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Research Article

Analysis of Flavor in Roasted Coffee Using Temperature Programmable Injection (TPI) at GC/MS Method

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Abstract: An analytical method was carried out to identify volatile compounds that play a role in the coffee aroma and their stability. Because it is unstable at high temperatures, the effect of the injection temperature on the GC/MS column on changes in compound profile was observed. This research aims to develop analytical methods for coffee analysis using TPI-GC/MS method. Samples of Lemar Arabica coffee were taken from the Wonosantri Abadi plantation, Singosari, Malang, and roasted at 210°C. The roasted coffee was extracted using the soxhletation method and methanol as solvent. The compound profiles were analyzed using the GC/MS method with injection temperatures of 40°C, 140°C, and 240°C. The results showed that ketones, esters, furans, and thiazoles play a role in the aroma of coffee. The compounds present in roasted coffee injected at 40°C were less than those at injection temperatures of 140°C and 240°C based on the chromatograms. The profile of the compound at the injection temperature of 240°C is also more diverse than the others because the large injection temperature allows decomposition to occur so that there are many fractional compounds from the thermal decomposition. Non-volatile caffeine compounds were also detected at an injection temperature of 240°C.

Keywords: roasted coffee, coffee flavor, injection temperature, TPI-GC/MS

Introduction

Gass Chromatography/Mass Spectrometry (GC/MS) is a modern analytical method used to separate and identify sample components. GC/MS consists of two parts, gas chromatography, and mass spectroscopy. Gas chromatography separates molecules based on differences in the migration rates of components while mass spectroscopy is a method for measuring and determining the mass of a molecule by finding the mass-to-charge ratio of the ions [1].

This method is quite popular for analysis in various fields such as food, medicine, contaminants, pollutants, and even forensics. The GC/MS method is used for the analysis of volatile compounds or compounds that can be used as volatiles [2]. The components in the sample must be volatile to move through the column. Substances with low volatility can be retained by the column and will be eluted during analysis, but substances that are not volatile will condense in the column. Nonvolatile compounds must be made volatile before being analyzed by GC/MS.

The Temperature Programmable Injection (TPI) method in GC-MS is a method for controlling the injection temperature during analysis. The injection temperature was made several variations to see the optimal temperature in the analysis of a sample. Making this injection temperature variation is also useful in the analysis of compounds in complex samples that have a wide range of boiling points.

Setting the injection temperature is one of the important points when GC/MS analysis. Column temperature settings are carried out so that the optimal temperature is reached to separate the components

in the sample. The injection temperature affects the volatility of the substance, the higher temperature, the higher the vapor pressure. Substances with high volatility can be analyzed at lower temperatures than substances with low volatility. However, too high a temperature can also cause some of the substances in the sample to decompose. Therefore, the analysis of substances using the GC/MS method depends on their volatility and thermal stability [2]. If the sample analyzed by GC contains components whose boiling points have a wide range, it is often not possible to select a suitable temperature for carrying out the analysis [3]. So increasing the column temperature will optimize analysis and reduce analysis time. Japanese researchers succeeded in analyzing carbofuran, benfuracarb, and carbosulfan in tap water which is easily hydrolyzed and decomposed by heat using the TPI-GC/MS method [4]. This research is also expected to be able to use this method for the analysis of unstable compounds in coffee.

Coffee is one of the most popular drinks in the world besides tea because it has a distinctive aroma and taste. The flavor of the coffee depends on the components and the presence of chemical compounds in coffee [5]. More than 1000 chemical compounds in coffee [6] consist of carbohydrates, proteins, lipids, caffeine, aromatic compounds, minerals, and acids [7]. Aroma is one of the important attributes of roasted coffee resulting from many mixtures of volatile compounds in which more than 800 volatile compounds have been identified in roasted coffee and ground coffee [8]. Coffee volatile compounds comprise several chemical classes including hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, pyrazines, pyrroles, pyridines, other bases (e.g. quinoxalines, indoles), sulfur compounds, furans, furanone, phenols, oxazoles among others [9]. The chemical compounds responsible for the aroma of coffee are very complex, so they are not easy to fully understand.

Research to identify compounds in coffee using the GC/MS method has previously been carried out by Wonorahardjo et al., 2019 [10]. Coffee beans were extracted using a liquid-liquid extraction method with chloroform and n-hexane then tested by GC/MS with an oven temperature setting of 70°C for 5 minutes and then raised to 300°C at a rate of 5°C/minute and held for 19 minutes. minute. The injection temperature is 300°C and the flow rate is 25.5 mL/sec. The chromatogram results almost entirely indicate polar compounds. But some components have not been revealed, namely, there is a very broad peak around the retention time of 50 minutes where all the chromatograms show the complexity of separating components. Therefore, further research is needed to describe these components, where the possible properties inherent in coffee depend on these components.

The purpose of this study was to identify compounds that play a role in the coffee flavor and their stability and to develop analytical methods for coffee analysis using TPI-GC/MS method [4], [11]. Compounds in roasted coffee were extracted using the soxhletation method with methanol as a solvent to extract both polar and non-polar compounds. After that, it was analyzed using GC/MS at injection temperatures of 40°C, 140°C, and 240°C. The novelty in this study is the use of the TPI-GC/MS method to avoid thermal decomposition of the compounds present in roasted coffee, where this method has been successfully done by Japanese researchers in the analysis of carbofuran and its derivatives in tap water. This TPI method is also now applied to natural samples and investigated its effect on the presence of compounds in the sample.

Materials and Methods

Materials

A set of coffee roaster machines (NOR coffee roaster machine N5000i), coffee grinder, rotavapor, 50 kg digital sitting scale, analytical balance, soxhlet set, GC/MS (QP2010Plus Shimadzu), and other research glassware according to work procedures.

The materials used in this study were Arabica Lemar coffee beans harvested from the Wonosantri Abadi Plantation, Singosari, Malang, and methanol p.a from Merck.

Sample Preparation

Lemar Arabica coffee beans are dried by a natural dry process. The coffee beans picked from the tree are spread and dried on an open floor/patio for more than 21 days to reduce the moisture content in the coffee beans. Then, the coffee beans are sorted based on the rice coffee beans' size (grading) and

quality. It was roasted using NOR coffee roaster machine N5000i, and samples were taken at 210℃. Then each sample that has been taken is cooled and mashed with a grinder so that it becomes coffee powder.

Extraction of Compounds from Roasted Coffee

Extraction of roasted coffee was used the soxhletation method. A total of two grams of roasted coffee beans are wrapped in filter paper. The soxhlet apparatus is set and the bag containing the sample is inserted into the siphon tube. The methanol solvent was put into a round tube of 250 mL. The heating mantle is then turned on. The sample was extracted until it was clear. The extract obtained was then concentrated with a rotary evaporator and stored in the refrigerator.

Identification of Volatile Compounds in Roasted Coffee Using GC/MS

The extract was identified using GC–MS to identify any compounds contained in the extract. In this study, samples were tested in the GC/MS port (Shimadzu, type QP2010 Plus) with column type RTX-5MS. The injection temperatures were chosen at 40°C, 140°C, and 240°C to see the differences in the compounds that could be detected at low, medium, or high temperatures. The temperature range is quite large because the compounds in coffee are very complex and have a wide range of boiling points.

Chromatography experiments were set up with this condition: The injection method was conventional splitless injection. A total of 1 µL samples were inserted into the GC/MS port with an injection temperature of 40°C. The oven temperature was 40°C for 4 minutes and increased to 280°C with a 20°C/min rate, then holding it until 13 minutes. Ion source and interface temperatures were 200°C. The carrier gas was helium with a flow rate of 45.3 cm^3/sec. The MS mode was a full scan with a mass range of 50 – 600 m/z. Further analysis was carried out at injection temperatures of 140°C and 240°C with the same procedure and then all the data.

Data Analysis

Data analysis was carried out based on the data obtained at each stage of the study. The data obtained came from 1) the chromatograms at injection temperatures of 40°C, 140°C, and 240°C and 2) the mass spectrum which was matched with the Willey 8 library. Chromatogram data was used to see the difference in peaks between injection temperatures. While the mass spectrum data is used to see what compounds appear in all chromatograms. The compounds used are compounds with a Similarity Index (SI) > 80.

Result and Discussion

In this study, the coffee used for the sample was Arabica coffee from the Lembah Arjuno (Lemar) which was grown in the Wonosantri Abadi Plantation, Singosari, Malang. Arabica coffee was chosen because it contains more complex chemical components than Arabica coffee [10].

Lemar coffee beans have been harvested through several preparation processes before roasting. First, sorting is done manually by color, namely separating green and red coffee beans. Green seeds are sorted because they are considered less good. The second process is drying the coffee beans and skins on an open floor for more than 21 days until the water content in the beans decreases. Initially, the water content of coffee beans after harvesting is about 55 – 60% and after drying the water content drops to 12% [9]. Then, the seeds were separated from the skin (hulling). The next process is grading, which is the process of sorting based on the size of the coffee beans, where the coffee beans are grouped into 3 sizes, namely 7 mm, 6 mm, and 5 mm. This process needs to be done carefully and correctly because it will affect the roasting time. Roasting time will vary according to size. After that, the defect process is carried out or sorting the coffee that is considered bad. If something is black or does not match, it will be sorted. In this study, coffee was roasted at a temperature of 210°C using a set of coffee roaster machines (NOR coffee roaster machine N5000i).

Roasting at a temperature of 210°C is included in medium roasts where the first crack is complete but the second crack has not yet occurred, the caffeine content is lower, producing coffee with a balanced aroma and a lot of flavors. The coffee beans are dark brown and not shiny. The roasted coffee beans that

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have been ground are extracted by the soxhletation method with methanol solvent and the extract can be seen in Figure 1. The purpose of this extraction is to take all the components in the roasted coffee matrix, both polar and non-polar compounds for further processing analyzed by GC/MS method.

(a) (b)

Figure 1. (a) Extraction Process and (b) Roasted Coffee Extract

The extracts were tested in the GC/MS port with injection temperatures of 40°C, 140°C, and 240°C. In this study, the carrier gas used was helium with a total rate of 78.5 mL/minute and the oven temperature started at 40°C and held until 280°C. The analysis with GC/MS obtained a chromatogram and mass spectrum at each injection temperature. The chromatogram showed that the compounds can be separated during the elution process, and the mass spectrum showed the type of compound by matching it with the GC/MS database based on density and retention time. The results of GC/MS analysis results obtained two data, namely the chromatogram derived from the gas chromatography analysis and the mass spectra from the MS analysis results.

Chromatograms produced from roasted coffee at 210°C at injection temperatures of 40, 140, and 240°C are shown in Figure 2.

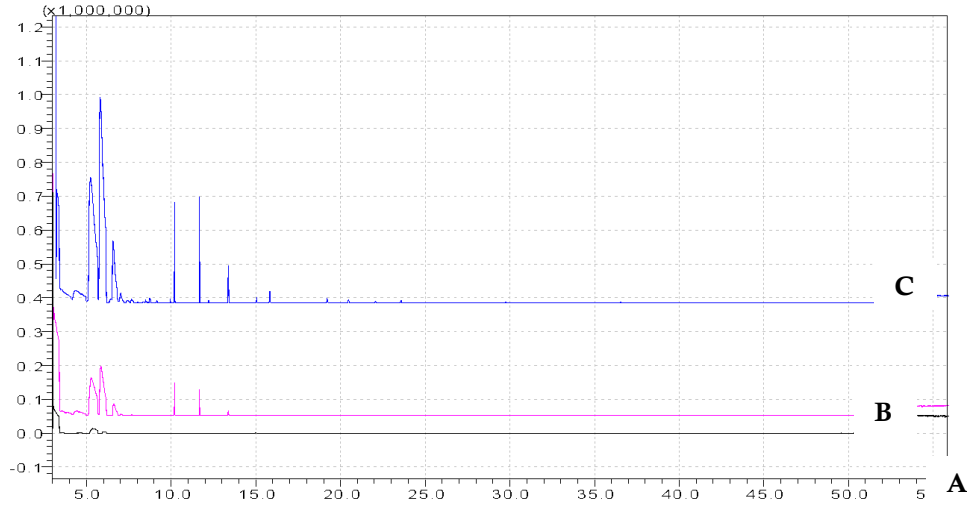

Figure 2. Chromatogram of Roasted Coffee at Injection Temperature of (A) 40°C (Black), (B) 140°C (Pink), and (C) 240°C (Blue)

Based on the chromatogram at injection temperatures of 140°C (pink) and 240°C (blue) in Figure 2, it can be seen that the peaks that appear are in similar areas. However, on the chromatogram at the injection temperature of 40°C, only a few peaks appeared with less intensity than the other two peaks. The peak intensity increases with the increase of GC temperature [12].

The injection temperature of 40°C has not been able to separate the components in the sample. The components in the sample have not evaporated and entered the column so they have not produced

peaks. The higher temperature, the number of peaks and intensity are also greater because the components with higher boiling points begin to evaporate and produce peaks.

Furthermore, identification to determine the components of the compounds in the extract at all injection temperatures based on data on Similarity Index (SI), base peak, and mass spectra compared to the spectra from the library, namely Willey 8. LIB. Some compounds have high suitability based on their Similarity Index (SI), and others have very low matches. At a low level of compatibility, the compounds have not been completely separated so a pure compound has not been produced. On the other hand, larger compounds such as polysaccharides cannot be analyzed by this method, so it is possible that the results also contain fractional compounds from larger compounds.

Table 1 showed the similarities and differences in the component profiles in roasted coffee at injection temperatures of 40°C, 140°C, and 240°C. In general, polar and semi-polar compounds in roasted coffee can be extracted by methanol solvent, but the presence of polar compounds has not been seen. Maybe it is in the mass spectra where the SI is low so it has not been completely separated. Or because the boiling point is high so it has not evaporated. The extraction method used is only a single extraction, so in future research, it is necessary to carry out further extraction to separate polar and non-polar compounds.

Table 1. Results of Mass Spectra Analysis of Compounds in Roasted Coffee at Injection Temperatures of 40°C, 140°C, and 240°C

40°C	140°C	240°C
Alkanes n-hexane	**Ketones** 4-methyl-3-penten-2-one 4-hydroxy-4-methyl-2-pentanone	**Ketones** 4-methyl-3-penten-2-one 4-hydroxy-4-methyl-2-pentanone
Aromatics toluene 1-phenyl-1-trimethylsilyloxyethylene	**Aromatics** toluene 1,1-dichloro-2-phenyl-2-trimethylsiloxypropane	**Aromatics** toluene
Esters 2-methyl-(2-(trimethylsilyloxy)ethoxy) acetate	**Heterocyclics** 2-ethyloxetane 2-benzylsulfonyl-1*H*-benzimidazole	**Heterocyclics** 2-ethyloxetane 5,6-dihydro-2H-pyran-2-one 3-methyl-1H-1,2,4-triazole Caffeine
Furans 1-furan-2-ylmethyl-2,3-dimethyl-p**iperidine**-4-one,	**Siloxanes** decamethylcyclopentasiloxane dodecamethylcyclohexasiloxane 2-(2',4',4',6',6',8',8'-heptamethyl tetrasiloxane-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	**Siloxanes** decamethylcyclopentasiloxane dodecamethylcyclohexasiloxane
Thiazoles Thiazolidine-2,5-dione	**Carboxylic Acids** Propanoic acid, anhydride	**Carboxylic Acids** 2-*O*-(4-bromophenyl) 1-*O*-ethyl benzene-1,2-dicarboxylate 4,4-Dimethyl-2-pentanol, heptafluorobutyrate
	Esters tris(trimethylsilyl) arsorite	
	Thiazoles 2-1-Phenyl ethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole	

Based on Table 1, it can be seen that the sample of roasted coffee at 40°C injection temperature did not contain many compounds with a Similarity Index (SI) > 80. The profiles of the compounds at injection temperatures of 140°C and 240°C were more diverse than those at 40°C. This is possible because other compounds at an injection temperature of 40°C have not evaporated so they cannot be detected. The compounds that appear at the injection temperature of 40°C also do not appear again at the injection temperature of 140°C and 240°C, these compounds may be unstable and decompose into other compounds.

Meanwhile, the compound profiles at injection temperatures of 140°C and 240°C produced the same compounds as in the similar peaks of the chromatogram. There were 4-methyl-3-penten-2-one; 2-ethyl-oxetane; 4-hydroxy-4-methyl-2-pentanone; decamethylcyclopentasiloxane; and, dodecamethyl cyclohexasiloxane. These compounds increased in percent area from the injection temperature of 140°C to 240°C, except for 2-ethyl-oxetane was decreased. It experienced a very significant decrease in the area from the injection temperature of 140°C to 240°C, which is 44.69% to only 9.7%. This compound is indeed unstable at high temperatures because it is a non-polar compound with a low molecular weight.

The 4-methyl-3-penten-2-one and 4-hydroxy-4-methyl-2-pentanone are compounds belonging to the ketone group that gives coffee aroma and also experienced an increase in the percent area, respectively, from 21.63% to 38.87. % and 3.29% to 7.24%. In the other research, the 3-penten-2-one compound gives a fruity aroma while 3-pentanone gives an ethereal aroma [13] so their almost similar structures will give the same aroma.

The compound in roasted coffee that appeared at all three injection temperatures was only toluene with the percent area increasing as the injection temperature increased. From these results, it can be seen that toluene is quite stable to temperature. At the injection temperature of 40°C, the percent area of toluene was 32.95% and increased along with the increasing injection temperature, were 34.31% at 140°C and 36.82% at 240°C. Toluene is recognized as a very thermally stable compound with an assumed thermal stability limit of 480°C [14].

The existence of toluene and n-hexane may be due to the structural analysis only based on the Willey 8 Library. Many compounds were detected in the results of their m/z being similar to each other, so there showed compounds that could not be present in the sample. This is one of the weaknesses of this research, so it needs to be considered for further spectrum analysis other than based on the library.

The compound profile at the injection temperature of 40°C showed a furan group compound, namely 1-furan-2-ylmethyl-2,3-dimethyl-piperidine-4-one with a 1.39% area that gives a coffee aroma. This compound produces a burnt and smoky aroma [10]. The top two classes in coffee are furans and pyrazines, quantitatively [9] which make coffee flavors complex and have their characteristics. There is thiazolidine-2,5-dione which also gives the coffee a smoky aroma with a 1.57%.

The group of thiazole also showed at injection temperature of 140°C, was 2-1-Phenyl ethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole with just a 0.03%, which plays a role in the coffee aroma too. This compound gives a burnt and nutty aroma [15] and does not appear at injection temperatures of 240°C. Other compounds that only appear at one injection temperature, such as esters, will give the coffee a fruity aroma, while acids will give the coffee a sour taste. Almost all groups of volatile compounds affect the aroma of the coffee, making the aroma very complex.

More interestingly, at the injection temperature of 240°C, caffeine appears at 0.13%. Caffeine was a non-volatile compound that may be important to coffee flavor [9]. Caffeine is usually analyzed using UV-Vis spectrophotometry or HPLC because of its non-volatile nature. However, in this study, using several injection temperatures, it was found that non-volatile compounds also appeared. So the TPI-GC/MS method may also be used as an alternative method of analyzing other compounds in coffee. As we know that GC/MS is limited to analytes that are not only volatile and thermally labile but can also withstand the harsh partitioning conditions of the gas chromatograph [16]. Even with this limitation, many analytes can only be separated from complex mixtures and identified by GC /MS. The GC/MS method may be suitable for the analysis of these compounds, although it is necessary to optimize the injection temperature and validate it for further analysis. This method is expected to be an alternative

method for other compounds in coffee such as caffeine, trigonelline, and contaminant compounds in coffee.

From the data above, it turns out that the injection temperature in the GC/MS column greatly affects the compounds profile in roasted coffee. A small temperature does not produce enough peaks, but more peaks will appear as the injection temperature increases. It is also possible that the compound that appears is the result of the thermal decomposition of a larger compound. This is an interesting thing when researching something from natural materials whose compound profiles are very numerous and changeable and are influenced by various factors. Starting from the type of coffee, the roasting process, processing, and how to make coffee drinks also affect the profile of its chemical compounds [17]–[20]. Therefore, the above data still requires further research.

Conclusion

The group of ketones, esters, furans, and thiazoles play a role in the aroma of coffee. The injection temperature in GC/MS affects the profile of compounds in roasted coffee and their stability. By using the TPI-GC/MS method, data on the profile of compounds in roasted coffee will be obtained which can be developed for further analysis of single compounds. The TPI-GC/MS method can be used as an alternative analytical method for non-volatile compounds in coffee such as caffeine, although validation needs to be done.

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