Effect of different aroma extraction methods combined with GC-MS on the aroma profiles of coffee

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Abstract. Aroma is arguably the most important factor in the determination of coffee quality. The aroma profiles of coffee could be evaluated by application of gas chromatography-mass spectrometry (GC-MS). Prior to GC-MS analysis, those aroma compounds need to be extracted. This extraction method can be carried out with or without solvent. This research aimed to know the effect of different aroma extraction methods combined with Gas Chromatography-Mass Spectrometry (GC-MS) on the aroma profiles of medium-roasted coffee (Java Arabica and Java Robusta). The solvent extraction applied Ultrasonic Assisted Extraction (UAE) method with three different solvents (dichloromethane, ethyl acetate, hexane), while Headspace-solid phase microextraction (HS-SPME) was employed as a solvent-free extraction method. The results showed that different solvents used for extraction produce significantly different yield of aroma extracts ($\alpha = 0.05$). Aroma compounds extracted using solvents combined with GC-MS consisted of 3 groups, i.e. acid, ester, and alcohol, where acid group dominated in all treatments. Meanwhile, HS-SPME GC-MS analysis resulted in an identification of 12 groups, such as aldehyde, pyrazine, pyrrole, pyrimidine, ketones, phenol, ester, acid, furan, alcohol, hydrocarbon, and sulfur compound. Aroma compounds of Java Arabica coffee was dominated by aldehydes, while phenols was found to be the major compounds identified in Robusta.

1. Introduction

Coffee production in the world reached 155.4 million of 60 kg bags in 2017 [1]. Meanwhile in Indonesia, coffee consumption reached 0.896 kg/capita/year in 2015 [2]. These markets certainly demand good quality coffee. Aroma is one of the determinants of coffee quality. Aroma has a large impact on the perceived flavour. Good quality coffee flavour has been described as a pleasant sensation, a balanced combination on flavour, body, and aroma in the absence of faults [3].

More than 800 volatile components have been identified through extensive research on coffee. Volatile components in coffee have been studied extensively using gas chromatography-mass spectrometry (GC-MS), such as in Lee and Shibamoto [4], Fisk et al. [5], Sunarharum [6], and Yang et al. [7]. In Indonesia, there are only few reports on the identification of volatile components from local coffee, especially from Malang’s coffee, Indonesia.

Before being analyzed, aroma compound has to be extracted. Extraction method can be carried out with solvent, such as Ultrasonic Assisted Extraction, and solvent-free, such as Headspace-solid phase microextraction (HS-SPME). UAE is a type of extraction that use ultrasound wave. The advantage of
using UAE are fast and medium temperature (can be used for heat sensitive compound) [8]. SPME is a type of a headspace sampling, which studies the volatile compounds in the space above a sample in a sealed container [9]. SPME is a fast, solvent-free alternative to conventional sample extraction techniques. SPME uses a fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both. The fiber coating extracts the compounds from sample by absorption in the case of solid coatings [9]. The objectives of this study were to use GC-MS paired with UAE and HS-SPME to identify volatile compounds in roasted coffee from Malang.

2. Materials and Methods

2.1. Materials

Coffee beans (1 kg) used in this research were medium roasted Java Arabica and Java Robusta coffee from Arjuno Mountain, UB Forest Malang, East Java. Both coffee beans were processed following full-wash method. Chemicals used include ethyl acetate, hexane, and dichloromethane pa. (Merck).

2.2. Methods

Two factors being studied were two different coffee species (Arabica and Robusta) and aroma extractions methods, with and without solvents. The solvents used were ethyl acetate, hexane, and dichloromethane. The solvent-free method employed Headspace Solid Phase Microextraction (HS-SPME) coupled with Gas Chromatography-Mass Scpectrometry (GC-MS).

2.2.1. Ultrasonic assisted extraction

The method used was modified from Kuś et al. [10]. As many as 1.5 g of coffees were extracted with 30 ml solvent (ethyl acetate; hexana; dicloromethane). The extraction was supported by ultrasound. Indirect sonication mode of ultrasonic bath (Elmasonic Type S 40 H) at 25 ± 5°C was applied. The extracts were sentrifuge at 5000 rpm for 10 min. 1μl was used for GC-MS analyses.

2.2.2. Headspace solid-phase microextraction (HS-SPME)/ GC-MS condition

The headspace microextraction was performed with divinylbezene -Carboxen- polydimethylsiloxane (DVB-Carboxen-PDMS) fibre. After extraction, the SPME fiber was moved to the splitless injection port of GC for manual injection. An Varian CP-3800 gas chromatograph coupled with an MS Saturn 2200 mass spectrometer, equipped with VF-5ms column (30 length x 0.25 mm internal diameter). Inlet temperature of GC was set at 300°C with constant column flow 0.7 ml/min. The transfer line temperature was set at 250°C. The oven parameters used were 40°C with holding for 2 min, rising to 240°C at a rate of 15.0°C/min, holding for 14.0 min.

Aroma compounds were identified based on NIST MS library database. Qualitative and quantitative data were tabulated in MS Excel 2013. Statistical analysis was performed using Minitab 17.0 Statistical Software (Minitab Inc., State College, Pennsylvania, USA).

3. Results and Discussion

3.1. Chemical properties

Chemical properties of coffee such as starch, protein, lipid, ash, and caffeine may be important to coffee flavour. These components contribute to volatile appearance while roasting. Table 1 shows chemical properties in Arabica and Robusta.

Arabica and Robusta were compared to see their differences. It can be seen from Table 1 that those two species had different chemical properties. Arabica was found to have less starch, protein, ash and caffeine than the Robusta counterpart. However, Arabica contains more lipid than Robusta. Those chemical differences were thought to have an influence on the characteristics of the brewed coffee.
Table 1. Chemical Properties in Arabica and Robusta.

| Component (%) | Arabica       | Robusta       |
|---------------|---------------|---------------|
| Starch        | 0.82 ± 0.03   | 1.16 ± 0.09   |
| Protein       | 12.48 ± 0.59  | 13.13 ± 0.12  |
| Lipid         | 12.79 ± 1.04  | 11.26 ± 0.20  |
| Water         | 3.18 ± 0.03   | 2.60 ± 0.16   |
| Ash           | 3.91 ± 0.14   | 4.07 ± 0.32   |
| Caffeine      | 1.01 ± 0.04   | 1.53 ± 0.01   |

3.2. Aroma compounds

3.2.1. Ultrasonic assisted extraction

There are three groups of aroma compounds were identified. They were 3 acids, 2 esters, and 1 alcohol. Aroma compound from alcohol and ester groups were found in arabica’s extract with ethyl acetate solvent. It were furfuryl alcohol (0.64%) and propanoic acid, ethyl ester (3.80%). Ester group was also found in robusta’s extract with hexana solvent. It was 2,3-dihydroxypropyl ester (0.15%). Meanwhile, aroma compound from acid group was found in all the extract. It were hexadenoic acid, acetic acid, octadecanoic acid.

3.2.2. HS-SPME

In Arabica, among 65 peaks observed in a chromatogram, 47 volatile compounds were identified. They were nine aldehydes, eleven pyrazines, seven ketones, four phenols, three esters, three pyroles, 3 acids, 2 furanones, 3 alcohols, 1 sulfur-containing compound, and 1 pyridine. The major constituents in Arabica were 5-methyl-furfural (14.01%), 2-furancarboxyvaldehyde (11.83%), and 2-furanmethanol acetate (11.74%). Meanwhile, in Robusta, among 80 peaks observed in a chromatogram, 57 volatile compounds were identified. They were eleven aldehydes, thirteen pyrazines, eleven ketones, five phenols, three esters, three pyroles, two acids, three furanones, one alcohols, three hydrocarbons, and one pyridine. The major constituents in Robusta were 2-methoxy-4-vinylphenol (12.24%), 2-furanmethanol acetate (9.27%), and 5-methylfurfural (8.46%). Table 2 shows volatile compounds identified from Arabica dan Robusta.

Table 2. Volatile compounds identified from Arabica and Robusta.

| No | Volatile Compound                        | RT % | Area | Flavour description [literature]                      |
|----|-----------------------------------------|------|------|------------------------------------------------------|
|    |                                          |      |      |                                                      |
| Arabica |                                              |      |      |                                                      |
| 1   | Isobutyraldehyde                        | 2.270| 0.70 | Burnt, Caramel, Cocoa, Malt [11]                     |
| 2   | Butanal,3-methyl-                       | 3.213| 0.95 | Malty [3]                                           |
| 3   | Butanal,2-methyl-                       | 3.348| 1.66 | Almond, cocoa, fermented, malt [3]                   |
| 4   | Hexanal                                 | 6.933| 0.43 | Green [12]                                          |
| 5   | 2,4-pentadienal                         | 7.158| 11.83| Fruity [12]                                         |
| 6   | 2-Furancarboxaldehyde                   | 8.079| 11.83| Almond like, caramel, spice [12]                    |
| 7   | 5-methyl-furfural                       | 13.339| 14.01| Caramel [12]                                        |
| 8   | 1H-pyrrrole-2-carboxaldehyde, 1-methyl  | 15.113| 1.58 | Roasted nut [12]                                   |
| 9   | Benzeneacetaldehyde                     | 16.764| 0.55 | Green, sweet, floral [11]                           |
| Pyrazine |                                              |      |      |                                                      |
| 10  | Pyrazine,methyl-                        | 7.837| 1.45 | Nutty [11]                                          |
| No | Volatile Compound                     | RT  | % Area | Flavour description [literature]                                      |
|----|--------------------------------------|-----|--------|---------------------------------------------------------------------|
| 11 | Pyrazine,2,5-dimethyl-               | 11.329 | 2.43   | Roasted nut [11]                                                    |
| 12 | Pyrazine,2,6-dimethyl-               | 11.490 | 0.50   | Nutty [11]                                                          |
| 13 | Pyrazine,ethyl-                      | 14.856 | 2.13   | Potato-like, nutty [11]                                             |
| 14 | Pyrazine,2-ethyl-6-methyl-           | 15.024 | 2.74   | Nutty [11]                                                          |
| 15 | Pyrazine,2-ethyl-3-methyl-           | 18.006 | 2.42   | Earthy, roast [11]                                                  |
| 16 | Pyrazine,2-ethyl-3,5-dimethyl-       | 18.266 | 0.33   | Chocolate, musty [11]                                               |
| 17 | Pyrazine,3-ethyl-2,5-dimethyl-       | 19.757 | 0.10   | Nutty [11]                                                          |
| 18 | 2-Acetyl-3-methylpyrazine            | 20.878 | 0.21   | Musty [11]                                                          |
| 19 | Pyrazine,2,3-diyethyl-5-methyl-      | 21.007 | 0.61   | Nutty [11]                                                          |
| 20 | Pyrazine,3,5-diyethyl-2-methyl-      | 11.655 | 0.69   | Woody, berry, floral, and fruity [12]                               |
|    | Trans.beta-Ionon,5,6-epoxide          | 11.176 | 2.12   | Balsamic-sweet, Nutty [12]                                          |
|    | Ethanone,1-(2-furyl)-2-propanone      | 12.978 | 0.39   | Herbal, green [12]                                                  |
|    | Bicyclo[4.1.0]heptan-2-one,6-methyl- | 14.489 | 0.18   | Mint [12]                                                          |
|    | 1-Propane,1-(2-furanyl)-             | 15.237 | 0.53   | Herbal, green [12]                                                  |
|    | Ethanone,1-(1-methyl-1H-pyrrolo-2-yl)| 17.934 | 0.50   | Earthy, Nutty [12]                                                  |
|    | beta.Damascenone                     | 28.944 | 0.15   | Honey-like, fruity [6]                                              |
| 28 | Phenol                               | 4.504 | 0.48   | Sweet [12]                                                          |
| 29 | Phenol,3,5-dimethyl-                 | 13.230 | 0.21   | Smoky, sweet [12]                                                   |
| 30 | Phenol,4-ethyl-2-methoxy-            | 25.369 | 0.30   | Spicy, woody [12]                                                   |
| 31 | 2-Methoxy-4-vinylphenol              | 26.662 | 2.54   | Woody, smoky [12]                                                   |
| 32 | Propanoic acid,2-oxo                 | 1.979 | 1.33   | Sour-acetic odor, Caramelic sweetness                               |
| 33 | Acetic acid                          | 2.410 | 1.09   | Pungent, vinegar [12]                                               |
| 34 | Acetic acid, anhydride               | 2.457 | 0.77   | Strong acetic [11]                                                  |
| 35 | Furan,2-methyl-                      | 2.566 | 1.40   | Cocoa [12]                                                          |
| 36 | 2-furfuryl furan                     | 18.184 | 0.88   | Roasted [12]                                                        |
| 37 | Acetic acid, methyl ester            | 2.116 | 0.68   | Roasted nut [11]                                                    |
| 38 | Furfuryloformate                     | 11.023 | 1.00   | Floral [12]                                                         |
| 39 | 2-Furanmethanol, acetate             | 14.647 | 11.74  | Fruity [12]                                                         |
| 40 | 2-Furanmethanol                      | 8.934 | 7.16   | Burnt, caramel [12]                                                 |
| 41 | Linalool oxide                       | 17.790 | 0.49   | Floral [12]                                                         |
| 42 | Linalool L                           | 18.917 | 1.00   | Floral [12]                                                         |
| 43 | 1H-Pyrrole,1-methyl-                 | 4.907 | 0.53   | Herbal                                                              |
| 44 | 1H-Pyrrole,1-(2-furanymethyl)-       | 21.943 | 1.98   | Earthy-green [12]                                                   |
| 45 | N-furfuryl pyrrole                   |       |        | Vegetable [12]                                                      |
| 46 | 3-Acetyl-2,5-dimethylthiophene       | 25.581 | 0.14   | Burnt, nutty                                                        |
| 47 | Pyridine                             | 5.379 | 0.95   | Sour, smoky, warm [11]                                              |

Robusta
| No | Volatile Compound | RT (min) | % Area | Flavour description [literature] |
|----|------------------|---------|--------|---------------------------------|
| 1  | Isobutyraldehyde | 2.270   | 0.12   | Burnt, Caramel, Cocoa, Malt      |
| 2  | Butanal,3-methyl-| 3.213   | 0.31   | Fruity, peach-like [3]           |
| 3  | Butanal,2-methyl-| 3.348   | 0.46   | Almond, cocoa, fermented, malt [3] |
| 4  | 2,4-pentadienal  | 6.927   | 0.09   | Fruity [12]                      |
| 5  | 2-Furancarboxaldehyde | 8.095 | 3.82   | Almond like, caramel, spice [12] |
| 6  | 5-methyl-furfural | 13.352  | 8.46   | Caramel [12]                     |
| 7  | 1H-pyrole-2-carboxaldehyde, 1-methyl | |      | [literature] |
Table 2: Aroma compounds identified by GC-MS and their percentage area and retention time (RT).

| No | Volatile Compound                                | RT  | Area % | Flavour description [literature] |
|----|-------------------------------------------------|-----|--------|---------------------------------|
| 43 | Furan, 2-methyl-                                | 2.566 | 0.63   | Cocoa [12]                      |
| 44 | Furan, 2,5-dimethyl                             | 4.077 | 0.07   | Meaty [12]                      |
| 45 | 2-furfuryl furan                                | 18.196 | 1.44   | Roasted [12]                    |
| 46 | Difurfuryl ether                                | 26.212 | 1.94   | Coffee, earth [12]              |
| 47 | Furfurylformate                                  | 11.037 | 0.29   | Floral [12]                     |
| 48 | 2-Furanmethanol, acetate                        | 14.658 | 9.27   | Fruity [12]                     |
| 49 | Furfurylpentanoate                              | 23.445 | 0.56   | Sweet, fruity, pineapple-like [12] |

| Alcohol | 2-Furanmethanol                           | 8.950 | 6.91   | Burnt, caramel [12]          |
| Hydrocarbon | 1H-Indole                              | 26.095 | 0.35   | Burnt, floral [12]           |
|         | 1H-Indole, 3-methyl                        | 29.226 | 0.15   | Animal [12]                   |
|         | 3,4-dimethoxystyrene                       | 28.479 | 0.54   | Floral, green [12]           |
| Pyrrole | 1H-Pyrrole, 1-methyl-                       | 4.907 | 0.08   | Herbal                        |
|         | N-furfuryl pyrrole                        | 21.954 | 3.59   | Vegetable [12]                |
|         | 1H-pyrrole, 1-(2-furanylmethyl)-            | -     | -      | Earthy-green [12]            |
| Pyridine | Pyridine                                 | 5.223 | 1.44   | Sour, smoky, warm [11]        |

Based on Table 2, aroma compounds of aldehyde group (38%) dominated in arabica, while phenol group (25%) dominated in Robusta. Several key flavour compound were identified by GC-MS. They were 2-methylbutanal (1.66% in Arabica; 0.46% in Robusta), 3-methylbutanal (0.95% in Arabica; 0.31% in Robusta), 2,5-dimethylpyrazine (2.43% in Arabica), 2-ethyl-3,5-dimethylpyrazine (2.42% in Arabica; 2.09% in Robusta), β-damascenone (0.15% in Arabica; 0.40% in Robusta), Linalool (1.00% in Arabica), and 1-methyl pyrrole (0.53% in Arabica; 0.08% in Robusta).

4. Conclusion
In conclusion, aroma extracts produced through UAE method consist of aroma compounds that majorly belong to 3 groups, i.e. acid, ester, and alcohol, where acid group dominated in all treatments. Meanwhile, the HS-SPME GC-MS analysis or the extraction from the headspace of samples could identify 12 groups of aroma compound, such as aldehyde, pyrazine, pyrrole, pyrimidine, ketones, phenol, ester, acid, furan, alcohol, hydrocarbon, and sulfur compound. Aroma compounds of Java Arabica coffee was dominated by aldehydes, while phenols were found to be the major compounds identified in Robusta.

Acknowledgments
We acknowledge the Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia and UB Forest for their ongoing support on this research.

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