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Bioactive Compounds Profile of Solok Arabica Coffee Analyzed by GC-MS Method

Rince Alfia Fadri [a,*], Kesuma Sayuti [b], Novizar Nazir [b], Irfan Suliansyah [c], Henny Fitri Yanti [a]

[a] *Study Program of Food Technology, Payakumbuh State Agricultural Polytechnic,26271, Indonesia*
[b] *Agricultural Technology Faculty, University of Andalas, 25163, Indonesia*
[c] *Agriculture Faculty, Andalas University, 25163, Indonesia*
*Corresponding author: *rincealfia.fadri@politanipyk.ac.id*

***Abstract*—The type of coffee affects the compounds in it, the environment, and the soil. One of the areas in West Sumatera where a Coffee Producer is Solok Regency. The kind of coffee that is cultivated in Solok is Arabica coffee. The height of the planting point has an influence on the taste, so Solok Arabica coffee has a different taste from the flavors of other Arabica coffees that are widely spread throughout Indonesia. This study aims to determine the compounds contained in Solok Arabica coffee, which was roasted at 200ºC for 10 minutes. The compound detection in Solok Arabica coffee was carried out using gas chromatography-mass spectrometry (GC-MS). The results of GC-MS analysis detected 25 compounds in *Solok Arabica* coffee at 200ºC for 10 minutes, and 4 of them were detected in large quantities, namely pyridine, caffeine, *n-hexadecanoic* acid, and butyl 9,12-octadecadienoate with amounts between 70-97 m / z. Pyridine is a benzene derivative by replacing CH groups with N atoms, which are toxic to humans because they can cause nausea, vomiting, headache, dizziness, and irritation when in contact with the skin. Caffeine is the main bioactive component of the purine ring system in coffee. The sensory test method used to determine the typical Arabica Coffee of "Ranah Minang" is cupping to assess the taste of the coffee. *n-hexadecanoic* acid is a saturated fatty acid with *antioxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic*, and *anti-inflammatory* activity.**

***Keywords*—Arabica; coffee; bioactive; compounds.**

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I. INTRODUCTION

Coffee is one of Indonesia's leading export commodities. The Central Statistics Agency (BPS) data shows that Indonesia exported 277,411.2 tons of coffee in 2018. One region that produces coffee in Indonesia is Solok Regency, West Sumatra. The coffee varieties cultivated in the Solok area are Arabica coffee and Robusta coffee. Solok arabica coffee production was 657.77 tons from the planted area of 6,630 hectares, while Robusta coffee production was 1,388 tons from an area of 16,996 hectares [1].

The place of growth and type of coffee affect the taste and aroma of the coffee produced. Soil elevation, temperature, humidity, type of coffee, and processing method affect the chemical composition, aroma, and taste of the coffee produced. The chemical composition of coffee beans depends on species, variety, and fruit ripeness. The environment also influences the method of harvesting the seeds and the conditions for which they are stored [2]. The value of a coffee bean is not only determined by its physical appearance, but also by its flavor characteristics. Coffee is consumed because of its distinctive taste and physiological effects as a refreshing drink. Given that coffee is an agricultural product that relies on aspects of flavor quality, the ultimate goal of coffee cultivation is a high-flavored bean product, which is determined by sensory testing [3].

The sensory test method carried out to determine the typical Arabica Coffee of "Ranah Minang" is cupping. This method used to assess the taste of the coffee. Due to base on its type, coffee has several different characteristics, that's why this method is considered good enough to distinguish the coffee characteristic. And the assessment characteristics is the aroma of dry coffee beans that have not been brewed but have been finely ground and wet coffee beans that have been brewed. Taste detection by the tongue is useful for translating what the coffee smells like. The flavor of coffee is a combination of perceptions recognized by the tongue and overall sense of smell. The main element of the value of brewed coffee is the flavor, which includes two elements at once. In general, organoleptic assessment, the flavor elements

are usually associated with other impressions such as temperature, coarseness/subtleness, etc. Flavor and aroma together form the basis of a complete coffee flavor assessment. It is a fact that other elements, such as the level of steeping heat, also determine aroma concerning aroma–forming compounds. The colder it is, the weaker the aroma value due to the low quantity of volatile compounds in the steeping water vapor [4].

The results of research on the Bourbon variety Arabica coffee showed that the higher the growing area, the higher the trigonelline content [5], [6], [7]. The same thing happened to the Robusta breed. Trigonelline is an alkaloid that causes many flavor compounds in coffee, such as alkyl-pyridines and pyrroles [8]. The results of other studies on Arabica coffee also showed an increase in glucose content and flavor attributes in line with the increasing place to grow [9].

Arabica coffee (Coffea arabica) has a better taste quality than other types of coffee. This type of coffee has a flat bean shape, dark green leaf color, and is wavy. Arabica coffee beans have characteristics in the form of a large enough bean size weighing 0.18 - 0.22 g/bean and a slightly brown bean color [10]. Solok arabica coffee is grown at an altitude of 1000 asl. The height of the planting point influences the taste so that *Solok Arabica* coffee has a different taste from the flavors of other Arabica coffees that are widely spread throughout Indonesia [11]. The process of roasting coffee not only changes the physical appearance of the coffee beans but also rearranges their chemical makeup. Roasting plays a huge role in determining the taste of the coffee. Unroasted coffee beans contain more sugar, protein, fat, and caffeine than roasted beans. The substances lost during the roasting process are replaced by other compounds that form the basic character of a cup of coffee [12].

There is a significant effect of air temperature factors as a result of differences in altitude on the proteinase enzyme activity and the quality of Arabica coffee. Arabica coffee cultivation in locations with lower average temperatures and higher altitudes generally undergo a period of perfect fruit ripening. Such conditions can support the biosynthesis of substances related to aroma and flavor characteristics [13]. The higher the growing place, the value of the total score of Arabica coffee quality tends to increase [14], [15]. The increase in the total score is also associated with changes in other climatic characteristics, such as mean temperature and precipitation rate [15].

One of the important stages in coffee processing is roasting because it can increase aroma formation and influence changes in the composition of biologically active compounds in coffee [12]. The aroma of a product, in many ways determines whether a product smells; even smell or smell is more complex than taste. The sensitivity of the sense of smell is usually higher than that of taste. Even the food industry considers odor tests very important because they can quickly assess a product [16]. The aroma of coffee arises as a result of volatile compounds that are captured by the human sense of smell. Volatile compounds that affect the aroma of roasted coffee are formed from the Maillard reaction or non-enzymatic browning reactions, free amino acid degradation, trigonelline degradation, sugar degradation, and degradation of phenolic compounds. This is because the distinctive aroma in coffee will slowly appear after the roasted beans are cooled. The longer it roasts, the more volatile compounds will evaporate, affecting ground coffee's aroma [11]. Based on this background, this study was carried out to find out the bioactive components contained in Solok Arabica coffee, which was roasted at 200°C for 10 minutes.

II. Materials and Method

A. Material

The material used in this research is arabica coffee from Solok. Sep Pak Plus C18 cartridges of the Waters brand (Milford, MA, USA) were used for solid-phase extraction. *Zinc sulfate heptahydrate* (*Carrez I*), potassium *hexacyanoferrate* (II) *trihydrate* (*Carrez II*), acrylamide standard, *methanol* (*HPLC* grade), and *acetone* (*GC grade*) were obtained from *Sigma Aldrich* (St. Louis, MO, USA).

B. Method

1) Sample Isolation Technic: The method reported by Şenyuva and Gökmen [17] was used for the isolation of acrylamide from samples. Each sample (5 g) was dissolved in a mixture of water (10 mL) and absolute ethanol (15 mL) by shaking vigorously for a minute, and then the mixture was kept at -20°C for 15 min. Each mixture was centrifuged at 15,000 g for 5 min at 4°C. Supernatants were acidified with glacial acetic acid until pH reached about 4–5. Afterward, Carrez I (1 mL) and Carrez II (1 mL) clearing solutions were added to the flasks, and then the mixture was shaken vigorously and kept at 4°C for 30 min. This solution was centrifuged at 15,000 g for 5 min at 4°C, and the supernatant was filtered through a 0.45 µm syringe nylon filter (*Sartorius, Goettingen*, Germany). The solvent was partially removed by a rotary evaporator (*Heidolph, HL/HB G3*) at 55°C, and then the evaporator vessel was washed with 2 mL of water, which was added to the solution in the vial.

2) SPE clean-up procedure: Sep Pak Plus C18 cartridges were placed in a manifold system and activated with 10 mL methanol and finally 10 mL rinsing water. The sample solution (5 mL) was loaded onto the column, and then the sorbents were dried. Acrylamide was eluted from the cartridges using 2 mL acetone.

3) Calibration standard: Stock standard solution of acrylamide (10 mg/mL) was prepared in acetone, and six different concentrations were used for the calibration curve. The calibration curve was obtained by plotting the peak areas against the concentration of standard acrylamide solutions. The LOD value was three times the chromatographic instrument's background noise. The extraction recovery was determined by spiking samples with acrylamide in three replicates, and they were extracted as previously described.

4) Chromatography and apparatus: An Agilent 7890A gas chromatography unit equipped with a 5975-mass detector (MSD), a 7693B automatic sampler, and an MSDCHEM (Agilent, Santa Clara, CA, USA) data system was used for the determination of acrylamide in coffee and tea samples. Analytes were separated in a fused silica capillary column DB-Wax. The carrier gas (helium) flow rate was 1 mL/min. The oven temperature program was as follows: initial temperature of 60°C, held for a minute, increased to 240°C at

20°C/min, held at 20 min. The injection port, detector, and ion source temperatures were 240, 250, and 230°C, respectively. The injection volume was 1 µL, and identification was determined using the selective ion monitoring (SIM) mode (m/z = 71).

III. Results and Discussion

Based on the analysis using GC-MS in Fig. 1. It can be seen that 25 bioactive compounds were detected in Solok arabica coffee, which was roasted at 200°C for 10 minutes. Of the 25 compounds detected, there were four main compounds: pyridine, caffeine, *n-hexadecanoic* acid, and butyl 9,12-octadecadienoate.

In this study, Solok arabica coffee was roasted at 200°C for 10 minutes, which is the optimum temperature for roasting coffee. The roasting at a temperature of 200°C with a time of about 10 minutes greatly affects the beans' physical, chemical, and flavor quality changes, which are also influenced by the operating conditions of the roaster machine. Also, it is influenced by the characteristics of coffee beans, including the type of coffee, moisture content, size, processing methods, and bioactive components in coffee [18].

A. Pyridine

Pyridine is one of the compounds detected in large quantities in Solok Arabica coffee, which was roasted at 200°C for 10 minutes using GC-MS. The pyridine ion measured by Solok Arabica coffee was detected as much as 90 m/z.

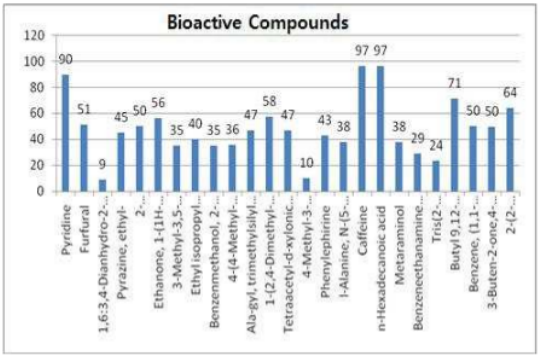

Fig. 1 The Bioactive Compounds in Solok Arabica Coffee that roasted in 200°C at 15 min

Pyridine is included in a group of aromatic heterocyclic amines which have the chemical structure of C_5H_5N with a molecular mass of 79.1 g/mol, boiling point 115°C, and density of 982 kg/m^3. Pyridine has synonyms azobenzene, azine, NCl-C55301, RCRA waste number U196[19]. The structure of the pyridine molecule can be seen in Fig. 2.

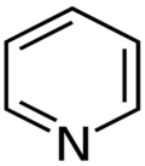

Fig. 2 Pyridine

Pyridine is a benzene derivative by replacing the CH group with an N atom, a hygroscopic, colorless liquid with a characteristic odor. This compound is similar to water, so it is often mixed with water or other organic solvents to manufacture various products such as medicines, vitamins, food flavorings, pesticides, paints, dyes, rubber products, adhesives, waterproofing fabrics, and nitrogen-containing plant [20]. However, pyridine will be dangerous in direct contact with humans. People who inhale pyridine will experience nausea, vomiting, headache, and dizziness. In addition, pyridine can also cause irritation, photosensitization, and contact dermatitis [19].

B. Caffeine

Caffeine is the main compound in coffee. Solok arabica coffee roasted at 200°C for 10 minutes detected 97 m / z of caffeine. Analysis of the compounds contained in Solok Arabica coffee used GC-MC.

Caffeine is an alkaloid from the methylxanthine group, which has the chemical structure of $C_8H_{10}N_4O_2$. Caffeine has a molecular weight of 194.19 g, a melting point of 236°C, a vapor point of 178°C of atmospheric pressure, and its solubility in water 2.17% [21]. The pyridine framework formula can be seen in Fig. 3.

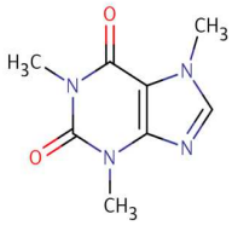

Fig. 3 Caffeine

Caffeine is the most important component of coffee's purine ring system [22]. The caffeine content in roasted coffee is higher than that of raw coffee [21]. During the roasting process, raw green beans, which have the characteristics of a soft, "grassy" smell and little / no taste, are transformed (transform) into roasted beans, which have unique aromatic characteristics and are rich in flavor. Coffee will also change from absorbing heat (endothermic) to producing heat (exothermic) during the roasting process. Various components that affect the taste of coffee created by the roasting process. During the roasting process, coffee beans undergo two processes: water evaporation at 100°C and pyrolysis reaction at 180-225°C. This reaction is a decomposition reaction of hydrocarbons, including carbohydrates, hemicellulose, and cellulose, in coffee beans. This reaction generally occurs after the roast temperature is above 180°C. At the pyrolysis stage, coffee undergoes chemical changes, including the charcoal of crude fiber, the formation of volatile compounds, the evaporation of acidic substances (the evolution of large amounts of CO_2 gas from the white roasting room), and the formation of a distinctive aroma of coffee [23].

Several studies have shown that caffeine affects human health, including being able to stimulate A1 and A2A adenosine receptors in the brain, reduce the potential for heart disease, affect cognition and mood, maintain liver and gastrointestinal health, and reduce the risk of developing Parkinson's disease and neurologic disease [24], [25].

C. n-Hexadecanoic acid

Another compound that was detected in large quantities in *Solok Arabica* coffee, which was roasted at 200°C for 10 minutes, besides pyridine and caffeine, was *n-hexadecanoic* acid. The amount of *n-hexadecanoic* acid detected on GC-MS was 97 m / z.

It is a saturated fatty acid that is found in animals, plants, and microorganisms. *n-Hexadecanoic* acid has a chemical structure of $C_{16}H_{32}O_2$ with a molar mass of 256.42 g / mol, a melting point of 62.9°C, a boiling point of 351°C, and a density of 853 kg / m3 [26]. The molecular structure of *n-Hexadecanoic* acid can be seen in Fig. 4.

The fat content in Arabica coffee is in the protective wax coating of the beans and in coffee oil. There are 5-hydroxytryptamine fatty acids in the wax layer from palmitic, arachidic, behenic, and lignoceric acids. Fat in coffee is one of the chemical compositions that makes coffee taste. The total fat content in Arabica coffee is between 2-6%, which is found in the protective wax layer of the beans. The increase in free fatty acids during storage will cause rancidity in the coffee grounds, affecting the taste and decreasing the quality of the coffee grounds. The resulting fat content is the same as that produced in rice coffee beans [27].

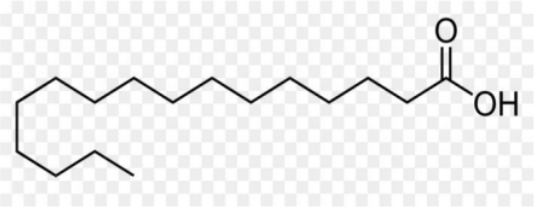

Fig. 4 n-Hexadecanoic Acid

Several types of saturated fatty acids are known to have antibacterial and antifungal properties [28]. *n-hexadecanoic* acid can function as an anti-inflammatory agent. This is evidenced by the ability of *n-hexadecanoic* acid to inhibit PLA2 (Phospholipase A2, E.C.3.1.1.4) [29]. *n-hexadecanoic* acid is a saturated fatty acid that has *antioxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic* activity [30], [31].

D. Butyl 9,12-octadecadienoate

In *Solok Arabica* coffee, which was roasted at 200°C for 10 minutes, Butyl 9,12-octadecadienoate compounds were also detected. The number of *Butyl 9,12-octadecadienoate* detected on *GC-MS* was 71 m / z. *Butyl 9,12-octadecadienoate* or *butyl linoleate* is a compound that has the molecular formula $C_{22}H_{40}O_2$, molecular weight 336.6 g / mol, boiling point 417.1oC at 760 mmHg, density 0.88 g / cm3, and flash point 94.1oC. Apart from *butyl linoleate, butyl 9,12-octadecadienoate* has other names, namely *linoleic acid, butyl ester (7ci, 8ci)* | *(9z, 12z) -octadecane-9,12-dienoic acid butyl ester* | *9,12-octadecadienoicacid (z, z) -, butyl ester* | *butyl (9z, 12z) -octadeca-9,12-dienoate* | *n-butyl linoleate* | *9,12-octadecadienoicacid (9z, 12z) -, butyl ester* | *9,12-octadecenoicacid (z, z) -, butylester* | *einecs 220-121-4* | *12-octadecadienoicacid (z, z) -butylester*. The molecular structure of *butyl 9,12-octadecadienoate* can be seen in Fig. 5.

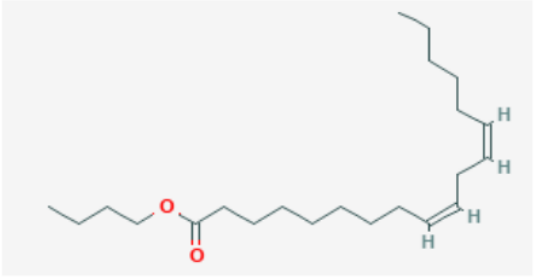

Fig. 5 Butyl 9,12-octadecadienoate

Butyl 9,12-octadecadienoate belongs to the group of heterocyclic organic compounds. Heterocyclic compounds are cyclic compounds in which the ring contains hetero atoms such as N, O, S, B, P and some metalloids such as Al, Sn, As, Cu. Based on the aroma, heterocyclic compounds are aromatic and non-aromatic. The most abundant heterocyclic compounds are heterocyclic compounds that bind to N (aza group), O (oxo group), and S (thio group).

The results of GC-MS analysis of *Solok Arabica* coffee roasted at 200°C for 105 minutes showed that 25 compounds were detected. Of the 25 compounds, four compounds were detected in large amounts, around 70-97 m / z. The four compounds are pyridine, caffeine, n-hexadecanoic acid, and butyl 9,12-octadecadienoate. Pyridine is a benzene derivative by replacing CH groups with N atoms, which are toxic to humans because they can cause nausea, vomiting, headache, dizziness, and irritation when in contact with skin. Caffeine is the main bioactive component of the purine ring system in coffee. The effects of caffeine on human health include stimulating A1 and A2A adenosine receptors in the brain, reducing the potential for heart disease, affecting cognition and mood, maintaining liver and gastrointestinal health, and reducing the risk of developing Parkinson's disease and neurologic disease. *n-hexadecanoic* acid is a saturated fatty acid with *antioxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic*, and *anti-inflammatory* activity. In addition to pyridine, caffeine, and *n-hexadecanoic* acid, one more compound was detected in large quantities, *butyl 9,12-octadecadienoate*. This compound is a heterocyclic organic compound.

IV. Conclusions

The results of GC-MS analysis of *Solok Arabica* coffee roasted at 200°C for 105 minutes showed that 25 compounds were detected. Of the 25 compounds, four compounds were detected in large amounts around 70-97 m / z. The four compounds are pyridine, caffeine, *n-hexadecanoic* acid, and *butyl 9,12-octadecadienoate*. Pyridine is a benzene derivative

by replacing CH groups with N atoms, which are toxic to humans because they can cause nausea, vomiting, headache, dizziness, and irritation when in contact with the skin. Caffeine is the main bioactive component of the purine ring system in coffee. The effects of caffeine on human health include stimulating A1 and A2A adenosine receptors in the brain, reducing the potential for heart disease, affecting cognition and mood, maintaining liver and gastrointestinal health, and reducing the risk of developing Parkinson's disease and neurologic disease. *n-hexadecanoic* acid (palmitic acid) is a saturated fatty acid that is found in animals, plants, and microorganisms. *n-hexadecanoic* acid is a saturated fatty acid that has antioxidant, *hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic*, and *anti-inflammatory* activity. In addition to *pyridine*, *caffeine*, and *n-hexadecanoic* acid, one more compound was detected in large quantities, butyl 9,12-octadecadienoate. This compound is a heterocyclic organic compound.

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