THESIS

EFFECTS OF FORM AND STORAGE TEMPERATURE ON ANTIOXIDANT ACTIVITY AND SANSHOOL CONTENT OF DRIED ANDALIMAN (*Zanthoxylum acanthopodium* DC.)

Written as partial fulfillment of the academic requirements to obtain
the degree of *Sarjana Teknologi Pertanian Strata Satu*

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STATEMENT OF THESIS AUTHENTICITY

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Hereby declare that my thesis, entitled **"EFFECTS OF FORM AND STORAGE TEMPERATURE ON ANTIOXIDANT ACTIVITY AND SANSHOOL CONTENT OF DRIED ANDALIMAN (*Zanthoxylum acanthopodium* DC.)"**:

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ABSTRACT

Amanda Inggita (03420110008)

EFFECTS OF FORM AND STORAGE TEMPERATURE ON ANTIOXIDANT ACTIVITY AND SANSHOOL CONTENT OF DRIED ANDALIMAN (*Zanthoxylum acanthopodium* DC.

(xiii + 120 pages: 5 tables, 6 figures, and 22 appendices)

Andaliman is one of Indonesian traditional spices well known in North Sumatra with unique flavor with pleasant citrus-like aroma and a tongue-numbing trigeminal sensation. Fresh andaliman is very susceptible to deterioration. Drying was applied to fresh andaliman in order to determine the effect of form and storage temperatures within six weeks, including the initial point. Dried andaliman were divided into two forms (whole and ground) and stored into three levels of temperatures (25 °C, 35 °C and 45 °C) with vacuum packaging. Dried andaliman samples were taken out every week for carrying out antioxidant activity assay (DPPH radical scavenging assay), moisture content, and attribute rating test to sixteen-trained panelist. Furthermore, high performance liquid chromatography (HPLC) coupled with DAD detector was used to identify and quantify the active compounds (specifically α-sanshool). After storage treatments whole andaliman significantly had higher tingling sensation and lower moisture content, while ground andaliman significantly had higher antioxidant activity and α-sanshool content. Lower storage temperature significantly maintain the qualities of dried andaliman better. The moisture content and antioxidant activity of dried andaliman in vacuum packaging changed significantly on the second week and the tingling sensation had reduced significantly on the third week. According to its moisture content, whole andaliman fulfilled the SNI standard requirements of dried spices (12%) after storage treatments meanwhile ground andaliman stored in room temperature had more than 12% moisture content starting from the fourth week. Dried andaliman still possed its tingling sensation after storage treatments (77.78%).

Keywords : Andaliman, storage, attribute rating test, antioxidant activity, trigeminal sensation
References : 41 (1981-2014)

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The author is aware that this report is far from perfect and it may contain many mistakes. The author would like to deliver apology and welcome any critics and/or suggestions given to this report with great pleasure. Finally, the author hopes that this report would be useful for the readers. God Bless You

Karawaci, July 2015

Writer

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CHAPTER I

INTRODUCTION

1.1 Research Background

Flavor is a primary consideration for food selection. Therefore, people strive to enhance desirable flavor, avoid off-flavors and minimal or delay flavor loss (Mussinan and Morello, 1998). Flavor chemistry does not only covers compounds that transmit taste and aroma, but it is a system that involves combination of gustatory, olfactory and trigeminal sensations (Jelen, 2012). Continuous development on flavor chemistry is being done to enhance food products. Flavor ingredients should not only posses distinct organoleptic properties, but it also needs to remain stable during long period of storage in order to be optimally utilized (Mussinan and Morello, 1998).

Andaliman (*Zanthoxylum acanthopodium* DC.) is one of Indonesian wild herb originated from Northen Sumatra. This plant belongs to Rutaceae family and is commonly used in Batak cuisine, such as *sambal tinombur, naniura*, and *saksang*. Himalayan people have been using andaliman as aromatic ingredients, to sooth stomachace and to increase appetite. Andaliman exhibits a distinct citrus-like and peppery flavor with a unique trigeminal sensation, also known as numbing and biting effect to the tounge (Wijaya *et al.,* 2002). Isolation and identification of andaliman chemical composition has been succeeded by Wijaya *et al.,* (2002), which includes its potent odorant and volatile aroma compounds. Besides its flavor characteristic, other researches have also been able to prove

andaliman to become a functional spice due to its antioxidant and antimicrobial activity. Andaliman has been implemented to preserve food such as raw fish and tofu (Parhusip *et al.*, 2007). Based on its characteristic, andaliman has a great potential to improve flavors in food as well as being used for its functions. In spite of that, limited researches have been done on the specific flavor and trigeminal characteristics of andaliman and its potential as a functional flavoring has yet to be studied further.

Fresh andaliman could not be stored in several days because it is very susceptible to deterioration. Commonly, fresh andaliman undergo conventional sun drying method to prolong its shelf life, but the strength of flavor will be reduced greatly due to its unstable flavor compounds. According to Wijaya *et al*., (2002) the isolated and identified the trigeminal active compound of andaliman which is a substituted amide named as sanshool (2E, 6Z, 8E, 10E-N-(2'-methylpropyl)-dodecatetraenamide). The pungent sanshool compound isolated from *Zanthoxylum* is an unsaturated aliphatic amide which is unstable and has the tendency to be easily polymerized (Galopin *et al.*, 2004). It has been reported that the tingling trigeminal effect of Zanthoxylum fruits is caused by series of polyunsaturated alkylamieds. These compounds are not stable and deteriorates easily under UV light and hydrolytic conditions (Yang, 2008). This will lead to difficulties when being stored over prolonged period of time and could promote instability in food products. Consequently, a suitable storage condition and packaging material that can prolong the shelf life is needed in order to retain its flavor, trigeminal sensation and it functional properties.

There are three factors that controls the shelf life of a food product, which are the product characteristic (intrinsic factors), environment to which the product is exposed during distribution and storage (extrinsic factors) and properties of the food packaging. Intrinsic factors include water activity, enzymes, pH and concentration of reactive compounds. Extrinsic factors include temperature, RH, partial pressures of different gases, light, and mechanical stresses. The combination of these factors greatly affects deterioration rate and reactions during food product storage. The properties of the package can have imporant effect on extrinsic factors thus consequentially reduce the rate of deteriorative reactions (Robertson, 2010).

Packaging is essential and pervasive for food products; essential because food safety and quality will be compromised without packaging, and pervasive because almost all food is packed in some way. Food packaging could functions different tasks, among them are: to protects food from spoilage and contaminats, to equipt identical measurement of contents, and to ease transportation and storage of foods. Various materials of food packaging are available, including metal, glass, plastic and paper. The protection provided by the package is an essential part of preservation process for the majority of food products (Robertson, 2010).

1.2 Research Problem

Andaliman posses a highly favored compound known as sanshool. This compound is found to be an unsaturated and unstable aliphatic amide, thus the strength of its flavor could be reduced during storage period. However, studies regarding flavor stability of dried andaliman are still limited. Food packaging and

storage conditions play an important role in the stability of food products. However, the effect of storage temperature and form of dried andaliman towards its antioxidant activity and sanshool compound has not yet to be studied. Therefore this research focuses on evaluating the effect of storage temperature on the antioxidant capacity and sanshool compound of dried andaliman in whole and ground form.

1.3 Research Objectives

1.3.1 General Objectives

The general objective of this research was to evaluate the stability of dried andaliman in different storage temperature and form conditions.

1.3.2 Specific Objectives

The specific objectives of this research were:

1. To determine the effect of storage temperature towards sanshool compounds and antioxidant capacity of dried andaliman.
2. To determine the effect of form (whole/ground) towards sanshool compounds and antioxidant capacity of dried andaliman.

CHAPTER II

LITERATURE REVIEW

2.1 Flavor

Taste and odor are the most influencing factors for food selection by humans. Taste are perceived in oral cavity when non-volatile substances are dissolved in saliva, can be detected by taste buds receptors on the tongue and soft palate. Five basic tastes are distinguished: sweet, salty, sour, bitter and umami. Salty taste incorporate in iron regulation and water homeostasis in the body, sweet and umami tastes are capable for estimating the energy content of food, whereas sour and bitter taste may act as caution system to avoid consumption of unripe fruits or toxins intake. Aroma is perceived when volatile compounds are detected by olfactory receptor on the nasal epithelium, at the roof of nasal cavity. Flavor perception does not only involves taste and aroma compounds, but it is a complex system of the gustatory, olfactory and trigeminal sensations (Jelen, 2012).

Most flavors are volatile compounds which are delicate and unstable. However, flavor has a high influence in creating consumer satisfaction and inducing further consumption of food, thus there have been interest to preserve them. Flavor isolation commonly used distillation and extraction, other production of synthetic flavor have been avoided because of its reputation as being harmful to health. Flavor is a distinctive quality of a particular food and drink as perceived by taste buds and sense of smell. Food flavor is a fundamental aspect not only for consumers at the moment of choosing a particular product, but also an important

feature for breeders of fruit and vegetable in selection of raw materials used for food production. Flavor is always a subject for food technologist in developing new products, meeting consumer requirements and controlling it during processing and storage. Finally, flavor is one of the main factors that determine shelf life of a food product. Chemical, enzymatic and microbial changes could develop off-flavors, hence the food product is unpalatable. Nevertheless, proper flavor maintenance is in the interest of both consumers and producers (Jelen, 2012).

2.1.1 Trigeminal Sensation

Our chemical senses include gustation, olfaction and somatosensation. Trigeminal somatosensory neurons transmit the detection of thermal, mechanical, and chemical stimuli in the head and neck region. Nasal and oral cavities are areas of particularly dense innervation because olfaction and taste are are the main chemical senses (Gerhold and Bautista, 2009).

Heating, warming, and pungent sensations are caused mostly by substances transmitting the vanilloid receptor TRPV1 or the pain receptor TRPA1 indicated on free nerve endings of the trigeminal nerve system. These effects are not drawn out by real increase in physical temperature but by lowering the temperature threshold of the free nerve endings. Therefore, the nerves are firing at body temperature and not only at moderate or dangerous heat as in the nonactivated state. The exact description of chemestetic effects depends on the compound and its concentration; fox example, low amounts capsicum extract brings out only a mild and pleasant warming effect, while high concentrations is described as “pungent”, “hot”, or even “burning like fire”. Some of the most

important flavor compounds that elicits these effects are capsaicin and nonivamide from *Capsicum* extracts, piperine from pepper, gingerol and related compounds from ginger. The alpha-*hydrozy* sanshool from *Zanthoxylum schinifolium* exhibits a “tingling” effect on the tounge. This is perceived as an irritating sensory experience and accompanied by a numbing feeling. In addition, they can induce salivation (Jelen, 2012).

2.1.1.1 Sanshool

Sichuan peppers and other members of the *Zanthoxylum* genus have been traditionally used as folk medicine to treat toothache and other types of trigeminal pain. Opposite to the intense burning paoin associated with hot peppers of *Capsicum* genus, *Zanthoxylum* members brings out a robust, benign buzzing and tingling paresthesia, suggestive of an interataction with neurons involved in tactile sensation. Psychophysical studies in humans have shown that the alkylamide, hydroxyl-α-sanshool is the active compound in *Zanthoxylum piperitum* (Sanshoo pepper) (Gerhold and Bautista, 2009).

Multifarious molecular mechanisms were proposed to account the sensations drawn out by hydroxyl-α-sanshool, including TRPV1 and TRPA1 activation. However, two findings suggest that hydroxyl-α-sanshool has a distinct molecular target. First, hydroxyl-α-sanshool triggers somatosensory neurons that are insensitive to capsaicin and mustard oil. Second, mice lacking functional TRPV1 and TRPA1 channels exhibits the same distaste towards hydroxyl-α-sanshool-containing water as their wild-type littermates. It is found that three members of the KCNK two-pore potassium channel family serves as hydroxyl-α-sanshool receptors. Unlike the opening or TRP channels by irritants, hydroxyl-α-

sanshool inhibits KCNK3, 9 and 18. This inhibition of hackground potassium conductance produce the somatosensory neuron activation. KCNK18 seems to be the primary target of sanshool action in somatosensory neurons. However, KCNK an analysis of KCNK-deficient animals must be conducted to prove whether KCNK channels embody the sole molecular target by which sanshool exerts its tingling sensation (Gerhold and Bautista, 2009).

2.2 Andaliman

Andaliman was named *Zanthoxylum acanthopodium* DC. in which DC stands for de Candolle who was a renowed swiss botanist. Andaliman is one of Indonesia's wild spice that is originated from North Sumatera. It is commonly known as '*merica Batak*' and is widely consumed in Batak cuisines. Andaliman is used by local people to mask the fishy odor of raw fish or meat for years (Wijaya, 1999). It has the characteristic of a fresh citrusy and sweet peppery odor. Contrasting to spicy sensation given by chili or pepper, andaliman elicits numbing effect to the tongue due to its unique trigeminal sensation, which is similar to sansho (well known as Japanese pepper) (*Zanthoxylum piperitum* DC). The fruit has a small and sphere form. The fruit will be green in color when it is fresh and young, red when it is mature and black when it is dried. The flavor intensity and aroma of dried andaliman is stronger than fresh andaliman. Andaliman plant can be seen in Figure 2.1.

Andaliman is potential in inhibiting some pathogen bacteria and molds thus it could be an alternative source of natural antimicrobial ingredient.

(a)

(b) (c)

Figure 2.1 Andaliman Plant; (a) Andaliman tree (b) Andaliman leaves (c) Andaliman fruits
Source: Datta S. *et al*., (2013); Parhusip *et al*., (2007)

According to Parhusip *et al*., (2003), andaliman extract by maceration method and methanol solvent showed the highest inhibition towards *Salmonella typhiurium*, which is 12.61mm/g extract, while andaliman extract by reflux method with methanol sovent showd the highest inhibition towards *Escherichia coli*, which is 7.61 mm/g extract. Andaliman extract could also be applied in tofu preservation (Parhusip *et al.,* 2007). Atsiri oil from andaliman also has the potential to inhibit *B. cereus, S. aureus* and *Pseudomonas* (Mulia, 2000). Nevertheles, studies regarding andaliman is very limited and its potential as natural flavorings and has not been widely acknlowledged (Wijaya *et al.*, 2002).

Wijaya *et al*., (2002) has succeeded to identify the volatile compounds and key aroma of andaliman fruit. The identification were conducted by maceration

extract using GC-MS, GC/O and aroma extract dilution analysis (AEDA). The best extraction method to identify the compounds was maceration method with diethyl ether solvent, followed by chloroform, ethanol and acetone, respectively. GS-MS of maceration extract using diethyl ether results in 24 volatile components, which includes oxygenated monoterpenes (α-pinene, β-myrcene, limonene) and hydrocarbon monoterpenes (α-terpineol, β-citronellol, geraniol) as the main constituents. As for the major volatile compounds in andaliman extract is geranyl acetate (32.04%) and limonene (15.8%), which promotes floral citrusy and sweet orange peel aroma, respectively. According to AEDA (aroma extract dilution analysis) analysis, citronellal and limonene were the most impacting compounds on the aroma of andaliman. Limonene is also the major compound in sansho, which is a dominant compound in Japanese pepper (*Zanthoxylum piperitum* DC.). Citronellal has the characteristic of strong and warm citrusy aroma, while limonene has a citrus peel sweet aroma (Wijaya *et al.*, 2002).

Andaliman posses a 'tingling', biting effect produced by a compound namely (2E, 6Z, 8E, 10E-N-(2'-methylpropyl)-dodecatetraenamide), which is commonly found in the pericarp fruit of *Zanthoxylum* sp. Other members of Zanthoxylum have also been used as traditional spices including Z. *sansho* (Japan), Z. *schinifolium* (Korea), Z. *simulans* (Taiwan) and Z. *rhetsa* (India). Andaliman extract also showed to hav immunomodulator effect in moderate concentration. Furthermore, oral intake of andaliman also proves to have antidiabetic, anti-hypertensive and relaxation actions, improves bowel evacuation and increases appetite. However, the scent simulation reduce the appetite and

indicated anti-obesity activity due to its ability to keep the nerve active (Wijaya, 1999)

According to Galopin *et at.,* (2004), the pungent and tingling compounds comes from sanshool which is isolated from Zanthoxylum. This highly flavored compound is found to be an unsaturated and unstable aliphatic amide, thus it has the tendency to be easily polymerized. Furthermore, it was also found that the tingling compound in Zanthoxylum species is an alkylamide compound, which deteriorates easily under UV light and hydrolytic conditions (Yang, 2008). Thus with this characteristic, andaliman tends to be difficult to be stored over prolonged period of time due to its influence to promote food quality instability. Wijaya (2002) has succeeded extracting the trigeminal active compound by maceration method with the solvent of ethyl acetate-ethanol (10:1). From the research, it was reported that the trigeminal active componenet in andaliman is an amide compound.

Terpenoid compounds such as linalool, lemonene and geraniol are one of the compounds that are found in andaliman. These compounds are reported to have antioxidant activity, and antioxidant compounds have been known to protects human immune cells from oxidative stress. Furthermore, Wijaya *et al*., (1999) has proven that andaliman that is extracted with soxhlet method in 200 ppm could elicits antioxidant activity higher than alpha-tocopherol, although it is slightly lower than BHT. The protection value of andaliman extract, alpha-tocopherol and BHT is 4.34, 3.99, and 4.73, respectively. The research also reported that andaliman extract and sanshoo compound could improve sel defence towards a paraquat toxin that is given in the cell cultures. Testing on mice

macrophague also shown that andaliman extract could improve the production of free radicals that is produce by macrophague. The induce production of free radicals is bactericidal thus it will promote good health, due to its higher ability to protect from microorganism and pathogen attack (Wijaya, 1999).

2.3 Parameters Affecting Storage Stability

The nature of food could undergo physical, chemical, enzymatic and microbial changes. The relevance of such changes in influencing storage stability will be influenced by treatment of food prior to storage. Processed foods (e.g. heated, frozen, dried, salted and smoked) generally will have no active microbial populations and minimal enzymatic activity prior to storage. While on the other hand, fresh foods (e.g not processed and stored at ambient or chill temperatures) will have active microbial populations. More problems could exist in the form of fresh fruits due to increased enzymatic reactions, e.g., discolorations and increased susceptibility to microbial growth (Hole, 2003).

The parameters that highly influences storage stability are temperature, moisture content, packaging and food additives. Each of these parameters will have its function, but it should be known that the combined use of the above parameters is usually beneficial in practice (Hole, 2003).

2.3.1 Temperature

The temperature of food storage is the most important parameter which influence storage stability of fresh fruits. Respiration process of fruits and vegetables, enzymatic activity and microbial growth could be greatly reduced in rate by the use of chill or refrigerated conditions (0-5 °C**),** while frozen conditions

(-20 °C) could effectively decrease biological processes. Chemical reactions are reduced by 50% with lowering 10 °C of their ambient (20-25 °C) rate. Physical effects results in textural changes are one potential disadvantages of frozen storage for intact tissue systems due to the loss of ‘fresh’ image for such tissue systems, such as vegetables, fruit, meat, and fish. The use of chilled storage and modified-atmosphere packaging can extend the storage period significantly, but some fruits and vegetables could suffer chilling injury (Hole, 2003).

Chill storage could not inhibit all pathogens, for example, *Listeria monocytoges* can grow at 0 °C. However, there is an increased demands for ‘fresh’ foods therefore there should be a development on storage periods at chill conditions by utilizing specific packaging to obtain the longest shelf life (Hole, 2003).

There is considerable evidence that temperature plays major role in causing changes in food quality during storage. Higher storage temperatures generally lead to increased quality deterioration. High temperature condition could accelerate lipid oxidation, chemical reaction, and microbial growth and color deterioration. The increase in storage temperature will decrease the shelf life of foods, especially refrigerated foods. Herbs and spices should avoid high temperature storage, utilize packaging with low oxygen permeability and gas flush with controlled atmosphere or modified atmosphere conditions in order to reduce the oxidation reactions. The higher the temperature, the more permissive conditions are for microbial growth and chemical reactions. Food products should be kept in low relative humidity and cool temperature to preserve the quality for food making (Man and Jones, 1994).

2.3.2 Packaging

As well as the general function of containing food and preventing contamination, packaging can be an effective barrier against transfer of microorganism, oxygen and moisture. In addition, exclusion of light and increasingly the control of gaseous content of the pack are important (Hole, 2003).

Sterilized food prevention of leakage of microorganisms is the major requirement, and metal containers (cans) have been used for over 100 years for this reason. Rigid plastic containers and flexible pouches are increasingly used. With oxygen removal, such sterilized foods could be stored in room temperature for up to 1 year. Detrimental oxidation of lipids, color, and flavors is accelerated by light and hence opaque packaging, e.g., metallized films, is favorable. Discoloration in metal cans with high-pH protein-rich foods (meat and fish) is avoided by the use of specific lacquers on the inside surface of the can. Vacuum packaging is beneficial for food that is prone to lipid oxidation (Hole, 2003).

For fresh fruit and vegetables, the continuous respiration will change carbon dioxide and oxygen concentrations in the pack thus the permeability of the packaging film to these gases must be considered. The remaining and rapid respiration after harvesting fruits presents problems in extending their storage lives. The use of relatively low oxygen concentrations (2-3%) and high carbon dioxide concentrations (approximately 5%), integrated with chill temperatures (0-5 °C) can reduce the rate of respiration and give a useful extension of the storage period (Hole, 2003).

2.3.2.1 Glass Containers

Glass is an advantageous packaging material due to its chemical inertness, clarity, rigidity, heat resistance, resistance to internal pressure and low cost. However, it also has disadvantages due to its fragility and heavy weight.

Glass is justified as being unaffected by, and having no effect on, most food ingredients that are packed within. Hydrofluoric acid is the only liquid which has rapid reaction with glass material at room temperature. Other aqueous solutions and water may react with glass in an extremely low rate at room temperature. Glass also functions as a complete barrier to water vapor and to gasses. Nevertheless, there is still a possibility of pick-up and loss of gases or vapors via the bottle closure (Paine and Paine, 1992).

2.3.2.2 Plastic Vacuum Packaging

Vacuum packaging is an alternative to controlling or modifying the atmosphere level in which involves removal of all of the gas in the package. This can be a very effective way of retarding chemical changes such as oxidative rancidity development. Vacuum packaging that is combined with mild heat and chilled storage will have higher extended shelf lives. This packaging is highly necessary for food products that have high fat content because it provides packaging material with low oxygen permeability. The use of vacuum packaging that excludes oxygen will also prevent the growth of bacteria, especially *Pseudomonas* species which is the major spoilage group in chilled foods. The types of material used for vacuum packaging is PET/PE packaging and it has good barrier towards gas and moisture (Coles *et al.,* 2003).

2.3.2.3 Polypropylene

Polypropylene is a linear polymer, which contains little until no unsaturation. It has similar chemical characteristic with high-density polyethylene. PP has a lower density (900 kg m^{-3}) and a higher softening point (140 – 150 °C) than the PEs. PP has low water vapor transmission, medium gas permeability, good resistance to chemicals, good abrasion resistance, high temperature stability, good gloss and high clarity, with the later two factors making it ideal for reverse printing (Robertson, 2010). Polypropylene could be injection molded, blow molded, and extruded into film and sheet. The sheet can be thermoformed to give thin-walled trays of excellent stiffness (Paine and Paine, 1992).

2.3.3 Moisture Content

Water is present in all foods, ranging from trace amounts in dried food products to very high amounts in beverages. The stability and shelf life of foods are highly dependent on the water content since it directly affects the rate of food deterioration reactions. Relative humidity of the immediate environment directly affects the moisture content and water activity of food. Rates of deteriorative changes and microbial growth under normal food storage conditions often depend on moisture content and water activity. Food deterioration due to microbial growth is not likely to occur at aw < 0.6. However, chemical reactions and enzymatic changes may occur at considerably lower water activities (Steele, 2004).

Moisture removal or dehydration has long been used as a technique for improving food storage stability. Small increases in the moisture content of low and intermediate moisture foods can significantly reduce their shelf life. In

addition, moisture content influences textural properties of low moisture food (Nollet, 2004).

2.4 High Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is the most frequently used column chromatographic technique due to its high separation power, excellent selectivity, and high diversity of solutes that can be separated with this method. HPLC offers a unique possibility to employ supports with different separation mechanisms, such as ion-exchange, size exclusion, and gel permeation chromatography (Cserhati and Forgacs, 1999).

Separation in ion-exchange HPLC is based on the interaction of charged solute with the oppositely charged surface of the stationary phase. The retention of electrostatically bonded solutes can be easily influenced by addition of salt into the mobile phase or by modifying its pH value. Size exclusion chromatography (SEC) is a technique for separating molecules according to their effective shape and size in the aqueous mobile phase. Gel permeation chromatography (GPC) uses organic solvents for the separation of water-insoluble macromolecules. The stationary phases used in these separation modes care porous particles with closly controlled pore size. The molecules of solute can enter the pores of the support accrding to their size and shape (Cserhati and Forgacs, 1999).

HPLC system commonly consists of an injection device, a column, a mobile phase delivery system, a detector and a signal output device. The injection device is an important part of the HPLC system because it has to inject a precise volume of solute solution into the mobile phase under high pressure. The

dimensions of HPLC columns and the character of supports vary considerably according to the retention behavior of the analytes. The mobile-phase delivery system includes a pump with filter, a degasser, and transfer tubing. It transfers the mobile-phase components into the separation system with a constant, precise and reproducible flow rate. The composition of the mobile phase is held constant during the separation process and this method is called isocratic elution. The composition of the mobile phase can be continuously changed in a predetermined manner in order to increase the efficiency of the separation or eluting solutes with highly different physicochemical characteristics in one run. A broad variety of detectors are used in HPLC, applying different physicochemical principles or exploting different molecular characteristics of solutes. The most common detector used are ultraviolet-visable (UV-VIS), fluorescence, refractive index (RI detector) and various electrochemical detectors (ECD) (Cserhati and Forgacs, 1999).

2.5 Sensory Evaluation

Sensory evaluation is a set of techniques for accurate measurement of human responses to foods. The evaluation attempts to isolate the sensory properties of foods themselves and provides important and useful information to product developers about the sensory characteristics of their products. Sensory evaluation helps to determine important sensory attributes that may increase product acceptability, identify consumer segments, evaluate new concepts and measure sensory changes in products with altered processing or ingredients. Sensory evaluation is divided into subjective and objective testing. A subjective

or affective test focus on measuring consumer's acceptability, liking or preference towards one or more product's sensory attributes. On the other hand, objective testing focus on evaluating product's sensory attributes by a selected or trained panel. Objective test can be divided into discriminative, scoring, ranking and descriptive test (Kemp *et al.,* 2009).

Table 2.1 Classification of test methods in sensory evaluation

Class	Question of interest	Type of test	Panelist type
Discriminative	Are products perceptibly different in some way?	Analytic	Screened for sensory acuity, oriented to test method, in some cases are trained
Descriptive	How do samples differ in specific sensory characteristics?	Analytic	Screened for sensory acuity and motivation, trained or highly trained
Affective	How well are products liked or preferred?	Hedonic	Untrained

Source: Lawless and Hildegarde (2010)

2.5.1 Discriminative Test

Discrimation test is used to determine a difference or similarity that exist between two or more samples. The results data is analyzed by statistical significance testing to determine whether or not the samples are perceived to be different or similar. This test is commonly used in screening and training of panelist, determination of threshold sensitivity, quality control, investigation the effect of altered processing or ingredients and in preliminary assessments. There is an option on whether or not 'no difference' answer is allowed or forced to the panelist to make their decision. When 'no difference' option is allowed, there are two common approaches to the data analysis; the 'no difference' responses are ignored or the 'no difference' response is split proportionally between the products (Kemp *et al.,* 2009).

Discriminative test is categorized into overall difference test and attribute specific test. An overall difference test uses all available information to determine

the difference between two or samples. In some cases, the test might also be restricted to one specific attribute, but this will require the disguise of other sample attributes, e.g. by colored light to mask the appearance color. Several forms of overall difference test are triangle test, duo-trio, difference from control test, same-different test and A-not A test (Kemp *et al.*, 2009).

2.5.2 Scaling Test

Interval scale is one in which the distance between points on the scale is assumed to be equal and the scale has an arbitrary zero point, thus it eliminates the "absolute" magnitude claim of the attribute measured. Interval scales could be presented in a paired comparison, rank, or by rating scale procedures. (Stone and Sidel, 2004).

A graphic rating scale was developed utilizing a procedure described as functional measurement. The subjects are exposed to the stimuli and are provided practice with stimuli of the end anchors, which are the examples of scale extremes. When these two steps is used, and combined with line scale, the result can be mathematically as equal interval. Line scale is a part of graphic scale in which a line is anchored labeled at two endpoints. Line scale is commonly used to quantify characteristics. It measures the respond intensity of a sensory characteristics and the amount of difference between samples. Often, the intensity is established as a left to right reading continuum with low or bad on the left side and high or good on the right side of the page. Line scale data is analyzed by converting panelists' marks to numerical scores. This is done by measuring the distance from the lowest intensity point to the panelists' mark in cm (Stone and Sidel, 2004).

The use of line scale is proven to be very effective in descriptive analysis. The greatest advantage of line scale is in the absence of any numerical value associated with response of the panelist, and the limited application of words thus minimizing word bias (Stone and Sidel, 2004).

Line scales eliminates the use of number from the scale and the panelists' responses in scaling method, thus it has the advantages to eliminate two sources of bias. First, it eliminates the bias from panelist avoiding (or preferring) a particular number with negative (or positive) connotations; secondly it also eliminates the bias from panelists who changed use of number over time. This can be a problem since it is unknown if the change is related to the sample's difference or true bias (Stone and Sidel, 2004).

2.5.3 Descriptive Test

Descriptive analysis results in a more precise sensory description of a product since the panelist has to describe and quantify the sensory attributes of the product. This test usually uses trained panelist. (Kemp et al., 2009). In descriptive test, the vocabulary description of each attribute, characteristics, character notes and descriptions must be determine through a consensus among the panel members. The intensity and quantification of each descriptive analysis must be ensured to reflect the scale, category and ratio of each parameter (Lawless and Heymann, 1998). The efficiency of a descriptive analysis depends on four factors; the panel leader, panelist training and experience, commitment of the panel and analysis implementation (Gacula, 1997).

Attribute Rating Method is based on the principle of panelist ability to verbalize their perception of a certain characteristic in a reliable consistency

(Hootman, 1992). This method measures intensities of specified characteristics (attributes). The characteristics may be defined through flavor or texture profile analysis or by a person or persons thoroughly familiar with a product's sensory attributes. Trained panelist analytically discriminate intensity differences among the samples presented. The attribute rating method employs either one or two scaling approaches – category scaling or ration scaling (magnitude estimation). Category scaling is categorized into structured and unstructured scales. Structured category scales utilize a series of equidistant scalar intervals, anchored with an appropriate descriptive terms at each intervals. Unstructured scales have descriptive quantitative points at each end; the anchor points indicate extremes of the characteristics to be measured. Regardless of the scaling approach used, the selection process should begin with a large group of panelist; which are twenty-two to thirty-six prospective judges. The larger the number of candidates, the greater the probability of finding persons of superior ability (ASTM, 1981).

The panel leader is a sensory professional, who is in charge to manage the panelist screening process, organizing and implementing the screening test and selection subjects for training. However, panel leader is not the participant in any screening or training. The panel leader will assist all panelist to clarify a specific attribute or sensation, obtain suitable references and determining the completion of the training session. (Hootman, 1992).

Panelist screening procedure consist of three basic steps, which are selection of panelist, product usage and familiarity, and discrimination ability and task comprehension. These steps will create a group of panelist that are able to significantly differentiate and quantify a characteristic of a product, and eliminate

those who have difficulties in discrimination ability. Twenty five individuals is a sufficient number of panelists to start with and it will result in 12 to 25 qualified discriminators. The selected panelist will be trained in order to create a scorecard that includes sensory language, which describes the panelists' perception of the product. The description and the value must be a result of a consensus that is agreed by the entire panel. The scorecard includes explanation of each word and standardizes procedure of product evaluation, and scoring practice to familiarize the panelist with the scale. Panel leader may use references to create the same perception of a sensory terminology when there is confusion during training session. In special cases, discrimination ability trial might use the product, which is going to be described (Hootman, 1992; Stone and Sidel, 2004).

The procedure of product evaluation will be conducted without a reference standard, in individual manner in separate booths. The panelist will have to quantify the intensity of the characteristic on a line scale. A line with 15 cm length, with two anchors located at approximately 1.5 cm from each end is considered to be fully effective (Stone and Sidel, 2004). The difference among products produce by Attribute Rating Test will be a relative measurement since panelist could mark in any part of the scale.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Materials and Equipments

The materials for this research include fresh andaliman (*Zanthoxylum acanthopodium* DC.*)* originated from plantation in Medan which was transported to Pasar Senen, Jakarta, glass bottle, polypropylene plastic packaging, vacuum plastic packaging, cocoa powder, capulaga, turmeric, coffee, pepper, garlic, cinnamon, clove, onion, orange, wasabi, and other materials for sensory analysis.

Equipments used in this research include analytical balance, dry blender, refrigerator, freezer, pipette, spatula, tongs, dark bottles, funnel, graduated cylinder, beaker glass, erlenmeyer flask, evaporating dish, cabinet dryer, HPLC (Hitachi LaChrom LC System, USA), DAD detector (Agilent L7400, USA), column oven (Agilent L300, USA), separation column (Agilent Zorbax Eclips Plus C18, 4.6 mm id x 250 mm), magnetic stirrer, desiccator, vacuum packaging machine and other equipment for physical and sensory analysis.

3.2 Research Methods

The research was conducted in two parts including the preliminary stage and the main research. The preliminary stage of the research was to determine the suitable packaging to maintain sanshool and antioxidant activity of dried andaliman. Meanwhile the main stage of the research was to determine the effect

of different storage temperature towards dried andaliman in whole and ground form, as well as Attribute Rating Test to dried andaliman.

3.2.1 Preliminary Stage

The preliminary stage of this research was to determine the suitable packaging to maintain sanshool and antioxidant activity of dried andaliman. The result of this step was used in the main research. The types of packaging used in preliminary step were polypropylene plastic bag, vacuum packaging and glass bottle. All samples were kept in a place that is not exposed to light.

For the preliminary stage, 600 g of andaliman fruits were dried under warm air of 54 °C for 8 hours as the optimum condition for drying andaliman (Napitupulu, 2014). The dried andaliman were kept inside three types of packaging, which were polypropylene plastic bag, vacuum packaging and glass bottle. Polypropylene plastic used for preliminary research was 110 x 150mm, 29 µm. Vacuum packaging material was PET/PE plastic with dimension 142 x 200 mm, 90 µm. Vacuum packaging was vacuumed under 10^{-5} Pa, categorized as high vacuum condition, and sealed with double heat sealer. Glass packaging used was a glass jar obtained from IKEA, Alam Sutra, Tangerang. It had 50 mm diameter, 60 mm height and closed with a tin cap. Three types of packaging used in preliminary research can be seen in Figure 3.1. All packaging contained 60 g of ground andaliman

Dried andaliman were kept for 1 week to determine which packaging could maintain sanshool compounds best. The analysis for this preliminary stage was only by sensory analysis. The dried andaliman from three types of packaging

were tasted by the trained panelist and mentioned whether or not the dried andaliman still have its tingling sensation characteristic. Sensory analysis based on consensus of 20-trained panelist was used to determine the type of packaging that could retain the tingling sensation of dried andaliman. Two of the best packaging that could retain the tingling sensation of dried andaliman after 1-week storage, was further analyzed using paired comparison test (2-AFC) to determine the significance difference of 2 types of packaging.

(a) (b) (c)

Figure 3.1 Three types of packaging used for preliminary research.
(a) Polypropylene (b) Vacuum Pack (c) Glass Jar

3.2.2 Main Research

The main research was to determine the effect of different storage temperature towards dried andaliman in whole and ground form. Whole andaliman was grounded with dry blender (Panasonic Blender MX-J1GWSR) for 1 minute to produce ground andaliman. Whole andaliman was put in the glass container until half full for every grinding process. The temperatures conditions used as treatments were 25 °C, 35 °C and 45 °C. All samples were kept in a place that was not exposed to light.

For the main stage of the research, 13 kg andaliman fruits were dried under warm air of 54 °C for 8 hours as the optimum condition for drying andaliman. The moisture content was recorded. The dried andaliman was divided into two subgroups (whole and ground), and it was put into three subsamples in one type of packaging, which was vacuum packaging. Analysis for moisture content, sanshool compound and antioxidant capacity was conducted on each subsample once a week, for 6 weeks including the initial point.

3.3 Experimental Design

For the preliminary stage of this research, the samples were subjected to 20-trained panelist and analyzed using binomial testing by to state whether or not the stored andaliman has its tingling sensation. The difference of glass and vacuum packaging was further analyzed by simple paired comparison test. As for the main research, the experimental group design is shown on Table 3.1

In every period of time, the dried andaliman were subjected to sensory analysis using scaling test towards 16 panelists. The result of the all the analysis test was statistically analyzed using ANOVA in SPSS software. The research design was a completely randomized design with three factors. The variables used are the storage temperature (25 °C, 35 °C, 45 °C), form (whole/ground) of dried andaliman and storage time (0, 1, 2, 3, 4, 5 weeks).

Table 3.1 Reseach Stage I Group Design

		Temperature	Form	
			Whole (C_1)	Ground(C_2)
Time	Week 0 (A_1)	25 °C (B_1)	$A_1B_1C_1$	$A_1B_1C_2$
		35 °C (B_2)	$A_1B_2C_1$	$A_1B_2C_2$
		45 °C (B_3)	$A_1B_3C_1$	$A_1B_3C_2$
	Week 1 (A_2)	25 °C (B_1)	$A_2B_1C_1$	$A_2B_1C_2$
		35 °C (B_2)	$A_2B_2C_1$	$A_2B_2C_2$
		45 °C (B_3)	$A_2B_3C_1$	$A_2B_3C_2$
	Week 2 (A_3)	25 °C (B_1)	$A_3B_1C_1$	$A_3B_1C_2$
		35 °C (B_2)	$A_3B_2C_1$	$A_3B_2C_2$
		45 °C (B_3)	$A_3B_3C_1$	$A_3B_3C_2$
	Week 3 (A_4)	25 °C (B_1)	$A_4B_1C_1$	$A_4B_1C_2$
		35 °C (B_2)	$A_4B_2C_1$	$A_4B_2C_2$
		45 °C (B_3)	$A_4B_3C_1$	$A_4B_3C_2$
	Week 4 (A_5)	25 °C (B_1)	$A_5B_1C_1$	$A_5B_1C_2$
		35 °C (B_2)	$A_5B_2C_1$	$A_5B_2C_2$
		45 °C (B_3)	$A_5B_3C_1$	$A_5B_3C_2$
	Week 5 (A_6)	25 °C (B_1)	$A_6B_1C_1$	$A_6B_1C_2$
		35 °C (B_2)	$A_6B_2C_1$	$A_6B_2C_2$
		45 °C (B_3)	$A_6B_3C_1$	$A_6B_2C_3$

The mathematical model of completely randomized design with three factors is as follows:

$$Y_{ijk} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijkm}$$

where:

Y_{ijk} = Observation value of dried andaliman stored in temperature condition on level i, form on level j, with n replication

μ = Real mean data

A_i = Effect of storage temperature on level i (i = 25 °C, 35 °C, 45 °C)

B_j = Effect of form on level j (j = whole, ground)

C_k = Effect of storage time on level k (k = 0, 1, 2, 3, 4, 5 weeks)

AB_{ij} = Effect of interaction between storage temperature on level i and form on level j

AC_{ik} = Effect of interaction between storage temperature on level i and storage time on level k

BC_{jk} = Effect of interaction between form on level j and storage time on level k

ABC_{ijk} = Effect of interaction between storage temperature on level i, form on level j and storage time on level k

ε_{ijk} = Error

The hypotheses of this research were:

H_0 =

1. There was no effect of storage temperature condition on sanshool compound and antioxidant activity of dried andaliman
2. There was no effect of form of dried andaliman on sanshool compound and antioxidant activity of dried andaliman.
3. There was no effect of storage time on sanshool compound and antioxidant activity of dried andalimana
4. There was no effect of interaction between storage temperature and form of dried andaliman on sanshool compound and antioxidant activity of dried andaliman.
5. There was no effect of interaction between storage temperature and time on sanshool compound and antioxidant activity of dried andaliman.
6. There was no effect of interaction between form of dried andaliman and time on sanshool compound and antioxidant activity of dried andaliman.

7. There was no effect of interaction between storage temperature, form and time on sanshool compound and antioxidant activity of dried andaliman.

H_1 =

1. There was effect of storage temperature condition on sanshool compound and antioxidant activity of dried andaliman
2. There was effect of form of dried andaliman on sanshool compound and antioxidant activity of dried andaliman.
3. There was effect of storage time on sanshool compound and antioxidant activity of dried andaliman
4. There was effect of interaction between storage temperature condition and form of dried andaliman on sanshool compound and antioxidant activity of dried andaliman.
5. There was effect of interaction between storage temperature and time on sanshool compound and antioxidant activity of dried andaliman.
6. There was effect of interaction between form of dried andaliman and time on sanshool compound and antioxidant activity of dried andaliman.
7. There was effect of interaction between storage temperature, form and time on sanshool compound and antioxidant activity of dried andaliman.

3.4 Procedures

3.4.1 Panelist selection (ASTM, 1981)

Panelist selection was firstly conducted in order to select panelists with good sensory sensitivity. The panelist candidates were 35 students of Food

Technology Universitas Pelita Harapan who were in their 6th semester. The candidates were selected because they have passed the basic of sensory evaluation in previous class. Preliminary screening was done by asking the panelists to fill questionnaire regarding time spare, willingness and commitment of each panelists to participate in the training continuously. The questionnaire also included medical history and eating habits of the panelist. Furthermore, panelists were asked to do scaling exercise in order to obtain information regarding panelists' scaling accuracy. The panelist pre-screening questionnaire can be seen in Appendix A.

The next part of panelist selection was performing triangle test for basic taste. The objective was to know the ability of each panelist in differing taste and aroma, as well as to measure their sensitivity towards the characteristics of each flavor. Standard solutions that were prepared in testing the basic taste include sucrose (sweet), citric acid (sour), NaCl (salty) and caffeine (bitter) as mentioned in Table 3.2. The panelist candidates were asked to taste the presented sample and then select one that is different between the three samples (Appendix B).

Table 3.2 Standard solution concentration used in triangle test

Ingredients	Concentration		
Sucrose	1 %	2%	4%
Citric acid	0.035 %	0.07%	0.14%
Salt (NaCl)	0.1 %	0.2%	0.4%
Caffeine	0.035 %	0.07%	0.14%

Source: ASTM (1981)

Aroma identification test (Appendix C) was conducted in order to measure the ability of panelist candidates in differentiating characteristics of similar aroma properties. The standard odorants that were prepared includes cocoa powder, capulaga, turmeric, coffee powder, pepper, garlic, cinnamon, clove, onion and

orange. The candidates were asked to smell a coded jar with three short sniffs and identify the possible correct answer of the samples.

The last test was trigeminal sensation rating test. The trigeminal sensation questionnaire can be seen in Appendix D. Panelists were given 5 samples of wasabi in different concentration. Panelists must give an assessment on the sample trigeminal sensation by placing a vertical mark on a 15 cm line according to the perceived intensity. Panelist must at least had 60% correct answers out of all tests to be selected in the panelist training.

3.4.2 Panelist training (ASTM, 1981)

Panelist training was conducted to grow the panelists' sensitivity and to maintain decision consistency, thus panelist are eligible as 'trained panel'. Selected panelists received trainings related to terminology, definition and evaluation procedures; as well as to increase their skills in discriminating flavor intensity. This was done through scaling and ranking test for trigeminal sensation of samples with different intensity. Trainings were conducted in a conference style room. Panel leader led the session and provided all training products and reference material necessary, without participating in the product description analysis. Training was conducted for a period of approximately three weeks, consisting of 14 sessions with each session conducted for one hour. The training activities for each session are shown in Table 3.3.

Panelists were firstly introduced to fresh andaliman sample, as well as terminologies of flavor that may possibly occur in fresh andaliman sample and strored andaliman. Then panelists were familiarized with rating and scaling

method for trigeminal sensation. Flavor terminology training objective was to standardize the concept of terminology that can be communicated between panelists (Lawless & Heymann, 1998). Then, panelists were given fresh andaliman everyday to train their sensitivity towards the reference value for Attribute Rating Test. Panelist were trained for rating and scaling test for trigeminal sensation in a focus group consensus. The rating and scaling test were done in replicates until the panelists were considered as able to make correct and consistent judgment.

Table 3.3 Panelist's training schedule for Attribute Rating Test

Session	Training activity
1	Introduction to QDA training, scaling and ranking test, tasting technique Training session scheduling
2	Introduction to andaliman sample and discussion of sample's flavor attributes
3	Daily fresh andaliman intake as the reference
4	Daily fresh andaliman intake as the reference
5	Focus group consensus for trigeminal sensation of dried andaliman
6	Daily fresh andaliman intake as the reference
7	Daily fresh andaliman intake as the reference
8	Daily fresh andaliman intake as the reference
9	Daily fresh andaliman intake as the reference
10	Focus group consensus for trigeminal sensation of dried andaliman
11	Daily fresh andaliman intake as the reference
12	Daily fresh andaliman intake as the reference
13	Daily fresh andaliman intake as the reference
14	Scaling test for trigeminal sensation of dried andaliman

3.4.3 Paired Comparison Test (2-AFC) (Kemp, Hollowood and Hort, 2009)

The difference of vacuum packaging and glass bottle packaging in preserving the tingling sensation of stored andaliman was analyzed by using paired comparison test (2-AFC). This method was used to determine if a difference exists between two samples with regard to a specified attribute, which was the tingling sensation of stored andaliman. Twenty trained panelist were presented with two blind coded samples as it can be seen in Appendix F. They were asked to asses the samples and determine which of the two has the greatest

intensity of a tingling sensation. The result was compared to statistical tables which states the minimum number of responses required before a significant difference can be concluded from the test ($p < 0.05$).

3.4.4 Attribute Rating Test (ASTM, 1981; Chambers, 1996)

Determination of the effect of storage condition, storage temperature and wholeness of dried andaliman, was based on Attribue Rating Test towards 16 trained panelists (with replication). Preparation of the fresh andaliman sample and dried andaliman were done by crushing it using dry blender. Fresh andaliman and stored andaliman were assessed for one parameter, which was the tingling sensation (tongue-numbing) only.

The stored andaliman scaling test questionnaire can be seen in Appendix G. Panelists were instructed to place fresh andaliman sample on their tongue for 30 seconds and then spit out, while the stored andaliman were presented using tooth pick which was dipped into water then dipped into the dried andaliman sample, it is approximately the same amount as 5 mg of sample. Panelists were then asked to place a mark on an unstructured 15 cm line scale with intensity words (none to strong) on each end, according to the perceived intensity of the parameter. The trigeminal sensation parameter were assessed 30 to 60 seconds after the sample were placed on tongue to provide time for the trigeminal sensation to develop. Milk and white bread were provided to neutralize panelists' tongue as well as to reduce carry over and fatigue.

3.4.5 Moisture Content (AOAC, 2000)

The method used in moisture content determination is AOAC direct gravimetric method with slight modification. Empty dishes were placed in the oven at 105 ˚C for 3 hours for drying, and then transferred to the desiccators. After cooling down, the dish is weighed. Three grams of sample was weighed into the dish. The dish containing sample was then placed in the oven at 105˚C for 3 hours. The dish and sample were weighed again. The moisture content was calculated using the formula:

$$\text{Moisture (\%)} = \frac{(\text{weight of sample before drying (g)} - \text{weight of sample after drying (g)})\text{x}100\%}{\text{weight of sample before drying (g)}}$$

3.4.6 Measurement of the DPPH radical-scavenging activity (Yamazaki *et al*., 2007)

Different concentration of the andaliman extract (200, 400, 600, 800, 1000 and 1000 ppm) was dissolved in 25 ml of methanol and was taken 1.0 ml into different test tubes. Then 1.0 ml of 0.2 mM DPPH in methanol was added to each test tube. The reaction mixtures were let to stand for 30 minutes in a dark place with room temperature. The control was prepared without any andaliman extract and methanol was used as the baseline correction. Absorbance of the reaction mixture are measured at 517 nm using a Model 550 Microplate Reader. Measurements were performed at least in duplication. DPPH radical scavening activity was expressed as the percentage and was calculated using the following formula:

$$\text{\% DPPH radical scavenging activity} = \frac{(\text{Ab} - \text{As})}{Ab} \times 100\ \%$$

**Ab* is the absorbance of the control, and *As* is the absorbance of the sample

The inhibition concentration (IC_{50}) was calculated as the concentration of antioxidant compounds, which was required to inhibit 50% of DPPH radical at 517 nm.

3.4.7 Quantitative analysis of sanshool compound (Sugai *et al.*, 2005)

Dried ground products of Andaliman plant (0.5 g) were mixed at room temperature for 1 hour in alcohol 50% w/w (25 g). Filtration was done by using filter paper to separate the solid samples and filtrates. Filtrates were collected into 100 ml measuring flask. Alcohol 50% w/w were added until 100 ml. An aliquot (10 µl) was subjected to high-performance liquid chromatography (HPLC) equipped with DAD detector (Agilent, USA) at Ogawa Flavor and Fragrance Company in Karawang, Indonesia. Chromatograpic separation was performed in a column (Agilent Zorbax Eclips Plus C18, 4.6 mm id x 250 mm) with a detection wavelength of 280 nm. The column temperature was maintained at 40 ˚C with a Hitachi L300 column oven (Agilent L300, USA). The mobile phase used for analyzing the sanshool compounds by HPLC was CH_3CN/H_2O = 10:90 then holding for 30 minutes. The amount of sanshool compound was quantified with HPLC by comparing the measured peak area with calibrated curves obtained from α-sanshool as standards.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Andaliman

Andaliman (*Zanthoxylum acanthopodium* DC.) used in this research were obtained from Medan and transported to Pasar Senen, Jakarta. Andaliman were transported using a perforated sack as the primary packaging and a cartoon box as the secondary packaging. The condition of andaliman was fresh with light green to dark green color, with a few of ripe andaliman with red to dark brown color. Andaliman, which were picked from the plantation in Medan, were air dried for one night and transported to Pasar Senen early in the next morning, then it was taken on the same day it arrived at Pasar Senen. Andaliman that were used in this research were unripe andaliman, with light green to dark green color. Andaliman were separated from leave and twigs, cleaned, and then stored in freezer overnight before being dried on the next day. Fresh andaliman in this research refers to andaliman fruits that have been frozen for maximum three days. Fresh andaliman that had been stored in refrigeration temperature for four days had lost its trigeminal sensation.

Figure 4.1 Andaliman after cleaned and separated from leaves and twigs

4.2 Dried Andaliman

Fresh andaliman was dried under oven drying method using a cabinet dryer. This method was used based on the previous research. According to Napitupulu (2014), oven drying has good drying results as compared with fluidized bed, sun, air, far infrared and freeze drying method based on its moisture content and water activity. Although freeze-drying method was the best method among all, oven drying was the second best, and it has similar results as compared to freeze-drying results. Based on the drying results, economic consideration, and the availability of the equipment, the oven drying method was selected as the suitable drying method to be conducted in this research.

The yield of drying andaliman was 25,33% ± 0.34 (Appendix H). According to Napitupulu (2014), oven drying method obtained the second best result with yield 26.01%, after freeze-drying method (31.41%). The result gained the nearly highest yield as Napitupulu did.

For the total 6 treatments, dried andaliman was divided into two groups. The first group was grounded into powder form, and the second group was remained whole. Both groups was divided into 18 packs, resulting in 36 packs which each contained 60 gram of dried andaliman, for applying the storage methods in three different temperatures. Weekly observations were taken from the new packs taken from its chamber.

4.3 Effect of Different Packaging

Dried andaliman were put into three types of packaging, polypropylene plastic bag, vacuum packaging and glass bottle. Table 4.1 shows the result of

dried andaliman stored in different types of packaging after 1 week stored at 45°C.

Table 4.1 Result of dried andaliman stored in three types of packaging

Item	Dried andaliman	Polypropylene bag	Vacuum bag (PET/PE)	Glass Bottle
Presence of Trigeminal Sensation	+	-	+	+

The dried andaliman stored in three types of packaging was subjected to twenty-trained panelist and the final result was obtained by consensus in a focus group discussion. Dried andaliman in polypropylene bag lost their pungent characteristic after being stored in 45°C for 1 week while vacuum packaging and glass bottle could retain the pungent characteristic of dried andaliman.

Andaliman is prone to oxidation, thus the oxygen transmission rate of each packaging plays an important role on maintaining the pungent characteristic of dried andaliman. Polypropylene plastic bag has relatively high oxygen transmission rate, which is 23 O_2 cm^3 . mm cm^{-2} s^{-1} (cm Hg)$^{-1}$ at 30 °C while PET has lower oxygen transmission rate rather than PP plastic, which is 0.22 O_2 cm^3 . mm cm^{-2} s^{-1} (cm Hg)$^{-1}$ at 30 °C (Mathlouthi, 1994). Glass bottle has complete barrier to water vapor and to gases (Paine and Paine, 1992). The result is in accordance to the theory; in which dried andaliman stored in packaging with higher oxygen transmission rate (PP) lost its tingling sensation after one week while dried andaliman stored in packaging with lower oxygen transmission rate (PET and glass bottle) could retain the tingling sensation of dried andaliman.

Vacuum packaging has PET/PE as the material, which has good barrier towards gas and moisture. PET film’s outstanding properties as a food packaging

material are its great tensile strength, light weight, elasticity, stability over a wide range of temperatures (-60 °C – 220 °C) and excellent chemical resistance. Furthermore, vacuum packaging is a packaging design that reduced the oxygen content within a packaging until less than 1%. The elimination of oxygen within the packaging causes slow deterioration process on dried andaliman.

A similar result was obtained from the dried andaliman stored in glass bottle. Although glass bottle could not eliminate the oxygen content within the packaging, glass bottle offers very good barrier characteristics. Glass as a packaging material has the advantages of chemical inertness, rigidity, resistance to internal pressure and heat resistance (up to about 500 °C). Glass is also a complete barrier to water vapor and to gases (Paine and Paine, 1992). Both vacuum packaging and glass bottle effectively prevent the interchange of gasses between the food and the atmospheres thus retain the pungent characteristic of dried andaliman in one-week period.

Both result of dried andaliman stored in vacuum packaging and glass bottle were further assessed by 20-trained panelist by paired comparison test. Based on the result, 11 panelists stated that andaliman stored in glass bottle packaging has higher pungent characteristic rather than the andaliman stored in vacuum packaging. There was no significant difference from the dried andaliman stored by vacuum packaging and glass bottle ($p > 0.05$), because based on the binomial significance table there must be a minimum of 15 panelist that could state the difference in two samples in order to reject the null hypothesis.

Based on the result of the preliminary research, economic, practical, and

space consideration, vacuum packaging (142 x 200 mm) with PET/PE material was used as the method to store the dried andaliman in the main research to be stored in 25 °C, 35 °C and 45 °C for 6 observation time, including the initial measurements of sample before applying storage methods.

4.4 Effects of Different Form and Storage Temperature Over Time

4.4.1 Changes in moisture content after storage treatments

Dried andaliman were subjected to moisture content analysis as the physical characteristic of the samples. Each of the samples was analyzed with four replications to test the significance of various variables. The changes of Moisture Content of stored samples in different temperature scales can be seen in Appendix I and Figure 4.2.

Andaliman, which was grounded after drying process, had significantly higher moisture content ($p > 0.05$) than whole dried andaliman, as it is reported in Appendix J. Dry andaliman in whole form had 5.4% ± 1.82 moisture content while dry andaliman in ground form had 8.83% ± 1.78 moisture content. Moisture content was at significant difference as the effect of different form in the initial point prior to storage treatments. The result indicates that size reduction process could significantly affect the physical properties of dried andaliman. According to Peter (2006), as soon as dried spices are size reduced this increases the surface area exposed to atmospheric conditions. The smaller the particle size in ground spices the larger the surface area are exposed to atmospheric conditions and more susceptible the product is to moisture penetration, oxidation reactions and flavour

loss. To limit these reactions, it is important to control the temperature, contact with oxygen, the humidity surrounding the product and reduce the contact with light.

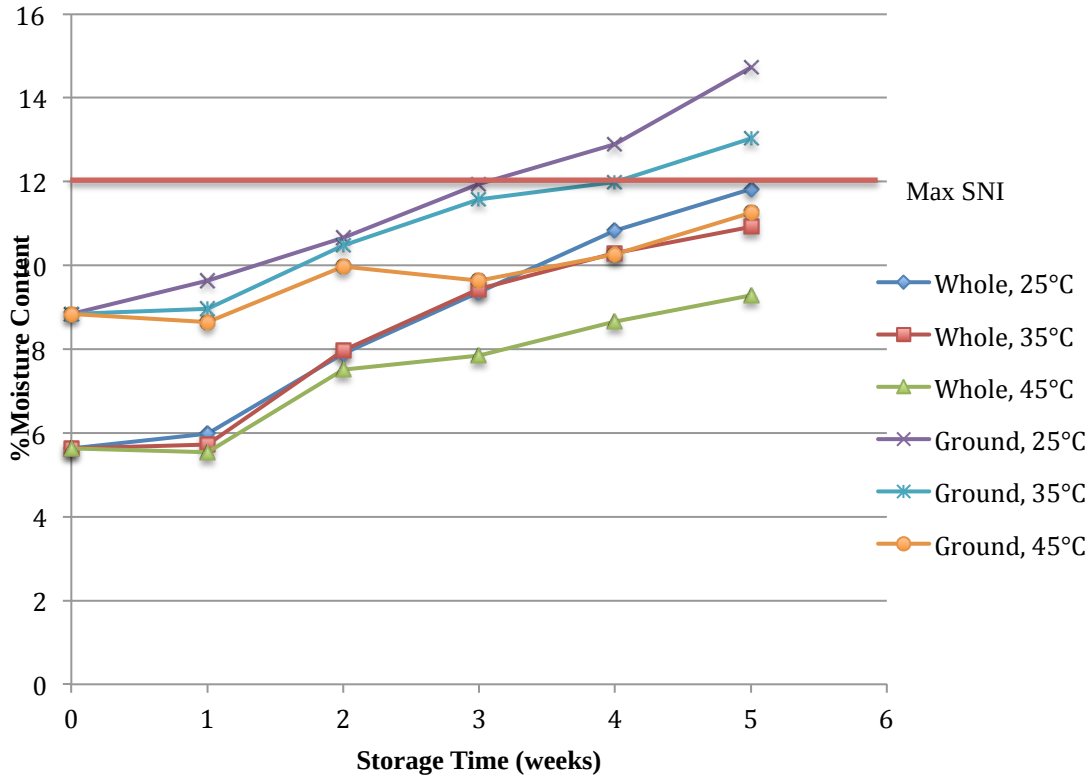

Figure 4.2 Changes in moisture content of whole and ground andaliman in different temperature scales after storage treatments

Andaliman in whole form has the lowest moisture content (5.4% ± 1.82) and as the storage treatment continues, andaliman in ground form that is stored in room temperature (25 °C) has the highest moisture content (14.72% ± 1.31). The moisture content of dried spices generally ranges from 6.27% to 12.44% (Nely, 2007). According to SNI 01-3717-1995, the maximum moisture content of white pepper is 12%. The same requriement applies as the standard quality of mixed white pepper based on SNI 01-0004-1995. According to this standard, ground andaliman stored in room temperature (25 °C) has failed to fulfill the requirement

of dried spices after being stored for 5 weeks due to its high moisture content (12.89% ± 1.23). The similar phenomena happened to ground andaliman stored in 35 °C, which failed to fulfill the requirement of dried spices after being stored for 6 weeks because it exceed the moisture content limit (13.03% ± 1.78). Moisture content of dried spices should be maintained to be below 12% because changes in moisture content of dried food can affect its nutritional quality. Increase in moisture content of dried food will promote microbial deterioration and accelerate rancidity and oxidation of vitamins and active compounds (Deepa, *et al*., 2011). According to Peter (2006), increasing the moisture content of the spices can also lead to problems with insect damage and possibly potential microbial risk factors if the water activity of the product medium reaches high levels.

The results were analyzed with three factors ANOVA to determine the effect of form, temperature and storage time towards moisture content of dried andaliman. The statistical result of three factors ANOVA can be seen in Appendix K. The result shows that there was no interaction between form and temperature and storage time; form and temperature; form and storage time; and temperature and storage time. However, there is a significant difference due to the effect of form, effect of temperature and effect of storage time.

After six-week storage treatment in different temperature scales, whole andaliman continue to have significantly ($p < 0.05$) lower moisture content as compared to ground andaliman. This result was the continuation from its initial moisture content. Due to the significant difference of moisture content prior to storage treatment, the storage treatments continue to give significant differences

of moisture content as the effect of form of andaliman. This result indicates that size reduction process could significantly affect the moisture content within sample because smaller particles have larger surface area to absorb moisture from the atmosphere thus results in higher moisture content after storage period. Therefore ground andaliman has higher moisture increase and more susceptible to oxidation reactions and flavor loss. According to Peter (2006) grinding process will lower the shelf life of dried spices. Shelf life of ground spices is about three to four months at refrigeration temperature and whole spices will have about one and a half years of shelf life. Whole spices are much more robust and deteriorates much slower than spices that have been sized reduced (Peter, 2006).

The storage temperature also gave significant effect towards moisture content of stored andaliman. Lower storage temperature produced higher moisture content of dried andaliman. The slope of the linear regression equation indicates the increase of moisture content. The linear regression equation of moisture content changes over time can be seen in Appendix W. Whole andaliman stored in room temperature (25 °C) increases its moisture content 0.1917% each day, while whole andaliman stored in higher temperature (35 °C and 45 °C) had smaller increase rate of moisture content (0.17% and 0.1143%, respectively) each day. The same phenomena also happened in dried andaliman with ground form. Ground andaliman stored in higher temperature has lower increase of moisture content. The increase rate of moisture content decreases as the temperature increases. This result indicates the effect of temperature and relative humidity. According to Lawrence (2004) and University Corporation for Atmospheric

Research (2008), as the temperature increases, relative humidity decreases. Thus there is less moisture available in the atmosphere that could penetrate though the packaging and into the samples. Therefore dried andaliman that is stored in higher temperature absorbs less moisture from the atmosphere due to the lower humidity. High temperature conditions could maintain the moisture content of dried andaliman, but it might accelerate the loss of active components in spices.

Duncan Test as the Post Hoc further analyzed this effect of different storage temperature. The result in Appendix K and Figure 4.2 shows that there was a significant difference of moisture content of stored andaliman when it was stored in room temperature (25 °C) as compared to 45 °C, while there was no significant difference of moisture content when dried andaliman was stored in room temperature (25 °C) as compared to 35 °C. This result indicates that moisture content of stored andaliman changes significantly when it is stored with 20 °C interval. According to Utah State University (2008), the absolute maximum humidity doubles for every 20 °F or 10 °C increase in temperature, therefore the relative humidity will become halves for each 20 °F or 10 °C increase in the temperature, assuming conversion of absolute moisture. According to Valsson and Bharat (2011), even though there is no water source and no water vapour is added, a lowering of air temperature results in a rise of relative humidity. The amount of water vapor that could be present at saturation is less at the lower temperature, so the existing amoung of water vapor represents a higher percentage of the saturation level of the air. The moisture holding capacity of air depends on the air's temperature. It increases with increase in temperature. As the moisture

holding capacity increases, the relative humidity decreases, provided no moisture is added into the air. Due to this reason, dried andaliman should be kept in whole form, which have lower moisture content as compared to ground, and in a dry storage condition to keep it from absorbing too much moisture.

As the dried andaliman were stored in different temperature and form, the moisture content of stored andaliman increases over time. Storage time also gives significant effect towards moisture content of stored andaliman. A post hoc analysis was done in order to determine the significant difference of storage time towards moisture content of stored andaliman. The result shows that moisture content of stored andaliman increased significantly on the second week. Therefore dried andaliman that is stored in vacuum packaging could have stable moisture content until 14 days. This result shows that the moisture content of dried andaliman stored in vacuum packaging increased in a short period of time. The reason of this result is due to the high water transmission rate of PET/PE as the material packaging. According to Mathlouthi (1994) the water transmission rate of PET is 1300 H_20 cm^3 . mm cm^{-2} s^{-1} $(cm\ Hg)^{-1}$, 90% RH, 25 °C. This shows that although the dried samples are packed inside a vacuum packaging with low oxygen available within, it still has high water transmission rate therefore the samples deteriorates in a short period of time. According to Peter (2006), under good storage conditions it is reported that coriander seed will retain flavor and color for 6-9 months. Thus this vacuum packaging has not fulfill the need of a suitable packaging that could retain dried andaliman physical properties.

Aluminium pouches and a storage condition of 37 °C and 70% relative humidity are an ideal storage condition for dried herbs.

4.4.2 Changes in IC_{50} value after storage treatments

To measure the antioxidant activity of stored andaliman, samples were subjected to be analyzed for its IC_{50} value. The optical density difference of the samples was measured at 517 nm. From the data, the samples' antioxidant capacities were expressed % DPPH Radical Scavenging Activity in ppm.

An IC_{50} value indicates the concentration of antioxidant compounds, which are required to inhibit 50% of DPPH. The data of changes in IC_{50} value of stored samples in different temperature scales after 6 weeks storage can be seen in Appendix L and Figure 4.3.

In general, all of the samples experienced an increase in IC_{50} values after 6 weeks storage time, as reported in Figure 4.3. Ground andaliman had significant lower initial IC_{50} value than ground andaliman (Appendix M). However, this is a contrary result from the theory. Samples with lower moisture content should produce lower IC_{50} values. The reason for this deviation might be due to the extraction process. Although the whole andaliman were also grounded before being extracted with methanol for 24 hours, all samples were not sized into the same particle size before the extraction process, they were only grounded with the same machine and the same amount of time. Due to this reason, the ground andaliman might have smaller particle size than the whole andaliman, therefore it was extracted better and eventually produced lower IC_{50} value at the initial point.

During storage time, the antioxidant capacity of a sample decreased which means that the antioxidant content decreased thus it needs higher concentration of a sample to inhibit 50% of DPPH, leading to an increase in IC_{50} value.

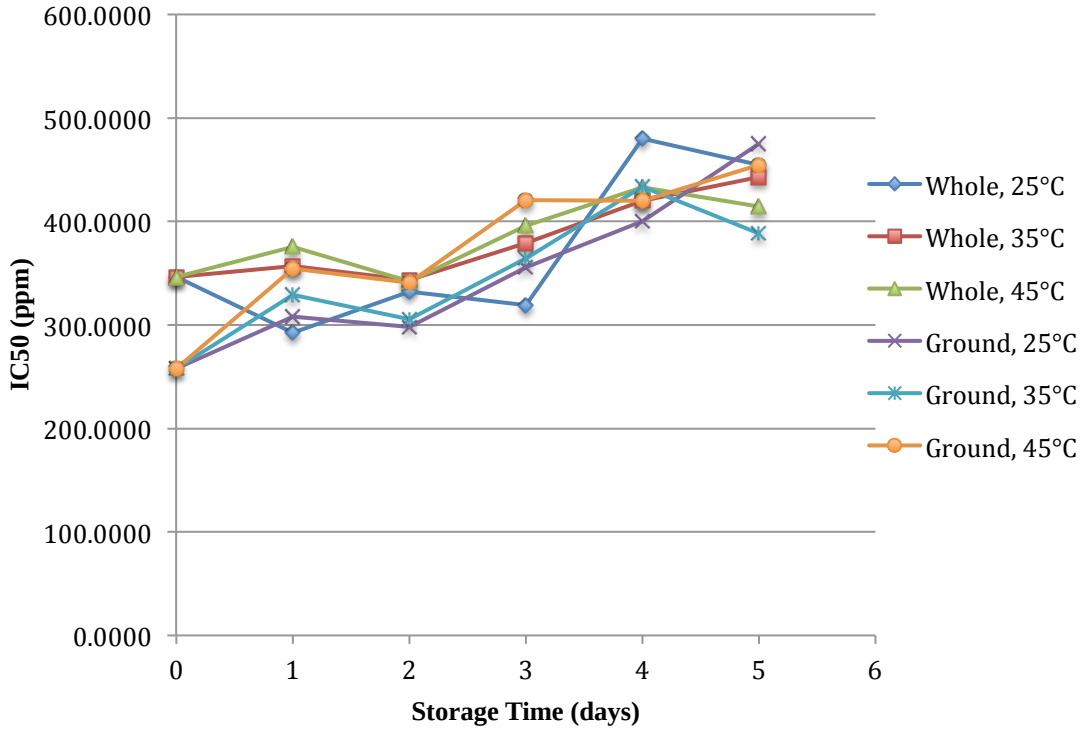

Figure 4.3 Changes in IC50 value of whole and ground andaliman in different temperature scales after storage treatments

The effects of different form and storage temperature over time is analysed with three factors ANOVA to detemine whethter the treatments effect to dried andaliman over storage time period. The result in Appendix N shows that there was no interaction of form, temperature and storage time. There was no interaction of form and temperature, form and storage time, as well as temperature and storage time. The result shows that only form and time gives significant difference toward antioxidant activity of dried andaliman.

Whole andaliman has significantly ($p < 0.05$) higher IC_{50} value thus it shows that whole andaliman has lower antioxidant activity as compared to ground andaliman. However, the result is contrary to the theory. Samples with lower moisture content should have higher antioxidant activity. This result might occurred due to the various particle sizes of whole andaliman which were not sized into a particular size prior extraction process. Thus the particle sizes distributions of whole dried andaliman were not unimodal. Ground andaliman might have smaller particle size and extracted better throughout the 24 h extraction process in methanol solution. Thus the extracts of ground andaliman has more active compound and has higher antioxidant activity. The similar result happened in sanshool content determination in which ground andaliman had higher sanshool content as compared to whole andaliman.

According to Rosa, et al. (2012), the antioxidant capacity of wheat bran was linearly correlated with the specific surface. Finely ground bran inhibited the accumulation of conjugated dienes more efficiently than coarse bran. The antioxidant capacity could be related to the release of some intracellular compounds that acts as antioxidant. The result of this research might be simillar to the related research by Rosa, et al. (2012). The reduction of the particle size and/or opening of the dried andaliman lead to higher exposition of the phenolic compounds as the main contributor to the increase of antioxidant activity of dried andaliman.

After being stored for six weeks, the storage time gave significant effect towards antioxidant activity of stored andaliman. Post Hoc analysis was done

using Duncan's Test. According to the result, there was a siginificant difference of dried andaliman starting from the second week. Therefore it can be concluded that dried andaliman can retain its antioxidant activity for 14 days. This result is in accordance to moisture content result. Both moisture content and antioxidant activity of dried andaliman in vacuum packaging is stable for 14 days. A similar result is reported by Arabshahi-D, et al. (2005) that mint leaves extracts did not show significant changes of antioxidant activity after a 15 days period storage at room temperature.

This result shows that the stability of antioxidant activity of dried andaliman is low. Dried herbs generally have shelf life of four to six months. According to Hossain, et al. (2010), dried Lamiacea herbs (rosemary, oregano, marjoam, sage, basil and thyme) did not show a significant decrease of antioxidant activity after 60 days storage in chilling condition. Dried andaliman might have lower shelf life due to the high increase of moisture content and the external environment. As the moisture content increase, dried spices are more susceptible to oxidation and enzymatic reations that might lead to deterioration of antioxidant activities (Peter, 2006).

4.4.3 Changes in tingling sensation after storage treatments

Dried andaliman samples were subjected to sixteen trained panelist for Attribute Rating Test. Six random samples were being tasted by the panelist every week. Panelists were asked to rate the trigeminal sensation intensity, which is the tingling sensation characteristic of the samples. All samples were being compared

to fresh andaliman, which was valued by a 7.5 score. The changes of intensity scores of dried andaliman in different temperature scales after 6 weeks storage can be seen in Figure 4.4 and Appendix O.

According to the result in Appendix P, the initial scores of dried andaliman are significantly higher than fresh andaliman ($p < 0.05$) with whole dried andaliman having the highest intensity score (9.46 ± 1.08), followed by ground andaliman and fresh andaliman (8.46 ± 1.71 and 7.5 ± 0.00). The result indicates that drying method could enhance the trigeminal sensation of Andaliman sample. The result of flavor intensification through drying was due to the elimination of most moisture, which resulted with a greater concentration of the low volatile compounds that give stronger flavor but less aroma due to the loss of volatile constituents. Most dried spices retain a higher overall flavor concentration than fresh spices. Furthermore, dried spices are more stable in high temperatures and processing conditions as compared to fresh spices (Raghavan, 2007). Whole dried andaliman also posses significantly higher trigeminal sensation as compared to ground dried andaliman. This result indicates that andaliman with lower moisture content has concentrated volatile compounds due to the elimination of moisture during drying process.

Whole and ground andaliman lost their trigeminal sensation through six weeks storage period in different temperature scales as seen in Figure 4.4. Three factors ANOVA were used to analyze the effect of form, temperature and storage time towards dried andaliman. The statistical result can be seen in Appendix Q. According to the result, there was no significant effect ($p > 0.05$) of interaction

between form, temperature and storage time; form and temperature; form and storage time; as well as temperature and storage time. In the other hand, different form, storage temperature and storage time gave significant effect towards tingling senstion ($p < 0.05$).

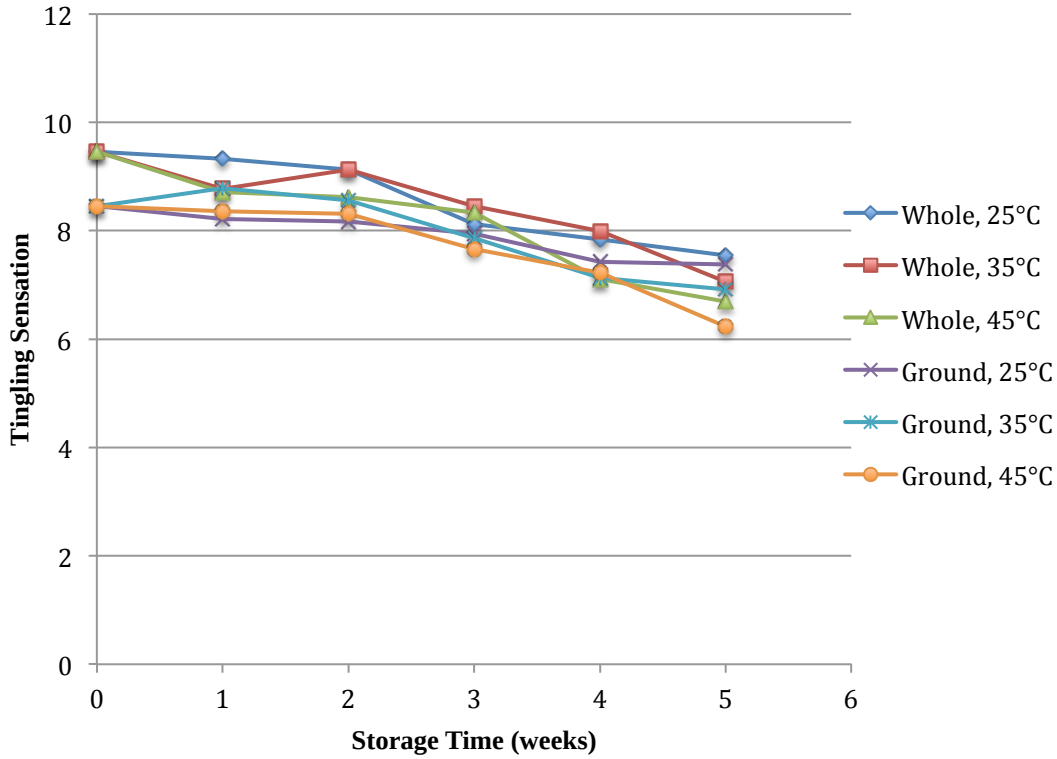

Figure 4.4 Changes in tingling sensation of whole and ground andaliman in different temperature scales after storage treatments

Whole andaliman (8.40 ± 0.88) had significantly higher tingling sensation as compared to ground andaliman (7.86 ± 0.68). This result occurred due to lower moisture content of whole andaliman. Andaliman with lower moisture content has more concentrated flavor due to the elimination of moisture. The result from the initial point prior storage treatments continue to have the same result after the storage treatments ends. Ground andaliman has smaller particle size, larger surface area and higher moisture content thus it deteriorates faster as compared to

whole andaliman. According to Raghavan (2007), moisture could cause accelerate the loss of flavour, aroma and active components due to the oxidation and enzymatic reaction that might occur. Furthermore, moisture will help mold growth that will cause spoilage. High moisture content could also create caking and hardening of the ground spices, thus it has less desirability.

As it is reported in Figure 4.4, andaliman that was stored in higher temperature has higher deterioration rate, indicated with higher slope in the linear regression equation. The slope of the equation indicates the loss rate of trigeminal intensity in the samples. The linear regression equation of tingling sensation changes over time can be seen in Appendix X. The negative mark shows that the intensity of stored andaliman decreases over storage time. Whole andaliman stored at 45 °C has gradient value of -0.0774 which means that the trigeminal sensation intensity of dried andaliman in whole form decreases 0.0774 of its value every day, when being stored in 45 °C storage temperature. The deterioration rate of whole andaliman stored in lower temperature (35 °C and 25 °C) is smaller than the deterioration rate of whole andaliman stored in high temperature (45 °C), which is showed in a smaller gradient value (0.0615 and 0.0614, respectively). The same gradual changes of gradient value also occur in dried andaliman stored in ground form. The deterioration rate of ground andaliman, which is figured as the value of gradient value in the equation, increase (0.0328, 0.0546, and 0.062) as the temperature increase (25 °C, 35 °C, and 45 °C, respectively). The result shows that heat could accelerate the loss of the components of spices. According to Peter (2006), the essential oil components naturally present in most of the

spices are subject to oxidation by atmospheric oxygen, particularly in high storage temperature resulting in flavor loss and development of off-flavours. According to Raghavan (2007), high temperature storage could cause flavor loss and color changes of dried spices. It is recommended to store dried spices in a tightly closed container that is not exposed to light, high temperatures, or high humidity conditions.

A post hoc analysis of temperature difference was done to indicate the significant differences of the temperature scales. The result shows that samples stored in high temperature (45 °C) had significantly lower trigeminal sensation intensity as compared to samples stored in room temperature (25 °C), but there was no significant difference of samples stored in 25 °C as compared to 35 °C. Therefore the result indicates that andaliman samples deteriorate faster if being stored in higher temperature with an interval of 20 °C. High temperature storage condition accelerate the loss of trigeminal sensation, regardless the form of andaliman that was being stored (whole or ground). According to Raghavan (2007), essential oils in spices dissapear quicly at high temperature storage. High temperature will help spoilage due to mold growth and cause flavor loss, color changes.

The tingling sensation of stored andaliman decreased significantly ($p < 0.05$) over storage time. This result happened due to the oxidation of samples through storage due to the permeability of PET/PE packaging. Andaliman samples were very prone towards oxidation and it deteriorates over time. Post hoc analysis was done in order to determine the significant differences of storage time.

The result shows that stored andaliman experienced a significantly decrease in tingling sensation starting from the third week. Therefore it can be concluded that dried andaliman stored in vacuum packaging could retain its tingling sensation for 21 days. The result had 7 days difference as compared to moisture content and antioxidant activity changes. Thus the panelist could not differentiate the difference in tingling sensation although the moisture content had increased significantly. Although the tingling sensation has better stability, it is categorized into low shelf life as compared to other dried spices that could retain its flavor characteristics for four to six months in good storage conditions. Therefore the vacuum packaging has not succeeded in retaining the tingling sensation of dried andaliman throughout storage period.

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4.4.4. Changes in sanshool content after storage treatments

Dried andaliman were subjected to HPLC analysis to determine the changes of sanshool content during storage with different temperature scales and form of andaliman. All samples were analyzed at Ogawa Flavor & Fragrance in Karawang Barat, Karawang.

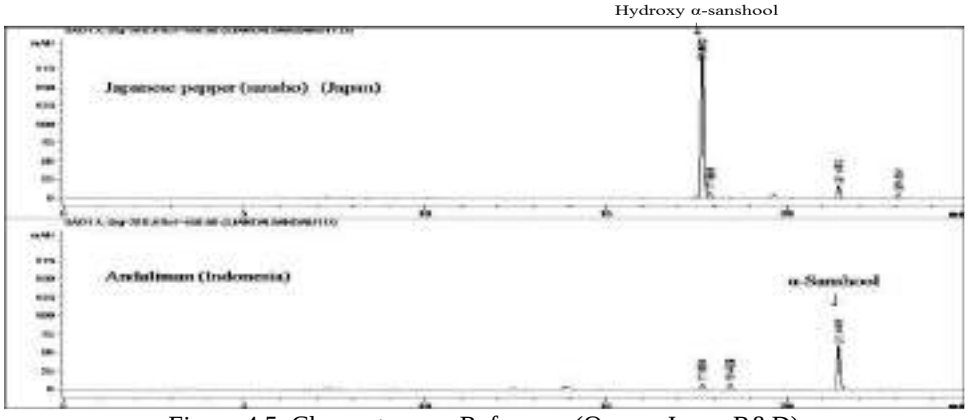

Figure 4.5. Chromatogram Reference (Ogawa Japan R&D)

According to Chromatogram Reference (Ogawa Japan R&D) in Figure 4.5, it could be predicted that α-sanshool peak appeared in around 22 minutes of the retention time. Therefore data of each sample was accountable as a reliable data if the peak appeared in around 22 minutes of retention time. The peak area was determined by the absorbance (optical density) by the spectrophotometer of the HPLC device. Pure-diluted hydroxyl α-sanshool was injected prior to every analysis of sample to produce the standard curve of sanshol content in different concentration. Peak area of the samples were compared to the standard curve that has known concentration, therefore sanshool content of each samples can be obtained.

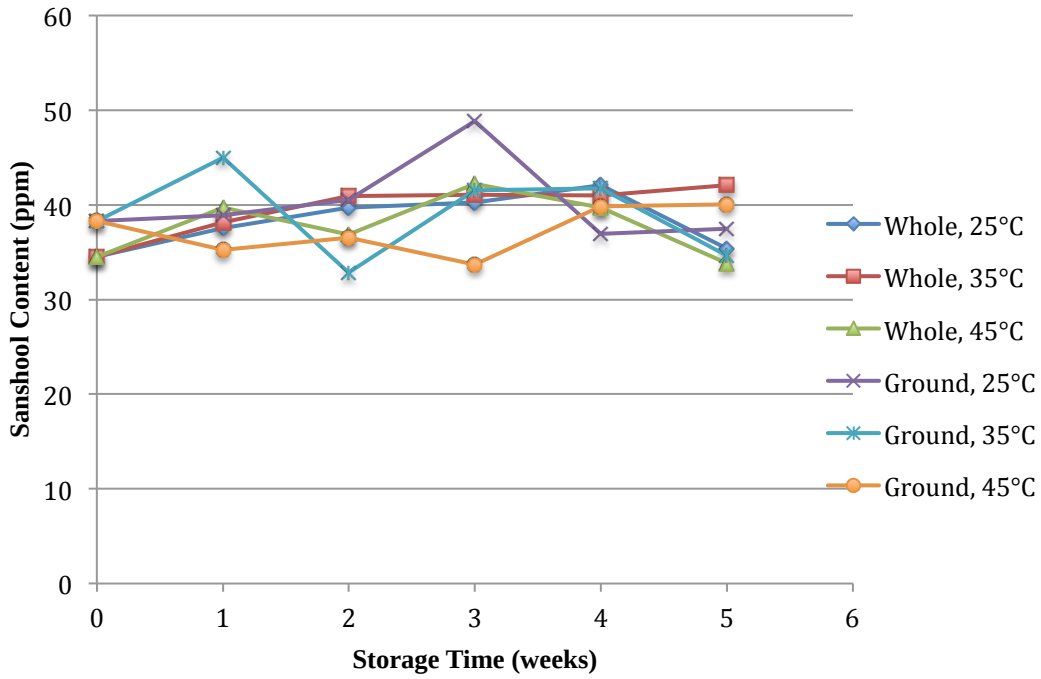

Figure 4.6 Changes in sanshool content of whole and ground andaliman in different temperature scales after storage treatments

The changes of sanshool content of stored samples in different temperature scales can be seen in Appendix R. The data reported in Figure 4.6 shows the

changes of sanshool content of whole and dried andaliman in different temperature scales after storage treatments. The chromatogram result from HPLC analysis is reported in Appendices V.

Based on the HPLC analysis, dried andaliman in ground form had significantly higher sanshool content (38.1138 ppm ± 0.288) as compared to andaliman in whole form (34.501 ppm ± 0.13) as it is reported in Appendix S. The result shows that there was significant difference in samples of andaliman in different form. According to Rosa, et al. (2012) grinding process could lead to reduction of particle size and/or opening of the active compounds that leading to higher exposition during extraction process and resulted in the increase of sanshool content of ground andaliman. The same phenomena happened to antioxidant activity analysis on its initial point too. Based on the result, ground andaliman has higher sanshool content and antioxidant activity prior storage treatments. This result shows that sanshool content contributes to the antioxidant activity of the sample. In general, sanshool was a kind of potential antioxidant compounds, but the ability in specific free radicals was not strong (Zhou, 2015).

After being stored in for six weeks in different form and different temperature scales, the result of sanshool content on the sixth weeks was analyzed using ANOVA with two factors; form and storage temperature. The statistical result in Appendix T shows that there was significant effect of the interaction of different form and storage temperature. The result showed that whole andaliman stored in 35 °C and ground andaliman stored in 45 °C has possessed the most sanshool content after storage treatments. The result happened due to the lower

moisture content of samples that were stored in higher storage temperature. Ground andaliman stored in 25 and 35 °C had the least sanshool content. It is in accordance to the moisture content result in which both samples had more than 12% moisture content on the fifth week and had failed to fulfill the requirements of SNI standard. Therefore ground andaliman could not be stored for long period of time due to its high moisture content and significant loss of sanshool content after storage. This result happened due to the vacuum packaging material that has high water transmission rate and affected the samples. The contrary result happened with dried chili stored in vacuum packaging. As it was reported by Chetti, et al. (2012), there was no significant decline in the capsaicin content up to 6 months was observed in all the vacuum packed bags regardless of their storage conditions, i.e. light, dark, or cold storage and the moisture content before packaging. Similar results were observed in chilli seeds by Deepa, et al. (2011). The samples held under vacuum packaging were unlikely to suffer from oxidation due to the property of the films used for packaging.

The difference of the result from this research as compared to Chetti, et al. (2012) might be caused from the variety of spice and the material used as vacuum pacakging. According to Raghavan (2007), different variety of spices possesed different deterioration rate. Over a six month of storage period for ground material, one variety can retain its 78% of its active compounds, whereas another can suffer a complete loss. Another factor that could differ the result was the materials used for vacuum packaging. Materials may consist single components such as polethylene or polyvinyl chloride, or of multiple components where the

properties required of the pcakgaing material cannot be satisfied by a single film. Therefore the properties of the vacuum packaging might differ according to its material Mathlouthi (1994)

Samples were being subjected to HPLC analysis in simplo due to the equipment availability in Ogawa Flavor & Fragrance. Only samples in the initial point and after being stored on the sixth week was analyzed with duplo. Andaliman samples lost approximately 10% of its sanshool content after being stored for six weeks. Before storage tretment are applied, the mean value of sanshool content is 36.31 ppm ± 1.89, and on its sixth week the mean value of sanshool content is 33.58 ppm ± 2.911. After applying paired sample t-test to the data from the first and the sixth week, the statistical result in Appendix U shows that there is significant difference in samples after being stored for 6 weeks ($p < 0.05$). Therefore it can be concluded that the sanshool content deteriorates over time and that the storage time exerts the highest impact on the decomposition rate while the effect of oxygen is relatively low. But due to the limited data, it could not be stated on which week the sanshool content gives significance difference as compared to the initial point. A similar reseach by Chuong (2014) also stated that dried andaliman stored in refrigeration temperature also decreased significantly after 4 weeks of storage period of time as compared to its initial point. Vacuum pacakging have not succeeded in maintaining the sanshool content of dried andaliman. It is suggested for the further research to have more datas in every weekly observation in order to determine the significant changes of sanshool content at a certain period of time.

CHAPTER V

CONCLUSION AND SUGGESTIONS

5.1 Conclusion

Fresh andaliman deteriorated after 4 days in refrigeration temperature. Dry andaliman stored in polypropylene bag lost its trigeminal sensation after being stored 1 week in high temperature (45 °C) while andaliman stored in vacuum packaging and glass bottle could retain its trigeminal sensation after 1 week stored in high temperature (45 °C).

Whole andaliman had significantly higher tingling sensation and lower moisture content. Higher temperature storage (45 °C) accelerated the decrease rate of tingling sensation and antioxidant activity. Moisture content, antioxidant activity, and tingling sensation scores of dried andaliman changed over time. The moisture content and IC_{50} value of antioxidant activity increased while tingling sensation scores decreased during storage treatments. The moisture content and antioxidant activity of dried andaliman had changed significantly on the second week, and the tingling sensation had decreased significantly on the third week.

Sanshool content of dried andaliman kept in different storage temperatures and different forms (whole and ground) showed a significant interaction between form and storage temperature. Whole andaliman stored at 35 °C has the highest sanshool content after storage treatments. Furthermore, sanshool content

deteriorated significantly over time. Dried andaliman lost approximately 10% of its sanshool content after being stored for six weeks.

Dried andaliman is recommended to be stored in whole form. The storage temperature that is sufficient for dried andaliman is 35 °C due to the ability to retain trigeminal sensation and sanshool content of dried andaliman until 6 weeks storage treatments.

5.2 Suggestions

The stability of dry andaliman should be further studied since it has many advantages as natural flavoring, functional food, and antimicrobial agent. The use of other packaging material, such as aluminum pouch might be used to prolong the shelf life of dried andaliman. The effect of different concentration of nitrogen gasses injected into glass packaging might be used as a factor to determine the stability of dried andaliman. Longer storage time and larger sample sizes in analysis measurement could also improve the analysis of sanshool content and antioxidant activity stability in dried andaliman.

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