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

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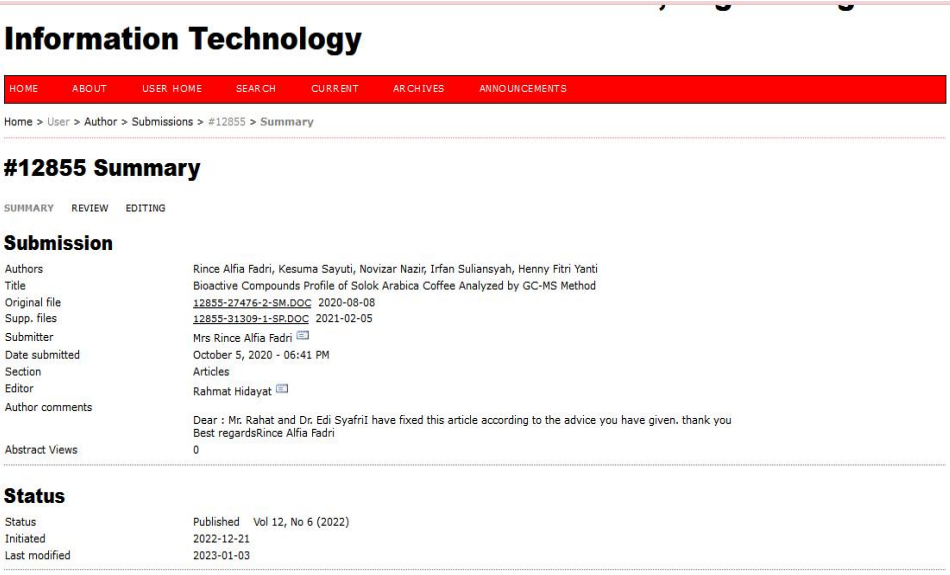

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#12855 Summary

SUMMARY REVIEW EDITING

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Title and Abstract

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

Indexing

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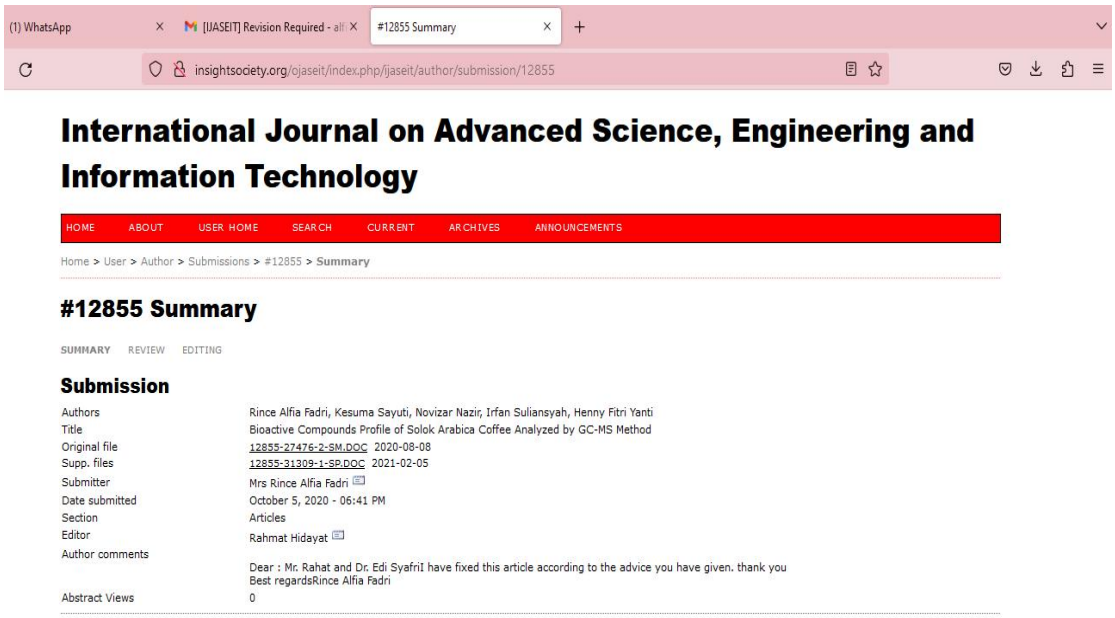

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Manuscript (Original File)

Aroma Compounds of Solok Arabica Coffee Analyzed by GC-MS Method

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Abstract— The compounds in coffee depend on the type of coffee, the environment, and the soil. Solok is one of the coffee-producing areas in West Sumatra. A kind of coffee that is cultivated in Solok is Arabica coffee. The purpose of this study was to determine the compounds contained in Solok Arabica coffee, which was roasted at 200°C 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.

***Keywords*— Kopi Arabica, Solok, pyridine, caffeine, n-hexadecanoic acid.**

I. INTRODUCTION

Coffee is one of Indonesia's leading export commodities. Data from the Central Statistics Agency (BPS) shows that Indonesia exported 277,411.2 tons of coffee in 2018. One of the regions that produce 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 16,396 hectares (BPS, 2018)

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 [1]. The chemical composition of coffee beans depends on species, variety, and fruit ripeness. The environment also influenced 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 to determine the profile of Arabica coffee in the specialty of the Minang realm was carried out by the cupping method. Coffee cupping is a method used to assess the taste of the coffee. Because each type of coffee has several different characteristics, that's why this coffee cupping is considered good enough to distinguish the characteristics of the coffee. Some of the coffee cupping method assessment characteristics are fragrance, the aroma of the coffee that will be smelled, namely the dry smell of coffee beans that have not been brewed but have been finely ground, and also the wet smell of brewed coffee beans. Flavor, this process the tongue is used to translate what has been smelled from the coffee was detected by the tongue or not. The flavor is a combination of perceptions between taste recognized by the tongue and aroma that is recognized by the olfactory organ as a whole. The flavor component of coffee is the main element of the value of the brewing coffee because it includes two elements at once. In general organoleptic assessment, the flavor elements are usually associated with other impressions such as temperature, coarseness/subtleness, etc. In the assessment of coffee flavor usually only includes taste and aroma elements simultaneously and intact. It is true that other elements such as the heat level of the brew also determine especially the aroma, which is related to the level of volatility of the aroma-forming compounds. The colder usually, the weaker the value of the aroma, as a result of the lower the 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) is a coffee that has a better taste quality than other types of coffee. This type of coffee has a flat bean shape, dark green leaf color, and 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 has an influence on 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, 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 or not, 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 to be very important because they can quickly provide an assessment of 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, which will affect the aroma of ground coffee[11].

Based on this background, this study was carried out with the aim of knowing the bioactive components contained in Solok Arabica coffee, which was roasted at 200ºC for 10 minutes.

II. THE MATERIAL AND METHOD

A. Material and Method

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).

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 supernatant were 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.

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 sorbents were dried. Acrylamide was eluted from the cartridges using 2 mL acetone.

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 defined as three times the background noise of the chromatographic instrument. The extraction recovery was determined by spiking samples with acrylamide in three replicates, and they were extracted as previously described.

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

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 200ºC for 10 minutes. Of the 25 compounds detected, there were 4 main compounds, namely pyridine, caffeine, n-hexadecanoic acid, and butyl 9,12-octadecadienoate.

In this study, Solok arabica coffee was roasted at 200ºC for 10 minutes, which is the optimum temperature for roasting coffee. The roasting at a temperature of 200ºC with a time of about 10 minutes greatly affects the physical, chemical, and flavor quality changes of the coffee beans, which are also influenced by the operating conditions of the roaster machine, as well as the characteristics of coffee beans including the type of coffee, moisture content, size. , and 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 200ºC for 10 minutes using GC-MS. The pyridine ion measured by Solok Arabica coffee was detected as much as 90 m/z.

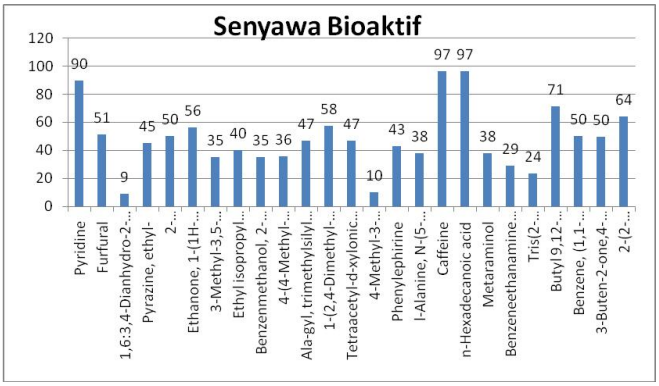

Fig. 1 The Bioactive Compounds in Solok Arabica Coffee that roasted in 200ºC at 15 min

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 115ºC, and density 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.

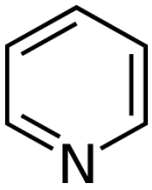

Fig. 2 Pyridine

Pyridine is a derivative of benzene by replacing the CH group with an N atom, which is 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 in the manufacture of 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 200ºC 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 236ºC, its vapor point at 178ºC of atmospheric pressure, and its solubility in water 2.17%[21]. The pyridine framework formula can be seen in Fig. 3.

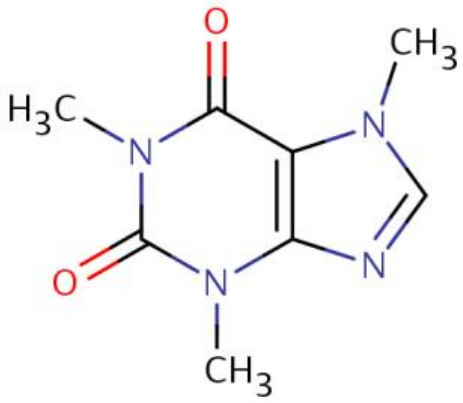

Fig 3. Caffeine

Caffeine is the most important component of the purine ring system in coffee to date[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, namely water evaporation at 100ºC and pyrolysis reaction at 180-225ºC. 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 180ºC. 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 200ºC 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.

n-Hexadecanoic acid (palmitic acid) 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 62.9ºC, a boiling point of 351ºC, 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. In the wax layer, there are 5-hydroxytryptamine fatty acids from palmitic, arachidic, behenic, and lignoceric acids. Fat in coffee is one of the chemical compositions of coffee 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, which will affect the taste and decrease the quality of the coffee grounds. The resulting fat content is the same as that produced in rice coffee beans [27].

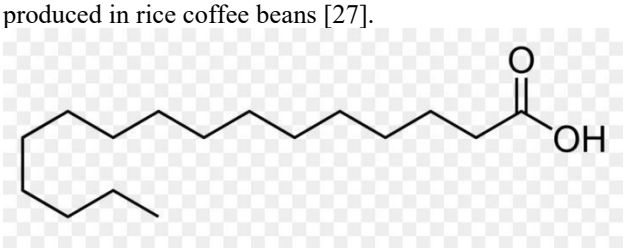

Fig 4. n-Hexadecanoic Acid

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 anti-

oxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic activity[30].

D. Butyl 9,12-octadecadienoate

In solok arabica coffee, which was roasted at 200ºC 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.

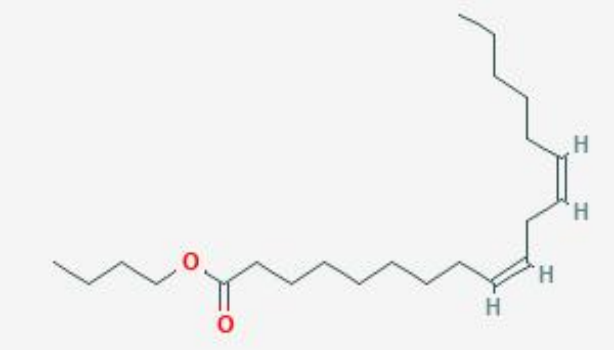

Fig 3. Butyl 9,12-octadecadienoate

Butyl 9,12-octadecadienoate termasuk dalam kelompok senyawa organik heterosiklik. Senyawa heterosiklik adalah senyawa siklik yang pada cincinnya terdapat atom hetero seperti N, O, S, B, P dan beberapa metaloid seperti Al, Sn, As, Cu. Berdasarkan aroma, senyawa heterosiklik ada yang aromatis dan non-aromatis. Senyawa heterosiklik yang paling banyak adalah senyawa heterosiklik yang berikatan dengan N (golongan aza), O (golongan okso), dan S (golongan tio).

IV. CONCLUSIONS

The results of GC-MS analysis of solok arabica coffee roasted at 200ºC for 105 minutes showed that 25 compounds were detected. Of the 25 compounds, 4 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 derivative of benzene by replacing CH groups with N atoms, which have toxicity 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 (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 anti-oxidant, 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, there is one more compound that was detected in large quantities, namely butyl 9,12-octadecadienoate. This compound is a heterocyclic organic compound.

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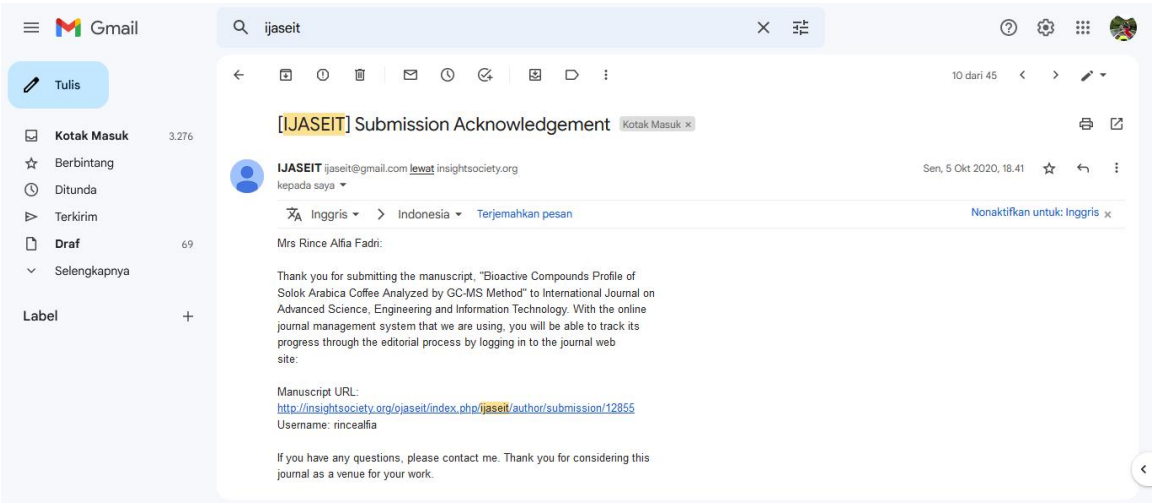

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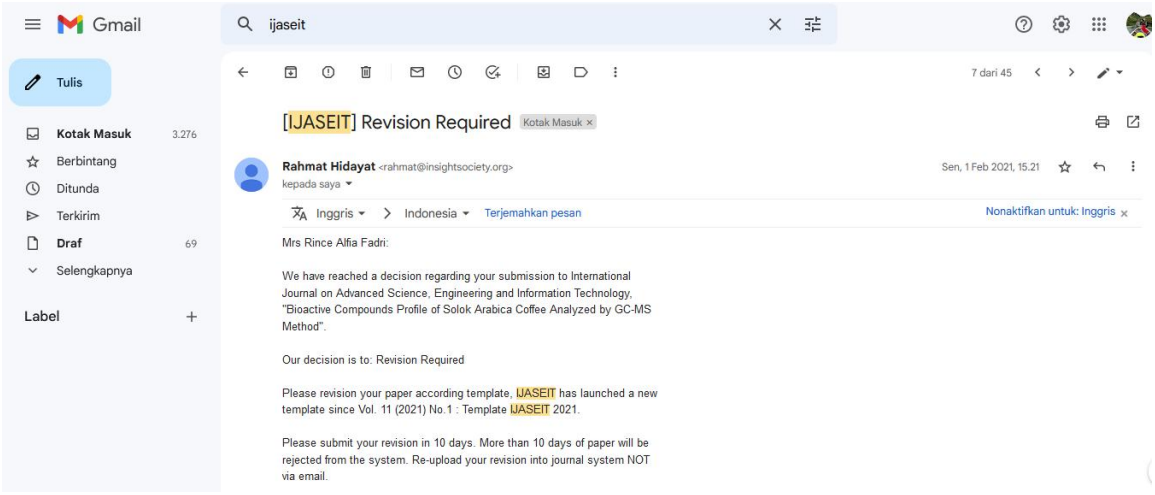

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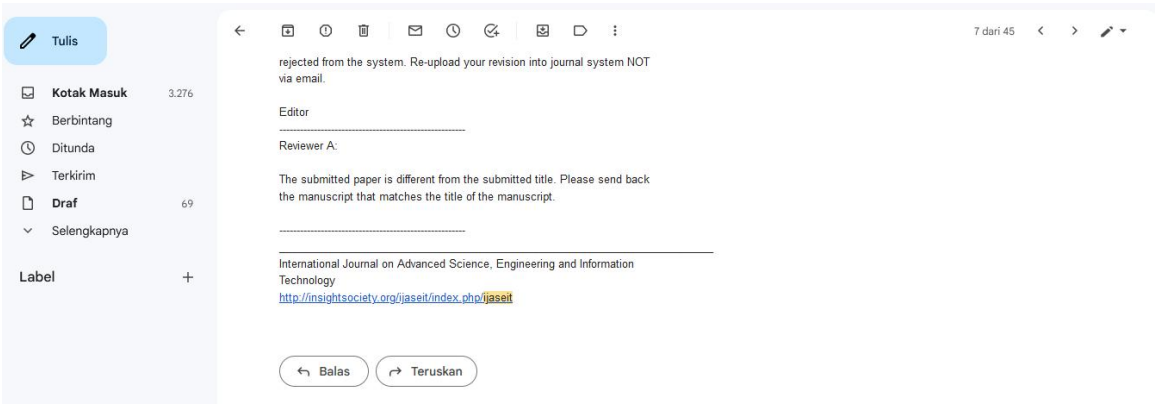

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4. Hasil Revisi Pertama

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

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Abstract— The compounds in coffee depend on the type of coffee, the environment, and the soil. Solok is one of the coffee-producing areas in West Sumatra. A kind of coffee that is cultivated in Solok is Arabica coffee. The purpose of this study was 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.

***Keywords*— Kopi Arabica, Solok, pyridine, caffeine, n-hexadecanoic acid.**

INTRODUCTION

Specialty coffee quality is focused on the texture, aroma and taste of the drink, but focusing mainly on the flavour compounds, the balance of biologically active compounds is disrupted. The coffee composition of the biologically active compounds and flavour compounds are influenced differently by almost all technological processes. It has been proven that green coffee has approximately 100 different volatile compounds and 950 volatile compounds in roasted coffee. However, only about 20 of them can significantly affect the formation of flavour and aroma.

The sensory test to determine the profile of Arabica coffee in the specialty of the Minang realm was carried out by the cupping method. Coffee cupping is a method used to assess the taste of the coffee. Because each type of coffee has several different characteristics, that's why this coffee cupping is considered good enough to distinguish the characteristics of the coffee. Some of the coffee cupping method assessment characteristics are fragrance, the aroma of the coffee that will be smelled, namely the dry smell of coffee beans that have not been brewed but have been finely ground, and also the wet smell of brewed coffee beans. Flavor

Coffee is one of Indonesia's leading export commodities. Data from the Central Statistics Agency (BPS) shows that Indonesia exported 277,411.2 tons of coffee in 2018. One of the regions that produce 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 16,396 hectares (BPS, 2018)

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[1]. The chemical composition of coffee beans depends on species, variety, and fruit ripeness. The environment also influenced 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]. , this process the tongue is used to translate what has been smelled from the coffee was detected by the tongue or not. The flavor is a combination of perceptions between taste recognized by the tongue and aroma that is recognized by the olfactory organ as a whole. The flavor component of coffee is the main element of the value of the brewing coffee because it includes two elements at once. In general organoleptic assessment, the flavor elements are usually associated with other impressions such as temperature, coarseness/subtleness, etc. In the assessment of coffee flavor usually only includes taste and aroma elements simultaneously and intact. It is true that other elements such as the heat level of the brew also determine especially the aroma, which is related to the level of volatility of the aroma-forming compounds. The colder usually, the weaker the value of the aroma, as a result of the lower the 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) is a coffee that has a better taste quality than other types of coffee. This type of coffee has a flat bean shape, dark green leaf color, and 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 has an influence on 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, 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 or not, 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 to be very important because they can quickly provide an assessment of 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, which will affect the aroma of ground coffee[11].

Based on this background, this study was carried out with the aim of knowing the bioactive components contained in Solok Arabica coffee, which was roasted at 200ºC for 10 minutes.

THE MATERIAL 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

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 supernatant were 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.

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 sorbents were dried. Acrylamide was eluted from the cartridges using 2 mL acetone.

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 defined as three times the background noise of the chromatographic instrument. The extraction recovery was determined by spiking samples with acrylamide in three replicates, and they were extracted as previously described.

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).

V. 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 200ºC for 10 minutes. Of the 25 compounds detected, there were 4 main compounds, namely pyridine, caffeine, n-hexadecanoic acid, and butyl 9,12-octadecadienoate.

In this study, Solok arabica coffee was roasted at 200ºC for 10 minutes, which is the optimum temperature for roasting coffee. The roasting at a temperature of 200ºC with a time of about 10 minutes greatly affects the physical, chemical, and flavor quality changes of the coffee beans, which are also influenced by the operating conditions of the roaster machine, as well as the characteristics of coffee beans including the type of coffee, moisture content, size. , and 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 200ºC for 10 minutes using GC-MS. The pyridine ion measured by Solok Arabica coffee was detected as much as 90 m/z.

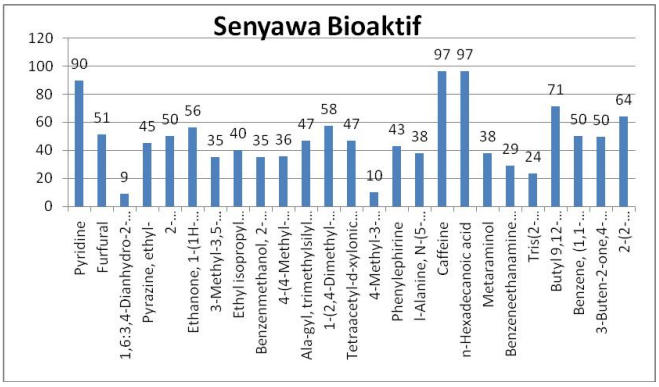

Fig. 1 The Bioactive Compounds in Solok Arabica Coffee that roasted in 200ºC at 15 min

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 115ºC, and density 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.

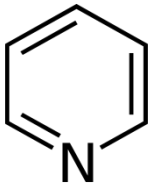

Fig. 2 Pyridine

Pyridine is a derivative of benzene by replacing the CH group with an N atom, which is 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 in the manufacture of 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 200ºC 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 236ºC, its vapor point at 178ºC of atmospheric pressure, and its solubility in water 2.17%[21]. The pyridine framework formula can be seen in Fig. 3.

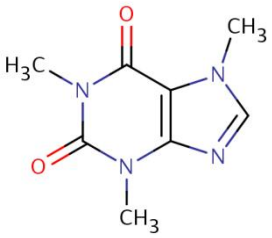

Fig 3. Caffeine

Caffeine is the most important component of the purine ring system in coffee to date[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, namely water evaporation at 100ºC and pyrolysis reaction at 180-225ºC. 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 180ºC. 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 200ºC 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.

n-Hexadecanoic acid (palmitic acid) 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 62.9ºC, a boiling point of 351ºC, 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. In the wax layer, there are 5-hydroxytryptamine fatty acids from palmitic, arachidic, behenic, and lignoceric acids. Fat in coffee is one of the chemical compositions of coffee 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, which will affect the taste and decrease the quality of the coffee grounds. The resulting fat content is the same as that produced in rice coffee beans [27].

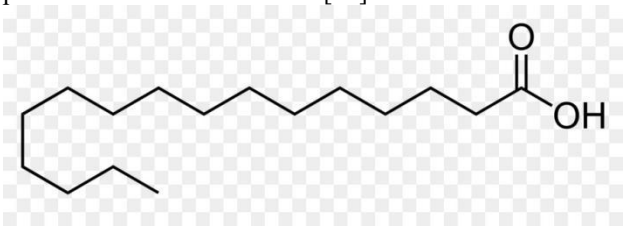

Fig 4. n-Hexadecanoic Acid

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 anti-oxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic activity[30].

D. Butyl 9,12-octadecadienoate

In solok arabica coffee, which was roasted at 200ºC 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.

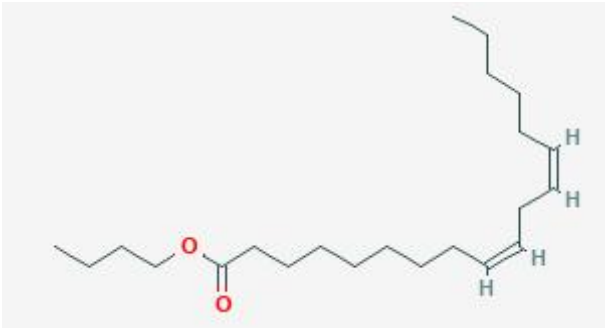

Fig 3. Butyl 9,12-octadecadienoate

Butyl 9,12-octadecadienoate termasuk dalam kelompok senyawa organik heterosiklik. Senyawa heterosiklik adalah senyawa siklik yang pada cincinnya terdapat atom hetero seperti N, O, S, B, P dan beberapa metaloid

seperti Al, Sn, As, Cu. Berdasarkan aroma, senyawa heterosiklik ada yang aromatis dan non-aromatis. Senyawa heterosiklik yang paling banyak adalah senyawa heterosiklik yang berikatan dengan N (golongan aza), O (golongan okso), dan S (golongan tio).

CONCLUSIONS

The results of GC-MS analysis of solok arabica coffee roasted at 200ºC for 105 minutes showed that 25 compounds were detected. Of the 25 compounds, 4 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 derivative of benzene by replacing CH groups with N atoms, which have toxicity 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 (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 anti-oxidant, 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, there is one more compound that was detected in large quantities, namely butyl 9,12-octadecadienoate. This compound is a heterocyclic organic compound.

ACKNOWLEDGMENT

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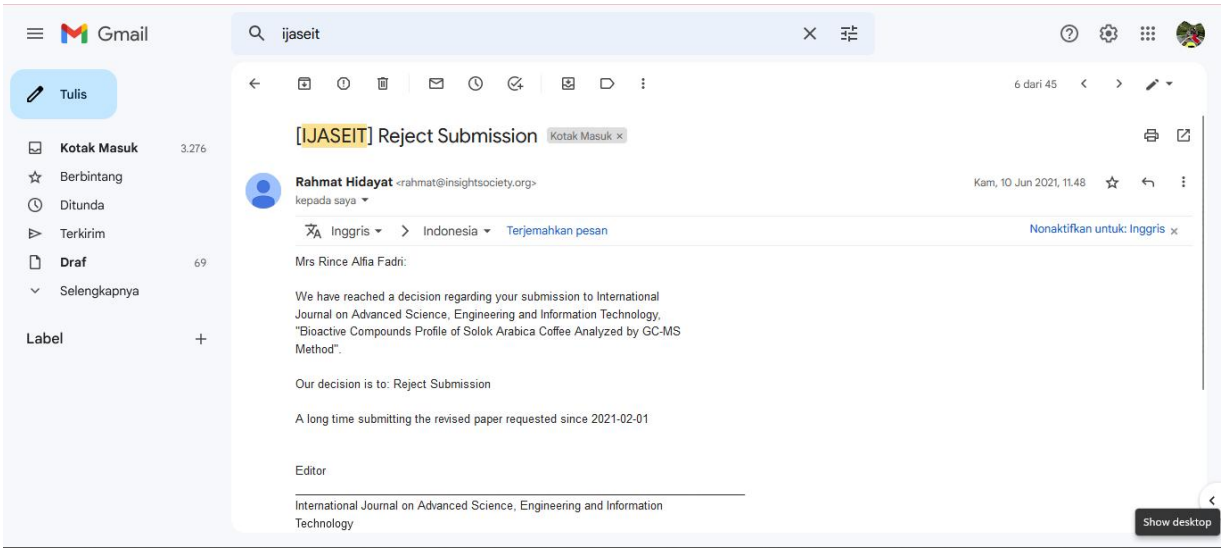
5. Reject Submission Pertama, 10 Juni 2021 Jam 11.48 WIB



Gambar 5. Reject Submission Pertama, 10 Juni 2021 Jam 11.48 WIB

Narasi dari Editor

Mrs Rince Alfia Fadri:

We have reached a decision regarding your submission to International Journal on Advanced Science, Engineering and Information Technology, "Bioactive Compounds Profile of Solok Arabica Coffee Analyzed by GC-MS Method".

Our decision is to: Reject Submission

A long time submitting the revised paper requested since 2021-02-01

Editor

International Journal on Advanced Science, Engineering and Information Technology
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6. Submission manuscript kedua Tanggal 10 Oktober 2021

Manuscrip Kedua

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

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Abstract— The compounds in coffee depend on the type of coffee, the environment, and the soil. Solok is one of the coffee-producing areas in West Sumatra. A kind of coffee that is cultivated in Solok is Arabica coffee. The purpose of this study was to determine the compounds contained in Solok Arabica coffee, which was roasted at 200^{o}C 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 200^{o}C 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.

***Keywords*— Kopi Arabica, Solok, pyridine, caffeine, n-hexadecanoic acid.**

INTRODUCTION

Specialty coffee quality is focused on the texture, aroma and taste of the drink, but focusing mainly on the flavour compounds, the balance of biologically active compounds is disrupted. The coffee composition of the biologically active compounds and flavour compounds are influenced differently by almost all technological processes. It has been proven that green coffee has approximately 100 different volatile compounds and 950 volatile compounds in roasted coffee. However, only about 20 of them can significantly affect the formation of flavour and aroma.

The sensory test to determine the profile of Arabica coffee in the specialty of the Minang realm was carried out by the cupping method. Coffee cupping is a method used to assess the taste of the coffee. Because each type of coffee has several different characteristics, that's why this coffee cupping is considered good enough to distinguish the characteristics of the coffee. Some of the coffee cupping method assessment characteristics are fragrance, the aroma of the coffee that will be smelled, namely the dry smell of coffee beans that have not been brewed but have been finely ground, and also the wet smell of brewed coffee beans. Flavor

Coffee is one of Indonesia's leading export commodities. Data from the Central Statistics Agency (BPS) shows that Indonesia exported 277,411.2 tons of coffee in 2018. One of the regions that produce 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 16,396 hectares (BPS, 2018)

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[1]. The chemical composition of coffee beans depends on species, variety, and fruit ripeness. The environment also influenced 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]. , this process the tongue is used to translate what has been smelled from the coffee was detected by the tongue or not. The flavor is a combination of perceptions between taste recognized by the tongue and aroma that is recognized by the olfactory organ as a whole. The flavor component of coffee is the main element of the value of the brewing coffee because it includes two elements at once. In general organoleptic assessment, the flavor elements are usually associated with other impressions such as temperature, coarseness/subtleness, etc. In the assessment of coffee flavor usually only includes taste and aroma elements simultaneously and intact. It is true that other elements such as the heat level of the brew also determine especially the aroma, which is related to the level of volatility of the aroma-forming compounds. The colder usually, the weaker the value of the aroma, as a result of the lower the 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) is a coffee that has a better taste quality than other types of coffee. This type of coffee has a flat bean shape, dark green leaf color, and 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 has an influence on 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, 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 or not, 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 to be very important because they can quickly provide an assessment of 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, which will affect the aroma of ground coffee[11].

Based on this background, this study was carried out with the aim of knowing the bioactive components contained in Solok Arabica coffee, which was roasted at 200^{o}C for 10 minutes.

THE MATERIAL 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

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 supernatant were 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.

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 sorbents were dried. Acrylamide was eluted from the cartridges using 2 mL acetone.

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 defined as three times the background noise of the chromatographic instrument. The extraction recovery was determined by spiking samples with acrylamide in three replicates, and they were extracted as previously described.

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).

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^{o}C for 10 minutes. Of the 25 compounds detected, there were 4 main compounds, namely pyridine, caffeine, n-hexadecanoic acid, and butyl 9,12-octadecadienoate.

In this study, Solok arabica coffee was roasted at 200^{o}C for 10 minutes, which is the optimum temperature for roasting coffee. The roasting at a temperature of 200^{o}C with a time of about 10 minutes greatly affects the physical, chemical, and flavor quality changes of the coffee beans, which are also influenced by the operating conditions of the

roaster machine, as well as the characteristics of coffee beans including the type of coffee, moisture content, size. , and processing methods and bioactive components in coffee[18].

E. Pyridine

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

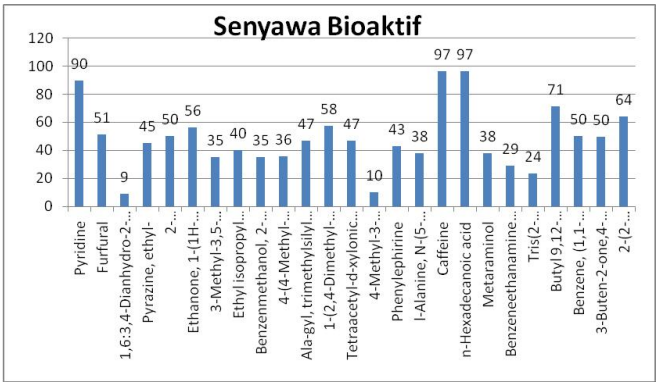

Fig. 1 The Bioactive Compounds in Solok Arabica Coffee that roasted in 200ºC at 15 min

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 115ºC, and density 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.

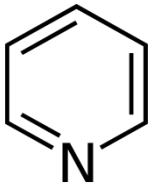

Fig. 2 Pyridine

Pyridine is a derivative of benzene by replacing the CH group with an N atom, which is 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 in the manufacture of 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].

F. Caffeine

Caffeine is the main compound in coffee. Solok arabica coffee roasted at 200ºC 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 236ºC, its vapor point at 178ºC of atmospheric pressure, and its solubility in water 2.17%[21]. The pyridine framework formula can be seen in Fig. 3.

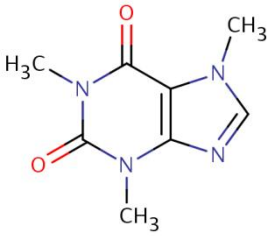

Fig 3. Caffeine

Caffeine is the most important component of the purine ring system in coffee to date[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, namely water evaporation at 100ºC and pyrolysis reaction at 180-225ºC. 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 180ºC. 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].

G. n-Hexadecanoic acid

Another compound that was detected in large quantities in solok arabica coffee, which was roasted at 200ºC 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.

n-Hexadecanoic acid (palmitic acid) 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 62.9ºC, a boiling point of 351ºC, 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. In the wax

layer, there are 5-hydroxytryptamine fatty acids from palmitic, arachidic, behenic, and lignoceric acids. Fat in coffee is one of the chemical compositions of coffee 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, which will affect the taste and decrease the quality of the coffee grounds. The resulting fat content is the same as that produced in rice coffee beans [27].

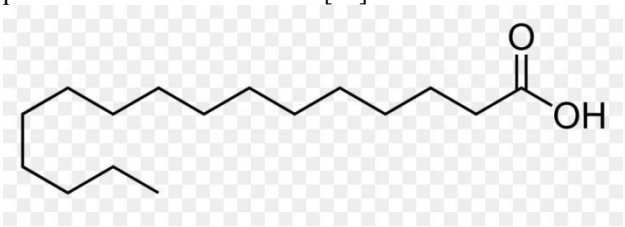

Fig 4. n-Hexadecanoic Acid

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 anti-oxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic activity[30].

H. Butyl 9,12-octadecadienoate

In solok arabica coffee, which was roasted at 200ºC 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.

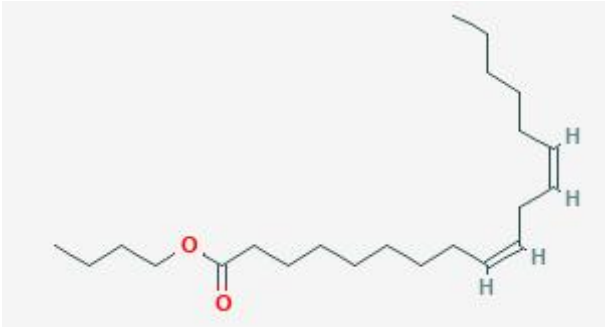

Fig 3. Butyl 9,12-octadecadienoate

Butyl 9,12-octadecadienoate termasuk dalam kelompok senyawa organik heterosiklik. Senyawa heterosiklik adalah senyawa siklik yang pada cincinnya terdapat atom hetero seperti N, O, S, B, P dan beberapa metaloid seperti Al, Sn, As, Cu. Berdasarkan aroma, senyawa heterosiklik ada yang aromatis dan non-aromatis. Senyawa heterosiklik yang paling banyak adalah senyawa heterosiklik yang berikatan dengan N (golongan aza), O (golongan okso), dan S (golongan tio).

CONCLUSIONS

The results of GC-MS analysis of solok arabica coffee roasted at 200ºC for 105 minutes showed that 25 compounds were detected. Of the 25 compounds, 4 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 derivative of benzene by replacing CH groups with N atoms, which have toxicity 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 (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 anti-oxidant, 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, there is one more compound that was detected in large quantities, namely butyl 9,12-octadecadienoate. This compound is a heterocyclic organic compound.

ACKNOWLEDGMENT

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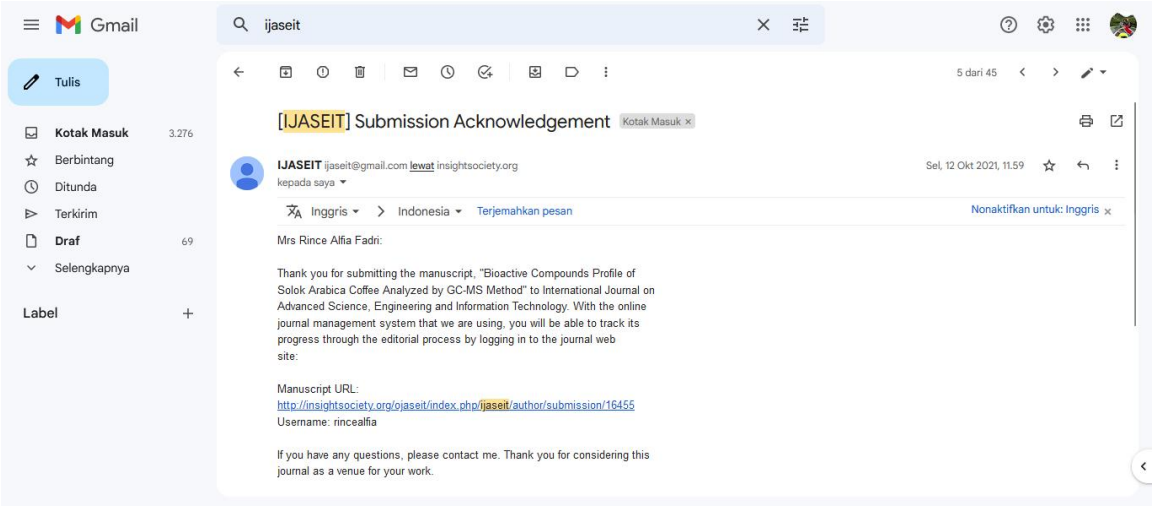
7. Balasan submission manuscript kedua diterima 12 Oktober 2021 Jam 11.59 WIB



Gambar 6. Balasan dari editor : submission manuscript II Tanggal 12 Oktober 2021 Jam 11.59 WIB

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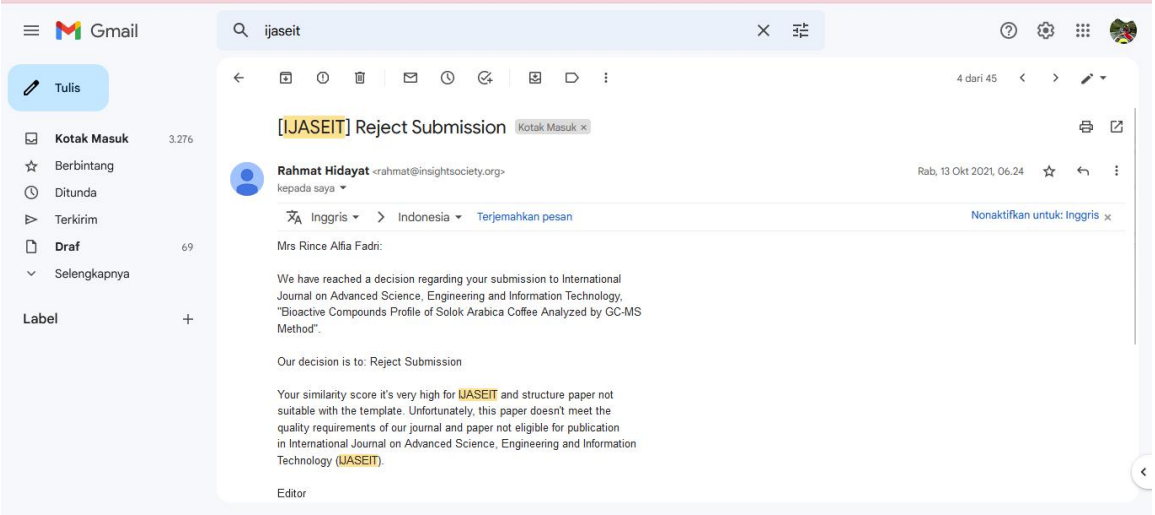

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Bioactive Compounds Profile of Solok Arabica Coffee Analyzed by GC-MS Method

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***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.

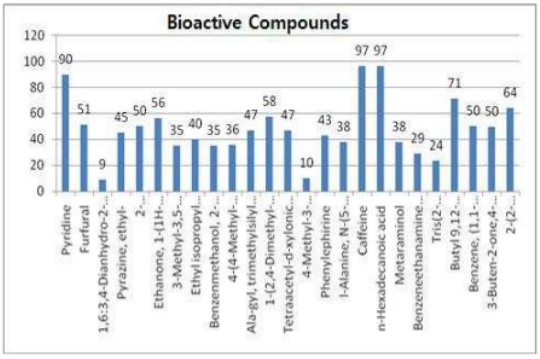

Fig. 1 The Bioactive Compounds in Solok Arabica Coffee that roasted in 200°C at 15 min

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.

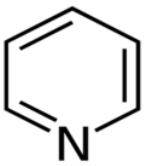

Fig. 2 Pyridine

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.

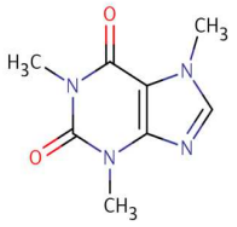

Fig. 3 Caffeine

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].

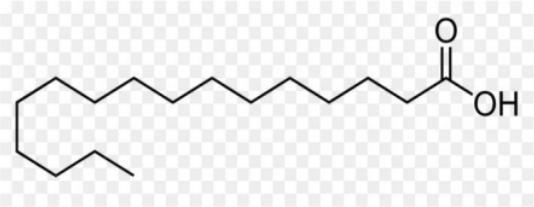

Fig. 4 n-Hexadecanoic Acid

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.

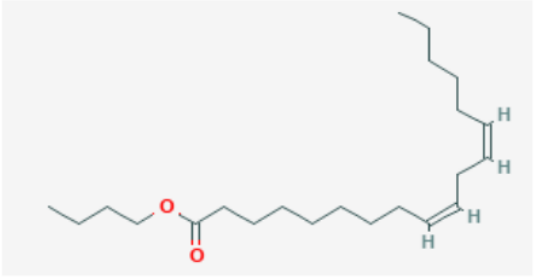

Fig. 5 Butyl 9,12-octadecadienoate

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.

ACKNOWLEDGMENT

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Dear **Rince Alfia Fadri**
Food Technology Politeknik Pertanian Negeri Payakumbuh, Indonesia
Coresponding Author: alfiarince@gmail.com

Title:	Bioactive Compounds Profile of Solok Arabica Coffee Analyzed by GC-MS Method
Author(s):	Rince Alfia Fadri, Kesuma Sayuti, Novizar Nazir, Irfan Suliansyah, Hanny Fitri Yanti
Paper-ID	12855

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The novelty: This research has successfully the bioactive components contained in Solok Arabica coffee, which was roasted at 200 ºC for 10 minutes. Solok arabica coffee is grown at an altitude of 1000 asl. The height of the planting point has an influence on the taste so that Solok Arabica coffee has a different taste from the flavors of other Arabica coffees that are widely spread throughout Indonesia. 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.

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Abstract— **ABSTRACK**

Abstract – The compounds in coffee depend on the type of coffee, the environment, and the soil. Solok is one of the coffee-producing areas in West Sumatra. A kind of coffee that is cultivated in Solok is Arabica coffee. The purpose of this study was to determine the compounds contained in Solok Arabica coffee, which was roasted at 200 ºC 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 200 ºC 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.

Keywords— arabica, Solok, pyridine, caffeine, n-hexadecanoic acid.

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11. Hasil Revisi Pertama Setelah Submission Kedua diterima

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

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Abstract— The compounds in coffee depend on the type of coffee, the environment, and the soil. Solok is one of the coffee-producing areas in West Sumatra. A kind of coffee that is cultivated in Solok is Arabica coffee. The purpose of this study was 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.

***Keywords*— Kopi Arabica, Solok, pyridine, caffeine, n-hexadecanoic acid.**

I. INTRODUCTION

Coffee is one of Indonesia's leading export commodities. Data from the Central Statistics Agency (BPS) shows that Indonesia exported 277,411.2 tons of coffee in 2018. One of the regions that produce 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 16,396 hectares (BPS, 2018)

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 [1]. The chemical composition of coffee beans depends on species, variety, and fruit ripeness. The environment also influenced 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 to determine the profile of Arabica coffee in the specialty of the Minang realm was carried out by the cupping method. Coffee cupping is a method used to assess the taste of the coffee. Because each type of coffee has several different characteristics, that's why this coffee cupping is considered good enough to distinguish the characteristics of the coffee. Some of the coffee cupping method assessment characteristics are fragrance, the aroma of the coffee that will be smelled, namely the dry smell of coffee beans that have not been brewed but have been finely ground, and also the wet smell of brewed coffee beans. Flavor, this process the tongue is used to translate what has been smelled from the coffee was detected by the tongue or not. The flavor is a combination of perceptions between taste recognized by the tongue and aroma that is recognized by the olfactory organ as a whole. The flavor component of coffee is the main element of the value of the brewing coffee because it includes two elements at once. In general organoleptic assessment, the flavor elements are usually associated with other impressions such as temperature, coarseness/subtleness, etc. In the assessment of coffee flavor usually only includes taste and aroma elements simultaneously and intact. It is true that other elements such as the heat level of the brew also determine especially the aroma, which is related to the level of volatility of the aroma-forming compounds. The colder usually, the weaker the value of the aroma, as a result of the lower the 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) is a coffee that has a better taste quality than other types of coffee. This type of coffee has a flat bean shape, dark green leaf color, and 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 has an influence on 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, 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 or not, 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 to be very important because they can quickly provide an assessment

of 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, which will affect the aroma of ground coffee[11].

Based on this background, this study was carried out with the aim of knowing the bioactive components contained in Solok Arabica coffee, which was roasted at 200ºC for 10 minutes.

II. THE MATERIAL AND METHOD

A. Material and Method

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).

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 supernatant were 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.

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 sorbents were dried. Acrylamide was eluted from the cartridges using 2 mL acetone.

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 defined as three times the background noise of the chromatographic instrument. The extraction recovery was determined by spiking samples with acrylamide in three replicates, and they were extracted as previously described.

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

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 200ºC for 10 minutes. Of the 25 compounds detected, there were 4 main compounds, namely pyridine, caffeine, n-hexadecanoic acid, and butyl 9,12-octadecadienoate.

In this study, Solok arabica coffee was roasted at 200ºC for 10 minutes, which is the optimum temperature for roasting coffee. The roasting at a temperature of 200ºC with a time of about 10 minutes greatly affects the physical, chemical, and flavor quality changes of the coffee beans, which are also influenced by the operating conditions of the roaster machine, as well as the characteristics of coffee

beans including the type of coffee, moisture content, size. , and 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.

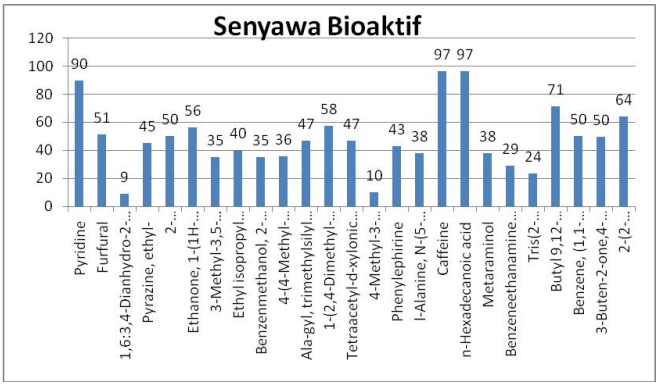

Fig. 1 The Bioactive Compounds in Solok Arabica Coffee that roasted in 200°C at 15 min

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 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.

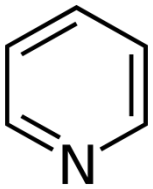

Fig. 2 Pyridine

Pyridine is a derivative of benzene by replacing the CH group with an N atom, which is 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 in the manufacture of 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, its vapor point at 178°C of atmospheric pressure, and its solubility in water 2.17%[21]. The pyridine framework formula can be seen in Fig. 3.

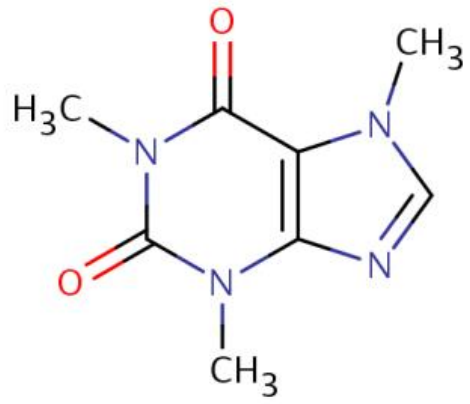

Fig 3. Caffeine

Caffeine is the most important component of the purine ring system in coffee to date[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, namely water evaporation at 100ºC and pyrolysis reaction at 180-225ºC. 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 180ºC. 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 200ºC 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.

n-Hexadecanoic acid (palmitic acid) 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 62.9ºC, a boiling point of 351ºC, 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. In the wax layer, there are 5-hydroxytryptamine fatty acids from palmitic, arachidic, behenic, and lignoceric acids. Fat in coffee is one of the chemical compositions of coffee 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, which will affect the taste and decrease the quality of the coffee grounds. The resulting fat content is the same as that produced in rice coffee beans [27].

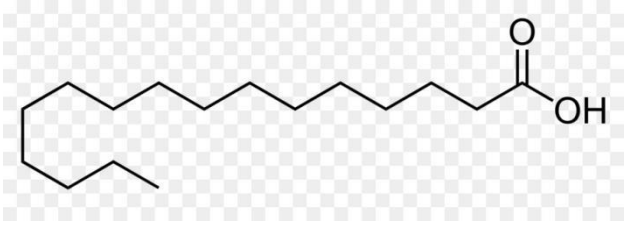

Fig 4. n-Hexadecanoic Acid

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 anti-oxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic activity[30].

D. Butyl 9,12-octadecadienoate

In solok arabica coffee, which was roasted at 200ºC 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.

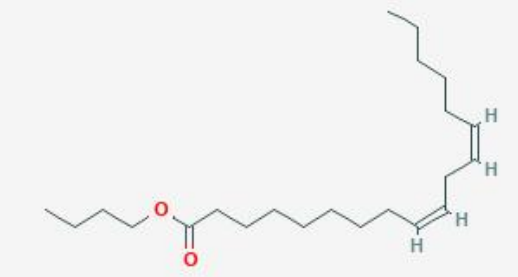

Fig 3. Butyl 9,12-octadecadienoate

Butyl 9,12-octadecadienoate termasuk dalam kelompok senyawa organik heterosiklik. Senyawa heterosiklik adalah senyawa siklik yang pada cincinnya terdapat atom hetero seperti N, O, S, B, P dan beberapa metaloid seperti Al, Sn, As, Cu. Berdasarkan aroma, senyawa heterosiklik ada yang aromatis dan non-aromatis. Senyawa heterosiklik yang paling banyak adalah senyawa heterosiklik yang berikatan dengan N (golongan aza), O (golongan okso), dan S (golongan tio).

IV. CONCLUSIONS

The results of GC-MS analysis of solok arabica coffee roasted at 200ºC for 105 minutes showed that 25 compounds were detected. Of the 25 compounds, 4 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 derivative of benzene by replacing CH groups with N atoms, which have toxicity 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 (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 anti-oxidant, 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, there is one more compound that was detected in large quantities, namely butyl 9,12-octadecadienoate. This compound is a heterocyclic organic compound.

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REVIEW FORM

14 th November 2021 y
Ref. No. 7/ReV/IJASEIT/VI/2021

Dear **Rince Alfia Fadri**
Food Technology Politeknik Pertanian Negeri Payakumbuh, Indonesia
Coresponding Author : alfiarince@gmail.com

Title:	Bioactive Compounds Profile of Solok Arabica Coffee Analyzed by GC-MS Method
Author(s):	Rince Alfia Fadri, Kesuma Sayuti, Novizar Nazir, Irfan Suliansyah, Hanny Fitri Yanti
Paper-ID	12855

A. Technical aspects

	0	1	2	3	4	5
1. The paper is within the scope of the Journal.	□	□	□	□	■	□
2. The paper is original.	□	□	□	□	□	■
3. The paper is free of technical errors.	□	□	□	□	■	□

B. Communications aspects

	0	1	2	3	4	5
1. The paper is clearly readable.	□	□	□	□	■	□
2. The figures are clear & do clearly convey the intended message.	□	□	□	□	□	■
3. The length of the paper is appropriate.	□	□	□	□	■	□

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C. Comments to the authors (You may use another sheet of paper.)

Thank you very much for the submission through the online system. The manuscript has been reviewed. Please look into this and resubmission your manuscript after revision. Please find the revision in the attachment!

The novelty: This research has successfully the bioactive components contained in Solok Arabica coffee, which was roasted at 200ºC for 10 minutes. Solok arabica coffee is grown at an altitude of 1000 asl. The height of the planting point has an influence on the taste so that Solok Arabica coffee has a different taste from the flavors of other Arabica coffees that are widely spread throughout Indonesia. 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.

The Title: The title should be made more attractive and have novelty and avoid writing research locations. The title of the article should be written briefly and clearly. The title of the article should first state the main idea of the new article, followed by other explanations. The title of the article must indicate exactly the problem to be raised. The **title** summarizes the main idea or ideas of your study. A good **title contains** the fewest possible words that adequately describe the contents and/or purpose of your research paper. The **title** is without doubt the part of a paper that is read the most, and it is usually read first. The title of this paper is good and informative.

The abstract: has already explained, "What is the importance of research". The abstract should contain: the problem behind the research; the main purpose of research; types of research; subjects involved in the research (population/sample for experimental research); data collection methods and instrumentation; data analysis method used; the main results of the research; conclusions and research implications

The research objective should use operational verbs that are high order thinking (avoid: knowing, use: analyzing, exploring, testing.[An abstract should be between 150-250 words]. Please improve the English, use simple sentence and provide the implication of research

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Abstract— **ABSTRACK**

Abstract – The compounds in coffee depend on the type of coffee, the environment, and the soil. Solok is one of the coffee-producing areas in West Sumatra. A kind of coffee that is cultivated in Solok is Arabica coffee. The purpose of this study was 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.

Keywords— arabica, Solok, pyridine, caffeine, n-hexadecanoic acid.

The Introduction typically occupies 10-15% of the paper. GAP Analysis: Explaining the gap between the expectations to be achieved and the facts/circumstances that are currently happening (the gap between das sollen and das sein). So that raises an urgency to carry out research on the chosen topic. The **introduction should** consists of two parts: It **should** include a few general statements about the subject to provide a background to your paper and to attract the reader's attention. It **should** try to explain why you are writing the paper. The introduction section has included a general introduction, problem definition, problem solution, study motivation, aims and objectives, gaps in the literature.

The introduction is already mentioned in the Introduction.

The Materials and methodology is good. The methods have described how the research question was answered, explain how the results were analysed. Method. In general, the submission contains 4 main aspects, namely the type, approach, and brief research procedure used; research subjects/participants; data collection methods & instruments; and data analysis methods. In the methodology, avoid writing down the context (for example; interviews are... quantitative is... etc.). Illustrate the research design with a chart or picture. Include the instrument grid used, and provide an explanation of the instrument validation process.

Materials and methods has been written in more detailed

Results and Discussion in the discussion avoid using Numbering and Bulleting. Please make it into a paragraph by adding a connecting sentence. Have included findings, comparison with prior studies, causal arguments, and deductive arguments. Data on the results should be presented with graphs or tables to make it more interesting. avoid anything conceptual. The conclusion is in the form of a brief description of the findings / research questions and not rewriting the data on the results and discussion with a solution sentence.

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Result and discussion has been written in accordance with scientific principles

Conclusion:

The Conclusion has been written in relation to the objectives included in the introduction.

Reference:
The author has added references to the publication, which has been published for the past three years, according to the reviewer's advice.

Decision: As a result of research with an appropriate methodology, **this paper is ACCEPTED for publication**

Additional Comments:

There are some grammatical mistakes and some mistakes in Punctuation.

D. Recommendation (Tick one)

1. Accepted without modifications. ☐
2. Accepted with minor corrections. ■
3. Accepted with major modification. ☐
4. Rejected. ☐

E. Comments to the editors (These comments will not be sent to the authors)

Please makes sure that all reviewers comment already answered by the author and fixed it in the manuscript.

Sincerely,

Regards,

Rahmat Hidayat

Editor in Chief
International Journal on Advanced Science, Engineering and Information Technology
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Bioactive Compounds Profile of Solok Arabica Coffee Analyzed by GC-MS Method

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Abstract— The compounds in coffee depend on the type of coffee, the environment, and the soil. Solok is one of the coffee-producing areas in West Sumatra. A kind of coffee that is cultivated in Solok is Arabica coffee. The purpose of this study was 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.

***Keywords*— Kopi Arabica, Solok, pyridine, caffeine, n-hexadecanoic acid.**

I. INTRODUCTION

The sensory test to determine the profile of Arabica coffee in the specialty of the Minang realm was carried out by the cupping method. Coffee cupping is a method used to assess the taste of the coffee. Because each type of coffee has several different characteristics, that's why this coffee cupping is considered good enough to distinguish the characteristics of the coffee. Some of the coffee cupping method assessment characteristics are fragrance, the aroma of the coffee that will be smelled, namely the dry smell of coffee beans that have not been brewed but have been finely ground, and also the wet smell of brewed coffee beans. Flavor

Coffee is one of Indonesia's leading export commodities. Data from the Central Statistics Agency (BPS) shows that Indonesia exported 277,411.2 tons of coffee in 2018. One of the regions that produce 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 16,396 hectares (BPS, 2018)

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[1]. The chemical composition of coffee beans depends on species, variety, and fruit ripeness. The environment also influenced 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]. , this process the tongue is used to translate what has been smelled from the coffee was detected by the tongue or not. The flavor is a combination of perceptions between taste recognized by the tongue and aroma that is recognized by the olfactory organ as a whole. The flavor component of coffee is the main element of the value of the brewing coffee because it includes two elements at once. In general organoleptic assessment, the flavor elements are usually associated with other impressions such as temperature, coarseness/subtleness, etc. In the assessment of coffee flavor usually only includes taste and aroma elements simultaneously and intact. It is true that other elements such as the heat level of the brew also determine especially the aroma, which is related to the level of volatility of the aroma-forming compounds. The colder usually, the weaker

the value of the aroma, as a result of the lower the 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) is a coffee that has a better taste quality than other types of coffee. This type of coffee has a flat bean shape, dark green leaf color, and 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 has an influence on 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, 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 or not, 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 to be very important because they can quickly provide an assessment of 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, which will affect the aroma of ground coffee[11].

Based on this background, this study was carried out with the aim of knowing the bioactive components contained in Solok Arabica coffee, which was roasted at 200ºC for 10 minutes.

II. THE MATERIAL 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

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 supernatant were 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.

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 sorbents were dried. Acrylamide was eluted from the cartridges using 2 mL acetone.

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 defined as three times the background noise of the chromatographic instrument. The extraction recovery was determined by spiking samples with acrylamide in three replicates, and they were extracted as previously described.

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 200ºC for 10 minutes. Of the 25 compounds detected, there were 4 main compounds, namely pyridine, caffeine, n-hexadecanoic acid, and butyl 9,12-octadecadienoate.

In this study, Solok arabica coffee was roasted at 200ºC for 10 minutes, which is the optimum temperature for roasting coffee. The roasting at a temperature of 200ºC with a time of about 10 minutes greatly affects the physical, chemical, and flavor quality changes of the coffee beans, which are also influenced by the operating conditions of the roaster machine, as well as the characteristics of coffee beans including the type of coffee, moisture content, size. , and 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 200ºC for 10 minutes using GC-MS. The pyridine ion measured by Solok Arabica coffee was detected as much as 90 m/z.

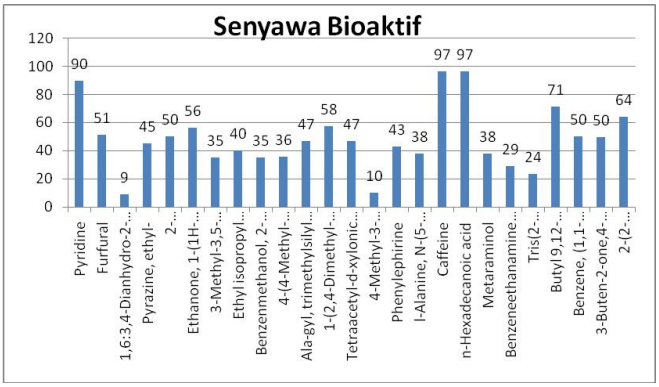

Fig. 1 The Bioactive Compounds in Solok Arabica Coffee that roasted in 200ºC at 15 min

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 115ºC, and density 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.

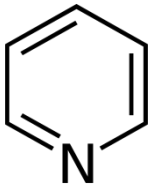

Fig. 2 Pyridine

Pyridine is a derivative of benzene by replacing the CH group with an N atom, which is 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 in the manufacture of 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 200ºC 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 236ºC, its vapor point at 178ºC of atmospheric pressure, and its solubility in water 2.17%[21]. The pyridine framework formula can be seen in Fig. 3.

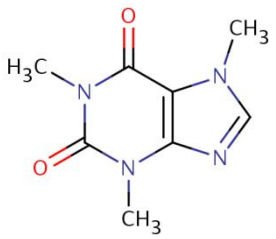

Fig 3. Caffeine

Caffeine is the most important component of the purine ring system in coffee to date[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, namely water evaporation at 100ºC and pyrolysis reaction at 180-225ºC. 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 180ºC. 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 200ºC 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.

n-Hexadecanoic acid (palmitic acid) 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 62.9ºC, a boiling point of 351ºC, 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. In the wax layer, there are 5-hydroxytryptamine fatty acids from palmitic, arachidic, behenic, and lignoceric acids. Fat in coffee is one of the chemical compositions of coffee 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, which will affect the taste and decrease the quality of the coffee grounds. The resulting fat content is the same as that produced in rice coffee beans [27].

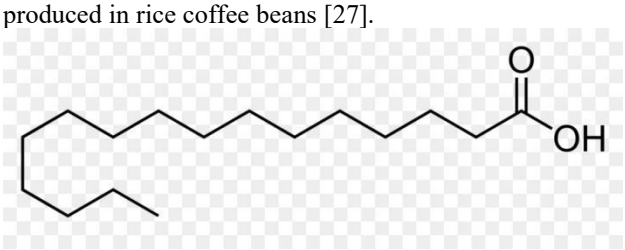

Fig 4. n-Hexadecanoic Acid

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 anti-oxidant, hypocholesterolemic, nematicide, anti-androgenic, hemolytic, pesticide, lubricant, 5-alpha reductase inhibitor, antipsychotic activity[30].

D. *Butyl 9,12-octadecadienoate*

In solok arabica coffee, which was roasted at 200ºC 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.

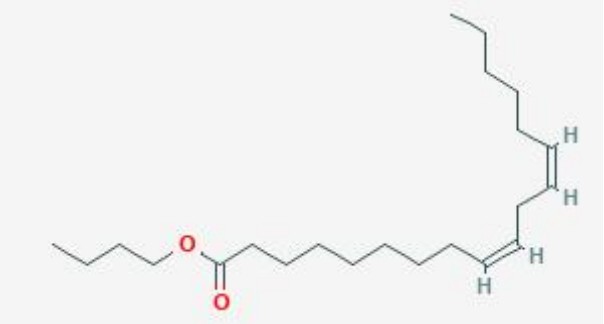

Fig 3. Butyl 9,12-octadecadienoate

Butyl 9,12-octadecadienoate termasuk dalam kelompok senyawa organik heterosiklik. Senyawa heterosiklik adalah senyawa siklik yang pada cincinnya terdapat atom hetero seperti N, O, S, B, P dan beberapa metaloid seperti Al, Sn, As, Cu. Berdasarkan aroma, senyawa heterosiklik ada yang aromatis dan non-aromatis. Senyawa heterosiklik yang paling banyak adalah senyawa heterosiklik yang berikatan dengan N (golongan aza), O (golongan okso), dan S (golongan tio).

IV. CONCLUSIONS

The results of GC-MS analysis of solok arabica coffee roasted at 200ºC for 105 minutes showed that 25 compounds were detected. Of the 25 compounds, 4 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 derivative of benzene by replacing CH groups with N atoms, which have toxicity 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 (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 anti-oxidant, 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, there is one more compound that was detected in large quantities, namely butyl 9,12-octadecadienoate. This compound is a heterocyclic organic compound.

ACKNOWLEDGMENT

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REVIEW FORM

1th Desember 2022
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Dear **Rince Alfia Fadri**
Food Technology Politeknik Pertanian Negeri Payakumbuh, Indonesia
Coresponding Author: alfiarince@gmail.com

Title:	Bioactive Compounds Profile of Solok Arabica Coffee Analyzed by GC-MS Method
Author(s):	Rince Alfia Fadri, Kesuma Sayuti, Novizar Nazir, Irfan Suliansyah, Hanny Fitri Yanti
Paper-ID	12855

A. Technical aspects

	0	1	2	3	4	5
1. The paper is within the scope of the Journal.	□	□	□	□	■	□
2. The paper is original.	□	□	□	□	□	■
3. The paper is free of technical errors.	□	□	□	□	■	□

B. Communications aspects

	0	1	2	3	4	5
1. The paper is clearly readable.	□	□	□	□	■	□
2. The figures are clear & do clearly convey the intended message.	□	□	□	□	□	■
3. The length of the paper is appropriate.	□	□	□	□	■	□

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C. Comments to the authors (You may use another sheet of paper.)

Thank you very much for the submission through the online system. The title of the article should be written briefly and clearly. The title of the article should first state the main idea of the new article, followed by other explanations. The title of the article must indicate exactly the problem to be raised. From this research you are trying to display data about the bioactive components present in Arabica coffee

The novelty: This research has successfully the bioactive components contained in Solok Arabica coffee, which was roasted at 200ºC for 10 minutes. Solok arabica coffee is grown at an altitude of 1000 asl. The height of the planting point has an influence on the taste so that Solok Arabica coffee has a different taste from the flavors of other Arabica coffees that are widely spread throughout Indonesia. 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.

The Title: The **title** summarizes the main idea or ideas of your study. A good **title contains** the fewest possible words that adequately describe the contents and/or purpose of your research paper. The **title** is without doubt the part of a paper that is read the most, and it is usually read first. The title of this paper is good and informative.

The abstract: has already explained, "What is the importance of research". The abstract should contain: the problem behind the research; the main purpose of research; types of research; subjects involved in the research (population/sample for experimental research); data collection methods and instrumentation; data analysis method used; the main results of the research; conclusions and research implications. The research objective should use operational verbs that are high order thinking (avoid: knowing, use: analyzing, exploring, testing.[An abstract should be between 150-250 words]. Please improve the English, use simple sentence and provide the implication of research

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Abstract— **ABSTRACK**

Abstract – The compounds in coffee depend on the type of coffee, the environment, and the soil. Solok is one of the coffee-producing areas in West Sumatra. A kind of coffee that is cultivated in Solok is Arabica coffee. The purpose of this study was 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.

Keywords— arabica, Solok, pyridine, caffeine, n-hexadecanoic acid.

The Introduction typically occupies 10-15% of the paper. GAP Analysis: Explaining the gap between the expectations to be achieved and the facts/circumstances that are currently happening (the gap between das sollen and das sein). So that raises an urgency to carry out research on the chosen topic. **The Materials and methodology** is good. The methods have described how the research question was answered, explain how the results were analysed.

The **introduction should** consists of two parts: It **should** include a few general statements about the subject to provide a background to your paper and to attract the reader's attention. It **should** try to explain why you are writing the paper. The introduction section has included a general introduction, problem definition, problem solution, study motivation, aims and objectives, gaps in the literature.

Noted: I Please clearly mention the objective of study in Introduction/ Citation in paragraph 1, please use [number]. Add some recent literature to strengthen the need for this research to be carried out

The Materials and methodology is good. The methods have described how the research question was answered, explain how the results were analysed. Method. In general, the submission contains 4 main aspects, namely the type, approach, and brief research procedure used; research subjects/participants; data collection methods & instruments; and data analysis methods. In the methodology, avoid writing down the context (for example; interviews are... quantitative is... etc.). Illustrate the research design with a chart or picture. Include the instrument grid used, and provide an explanation of the instrument validation process.

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Results and Discussion have included findings, comparison with prior studies, causal arguments, and deductive arguments. The discussion has been good although there are some that lack detail in discussing. Explain how existing research developments (which have been published in articles in reputable journals) are related to the topic under study. Then explain the novelty/novelty value of the research carried out. Research objectives, explaining the purpose of carrying out research in a straightforward and firm manner, accompanied by the delivery of things that are expected from the results of the research conducted. in the discussion avoid using Numbering and Bulleting.

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Result and discussion has been written in accordance with scientific principles

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The Conclusion has been written in relation to the objectives included in the introduction.

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Decision: As a result of research with an appropriate methodology, **this paper is ACCEPTED for publication**

Additional Comments:

There are some grammatical mistakes and some mistakes in Punctuation.

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1. Accepted without modifications. ☐
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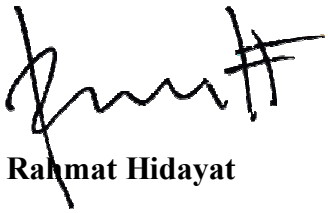
Regards,



Rahmat Hidayat

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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 FitriYanti[a]

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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 16,396 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^{o}C for 10 minutes.

II. The 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).

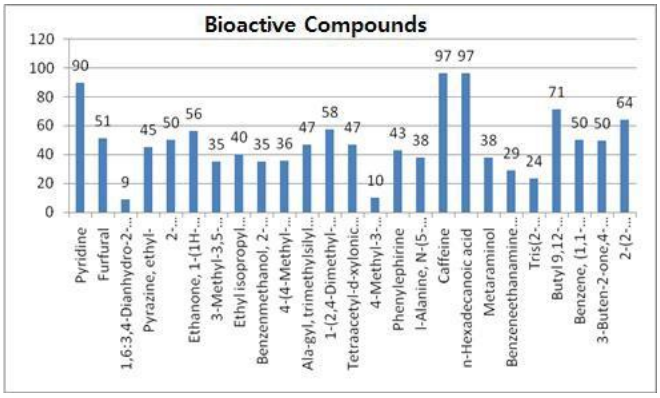

Fig. 1 The Bioactive Compounds in Solok Arabica Coffee that roasted in 200° C at 15 min

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.

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.

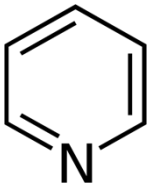

Fig. 2 Pyridine

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.

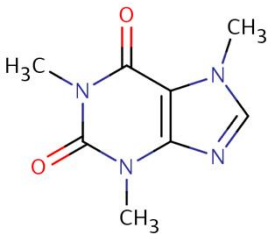

Fig 3. Caffeine

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 200ºC 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 62.9ºC, a boiling point of 351ºC, 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].

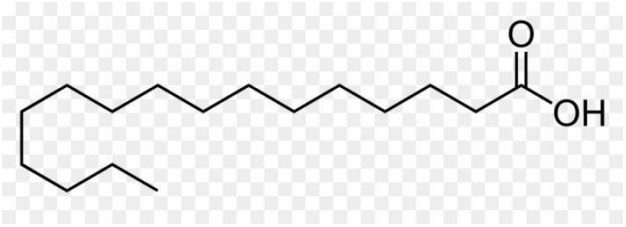

Fig 4. n-Hexadecanoic Acid

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 200ºC 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.

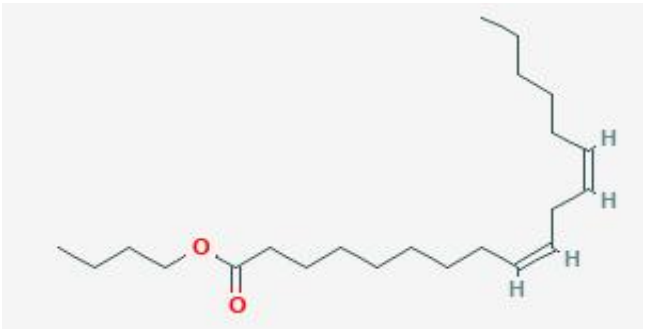

Fig 3. Butyl 9,12-octadecadienoate

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 200ºC 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 200ºC 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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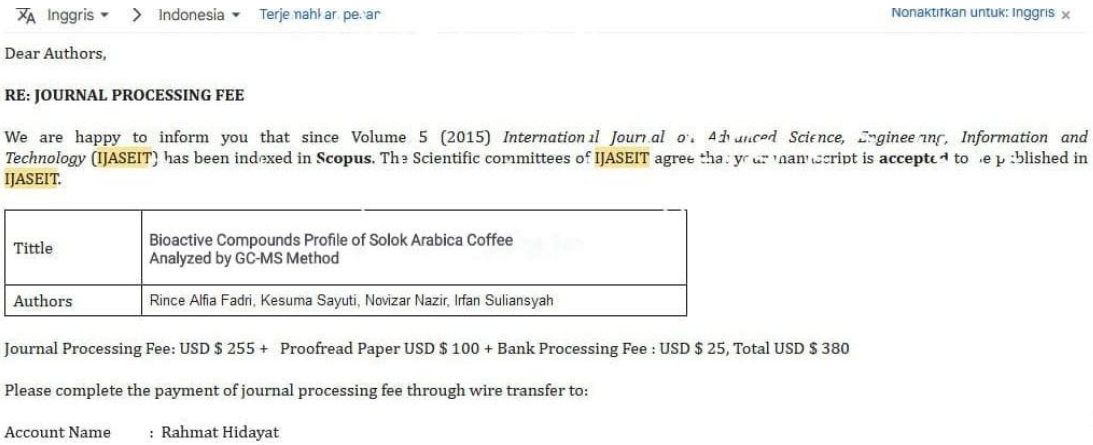

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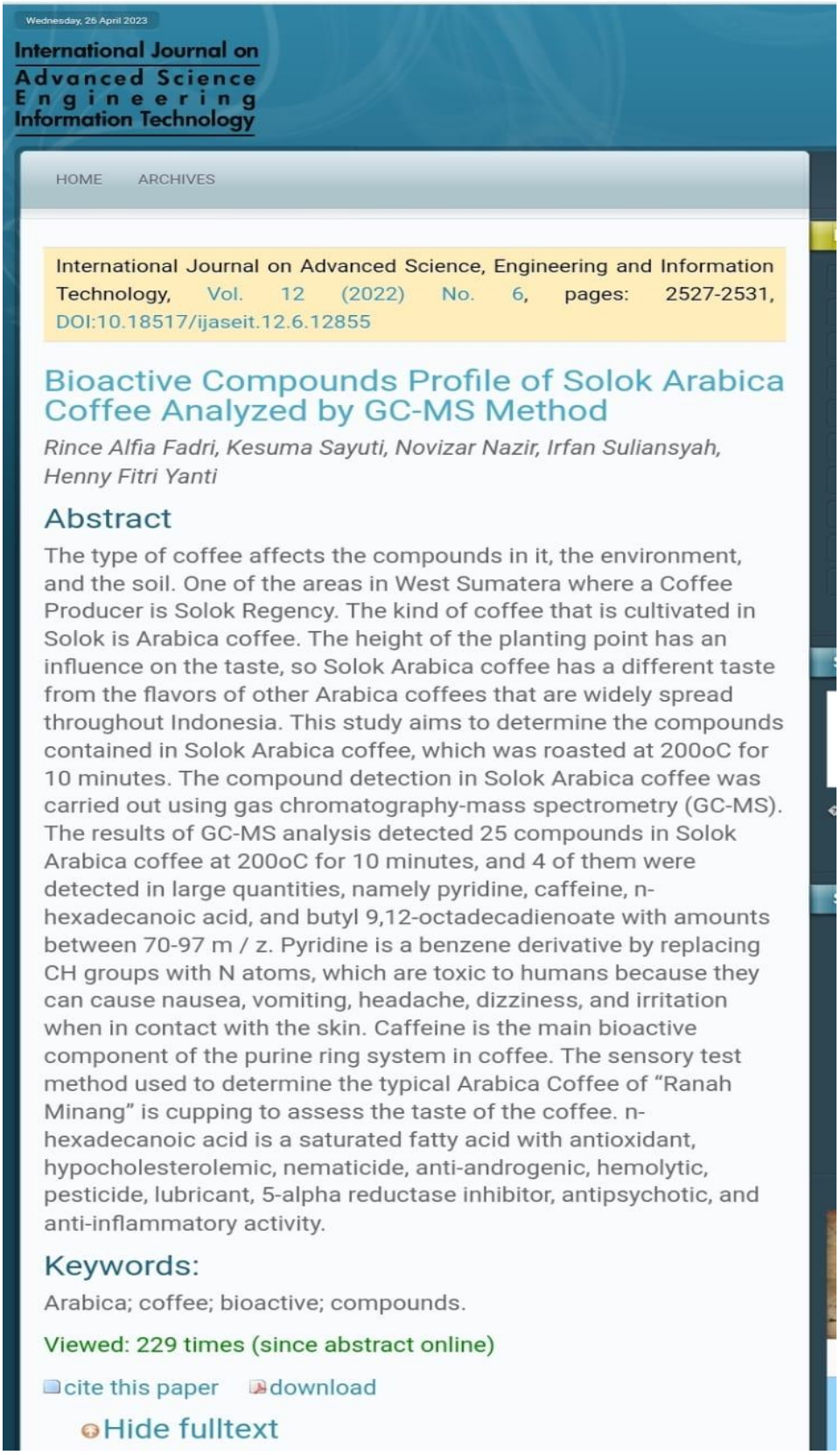

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