

Authentication of volatile and non-volatile compounds in Robusta Java Bogor as a differentiator in post-harvest processes

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Abstract. Whether the post-harvest process will greatly influence volatile or non-volatile coffee chemical compounds. Four post-harvest coffee processing techniques, namely natural, honey, fullwash, and wine, were evaluated in this study. This research aims to authenticate the volatile and non-volatile compounds of Robusta Jawa Bogor green bean as a differentiator in natural, fullwash, honey, and wine processing. Using HS-SPME-GC-MS and LC-MS, we identified a total of 128 volatile compounds (113 in natural, 111 in honey, 100 in fullwash, and 126 in wine), as well as 105 non-volatile compounds (77 in natural, 73 in honey, 66 in fullwash, and 93 in wine). The study found volatile compounds like ethyl cinnamate potential marker for honey processing. A potential marker for natural and wine processing is 1- isopropyl-3 methylbenzene. Some potential markers for wine processing are (E)-4-hexen-1-ol, 5-methyl-2-hexanol, diethyl succinate, ketoisophorone, and 4-ethyl-2-methoxyphenol. Non-volatile compounds like 1-naphthoic, [4]-gingerol, and theophylline are non-volatile markers for natural processing. Succinic acid is a non-volatile marker for natural and wine processing. While maleic acid and adenosine are markers for honey processing, adenine is a marker for wine processing. In contrast, fullwash does not have any volatile and non-volatile marker. Due to post-harvest-process variations, the obtained results assist in authenticating the chemical compounds of Robusta Java Bogor green beans.

1 Introduction

Robusta Java Bogor is a type of robusta coffee from Indonesia that has been certified as a geographical indication originated from five sub-districts in Bogor Regency (Sukmaktur, Cariu, Tanjung Sari, Cisarua, and Babakan Madang). Single-origin coffee is referred to as coffee with a geographical indicator certification, and specialty coffee is frequently

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distinguished from regular coffee using geographical indications [1]. Sukamakmur District, one of the Robusta Java Bogor producing districts, processes coffee using natural, honey, fullwash and wine methods.

Natural processing is characterized by fresh coffee cherries placed on a drying tray and left exposed to direct sunlight until dry [2, 3]. Water use distinguishes fullwash processing since a washing procedure is performed following the removal of the fruit flesh (pulping) and the fermentation process to eliminate the mucilage [4, 5]. Honey processing is a combination of fullwash and natural processing, sometimes referred to as pulped natural or semi-wet processing. Honey processing begins with removing around 50-60% of the fruit flesh, leaving only some parts of the mucilage and continues with the drying process. The method of wine processing originated from natural processing. Two fermentation processes aerobic and anaerobic are involved in the characteristics of wine processing. A lengthy fermentation can result in coffee drinks with the taste and aroma of wine [6]. An aerobic fermentation process takes place naturally throughout the drying process. The presence of fruit flesh during the fermentation and drying processes reveals variances in coffee processing based on the process stages [7], water-related processes and the duration of the processing cycle.

The physical and chemical qualities of the coffee beans produced, as well as the cup quality and sensory aspects of the coffee drink created, are all directly impacted by the same coffee beans conducted using various post-harvest processing techniques [8–10]. Because of variations in the microbial ecological structure during the fermentation process, the composition of volatile and non-volatile compounds in coffee varies significantly depending on the method used [11, 12]. During the drying and fermentation stages of the post-harvest process, volatile compounds will occur [13], and non-volatile compounds derived from carbohydrates, carboxylic acids, and alkaloids are reported [14] to be affected by the fermentation process. This research was designed to authenticate markers for volatile and non-volatile compounds in robusta green coffee beans for each processing method. The chemical properties of volatile and non-volatile compounds in Bogor robusta green coffee beans using four processing methods have never been reported. This effort was carried out to provide data that can be used to evaluate coffee quality in the future. Potential marker compounds can provide information to identify coffee products experiencing adulteration.

2 Materials and method

The primary material is robusta coffee green beans (*Coffea canephora*) obtained from farmers at the Perhutani coffee plantation in Sukamulya Village, Sukamakmur District, Bogor Regency, West Java Province, Indonesia, harvested in July 2021. The chemicals used were internal standard alkanes C9–C32 (Sigma–Aldrich, USA), LC-grade water, and LC-grade methanol.

2.1 Post-harvest processing

The harvesting process is carried out by farmers hand-picking whole, red-ripe coffee cherries. After harvesting, the coffee cherries are sorted by floating in a tank filled with water. The floating coffee cherries and dirt are separated from the coffee cherries through the sorting. Coffee cherries that pass the sorting process are processed into four processing methods, namely natural, fullwash, honey and wine. Characteristics of each technique can be seen in Table 1 and the processing stages in Figure 1. The green coffee beans of each processing are sorted manually to obtain fine-grade robusta coffee, according to the fine robusta standards and protocols of UGDA and the Coffee Institute [15].

Table 1. Post-harvest processing characteristics of natural, honey, fullwash, and wine

Section	Post-harvest processing			
	Natural	Honey	Fullwash	Wine
Raw material	Coffee cherries	Pulping remains 50-60% skin and fruit flesh	Pulping remains mucilage	Coffee cherries
Fermentation	During drying	During drying	1. In plastic sack (16 hours) 2. During drying	1. Anaerob in plastic drum (30 days) 2. During drying
Washing	None	None	Yes	None
Drying	Sun (20 days, 8 hours/day)	Sun (10 days, 8 hours/day)	Sun (10 days, 8 hours/day)	Sun (30 days, 8 hours/day)
Hulling	Yes	Yes	None	Yes



Fig. 1. Robusta coffee processing methods, A) fresh coffee cherries; B) and C) the process of stripping the fruit skin (pulping) in honey and fullwash processing; D) fresh coffee cherries after the pulping; E) fermentation process in fullwash processing; F) anaerobic fermentation process of fresh coffee bean in wine processing; G) honey processing of drying coffee cherries; H) natural processing of drying coffee cherries; I) coffee drying process; and J) drying coffee cherries in wine processing.

2.2 Sample preparation and extraction

Green bean sample preparation refers to [16] with modification. The process begins by removing the silver skin, soaking it in liquid nitrogen for 5 seconds, then crushing it using a blender to produce green bean powder. Green bean powder is extracted using the method [17] with modification. Boiling water (100 mL) was added to an Erlenmeyer flask containing 5 g of green bean powder heated to the hot plate at a temperature of 90°C and stirred for 1 minute with a magnetic stirrer. The solution was then transferred to an ice bath and stirred for 2 minutes. The activity continued by filtering the sample using a vacuum filter with Whatman paper no. 1 to produce green bean brew.

2.3 Volatile compound analysis with HS-SPME-GCMS

Volatile compound analysis refers to [18] with modification. Extraction of volatile components used *Headspace-Solid Phase Microextraction*. Green bean powder (2 g) was placed in a 22 mL vial and heated at 70°C for 10 minutes to reach equilibrium. The volatile compounds extraction process was carried out by inserting 2 cm DVB/CAR/PDMS fiber (*divinylbenzene-carboxy-polydimethylsiloxane*) into the headspace of the vial and leaving it at a temperature of 70°C for 20 minutes. Volatile component analysis used GC (Agilent 7890A) and MS (Agilent 5975C XL EI/CI). The GC-MS injector was operated in splitless mode at 250°C with a DB-Wax column (30 m x 250 µm x 0.25 µm). Volatile compounds were identified based on RI or retention index (retention index calculated by comparing the retention times of C9–C32 alkanes) with a comparison of mass spectra with those published in the NIST-14 database. The percent contribution value was calculated by comparing the compound area with the total detected area.

2.4 Volatile compound analysis with HS-SPME-GCMS

Non-volatile compound analysis refers to [19] used LC-MS UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific. A total of 1 ml of green bean brew was diluted using water and LC grade methanol (1:1), then filtered using PTFE 0.22 µm (Merck, Germany). A 5.0 µL aliquot was injected into an Accucore C18 column, 100 x 2.1 mm, 1.5 µm (ThermoScientific) at a column temperature of 30°C. A mixture of water and 0.05% LC grade (A) formic acid and LC grade (B) methanol as the mobile phase. Flow rate of 0.20 mL/minute followed by a gradient elution system: 0-17 minutes (5-90% B), 17-20 minutes (90% B), 20-23 minutes (90-5% B), and 23-30 minutes (5% B). Electrospray ionization (ESI) ionization mode on positive and negative. Analysis was conducted in the 100-1500 m/z mass range with UV detection at 220, 265, 272, and 320 nm wavelengths. The suspected identified compounds were then compared with MS/MS several databases (Pubchem, NIST, and FoodB). Quantifying the relative area value of the alleged compound was measured by comparing the compound's relative area value with the total area of the eluted compound in the form of percent relative area.

2.5 Data analysis

Data on the percent contribution of compounds from GC-MS and the relative percent of compounds from LC-MS were analyzed multivariate using OPLS-DA modeling to show compound groupings and detect potential discriminant marker compounds responsible for the differences in roasted coffee groups at the four processing levels. Marker compounds that have a VIP (Variable importance plot) value > 1 were designated as compounds that have the highest discrimination potential [20]. Modeling is considered valid if it has a CV ANOVA value $p < 0.05$ and permutation test results show that the predicted Q^2 intercept value is lower than the analysis Q^2 . OPLS-DA modeling uses SIMCA software version 14.1 (Umetrics, Sweden). Potential marker compounds are selected based on their presence in one or two processes, having a percent contribution or relative percent area value > 0.1 and a VIP value > 1. Results were expressed as means of duplicate replication.

3 Results

3.1 Volatile compound profile of green coffee bean

Volatile compounds from green beans were successfully extracted using HS-SPME GC-MS, and a total of 128 compounds were identified and quantified, respectively 113 compounds in natural, 111 compounds in honey, 100 compounds in fullwash, and 126 compounds in wine. Based on the compound chromatogram (Figure 2), the ten dominant compounds in coffee include ethyl acetate, acetic acid, ethyl lactate, isovaleric acid, toluene, acetaldehyde, ethyl salicylate, alpha-terpineol, isoamyl alcohol, and acetoin.

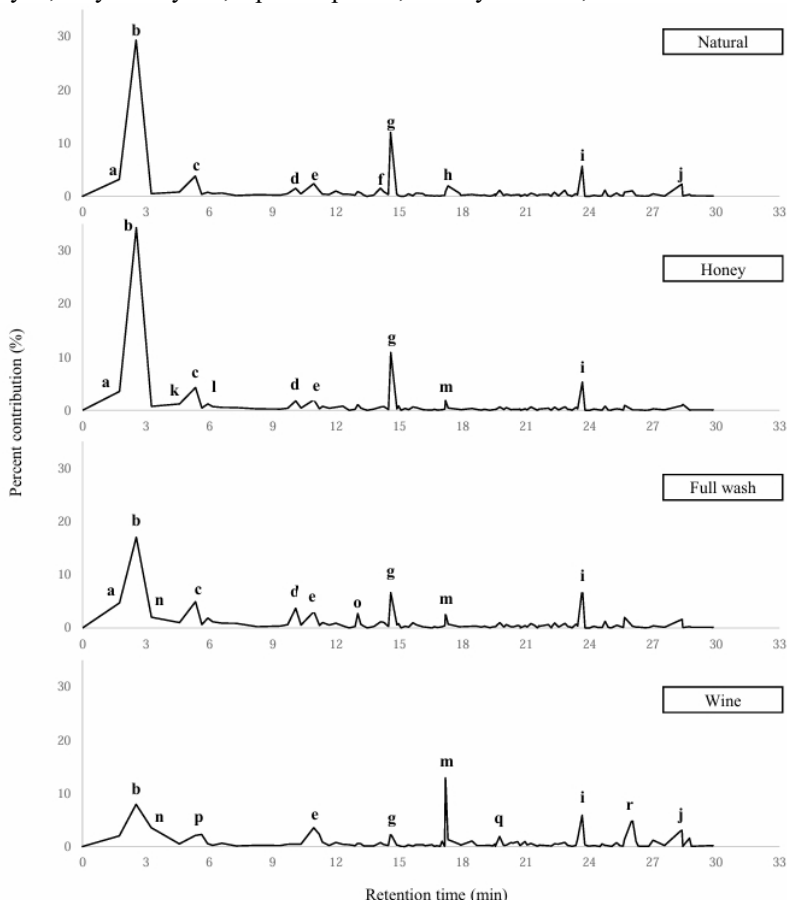


Fig. 2. GC-MS chromatogram of identified volatile compounds from four processing methods. (a) acetaldehyde, (b) ethyl acetate, (c) toluene, (d) D-limonene, (e) isoamyl alcohol, (f) 2-heptanol, (g) ethyl lactate, (h) 1-octen-3-ol, (i) isovaleric acid, (j) phenylethyl alcohol, (k) (+)-alpha-pinene, (l) 2,2-dimethylpentane, (m) acetic acid, (n) ethanol, (o) acetoin, (p) ethyl isovalerate, (q) ethyl isobutenoate, (r) ethyl salicylate.

3.2 Volatile potential markers of green coffee bean

OPLS-DA modeling of volatile compounds produced a total diversity of 70.90% (PC1: 49.20%, PC2: 21.70%), which indicates that each processing method is well separated. OPLS-DA modeling has an R^2X value of 0.848, R^2Y 1 and Q^2 0.976. Regression value > 0.4 ,

CV ANOVA 0.02. Two hundred permutation tests were carried out. The predicted Q^2 value $< Q^2$ analysis was obtained so that the OPLS-DA modeling was valid. The biplot image in Figure 3 and Table 2 displays potential markers of volatile compounds.

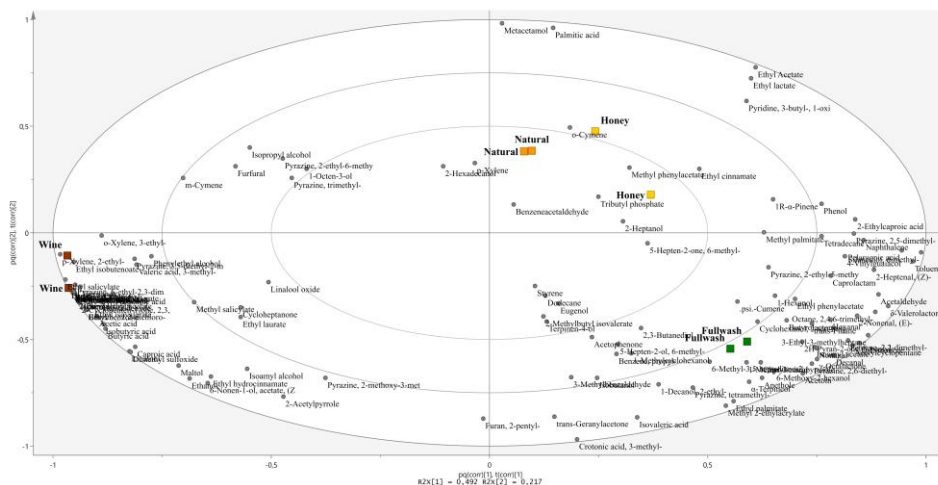


Fig. 3. OPLS-DA biplot of volatile compound profiles with four processing methods.

Table 2. Potential markers for volatile compounds were identified from four processing methods

Compounds	Post-harvest processing (percent contribution %)				VIP
	Natural	Honey	Fullwash	Wine	
Ethyl cinnamate	nd	0.12	md	nd	1.11
1-Isopropyl-3 methylbenzene	0.37	nd	nd	0.36	1.05
(E)-4-hexen-1-ol	nd	nd	nd	0.12	1.05
5-methyl-2-hexanol	nd	nd	nd	0.11	1.06
Diethyl succinate	nd	nd	nd	0.36	1.05
Ketoisophorone	nd	nd	nd	0.10	1.05
4-ethyl-2-methoxyphenol	nd	nd	nd	0.21	1.05

Values are the mean of the percent contribution from 2 replicates. nd: not detected and md: minor detected (percent contribution $< 0,1$), VIP: variable importance plot

3.3 Non-volatile compound profile of green coffee bean

Non-volatile compounds from green coffee beans were successfully extracted using LC-MS. A total of 105 compounds were identified, including 77 compounds in natural, 73 in honey, 66 in fullwash, and 93 in wine processes. The chromatogram of the detected compounds can be seen in Figure 4. The ten dominant non-volatile compounds in coffee include caffeine, 5-CQA (5-O-caffeoylquinic acid), trigonelline, 5-FQA (5-feruloylquinic acid), L-(+)-leucine, DL-phenylalanine, citric acid, 4-CQA (4-O-caffeoylquinic acid), choline, and N-caffeoyltryptophan.

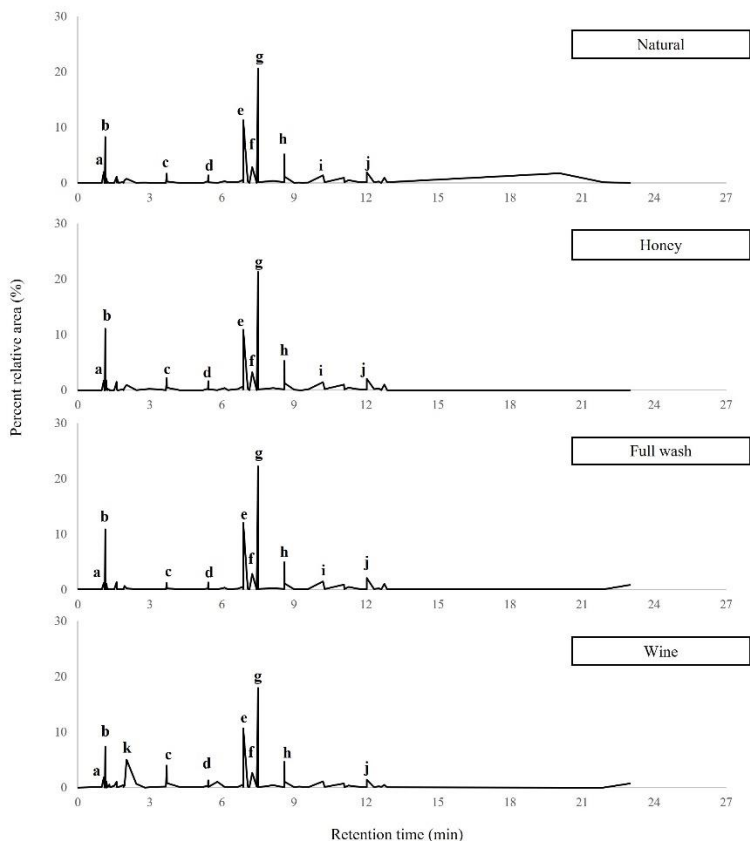


Fig. 4. LC-MS chromatogram of identified non-volatile compounds from four processing methods. (a) choline, (b) trigonelline, (c) citric acid, (d) DL-phenylalanine, (e) 5-CQA, (f) 4-CQA, (g) caffeine, (h) 5-FQA, (i) isovaleric acid, (j) N-caffeoyltryptophan, (k) L-(+)-leucine.

3.4 Non-volatile potential markers of green coffee bean

OPLS-DA modeling of non-volatile compounds produced a total diversity of 60.26% (PC1: 58.70%, PC2: 1.56%), which indicated that each processing method was separated well. OPLS-DA modeling has an R^2X value of 0.864, R^2Y 1 and Q^2 0.886. Regression value >0.4 , CV ANOVA 0.01. Carrying out 200 permutation tests, the predicted Q^2 value $<Q^2$ analysis was carried out so that the OPLS-DA modeling was valid. The biplot image in Figure 5 and Table 3 displays potential markers of non-volatile compounds.

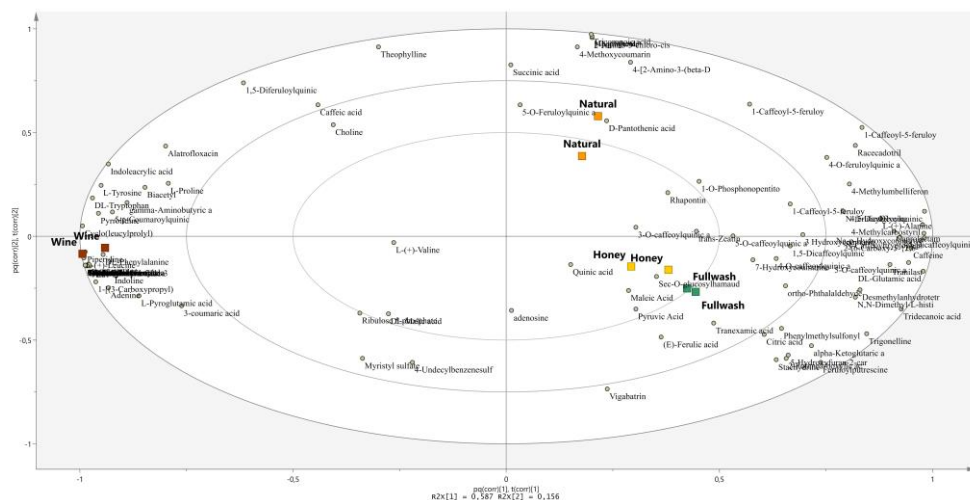


Fig. 5. OPLS-DA biplot of non-volatile compound profiles with four processing methods.

Table 3. Potential markers for non-volatile compounds were identified from four processing methods

Compounds	Post-harvest processing (percent relative area %)				VIP
	Natural	Honey	Fullwash	Wine	
1-naphthoate	0.19	nd	md	nd	1.26
[4]-gingerol	1.96	nd	nd	nd	1.26
Theophylline	0.10	nd	nd	nd	1.21
Succinic acid	0.68	nd	nd	0.14	1.06
Maleic acid	nd	0.11	nd	nd	1.05
Adenosine	nd	0.24	nd	md	1.33
Adenine	nd	nd	nd	0.16	1.00

Values are the mean of the percent relative area from 2 replicates. nd: not detected and md: minor detected (percent relative area <0,1), VIP: variable importance plot

4 Discussion

The ten dominant volatile compounds in coffee include ethyl acetate, acetic acid, ethyl lactate, isovaleric acid, toluene, acetaldehyde, ethyl salicylate, alpha-terpineol, isoamyl alcohol, and acetoin. Acetic acid is associated with an acidic aroma, while isovaleric acid is associated with a cheesy sour aroma; both have a fermented sour taste [21]. The presence of this compound in high concentration suggests that the wine-based green beans have a distinct fermented flavor. The length of fermentation also influences the activity of microorganisms like acetic acid bacteria and Enterobacteriaceae; the longer the fermentation period, the greater the likelihood of acetic acid formation [22–24]. Isoamyl alcohol is the dominant volatile compound in Arabica coffee green beans and the fermentation process increases its concentration [25, 26]. Phenylethyl alcohol compound is associated with a sweet aroma and floral, honey-like flavor [21, 27]. It is said to be the highest alcohol group compound in green

beans and the highest alcohol group compound in wine-processed [27, 28]. The fermentation process produces higher ethanol than without the fermentation process, and high concentrations of phenylethyl alcohol and ethanol are found in coffee that undergoes the fermentation process for the most prolonged duration [25, 27]. These findings are consistent with another study that found that the coffee with the most prolonged fermentation period had the greatest phenylethyl alcohol and ethanol contents. Coffee beans from the same fruit that are processed differently will produce distinct acid and alcohol components and different pectinase enzymes [29]. This volatile compound, ethyl 2-hydroxybenzoate or ethyl salicylate, is not created during fermentation. This compound is a phenolic group member, mainly in fresh coffee pulp and produced by chemical processes [30]. Coffee that undergoes fruit peeling processes such as honey processing and fullwash is assumed to have lower concentrations of the ethyl salicylate compound. Toluene was reported by [31] to be found in green coffee beans and will experience a decrease in concentration after roasting. Meanwhile, benzaldehyde is a group of aldehydes whose presence is influenced by the oxidation process of unsaturated fatty acids in coffee [32] and was reported as one of the marker compounds for defective coffee [33, 34]. Ethyl acetate and ethyl lactate are volatile compounds from the ester group found in fruit and have a fruity aroma that can be produced through fermentation [35–37]. Research [25] showed that the ethyl acetate compound increased drastically during fermentation.

Efforts to detect discriminant volatile compounds and potential markers responsible for differences between the four processing methods were conducted using multivariate analysis with OPLS-DA modeling on percent contribution data. The data display is a biplot, a combination of a score plot and a loading plot (Figure 3). Figure 3 shows that natural and honey processing is on the same plane, namely positive y and x , which means that the volatile compound contribution from natural and honey processing of coffee is very similar. Fullwash processing is in the positive x and negative y planes. The wine process tends to separate away in the negative x and y planes, indicating that this processing contributes to volatile compounds that are very different from the other three processes. The results show that honey processed, which has undergone partial pulp removal, has a composition of percent contribution of volatile compounds similar to natural, which has not undergone pulp removal. Even though wine-processed coffee still has fruit pulp, the results show that the percent contribution of the volatile compound appears to be very different from that of natural and honey. The fermentation process tremendously influences the composition of the volatile compounds formed. Natural and honey undergo a spontaneous fermentation process during drying, while processed wine undergoes an anaerobic fermentation before entering the drying process. Fullwash, which undergoes a total process of peeling the pulp, washing, and fermenting in a plastic sack, produces a different percentage of volatile compound contribution.

Table 2 displays potential marker volatile compounds that satisfy the criteria. Potential marker compounds include the alcohol group ((E)-4-hexen-1-ol and 5-methyl-2-hexanol), the ketone group (ketoisophorone), the ester group (ethyl cinnamate and diethyl succinate), benzene group (1-Isopropyl-3 methylbenzene) and phenolic group (4-ethyl-2-methoxyphenol). Ethyl cinnamate is thought to be a potential marker for honey processing. This compound is also found in fullwash processing but is classified as minor. The compound 1-Isopropyl-3 methylbenzene is a marker compound for natural and wine processing. Long-lasting anaerobic fermentation in wine processing can form several volatile compounds not found in other processes. This can be seen from the total number of volatile compounds identified and quantified in wine processes, greater than in natural, honey, and fullwash processes. The large number of compounds formed means that wine processed tends to have more potential marker volatile compounds. Five compounds are thought to be potential volatile markers for wine processing, including (E)-4-hexen-1-ol, 5-methyl-2-hexanol,

ketoisophorone, diethyl succinate, and 4-ethyl-2-methoxyphenol. Based on the *yeast metabolome database*, the compounds diethyl succinate and 4-ethyl-2-methoxyphenol can be produced by *Saccharomyces cerevisiae* and are microorganisms commonly found in coffee fermentation [38]. No potential marker volatile compounds were found in fullwash. Fullwash processing does not necessarily contain potential marker compounds, even though its volatile compound contributions are substantially different from those of the other three processes. Most volatile compounds in fullwash processed are also found in natural, honey or wine processed, although the contribution is more minor. In addition, determining the criteria for potential marker compounds with a percent contribution value > 0.1 has eliminated compounds that could potentially be potential marker compounds for fullwash processing.

The chromatogram of non-volatile compounds (Figure 4) showed that the ten dominant compounds are caffeine, 5-CQA, trigonelline, 5-FQA, L-(+)-leucine, DL-phenylalanine, citric acid, 4-CQA, choline, and N-caffeoyltryptophan. Caffeine and trigonelline compounds belong to the alkaloid group, while 5-CQA, 4-CQA, and 5-FQA belong to the chlorogenic acid compound group. Alkaloid and chlorogenic acid are coffee's dominant compounds [39–44]. Chlorogenic acid is influenced by the drying method applied [45]. In contrast to volatile compounds, the dominant non-volatile compounds in green beans do not appear to differ between processing except for the compound L-(+)-leucine, which is dominant in green beans processed in wine. L-(+)-leucine is a group of essential amino acids reported to be produced by microorganisms through fermentation [46, 47].

The biplot image (Figure 5) shows natural processing in the positive x and y planes. The honey and fullwash processes are on the same plane, namely x is positive and y is negative, which means that the percent relative area of non-volatile compounds in honey and fullwash is very similar. The wine process tends to separate in the negative x and y planes, indicating that this processing has a percent relative area of non-volatile compound areas that are very different from the other three processes. In contrast to volatile compounds, the percent relative area of non-volatile compounds from the natural process appears to differ from that of the honey process. Interestingly, honey tends to have a percent relative area of non-volatile compounds similar to fullwash. The fermentation process in the plastic sack and the washing process does not significantly influence the composition of the percent relative area of non-volatile compounds. In this condition, partial pulp removal in honey processing cannot form non-volatile compounds similar to natural processing and tends to be more like fullwash process which undergoes total pulp stripping. As with volatile compounds, anaerobic fermentation significantly influences the percent contribution of non-volatile compounds in wine processing.

Table 3 displays potential markers for non-volatile compounds that satisfy the requirements. Theophylline, [4]-gingerol, and 1-naphthoate are potential markers of non-volatile compounds in natural processes. Bacteria, algae, or fungi oxidize 1-methylnaphthalene by hydroxylating the side chain or aromatic ring to generate the 1-naphthoate molecule [48]. The presence of phenolic compounds, the dominant compounds in ginger rhizomes, namely [4]-gingerol, is interestingly only detected in the natural process. Theophylline, a group of alkaloids, is reported to have higher levels in Luwak coffee than coffee with a washing process [49]. This compound was also detected in wine processing but was classified as minor. Succinic acid is a marker compound in natural and wine processing. The presence of succinic acid is a sign of the activity of yeast and bacterial microorganisms [50]. Maleic acid and adenosine are potential marker compounds in honey processing. Yeast such as *Saccharomyces cerevisiae* can produce malic acid during fermentation, which can be degraded to form maleic acid [51, 52]. While adenosine concentration can be influenced by the sugar concentration during fermentation by microorganisms [53]. Wine processing has one potential marker compound, namely adenine. The concentration of purine group

compounds such as adenine is influenced by processing methods due to yeast purine metabolic activity [54]. The adenine is also present in honey processing but is classified as a minor compound. During fermentation, fungi can produce catalysts and compounds [55], so differences in fermentation media allow differences in the types and activities of microorganisms to influence the types of compounds contained in coffee. Fullwash coffee lacks potential marker compounds, comparable to how volatile compounds do.

5 Conclusion

The efforts to authenticate volatile and non-volatile compounds as differentiators of post-harvest processing were successfully carried out. While the anaerobic fermentation process significantly impacts the content of volatile and non-volatile compounds. The presence of pulp during the fermentation and washing processes significantly influences the composition of volatile compounds. Meanwhile, non-volatile compounds are more influenced by anaerobic fermentation and the presence of fruit flesh during fermentation. The fermentation process in plastic sacks and washing does not have a major effect on the creation of non-volatile compounds. The potential marker volatile compound in honey processing is Ethyl cinnamate. The compound 1-Isopropyl-3 methylbenzene is a potential marker for natural and wine processing. While potential marker volatile compounds for wine processing include (E)-4-hexen-1-ol, 5-methyl-2-hexanol, ketoisophorone, diethyl succinate, and 4-ethyl-2-methoxyphenol. Potential marker non-volatile compounds in natural processing include 1-naphthoate, [4]-gingerol and theophylline. Succinic acid is a potential marker compound in natural and wine processing. Maleic acid and adenosine are potential marker compounds in honey processing, while adenine is a potential marker compound in wine processing. Fullwash processing does not have potential marker for volatile and non-volatile compounds. More investigation is required into the mechanisms underlying the synthesis of chemical compounds and the assessment of the kinds of microorganisms involved in each process.

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