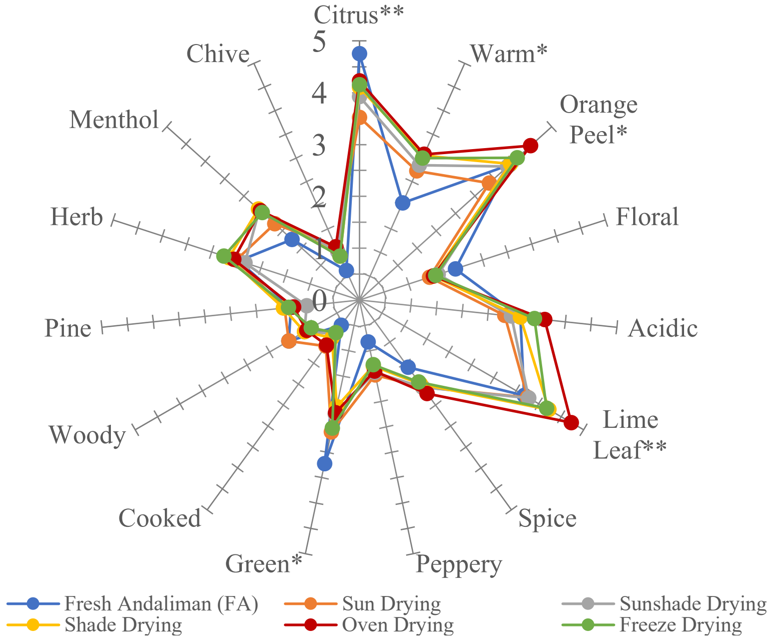

Figure 35. Aroma profile of fresh and dried andaliman.
0 (absent), 1 (very weak) to 6 (very strong). * : p-value < 0.1, ** : p-value < 0.05.

When the overall aroma intensity was evaluated, oven-dried andaliman had the highest aroma intensity, followed by freeze-dried, shade-dried, fresh, sun-dried, and sunshade-dried andaliman. However, there was no significant difference (p-value of 0.613) in the overall aroma intensity even though some noticeable differences in each aroma attribute were observed. This lack of significance could be explained by the variation in the intensities of the individual aroma attributes amongst all samples and the similar total volatiles content of all andaliman samples from the previous analysis.

Table 10. Overall aroma intensity and overall liking of all andaliman samples

Sample	Overall Aroma Intensity	Overall Liking
FA	4.05 ± 1.50	6.40 ± 2.16^{bc}
SD	3.95 ± 1.26	4.93 ± 1.90^{a}
SSD	3.83 ± 1.24	5.58 ± 1.87^{ab}
SHD	4.10 ± 1.17	5.90 ± 1.58^{bc}
OD	4.33 ± 1.33	6.50 ± 1.41^{c}
FD	4.18 ± 1.30	6.38 ± 1.78^{bc}

a, b, c, d – mean sharing similar letters were not significantly different at a 95% confidence interval based on the Duncan posthoc test.

Oven-dried andaliman resulted in the highest acceptance score compared to all andaliman samples with a score of 6.50, which could be described that the panelist tended to like slightly to like moderately. This liking score was not significantly different compared to fresh, freeze, and shade dried andaliman with scores of 6.40, 6.38, and 5.90 respectively.

To better visualize the variations of andaliman aroma intensities, an unsupervised PCA was conducted using the average scores of each aroma intensity from each sample. As could be seen in Figure 36, the two principal components explained 79.11% of the total variance (PC1: 51.35%; PC2: 27.76%). Fresh andaliman was separated from the other samples indicating that no drying techniques yielded a similar sensory profile of fresh andaliman, which agreed with the PCA plot of volatiles content. Fresh andaliman was correlated with a citrus, floral, and green aroma, which agreed with the previous spider chart.

OD was also separated from others, indicating its unique aroma profile. It was also correlated with lime leaf, acidic, orange peel, and overall aroma. FD had a similar aroma profile to SHD, both of which were low temperature drying. FD was correlated with acidic, orange peel, and overall andaliman aroma. SD and SSD were located close to each other, indicating that both had a similar aroma profile; however, no aroma attributes were associated with both samples.

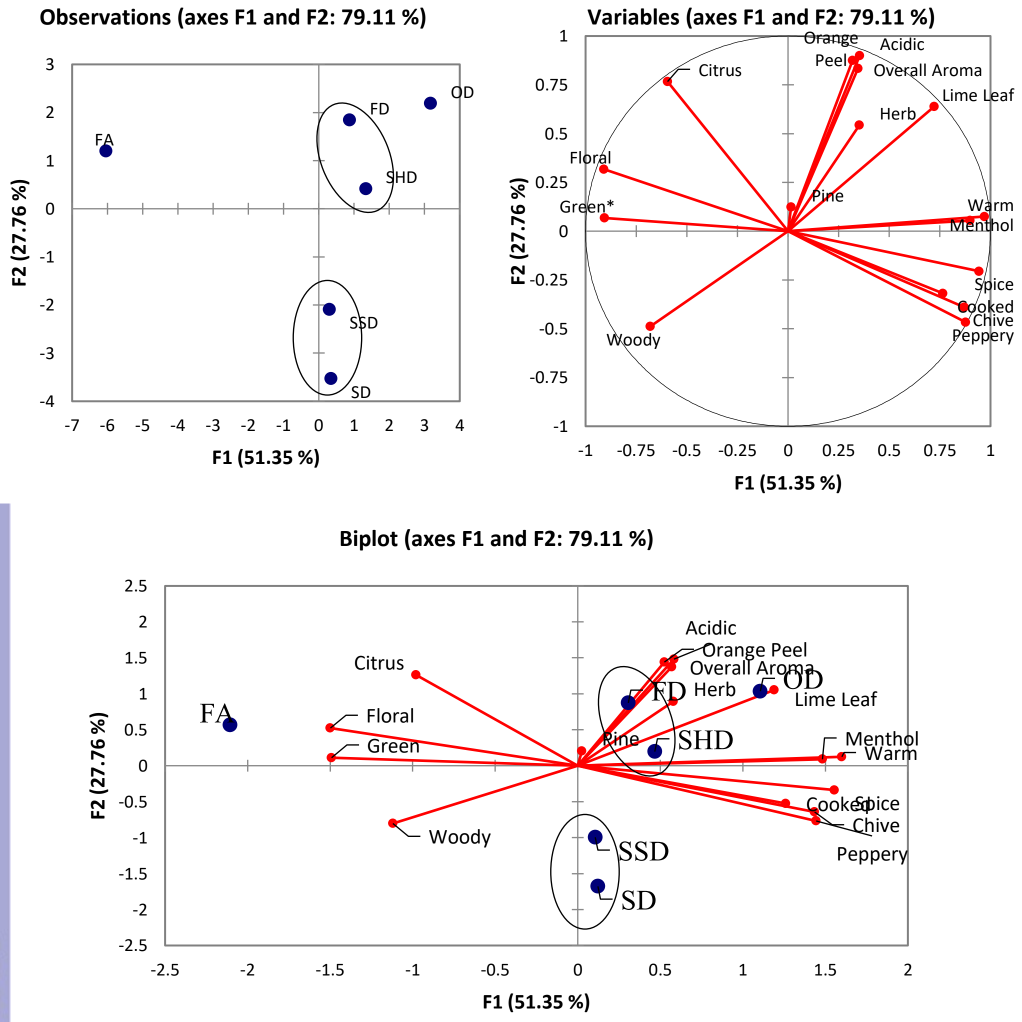

Figure 36. PCA biplot of the average of andaliman aroma intensities

PCA could also explain the relationship between each aroma attributes. Floral and green are positively correlated with each other while negatively correlated with aroma attributes such as warm, spice, peppery, cooked, menthol, and chive. The overall aroma intensity correlated with orange peel, lime leaf, and acidic aroma, which indicated that these aroma attributes would contribute enormously to the overall aroma impression of andaliman. Menthol and warm aroma were strongly correlated while negatively correlated with floral and green.

Overall, the PCA managed to yield clear separation and grouping of andaliman samples. This result agreed with the PCA of volatiles that grouped sun and sunshade drying together and grouping freeze-dried and shade dried together while separated the oven-dried and fresh andaliman from the other treatments. However, this separation contradicted with the result of a similar study that found all essential oil samples of fresh and dried makwhaen (*Zanthoxylum myriacanthum)* could not be differentiated by descriptive analysis (Sriwichai et al. 2019). This contradiction could be caused by the different types of samples used in the study, which used essential oils extracted from the fresh and dried makwhaen. On the other hand, this study used the unaltered samples of andaliman. The extraction process could result in less difference amongst the samples due to high heat in the extraction. Based on previous research, the vacuum distillation of andaliman yielded a different aroma compare to fresh andaliman (Wijaya et al. 2001).

Pearson correlation analysis was conducted between the aroma intensities and the overall liking score to determine the favorable attributes that could drive the liking score. It was found that three attributes, namely, citrus, orange peel, and acidic, were the favorable attributes indicated by the significant correlation between their intensities and liking score, as shown in Table 11. These three attributes were determined as the most crucial factor of andaliman aroma qualities based on their correlation to the overall liking score. Additionally, oven-dried andaliman had the highest intensities of those three attributes. Therefore, a further investigation was conducted to identify the volatiles that significantly contributed to each attribute.

Table 11. Pearson correlation matrix between aroma intensities and liking score

Variables	Liking	Variables	Liking
Orange Peel	**0.8784**	Warm	-0.0471
Acidic	**0.8579**	Pine	-0.0590
Citrus	**0.8459**	Spice	-0.2821
Lime Leaf	0.5337	Woody	-0.4433
Floral	0.4631	Cooked	-0.4440
Herb	0.3709	Chive	-0.4672
Green	0.1571	Peppery	-0.5452
Menthol	-0.0235		

Values in bold are different from 0 with a significance level alpha=0.05

4.8 Relationship Between the Volatiles and Sensory Characteristics

Partial Least Square Regression (PLSR) analysis is one of the most used tools to analyze the relationship between chemometrics and sensory data. An initial PLSR analysis was conducted to the means of each identified compounds and aroma attribute scores. Volatiles that have been previously reported as aroma-active compounds in andaliman was automatically added into the final PLSR analysis. Additionally, volatiles with VIP scores of less than 1 and compounds known to be odorless was excluded from the model to reduce the noise, improve prediction quality and reduce complexity (Seisonen et al. 2016). After that, another PLSR was conducted with the reduced volatiles to determine the sensory attributes that could be predicted by the model. Sensory attributes with cumulative Q^2 of less than <0.4 were excluded from the model. In the end, 23 volatiles and five sensory attributes were included in the PLSR analysis, as shown in Table 13.

The analysis was conducted based on a maximum of two components resulting in a biplot shown in Figure 37. The overall model quality could be explained by the Q^2 value of 0.7627, R^2Y value of 0.9153, and R^2X value of 0.8744, as shown in Figure 36. These values indicated that the model could predict 76.27 % variances of the sensorial data based on the volatile data, as well as explain 91.53 % variances of sensory data and 87.44 % of volatiles data variance.

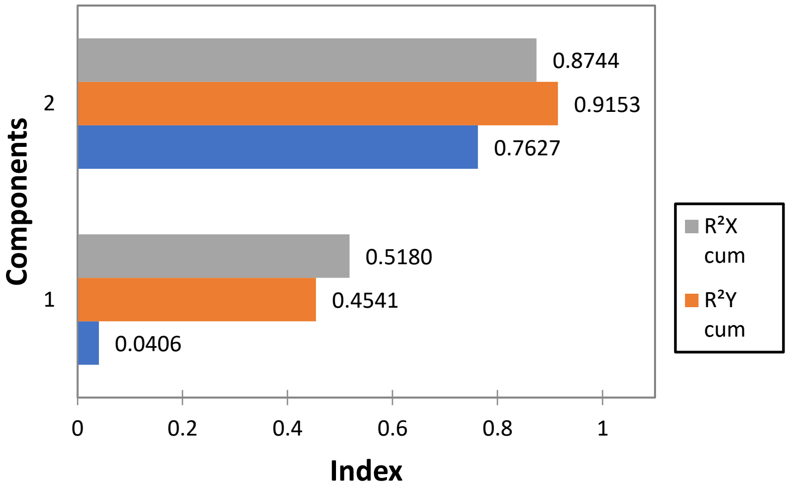

Figure 37. PLSR model quality based on 1 and 2 components

The samples' separation was similar to the previous two PCAs (volatiles and sensory), which showed clustering of shade dried and freeze-dried andaliman. Similarly, sun and sunshade-dried andaliman were also clustered. This result confirmed that sunshade usage did not substantially alter the sensorial and volatile properties of andaliman compared to regular sun drying. SHD and FD, which processed the berries without heat, resulted in comparable properties while OD and FA were separated from the other drying process. This separation indicated that no drying process resulted in a similar sensorial and volatile profile to fresh andaliman.

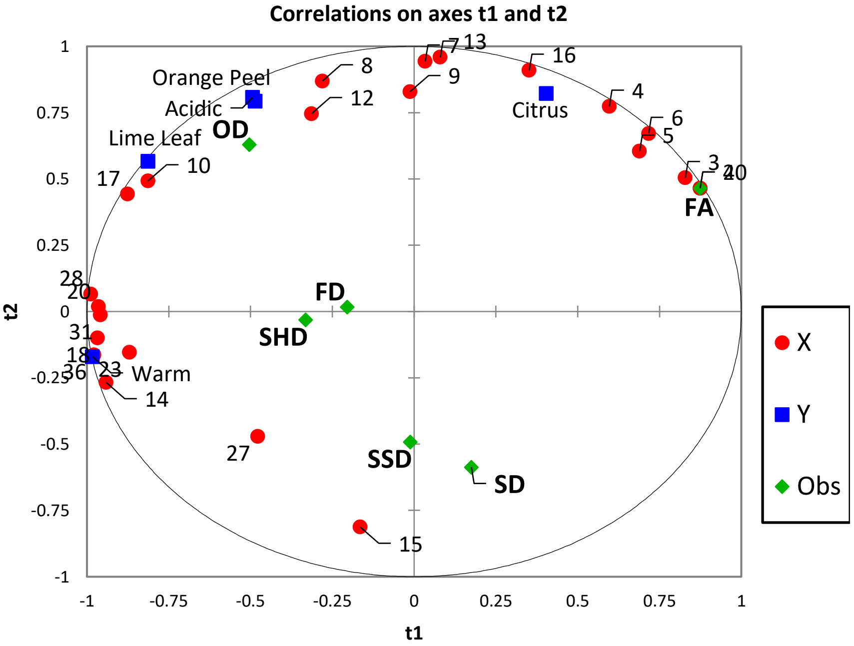

Figure 38. Biplot PLSR analysis on volatiles (X) and sensory intensities (Y)

A Pearson correlation analysis was conducted to further confirm the correlation between sensory attributes to the volatiles, as shown in Table 13. The summary of positively correlated attributes and volatiles could be seen in Table 12. As indicated from previous analysis, citrus, orange peel, and acidic were the most critical attributes that drove the overall liking score of andaliman aroma.

Table 12. Summary of correlated volatiles and attributes

Attribute	Positively Correlated Volatiles
Citrus	Sabinene, Limonene, (Z)-β-Ocimene, β-Citronellol, Geranial
Orange Peel	(E)-β-Ocimene, Citronellal
Acidic	(Z)-β-Ocimene, (E)-β-Ocimene, Citronellal
Lime Leaf	Citronellal, Geranyl formate, (E,E)-α-Farnesene
Warm	Neral, Citronellyl acetate, Geranyl acetate, (E)-β-Farnesene, (E,E)-α-Farnesene, (E)-Nerolidol, (2E,6E)-Farnesyl acetate

The citrus aroma was significantly correlated with sabinene, limonene, (Z)-β-ocimene, β-citronellol, and geranial (p-value <0.05). All of these compounds, except sabinene, have been previously reported as the aroma-active compounds of andaliman (Wijaya et al. 2001). Citrus was the primary aroma of limonene and geranial, which explained their correlation with the citrus attribute in the PSLR analysis. Limonene was also reported as the major aroma constituent (>90 %) of orange peel oil (Burdock 2010). Sabinene was not detected in the previous aroma study of andaliman (Wijaya et al. 2001), but during this study, it was correlated with the citrus aroma of andaliman. Woody is the dominant aroma of sabinene; however, it could also be described as spicy, citrus, and terpenic (TGSC 2019). The aroma of Z-β-ocimene has been described as floral and sweet (TGSC 2019). However, during the GC-O analysis of andaliman extract, this compound was described as citrus and sweet (Wijaya et al. 2001). Overall, all correlated compounds had a citrus aroma, explaining its association with the citrus aroma.

Orange peel aroma was strongly correlated with (E)-β-ocimene (sweet herbal) and citronellal (floral, sweet, and citrus); at the same time, the acidic aroma was significantly correlated with (Z)-β-ocimene, (E)-β-ocimene, and citronellal (TGSC 2019). Citronellal was reported to be key aroma compounds of andaliman that elicited strong citrus aroma by GC-O analysis (Wijaya et al. 2001) and intense lemon type aroma (Burdock 2010). This combination might be perceived as orange peel and acidic aroma by the panelists.

Table 13. Pearson correlation matrix of volatiles and sensory attributes

No.	Volatiles	Citrus	Orange Peel	Acidic	Lime Leaf	Warm
2	(E)-2-Hexenal	0.780	-0.047	-0.078	-0.462	**-0.939**
3	α-Pinene	0.699	-0.030	-0.055	-0.374	**-0.917**
4	Sabinene	**0.888**	0.287	0.263	-0.051	-0.726
5	β-Myrcene*	0.712	0.093	0.041	-0.201	-0.808
6	Limonene*	**0.864**	0.175	0.133	-0.211	**-0.828**
7	(Z)-β-Ocimene*	**0.832**	0.765	**0.832**	0.497	-0.171
8	(E)-β-Ocimene	0.595	**0.845**	**0.956**	0.727	0.153
9	Linalool*	0.591	0.742	0.703	0.501	-0.150
10	Citronellal*	0.043	**0.847**	**0.838**	**0.948**	0.708
12	α-Terpineol	0.614	0.686	0.643	0.638	0.184
13	β-Citronellol*	**0.815**	0.792	0.804	0.478	-0.238
14	Neral*	-0.535	0.248	0.222	0.593	**0.973**
15	Geraniol*	-0.524	-0.408	-0.496	-0.395	0.323
16	Geranial*	**0.892**	0.535	0.495	0.226	-0.514
17	Geranyl formate	0.018	0.731	0.738	**0.961**	0.782
18	Citronellyl acetate	-0.391	0.451	0.437	0.704	**0.980**
20	Geranyl acetate*	-0.352	0.436	0.480	0.791	**0.956**
23	(E)-β-Farnesene	-0.576	0.280	0.230	0.646	**0.850**
27	(Z,E)-α-Farnesene	-0.749	-0.184	-0.260	0.169	0.501
28	(E,E)-α-Farnesene	-0.386	0.497	0.508	**0.854**	**0.952**
31	(E)-Nerolidol	-0.348	0.432	0.386	0.756	**0.937**
36	(2E,6E)-Farnesyl acetate	-0.536	0.331	0.312	0.703	**0.981**
40	Ethyl palmitate	0.780	-0.047	-0.078	-0.462	**-0.939**

* Previously identified as aroma-active compounds of andaliman

The warm aroma was associated with numerous volatiles such as neral (citrus), citronellyl acetate (floral), geranyl acetate (floral), (E)-β-farnesene (woody), (E,E)-α-farnesene (woody), nerolidol (floral), (2E,6E)-farnesyl acetate (oily) (TGSC 2019). It was possible when those compounds were combined, they gave a warm type sensation to the panelists.

Lime leaf aroma was also correlated with citronellal (citrus), geranyl formate (floral, green aroma), (E,E)-α-farnesene (woody, green aroma). Citronellal was found to be the major key odorants of kaffir lime leaves that elicit intense citrus and kaffir lime-like aroma by GC-O analysis (Jirapakkul et al. 2013). Therefore, it could be hypothesized that the combination of volatiles with intense citrus, floral, green, and woody aroma could contribute to the lime leaf aroma of andaliman.

Out of the ten potent odorants previously identified, β-myrcene, linalool, and geraniol were not correlated with any sensory attributes in this study. This lack of correlation might have been caused by the similarities in content amongst all andaliman samples for those three volatiles. Limonene, (Z)-β-ocimene, citronellal, β-citronellol, neral, and geranial were correlated with aroma attributes such as citrus, warm, orange peel, and acidic. This research managed to tentatively identify 13 additional compounds that could elicit aroma attributes in andaliman.

In general, this study showed that a high content of sabinene, limonene, β-citronellol, geranial, (Z)-β-ocimene, (E) β-ocimene, and citronellal is necessary to achieve an acceptable andaliman product based on its aroma profile. This finding could simplify the selection of other andaliman varieties, and a new processing method based on a targeted analysis of the seven identified volatiles.

V CONCLUSION AND SUGGESTIONS

5.1 Conclusion

Andaliman drying processes, namely sun (SD), sunshade (SSD), shade (SHD), oven (OD), and freeze drying (FD) could be best described by the logarithmic thin-layer drying model. All methods resulted in a similar yield. SHD resulted in the highest yield, moisture, and water activity, while SD and FD resulted in the lowest values. Drying increased the a* value significantly without any significant difference amongst all dried andaliman samples.

Forty-two compounds consisting of volatiles, α-sanshool, hydroxy α-sanshool, and others were tentatively identified from all andaliman extracts. Fresh and dried andaliman had similar total relative content of volatiles with variations in the relative quantities of each compound. The content of limonene, (E)-2-hexenal, geranial, α-pinene, sabinene, and β-myrcene as well as the intensities of citrus and green aroma tended to decrease. In contrast, the content of geranyl acetate, citronellal, neral, α-sanshool as well as the intensities of warm and lime leaf aroma tended to increase upon drying. PCA on volatiles and aroma intensity resulted in a similar separation of samples. SHD and FD were grouped while SD and SHD dried andaliman were clustered. OD and FD were separated from the other samples, indicating their unique aroma profile.

The overall aroma intensities of all samples were not significantly different. OD had the highest liking score, similar to FA, FD, and SHD. Orange peel, citrus, and acidic aroma were identified as the favorable aroma attributes based on their correlation to the overall liking score with OD resulted the highest intensities of those three favorable attributes compared to other drying methods. The citrus aroma was correlated with sabinene, limonene, (Z)-β-ocimene, β-citronellol, and geranial. Orange peel aroma was correlated with (E)-β-ocimene and citronellal, while acidic aroma was correlated with (Z)-β-ocimene, (E)-β-ocimene, and citronellal. The acceptance of andaliman aroma could be indicated by the high content of citronellal, limonene, β-citronellol, geranial, (Z)-β-ocimene, (E)-β-ocimene, and sabinene.

No drying methods could produce dried andaliman with similar profiles to fresh andaliman. Oven drying was proposed to be the ideal drying method because of its high acceptability, intensities of favorable aroma attributes, volatiles, and sanshools content as well as its short duration and low water activity.

5.2 Suggestion

Further research should be conducted to profile other drying methods, different oven drying temperatures, growing locations, and varieties as well as to analyze the glycosidically bound volatiles in fresh andaliman and compare the mixture of solvents to extract volatiles in fresh andaliman efficiently. Additionally, future studies should identify other sanshool isomers in andaliman, confirm α-sanshool peak with the pure standard, compare the quantities of sanshools obtained by GC-MS and HPLC. Sensory descriptive analysis using trained panelists or andaliman heavy users should be conducted to analyze the aroma and numbing intensities of fresh and dried andaliman.

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BIOGRAPHY

Born in Jakarta on October 12, the author pursued his secondary education in IPEKA Tomang II Junior High School and SMA Kristen 1 Penabur Jakarta. The author continued to pursue his Bachelor's in food science (with distinction) from Purdue University, West Lafayette, USA, and a certificate in Culinary Arts from the Institute of Culinary Education, New York, USA. The author entered IPB University graduate school in the Department of Food Science in 2018 and participated in the Hokkaido University PARE (Population Activities Resources Environment) Summer School Program in 2019, receiving a Japan Student Services Organization (JASSO) Student Exchange Support Program. Additionally, during his study at IPB University, the author has written part of a published review article titled "Traditional fermented foods with anti-aging effect: A concentric review" published in the Food Research International journal (Q1). He also wrote a concise review titled "Plant-derived exosome-like nanoparticles: a concise review on its extraction methods, content, bioactivities, and potential as functional food ingredient" currently under review in the Journal of Food Science (Q2). Part of this thesis was submitted to Food Chemistry journal (Q1) titled "Changes in Volatiles and Aroma Profile of Andaliman (*Zanthoxylum acanthopodium* DC.) Upon Various Drying Techniques" and was currently under review.