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LCMS Analysis of Biochemical Composition in Different Kenyan Coffee Classifications

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Abstract
Kenyan coffee is classified by defects after grading by the 'Devonshire method.' The method involves classification of the coffee beans into different classes based on the raw and roasted coffees and cup quality, with class one being the best and ten the poorest. In this study, the relationship between classification of the coffee and the content of sucrose, trigonelline, caffeine and chlorogenic acids was determined by simultaneous LC-MS analysis. By using the sensory variables the class 3 coffee portrayed the best quality, followed by class 4 and 5 which were placed further distinctively from the other classes (6, 7, 8 and 9). The class 3 coffee had a high percent of non-defective beans with 94.31% and class 9 had a high defect count at 79.53% hence the defective beans increased with decrease in coffee class. The caffeine concentration in green coffee for class 3 coffee was significantly different from the rest of the coffees at 1.23 ± 0.00g/100g. A higher content of trigonelline levels was observed as the quality of the green coffee decreased in classes 6, 7 and 8. The highest level of sucrose in green coffee beans was observed in class 3 coffee and the lowest in the class 9. Lower content of chlorogenic acids were observed among the high quality coffees i.e. class 3, 4 and 5 with class 3 having the lowest while a higher content was observed among the lower quality coffees i.e. classes 6, 7 and 9 with class 6 having a higher content was it significantly different in class 6 compared to 7 and 9?. Quantities of different chemical components among the classes are clear indicators that the classification method used on the Kenyan coffees brings out the differences in coffee quality based on the analysis of the green and the roasted coffees.
Keywords: biochemical composition, coffee classification, LCMS

1. Introduction

Kenya grows one of the most flavorful Arabica coffee that is classified under the top quality group called the Colombian milds (Ponte, 2002; Mureithi, 2008). Most of the Kenyan coffee is wet processed and this has been found to contribute to flavour improvement due to the production of microbial volatile compounds and metabolites which are precursors of flavour volatiles formed during the roasting process (Gonzalez-Rios *et al.,* 2007). After wet processing, the coffee is sun dried to a moisture content of 11% after which the dried parchment is removed in a dry mill releasing the green coffee beans (Clarke, 1985; Vincent, 1987). The green beans are graded according to the bean size and percentage of imperfections leading to seven major grades identified by letters as follows E, AA, AB, PB, C, TT and T(Ramalakshmi *et al.*, 2007). The graded coffees are then classified based on the degree of defects using the 'Devonshire method.' The method classifies the coffee beans into ten classes based on the raw, roast and cup quality, with one being the best and ten the poorest (Devonshire, 1935; Njoroge *et al.*, 1993; Kuri, 2000; Agwanda *et al.*, 2003). The coffee is described as fine, good, fair to good, fair average quality, about fair average quality, fair, poor to fair, poor and extremely poor. This classification system is mainly used by the millers in analyzing the coffees delivered to them by the farmers but it is hardly used by the coffee traders who have devised their own methods of assessing coffee quality. (FAO, 2006).

The quality of coffee is greatly associated with a set of factors that include physical-chemical and sensory aspects which in turn depend on the conditions under which the coffee was dried and stored (Coradi et al., 2007). The main families of chemicals associated with the volatiles in the roasted coffees are alkaloids namely trigonelline, lipids, chlorogenic acids and proteins in addition to other substances which are associated with the coffee roasting process (Kathurima *et al.*, 2012). These chemicals affect the final cup quality depending on their concentration in the coffee and may have a favorable effect on the beverage quality. The components interact in all stages of coffee processing producing a product which has great diversity as well as complexity of structure. (Gichimu *et al.*, 2014; Gimase *et al.*, 2014). Caffeine contributes to the bitter taste of coffee flavour and is responsible for not more than 10% of the perceived bitterness of the coffee beverage though it has been found to influence the perceived strength, bitterness and body of a brewed cup (Clarke, 1985; Farah *et al.*, 2006; Farah, 2012). Trigonelline content is most important due to its contribution to overall aroma perception of both roasted coffee and brewed coffee beverage and nutrition (Gimase *et al.*, 2014; Sunarharum *et al.*, 2014). Sucrose occurs in high concentration in coffee and acts as aroma precursor during coffee roasting, leading to formation of substances such as furans, aldehydes and carboxylic acids that affect both the flavor and aroma of the coffee (Farah *et al.*, 2006). Chlorogenic acids on the other hand contribute to the final acidity of the coffee as well as formation of phenols derivatives and other lactones

which are responsible for aroma and flavour hence may be used in determination of quality and acceptance of coffee (Gichimu et al., 2014). The main chlorogenic acid sub groups are caffeoylquinic acids, feruloylquinic acids and dicaffeoylquinic acids (Farah *et al.*, 2006). According to Farah, caffeoylquinic acids account for about 80% of the total chlorogenic acids, with 5-caffeolyquinic acid taking a higher content of 60% hence it is the most studied isomer representing chlorogenic acids.

Much research has been done on chemical composition of green and roasted coffee beans and its influence on cup quality (Franca *et al.*, 2005; Farah *et al.*, 2006; Kathurima *et al.*, 2010; Tessema *et al.*, 2011; Gichimu *et al.*, 2014; Gimase *et al.*, 2014). The biochemical components and sensory attributes have been used to discriminate between related hybrids of Arabica coffee in three different geographical regions in Kenya (Kathurima *et al.*, 2010). While in Brazil, a possible correlation between the cup quality and the content of the most important compounds in coffee namely sucrose, caffeine, trigonelline and chlorogenic acids was reported (Farah *et al.*, 2006). Similarly, the relationship between biochemical composition and cup quality has also been carried out among different Ruiru 11 sibs grown in varying environments in Kenya (Gichimu *et al.*, 2014), where significant difference were reported among the four biochemical components namely trigonelline, caffeine, lipids and chlorogenic acids across the different locations and seasons.

LC/MS is a powerful analytical technique for quantitative analysis especially due to its inherent selectivity and sensitivity (Chambers et al., 2007; Van Eeckhaut et al., 2009). Liquid chromatography coupled by an atmospheric pressure ionization (API) source to tandem mass spectrometric (MS/MS) detection has been considered as the method currently specific for quantitative analysis of compounds in biological matrices. The main advantage of using detection by MS/MS in the selected reaction monitoring (SRM) mode are the increased specificity, sensitivity and throughput. Studies on biochemical components and sensory attributes to discriminate between related hybrids of Arabica coffee in three different geographical regions in Kenya and an investigation on the relationship between biochemical composition and cup quality among different Ruiru 11 sibs grown in varying environments in Kenya have been undertaken. However, the link between such studies on biochemical composition and the classifications of coffee in Kenya is lacking. Hence in this research, a relationship between the classification of the Kenyan coffee and the biochemical components was studied.

2. Materials and methods

2.1 Standards and chemicals

Trigonelline, sucrose, caffeine and chlorogenic acids were purchased from Sigma Aldrich (Munich, Germany). All solvents were HPLC grade from Carl Roth (Karlsruhe, Germany). HPLC grade water was used throughout the experiments from Carl Roth (Karlsruhe Germany).

2.2 Samples

Coffee samples were randomly sampled from the Nairobi Coffee Exchange (NCE) sample room as raw coffee beans of grade AB in four consecutive sales. The NCE offers coffees from all over the country and it is sold based on quality and no consideration on the variety. The samples were classified by six professional cuppers and grouped into seven trade categories namely: fair average quality (class 3), about fair average quality (class 4), fair (class 5), about fair (class 6), poor to fair (class 7), poor (class 8) and extremely poor (class 9) coffees as described by the Devonshire method.

2.3 Water content

To express the amount of trigonelline, caffeine, nicotinic acid and sucrose on a dry weight basis (dwb), the water content of all ground coffee samples was determined according to the AOAC method (AOAC, 2005).

2.4 Extraction of caffeine, trigonelline & sucrose

The samples were extracted and purified according to a modified method of Ky et al. (2001). A sample of 5g of green coffee beans were frozen at -80 C overnight before grinding to (<0.5 mm particle size). In a 100 ml round bottomed flask, 100mg of the sample was added together with 100mg Magnesium Oxide and 30ml of distilled water. The mixture was refluxed for 25 minutes, cooled and vacuum filtered using a filter paper (Whatman No. 42). The filtrate was transferred to a 50 ml volumetric flask and topped up to the mark with distilled water. A sample 50µl was transferred to 950µl of LCMS grade water and then filtered with centrifuge filters (750µl, PTFE 0.2, 100/PK, United States) before injecting 3µl to the LCMS.

2.5 Extraction of chlorogenic Acid (CGA)

Chlorogenic acid was extracted from the green coffee using a modified method by (Ky et al., 1997; Ky et al., 1999). A sample of 50mg green coffee beans frozen at -80°C overnight were ground (<0.5 mm particle size). In a 50 ml beaker, the sample was mixed with 25 ml of 80% methanol and sonicated for 20 minutes. The mixture was vacuum filtered using a filter paper (Whatman No. 42) and then evaporated to dryness by a rotary vacuum

evaporator. The filtrate was reconstitute with 25ml distilled water and 100µl of it was transferred to 900µl of LCMS grade water and then filtered with centrifuge filters (750µl, PTFE 0.2, 100/PK, United States) before injecting 3µl to the LCMS.

2.6 Coffee Roasting

The green coffee beans (100g) were roasted using the Genes Cafe CBR-101 roaster. The roaster was preheated to 200 C before introducing the coffee. Roasting was done for 10 minutes at a constant temperature of 240 C. The roasted coffee samples (80g) were degassed for 12h at -20 C prior to grinding (<0.5 mm particle size). The samples were then frozen at -80 C in plastic containers pending their use.

2.7 Extraction of trigonelline, sucrose, caffeine and chlorogenic acids from roasted coffees

Samples were extracted in triplicate according the method described by deMaria et al. (1996) where 0.2g of ground coffee was suspended in 50mL of boiling water and shaken at room temperature for 15min at 300rpm. The mixture was then vacuum filtered through a filter paper (Whatman No. 42). The final extract was diluted ten times and filtered with centrifuge filters (750µl, PTFE 0.2, 100/PK, United States) prior to injecting 3µl to the LCMS.

2.8 LCMS Analysis

An optimized multiple reaction monitoring (MRM) method was developed using ultra-fast liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). A UFLC system comprising of two LC-20ADxR pumps, a CTO-20AC column oven and an SIL-30AC auto sampler (Shimadzu Corporation, Japan) was interfaced with an LCMS-8040 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan) fitted with an electrospray ion source. Chromatographic separations were carried out using an EC 125/4 Nucleodur 100-5C column (5.0µm particle size, length of 125mm and internal diameter 4.0mm; Macherer-Nagel, Germany) maintained at a constant temperature of 40 C. Caffeine, trigonelline, sucrose and chlorogenic acid were eluted using a gradient elution by a mobile phase consisting of 0.1% aqueous formic acid (eluent A) and methanol (eluent B), delivered at a flow rate of 0.8 mL/min. Elution began with 100:0 (A:B) at 0 min, increasing linearly to 0:100 over 4 min, maintained 2 min at 0:100 and then decreased linearly to 100:0 in 1 min for a total run time of 7 min. The instrument was operated in the positive electrospray ionization mode. Multiple reaction monitoring mode was used for the quantification. The selected transitions were m/z 194.9 → 138.0 for caffeine (positive ion), 355.1 → 163.0 for trigonelline (positive ion), 365.1 → 203.0 for sucrose (positive ion) and 137.7 → 92.1 for chlorogenic acid (positive ion). The electrospray parameters were as follows: interface temperature 250°C, gas flow 3.0 l/min, heat-block temperature 400°C, and drying gas flow 15 l/min. Identification of compounds of interest was performed by comparison with retention time and molecular weight of the respective standard. Data was acquired by LC-MS solution software (Shimadzu Corp., version 5.60 SP2) for the mass spectrometer.

2.9 Statistical analysis

Extraction of the samples were done in triplicates while the LC-MS measurements were done thrice on every replication. Data was evaluated using the statistical package for social scientist (SPSS version 20). Experimental design was a complete randomized design (CRD). Analysis of variance (ANOVA) was conducted, and the differences between group means analyzed using the least significant difference (LSD). Statistical significance was established at $p \leq 0.05$. Sensory analysis data was subjected to analysis of variance and multivariate analysis. Mean separation was done using Student-Newman-Keuls (SNK5%) test by Costat. Discriminant Function analysis was done using SPSS.

3. Results and Discussion

3.1 Discriminant function analysis of the sensory variables in coffee classifications

Discriminant function analysis defines the variables which discriminate between two or more naturally occurring groups and whether these groups contrast with regard to the mean of a variable to predict group membership. The sensory data generated from different coffee classifications was subjected to discriminant function analysis. The analysis revealed the variables (size of the bean, raw bean color, quality of green, type and center cut of the roast, acidity of the cup and flavour of the cup) contributed significantly to the discrimination.

Using sensory variables the class 3 coffee portrayed the best quality, followed by class 4 and 5 and were placed further distinctively from the other classes (6,7,8 and 9) (Fig 1). The factors which contributed significantly ($p \leq 0.001$) to the discrimination were quality of the roast (100.0), quality of the green (84.33), quality of the cup (53.68), acidity of the cup (19.9), colour of the green bean (12.14), size of the bean (11.5) and type and center cut of the roast (10.43). Body of the cup showed the least contribution to the discrimination (Table 1).

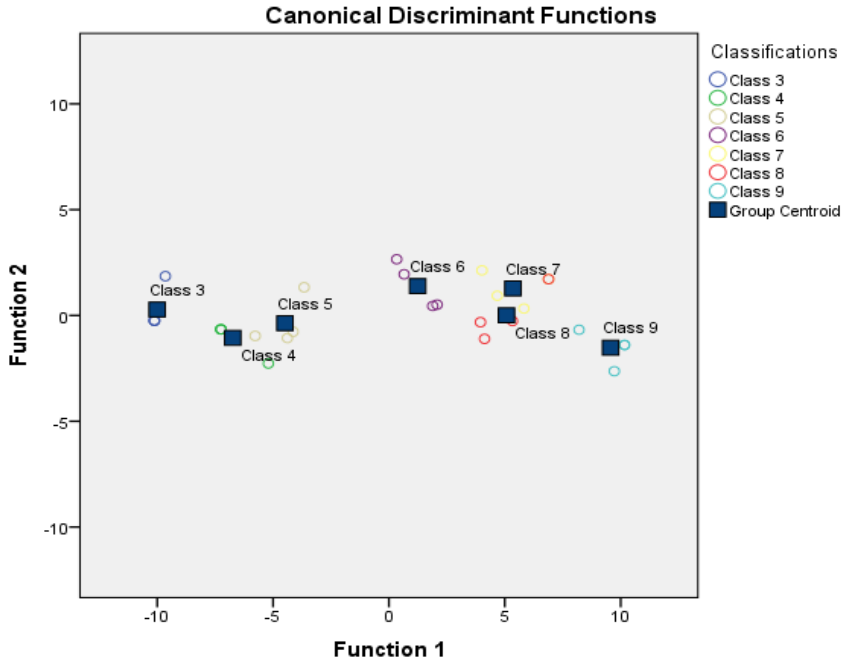

Figure 2: Discriminant factor analysis (DFA) based on sensory data in different coffee classifications

Table 1: Contribution of the specific quality variables to the discrimination

Tests of Equality of Group Means						
	Sensory variables	**Wilks' Lambda**	**F**	**df1**	**df2**	**Sig.**
Green Beans	Size	.233	11.500	6	21	.000
	Colour	.224	12.137	6	21	.000
	Quality	.040	84.333	6	21	.000
Roasted Beans	Type	.251	10.429	6	21	.000
	Color of Center cut	.251	10.429	6	21	.000
	Quality	.034	100.000	6	21	.000
Cup	Acidity	.150	19.900	6	21	.000
	Body	.513	3.324	6	21	.018
	Quality	.061	53.680	6	21	.000

3.2 Distribution of defective and non-defective beans

Average results for the distribution of defective and non-defective beans in the classified coffee samples are shown in table 2. The class three (3) coffee had a high percent of non-defective beans with 94.31% and class nine (9) had a high defect count with a 79.53%. The defective beans increased with decrease in cup quality as reported by Farah et al. (2006). The non-defective percentage for class four (4) and five (5) coffees was the same at 87.21 and 87.16 respectively, but the class 5 had a higher percentage of sour, pulper damaged, diseased and shell as compared to class 4 which had a higher quantity of insect damaged and amber beans. Amber beans have been reported to affect the acidity of the cup slightly and hence their large quantity in class four (4) coffee have a lower effect to the quality when compared to the defects of class five (5) (International Coffee Organization, 2006). The percentage of pulper damaged beans, shells, diseased beans and sour beans increased with decrease in quality from class 3 to 9. The class seven (7), eight (8) and nine (9) coffees also had a higher percentage of blacks which is lacking in the higher quality coffees. The black beans are considered as serious defects as they affect the quality by conferring a heavy negative flavour to the beverage (Mazzafera, 1999). Sour beans are associated with poor fermentation of beans and have been reported to play a role in downgrading coffee flavour (Mazzafera, 1999; Vasconcelos *et al.*, 2007) class 3 coffees had the lowest with 1.55% while the class 9 had the highest with 23.05%. The higher quality coffees that is class 3, 4 and 5 had lower percentages of the sour beans as compared to lower quality coffees of class 6, 7, 8 and 9 which had a higher percentage indicating a positive correlation with classification. The prevailing defect in all samples consisted of sour beans, pulper damaged, diseased, insect damaged and amber beans this could be an indication that their percentages could present a positive correlation with classification. These are the most common types of defects which are a consequence of problems occurring during harvesting and preprocessing operations (Vasconcelos *et al.*, 2007). Immature beans are high in lower quality coffees namely class 7, 8 and 9 they are those that come from immature fruits and have been reported to contributing to beverage

astringency hence negatively affecting the coffee flavour but them alone cannot be used in prediction of flavour quality in coffees (Vasconcelos *et al.*, 2007).

Table 2: Distribution of non-defective and defective beans in the coffee samples

Classifications	Non-Defective (%)	Defective beans (%)							
		Amber	Sour	Insect Damaged	Diseased	Shells	Pulper damaged	Immature	Blacks
Class 3	94.31	1.86	1.55	0.74	0.57	0.97	-	-	-
Class 4	87.21	2.02	2.57	2.08	3.27	1.29	1.46	0.10	-
Class 5	87.16	0.82	2.91	0.23	3.77	2.24	2.76	0.10	-
Class 6	59.21	1.00	17.64	2.79	6.10	10.32	2.74	-	0.19
Class 7	48.8	4.24	16.56	1.53	2.63	16.81	3.00	3.95	2.47
Class 8	43.56	3.88	14.58	1.64	10.40	16.05	3.76	2.68	3.44
Class 9	20.47	-	23.05	-	0.94	35.59	-	1.59	18.36

3.3 Chromatographic separation

Caffeine, trigonelline and sucrose were analysed from a single extraction by water while chlorogenic acids were extracted from the samples by methanol. Fig. 2 shows a typical chromatogram for separation of sucrose, trigonelline, caffeine and chlorogenic acids.

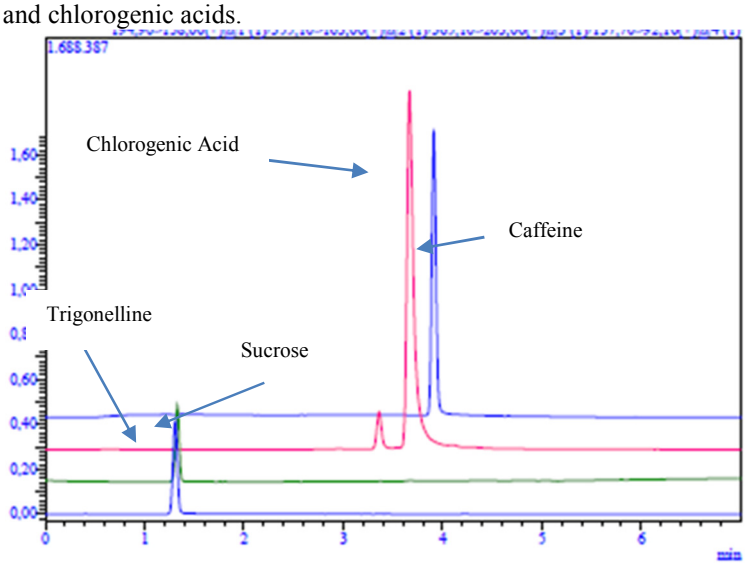

Figure 3: Typical chromatographic separation of sucrose, trigonelline, caffeine and chlorogenic acids

Quantification of the sucrose, trigonelline and caffeine in green coffees was done using the areas of chromatograms obtained from water extraction of coffee samples while quantification of CGA was done using the areas of chromatograms obtained from methanol extraction of the green coffee samples. Quantification of the analytes in the roasted coffees was done using the areas of chromatograms obtained from water extraction.

3.4 Caffeine

The caffeine content in the green beans among the classifications showed a small but significant difference. The caffeine concentration in class 3 coffee was significantly different from the rest of the coffees at 1.23 ± 0.00g/100g (Table 3). The highest content was observed in class 3 coffee and the lowest was observed in class 8 and class 9 coffees at 1.17 ± 0.01 g/100g. There was a slight negative correlation between coffee classification and the concentration of caffeine in the coffee beans r= -0.488, n=63, p=0.001. Higher content of caffeine has been reported in coffees of higher quality by Franca *et al.* (2005) as observed in the case of class 3. The observed values of caffeine content in the samples are within those reported in Arabica coffee with a range between 0.9 and 1.3g/100g (Ky *et al.*, 2001; Franca *et al.*, 2005; Perrone *et al.*, 2008; Farah, 2012; Gichimu *et al.*, 2014). In the roasted beans, there was no significance difference observed among the coffee classifications, with an average of 0.77g/100g (table 3). A 36.70% average loss of caffeine was noted in the roasted coffees which is slightly above the reported values of 30% loss Franca *et al.* (2005) which is attributed to loss of water vapour released during coffee roasting. The solubility of caffeine increased with increasing roasting temperatures.

Table 3: Bio-chemicals in green coffee beans

Classification	Sucrose	Trigonelline	Caffeine	Chlorogenic Acid
Class 3	9.22±0.15^{d}	1.22±0.01^{a}	1.23±0.00^{d}	4.01±0.12^{a}
Class 4	8.75±0.33^{d}	1.27±0.02^{ab}	1.19±0.01^{abc}	4.18±0.18^{a}
Class 5	8.80±0.06^{d}	1.24±0.02^{ab}	1.20±0.01^{bc}	4.77±0.15^{b}
Class 6	9.21±0.17^{d}	1.32±0.01^{c}	1.19±0.01^{abc}	5.49±0.17^{c}
Class 7	8.06±0.05^{c}	1.39±0.02^{d}	1.20±0.00^{c}	5.03±0.19^{bc}
Class 8	7.52±0.12^{b}	1.29±0.01^{bc}	1.17±0.01^{a}	5.46±0.14^{c}
Class 9	4.58±0.14^{a}	1.23±0.02^{a}	1.17±0.01^{ab}	5.12±0.06^{bc}

[1]values are means (± SE) of triplicate determinations.
[2]means designated by different small letters in a column for each compound are significantly different at (P <0.05).

*3.5 **Trigonelline***

There were significant differences observed from the trigonelline content of green coffee beans in all the classifications. The lowest concentration was observed in both class nine (9) and class three (3) coffees at 1.22 ± 0.01 g/100g and 1.23 ± 0.02 g/100g respectively (Table 3). The highest concentration was observed in class seven (7) coffee at 1.39 ± 0.02 g/100g. Farah *et al.* (2006) has reported a higher content of trigonelline levels as the quality of the coffee worsened a similar case observed in classes 6, 7 and 8. The lower content of trigonelline in class 9 in this study cannot be explained although higher contents of trigonelline have been observed among poor quality Brazilian hard and soft coffee which could be attributed to the presence of defective beans in lower quality coffee (Franca *et al.*, 2005). There was no correlation observed between the trigonelline content in green coffee beans and the classification. A similar observation was reported by Franca *et al.* (2005) among Brazilian coffees where there were no correlation in trigonelline concentration with coffees of different qualities. The concentrations obtained are within the range of 1.22 – 1.39g/100g in Arabica coffee reported by Koskei *et al.* (2015). There were significant differences observed from the trigonelline content of roasted coffee beans in all classifications. A lower content of trigonelline in roasted coffee was observed in higher quality coffee with class 3 coffee having 0.59±0.01g/100g compared to the lower quality with class 8 and 9 coffee having 0.71±0.01 g/100g and 0.66±0.04g/100g respectively. Coffee roasting breaks down trigonelline into nicotinic acid (3%) and various volatile compounds grouped as pyrrols (3%), pyridines (46%), pyrazines and methyl nicotinate (Farah, 2012). The breakdown of trigonelline in various constituents contributing to aromas can be attributed to lower content in roasted coffees. The values reported from the roasted coffee are within the range reported by Farah and Donangelo (2006) of 0.6 to 2.0g/100g. The percentage loss of trigonelline observed from the roasted coffee was averaging at 49.69% which is slightly below the reported percentage of between 50-80%. (Trugo *et al.*, 1983; Franca *et al.*, 2005; Franca *et al.*, 2005)

Table 4: Bio-chemicals in roasted coffee beans

Classification	Sucrose	Trigonelline	Caffeine	Chlorogenic Acid
Class 3	0.18±0.00^{g}	0.59±0.01^{a}	0.77±0.01	1.61±0.06^{e}
Class 4	0.18±0.01^{f}	0.66±0.02^{c}	0.76±0.01	1.67±0.05^{f}
Class 5	0.16±0.00^{e}	0.62±0.01^{b}	0.78±0.02	1.40±0.03^{c}
Class 6	0.13±0.01^{d}	0.63±0.03^{b}	0.76±0.02	1.23±0.06^{b}
Class 7	0.12±0.00^{c}	0.62±0.04^{b}	0.76±0.04	1.18±0.08^{a}
Class 8	0.09±0.00^{b}	0.71±0.01^{d}	0.75±0.00	1.51±0.03^{d}
Class 9	0.07±0.01^{a}	0.66±0.03^{c}	0.76±0.00	1.22±0.03^{ab}

[1]values are means (± SE) of triplicate determinations.
[2]means designated by different small letters in a column for each compound are significantly different at (P <0.05).

*3.6 **Sucrose***

The sucrose level observed in green coffee beans were significantly different among the different classifications. The highest level was observed in class 3 coffee (9.22 ± 0.15 g/100 g) and the lowest in the class 9 coffee (4.58 ± 0.14 g/100 g) (Table 3). Farah *et al.* (2006) has reported similar observations in Brazilian coffees at 7.85±0.26 g/100g for the highest quality and 4.88±0.10g/100g for the lowest quality coffee. Higher contents of sucrose in coffees are one of the reasons for the superior aroma and the overall flavor of Arabica coffees (Farah, 2012). A negative correlation was observed between the coffee classification and the concentration of sucrose in the coffee beans r= -0.77, n=63, p=0.01. The values were within the range reported by Farah (2012) averaging between 6.0 and 9.0g/100g in Arabica coffee with the exception of class 9 coffee. The lower concentration of sucrose in the lower quality coffees can be attributed to a higher percent of defective beans with class 9 having the highest with 79.53%. Mazzafera (1999) reported lower sucrose levels in defective green beans in comparison with non-defective green beans. There were significant differences observed in sucrose levels of the roasted coffees among the different coffee classifications. The highest concentrations were found among class 3 and class 4 at 0.18±0.00

and 0.18±0.01 g/100g respectively. A decline in concentration of the sucrose content of roasted coffee at an average of 98.2% was observed which can be attributed to the caramelization reactions taking place during roasting. A similar loss was also reported by Farah *et al.* (2006). A negative correlation was observed between the coffee classification and the concentration of sucrose in the roasted coffee beans r= -0.97, n=65, p=0.01. With an average of 0.13g/100g, sucrose was slightly below the values between 15.9 and 183.8mg/100g reported by Perrone *et al.* (2008), which can be attributed to the roasting method and degree of roasting.

3.7 Chlorogenic acids (CGAs)

There were significant differences observed in the chlorogenic acids content in the green coffee among the different classifications. Lower content was observed among the high quality coffees i.e. class 3, 4 and 5 with class 3 having the lowest at 4.01±0.12g/100g. Higher content was observed among the lower quality coffees i.e. classes 6, 7 and 9 with class 6 having a higher content at 5.49±0.17g/100g(Table 3). A slight positive correlation between coffee classification and the concentration of chlorogenic acids in the coffee beans r=0.635, n=63, p=0.01 was observed. Farah *et al.* (2006) reported a higher content of chlorogenic acids for the lower quality coffee in Brazil at 7.02±0.17g/100g and the lowest for the best quality sample at 5.78±0.09g/100g. The higher content of chlorogenic acids in green beans of the lower quality can be attributed to the presence of defective beans such as immature and black beans (Farah *et al.*, 2006). CGAs are known to be important determinants of coffee flavour, as they contribute to acidity, astringency and bitterness to the beverage but a relationship between them and coffee cup quality is still unclear and controversial (Farah and Donangelo, 2006). There were significant differences obsereved in the CGAs content among the different classifications of the roasted beans. A higher content was obsereved among classes 3, 4, 5 and 8, with classes 6, 7 and 9 having lower contents. A negative correlation between coffee classification and the concentration of chlorogenic acids in the roasted coffee beans r=-0.615, n=63, p=0.01 was found with the highest content class 4 at 1.67±0.05g/100g followed by class 3 coffee at 1.61±0.06g/100g and class 8 coffee at 1.61±0.03g/100g(Table 4). There was a decline in concentration for the roasted beans averaging at 70.46% which is lower than 90% reported by Trugo and Macrae (1984) . The decline is due to destruction of chlorogenic acids during roasting with an approximately 8 to 10% being lost for every 1% of dry matter. At the early stages CGA is isomerised and partially hydrolpysed yielding quinic acid and a variety of cinnamic acids (Clifford, 2000).

4. Conclusion

Analysis of caffeine, trigonelline, sucrose and chlorogenic acid was done on Kenyan coffee classifications by liquid chromatography-mass spectrometry. Using sensory variables the class 3 coffee portrayed the best quality, followed by class 4 and 5 which were placed further distinctively from the other classes (6, 7, 8 and 9). The best quality coffee had a low defect count as compared to the low quality hence the defective beans increased with decrease in coffee classifications. The caffeine concentration in green coffee for class 3 coffee was significantly different from the rest of the coffees. A higher content of trigonelline levels was observed as the quality of the green coffee worsened. The highest level of sucrose was observed among the best quality coffees while lower contents of chlorogenic acids coffees were observed among the high quality coffees. Quantities of different chemical components among the classifications are clear indicators that the classification method used on the Kenyan coffees brings out the differences in coffee quality based on the analysis of the green and the roasted coffees.

5. Acknowledgement

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6. References

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