Volatile Profile of Green Coffee Bean from *Coffea arabica* L. Plants Grown at Different Altitudes

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Abstract

This study was aimed to identify volatile compounds of 31 green coffee bean samples and evalute their correlation with altitude of the coffee plants grown in two zones (Gedeo and Jimma) in Ethiopia. A total of 81 different compounds were detected. The contents of dominant volatile compounds in green coffee beans were in the range: trans-linalooloxide (3.24–19.13%), linalool (1.56–21.76%), 2-methoxy-4-vinylphenol (2.34–15.08%) and cis-linalooloxide (1.03–13.27%). In addition, benzene acetaldehyde (0.45–10.97%), 2-heptanol (0.67–8.49%), α-terpineol (0.64–6.52%), phenylethyl alcohol (0.44–4.98%) and furfural (0.92–5.3%) were the next dominant compounds. The volatile compounds identified in the green coffee beans are groups of alcohols, aldehydes, ketones, pyrazines, pyridines, and furans. The volatile compounds in green coffee beans showed either weak positive or weak negative correlation with the altitude of coffee plants indicating that variation in altitude of the coffee plants does not significantly influence the volative compounds of green coffee beans.

Keywords: Coffea arabica L., Volatile compounds, Altitudes, Heterocyclic compounds

Introduction

The name of coffee is derived from the name of the province Keffa (birthplace of coffee) where shepherds from Abyssinia/Ethiopia discovered it in the world (Amamo, 2014 and Weldegebreal *et al*., 2017). Coffee has over sixty different species, only three of which (*Coffea arabica*, *Coffea robusta (canephora)*, and *Coffea liberica* global commercial values (Hagos *et al*., 2018) have. *Coffea arabica* and *Coffea canephora* are the most important commercial species (Yisak et al., 2018). Arabica coffee has a higher quality than Robusta coffee according its complex aroma and flavors (Mehari *et al*., 2016). It is the second largest traded commodity after oil in the world (Mussatto *et al*., 2011) and it is an important non-alcoholic beverage crop (Kathurima *et al*., 2000).

Coffee quality is defined by its sensorial aspects (Fassio *et al*., 2017). Quality and content of the chemical composition of coffee beans vary based on the species of coffee, altitude, soil, temperature variations and the place where it grows (Bertrand *et al*., 2006 ; Villarreal et al., 2006 ; Mehari *et al*., 2016 and 2019). Sherge et al. (2017) have studied the influence of growing altitude, shade and harvest period on quality and biochemical composition of Ethiopian specialty coffee. Mintesnot and Dechassa, (2018) and Adem et al. (2018) have reported the effect of altitude on biochemical composition (caffeine, chlorogenic acids and sucrose contents) and quality of green arabica coffee beans. Caffeine and chlorogenic acid contents were decreased with increasing altitude. According Worku et al. (2018), sucrose content was increased with increasing altitude. Tolessa et al. (2017) have reported the influence of growing altitude, shade on quality and biochemical composition of Ethiopian coffee. She concluded that coffee from high altitude with open or medium shade had a superior bean quality and favored the production of beans with lower caffeine.

Coffee taste is different due to the presence of different volatile and nonvolatile chemical constituents. Volatile compounds are fragrant components, generally odorous, which occur in certain indigenous plants or at specified parts of the plants. Their presence in green coffee beans is responsible for the aroma profile (Knysak, 2017). Their profile could be used to confirm the authenticity of *Coffea arabica*. Volatile compounds of coffee beans are diverse in their chemical characteristics. Numerous volatile chemicals have been identified in the green coffee beans (Saw *et al*., 2015).

The roasting process mainly induces the majority of volatile chemicals in coffee. However, there are only a few reports on the identification and quantification of volatile components in the green coffee beans (Czerny and Grosch, 2000). Researchers have identified about 100 volatile compounds and have reported the major constituents of volatile compounds found in green coffee beans (Toci and Farah, 2014 ; Bertrand *et al*., 2012; Dong *et al*., 2015). Toledo et al. (2015) have comprehensively reviewed the relationship between the different aspects related to coffee quality and their volatile compounds. This study has provided an overview of different aspects related to the quality of coffee beans and their volatile fractions: species/cultivars, geographic origins, bean defects, and types of beverages, processing, roasting, and storage.

Heterocyclic compounds are very important components in providing coffee with their characteristic flavors. Some of these compounds have been found useful in differentiating between Robusta and Arabica coffee varieties (Franca *et al*., 2009). Some volatile compounds found in green coffee bean have been indicated as characteristics of bean defects due to certain external causes like over fermentation during processing or insect attacks (Toci and Farah, 2008). However, the vast majority of volatiles compounds are only generated during the roasting of green coffee beans (Yeretzian *et al*., 2002).

Characterization of volatile compounds of coffee was used to identify coffee origin and production locations (Risticevic *et al*., 2008). Risticevic et al. have reported profile of volatile flavor chemicals obtained from three countries (Ethiopia, Nicaragua, and Indonesia). Characterization of volatile profiles from the various coffee cultivars of Ethiopia is very limited. Therefore, it is necessary to study systematically the volatile compounds of green coffee beans obtained from coffee plants grown at different altitudes in Ethiopia. The objectives of this study were to (i) identify and quantify the volatile compounds of green coffee bean samples from the coffee plants grown at different altitudes in Ethiopia by gas chromatography-mass spectrometry and (ii) evalute their correlation with altitude of the coffee plants.

Experimental

Chemicals

Dichloromethane ($\geq$ 97%, Sigma-Aldrich, USA) GC/MS grade was used for the extraction of volatile compounds after hydro-distillation of green coffee beans. Anhydrous sodium sulfate (Fluka, Buchs, Switzerland) was used for drying the dichloromethane extract.

Sample collection

Thirty-one green coffee bean samples were collected from Gedeo Zone, Yirgacheffe District (21) and Jimma Zone, Gomma District (10), Ethiopia, during December 2017 to March 2018 from the farmers who produce a special coffee under the supervision of agricultural experts for the export to international markets. The description of green coffee bean samples and sampling sites (sample number, sampling zone, sampling district/woreda and altitude) are given in Table 1. All the green coffee bean samples were obtained from the ripen coffee cherries and processed by sun drying. About 1 kg of green coffee beans was collected from different farmers from each sampling site and stored in paper bags under room temperature conditions. It was ground in an electronic grinder (Moulinex, SEB Group, Selongey, France) for the analysis.

Table 1. The description of green coffee bean samples and sampling sites

Sample Number	Sampling Zone	District/Woreda	Altitude (M)
J32	Jimma	Gomma	1515
J31	Jimma	Gomma	1579
J24	Jimma	Gomma	1627
J30	Jimma	Gomma	1727
J25	Jimma	Gomma	1737
J29	Jimma	Gomma	1782
G12	Gedeo	Yirgachefe	1840
G11	Gedeo	Yirgacheffe	1855
G13	Gedeo	Yirgacheffe	1860
G10	Gedeo	Yirgacheffe	1878
G03	Gedeo	Yirgacheffe	1891
G04	Gedeo	Yirgacheffe	1904
G09	Gedeo	Yirgacheffe	1916
G22	Gedeo	Yirgacheffe	1918
G02	Gedeo	Yirgacheffe	1922
G21	Gedeo	Yirgacheffe	1945
G01	Gedeo	Yirgacheffe	1971
J33	Jimma	Gomma	1972
J28	Jimma	Gomma	2010
G16	Gedeo	Yirgacheffe	2037
G20	Gedeo	Yirgacheffe	2044
G19	Gedeo	Yirgacheffe	2046
G15	Gedeo	Yirgacheffe	2050
G17	Gedeo	Yirgacheffe	2050
G18	Gedeo	Yirgacheffe	2063
G14	Gedeo	Yirgacheffe	2068
J26	Jimma	Gomma	2137
G06	Gedeo	Yirgacheffe	2139
J27	Jimma	Gomma	2163
G05	Gedeo	Yirgacheffe	2200
G07	Gedeo	Yirgacheffe	2220

Extraction of volatile compounds by hydro-distillation

The volatile compounds were extracted from green coffee beans by hydro-distillation according to the procedure of Saw et al. (2015) with minor modifications. Each ground sample of green coffee beans (200 g) was transferred into 2 L round bottom flask with 1 L of distilled water. The mixtures were distilled for 6 h at atmospheric pressure. The distillate was transferred to 50 mL of separatory funnel, and 3 mL of dichloromethane was added and shaken properly. The lower organic phase was separated and dried over anhydrous Na_2SO_4 to remove residual water content, and the dried extract was transferred into a 2.5 mL glass vial for gas chromatography-mass spectrometry (GC-MS, Agilent, 7890) analysis.

Analysis of volatile compounds

The extracted volatile compounds were analyzed by Agilent GC-MS 7890 (USA) gas chromatograph equipped with a mass spectrometer detector and Agilent automatic injector. The chromatographic conditions were set with 1 μL of injection volume (splitless) and injector temperature was set at 275 °C. Separation was achieved on HP-5MS 5% phenyl methyl silox capillary column (30 m x 250 µm, film thickness 0.25 µm) under temperature program conditions of 40 °C for 3 min then 5 °C/min to 230 °C and held at 230 °C for 20 min. Helium was used as a carrier gas at a flow rate of 1.68 mL/min with a pressure of 8 psi. Conditions used for the mass spectrometer were a temperature of 230 °C, scan range 40-650 m/z, operating in positive electron impact mode with ionization energy of 70 eV. Chromatographic and mass spectral data were processed by using the instrument built in software (MS ChemStation; Agilent Technologies, USA). The volatile components were identified from chromatograph library and by the MS fragmentation pattern of authentic chemicals. The method validation for the analysis by GC-MS was done by running the blank and standard methyl ester of decanoic acid before the analysis of samples.

Result and Discussion

Identification and quantification of volatile compounds of green coffee beans
Volatile components isolated from green coffee beans (*Coffea arabica* L.) were identified using the comparison of the corresponding mass spectra in GC-MS with data of NIST-14 mass spectral data library found in GC-MS database. Their quantification was carried out according to their peak area of the chromatogram.
Figure 1 shows chromatogram peak profile of volatile compounds presents in green coffee beans detected by GC-MS. Most of the compounds are found in the range of 4.2 min up to 28.5 min in all samples, but the compounds detected after 25.5 min were not identified. The chromatogram shows that the most intense compounds are translinalooloxide at a retention time of about 8.2 min, cis-linalooloxide (8.56 min), linalool (8.75 min), 2-methoxy-4-vinylphenol (12.77 min).

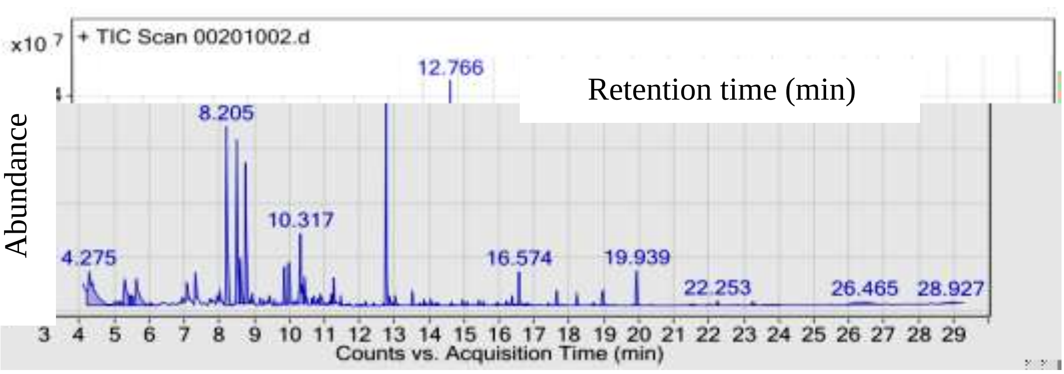

Figure 2. The chromatogram of volatile compounds of green coffee beans (sample number G04) from Gedeo zone.

In this study, 81 volatile compounds with 18 chemical classes were detected as shown in Figure 1. The peak number, name of volatile compounds, their chemical class, retention time (RT) and range of percentage peak area of the identified compounds in the 31 green coffee bean samples by GC-MS are given in Table 2. Some compounds in green coffee beans reported in the literature by Saw et al. (2015) and Bertrand et al. (2012) (such as α-terpineol, trans-linalool oxide, cis-linalool oxide, 2-heptanol, furfural, linalool, benzyl alcohol, indole, methyl stearate) were also identified in the green coffee bean samples of the present study. The components detected in roasted coffee beans like furans, pyrazines, and pyridines were also identified in the present study. Among these, the dominant compounds (mean/range) are: trans-linalool oxide (12.2%, 3.24–19.13%), linalool (9.28%, 1.56–21.76%), 2-methoxy-4-vinylphenol (8.47%, 2.34–15.08%), cis-linalooloxide (7.31%, 1.03–13.27%), with the retention time of 8.20, 9.28, 12.8 and 8.47 min, respectively. Benzeneacetaldehyde (5.18%, 0.45–10.97%), 2-heptanol (4.86%, 0.67–8.49%), α-terpineol (4.85%, 0.64–6.52%), phenylethyl alcohol (4.07%, 0.44–4.98%) and furfural (3.15%, 0.92–5.3%) are the next dominant compounds, respectively.

Table 2. The peak number, name of volatile compounds, their chemical class, retention time (RT) and range of percentage peak area of the identified compounds in the 31 green coffee bean samples by GC-MS.

Peak No.	Compound name	Class	RT	Range of peak area (%)
01	3-Methyl-2-butenal	Aldehyde	4.28	0.13–4.35
02	Ethyl-3-methyl, butanoate	Ester	4.80	0.63–3.23
03	2,6-Lutidine	Pyridine	5.01	0.16–0.94
04	3,5-Dimethylpyrazole	Pyrazole	5.28	0.53–3.69
05	Pyridine, 2,3-dimethyl-	Pyridine	5.30	0.61–1.37
06	Furfural	Furan	5.32	0.92–5.3
07	3-Furfural aldehyde	Furan	5.36	0.62–4.5
08	1H-Imidazole,1,4-dimethyl-	Imidazole	5.38	0.35–1.30
09	2-Heptanol	Alcohol	5.63	0.67–8.49
10	1,4-Hexadiene, 4-methyl	Hydrocarbon	6.36	0.13–2.5
11	Eucalyptol/1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane	Ether	6.94	0.34–5.27
12	Benzaldehyde	Aldehyde	7.08	0.65–3.95
13	1-Butanamine, 4[2,4-bis(1,1-dimethylpropyl)phenoxy]-N-propylidene	Amine	7.32	0.36–2.48
14	N-Ethylidene t-butylamin	Amine	7.36	0.34–1.85
15	1-Pyrolidineethaneamine/1-(2-aminoethyl)pyrrolidine	Pyrolidine	7.41	0.30–3.21
16	Cyclohexanol, 2,2-dimethyl-	Alcohol	7.72	0.25–1.77
17	3-Ethyl-4-methylpentan-1-ol	Alcohol	7.79	0.22-1.9
18	2-Ethyl piperidine	Piperidine	7.81	0.27-1.98
19	1-Pyrolid-2-one, N-carbamoyl-	Pyrolidine	7.84	0.21-1.90
20	1,6-Dioxaspiro[4.4]nonane, 2-ethyl	Hydrocarbon	7.96	0.20-1.64
21	Pyrazole, 3,5-dimethyl-1-allyl-	Pyrazole	8.12	0.16-5.01
22	1H-1,2,3-Triazole-5-carbxamide, 4-amino-	Triazole	8.01	0.20–4.22
23	Trans-linalooloxide	Furan	8.20	3.24–19.13
24	Cis-linalooloxide	Furan	8.49	1.03–13.27
25	Benzeneacetaldehyde	Aldehyde	8.60	0.45–10.97
26	Linalool	Alcohol	8.75	1.56–21.76
27	Benzyl alcohol	Alcohol	8.94	0.38–2.47
28	1,3-Dioxolane-2-propanal, 2-methyl	Aldehyde	9.16	0.17–0.67
29	2-Cyclohexene-1-ol, 1-methyl-4-[1-methylethenyl]-, trans-	Alcohol	9.57	0.23–1.39

30	Pyrazine, 3,5-diethyl-2-methyl-	Pyrazine	9.23	0.11–1.56
31	6-Pethyl-3,5-heptadiene-2-one	Ketone	9.44	0.53–1.25
32	1,3,8-*p*-Menthatriene	Hydrocaron	9.55	0.12–6.24
33	Pyrazine, 2-methoxy-3-[2-methylpropyl]-	Pyrazine	9.40	0.09–1.68
34	Benzen, 1,2-dimethoxy	Ether	9.86	0.32–4.73
35	Phenylethyl alcohol	Alcohol	10.0	0.44–4.98
36	2-Butanone, 4-[5-methyl-furanyl]	Furan	10.0	0.08–4.60
37	α-Terpineol	Alcohol	10.3	0.64–6.52
38	Methyl salicylate	Ester	10.4	0.81–4.98
39	S-Benzoyl(thiohydroxylamine)	Amine	10.5	0.08–10.51
40	Benzene, 1-methyl-4-[1-methylethylene]-/ p-Isopropenyl toluene	Hydrocarbon	10.7	0.07–0.53
41	Cyclopentane, 1,3-bis[methylene]	Hydrocarbon	10.7	0.1–0.90
42	Hexane, 1-chloro-5-methyl	Hydrocarbon	10.8	0.07–1.30
43	3-Carene/3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene	Hydrocarbon	10.89	0.35–1.88
44	1H-Imidazole, 2-ethyl-4-methyl-	Imidazole	10.93	0.06–4.17
45	Phenylephrine	Alcohol	10.93	0.58–0.86
46	3,4,5-Trimethylpyrazole	Pyrazole	10.94	0.18–0.97
47	(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	Alcohol	11.13	0.17–1.32
48	Benzene acetic acid, ethyl ester	Ester	11.23	0.22–2.68
49	2,6-Octadien-1-ol, 3,7-dimethyl-,(z)/trans-farnesol	Alcohol	11.28	1.22–3.08
50	Benzoic acid, 2-hydroxy-, ethyl ester	Ester	11.48	0.15–2.76
51	Citral	Aldehyde	11.75	0.22–1.23
52	Benzeneacetaldehyde, .alpha.-ethylidene	Aldehyde	12.19	0.08–0.84
53	2,4-Decadienal, (E,E)-	Aldehyde	12.41	0.19–0.54
54	2-Methoxy-4-vinylphenol	Alcohol	12.77	2.34–15.08
55	3- Methoxyacetophenone	Ketone	12.00	0.16–7.91
56	Phenol, *p*-tert-butyl-	Alcohol	12.88	0.46–1.12
57	2-Butene-1-one, 1-[2,6,6-trimethyl-1,3-cyclohexadien-1-yl]-, (E)-	Ketone	13.03	0.16–1.53
58	Indole	Indole	13.52	0.45–0.94
59	3-Amino-1,2,4-triazole-5-carboxlic acid	Triazole	13.85	0.20–0.50
60	2[3H]-Furanone, dihydro-5-pentyl-	Furan	14.04	0.12–0.50
61	Acetamide, N-phenyl-	Amide	15.47	0.15–0.25
62	α-Isomethyl ionone	Kitone	14.21	0.21–0.7
63	Lilial	Aldehyde	14.96	0.09–1.88
64	2,4-Di-tert-butylphenol	Alcohol	15.09	0.16–0.45
65	Octadecane	Hydrocarbon	16.36	0.08–0.79
66	Pentadecanal	Aldehyde	16.57	0.15–4.09
67	Hexadecanal	Aldehyde	16.57	0.15–3.01
68	Octanal, 2-{phenylmethylene}-	Aldehyde	17.65	0.84–1.74
69	2-Pentadecanone, 6,10,14-trimethyl	Kitone	18.22	0.36–1.16
70	Methylpent-4-enylamine	Amine	18.24	0.24–0.91
71	2,7-Diphenylindole	Indole	18.69	0.14–1.52
72	2-Butene-1,4-diamine, N,N'-dimethyl-	Amine	18.93	1.21–2.69
73	Hexadecanoic acid, methyl ester	Ester	19.93	1.04–12.71
74	Caffeine	Amide	21.36	0.50–1.60
75	7-Octadecenoic acid, methyl ester	Ester	21.98	0.13–3.5
76	9,12-Octadeadienoic acid, methyl ester	Ester	22.08	0.26–7.73
77	Methyl stearate	Ester	22.24	0.24–6.46
78	*p*-Hydroxynorephedrine	Ephedrine	23.15	0.18–0.72
79	Linoleic acid, methyl ester	Ester	23.25	0.20–3.51
80	*o*-Veratramide	Amide	23.26	0.26–3.6
81	Stigmasterol	Alcohol	25.99	0.58–2.73

According to Lee and Shibamoto, (2002) and Bertrand et al. (2012) about 74 compounds were identified in green coffee beans from other countries. Among the major components of volatile compounds found in green coffee beans were 3-methyl butanoic acid, phenyl ethyl alcohol, hexanol, 4-hydroxy-3-methylacetophenone, 3-methyl butanol, 2-methoxy-3-(2-methylpropyl)-pyrazine. Brevard and Yeretzian, (2001) also identified about 219 volatile compounds and approximately 100 different volatile compounds were identified in green coffee beans (Toci and Farah, 2014). Poyraz et al. (2016) have reported about 40 compounds in green coffee beans. In their report, the components of the green coffee beans were identified as isoamyl alcohol (10.4%), hexanal (10.4%) and hexacosane (8.2%) while furfuryl alcohol (13.6%), furfurylacetate (10.7%) and 5-methyl furfural (9.27%) were identified as the main components of the roasted coffee obtained from a local market in Turkey in 2014. While the major compounds identified in present study are: trans-linalool oxide (12.2%), linalool (9.28%), 2-methoxy-4-vinylphenol (8.47%), cis-linalooloxide (7.31%), benzene acetaldehyde (5.18%), 2-heptanol (4.86%), α-terpineol (4.85%), phenylethyl alcohol (4.07%) and furfural (3.15%) which are different from the compounds reported in the literature by Poyraz et al. (2016) .

Volatile compounds identified in the extracted green coffee beans of the present study are a group of alcohols, aldehydes, ketones, esters, hydrocarbons, pyrroles, furans, and other compounds as listed in Table 3, which are accountable to flavor profiles. In this study, the first dominant class of compounds in the green coffee beans is an alcohol with the average value of 31.1%. This is comparable with the results reported by Brevard and Yeretzian, (2001). Green coffee beans are also very rich in aldehydes and esters. Esters are important compounds in sensory point of view. The highest average value of chemical classes of volatile compounds present in 31 green coffee bean samples were shown in Figure 2. In another study, the major volatile compounds in green coffee beans were aldehydes (benzaldehyde) and alkanes (Poyraz *et al.,* 2016). Whereas the major volatile compounds in roasted beans determined were a group of heterocyclic compounds as furans, pyrazines, and pyridines (Somporn *et al*., 2011).

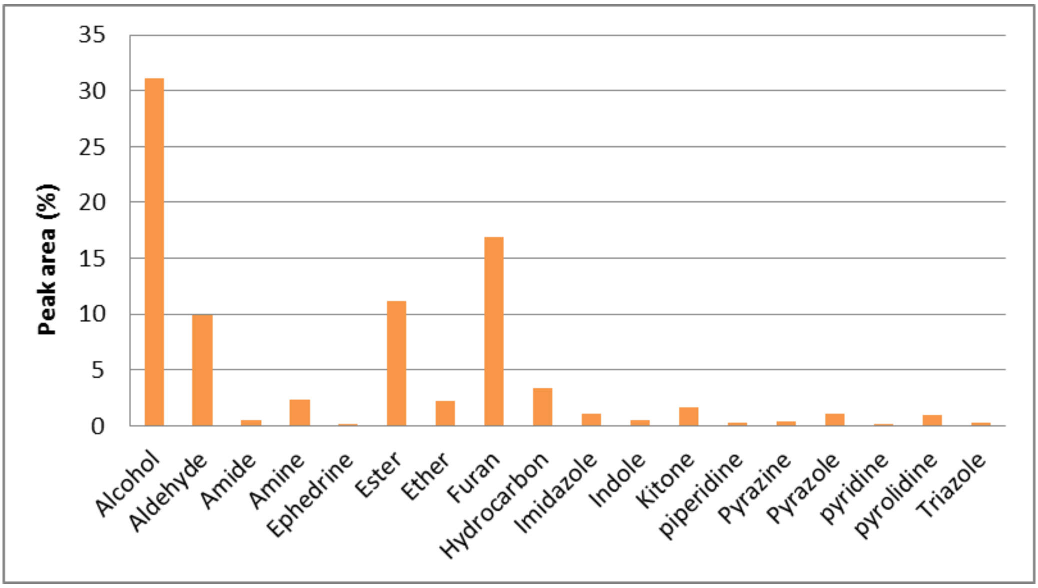

Figure 3. Average percentage peak area for chemical classes of volatile compounds that constitute in 31 green coffee bean samples.

Heterocyclic compounds are responsible for the characteristic flavor of coffee as flavor agents and they also used as stimulants and medication. Their existence in green coffee beans is somewhat different from roasted coffee beans. Over 350 heterocyclic compounds, including pyrroles, furans, pyrazines, thiazoles, oxazoles, thiophenes and imidazoles, have been identified in the roasted coffee beans. Lee and Shibamoto, (2002) have reported that heterocyclic compounds were not found in green coffee beans, except for 2-methoxy-3-(2-methylpropyl)-pyrazine. In the present study, furans, pyrazines, imidazole, indole, piperidine, pyrazole, pyridine, triazole and pyrrolidine, even 2-methoxy-3-(2-methylpropyl)-pyrazine were identified in the extract of green coffee beans. Their presence (or absence) in the individual green coffee bean samples are summarized in Table 3. These heterocyclic compounds are among the important contributors to the coffee flavor (Petisca *et al*., 2013) and they are used as flavoring agents like 2-methoxy-4-vinylphenol. Many compounds responsible to spicy, earthy (pyrazine, 2-methoxy-3-[2-methylpropyl]) and buttery flavor were also identified in the present study. Nitrogen-containing heterocyclic compounds are found more in green coffee beans collected from Jimma than from Gedeo. Therefore, it can be concluded that coffee plants grown in Jimma could be distinguished from coffee plants grown in Gedeo samples according to nitrogen heterocyclic compounds (Table 3).

Pyridine (in samples J25, J26, J29, J30), triazole (in samples J25, J26, J27, J29, J30, J31, J33) and piperidine (in samples J26, J27, J29, J30, J31, J33) are only found in green coffee bean samples from Jimma. In addition, pyrazine is only found in four samples from Gedeo (G10, G12, G20, G22) but in many sample from Jimma and ephedrine is only found in sample J24 and sample J33 (Jimma) (Table 3). Ephedrine is a medication, stimulant and it is used to prevent low blood pressure during spinal anesthesia. The volatile compounds of green coffee beans have an antioxidant activity (Lee and Shibamoto, 2002). The antioxidant activities of volatile compounds in green coffee beans are due to the presence of several components. Compounds responsible for antioxidants activities such as benzyl alcohol, methyl salicylate, and 4-hydroxy-3-methylacetophenone are identified in the research of Lee and Shibamoto, (2002) and these compounds are also identified in the present study.

Table 3. Total peak area (%) of each chemical class in individual green coffee bean samples

Sample	Altitude	Class of compounds*																	
number	(m)	Alc	Ald	Am	Ami	Eph	Est	Eth	Fur	Hyd	Ime	Ind	Ket	Pip	Pyr	Prz	Pyri	Pyro	Tri
J32	1515	31.4	10.38	1.80	0.94	-	23.9	0.81	13.8	2.72	2.88	-	0.95	-	-	-	-	-	-
J31	1579	42.9	18.03	0.26	11.03	0.72	8.17	2.66	21.7	1.12	0.86	1.52	2.22	1.56	0.79	0.50	-	1.90	0.98
J24	1627	7.50	3.92	-	-	0.25	37.4	3.71	5.40	0.38	4.17	0.14	0.63	-	-	-	-	-	-
J30	1727	40.0	14.96	0.26	3.33	0.72	7.19	7.21	10.2	3.87	1.60	0.75	3.36	1.56	0.79	4.46	0.94	4.38	0.98
J25	1737	29.2	18.40	-	-	0.25	6.25	1.58	14.4	5.91	4.17	0.50	1.71	-	-	0.68	0.73	-	0.96
J29	1782	52.5	11.47	0.51	4.13	0.72	6.87	3.77	8.94	6.14	2.42	0.72	3.45	1.56	0.79	4.42	0.64	1.90	1.31
G12	1840	26.0	6.64	1.77	2.18	-	11.0	1.39	14.3	2.24	-	-	-	-	1.89	-	-	-	-
G11	1855	19.5	6.37	-	1.60	-	5.52	1.22	12.9	5.20	1.84	-	0.59	-	-	-	-	-	-
G13	1860	29.1	11.9	-	2.06	-	3.24	1.28	16.8	2.70	2.43	-	1.05	-	-	-	-	-	-
G10	1878	22.2	7.17	-	1.04	-	10.3	1.35	10.3	2.44	0.61	-	2.40	-	0.99	-	-	-	-
G03	1891	35.5	12.9	-	0.91	-	1.26	2.54	32.1	3.48	-	1.98	1.12	-	-	-	-	-	-
G04	1904	40.9	12.9	-	2.05	-	2.24	2.49	31.6	3.80	-	1.08	1.51	-	-	-	-	3.67	-
G09	1916	40.2	13.8	-	3.83	-	15.2	2.75	21.0	4.14	-	2.58	2.21	-	-	2.34	-	3.67	-
G22	1918	26.1	6.13	-	2.22	-	11.2	1.35	19.0	5.09	-	-	1.44	-	1.08	-	-	-	-
G02	1922	42.3	10.1	1.33	2.74	-	5.07	2.06	26.8	5.71	3.72	-	-	-	-	-	-	-	-
G21	1945	10.7	2.51	0.30	0.88	-	17.7	0.60	8.58	2.53	0.64	-	2.54	-	-	-	-	-	-
G01	1971	24.3	5.00	-	-	-	9.50	1.34	14.6	1.72	-	-	1.67	-	-	-	-	-	-
J33	1972	29.8	12.80	0.26	1.27	0.72	27.2	3.76	9.08	1.68	0.86	0.83	2.57	1.56	0.79	0.50	-	1.90	0.98
J28	2010	15.9	7.94	-	-	-	20.5	3.53	-	-	-	-	0.84	-	-	2.22	-	-	-
G16	2037	36.8	12.1	-	3.90	-	8.54	2.53	26.7	3.35	-	0.80	2.50	-	-	1.31	-	0.65	-
G20	2044	26.5	8.92	-	1.23	-	7.47	1.77	16.5	4.37	-	-	0.52	-	1.49	-	-	-	-
G19	2046	38.1	15.3	-	4.30	-	9.54	2.53	34.7	3.62	-	0.93	1.73	-	-	3.07	-	3.21	-
G15	2050	27.9	15.4	-	4.00	-	12.8	2.33											

Correlations between the chemical classes of volatile compounds & altitude of the coffee plants

The Pearson correlation shows weak, moderate or strong relationship among variables in the positive or negative direction (Miller and Miller, 2005). When grouping the volatile compounds according to their chemical classes, correlations were evaluated with an altitude of Ethiopian coffee plants. The correlation coefficients of main chemical classes of alcohol, furan, and hydrocarbon with altitude have a weak significant positive correlation (p = 0.01) which indicates that the percentage of these compounds increases with increasing altitude. While aldehyde, amine, ester, and ether have a weak negative significant correlation (p = 0.01) which indicates that the percentage of these compounds decreases with increasing altitude as shown in Figure 3. The correlation coefficients of all chemical classes of volatile compounds in the green coffee beans with respect to the altitudes of coffee plants are listed in Table 4. Since the two zones (Jimma and Gedeo) have variable altitudes it is not possible to differentiate the compositions of the eight classes of compounds used to evaluate the correlation coefficients. There is lack of relevant literature on the correlation of volatile compounds in the green coffee beans with the altitude of the coffee plants. And to the best of our knowledge, this is the first investigation which deals on the correlation of volatile compounds in green coffee beans with altitudes of coffee plants.

Table 4. Correlation coefficients between the main chemical classes of volatile compounds and altitude of Ethiopian coffee plants

Compound	Alcohol	Aldehyde	Amine	Ester	Ether	Furan	Hydrocarbon	Ketone
Correlation coefficient (R)	0.111	-0.215	-0.095	0.237	-0.181	0.195	0.124	0.068

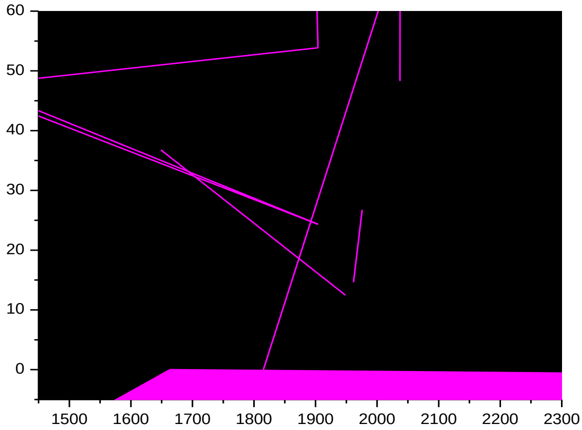

Figure 4. Correlation between the main eight chemical classes of volatile compounds in green coffee beans and altitude of coffee plants grown in Ethiopia.

Conclusions

A total of 81 different volatile compounds with a group of 18 chemical classes were identified in Ethiopian green coffee bean samples. The major volatile components identified in green coffee beans are trans-linalool oxide, linalool, 2-methoxy-4-vinylphenol, cis-linaloloxide, benzene acetaldehyde, 2-heptanol, and α-terpineol. About 80% of the total volatile compounds are a class of alcohols, furans, aldehydes and esters and very few heterocyclic compounds are identified in the green coffee beans. Nitrogen-containing heterocyclic compounds are found more in green coffee beans collected from Jimma than from other sites. Therefore, it can be concluded that coffee plants grown in Jimma could be distinguished from coffee plants grown in Gedeo samples according to nitrogen heterocyclic compounds. This could be due to genetics, soil composition, climate, and rainfall differences. Altitudes have a weak negative effect on the esters content of the Ethiopian green coffee beans. The quality of coffee depends on many parameters including chemical composition, soil characteristics, altitudes and climatic conditions. Therefore, it is not possible to determine the quality of coffee from the composition of volatile compounds in the green coffee beans only.

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