Volatile Organic Compounds in Brewed Kenyan Arabica Coffee Genotypes by Solid Phase Extraction Gas Chromatography Mass Spectrometry

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Abstract

Besides its stimulatory effect, coffee is appreciated and/or consumed for its pleasing aroma, which is a key quality index. The objective of this study was to characterize the volatile organic compounds in brewed Kenyan Arabica coffee genotypes. Solid phase Extraction (SPE) technique was used for the extraction of the organic compounds in the brewed coffee, while characterization of the compounds was done by gas chromatography mass spectrometry (GC-MS). Various volatile organic compounds were identified and classified into alcohols, aldehydes, carboxylic acids, furans, ketones, pyrazines and pyrroles. Differences were observed in the chromatographic profiles of the eluents from the seven coffee genotypes evaluated. Compounds such as 2, 6-dimethylpyrazine,5-methyl-1H-pyrole-2-carboxyaldehyde,2-furanmethanolacetate, 4-Ethylcatechol, Methoxy-4-vinylphenol, 2,6-Dihydroxyacetophenone and Ionone, were found to be present in all the coffee genotypes. This study demonstrated the presence of appreciable levels of volatile organic compounds in the coffee brew of the genotypes studied with variations in the types and concentrations being observed among the genotypes.

Keywords: Kenya, Coffee genotypes, Solid Phase Extraction (SPE), Gas Chromatography Mass Spectrometry (GC-MS) Volatile organic compounds.

Introduction

Coffee beans are the seeds of a perennial evergreen tropical plant, which belongs to the family Rubiceae and genus *Coffea.* The distinct flavor of brewed coffee is certainly the main reason for its wide popularity and almost universal appeal as a refreshing beverage (Petracco, 2001). *Coffea arabica* dominates the world trade due to its superior quality. Kenya has a reputation of producing some of the best mild coffees in the trade. Coffee was introduced into Kenya by French Missionaries around 1900 A.D. (Mwangi, 1983). The old cultivars grown in Kenya are K7 for low altitude areas with serious leaf rust, SL28 and SL34 for low to medium areas with good rainfall (Mwangi, 1983). The more recently released cultivar Ruiru 11, is suitable for all coffee growing areas because it is resistant to Coffee Berry Disease (CBD) and Coffee Leaf Rust (CLR) ((Nyoro and Sprey, 1986; Opile and Agwanda, 1993). The desirable quality attributes are derived from inherent genetic characteristics of selected coffee varieties, climatic conditions and proper field and post harvest management. Green coffee beans contain a wide range of different chemical compounds which react and interact at all stages of coffee processing to produce a final product with an even greater diversity and complexity of structure (Clifford, 1985). However, the desirable aroma and taste of brewed coffee is formed during roasting of green coffee beans.

The chemistry of coffee flavor is highly complex and is still not completely understood. The main families of chemical compounds found in green coffee, and responsible for the volatiles in roasted coffee, are alkaloids like trigonelline, chlorogenic acids, carbohydrates, lipids and proteins (Flament, 2002). During the roasting process, the composition of coffee beans is drastically changed and several hundreds of substances associated with coffee aroma and taste are formed (Nijssen *et al*., 1996). The main classes of compounds that have been identified in roasted beans are: furans, pyrazines, ketones, alcohols, aldehydes, esters, pyrroles, thiophenes, sulfur compounds, benzenic compounds, phenolic compounds, pyridines, thiazoles, oxazoles, lactones, alkanes, alkenes, and acids (Mondello *et al*., 2005). Gas Chromatography Mass Spectrometry (GC-MS) is commonly used for the analysis of volatile organics

in green beans, roasted beans and the final brewed coffee. The aroma of the brew is different from that of ground roasted coffee although the change in the aroma profile is not caused by the formation of new odorants but by a shift in the concentrations (Grosch, 2001). The main difference occurs in the analytical approach towards analyte extraction (Akiyama *et al*., 2003). The analysis of the freshly brewed coffee volatiles that linger in the air and reach the human nose could be a direct way to understand the factors that attract people to the pleasant coffee aroma. In Kenya, Ojijo (1993) made a review of some common aroma notes in coffee and their chemical origins. However, there is no report on the analysis of volatiles in different genotypes of Kenyan coffee. The objective of this study was to characterize volatile organic compounds of coffee brews of seven coffee genotypes comprising of two commercial varieties and five advanced breeding lines grown in Kenya.

2.0 Materials and Methods

2.1 Study Site

The coffee materials used in this study were obtained from Machakos Agricultural Training Centre (ATC) in Eastern Kenya. This site lies at latitude 1°31′S and longitude 37°16′E and has an altitude of 1600 Metres Above Sea Level. The area is semi-arid with mean annual rainfall of 750 mm and mean annual temperature of 20.9 ^{0}C. The soils are luvisols, well drained, moderately deep to deep, dark red to yellowish red, friable to firm, sandy clay often with a topsoil of loamy sand and are strongly leached soils. (Jaetzold *et al*, 2006).

2.2 Test Materials

Two commercial cultivars; SL28 and Ruiru 11 were assessed alongside five advanced breeding lines coded as Cross (Cr8), Cross 22 (Cr22), Cross 23 (Cr23), Cross 27 (Cr27) and Cross 30 (Cr30). The lines have been developed as individual tree selections from back-cross progenies involving SL4, N39, HDT and Rume Sudan as the donor varieties of disease resistance (CBD and CLR) and cultivars SL28, SL34, K7 as the recurrent parents.

2.3 Experimental layout

The coffee genotypes evaluated in this study were established in a Randomized Complete Block Design (RCBD) with three replications at Machakos ATC in 2007.

2.3.1 *Processing of the samples*

Ripe coffee berries were harvested from a sample size of 20 trees during the peak period in 2011. The cherries were bulked and wet processed using standard recommended procedures (Mburu, 2004). The cherry samples were pulped, fermented, washed and dried to a final moisture content of 10.5 to 11%. The parchment was then hulled and graded to seven grades based on size, shape and density. Grade AB was used as a representative grade for the characterization of volatile compounds.

2.3.2 *Roasting green coffee and brew preparation*

Roasting of the green coffee was done to attain a medium roast level using laboratory roaster (Probat BRZ 4, Rhein, Germany), within 24 hour of evaluation and allowed to rest for at least eight hours. The coffee brew was prepared as described by Lingle (2001). Samples were weighed out to the predetermined ratio of 8.25g per 150 ml of water. Each coffee genotype's batch was ground individually using a sample grinder (Probat vtv-633T, Rhein, Germany) for roasted coffee into the cup. Boiled deionised water was gently added to the cup taking care not to spill over while filling the cup. The brewed coffee was allowed to cool to room temperature (22-24^{0}C), filtered under vacuum through a Whattman filter paper (No. 42) and extracted immediately with C18 (reverse phase) Solid Phase Extraction cartridges.

2.4 Solid Phase Extraction Method Development and Optimization

2.4.1 *Solvent Choice determination*

Ten (10) ml of brewed coffee was each passed through two preconditioned 1000mg/6ml strata C18-E SPE (Phenomenex) cartridges at a flow rate of approximately 2ml/min in a vacuum manifold. Ten (10) ml of distilled water were ran through to wash away sugars and any other interfering matrices. The cartridges were dried by increasing the pressure in the manifold to 60 bars and later blowing a stream of nitrogen at high pressure. One cartridge was eluted with 10ml of Dichloromethane (DCM) while the other was eluted with 10ml hexane at a flow rate of 1ml/min followed by further pre-concentration to 1ml under a stream of nitrogen gas in a water bath at room temperature. Both eluents were injected into the GC-MS to determine the solvent that eluted a higher number of compounds.

2.4.2 *Sample volume optimization*

Brewed coffee volumes of 10, 20, 30, 40 and 50 ml (previously prepared) were passed through pre-conditioned 1000mg/6ml strata C18-E SPE (phenomenex) cartridges at a flow rate of approximately 2ml/min in a vacuum manifold. Ten (10) ml of distilled water was ran through each cartridge, dried and eluted with 10 ml of dichloromethane at a flow rate of 1ml/min followed by further pre-concentration to 1ml under a stream of nitrogen gas at room temperature. Prior to GC-MS analysis, the eluent obtained was spiked with 100µl of 400ppm of benzophenone (internal standard). The formula shown below was used to estimate the concentration of the various compounds present.

$$\mathbf{Concn_{ci} = (Concn_{is} \times PA_{ci}/PA_{is}) \times CF}$$

Where: $Concn_{ci}$ = Concentration of Compound of interest

$Concn_{is}$ = Concentration of internal standard

PA_{ci} = Peak area of compound of interest

PA_{is} = Peak area of compound of internal standard

CF = Concentration Factor

2.4.3 Chromatographic conditions

GC-MS analyses were performed in a Konic HRGC 400B Gas Chromatograph coupled to a Konic MSQ12 (Sant Cugat, Barcelona, Spain) quadrupole mass spectrometer. 1µl of each extracts were injected into the splitless mode in a TechnoKroma TRB5 (Cross-linked 5% Phenyl-95% Methyl Siloxane) capillary column (15m × 0.25mm i.d × 0.1µm film thickness). Helium was used as the carrier gas at a flow rate of 1ml/min. The injection temperature was maintained at 200^0 c, while the oven temperature was kept at 60^oc and programmed to rise at 4° c/min to 150^0 c and finally to 240^0 c at a rate of 6° c/min. Mass spectra were recorded in the Electron Ionization mode at 70 eV scanning from 35-450m/z range, the ion source and transfer line temperature were maintained at 200^oc and 250^oc respectively.

2.4.4 Compound Identification

Identification of the compounds in this study entirely relied on matching of the mass spectrometric fragmentation pattern corresponding to the various peaks in the samples total ion chromatogram with those present in the National Institute of Science and Technology (Gaithersburg , Maryland, USA) mass spectral database. Library searches were done using the Automatic Mass Spectral Deconvolution and Identification **S**ystem (AMDIS). Integration was done automatically for the individual peaks. In determining the best library hit the match factors were taken into consideration. The minimum user set match factor was set at 50 units below that of the internal standard (benzophenone).

2.4.5 *Data Analysis*

Principal Component Analysis was carried out using the software XL-STAT 2011.

3.0 Results and Discussion

In the SPE method development and optimization step, dichloromethane was found to be the most appropriate eluting solvent as it eluted the highest number of compounds from the cartridge as shown in Table 1. The variations observed in the quantities eluted by both solvents could be attributed to the differences in elution power between the two solvents. These results agreed with Snyder’s empirical eluant strength parameter (**εof)** which arranges solvents in increasing elution strength) hexane has eluant strength of 0.01 while DCM is 0.42 this implies that as an elution solvent DCM is much more powerful than hexane (Snyder, 1978). In the determination of the optimum sample volume, concentration of the eight compounds (identified to be present in all volumes) varied with increasing sample volume as shown in Figure 1. The optimum sample throughput volume was 40ml.

Chromatographic analysis of the eluents obtained by solid phase extraction from the brewed coffee of the seven coffee genotypes enabled the identification of 18 different volatile compounds. Figure 2 shows a typical gas chromatogram of an SPE brew eluent. There were observable differences in chromatographic profiles obtained, typically a chromatogram exhibited from 11 to 17 peaks. Table 2 shows the compounds identified and their relative concentration as determined using benzophenone as the internal standard. Results of the principle component analyses (PCA) for the volatile organic compounds indicated that the first two Principal Components (PC) explained

32.53% and 25.72% (a total of 58.25%) of the total variation (Figure 2). Ruiru 11 was placed away from the other genotypes in PC1 and this could be attributed to the fact that it had a higher number of volatile organic compounds than the other genotypes. The PC clustering showed distinctive diversity of the genotypes based on the volatile organic compounds fingerprints of the brewed coffees.

During roasting of coffee, many substances are formed due to reactions at high temperatures. These can contribute to the taste and aroma. One of the substances formed is 5-methyl-2-furancarboxyaldehyde (HMF) and the concentration in commercially available roasted coffee is in the range of 0.3–1.9 mg/g (Murkovic and Bornik, 2007). This compound was found to be present in all the analysed coffee genotypes. 5-methyl-2-furancarboxyaldehyde has a spicy, candy and slightly caramel odor (Arctander, 1969). 4 ethyl catechol was found to be in six coffee genotypes. This compound has been found to be generated exclusively upon thermal breakdown of caffeic acid moieties. Similar compounds have been investigated such as catechol and are primarily formed by degradation of caffeoylquinic acids from both parts of the molecule, the caffeic acid and the quinic acid moiety, as well as from Maillard-type reactions from carbohydrates and amino acids (Muller, 2006).

The alcohol 2-methoxy-4-vinylphenol (4-vinylGuaiacol) was found to be present in all the seven coffee genotypes but in different concentrations. This compound has been found to be formed during the coffee roasting process. Ralph *et al* (2003) proposed two mechanisms for the formation of this compound which were based on two connected reaction channels. One channel, termed the "low activation energy" channel, consists of ester hydrolysis of 5-O-Ferulyquinic acid followed by decarboxylation of the ferulic acid to form 4-vinylguaiacol. The second "high activation energy" channel opens up once the beans have reached higher temperatures. It leads to formation of guaiacol, via oxidation of 4-vinylguaiacol, and subsequently to phenol and other phenolic volatile organic compounds. This compound (2-Methoxy-4-vinylphenol) is associated with a smoky/phenolic odour and has been found to be present in medium roast Arabica coffee blends from Colombia (Mayer *et al.,* 2000). Similary, 4-Ethylguaiacol has a smoky and burnt flavor (Winter *et al*., 1976). It has been found that when 5-methyl-2-furancarboxaldehyde and 4-vinylguaiacol, furfural and furfuryl formate appear in higher amounts, the overall quality of the Arabica coffee is increased (Ribeiro *et al.,* 2009).

Three different pyrazines were identified in the brewed coffee extracts. 2,6 dimethyl pyrazine was found to be present in all the coffee genotypes evaluated. Pyrolysis of amino acids, especially in the presence of carbohydrates, gives rise to pyrazines that contribute to the "roasted" aromas of various food products (Rowe, 1998) including coffee. Pyrazine derivatives are formed by Maillard reactions, Strecker degradation and pyrolysis of hydroxyl amino acids and are considered as natural perfuming of foods (Baltes and Bochmann, 1987). 2-furanmethanol acetate was found to be present in all the coffee genotypes, this compound has been found to be presented in roasted Brazilian coffee. It has also been found that when compounds such as 2-furanmethanol acetate, 3-methylthiophen,2-ethyl-3,6-dimethylpyrazine and 1-(2-furanyl)-2-butanone are more abundant, the overall quality of the product drops (Ribeiro *et al.,* 2009).

The volatile groups reported in this study (pyrazines, pyrrole, furans, alcohols, aldehyde, ketone and carboxylic acid) were very few compared to what has been reported in the literature (Grosch, 2001). Solid-Phase Micro-Extraction (SPME) is known as a simple rapid and sensitive sampling method for liquid and gaseous volatile samples (Akiyama *et al*., 2003). However these were not available during the analysis and hence the use of SPE followed by solvent elution of the volatile compounds in coffee brew. Nevertheless, evaluation of volatile organic compounds showed that some appreciable levels of the volatiles were obtained in coffee brew.

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Table 1 **:** Identity of the compounds used in the optimization study of the solid phase extraction

Compound	**Concentration in Hexane eluent**	**Concentration in Dichloromethane eluent**
2,6-dimethyl pyrazine	Nd	84.4
5-methyl-2-furancarboxyaldehyde	26.8	53.3
2-acetoxymethylfuran	9.37	17.2
2-acetylpyrrole	Nd	10.5
Maltol	Nd	25.6
2,6-dihydroxy acetophenone	20.6	60.3
4-hydroxy-2-methylacetophenone	8.8	11.4
4-ethyl catechol	Nd	12.5

Key: **nd-** Not detected

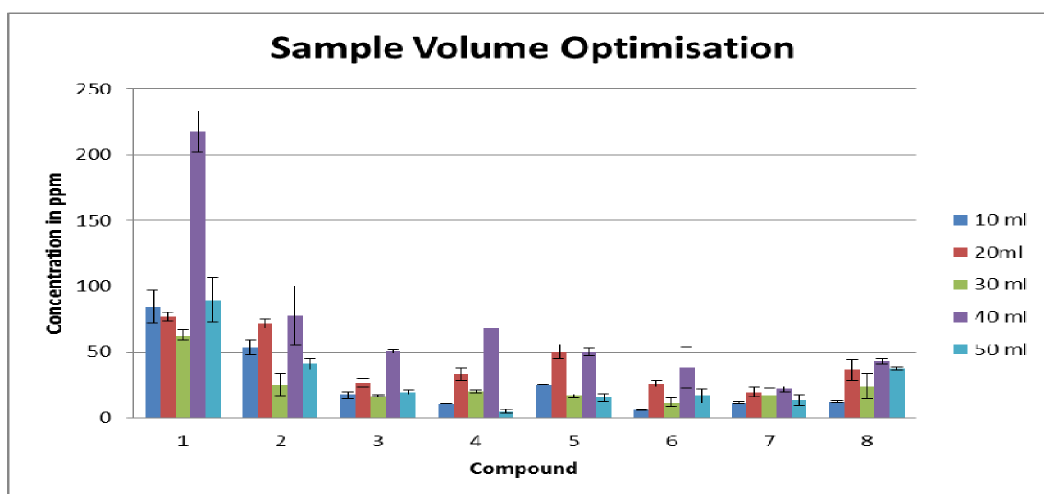

Figure 1: Comparison of concentration of compounds eluted with Dichloromethane with varying sample volume.

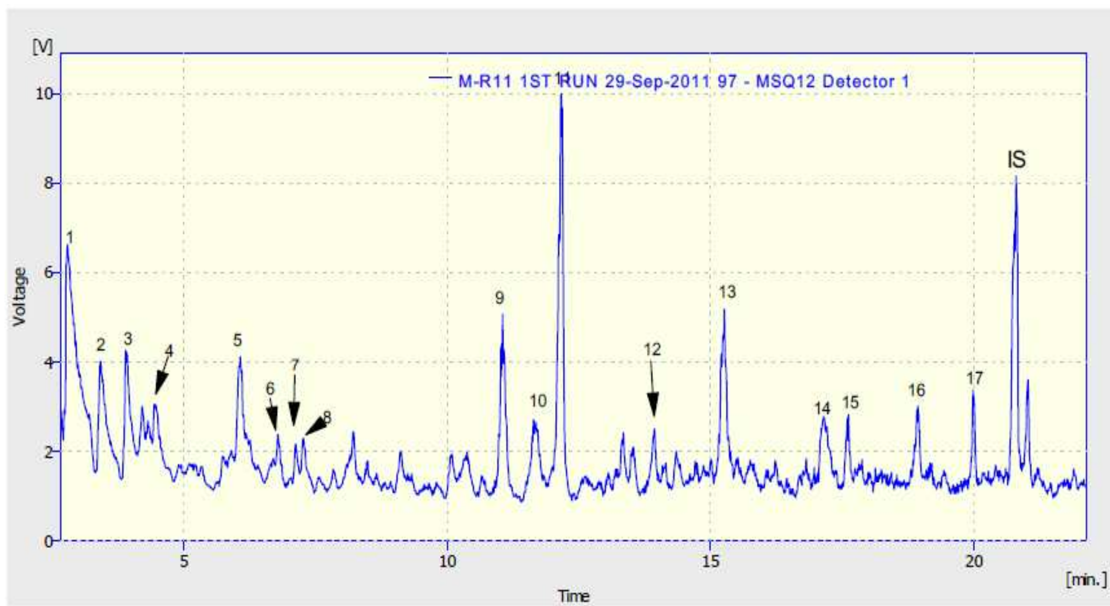

Figure 2: Typical gas chromatogram of SPE eluent of medium roasted Ruiru 11 brew

Table 2: Relative concentration of volatile organic compounds identified in SPE eluents of medium roasted coffee brews of different genotypes

Chemical compound	Cr30	Cr22	Cr23	Cr8	Ruiru 11	Cr27	SL28NS	Match factor
Pyrazines								
2-Ethyl-5-methylpyrazine	-	-	-	-	326.8	-	-	768
2,6-dimethyl pyrazine	1175.5	1147.9	868.5	1031.1	976.1	859.4	1571.5	785
2-Acetyl-3-methylpyrazine	-	-	-	-	480.5	443.0	665.9	793
Pyrroles								
1-methyl-1H-Pyrrole-2-carboxaldehyde	156.2	268.3	212.3	246.4	252.6	222.4	330.6	762
5-methyl-1H-pyrole-2-carboxyaldehyde	56. 9	90.4	86.6	140.0	82.0	102.4	107.0	826
Furans								
2-furanmethanol acetate	356.1	334.4	212.3	312.8	288.1	297.2	478.3	803
5-methyl-2-furancarboxyaldehyde	568.7	668.8	608.7	558.3	451.4	499.2	567.9	834
Alcohols								
Maltol	479.4	500.9	432.6	560.7	678.4	478.2	508.7	786
5-Isopropenyl-2-methyl-2-cyclohexen-1-ol	-	-	-	-	-	-	174.9	
4-Ethylcatechol	372.5	281.2	270.1	366.7	367.5	264.5	304.8	793
2-Methoxy-4-vinylphenol	527.4	409.5	413.2	487.0	600.7	425.3	383.0	932
Ketones								
2,6-Dihydroxyacetophenone	369.5	240.9	314.5	270.1	400.6	339.3	367. 6	797
Ionone	200.36	149.2	167.5	130.2	154.6	81.1	182.1	769
4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)-3-Buten-2-one,	183.0	172.8	-	-	145.0	-	191.8	774
3,4,5-Trimethyl-1H-pyrano[2,3-c]pyrazol-6-one	196.6	117.1	-	165.5	157.6	115.5	116.8	785
Aldehydes								
2-Hydroxy-4-methylbenzaldehyde		242.0						789
Carboxylic acids								
Oxiniacic Acid	-	-	-	-	-	-	235.5	793
Others								
1-[[(1,1-imethylethyl)imino]methyl]-Piperidine	105.0	-	-	-	-	115.9	-	
***Not identified**								
1	-	-	-	-	219.1	-	-	
2	-			11.9	71.5		-	
3	-	-	138.4	-	284.3			
4	182.8	217.1	164.5			214.6		
5			146.2					

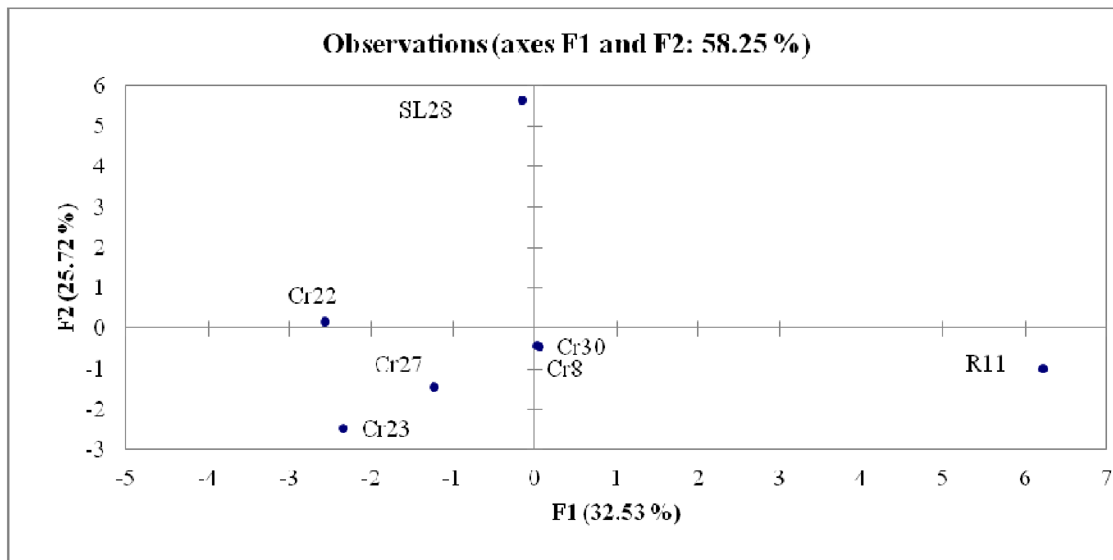

Figure 2 : Principal Component Analysis (PCA) clustering of the seven coffee genotypes as determined by the volatile organic compounds.

Key
R11-Ruiru 11

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