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Metabolite Signature of Fresh and Long-term Stored Coffee Pulp and Husk

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Abstract

Every product from the food and agriculture industry produces waste that can cause environmental pollution when carelessly discarded. Coffee husk and pulp are the main wastes generated by coffee processing. The secondary metabolites in these wastes can still be utilized, but their level can be affected by long storage. This research aims to determine the differences in the compounds obtained from old and fresh Gayo Arabica coffee pulp and husk. Coffee husk and pulp samples from Gayo Arabica coffee are extracted for GC-MS analysis to determine similarities between fresh and long-term stored Gayo Arabica coffee husk and pulp. Results show differences in level and type among the secondary metabolites. Among various compounds, caffeine is found in all the samples. The compounds obtained from Gayo Arabica coffee husk and pulp, such as caffeine, (Z,Z)-9,12-octadecadienoic acid, and palmitic acid, can be used in health and agriculture.

Keywords: gayo arabica coffee, GC-MS, husk, pulp

Introduction

The term coffee is derived from "qahva" or "qahwa," which means a drink made from plants. Initially, coffee cherry was consumed, and the seeds were crushed and mixed with animal fat to make energy-boosting supplements. Currently, 100 species of *Coffea* genus are found in tropical and subtropical regions. Two types of coffee, *Coffea arabica* and *Coffea canephora*, are commonly found in Indonesia [1, 2].

The largest producer of arabica coffee is in Northern Indonesia, namely, Gayo Lues, the central part of Aceh Province. The coffee produced in this area is called Gayo coffee. Cupping test ascribed a score of more than 80 for Gayo coffee, making it a specialty coffee [3]. The optimum condition for coffee growth can be found in the highland tropical region with altitude of 1.000–1.700 MASL and temperature of 18 °C–23 °C [4]. From a geographic perspective, Gayo highland fulfills this optimum condition [5]. Hence, the secondary metabolites from Gayo Arabica coffee are better than those from other types of coffee [6].

A natural product is a compound isolated from a living organism and is produced from primary and secondary metabolites. A metabolite is an intermediate or product of metabolism and can be classified as primary or secondary. Primary metabolites directly affect growth, development, and reproduction. Secondary metabolites do not directly affect these processes but are still important for plants. The most bioactive metabolites are alkaloids, tannins, flavonoids, and phenolic compounds [7]. Secondary metabolites in plants can be an alternative source for drug discovery [8].

The anatomy of the coffee fruit consists of the outer skin, pulp (mesocarp), pectin layer, parchment coat, silver skin, bean, and center cut [5]. Coffee processing is divided into two parts: dry and wet processing [9, 10]. Almost half of the coffee fruit will become waste. The husk will be produced in the dry process, and the pulp is the main waste from the wet process [11]. The coffee husk contains polyphenols, anthocyanins, vitamin C, and some secondary metabolites, such as caffeine, alkaloids, and tannins. Coffee pulp contains 4%–12% proteins, 1%–2% fats, 6%–10% minerals, and 45%–89% carbohydrates [12]. Ameca *et al*. [13] identified eight phenolic acids in coffee pulp: hydroxybenzoic acid, chlorogenic acid, ferulic acid, caffeine acid, syringic acid, gallic acid, vanillic acid, and coumaric acid. The secondary metabolites of coffee can inhibit metabolic

diseases, such as diabetes, obesity, hypertension, and neural diseases [14–18].

The storage period can affect the secondary metabolites in plants. A previous study on broccoli observed a reduction in vitamin C levels with prolonged storage time because of the increase in vitamin C-degrading enzyme activity during storage [19]. Another study on oat plants reported a consistent result of decreasing secondary metabolite concentration (total phenolic content and caffeic acid) with prolonged storage time [20].

As a compound targeted to produce a beneficial effect on health, any changes on secondary metabolites are crucial. Reduced secondary metabolites will not have an optimum impact on health, despite being extracted from the same plants. This study aims to identify differences among the secondary metabolites of husk and pulp of Gayo Arabica coffee under fresh condition and long storage.

Methods

Plant Materials. Husks and pulps of Gayo Arabica coffee were collected from arabica coffee plants (*Coffea arabica* L.) in Gayo Lues, Central Aceh, Indonesia. The variety of the coffee is Ateng super, which has a stem height of 1.5 m and planting height of 1.450 MASL (Mean Above Sea Level). Fresh Gayo Arabica husks and pulps were harvested in December 2021. Long-term stored husks and pulps were obtained from coffee that has been stored for 1 year (December 2020). This research was conducted in the chemical engineering faculty of Universitas Syiah Kuala, Aceh, Indonesia.

Extraction and Gas Chromatography Mass-Spectrophotometry (GC-MS). Husks and pulps of Gayo Arabica coffee were extracted by maceration using absolute n-hexane. Liquid extract was evaporated using a rotary evaporator at 40 °C for further analysis. The secondary metabolites of Gayo Arabica coffee husk and pulp were analyzed using GC with an Rtx-5MS column (30 cm length). In brief, 1 g of evaporated crude extract was dissolved with 3 ml of ethanol absolute and vortex for 3 minutes with an extension of 15 minutes. The homogenates were filtered using 0.45 µl filter paper, and 1 µL of the filtrate was used for injection at GC-MS (Shidmazu, QP2010) under the following conditions: temperature of 280 °C, splitless injector mode, column temperature of 40 °C, retention time of 40 min, and detector temperature of 280 °C. Helium was used as the carrier gas, and the pressure, total flow rate, column flow, and split ratio were set at 4,3367 psi, 8.4 mL/min, 0.9 mL/min, and 5:1, respectively. The MS was set to scan mode with a scan range of 30–500 m/z, ion source temperature of 200 °C, interface temperature of 300 °C, solvent cut time of 2.5 minutes, event time of 0.3 seconds, and a scan speed of 1666. Compounds were identified by comparing the obtained mass spectra of each compound with those from the WILLEY7 database. [2]

Results and Discussions

Several methods, such as GC-MS, Fourier transform infrared (FTIR) spectroscopy, and high-performance liquid chromatography (HPLC), are commonly employed for the detection of secondary metabolites. HPLC is not usual applied in detecting secondary metabolites, and FITR is not available in Aceh. Hence, GC-MS was selected for this study. Comparison of total ion chromatogram between fresh and long-term stored Gayo Arabica coffee husk is shown in Figure 1 and Figure 2. Differences were observed between the fresh and stored husk samples. The total compounds detected were 17, and 9 of which were not identified with different retention times shown in Table 1 and Table 2.

The first retention time of fresh husk was 11.194 minutes and the last was 34.168 minutes with total compounds 1.935% and 1.333% respectively. While the long-term stored husk showed the first retention time was 11.183 minutes and the last 24.321 minutes with total compound 3.265% and 4.394% respectively.

Differences of the compound were found between fresh and stored husk that shown in Table 3. Hydroxy-methylfurfural (HMF) and (Z,Z)-9,12-octadecadienoic acid were the compounds detected only in long-term stored husks. The formation of HMF depends on temperature, pH, and food composition, so this compound is often used as a marker of food quality. HMF is formed during heating through the Maillard reaction and sugar degradation [21]. Owing to the sugar content of the coffee husk, storage causes sugar degradation and HMF formation [22]. GC-MS analysis revealed that the fatty acids can be classified as saturated or unsaturated. The compounds included in the saturated fatty acids are palmitic acid and stearic acid. Linoleic acid and (Z,Z)-9,12-octadecadienoic acid belong to the unsaturated fatty acids. Saturated fatty acids are susceptible to oxidation during storage, and unsaturated fatty acids are resistant to oxidation [23].

Figures 3 and 4 show the comparison of the total ion chromatogram of the fresh and long-term stored pulps of Gayo Arabica coffee. Some differences in retention time and number of detected compounds were noted between the two samples. Among the 35 compounds detected, 7 were not identified. Figure 5 show three major compounds identified with the biggest area, caffeine, hexadecenoic acid, and elaidic acid. Each compound has retention time 15.363 minutes, 16.566 minutes, and 19.05 minutes sequentially. Caffeine was detected in the fresh and long-term stored pulp samples. The retention time of caffeine was 15.363 minutes in the fresh pulp and 21.48

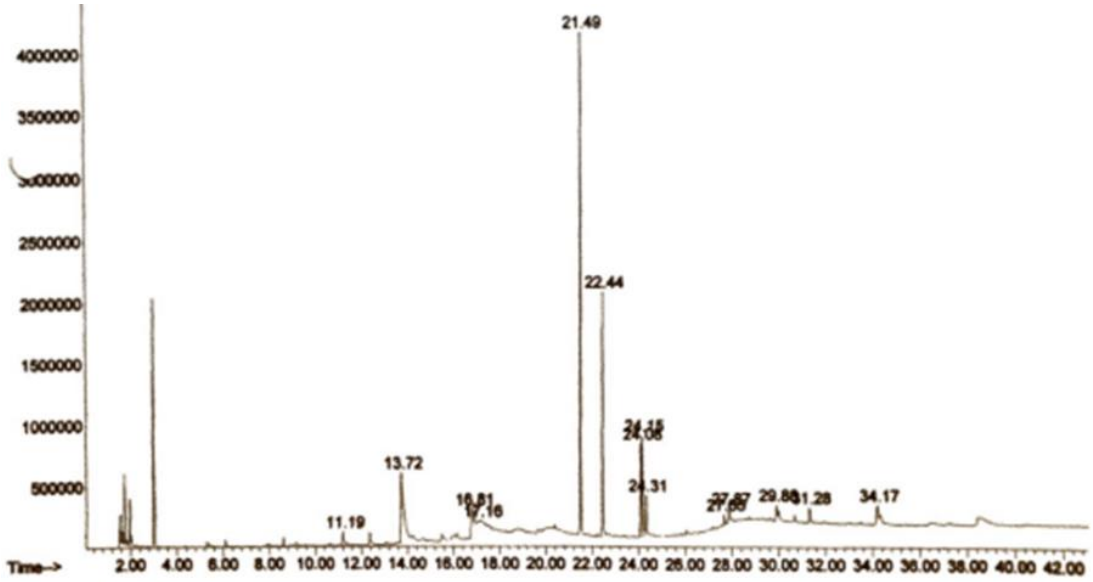

Figure 1. Total Ion Chromatogram of the Fresh Husk of Gayo Arabica Coffee

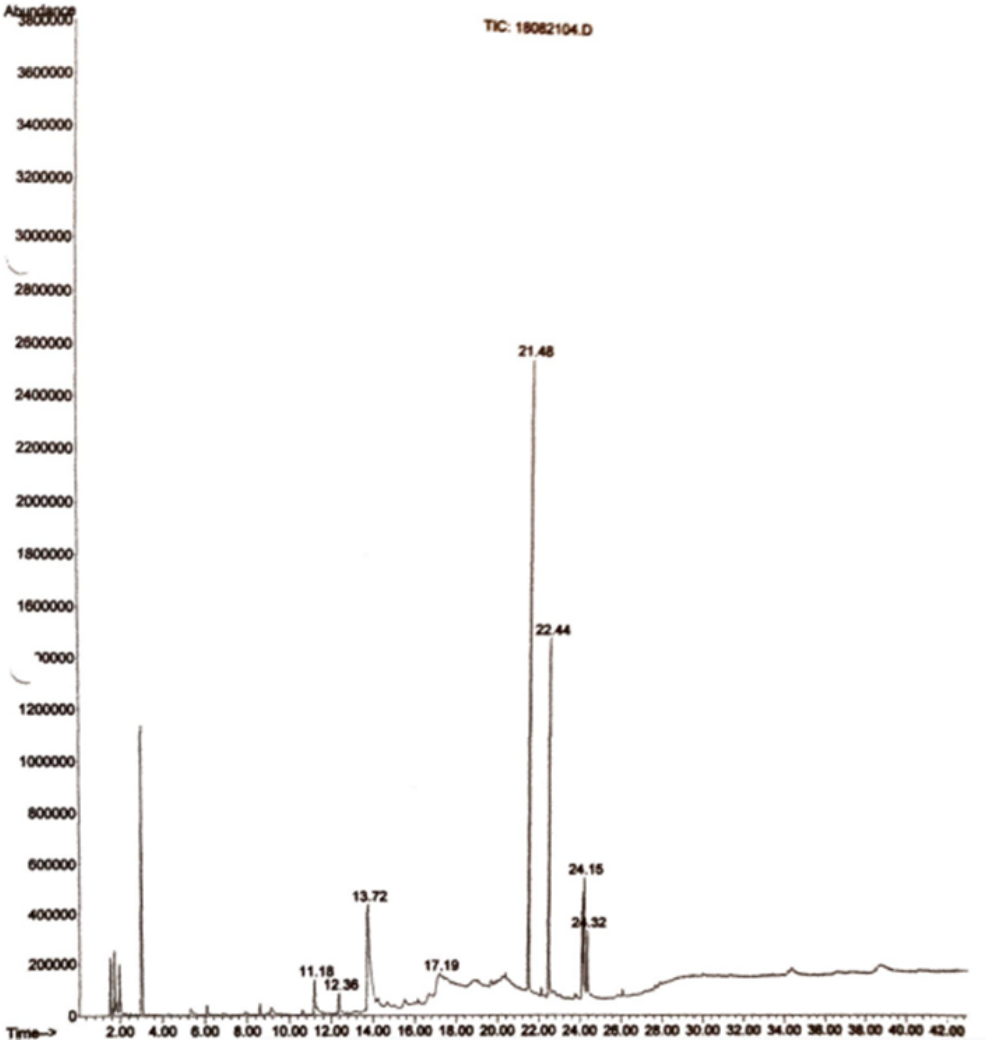

Figure 2. Total Ion Chromatogram of the Long-term Stored Husk of Gayo Arabica Coffee

Table 1. Mass Selective Detector (MSD) of the Fresh Husk of Gayo Arabica Coffee

Peak	Retention Time (Minute)	First Scan	Max Scan	Last Scan	PK TY		Peak Height	Corr. Area	Corr. % Max	% Of Total
1	11.194	1927	1941	1977	BB		113572	4998874	6.16%	1.935%
2	13.723	2317	2383	2460	BV		551336	44978770	55.42%	17.413%
3	16.813	2852	2923	2949	BV		204595	14193110	17.49	5.495%
4	17.168	2949	2985	3034	VB	9	72661	14986137	18.46%	5.802%
5	21.488	3705	3740	3783	BV		3985580	81161784	100.00%	31.421%
6	22.438	3890	3906	3942	BV		1933825	36135994	44.52%	14.990%
7	24.085	4175	4194	4199	BV		730854	14050215	17.31%	5.439%
8	24.148	4199	4205	4226	VV	2	803121	21456007	26.44%	8.306%
9	24.314	4226	4234	4267	VB		318991	8105089	9.99%	3.138%
10	27.645	4751	4816	4825	BV		83776	1632407	2.01%	0.632%
11	27.868	4825	4855	4896	VB	6	121133	6810521	8.39%	2.637%
12	29.882	5183	5207	5216	BV	3	96056	1684881	2.08%	0.652%
13	31.278	5421	5451	5483	BB		122636	4670221	5.75%	1.808%
14	34.168	5918	5956	5969	BV	3	114775	3443584	4.24%	1.333%

Table 2. MSD of Long-term Stored Husk of Gayo Arabica Coffee

Peak	Retention Time (Minute)	First Scan	Max Scan	Last Scan	PK TY		Peak Height	Corr. Area	Corr. % Max	% of Total
1	11.183	1923	1939	1983	BB		134371	5769343	11.67%	3.265%
2	12.362	2099	2145	2183	BB	3	78512	3644480	7.37%	2.063%
3	13.723	2334	2383	2461	BV		412014	38525802	77.90%	21.804%
4	17.185	2857	2988	3103	BB		74696	23317843	47.15%	13.197%
5	21.477	3687	3738	3797	BB		2540282	49456740	100.00%	27.991%
6	22.444	3872	3907	3942	BV		1406515	26769393	54.13%	15.151%
7	24.149	4172	4205	4227	BV	3	485776	21441406	43.35%	12.135%
8	24.321	4227	4235	4292	VB		246019	7763023	15.70%	4.394%

Table 3. Secondary Metabolites in the Fresh and Long-term Stored Husk of Gayo Arabica Coffee

Retention Time (Minutes)	Compound Name	Molecular Weight	% Area of Fresh *Husk*	% Area of Long-Term Stored *Husk*
11.18	Unknown	-	1.94	3.27
12.36	Unknown	-	Nd	2.06
13.72	HMF	126.11	Nd	21.80
13.73	Unknown	-	17.41	Nd
16.81	Unknown	-	5.50	Nd
17.19	Unknown	-	5.80	13.20
21.48	Caffeine	194	31.42	27.99
22.44	Palmitic Acid	256.42	13.42	15.15
24.09	Linoleic acid	280.4	5.44	Nd
24.15	(Z,Z)-9,12-Octadecadienoic acid	280.4	Nd	12.14
24.15	(Z,Z,Z)-9,12,15-Octadecatrienoic-1-ol	264.4	8.31	Nd
24.32	Stearic acid	284.5	3.14	4.39
27.65	Unknown	-	0.63	Nd
27.87	Unknown	-	2.64	Nd
29.88	Unknown	-	0.65	Nd
31.28	Unknown	-	1.81	Nd
34.17	Unknown	-	1.33	Nd

minutes in the stored pulp (Table 4). In contrast to husks, Gayo Arabica coffee pulps had various compounds as detected by GC-MS (Table 5).

The profiles and concentration of secondary metabolites in coffee depend on environmental factors, geographic origin, coffee genetics and variabilities, ripening stages, and pre- and postharvesting processing. [24]. Roasting affects the volatile compounds and aroma of coffee. [1, 13, 24–28]. Some of the volatile compounds identified in roasted coffee are aldehyde, ketones, ester, alcohols, furan, and phenolic volatiles. Drying also alters the compound contents of coffee [1, 24–26]. High temperature decreases the content of aldehyde, ester, acetic, and carboxylic compounds in coffee beans. [12, 13, 24, 26, 29, 30]. Caffeine, or 1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dion, is a methylxanthine alkaloid with a neuroprotective agent [14, 16, 18, 31–33]. Caffeine has been detected in coffee beans at high concentrations. However, some coffee bean processing techniques easily degrade caffeine [27, 33].

Coffee compounds promote some biological activities for human health. Chlorogenic acid, caffeine, and quinic acid exhibit anti-inflammatory activity through cyclooxygenase-2 inhibition [15, 34–37]. Caffeic acid stimulates PPAR activities and suppresses ACE2 activities [37, 38]. In addition, chlorogenic acid decreases blood tension [36].

(Z,Z)-9,12 Octadecadienoic acid has antioxidant activity [39], andacetic compounds, such as palmitic acid, exhibit anti-inflammatory and potential anticancer functions [40]. The concentrations of linoleic and palmitic acid in arabica coffee husk and pulp are high at approximately 48%–57% and 26%–28%, respectively [41]. 5-Hydroxymethylfurfural, which is found in coffee, shows antioxidant, antibacterial, and antiproliferative activities [42]. In addition, n-hexadecanoic acid promotes antioxidant activity [29, 43].

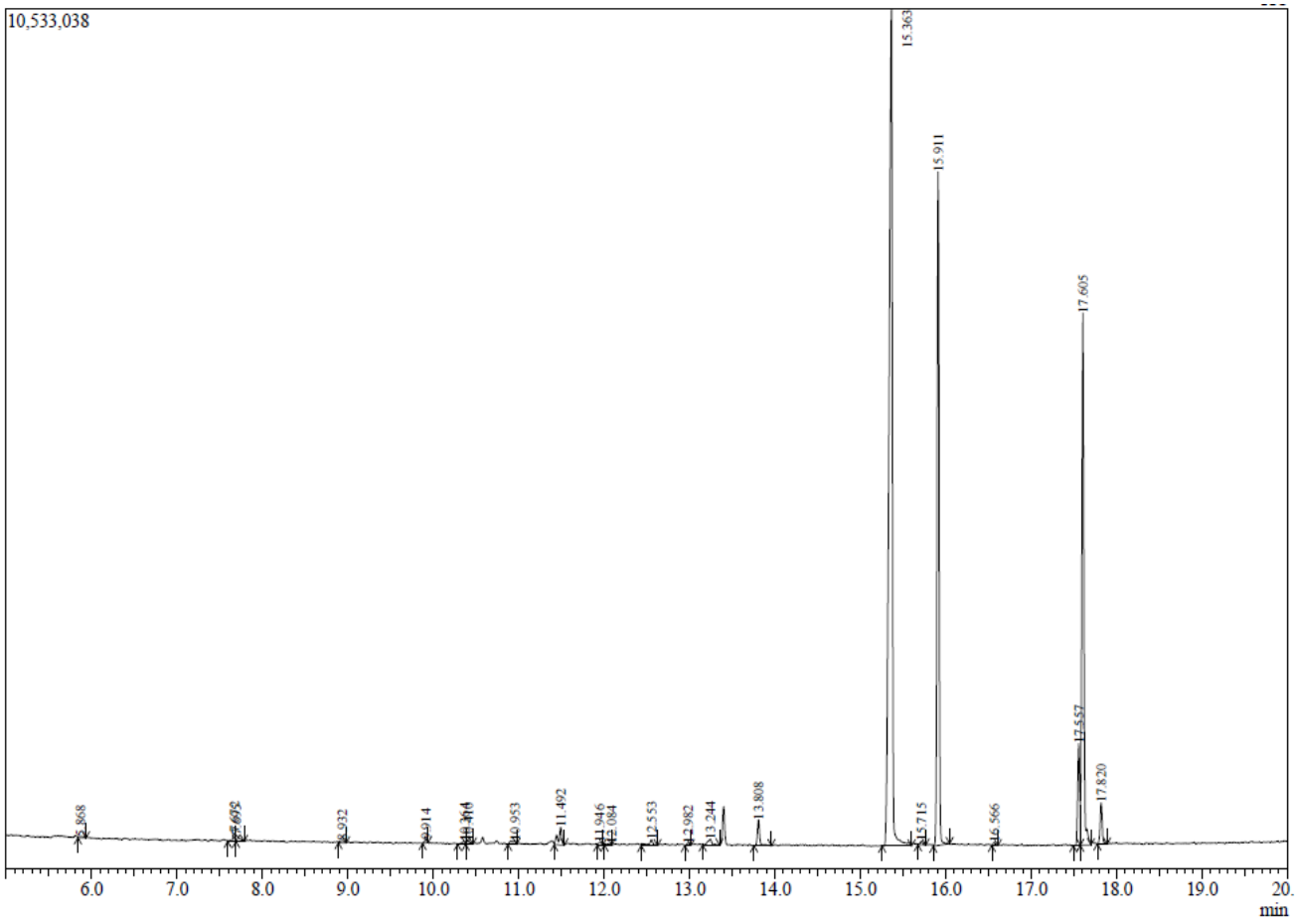

Figure 3. Total Ion Chromatogram of the Fresh Pulp of Gayo Arabica Coffee

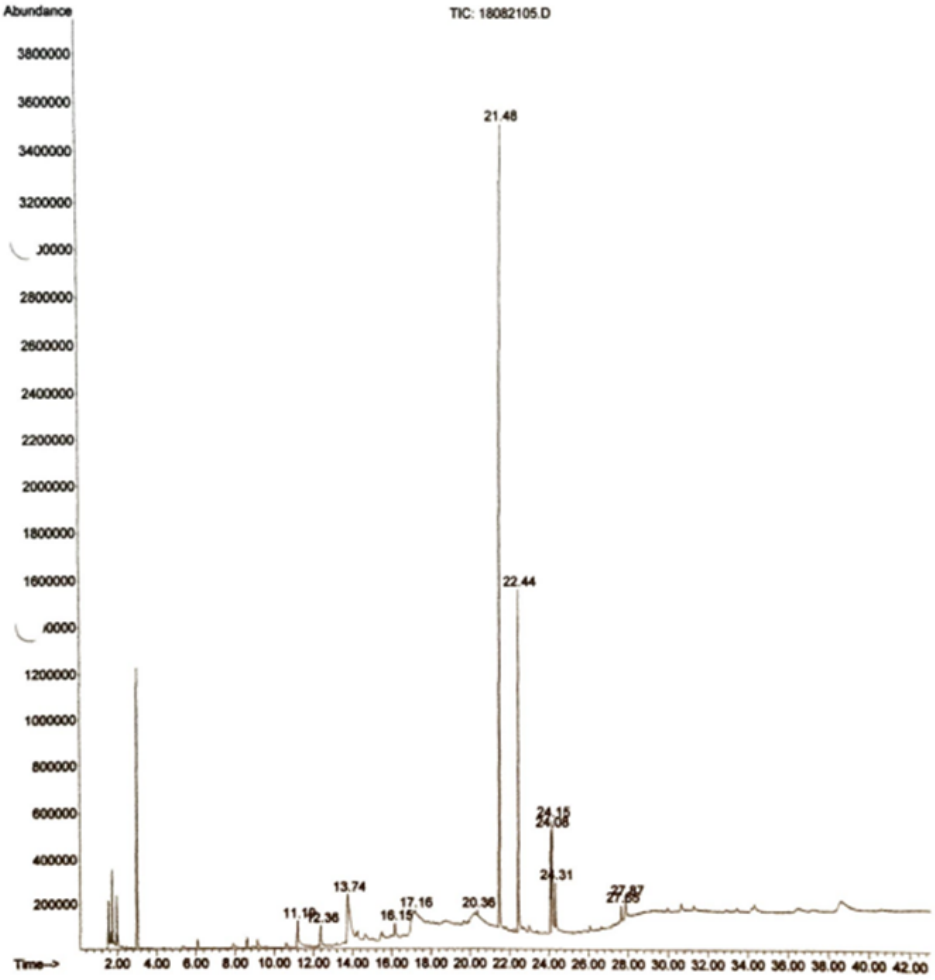

Figure 4. Total Ion Chromatogram of the Long-term Stored Pulp of Gayo Arabica Coffee

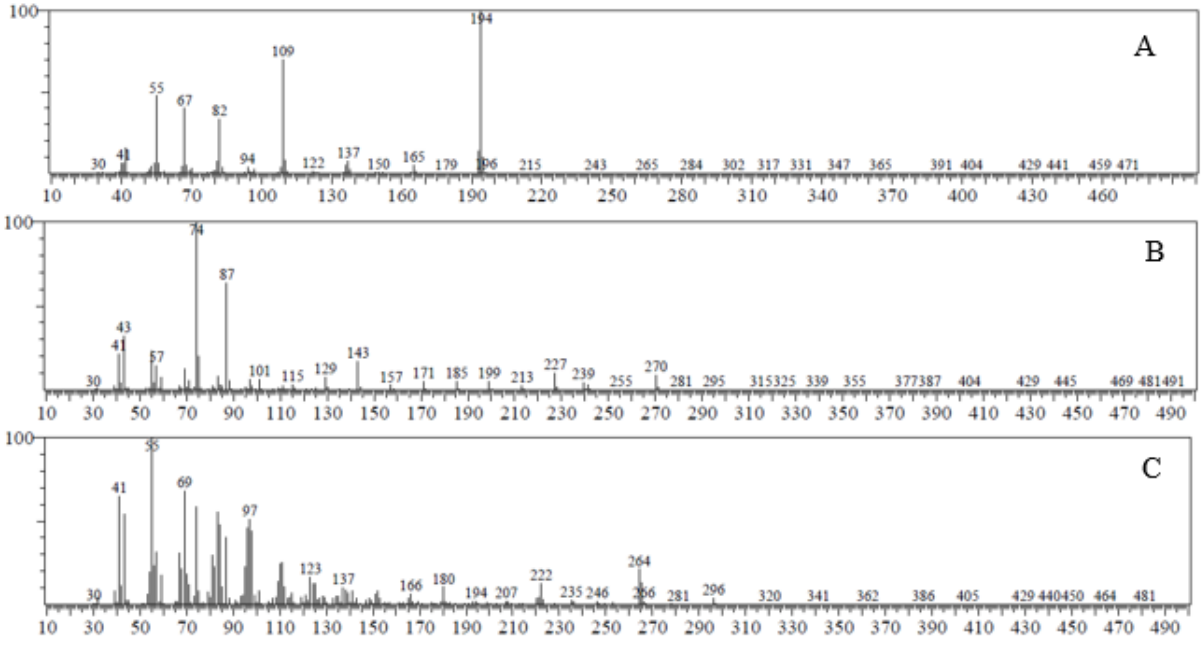

Figure 5. Mass Spectra Detected as Caffeine (A), Hexadecenoic Acid (B), and Elaidic Acid (C) in Gayo Arabica Coffee Fresh Pulp

Table 4. Mass Selective Detector (MSD) of the Long-term Stored Pulp of Gayo Arabica Coffee

Peak	Retention Time (Minute)	First Scan	Max Scan	Last Scan	PK TY		Peak height	Corr. Area	Corr. % max	% of total
1	11.188	1926	1949	1980	BB		115140	5125957	7.63%	2.865%
2	12.361	2101	2145	2179	BB		86635	3572047	5.32%	1.997%
3	13.740	2355	2386	2460	BV		200547	20505465	30.52%	11.462%
4	16.149	2753	2807	2843	BB	4	62127	3903332	5.81%	2.182%
5	17.157	2924	2983	3030	BB	8	66547	12614960	18.78%	7.051%
6	20.361	3441	3543	3576	BB	8	40836	7738156	11.52%	4.325%
7	21.482	3703	3739	3781	BB		3412235	67181158	100.00%	37.552%
8	22.438	3885	3906	3940	BV		1424703	26738585	39.80%	14.946%
9	24.080	4167	4193	4198	BV		428551	7561171	11.25%	4.226%
10	24.143	4198	4204	4227	VV		479638	13319097	19.83%	7.445%
11	24.315	4227	4234	4267	VB		211391	5358206	7.98%	2.995%
12	27.645	4711	4816	4828	BV	8	66694	1365740	2.03%	0.763%
13	27.868	4828	4855	4885	PB	3	87628	3918616	5.83%	2.190%

Table 5. Secondary Metabolite in the Fresh and Long-term Stored Pulps of Gayo Arabica Coffee

Retention Time (Minutes)	Compound	Molecular Weight	% Area of Fresh *pulp*	% Area of Long-Term Stored *Pulp*
5.868	2-Pyridinepropanoic acid, .alpha.-methyl-.b	207	0.13	Nd
7.672	5-Hydroxymethylfurfural	126.11	0.41	Nd
7.695	2,4(1H,3H)-Pyrimidinedione, 5-nitro-	306.23	0.34	Nd
8.932	Decanoic acid, methyl ester (CAS) Methyl	186.3	0.18	Nd
9.914	2-Cyclohexene-1-one, 3-(1,3-butadienyl)-2,4	206.33	0.15	Nd
10.364	Carbamic acid, Hydrazinecarboxamide	75	0.18	Nd
10.410	Stearic acid hydrazide	298.5	0.22	Nd
10.953	2H-2,4a-Ethanonaphthalene, 1,3,4,5,6,7-hex	204	0.32	Nd
11.492	Dodecanoic acid, methyl ester (CAS) Methy	214.34	1.01	Nd
11.946	8'-Apo.beta.-caroten-8'-al	416	0.13	Nd
12.084	3,6-DIHYDRO-3-METHYL-2H-PYRAN-2	112	0.10	Nd
12.553	.gama.-eudesmol	222.37	0.41	Nd
12.982	Ethanedioic acid, dihydrazide (CAS) Oxaly	118.10	0.12	Nd
13.244	.alpha.-Eudesmol, 2-Naphthalenemethanol	222.37	0.40	Nd
13.808	Tetradecanoic acid, methyl ester (CAS) Met	242.40	1.01	Nd
15.363	Caffeine	194	47.66	37.55
15.715	trans-13-Octadecenoic acid, methyl ester	296	0.16	Nd
15.911	Hexadecanoic acid, methyl ester (CAS) Me	270	22.86	Nd
16.566	Malonic acid, dihydrazide	132.12	0.12	Nd
17.557	Linoleic acid, 9,12-Octadecadienoic acid	280.4	3.56	Nd
17.605	Elaidic acid, 9-Octadecenoic acid, methyl e	296.5	19.05	Nd
17.820	Heptadecanoic acid, 16-methyl-, methyl ester	284.5	1.48	Nd
11.19	Unknown	-	Nd	2.87
12.36	Unknown	-	Nd	2.00
13.74	HMF	126.11	Nd	11.46
16.15	Unknown	-	Nd	2.18
17.16	Unknown	-	Nd	7.05
20.36	Unknown	-	Nd	4.33
21.48	Caffeine	194	Nd	37.55
22.44	Palmitic acid	256.42	Nd	14.95
24.08	Linoleic acid	280.4	Nd	4.23
24.15	(Z,Z,Z)-9,12,5-Octadecatrienoic-1-ol	280.4	Nd	7.45
24.31	Stearic acid	284.5	Nd	3.00
27.65	Unknown	-	Nd	0.76
27.87	Unknown	-	Nd	2.19

Nd = *not detected*

Conclusions

Metabolic investigation using GC-MS revealed that the postharvesting process impacts the secondary metabolites of coffee pulp and husk. Oxidation during storage possibly changes the detected metabolites. Major differences in several acetic acid and ester compounds are noted between the fresh and long-term stored byproducts of Gayo Arabica coffee. In particular, some of these compounds arise or disappear during storage, such as HMF, (Z,Z)-9,12-octadecadienoic acid, and (Z,Z,Z)-9,12,15-octadecatrienoic-1-ol.

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