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Research article

Semiquantification of volatile compounds and identification of potential volatile markers and dry aroma from robusta second-crack roasted coffee processed from several post-harvest processing

Nur Fajriani Suaib[1,2,3], Didah Nur Faridah[1,2], Dede Robiatul Adawiyah[1,2], and Nuri Andarwulan[1,2,*]

[1] Department of Food Science and Technology, IPB University, Bogor 16680, Indonesia
[2] South-East Asia Food & Agricultural Science and Technology (SEAFAST) Center, IPB University, Bogor 16680, Indonesia
[3] Department of Food Science and Technology, Halu Oleo University, Kendari, 93132, Indonesia

* **Correspondence:** Email: andarwulan@apps.ipb.ac.id; Tel: +62811118983.

Abstract: In Bogor, the farmers employed several methods for robusta post-harvest processing, including natural, honey, full wash, and wine processing. This research was conducted to examine the influence of the different post-harvest processing methods on volatile compounds and to identify volatile markers that can authenticate coffee roasted under second-crack roasting and characterize its dry aroma. The study identified and semiquantified 140 compounds. Post-harvest processing affected carboxylic acids, esters, alcohols, hydrocarbons, phenolics, thiophenes, and total volatile compounds. Principal component analysis (PCA) modeling showed that natural and honey processes had similar volatile compound compositions, while full wash and wine processes tended to differ. Based on the variable importance in projection (VIP) values from orthogonal partial least square discriminant analysis (OPLS-DA) modeling and percent contribution, two compounds (i.e., ethyl salicylate and 2-Methyl-5-methoxy-4H-pyran-4-one) were identified as potential markers for natural and wine processing. Ethyl acetate and 5-amino-2-methylbenzothiazole were identified as potential markers for wine processing. Honey and full wash processing did not have any distinct volatile marker. Natural processing exhibited a dry aroma of caramelly, roasted peanut, and chocolate, while honey processing had caramelly, nutty, and earthy aromas. Full wash processing had sweet nut, earthy, and herbal aromas with a hint of potato, and wine processing had fermented, winey, molasses, and chocolaty aromas. This research demonstrated that post-harvest processing influenced volatile compounds in second-

crack roasted coffee. Identifying potential markers provides valuable information for authenticating second-crack roasted coffee and differentiating it based on post-harvest processing and dry aroma.

Keywords: Robusta Bogor; post-harvest processing; second crack; volatile compound; volatile marker, dry aroma

1. Introduction

Bogor Regency is one of the areas in Indonesia that produces robusta coffee, and the processing methods used by farmers have undergone rapid development. Robusta coffee farmers commonly employ several post-harvest processing methods including natural, honey, full wash, and wine. The differences between these processes lie in the treatment of the coffee beans before processing, the fermentation methods used during processing, the presence of a washing stage, and the duration of the drying process [1]. In coffee beans, the composition of metabolites is influenced by genetic factors; agricultural practices, such as harvesting techniques and processing methods; and environmental factors, such as the climate where the coffee plants grow [2–4]. Previous research has shown variations in volatile compounds from robusta coffee beans processed using the natural, honey, and full wash methods. This study continues that research by focusing on coffee that has undergone the roasting process.

Roasting is a critical process in coffee processing because it is during roasting that volatile compounds and flavors are formed, which can become the distinctive characteristics of coffee brew [2,5,6]. During roasting, these compounds undergo significant changes due to the Maillard reaction, acid degradation, protein degradation and denaturation, caramelization, pyrolysis, and other reactions [6,7]. Temperature and roasting time are typically controlled during the roasting process, and parameters, such as color, aroma, flavor, pH, cracking sound, and mass loss, are often used by roasters to determine the degree of roasting in real time [8–10]. While color parameters have long been used to measure roasting degree, known as roast level, they are considered inadequate for providing a clear picture of the roasting process [11]. Cracking is a phenomenon that occurs in coffee beans during roasting, characterized by a popping sound audible to the human ear. It is caused by the accumulation of CO_2, which leads to steam pressure buildup. Two cracks occur during the roasting, the first crack, which produces a softer popping sound, and the second crack, which is characterized by louder and more frequent pops [8]. Based on the physical quality of the coffee beans produced using the four processing methods, the wine-processed coffee beans showed the most distinctive color characteristics, brownish hues. Due to this color difference, the roast color parameter was deemed less suitable; therefore, the second crack phase parameter was chosen for analysis in this study.

Variations in post-harvest coffee processing can lead to discrepancies in the aroma of roasted coffee, while the roasting procedure itself may affect the extraction of volatile compounds [12–14]. These volatile compounds contribute to the distinctive aroma of coffee. The profile of volatile compounds of Bogor robusta processed by natural, honey, full wash, and wine methods and roasted to the second crack phase has not been reported before. This study was designed to investigate whether the natural, honey, full wash, and wine coffee processing methods influence the concentration of volatile compounds produced. The study also aims to identify potential marker compounds of second-crack roasted coffee that can differentiate between post-harvest processing methods. Additionally, it

seeks to determine the aroma characteristics of second-crack roasted coffee produced from these four processing methods.

2. Materials and methods

2.1. Post-harvest processing methods

Robusta (*Coffea canephora*) beans were obtained from farmers in the Sukamakmur District of Bogor Regency, Indonesia. The coffee is grown at 900–1000 meters above sea level. Only ripe red coffee cherries were selected for post-harvest processing. The farmers used traditional methods for processing as shown in Figure 1. A total of 20 kg of coffee beans were collected per processing batch, then sorted according to the fine robusta standards and protocols established by the Uganda Coffee Development Authority and The Coffee Institute [15]. The coffee beans were manually sorted at each refining step to produce fine-grade robusta green coffee beans. The beans were then passed through sieves with 8 mm, 7 mm, and 6 mm diameters. Coffee beans that passed through the 8 mm sieve but could not pass the 7 mm sieve were collected and used in this study.

2.2. Roasting and sample preparation

A total of 500 g of coffee beans from each processing method were roasted using a Super Roaster roasting machine (Indonesia) with a capacity of 2.5 kg, featuring a gas burner heater and electric drive. The sound of cracking was carefully monitored during the roasting process. The endpoint of the roasting process was determined once the second crack sound was heard. Upon hearing the second crack, the heat source was immediately lowered to prevent a further increase in the final temperature. The roasted coffee was removed from the roasting machine and cooled for 3 minutes. The entire roasting process up until the second crack was recorded using Artisan 2.0.0 software (GPLv3.0 License, Germany) connected to a laptop. An experienced roaster carried out the roasting process to ensure accurate results. For volatile compound analysis, the roasted coffee was ground using a coffee grinder (Gemilai crm 9053, China).

2.3. Volatile compounds analysis by headspace solid-phase microextraction GC-MS

The volatile compounds were analyzed using GC (Agilent 7890A) and MS (Agilent 5975C XL EI/CI). The analysis of volatile compounds using HS-SPME-GCMS is based on the method referenced in [16], with minor modifications. A 22 mL vial containing 2 g of roasted coffee powder was filled with 5 µL of 0.01 concentration of the internal standard (3-heptanone). To achieve equilibrium, the vial was heated to 70 °C for 10 minutes. Subsequently, a 2 cm DVB/CAR/PDMS (*Divinylbenzene*/*Carboxen*/*Polydimethylsiloxane*) fiber was inserted into the headspace of the vial and incubated for 20 minutes at 70 °C. The volatile compounds were then desorbed for 5 minutes in the GC. Compound separation was carried out using a DB-wax column (30 m x 250 µm x 0.25 µm). The GC injector was operated in spitless mode at 250 °C, with helium as the carrier gas at a flow rate of 0.8 mL/min. The oven temperature was initially set to 50 °C for 5 minutes, then increased to 240 °C at a rate of 5 °C/min, where it was maintained for 10 minutes. The ion source (MS source was set to 230 °C, MS Quad was set to 150 °C), and the interface was set to 250 °C. Mass spectra were scanned

in the range of m/z 29–550 amu. Volatile compounds were identified by comparing their mass spectra and retention indices with the NIST-14 database. The retention times of C9-C32 alkanes were used to calculate the retention index.

Compounds were semiquantified based on the ratio of their peak area to the peak area of the 3-heptanone internal standard, which was used to calculate the relative amount of each compound in μg/g. The percent contribution of each compound was determined by dividing the peak area of that compound by the total peak area of all identified compounds. All measurements were conducted in duplicate.

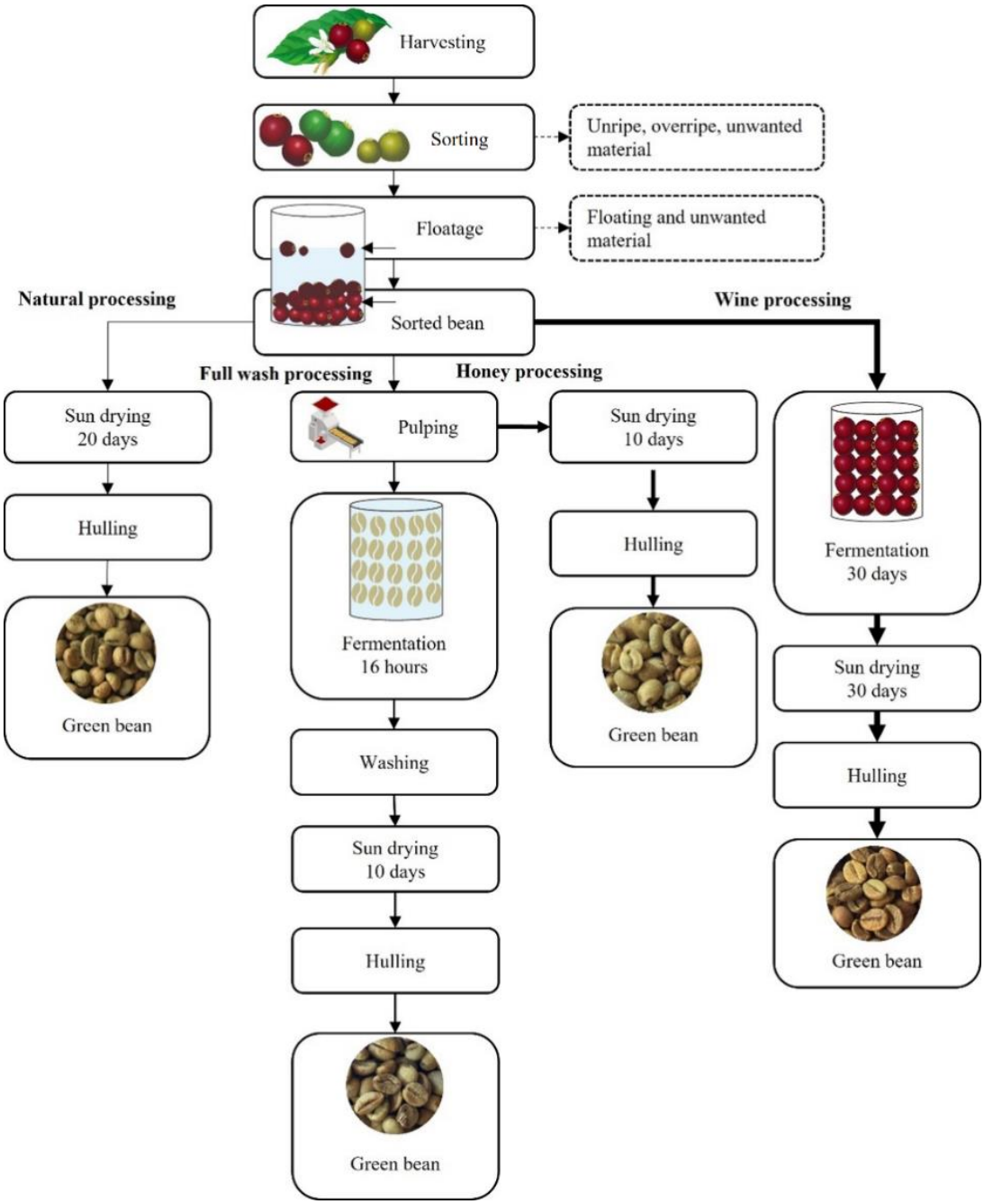

Figure 1. Stages of post-harvest coffee processing by farmers in Bogor Regency.

2.4. Sensory evaluation

2.4.1. Dry aroma evaluation

Dry aroma evaluation was conducted by six trained panelists, including one Q-grader, three baristas, and two roasters. Following the method outlined in [15], 8.75 g of ground coffee was placed in a porcelain container with a lid. The panelists were asked to smell the coffee and identify the type of dry aroma and then rate it on a scale from 1 to 6.

2.4.2. Ethics approval of research

This research has received ethical clearance for sensory evaluation from the Research Ethics Commission of IPB University, under approval number 613/IT3.KEPMSM-IPB/SK/2022.

2.5. Data analysis

An analysis of variance (ANOVA) and Duncan's multiple range test were used to assess the impact of post-harvest processing on relative amounts of volatile compounds. SPSS version 27 (SPSS Inc., Chicago, IL, USA) was employed to perform the ANOVA and Duncan tests. Multivariate analysis of the percent contribution data of compounds from GC-MS was conducted using principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) modeling to illustrate the grouping of compounds based on their post-harvest processing and to identify potential discriminant marker compounds using variable importance in projection (VIP) values. Compounds with a VIP value >0,8 were considered to have the highest potential for discrimination [17]. Compounds with VIP values >0,8 and percent contribution values >0,1 were identified as potential markers. PCA and OPLS-DA analysis were performed using SIMCA software version 14.1 (Umetrics, Umea, Sweden).

3. Results and Discussion

3.1. Roasting Profile

Based on the roasting process conducted (Figure 2), naturally processed coffee tends to reach the second crack earlier, followed by honey, full wash, and wine-processed coffee, with the latter experiencing the second crack last. The temperature at which the second crack occurred for each processing method was generally similar, ranging from 223 ℃ to 228 ℃.

Cracks in roasted coffee occur in two phases: the first crack and the second crack. The first crack typically occurs when the bean temperature reaches around 200 ℃ and is characterized by a loud, low-frequency popping sound lasting about two minutes. When the beans reach a temperature of approximately 230 ℃, the second crack occurs, characterized by a more frequent popping sound [18]. It has been reported that the roast profile is closely related to factors, such as coffee variety, temperature, time, airflow, and the roasting method, all of which influence the characteristics the roasted coffee produced [19].

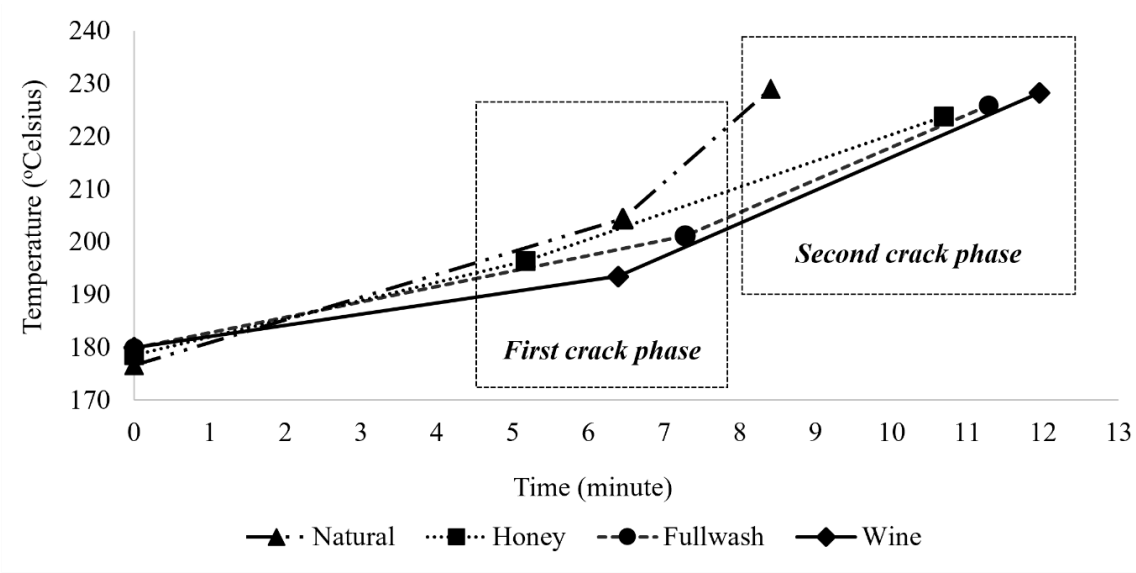

Figure 2. Graph of roasting process from four processing methods.

During the first crack phase, treatments had no significant temperature or first crack duration differences. Differences between treatments became apparent during the second crack phase. Naturally processed coffee entered the second crack phase more quickly than the other treatments, which resulted in a shorter roasting duration for natural processed coffee. This phenomenon was previously reported by [20], coffee processed using the natural method exhibited a denser bean structure, causing it to lose mass more rapidly during roasting than full wash processed coffee. In contrast, honey and wine processed coffees did not differ significantly from full wash processed coffee. The results of the second-crack roasting process (Figure 3) revealed the presence of oil on the surface of the coffee. Based on the physical characteristics of the coffee and the temperature, which ranged from 223 ℃ to 228 ℃, the second-crack roasted coffee produced by all processing was classified as a medium-to-dark roast level [21,22].

Figure 3. Appearance of second-cracked roasted coffee from four processing methods.

3.2. Volatile compound profile of second crack roasted coffee

HS-SPME GC-MS was used to extract volatile compounds from the second crack-roasted coffee, resulting in the identification and quantification of up to 140 compounds, which accounted for a total

contribution of over 87%. Analysis with PCA (Figure 4), based on the percent contribution value of volatile compounds, produced a model with a total variance of 70.10%, with PC1 contributing 49.90% and PC2 contributing 20.20%.

PCA analysis was used to examine the dominant volatile compounds and the grouping of processing methods based on the similarity of their volatile compounds. Figure 4 demonstrates a clear separation between the processing methods. Natural and honey processed coffee were located on the same plane, corresponding to positive PC1. Full wash processed coffee was positioned in the negative PC1 plane, while wine-processed coffee appeared in the farthest plane, negative PC2. The proximity of natural and honey-processed coffee on the same plane suggests that the volatile compound profiles of these two processes were similar. In contrast, full wash and wine processing differed significantly from the others. These results confirmed previous research [1], which reported that natural and honey-processed coffee beans shared similar volatile compounds, while full wash and wine-processed coffee beans were distinct. The influence of processing on the volatile compound profile remained consistent even after the second-crack roasting process. As reported by [4], coffee processing involving anaerobic fermentation affects the chemical components of the coffee fruit, leading to flavors that differ from those of other processing methods.

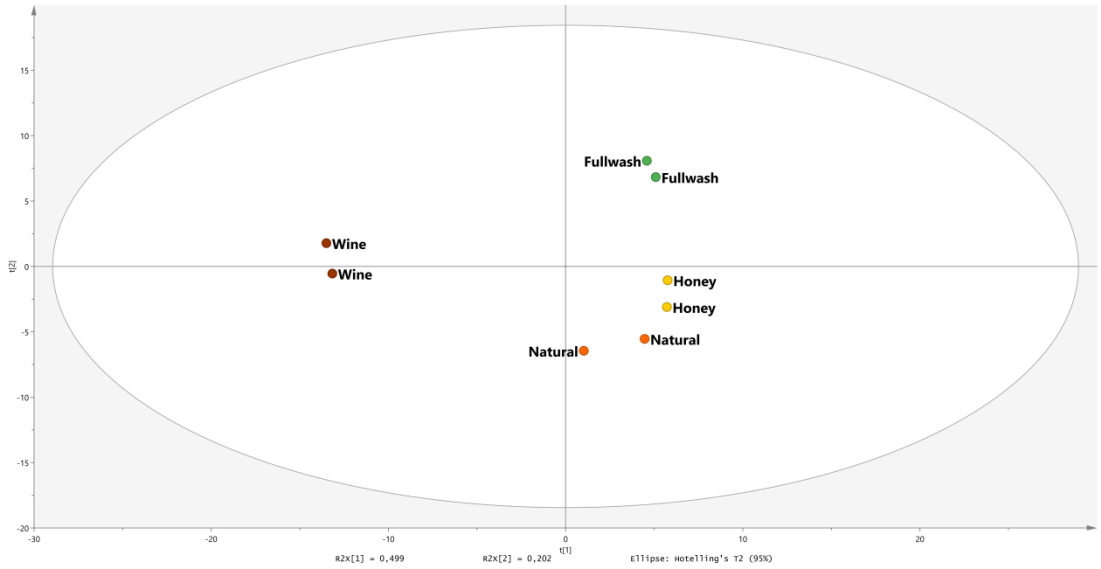

Figure 4. PCA score plot of volatile compounds of second-crack roasted coffee from four processing methods.

3.3. Effect of post-harvest processing on volatile compounds of second crack roasted coffee

The semiquantification of volatile compounds in roasted coffee is presented in Table 1. The analysis revealed that the dominant compounds in roasted coffee furan, pyrazine, phenolic, and carboxylic acid. The furan group, which was the most abundant compound group in second-crack roasted coffee, is formed during the roasting process due to interaction between high temperatures and sugar, amino acids, ascorbic acid oxidation, unsaturated fatty acids, and carotenoids [23,24]. The dominant compound within the furan group included 2-furanmethanol, furfuryl acetate, 5-methyl-furfural, and furfural. Among these, 2-furanmethanol was the predominant compound in Bogor robusta

coffee, a finding consistent with results reported by [4,25,26]. However, [27] reported that methylbutyrate, 2,3-dimethylpyrazine, and 3-hexanone were the dominant compounds in the robusta samples observed. This suggests that the compounds in roasted robusta coffee are influenced by various factors, including the treatment applied to the coffee beans. Furfuryl acetate, the second-highest compound, has been reported to be more prevalent in robusta coffee than in arabica coffee [28]. The analysis showed that the post-harvest processing did not affect the furan group compounds (Table 1). The concentration of furan in roasted coffee was reported to be more influenced by the coffee species. The degree of roasting, where robusta coffee tends to contain higher furan compounds compared to arabica, and coffee with a dark roast level contains more furan compared to the light level, dark roast level contains 5697 µg/kg of furan in robusta coffee. In comparison, arabica coffee contains 3809 µg/kg [29,30].

Table 1. The relative amounts of volatile compounds (µg/g) identified by HS-SPME-GCMS in second-crack roasted coffee from four post-harvest processing methods.

Compound	RT (min)	RI	Relative amount			
			Natural	Honey	Full wash	Wine
Aldehyde						
acetaldehyde	1.59	-	0.23 ± 0.04	0.31 ± 0.00	0.29 ± 0.01	0.23 ± 0.03
2-methylbutanal	2.57	912	0.49 ± 0.05	0.34 ± 0.01	0.39 ± 0.04	0.75 ± 0.10
3-methylbutanal	2.60	914	0.39 ± 0.02	0.28 ± 0.01	0.34 ± 0.03	0.50 ± 0.07
1-methyl-1H-pyrrole-2-carboxaldehyde	20.93	1619	0.69 ± 0.06	0.72 ± 0.02	0.95 ± 0.03	0.99 ± 0.13
2-phenyl-2-butenal	27.88	1931	0.30 ± 0.01	0.26 ± 0.02	0.41 ± 0.00	0.37 ± 0.08
Total Aldehydes			2.11 ± 0.06^{ab}	1.90 ± 0.05^{a}	2.38 ± 0.03^{ab}	2.83 ± 0.40^{b}
Furan						
furan	1.85	-	0.10 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
2-methylfuran	2.23	-	0.27 ± 0.01	0.22 ± 0.01	0.33 ± 0.01	0.22 ± 0.04
2.5-dimethylfuran	2.99	946	0.12 ± 0.01	0.10 ± 0.00	0.15 ± 0.01	0.14 ± 0.02
2-pentylfuran	10.41	1238	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.00	0.15 ± 0.01
dihydro-2-methyl-3-furanone	11.15	1262	0.51 ± 0.05	0.52 ± 0.01	0.51 ± 0.02	0.32 ± 0.02
2-methyl tetrahydrofuran	13.93	1354	0.14 ± 0.04	0.15 ± 0.02	0.15 ± 0.00	0.17 ± 0.01
furfural	16.92	1461	3.23 ± 0.21	2.53 ± 0.13	2.91 ± 0.24	2.41 ± 0.29
2-furfuryl methyl sulfide	17.56	1485	0.17 ± 0.00	0.20 ± 0.01	0.24 ± 0.00	0.25 ± 0.02
2-acetylfuran	18.02	1502	1.00 ± 0.01	1.08 ± 0.03	1.29 ± 0.01	1.02 ± 0.10
2-furfuryl-acetate	18.92	1538	5.84 ± 0.15	6.53 ± 0.55	6.69 ± 0.10	8.76 ± 0.04
2-furanmethanamine	19.54	1563	0.19 ± 0.02	0.19 ± 0.01	0.28 ± 0.01	0.30 ± 0.06
5-methylfurfural	19.76	1571	4.65 ± 0.14	3.70 ± 0.08	4.43 ± 0.21	3.73 ± 0.47
2-ethyl-5-methylfuran	19.84	1575	0.21 ± 0.00	0.23 ± 0.01	0.28 ± 0.01	0.16 ± 0.03
2.2-methylenebisfuran	20.71	1610	0.74 ± 0.05	0.75 ± 0.03	0.93 ± 0.01	0.73 ± 0.08
2-acetyl-5-methylfuran	20.80	1613	0.20 ± 0.01	0.21 ± 0.02	0.25 ± 0.01	0.19 ± 0.01
2-furanmethanol	22.02	1666	12.70 ± 0.43	11.87 ± 0.54	15.15 ± 0.65	14.90 ± 1.93
2-furfuryl-5-methylfuran	22.40	1681	0.38 ± 0.01	0.45 ± 0.02	0.68 ± 0.01	0.36 ± 0.05

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Compound	RT (min)	RI	Relative amount			
			Natural	Honey	Full wash	Wine
2-hexyl furan	23.12	1712	0.19 ± 0.02	0.12 ± 0.01	0.21 ± 0.01	0.23 ± 0.03
3.4-dimethylfuran-2.5-dione	23.46	1727	0.39 ± 0.02	0.36 ± 0.02	0.47 ± 0.02	0.44 ± 0.03
3-phenyl furan	26.24	1853	0.53 ± 0.00	0.44 ± 0.01	0.65 ± 0.03	0.72 ± 0.08
2-methyl-3-(2-furyl)propenal	26.71	1875	0.18 ± 0.00	0.17 ± 0.01	0.25 ± 0.01	0.18 ± 0.05
5-methyl-2-furanmethanethiol	26.93	1885	0.18 ± 0.01	0.17 ± 0.02	0.26 ± 0.01	0.29 ± 0.04
5-ethylfuran-2-carboxylic acid	27.02	1890	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.03	0.13 ± 0.04
difurfuryl disulfide	27.25	1900	0.15 ± 0.01	0.17 ± 0.03	0.28 ± 0.02	0.20 ± 0.06
2-propyl furan	27.29	1902	0.13 ± 0.02	0.14 ± 0.02	0.18 ± 0.02	0.12 ± 0.02
furfural acetone	27.35	1905	0.15 ± 0.01	0.15 ± 0.00	0.23 ± 0.01	0.24 ± 0.06
furfuryl ether	29.00	1985	0.47 ± 0.02	0.53 ± 0.04	0.79 ± 0.00	0.53 ± 0.07
furaneol	30.06	2038	0.12 ± 0.00	0.10 ± 0.00	0.16 ± 0.0-	0.11 ± 0.03
2-n-butyl furan	34.03	2245	0.14 ± 0.02	0.16 ± 0.02	0.29 ± 0.02	0.23 ± 0.04
2-ethylfuran	34.43	2267	0.10 ± 0.03	0.13 ± 0.02	0.16 ± 0.03	0.14 ± 0.03
Total Furans			33.39 ± 0.08[a]	31.71 ± 1.56[a]	38.60 ± 0.16[a]	37.64 ± 3.82[a]
Ketone						
acetone	1.94	-	0.43 ± 0.04	0.31 ± 0.01	0.44 ± 0.03	0.52 ± 0.06
2.3-butanedione	3.28	970	0.16 ± 0.00	0.12 ± 0.01	0.16 ± 0.00	0.13 ± 0.01
2.3-pentanedione	4.91	970	0.27 ± 0.02	0.20 ± 0.01	0.21 ± 0.01	0.18 ± 0.02
3-penten-2-one	6.82	1123	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.29 ± 0.04
hydroxyacetone	12.22	1296	0.62 ± 0.02	0.51 ± 0.02	0.56 ± 0.01	0.52 ± 0.05
1-acetoxy-2-butanone	18.82	1534	0.32 ± 0.01	0.28 ± 0.00	0.32 ± 0.01	0.27 ± 0.01
1-acetylcyclohexene	19.32	1554	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.00	0.15 ± 0.01
2-acetylpyridine	20.49	1600	0.18 ± 0.00	0.18 ± 0.01	0.21 ± 0.02	0.20 ± 0.01
2-acetyl-4-methylpyridine	21.15	1628	0.09 ± 0.01	0.12 ± 0.00	0.14 ± 0.01	0.11 ± 0.03
3-ethyl-2-hydroxy-2-cyclopentenone	22.44	1682	0.82 ± 0.03	0.80 ± 0.00	0.99 ± 0.03	0.85 ± 0.09
1-(6-methyl-2-pyrazinyl)-1-ethanone	22.66	1692	0.63 ± 0.03	0.56 ± 0.01	0.87 ± 0.04	0.87 ± 0.10
(+)-2-bornanone	23.18	1714	0.14 ± 0.03	0.12 ± 0.01	0.19 ± 0.03	0.21 ± 0.03
Total Ketones			3.78 ± 0.08[ab]	3.30 ± 0.03[a]	4.21 ± 0.12[ab]	4.29 ± 0.45[b]
Carboxylic acid						
acetic acid	16.62	1450	3.59 ± 0.15	3.09 ± 0.03	4.21 ± 0.08	6.37 ± 0.34
4-hydroxybutyric acid	21.05	1624	1.46 ± 0.15	1.31 ± 0.08	1.73 ± 0.14	2.63 ± 0.33
2-methyl-2-vinylmaleimide	21.32	1635	0.20 ± 0.01	0.18 ± 0.00	0.25 ± 0.01	0.27 ± 0.02
isovaleric acid	22.22	1673	1.26 ± 0.10	1.20 ± 0.06	1.70 ± 0.04	1.34 ± 0.15

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Compound	RT (min)	RI	Relative amount			
			Natural	Honey	Full wash	Wine
3-methylcrotonic acid	25.08	1799	0.53 ± 0.03	0.55 ± 0.03	0.75 ± 0.02	0.67 ± 0.08
2-furanpropionic acid	25.24	1806	0.24 ± 0.01	0.25 ± 0.03	0.36 ± 0.00	0.62 ± 0.09
Total Carboxylic acid*			7.27 ± 0.45[a]	6.58 ± 0.23[a]	9.00 ± 0.26[b]	11.89 ± 0.32[c]
Esther						
methyl acetate	1.99	-	0.12 ± 0.00	0.11 ± 0.01	0.18 ± 0.01	0.22 ± 0.04
ethyl acetate	2.34	-	nd	nd	nd	0.36 ± 0.05
ethyl formate	2.50	906	0.12 ± 0.01	0.34 ± 0.00	0.17 ± 0.00	0.24 ± 0.01
furfuryl propionate	20.45	1599	0.47 ± 0.02	0.49 ± 0.02	0.52 ± 0.05	0.38 ± 0.03
furfuryl isovalerate	22.75	1695	0.33 ± 0.02	0.40 ± 0.02	0.57 ± 0.00	0.34 ± 0.03
methyl salicylate	24.53	1774	0.47 ± 0.02	0.47 ± 0.03	0.59 ± 0.01	0.57 ± 0.05
methyl nicotinate	24.62	1778	0.27 ± 0.02	0.26 ± 0.02	0.36 ± 0.01	0.28 ± 0.04
ethyl salicylate	25.31	1810	0.17 ± 0.04	nd	nd	1.24 ± 0.15
methyl 3-methylfuroate	26.52	1866	0.47 ± 0.04	0.45 ± 0.03	0.56 ± 0.02	0.43 ± 0.07
methyl palmitate	33.50	2217	0.10 ± 0.00	0.10 ± 0.01	0.16 ± 0.00	0.09 ± 0.00
Total Esters*			2.53 ± 0.06[a]	2.62 ± 0.14[a]	3.11 ± 0.08[a]	4.15 ± 0.46[b]
Alcohol						
1.3-propanediol	2.43	-	5.21 ± 0.21	5.70 ± 0.04	6.84 ± 0.35	6.30 ± 0.26
ethyl alcohol	2.80	931	0.20 ± 0.00	0.12 ± 0.00	0.16 ± 0.01	0.89 ± 0.06
1-ethynylcyclohexanol	21.53	1644	0.23 ± 0.01	0.22 ± 0.02	0.29 ± 0.01	0.28 ± 0.01
benzyl alcohol	26.82	1880	0.07 ± 0.01	0.06 ± 0.01	0.11 ± 0.00	0.14 ± 0.02
phenylethyl alcohol	27.57	1916	0.25 ± 0.01	0.22 ± 0.02	0.28 ± 0.01	0.48 ± 0.02
2-thiophenemethanol	28.22	1947	0.13 ± 0.00	0.11 ± 0.00	0.16 ± 0.00	0.16 ± 0.04
4-pyridinemethanol	31.33	2103	0.28 ± 0.00	0.30 ± 0.01	0.45 ± 0.01	0.35 ± 0.05
Total Alcohols*			6.38 ± 0.21[a]	6.73 ± 0.03[a]	8.29 ± 0.35[b]	8.60 ± 0.45[b]
Hydrocarbons						
toluene	4.45	1033	0.13 ± 0.01	0.13 ± 0.00	0.18 ± 0.01	0.18 ± 0.02
dodecane	9.21	1199	0.18 ± 0.02	0.18 ± 0.02	0.19 ± 0.03	0.16 ± 0.02
2.7-dimethylundecane	15.25	1400	0.12 ± 0.02	0.09 ± 0.01	0.14 ± 0.01	0.16 ± 0.02
4-isopropyl-m-xylene	22.35	1679	0.23 ± 0.03	0.23 ± 0.03	0.29 ± 0.00	0.58 ± 0.07
decahydronaphthalene	22.59	1689	0.22 ± 0.01	0.19 ± 0.02	0.24 ± 0.02	0.24 ± 0.02
pulegone	22.89	1701	0.22 ± 0.02	0.22 ± 0.02	0.28 ± 0.00	0.36 ± 0.04
β-damascenone	25.60	1823	0.27 ± 0.01	0.26 ± 0.02	0.40 ± 0.01	0.38 ± 0.02
Total Hydrocarbons*			1.37 ± 0.19[a]	1.30 ± 0.11[a]	1.72 ± 0.03[ab]	2.06 ± 0.21[b]
Pyrrole						
1-ethylpyrrole	7.23	1137	0.12 ± 0.02	0.09 ± 0.00	0.13 ± 0.01	0.10 ± 0.00
1-butyl pyrrole	14.75	1382	0.06 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.20 ± 0.01
pyrrole	18.35	1515	0.51 ± 0.00	0.44 ± 0.05	nd	0.53 ± 0.09
1-methyl-2-pyrroleacetonitrile	21.59	1647	0.43 ± 0.03	0.39 ± 0.02	0.54 ± 0.05	0.59 ± 0.04
1-furfurylpyrrole	25.73	1829	1.45 ± 0.05	1.25 ± 0.01	1.92 ± 0.06	1.78 ± 0.21
2-acetylpyrrole	28.76	1974	0.83 ± 0.05	0.91 ± 0.04	1.35 ± 0.06	1.06 ± 0.12

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Compound	RT (min)	RI	Relative amount			
			Natural	Honey	Full wash	Wine
2-formylpyrrole	29.81	2026	0.51 ± 0.01	0.49 ± 0.00	0.76 ± 0.01	0.66 ± 0.07
Total Pyrroles			3.92 ± 0.06^{ab}	3.62 ± 0.13^{a}	4.74 ± 0.05^{ab}	4.92 ± 0.54^{b}
Pyridine						
pyridine	8.65	1182	0.67 ± 0.06	1.07 ± 0.03	1.24 ± 0.24	0.92 ± 0.11
2.6-dimethylpyridin-4-amine	15.39	1405	1.53 ± 0.18	1.36 ± 0.01	1.66 ± 0.10	2.47 ± 0.29
3-methoxypyridine	20.05	1583	0.16 ± 0.01	0.13 ± 0.01	0.17 ± 0.01	0.32 ± 0.02
3-methylpyridine 1-oxide	22.97	1705	0.08 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.14 ± 0.02
3-(3-thienyl)pyridine	28.09	1941	0.14 ± 0.00	0.13 ± 0.01	0.22 ± 0.00	0.27 ± 0.04
2-hydroxy-3-methylpyridine	34.99	2297	nd	nd	nd	0.11 ± 0.02
4-hydroxypyridine	37.02	2413	0.18 ± 0.01	0.20 ± 0.01	0.34 ± 0.00	0.22 ± 0.03
Total Pyridines			2.77 ± 0.26^{a}	3.00 ± 0.08^{a}	3.75 ± 0.37^{ab}	4.44 ± 0.49^{b}
Pyrazine						
pyrazine	9.54	1210	0.38 ± 0.05	0.27 ± 0.01	0.38 ± 0.01	0.36 ± 0.03
2-methylpyrazine	11.25	1265	3.49 ± 0.30	3.02 ± 0.02	3.88 ± 0.22	3.80 ± 0.47
2.5-dimethylpyrazine	12.98	1322	1.75 ± 0.12	1.69 ± 0.03	2.08 ± 0.16	2.51 ± 0.27
2.6-dimethylpyrazine	13.18	1329	1.87 ± 0.12	1.77 ± 0.02	2.32 ± 0.15	2.63 ± 0.33
2-ethylpyrazine	13.33	1334	1.30 ± 0.07	1.17 ± 0.01	1.45 ± 0.02	1.58 ± 0.20
2.3-dimethyl pyrazine	13.69	1346	0.48 ± 0.04	0.43 ± 0.02	0.53 ± 0.04	0.82 ± 0.09
2-ethyl-6-methylpyrazine	14.85	1386	1.77 ± 0.16	1.62 ± 0.01	2.13 ± 0.04	2.39 ± 0.27
2-ethyl-5-methylpyrazine	15.02	1392	1.30 ± 0.11	1.26 ± 0.01	1.47 ± 0.03	1.86 ± 0.21
2.6-diethylpyrazine	16.22	1436	0.47 ± 0.03	0.44 ± 0.02	0.55 ± 0.00	0.69 ± 0.08
3-ethyl-2.5-dimethylpyrazine	16.54	1447	1.89 ± 0.32	1.59 ± 0.07	1.83 ± 0.02	2.58 ± 0.30
2.3-diethyl pyrazine	16.78	1456	0.20 ± 0.01	0.17 ± 0.01	0.21 ± 0.01	0.23 ± 0.01
2-ethyl-3.5-dimethylpyrazine	17.03	1466	2.15 ± 0.10	1.80 ± 0.09	2.35 ± 0.09	2.94 ± 0.34
2-methyl-5-propylpyrazine	17.37	1478	0.18 ± 0.02	0.16 ± 0.01	0.22 ± 0.01	0.32 ± 0.02
2-ethenyl-6-methylpyrazine	17.65	1488	0.28 ± 0.02	0.24 ± 0.01	0.32 ± 0.01	0.46 ± 0.03
3.5-diethyl-2-methylpyrazine	17.85	1496	1.37 ± 0.10	1.21 ± 0.08	1.45 ± 0.05	1.74 ± 0.21
2.3.5-trimethyl-6-ethyl pyrazine	18.43	1519	0.47 ± 0.04	0.43 ± 0.03	0.53 ± 0.04	0.71 ± 0.09
2.5-dimethyl-3-(2-methylpropyl) pyrazine	18.70	1530	0.46 ± 0.02	0.34 ± 0.02	0.43 ± 0.01	0.58 ± 0.03

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Compound	RT (min)	RI	Relative amount Natural	Honey	Full wash	Wine
1-propenylpyrazine	19.21	1550	0.11 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.25 ± 0.02
isopropenyl pyrazine	20.39	1596	0.21 ± 0.00	0.21 ± 0.00	0.29 ± 0.04	0.24 ± 0.01
2-isoamyl-6-methylpyrazine	21.24	1632	0.14 ± 0.02	0.08 ± 0.01	0.13 ± 0.00	0.59 ± 0.07
2-methyl-6-(1-propenyl)pyrazine	21.89	1659	0.10 ± 0.01	0.09 ± 0.01	0.14 ± 0.00	0.19 ± 0.04
pyrazinamide	23.24	1717	0.96 ± 0.13	0.74 ± 0.02	1.13 ± 0.23	0.88 ± 0.08
2-acetyl-3.5-dimethylpyrazine	24.79	1786	0.18 ± 0.00	0.16 ± 0.02	0.24 ± 0.00	0.46 ± 0.07
Total Pyrazines			21.49 ± 1.54^{ab}	19.00 ± 0.44^{a}	24.17 ± 1.00^{ab}	28.80 ± 3.29^{b}
Phenolic						
2.3-dimethylphenol	11.96	1287	0.15 ± 0.03	0.12 ± 0.01	0.21 ± 0.01	0.31 ± 0.04
4-amino-3-methylphenol	20.66	1608	0.22 ± 0.06	0.22 ± 0.00	0.28 ± 0.02	0.37 ± 0.05
2-methoxyhydroquinone	23.72	1738	0.18 ± 0.00	0.18 ± 0.02	0.24 ± 0.01	0.24 ± 0.02
4-amino-2-hydroxytoluene	25.00	1795	0.16 ± 0.01	0.15 ± 0.02	0.26 ± 0.01	0.24 ± 0.03
guaiacol	26.41	1861	1.38 ± 0.08	1.17 ± 0.00	1.97 ± 0.04	1.88 ± 0.21
phenol	29.44	2007	0.66 ± 0.06	0.69 ± 0.04	1.25 ± 0.07	0.83 ± 0.11
4-ethyl-2-methoxyphenol	29.92	2031	1.49 ± 0.05	1.29 ± 0.10	2.17 ± 0.06	2.33 ± 0.28
p-cresol	31.10	2091	0.18 ± 0.00	0.19 ± 0.02	0.30 ± 0.02	0.25 ± 0.03
2-methoxy-4-vinylphenol	33.06	2193	5.60 ± 0.80	4.09 ± 0.14	6.94 ± 0.78	5.22 ± 0.66
Total Phenolics*			10.01 ± 0.52^{ab}	8.10 ± 0.36^{a}	13.61 ± 0.73^{c}	11.68 ± 1.43^{bc}
Thiopen						
3-ethyl thiophene	10.09	1228	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.14 ± 0.02
2-methylthiolan-3-one	18.62	1526	0.17 ± 0.00	0.16 ± 0.00	0.17 ± 0.00	0.15 ± 0.01
3-acetyl-2.5-dimethylthiophene	25.41	1814	0.28 ± 0.01	0.25 ± 0.03	0.33 ± 0.00	0.49 ± 0.05
Total Thiopens*			0.49 ± 0.00^{a}	0.44 ± 0.03^{a}	0.55 ± 0.00^{a}	0.77 ± 0.08^{b}
Miscellaneous						
sec-butylamine	1.36	-	0.54 ± 0.02	0.44 ± 0.03	0.59 ± 0.01	0.46 ± 0.05
1-methylpiperidine	4.29	1027	0.07 ± 0.00	0.09 ± 0.00	0.12 ± 0.01	0.15 ± 0.01
4-methylthiazole	11.73	1280	0.11 ± 0.01	0.08 ± 0.00	0.09 ± 0.00	0.19 ± 0.00
butyl ethyl ether	13.52	1340	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.01	0.12 ± 0.00
diisopropyl ether	14.02	1357	0.11 ± 0.00	0.11 ± 0.01	0.11 ± 0.00	0.23 ± 0.01
5-amino-2-methylbenzothiazole	19.04	1543	nd	nd	nd	0.25 ± 0.02
benzylhydrazine	21.39	1638	0.14 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.22 ± 0.05
p-aminoanisole	21.80	1655	0.56 ± 0.00	0.58 ± 0.03	0.78 ± 0.00	0.71 ± 0.08

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Compound	RT (min)	RI	Relative amount			
			Natural	Honey	Full wash	Wine
4-methoxyphenyl isothiocyanate	23.54	1730	0.22 ± 0.02	0.20 ± 0.01	0.31 ± 0.01	0.38 ± 0.06
4-methoxythiophenol	23.66	1736	0.84 ± 0.03	0.76 ± 0.02	0.89 ± 0.01	0.68 ± 0.09
2-methyl-5-methoxy-4h-pyran-4-one	25.90	1837	0.25 ± 0.00	nd	nd	0.33 ± 0.05
6-butyltetralin	32.02	2139	0.14 ± 0.00	0.12 ± 0.01	0.22 ± 0.00	0.18 ± 0.03
caprolactam	32.98	2189	0.09 ± 0.00	0.09 ± 0.01	0.16 ± 0.03	0.08 ± 0.00
indole	37.41	2433	0.15 ± 0.00	0.12 ± 0.02	0.21 ± 0.00	0.15 ± 0.01
Total Miscellaneous			3.34 ± 0.10^{ab}	2.82 ± 0.15^{a}	3.74 ± 0.04^{ab}	4.14 ± 0.46^{b}
Total Volatile Compounds*			99.91 ± 0.95^{ab}	92.10 ± 4.31^{a}	119.08 ± 0.38^{bc}	127.69 ± 11.08^{c}

Data were the averages ± standard deviation of two replicates. Numbers followed by different superscript letters in the same row indicate significant differences ($p < 0.05$) based on one-way ANOVA and Duncan test. Groups with (*) were affected by processing. RT (retention time). RI (retention index). Compounds grouping refers to pubchem.ncbi.nlm.nih.gov.

The pyrazine compound group was the second most abundant group of compounds found in roasted coffee. In addition to furan, pyrazine is produced during the Maillard reaction by interacting with reducing sugars and amino acids. The results of this analysis suggest that differences in coffee bean processing do not significantly affect the concentration of pyrazine compounds. Several studies have reported roasting temperature leading to higher pyrazine content [31–33]. However, it has been shown that not only the roasting process, but also post-harvest processing can produce physical and chemical differences in the coffee bean, which significantly influence the formation of aroma compounds, including those in the pyrazine group [12,34].

The fermentation process can influence the number and type of microorganisms in coffee beans, which affect the formation of compounds responsible for the aroma and flavor of coffee, such as pyrazine compounds [35–37]. In this study, roasted coffee processed using the wine and full wash methods tended to contain higher levels of 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and 2-methyl-3,5-diethylpyrazine compared to those processed using natural and honey processing. The different fermentation processes in full wash processing and the extended fermentation period in wine processing promote the growth of a greater variety and number of microorganisms, which can affect the formation of pyrazine compounds. Microorganisms, such as *Bacillus subtilis*, *Pseudomonas fluorescens* SBW25, and *Rhodococcus erythropolis* [38–40], produce compounds in the pyrazine group during fermentation.

Like furan and pyrazine, pyrrole compounds are formed during the roasting process. It has been widely reported that their presence is closely related to roasting conditions such as temperature and roasting intensity [32,41,42]. In this study, the processing method did not affect the relative amount of pyrrole compounds.

The processing method influenced the level of various volatile compound groups, including carboxylic acids, esters, alcohols, hydrocarbons, phenolics, thiophenes, and the total volatile compounds. Acetic acid and 4-hydroxybutyric acid, both carboxylic acid compounds, were found to be the highest in wine processing, followed by full wash processing. The level of acetic acid in wet-processed coffee is typically higher compared to that in natural processing [43]. The coffee soaking

process promotes a more consistent fermentation by microorganisms, as the beans have already undergone peeling, removing the fruit flesh. In contrast, natural processing does not involve peeling, and prevents microorganisms from efficiently degrading the sugar in the fruit flesh [44].

The anaerobic fermentation and its duration also influenced the formation of the carboxylic acid. As reported by [9], the post-harvest drying process in coffee processed using the natural method may to decrease in pH, which results from the absorption of acids produced during spontaneous fermentation during. Both the post-harvest processing and the treatment of coffee beans significantly impact the type and concentration of acid compound [45]. Coffee beans from the same fruit, when subjected to different processing methods, affect the production of pectinase enzyme and lead to the formation of varying acid components [46]. Anaerobic fermentation, in particular, fosters variations in microbial communities. It has been reported by [47], microorganisms, such as *Acetobacter*, *Leuconostoc*, and *Gluconobacter,* are dominant during anaerobic fermentation and are also present in aerobic fermentation. The concentration of acetic acid was higher in roasted coffee subjected to fermentation processes (full wash and wine), with higher concentrations observed in roasted coffee that underwent longer fermentation (wine). These results align with those reported by [26,37], where acetic acid concentrations in roasted coffee increased with longer fermentation times.

In the ester group, the presence of ethyl acetate, which was only identified in wine processing, and ethyl salicylate in natural and wine processing had a significant effect. The analysis showed that the post-harvest processing influenced the group of ester compounds. The volatile marker compounds section will discuss the ethyl acetate and ethyl salicylate.

1.3-propanediol is the main compound in all processing that belongs to the alcohols group. It is found in higher amounts in fermented coffee, like full wash and wine. According to the *yeast metabolome database*, this compound is identified as being produced by *Saccharomyces cerevisiae.* Similar to 1.3-propanediol, ethyl alcohol can be produced by *Saccharomyces cerevisiae* and is most prevalent in wine-processed coffee. The high relative amount of ethyl alcohol in wine processed coffee is an essential characteristic of the anaerobic fermentation process.

Similar to the carboxylic acid and alcohol groups, the highest relative amounts of phenolic group compounds, such as 2-methoxy phenol and 4-ethyl-2-methoxyphenol were found in roasted coffee that undergoes a fermentation process before the drying process (full wash and wine). Microorganisms such as *Saccharomyces cerevisiae* through the metabolic process can produce 4-vinyl to 4-ethyl derivatives of free phenolic acids naturally found in fruit [48]. During the fermentation of coffee fruit involving certain microorganisms, phenolic acid derivative compounds, such as 2-methoxy phenol, 4-ethyl-2-methoxyphenol, and 2-methoxy-4-vinylphenol, can be formed.

The hydrocarbon and thiophene groups were present in the slightest relative amounts compared to other groups. Hydrocarbons and thiophene were most abundant in coffee processed with fermentation processes, specifically full wash and wine processes. The results suggest that fermentation could affect the synthesis of hydrocarbon and thiophene components. The total relative amounts of volatile compounds indicates that post-harvest processing, particularly fermentation, induces the synthesis of chemicals and elevates their concentration.

3.4. Volatile marker compounds in second crack roasted coffee

The percent contribution data were analyzed using OPLS-DA modeling. The OPLS-DA analysis successfully separated and differentiated the samples based on dominant volatile compounds and

marker compounds associated with each processing method. The results indicated a total variance of 70.1%, with PC1 contributing 49.90% and PC2 contributing 20.20%. The model quality was validated using the R^2X, R^2Y, Q^2, and CV ANOVA values. The OPLS-DA modeling produced R^2X values of 0.81, R^2Y 1 and Q^2 0.846. Additionally, the CV ANOVA value was <0.05 (0.04) and the regression value was >0.4, confirming the validity of the model [49]. To further validate the statistical model, 200 permutation tests were conducted. The permutation test demonstrated the model's validity, as evidenced by a lower Q^2 prediction intercept (-0.701) compared to the Q^2 value obtained through analysis. These results indicated that the model can distinguish volatile compounds in second-crack roasted coffee from four different processing methods. The OPLS-DA loading plot is presented in Figure 5.

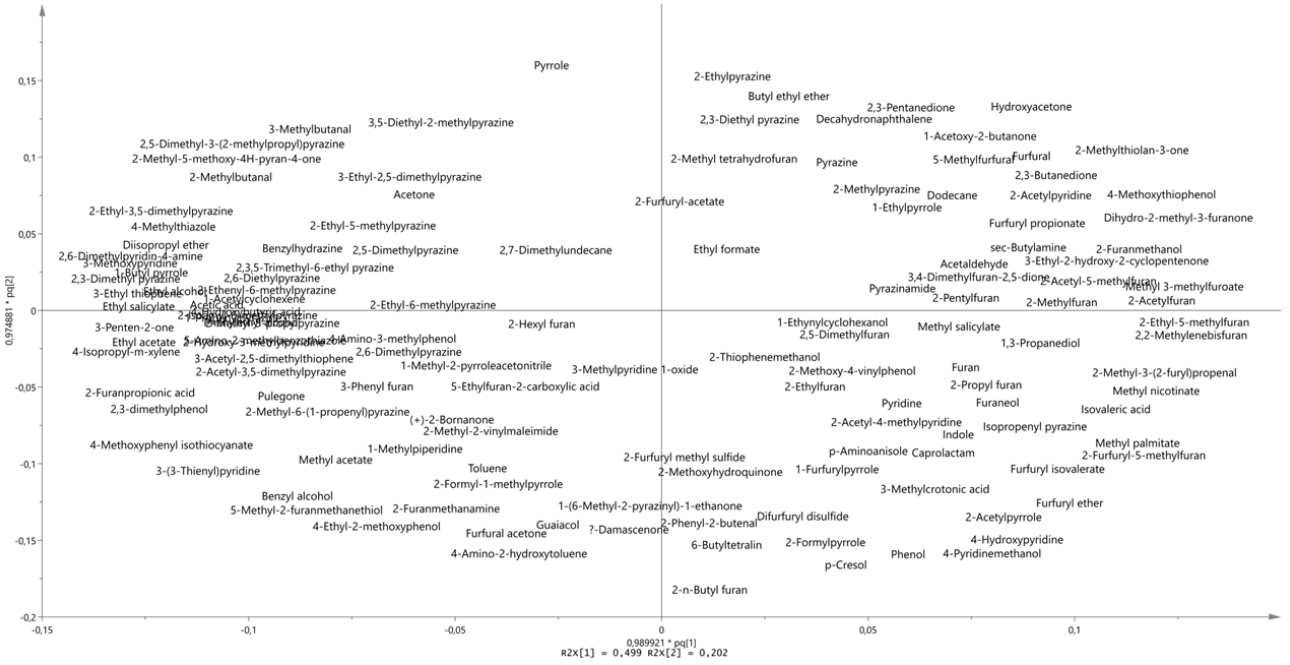

Figure 5. Loading plot OPLS-DA of volatile compounds identified in the second-crack roasted coffee from four processing methods using HS-SPME-GCMS.

OPLS-DA analysis successfully grouped compounds based on their contribution to each processing group. Several compounds were identified in one or more processes, indicating their potential as volatile markers of second-crack roasted coffee from each processing method, as shown in Table 2. The VIP value was used as a criterion to identify compounds with the best discriminant value. According to the results, all identified compounds had the potential to serve as marker compounds for each processing method, as they exhibited VIP values greater than 0.8 [50]. These marker compounds significantly influenced the discrimination of processing differences in second-crack roasted coffee. Additionally, the percent contribution value was used to assess the importance of each marker compound. Marker compounds with a VIP value >0.8 and a percent contribution value >0.1% were considered potential marker compounds.

Based on the VIP values and the percent contribution of volatile marker compounds successfully identified in second-crack roasted coffee, both natural and wine processing exhibited two potential

marker compounds: ethyl salicylate and 2-Methyl-5-methoxy-4H-pyran-4-one. Ethyl salicylate an ester derivative compound with a minty aroma, was reported to be slightly affected by temperature and roasting time due to its high boiling point [51]. It was also reported by [52] that this compound is found in fresh coffee pulp. Ethyl salicylate was only detected in coffee that did not undergo a fruit skin peeling process, such as natural and wine processing, and was thought to be absent in coffee subjected to fruit skin peeling processes, like honey and full wash processing.

Ethyl acetate and 5-amino-2-methylbenzothiazole were identified as markers in second-crack roasted coffee from wine processing. Ethyl acetate, a volatile compound from the carboxylic acid class, is associated with a fruity wine aroma, characteristic of fermented products [53]. One factor contributing to the increased concentration of ethyl acetate is the duration of the fermentation process. According to [54], increasing the duration of anaerobic fermentation of coffee enhances the production of ethyl acetate. Another marker compound in wine processing was 5-amino-2-methylbenzothiazole. While limited information is available on this compound, it has not been previously reported in coffee. However, it is known to belong to the benzothiazole group, a heterocyclic component likely found in roasted coffee. In this study, honey and full wash did not exhibit any potential marker compounds.

Table 2. Percent contribution of volatile potential markers of second-crack roasted coffee from four post-harvest processing methods.

Potential markers	Percent contribution (%)				VIP
	Natural	Honey	Full wash	Wine	
ethyl acetate	nd	nd	nd	0,26	0,98
5-amino-2-methylbenzothiazole	nd	nd	nd	0,18	0,98
ethyl salicylate	0,15	nd	nd	0,89	0,97
2-methyl-5-methoxy-4H-pyran-4-one	0,23	nd	nd	0,24	1,16

Data were the averages of two replicates. nd (not detected). VIP (variable importance projection).

3.5. Dry aroma second crack roasted coffee

Dry aroma refers to the aroma of ground coffee before brewing, which panelists evaluated by sniffing the roasted ground coffee. In coffee taste testing, both dry aroma and wet aroma (aroma after brewing) are used to determine the fragrance/aroma score [15]. The taste assessment conducted by the Indonesian Coffee and Cocoa Research Center indicated that robusta coffee from Sukamakmur District, Bogor, used in this study had characteristics of caramelly, chocolaty, spicy, and acidy aromas in coffee with a medium roast level [55]. The roast level greatly influences the characteristics of the coffee aroma.

This study selected 46 compounds with a percent contribution value of >0.5% for analysis to identify the dry aroma of second-cracked roasted coffee from each processing. The OPLS-DA analysis and sensory test are displayed in the loading plot shown in Figure 6. The dry aroma sensory test results revealed that natural roasted coffee exhibited caramelly, roasted peanut, and chocolaty aromas. Honey processed coffee had caramelly, nutty, and earthy aromas. Full wash processed coffee had sweet nut, earthy, and herbal aromas with a hint of potato. Wine-roasted coffee exhibited fermented, winey, molasses, and chocolaty aromas. The OPLS-DA analysis results (Figure 6) showed that natural and honey-processed coffee were positioned on the positive PC1 plane, alongside compounds contributing to sweet, nutty, and roasted aroma types. In both natural and honey processing, the most intense aroma was caramelly, associated with compounds, such as hydroxyacetone, furfural, 5-methylfurfural,

dihydro-2-methyl-3-furanone, 2-furanmethanol, and 3-Ethyl-2-hydroxy-2-cyclopentenone. The roasted peanut and chocolaty aromas in natural processed coffee were likely influenced by the presence of the 2-ethylpyrazine compound. The nutty aroma in honey processed coffee was probably due to the 2-methylpyrazine compound. Additionally, the 2-furanmethanol compound, which contributes to the caramelly aroma, was also a key contributor to the earthy aroma of honey-roasted coffee.

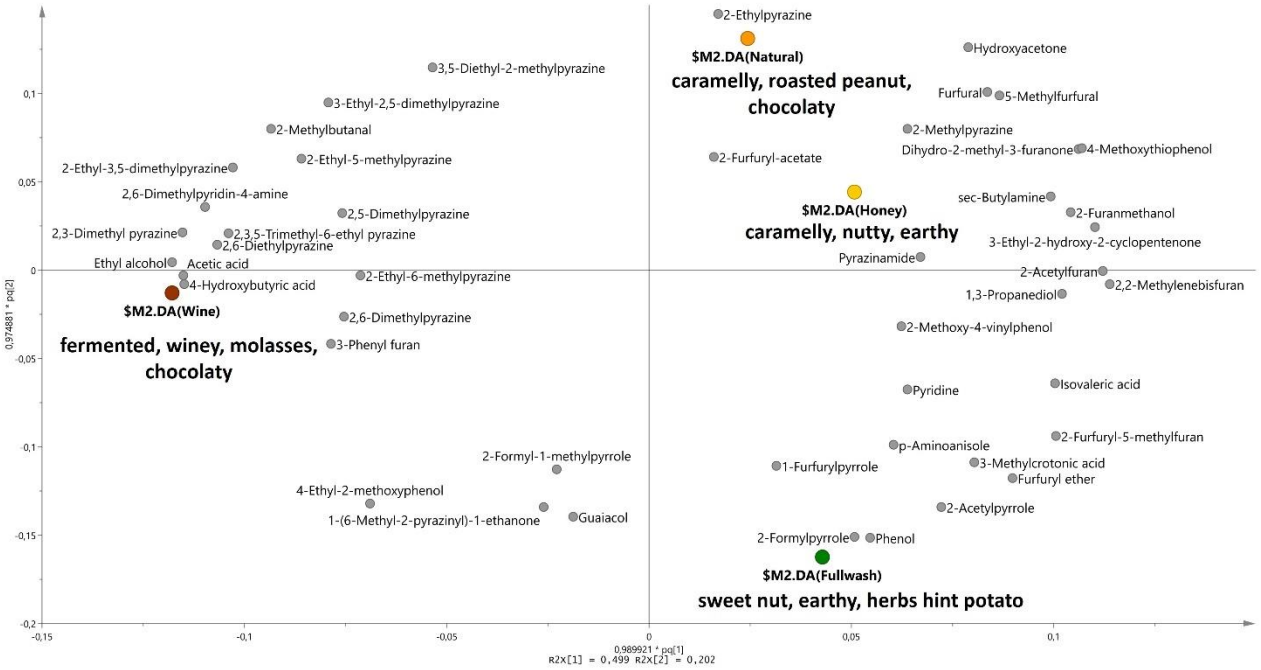

Figure 6. OPLS-DA loading plot of selected dominant volatile compounds and description of dry aroma of second-crack roasted coffee from four processing methods.

Full wash roasted coffee appeared on the negative PC1 plane, along with compounds that contributed to green, earthy, nutty aroma. The sweet nut aroma was likely influenced by compounds such as 2-acetylpyrrole and difurfuryl ether. The earthy aroma was thought to be influenced by the compounds like 2-formylpyrrole, phenol, and 3-methylcrotonic acid. Additionally, the aroma reminiscent of herb hint potato was attributed to the compound 1-furfurylpyrrole.

Wine-roasted coffee was positioned on the negative PC2 plane, associated with compounds contributing acidic, alcoholic, and chocolaty aroma. Acetic acid and ethyl alcohol were likely responsible for fermented, winey, and molasses aromas in wine-roasted coffee, while the chocolaty aroma was thought to be influenced by the compound 2,6-dimethylhydrazine. The description of these aroma compounds can be found in Table 3. The results indicated that the dry aroma of the second-crack roasted coffee from different processes (natural, honey, full wash, and wine) could be clearly distinguished.

Table 3. Compounds in each processing and aroma description.

Processing	Compounds	Aroma description*
Natural and Honey	2-ethyl pyrazine (Natural)	nutty, musty, fermented, coffee, roasted, cocoa, meaty
	hydroxyacetone (Natural)	sweet caramellic, ethereal
	furfural	sweet, brown woody, bready, caramellic, phenolic
	5-methylfurfural	sweet, caramellic, bready, brown, coffee-like
	dihydro-2-methyl-3-furanone	sweet, caramel, bread
	2-furfuryl-acetate (Honey)	fruity, roast, sweet
	2-methyl pyrazine (Honey)	nutty, cocoa roasted, chocolate, peanut, green
	2-furanmethanol (Honey)	alcoholic, chemical, musty, sweet caramel, bread, coffee
	3-Ethyl-2-hydroxy-2-cyclopentenone (Honey)	maple, caramel, coffee, smoke
Full wash	2-formylpyrrole	musty, beefy, coffee
	phenol	phenolic, plastic, rubbery
	2-acetylpyrrole	musty, nutty, coumarinic
	1-furfurylpyrrole	vegetative, cereal, bready, radish, mushroom, potato nuances
	difurfuryl ether	coffee, nutty, earthy
	3-methylcrotonic acid	green, phenolic, dairy
Wine	acetic acid	acidic, vinegar, sour, sharp
	ethyl alcohol	alcoholic, ethereal, medicinal
	2,6-dimethylpyrazine	chocolate, nutty, roasted

(*) The aroma description refers to Thegoodscentcompany.com.

4. Conclusions

The second-crack roast produced coffee at a medium-to-dark roast level across all processing methods. Out of 140 compounds identified and semiquantified, second-crack roasted coffee was dominated by furan, pyrazine, carboxylic acid, phenolic, and alcohol groups. Carboxylic acids, esters, phenolics, alcohols, hydrocarbons, and thiophenes were influenced by post-harvest processing. PCA modeling showed that natural and honey-processed coffee had similar volatile compositions, while full wash and wine-processed coffee exhibited distinct differences. Regarding the VIP value from OPLS-DA modeling and the percent contribution value, ethyl salicylate and 2-Methyl-5-methoxy-4H-pyran-4-one were identified as potential volatile marker compounds in natural and wine processing. In contrast, ethyl acetate and 5-amino-2-methylbenzothiazole were identified as potential marker compounds for wine-processed coffee. Honey and full wash processing did not present potential marker compounds. The dry aroma of natural roasted coffee was characterized by caramelly, roasted peanut, and chocolaty aromas. Honey processed coffee had caramelly, nutty, and earthy aromas. Full wash processed coffee exhibited sweet nut, earthy, herbal aromas with a hint of potato. Wine-processed coffee had fermented, winey, molasses, and chocolaty aromas. The evaluation of dry aroma through sensory analysis distinguished between natural, honey, full wash, and wine-roasted coffee.

Future research would focus on identifying types of microorganisms present in each processing

method and investigating the mechanisms behind the formation of potential marker compounds in natural and wine processed coffee.

Use of AI tools declaration

The authors state that they did not utilize Artificial Intelligence (AI) tools in creating this article.

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Conflict of interest

All authors declare that they have no conflict of interest.

Author contributions

Investigation: N.F.S.; Methodology: N.F.S and N.A.; Formal analysis: N.F.S; Project administration, N.F.S; Writing - Original draft: N.F.S.; writing—review and editing, D.N.F., D.R.A., and N.A. Supervision: D.N.F., D.R.A., and N.A. Funding acquisition: N.A. All authors have read and agreed to the published version of the manuscript.

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