Effect of Altitude, Shade, and Processing Methods on the Quality and Biochemical Composition of Green Coffee Beans in Ethiopia

Adugnaw Mintesnot[1]* and Nigussie Dechassa[2]

[1]Jimma University, Department of Horticulture and Plant Science, P. O. Box 307, Jimma, Ethiopia
[2]Haramaya University, Department of Plant Sciences, P.O. Box 138, Dire Dawa, Ethiopia

Abstract: There are growing demands for high quality coffee in the international market today. This has given coffee producing countries an impetus to increase the quality as well as the quantity of coffee they produce. For improving coffee quality and meet market demands, attention has been given to exploring genetic and environmental factors as well as agronomic and other coffee management practices. However, little information is available in Ethiopia regarding effect of environmental factors such as altitude and coffee management practices such as shading and processing methods on the quality and biochemical composition of green coffee beans. This problem has constrained efforts being made in the country to further exploit the growing demands for quality coffees in the international market. Therefore, a study was conducted during the 2010/11 main cropping season to determine coffee quality attributes as a function of altitude, shade, and processing methods. Red ripe coffee cherries were handpicked from three coffee farms in south-western Ethiopia located at altitudes of 1150, 1545 and 1802 meters above sea level, which represented lowland, midland, and highland coffee growing areas, respectively. The coffee cherries were obtained from both shaded and unshaded farms in each of the aforementioned coffee growing areas. The green coffee beans were subjected to both wet and dry coffee processing methods. A total of 36 coded samples (18 washed and 18 unwashed green coffee beans) with a moisture content of about 10.5% were subjected to cup and laboratory tests. Coffee cup test was done according to the procedure of Ethiopian Commodity Exchange (ECX) using 36 green coffee bean samples. Contents of caffeine, trigonelline, and chlorogenic acids were determined using HPLC/THERMO. Sucrose was determined using GC VARIAN 3800. Univariate analysis of variance and stepwise multiple regression analyses were conducted using SPSS 16 v2. The results revealed that coffee beans originating from the high altitude had significantly higher first grade and Q1 grade points than coffee beans originating from the low and middle altitudes. Unshaded and unwashed coffee grade was better than the washed and shaded coffee grade. Caffeine content of the beans was affected neither by altitude, shading, nor by the processing method. Shading affected only the content of bean caffeoylferuloylquinic acids (CFQA), which was found to be significantly higher for the unshaded coffee bean samples than the shaded coffee bean samples. Contents of 4,5-dicaffeoylquinic acid (4,5-DCQA), feruloylquinic acids (FQA), and total chlorogenic acid (TCGA) were significantly higher for coffee beans originating from the low and middle altitudes than those originating from the high altitude. Similarly, coffee beans that originated from the low altitude had significantly higher contents of 3,4-dicaffeoylquinic acid (3,4-DCQA), caffeoylferuloylquinic acids (CFQA), and trigonelline than coffee beans that originated from the high altitude. However, coffee beans that originated from the high and middle altitudes had significantly higher contents of chlorogenic acids, i.e., 3-caffeoylquinic (3-CQA) and 5-caffeoylquinic acid (5-CQA) than coffee beans that originated from the low altitude. The sucrose content of coffee beans that originated from the low latitude was significantly higher than the sucrose content of coffee beans that originated from middle and high altitudes. Washed coffee beans contained significantly higher amounts of dicaffeoylquinic acid (4,5-DCQA), caffeoylquinic acid (5-CQA), chlorogenic acid (TCGA), and trigonelline than unwashed coffee beans. However, unwashed coffee beans had significantly higher 3-caffeoylquinic (3-CQA) and caffeoylferuloylquinic acids (CFQA) contents than washed coffee beans. Unwashed coffee beans had higher values for primary defect, secondary defect, odour, total-point and preliminary grade whilst washed coffee beans had better scores for acidity, body, and flavour attributes, which distinctly influence the ultimate taste profile of coffee origins. It is concluded that coffee bean quality attributes and contents of the associated chlorogenic acids improved in response to increased altitude under both shaded and unshaded conditions regardless of the type of processing method used. The results imply that growing coffee plants in the highlands and midlands rather than in the lowlands as well as washing the beans results in the production of coffee beans with high quality attributes and chlorogenic acid contents that could meet the rising international market demands for high cup quality.

Keywords: Chlorogenic acid; Final grade; High altitude; Low altitude; Mid altitude

1. Introduction

It is documented that Arabica coffee has evolved in Ethiopian montane high rainforests where live genetic diversity exists (Aerts *et al.*, 2012). This natural endowment helps Ethiopia to grow coffee in a wide range of agro-ecologies (Adugnaw, 2014; Tadesse, 2015).

*Corresponding Author. E-mail: adugnawmintesnot@gmail.com

ISSN 1993-8195 (Online), ISSN 1992-0407(Print)

Climate (temperature, light and water) and altitude, among others, are well known to play an important role through affecting temperature and availability of light and water in the ripening process of coffee cherries (Decazy *et al.* 2003). The distribution of sunshine has also been identified as being a strong influential factor on flowering, bean expansion, and ripening. In line with this, the effect of shade was studied where some findings (Vaast *et al.* 2005) revealed a decrease in coffee tree productivity in response to shading by about 20%, thereby reducing the alternate bearing pattern. The finding by Vaast *et al.* (2005) also revealed positive effects of shade on bean size and composition as well as beverage quality by delaying berry flesh ripening by up to one month. In addition, the presence of shade trees, especially leguminous species, is known to improve soil fertility and enhance coffee plantation sustainability (Soto-Pinto *et al.* 2000). In traditional coffee production systems (Adugnaw, 2014) with different levels of shade, however, berries ripen more slowly and yields are lower but green coffee beans and cup quality are superior (Pohlan and Janssens, nd).

The flavour and aroma of coffee are believed to be affected by the presence of various volatile and non-volatile chemical constituents such as proteins, amino acids, fatty acids, and phenolic compounds, and also by the action of enzymes on some of these components (Gichimu *et al*., 2014). The role of biochemical composition of green coffee bean on cup quality was also reported by various researchers (Ky *et al*., 2001; Yigzaw *et al*., 2007; Gichimu *et al*., 2014). A review by Alonso-Salces *et al.* (2009) revealed that all of the cinnamoyl derivatives play an important role in coffee quality, being responsible for its organoleptic properties. For instance, according to the aforementioned authors, the quality of the beverage increased as the chlorogenic acid (CGA) content decreased. Beverage bitterness is assumed to associate with caffeine content.

Higher elevations produce hard, dense beans (higher quality than soft beans) that are more sought-after than beans grown at lower elevations, because they have a higher concentration of sugars, which produce more desired and nuanced flavors (Vaast *et al*., 2006; Scott, 2015). Several factors contribute to the increased concentration of sugars in coffee grown at high elevations. Higher elevations impose harsh growing conditions like lower temperatures, bean maturation process thereby providing time for full physiological process and complex sugars to develop (Scott, 2015). Higher elevations are well drained which reduces the amount of water the coffee plants can take up, and, in turn, their cherries show high dry matter content (Scott, 2015). Moreover, as the rate of plant growth is slower at higher elevations the competition for growth factor remains low, and the pressure from pests and diseases is low. In fact, elevation does not just have a generic positive effect on a coffee's quality. It may vary from region to region and, certain general flavors are associated with different elevations. According to Scott (2015) for instance, at 762 meters coffee beverage will be soft, mild, simple, and bland; at 914 meters it will be sweet and smooth; at ~1,200 meters, it may have citrus, vanilla, chocolate, or nutty notes, and at ~1,500 meters, it might be spicy, floral, or fruity.

The wet or the dry method of coffee processing strongly influences and determines the quality of green coffees, thereby establishing characteristic flavour differences. Coffee beverages prepared from coffees obtained from the wet process are characterized by their full aroma and pleasant acidity while dry-processed coffees typically exhibit a so-called full body (Mazzafera and Robinson, 2000).

Guyot *et al.* (1996) reported small losses of caffeine (3%) during the soaking phase of the wet process as compared to the dry process. Chlorogenic acid subgroups or individual chlorogenic acids were also proved to be affected by processing (Guyot *et al.,* 1995; Leloup *et al.,* 2005). In other work, trigonelline was found to be reduced by wet treatment (Leloup *et al.* 2005). The composition of numerous metabolites, particularly that of amino acids (Bytof *et al.* 2005), carbohydrates (Knopp *et al.,* 2006), and chlorogenic acids (Balyaya and Clifford 1995; Guyot *et al.,* 1996;) differ significantly in wet and dry processed green coffees even if entirely identical starting material was used.

Green coffee seeds contain up to 14 % of chlorogenic acids (CGA) which have a marked influence in determining coffee quality and play an important role in the formation of coffee acidity and bitterness (Selmar *et al.* 2000). The main groups of CGA found in green coffee beans are caffeoylquinic acids; 3- Caffeoylquinic acid (CQA) (10% of total CGA), 4- CQA(10% of total CGA), 5-CQA (56-62 % of total CGA); 3,4-dicaffeoylquinic acids (diCQA); 3,5- dicaffeoylquinic acids; 4,5-dicaffeoylquinic acids) all about 15-20% of total CGA; feruloylquinic acids (5-13% of total CGA); caffeoylferuloyl- quinic acids (the remaining percentage) (Clifford, 2003). The relatively high levels of chlorogenic acids and related compounds in coffee seeds reflect their physiological importance for the coffee plant, as well as their significant contribution to aroma and flavour formation of coffee beverage. Genetic factors, the degree of maturation, and to some extent environmental conditions and agricultural practices, are important determinants of the composition of chlorogenic acids in green coffee beans, and will also affect the final beverage. High levels of chlorogenic acid (CGA) were found to associate with low quality coffees and vice-versa (Selmar *et al.,* 2000). A study revealed a disagreeable flavour to coffee beverage due to addition of diCQA, which disappeared on subsequent addition of CQA (Selmar *et al.,* 2000). Moreover, Silva (1999) found higher CGA content in lower quality samples. Farah *et al.* (2006) also observed a strong association between higher levels of CQA (accounts for 60% of CGA contents in roasted coffee) and FQA and low cup quality. Coffee cup quality is reported to relate directly to polyphenol oxidase activity, and 5-CQA levels in mature coffee fruits seem to be inversely associated with polyphenol oxidase activity in coffee beans (Silva, 1999).

Arabica coffees from certain regions of Ethiopia (e.g. Sidamo, Yirgacheffe, and Harar) fetch premium prices (Stanculescu *et al.,* 2011). There are other hitherto unknown regions in the country where 'fine specialty coffees' are produced that are highly rated on the market (Adugnaw *et al.,* 2015b). It is generally understandable that inherent coffee quality could be influenced by environmental and management factors (Gichimu *et al.,* 2014). The role of chemical soil properties has been studied (Adugnaw *et al.,* 2015a). The diversity of suitable varieties, soil type, climate, cultivation methods, among others, enables Ethiopia to produce diverse and unique quality coffees for local and world markets (Tadesse, 2015). To cope with the growing consumption and demand of high quality coffee, improvement and valorisation of coffee quality in the coffee chain provides a new impetus, resulting in the segmentation of the market with substantial premium prices in the world (Hilina, 2010; Adugnaw *et al.,* 2015b). This scenario has led coffee-producing countries to give much attention to genetic, environmental, and management factors to cope with the market demands (Hilina, 2010). However, no sufficient information is available on the effect of altitude, shade and processing methods as well as associations of coffee quality attributes with biochemical composition of green coffee beans in Ethiopia. Thus, the objective of this study was to elicit information on the effect of altitude, shade, and processing methods on coffee quality attributes and their associations with the biochemical composition of green coffee beans.

2. Materials and Methods

2.1. Site Selection

The study was conducted on coffee beans collected from three farms in south-western Ethiopia located at altitudes of 1150, 1545 and 1802 meters above sea level, which are designated as lowland, midland, and highland coffee growing areas in the country, respectively, (MoA, 2003). The coffee beans were collected from both shaded and unshaded coffee trees in all three farms. According to the National Meteorological Services Agency (2010), the study region is characterized by a mono-modal rainfall pattern of about 1565 mm, maximum and minimum temperatures of 26.1 and 13.2^{0}C, respectively, relative humidity of 73.3%, and sunshine hours of 5.4 (Table 1). The farms were purposely selected considering the altitudinal ranges and uniformity in management methods. The selected farms belonged to the Coffee Plantation Development Enterprise. Nine sub-coffee farms were selected with shaded plots and unshaded plots.

2.2. Experimental Procedures

2.2.1. Coffee Berry Collection

Ripened coffee cherries were hand-picked at their peak ripening stages during the 2010/11 cropping season out of which washed and unwashed green coffee beans were carefully prepared without contamination in three replicates.

Table 1. Location of study sites.

Farm	Region	Latitude	Longitude	Altitude (meters above sea level)
Bebeka (N= 3)	Benchmaji/ SNNPRS[1]	6^{0}56.580'N	35^{0}30.607'E	1150
Goma (N= 3)	Jimma/ Oromia	7^{0}55.253'	36^{0}37.069'	1545
Kossa (N= 3)	Jimma/ Oromia	7^{0}57.223'N	36^{0}52.664'E	1802

Note: [1]SNNPRS = Southern Nations, Nationalities, and Peoples Regional State

2.2.2. Laboratory Analysis

Cupping analysis: A total of 36 coded samples (18 washed and 18 unwashed green coffee beans) with a moisture content of about 10.5% were used for the experiment. The coffee samples were handed over to ECX (100 g each), Jimma Centre.

Raw quality analysis (40%): The weight of 100 green coffee beans (HBW) for each sample was measured using a sensitive balance. Then the entire green coffee bean sample weighing 100 g for each sample was used for raw evaluation test before roasting. Then, primary and secondary defects, shape-make, colour, and odour of the coffee samples were assessed according to the procedure used by the Ethiopian Commodity Exchange (ECX) (ECX, 2010). According to ECX (2010) one full defect of Primary Defect is equated with one Full Black, one Full Sour, one Pod/Cherry, two Large Stones, five Medium Stones, two Large Sticks, or five Medium Sticks. Moreover, one full defect of secondary defect is equated with any of the following: Parchment (2-3), Hull/Husk (2-3), Broken/Chipped (5), Insect Damage (2-5), Partial Black (2-3), Partial Sour (2-3), Floater (5), Shell (5), Small Stones (1), Small sticks (1), or Water Damage (2-5).

Roasting and brew preparation: A batch roaster equipped with a cooling system, in which air was forced through a perforated plate, capable of roasting up to 500 g green coffee beans, was used for roasting the coffee beans. The entire 100 g of each bean sample was used and the beans were carefully roasted at the temperature of 170 - 200 ^{0}C to a medium brown roast colour (7 – 8 minutes).

The roasted beans were ground to a medium level using Guatemala SB coffee grinder. Then, the powder was brewed. The water used for brewing contained 0.3 mmol to 1.2 mmol of calcium carbonate ($CaCO_3$), which was free from chlorine or other foreign flavour-affecting factors. Using the preheating graduating cylinder, 150 ml of boiled water (93 ^{0}C) was poured into a cup containing 12 g of roasted coffee powder and the infusion was allowed to steep for approximately 4 minutes to settle. The cup was then evaluated for its aroma. Then, the surface of the beverage was skimmed off to remove foams after which it was cooled down to a comfortable temperature (55 ^{0}C) for tasting (ISO, 2004).

Cup quality analysis (60%): A panel of three trained, experienced, and internationally certified (Q graders) cuppers took 6 to 8 cc of the brew from 5 cups using soup spoons and forcefully slurped it to spread evenly over the entire surface of the tongue and palate and then expectorated onto a spittoon. Cup cleanness, acidity, body, and flavour were evaluated as per the standard method (ECX, 2010). Finally, the preliminary grade assessment was made based on the sum of scores of the raw and cup quality analyses which gives the total point. According to the definition by ECX (2010) raw value is the sum of points of primary defect, secondary defect, shape-make, colour, and odour, while cup quality is the sum of points of cup defect (number of cup defects out of five cups tested), acidity, body, and flavour (coffee brew taste in the mouth which indicates the natural and specific coffee characteristics).

Chemical analysis: Green coffee beans were subjected to freeze drying just before grinding to fine powder using a hand-held electrical Blade coffee grinder (Bosch MKM 6003 UC, Bean Container Capacity: 75 g, Power: 180 Watt). Grinding was assumed to be sufficient when the powder escaped to the ceiling of the cap of the grinder, and the powder was immediately packed in a plastic cup with a tight stopper, and kept in a desiccator until the laboratory analyses were conducted.

Caffeine, Trigonelline and Chlorogenic acids: The caffeine, Trigonelline and chlorogenic acid contents were determined using HPLC/THERMO following the method of Alonso-Salces *et al.* (2009): 0.1 g freeze dried green coffee powder was weighed in an Erlenmeyer flask of 50 ml. 10 ml of MeOH/Acetic Acid (30:7.5:2.5) containing 2 mg/ml ascorbic acid was added and then placed in an ultrasonic bath for 15 minutes. The extract was filtered using Whatman filter papers No.2, and subsequently over a 0.45 micrometer PTFE filter after which 1 ml of the filtrate was taken in a vial and injected into the HPLC/THERMO. The standard solutions of chlorogenic acid, caffeine and caffeic acid were mixed each at 0.5/1/1.5 mg/ml in one mixture in methanol and each solution was injected twice for calibration. A calibration curve was made using the standard concentration and area of sample and subsequently used to calculate the composition of the respective biochemical components using the area generated after the retention time. The detection was carried out at 278 nm (caffeine and trigonelline), and 324 nm (CGA). For the identification and quantitative analysis, a standard curve was prepared using standards of caffeine, trigonelline, and chlorogenic acids.

Sucrose measurement: Sucrose of the coffee beans was determined using GC VARIAN 3800 following the standard method. A sample of green coffee powder was freeze-dried and weighed (0.5 - 1 g) in 50 ml volumetric flask to which 30 ml distilled water plus 5 ml frozen Internal Standard Solution (ISS) (Phenyl-B-D-pyranoside) were added. The sample was placed at 60 ^{0}C for 30 minutes after which it was cooled. Next, 0.5 ml each of carre I (15 g $ZnSO4$ and 7.5 g Carre II $(K4FClCN)_6$ were added to de-protein the sample. The distilled water was then filled to the label of the mark on the 250 volumetric flasks and shaken well to homogenize the mixture. The solution was immediately filtered with Whatman filter papers, and subsequently 1 ml filtrate was taken in small bottles using a glass Pasteur pipette and dried under nitrogen drier using hollow needles to let nitrogen into the bottle. To this dry extract was added 1 ml STOX (2.5 g hydroxylamine hydrochloride diluted with dry pyridine to 100 ml) under hood and kept at 60 ^{0}C for 30 minutes after which it was cooled. Then, 1 ml of HMDS (hexamethyldisilazan) was added and subsequently 0.1 ml TFA (trifluor acetic acid) was added before sedimentation for 60 minutes to get a clear extract solution. From the clear extract solution, 1 ml sample was taken in a vial with rubber stopper to inject to GC VARIAN 3800.

2.3. Statistical Analysis

Frequency distribution of the preliminary grade was determined as a function of altitude, shade and processing methods. Covariance analysis, Univariate Analysis of Variance and Tukey HSD method of mean separation were applied to separate significant mean differences. Moreover, Pearson correlation and stepwise multiple regression analyses were conducted to examine the relationship between coffee quality attributes and various potential predictors using SPSS 16 v2 software (IBM, 2007).

3. Results

3.1. Preliminary Coffee Quality Attributes

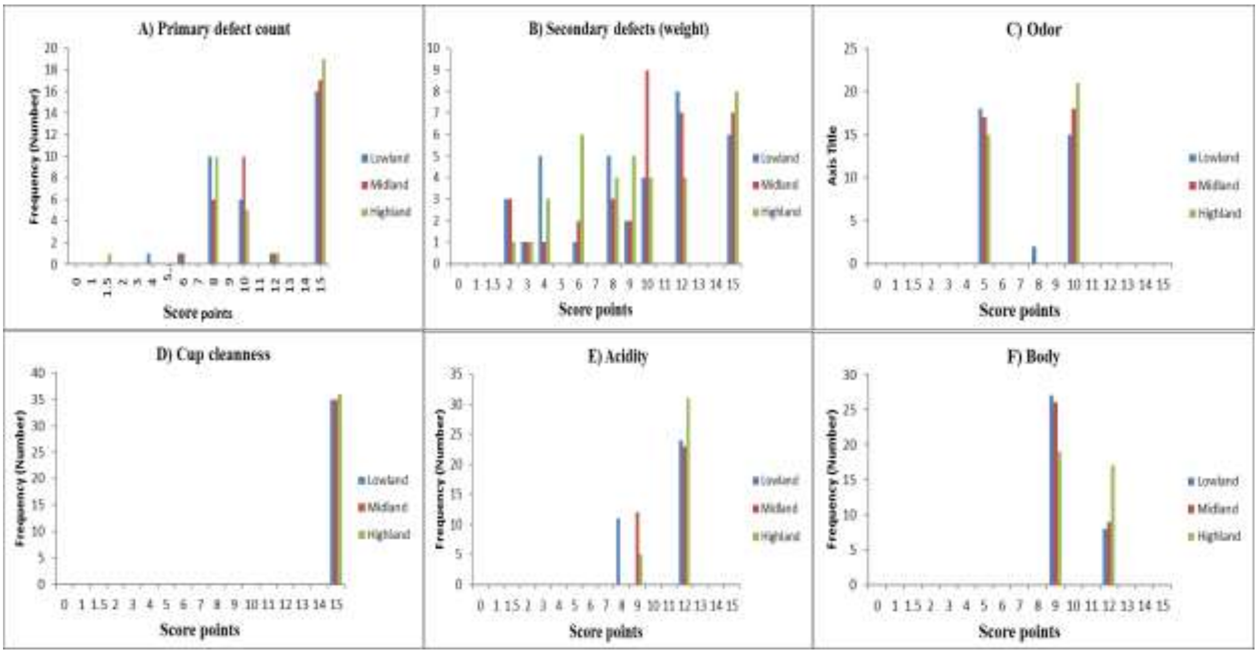
The preliminary coffee quality attributes are depicted in Table 2 and Figure 1A-G. The effect of processing methods was significant ($p \leq 0.05$) on primary defect, secondary defect, odour, total-point, and preliminary grade. With regard to primary defect, secondary defect, odour, total-point and preliminary grade, unwashed coffee had higher values than washed coffee. Although non-significant, washed coffee showed better score for acidity, body, and flavour attributes. Other results indicated no significant differences owing to altitude and shading on the attributes mentioned above. Although non-significant, subtle differences existed that may determine prices in the market. In line with this suggestion, with the exception of colour and secondary defect, and odour which were better at lowland and midland, respectively, the remaining attributes showed better score at highlands (Fig.1). As clearly depicted in Figure 1A-G, highland coffees dominantly scored higher values for preliminary coffee quality attributes. Colour and shape-make attributes were favoured under shaded condition whilst the remaining attributes, including secondary defect, primary defect, acidity, body, flavour, total-point, preliminary grade and specialty-total were favoured by unshaded condition.

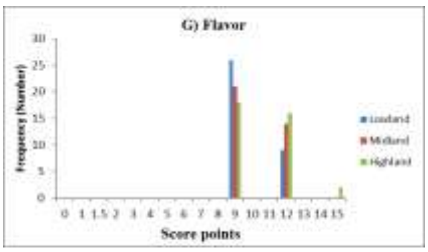
The combined analysis of altitude, shade and processing methods on preliminary coffee quality attributes revealed that none of the interactions was significant ($P > 0.05$). In fact, the subtle differences indicated that the highest total-point and preliminary grade were recorded for the highlands with shade and the least for the lowlands with shade. With regard to total-points and preliminary grade, washing favoured production of better coffee beans at the lowlands and midlands than not washing. However, the best unwashed coffees were obtained from the highlands. Both shaded and unshaded coffees had the highest quality score of preliminary grade under the unwashed method of preparation than under the washed method of preparation.

Table 2. Preliminary coffee quality attributes as affected by processing methods.

Sample lot	primary defect	secondary defect	Odour@	Acidity$^{\varepsilon}$	Body$^{\delta}$	Flavour	Total-Point	Preliminary Grade†	Specialty^{+}
Washed	8.3±0.3^{b}	4.9±0.8^{b}	5.0±0.1^{b}	11.5±0.3^{a}	10.2±0.4^{a}	10.5±0.4^{a}	75.8±1.6^{b}	2.9±0.2^{b}	81.5±0.9^{a}
Unwashed	14.8±0.3^{a}	11.6±0.9^{a}	9.8±0.1^{a}	10.8±0.3^{a}	9.9±0.4^{a}	10.3±0.4^{a}	82.4±1.6^{a}	2.3±0.2^{a}	80.0±0.9^{a}
P-value	<0.001	<0.001	<0.001	0.131	0.631	0.792	0.007	0.034	0.235
SD	3.5	4.7	2.5	1.4	1.4	1.8	7.5	0.8	3.8
CV%	30.8	57.6	33.6	12.5	14.5	17.5	9.4	29.3	4.7

Note: Means followed by the same letter within a column are not significantly different at 5% level of significance.
Preliminary grade is the ranking of the sum of raw and cup analysis points during preliminary assessment which helps to differentiate coffee that has potential for being sold as specialty coffee with other commercial coffees; $^{+}$Specialty coffee is distinctive because of its full cup taste and little to no defects and commands premium prices in the international coffee market; $^{\delta}$Body is the sensory perception of the mouth-feel of the brew. It is a combination of the fats, oils, and sediment swept off the surface of the freshly ground coffee particles and suspended in the unfiltered brew; $^{\varepsilon}$Acidity is the corporeal gustatory perception of the hydrogen ions present in the brew, an actual physical sensation on the tongue. @Odour is olfactory perception, olfactory sensation, smell, which is the sensation that results when olfactory receptors in the nose are stimulated by particular chemicals in gaseous form; Flavour is the perceived combination of aroma and taste, with the modulation of the basic tastes: sweet, sour, bitter, salty, achieving a distinctive cup characteristic. The aroma is experienced retro-nasally through the back of the palate as the coffee is aerated in the mouth while it is slurped (SCAA, 2004; ECX, 2010).





Note: The Altitudes
Lowland =1000 – 1550
Midland = 1551 – 1750
Highland = 1751 – 2150
Source: MoA, 2003

Figure 1. Frequency of preliminary coffee quality attributes as a function of altitude

The frequency of obtaining preliminary grade 1, 2, 3, 4, 5 and Q1, Q2, and commercial grade (3) was 2.8%, 41.7%, 44.4%, 5.6%, 2.8%, 8.3%, 41.7%, and 41.7%, respectively (Table 3). The high altitude contributed high number of first grade and Q1 grade point. Unshaded and unwashed coffee grade was better most likely owing to favourable climatic conditions prevailing in south-western Ethiopia. Unwashed coffee had superior quality attributes most likely due to better physiological development and uniform maturity of the fruit under optimal weather conditions

Table 3. Frequency of Preliminary and specialty grade by altitude, shade and processing methods in south-western Ethiopia.

Factor	Preliminary grade®					Specialty grade©			
	1	2	3	4	5	Q1	Q2	Commercial (C3)	Missing system
1150 m altitude (N=12)		5	6		1		3	8	1
1545 m altitude(N=12)		4	6		2		6	4	2
1802 m altitude (N=12)	2	6	4			3	6	3	
Shaded (N=6)	1	6	9	1	1			5	1
Unshaded(N=6)	1	9	7	1			3	3	
Washed (N=6)		6	9	2	1		2	3	1
Unwashed (N=6)	2	9	7				1	5	
Total (%) 97.2 (2.8 missing)	2.8	41.7	44.4	5.6	2.8	8.3	41.7	41.7	8.3

Note: ® Preliminary grade: According to ECX (2010) Grade 1 is the coffee that has a Total Value (Raw Value + Cup Quality value) of more than 85; Grade 2 between 75 -84; Grade 3 between 63 - 74; Grade 4 between 47 -62; and Grade 5 between 3 – 45 percent. ©Specialty Grade Green Coffee (Q1): According to SCAA (2004) specialty green coffee beans have no more than 5 full defects in 300 grams of coffee. No primary defects are allowed. A maximum of 5% above or below screen size indicated is tolerated. Specialty coffee must possess at least one distinctive attribute in the body, flavour, aroma, or acidity. It must be free of faults and taints. No Quakers (Unripened coffee beans, often with a wrinkled surface. Quakers do not darken well when roasted) are permitted. Moisture content is between 9-13%; Premium Coffee Grade (Q2): Premium coffee must have no more than 8 full defects in 300 grams. Primary defects are permitted. A maximum of 5% above or below screen size indicated is tolerated. It must possess at least one distinctive attribute in the body, flavour, aroma, or acidity. It must be free of faults and may contain only 3 Quakers. Moisture content is between 9-13%; Commercial Coffee Grade (C3): Commercial (Exchange grade) coffee must have no more than 9-23 full defects in 300 grams. It must be 50% by weight above screen size 15 with no more than 5% of screen size below 14. No cup faults are permitted and a maximum of 5 Quakers are allowed. Moisture content is between 9-13 % (SCAA, 2004; ECX, 2010).

The Variance Estimates of the Total-Point attribute indicated that on average, 7.1%, 2.3%, and 29.4% of the total variations were contributed by altitude, shade, and processing methods, respectively (Table 4).

Table 4 .Variance Estimates of Dependent Variables: Total-Point attributes.

Component	Estimate	Percent contributed for by
Var (Altitude)	4.825	7.1
Var (Shade)	1.554	2.3
Var (Processing methods)	19.919	29.4
Var (Error)	41.464	61.2
Total	67.762	100.0

Method: ANOVA (Type III Sum of Squares)

3.2. Biochemical composition of green coffee beans

The results of the biochemical compositions of green coffee beans are presented in Table 5.

Caffeine: The caffeine content of the coffee beans was unaffected by altitude, processing method, and shade.

3-Caffeoylquinic (3-CQA): The content of 3-CQA of the coffee beans was not affected by altitude and shade. However, it was significantly ($P < 0.05$) affected by coffee processing method. The content of this chemical composition in cofffee beans was found to be significantly higher for unwashed cofffe beans than washed coffee beans by about 11%.

4-Caffeoylquinic acid (4-CQA) and 5-Caffeoylquinic acid (5-CQA): The contents of 4-CQA and 5-CQA of the coffee beans were affacted by altitude, with significantly higher contents obtained for the high and middle altitudes than the low altitude. However, the contents of the two biochemical compositions were not affected by shade. On the other hand, coffee processing methods influenced the content of 5-CQA wheras it did not affect that of 4-CQA. Thus, the 5-CQA content of washed coffee was higher than that of unwashed coffee by about 3%.

Feruloylquinic acids (FQA): Altitude significantly ($P < 0.05$) influenced the coffee bean content of FQA, with significantly higher levels recorded for coffee beans originating from the low and middle altitudes than the high altitude. This means increasing the altitude at which coffee is grown reduces the FQA content of the bean. However, coffee processing method and shade did not affect this parameter.

3, 4-Dicaffeoylquinic acids (3,4-DCQA) and 4,5-Dicaffeoylquinic acid (4,5-DCQA): Altitude significantly ($P \leq 0.05$) influenced the contents of both 3, 4-DCQA and 4, 5-DCQA in the bean. But, shade did not affect the composition of both chemicals in the bean. Coffee beans originating from the low altitude areas contained significantly higher amounts of 3, 4-

DCQA than coffee beans that originated from the mid altitude and high altitude areas. Almost similarly, coffee beans that originated from low and mid altitude areas contained significantly higher amounts of 4, 5-DCQA than coffee beans that originated from the high altitude areas. The coffee bean content of 4, 5-DCQA was significantly influenced also by coffee processing method. Thus, washed coffee beans contained significantly higher amounts of 4, 5-DCQA than unwashed coffee beans. The 4, 5-DCQA content of washed coffee beans exceeded that of unwashed coffee beans by about 37%.

Caffeoylferuloylquinic acids (CFQA): The CFQA content of coffee beans was significantly ($P < 0.05$) affected by altitude, shade, and processing method. Thus, coffee beans that originated from the low and middle altitudes had significantly higher contents of CFQA than coffee beans that originated from the highlands. Unshaded coffee had significantly higher content of CFQA than shaded coffee. The content of CFQA in the bean obtained from coffee beans that originated from unshaded coffee trees was about 16% higher than the CFQA content of coffee beans that originated from shaded coffee trees. Unwahed coffee had significantly higher content of CFQA than shaded coffee. The amount of CFQA content obtained from unwashed coffee beans was about 46% significantly higher than that obtained from washed coffee beans.

Total chlorogenic acid (TCGA): The TCGA content of coffee beans was significantly ($P < 0.05$) influenced by altitude and processing method, but not by shade. The TCGA content of coffee beans originating from the low and middle altitude significantly exceeded the content of TCGA obtained from coffee beans originating from the high altitude by about 8%. Similarly, the TCGA content of washed coffee beans significantly exceeded that of unwashed coffee beans by about 3%.

Sucrose: The sucrose content of green coffee beans was significantly influenced by altitude. However, the content of this biochemical composition in the coffee bean was unaffected by both shade and processing method. The results clearly indicated that, increasing the altitude where coffee is grown consistently decreased sucrose content of the beans. Thus, the sucrose content of coffee beans originating from the low altitude areas was significantly higher than the sucrose contents of coffee beans that originated from middle altitude and high altitude areas by about 17% and 27%, respectively.

Trigonelline: The trigonelline content of coffee beans was significantly ($P \leq 0.05$) influenced by altitude and processing method. Similarly, this chemical composition of the bean was significantly influenced by processing method. However, shade did not influence the content of this chemical composition in the coffee bean. Increasing the altitude at which coffee is grown significantly decreased the trigonelline content of the bean. Thus, the trigonelline content of coffee beans originating from the low altitude areas significantly exceeded the trigonelline content of coffee beans obtained from coffee beans originating from the middle altitude and high altitude by about 2% and 7%. Similarly, the trigonelline content of washed coffee beans significantly exceeded that of unwashed coffee beans by about 2%.

Table 5. Effect of altitude, shade and processing methods on biochemical composition of green coffee beans in south-western Ethiopia.

Variables of study		Caffeine	3-CQA	4-CQA	5-CQA	FQA	3,4-DCQA	4,5-DCQA	CFQA	TCGA	Sucrose	Trigonelline
Altitude (m)	1150 (12)	13.6±0.2	3.9±0.2	5.7±0.1^{b}	27.2±0.3^{b}	4.5±0.2^{a}	4.1±0.2^{a}	8.1±0.3^{a}	4.0±0.1^{a}	57.4±0.6^{a}	58.8±2.2^{a}	87.6±0.3^{a}
	1545 (12)	14.4±0.2	4.0±0.2	6.4±0.1^{a}	28.9±0.3^{a}	4.8±0.2^{a}	2.9±0.2^{b}	7.1±0.3a	3.2±0.1^{b}	57.2±0.6^{a}	50.1±2.2^{b}	86.0±0.3^{b}
	1802 (12)	13.9±0.2	3.8±0.2	6.0±0.1^{a}	28.2±0.3^{a}	4.2±0.2^{b}	2.3±0.2^{b}	5.8±0.3b	3.1±0.1^{b}	53.2±0.6^{b}	46.2±2.2^{b}	81.6±0.3^{c}
	P-value	0.08	0.585	0.013	0.002	0.014	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Shade	Shaded	14.2±0.2	3.8±0.1	5.9±0.1	27.9±0.2	4.6±0.1	2.9±0.2	7.2±0.2	3.2±0.1^{b}	55.4±0.5	52.9±1.8	84.8±0.3
	Unshaded	13.7±0.2	4.1±0.1	6.1±0.1	28.2±0.2	4.4±0.1	3.2±0.2	6.8±0.2	3.7±0.1^{a}	56.5±0.5	50.5±1.8	85.3±0.3
	P-value	0.111	0.123	0.201	0.531	0.259	0.215	0.354	0.002	0.12	0.344	0.274
Processing	Washed	14.2±0.2	3.7±0.1^{b}	6.0±0.1	28.5±0.2^{a}	4.6±0.1	3.1±0.2	8.1±0.2^{a}	2.8±0.1^{b}	56.7±0.5^{a}	49.2±1.8	85.9±0.3^{a}
	Unwashed	13.7±0.2	4.1±0.1^{a}	6.0±0.1	27.6±0.2^{b}	4.3±0.1	3.1±0.2	5.9±0.2^{b}	4.1±0.1^{a}	55.2±0.5^{b}	54.2±1.8	84.2±0.3^{b}
	P-value	0.111	0.029	0.867	0.025	0.085	0.945	<0.001	<0.001	0.029	0.064	<0.001
	SD	0.9	0.6	0.5	1.5	0.6	1.0	1.7	1.0	3.1	11.0	3.9
	CV%	6.5	14.3	8.7	5.4	13.7	32.4	24.4	28.1	5.5	21.3	4.6

Note: Means followed by the same letter are not significantly different at 5% level of significance; 3-CQA = 3-Caffeoylquinic acid; 4-CQA = 4-Caffeoylquinic acid; 5-CQA = 5-Caffeoylquinic acid; FQA = Feruloylquinic acids; 3,4-DCQA = 3,4-Dicaffeoylquinic acids; 4,5-DCQA = 4,5-Dicaffeoylquinic acid; CFQA = Caffeoylferuloylquinic acids; TCGA = total chlorogenic acid.

3.3. Stepwise Multiple Regression

Stepwise multiple regressions were conducted to evaluate whether the biochemical compositions of the green coffee beans could predict preliminary coffee quality attributes. The regression analysis between biochemical compounds and preliminary coffee quality attributes (Tables 6 &7) showed significant contributions of the model to all attributes tested indicating significant positive regression weights. With regard to flavour, acidity, and shape-make, the predictors entered to first model included 4,5-DCQA, sucrose, and FQA, respectively (Table 6). The model contributed to about 16%, 14% and 11% of the total variations of flavour, acidity, and shape-make attributes, respectively. In this regard, the outcomes were negatively related to the respective predictors with the magnitude of 7.25%, 0.47%, and 5.85% of the variation in flavour, acidity, and shape-make accounted for by 4,5-DCQA, sucrose, and FQA, respectively (Table 6). With regard to odour attribute, three models (model 1, model 2 and model 3) contributed to about 44%, 67%, and 71% of the total variations, respectively. Among the predictors entered into the respective models, CFQA gave the best and positive prediction while 3,4-DCQA and 4,5-DCQA showed negative and respectively second and third most important prediction to the attribute. CFQA significantly affected odour attribute by 19.02% while 3,4-DCQA and 4,5-DCQA affected the attribute by 4.74% and 7.5% ($P \leq 0.001$). Regarding primary defect attributes, the model 1 & 2 showed significant contribution at the magnitude of 5.14% and 7.38%, respectively. In this regard, CFQA was found to be the best positive predictor followed by negative relation of 3, 4-DCQA with the magnitude of 21.19% and 10.63% of the variation of the outcome. Moreover, model 1($P \leq 0.002$) &2 ($P \leq 0.001$) had significant contributions to secondary defect attribute contributing 2.47% and 4.47% variation in the outcome. Positive effect of CFQA and negative effect of trigonelline showed about 31% and 6% of the variation of secondary defect attribute. The Total-Point attribute which determines the final grade for marketing was most negatively affected by FQA ($P \leq 0.013$) followed by 3, 4-DCQA ($P \leq 0.017$) predictors (Table 7). First and second models significantly ($p=0.001$ and $P \leq 0.001$) contributed for the variation of the outcome with the magnitude of about 2.6% and 3.8%, respectively. Thus, the final coffee grade could be most affected by increased level of the predictors in descending order of FQA and 3,4-DCQA composition of green coffee beans. This study did not reveal any association between the coffee quality attributes considered and biochemical compounds including caffeine, 3-CQA, 4-CQA, 5-CQA, and TCGA composition of green coffee beans.

Table 6. The model for stepwise regression of preliminary cup quality attributes on biochemical composition of green coffee beans in south-western Ethiopia.

Dependant variable	Model	R	R^2	Adjusted R^2	SE. of the Estimate	Model P-value (Regression)
Flavour	1	.397	.158	.133	1.701	0.016
Acidity	1	.379	.143	.118	1.273	0.023
Odour	1	.665	.442	.426	1.837	<0.001
	2	.812	.659	.639	1.457	<0.001
	3	.841	.708	.680	1.370	<0.001
Shape-make	1	.332	.110	.084	1.037	0.048
Primary defect	1	.717	.514	.500	2.506	<0.001
	2	.859	.738	.722	1.868	<0.001
Secondary defect	1	.497	.247	.225	4.045	0.002
	2	.692	.479	.447	3.416	<0.001
Total-point	1	.510	.260	.238	6.522	0.001
	2	.615	.378	.340	6.069	<0.001

Table 7. Regression of preliminary coffee qualities attributes on biochemical composition of green coffee beans in south-western Ethiopia.

Dependant variable	Variable in the model	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	T	Sig.
Flavour	4,5-DCQA	-.725	.287	-.397	-2.524	0.016
Acidity	sucrose	-.047	.020	-.379	-2.385	0.023
Odour	CFQA	1.902	.329	.755	5.780	≤0.001
	3,4-DCQA	-.474	.165	-.334	-2.871	0.007
	4,5-DCQA	-.749	.326	-.309	-2.301	0.028
Shape-make	FQA	-.585	.285	-.332	-2.053	0.048
Primary defect	CFQA	2.119	.344	.580	6.162	≤0.001
	3,4-DCQA	-1.063	.203	-.493	-5.231	≤0.001
Secondary defect	CFQA	3.066	.627	.642	4.892	≤0.001
	trigonelline	-.590	.154	-.503	-3.832	0.001
Total-point	FQA	-4.650	1.779	-.383	-2.614	.013
	3,4-DCQA	-1.602	.640	-.366	-2.503	.017

4. Discussion

The results indicated non-significant effect of altitude and shading on the studied coffee quality attributes. Consistent with the results of this study, findings by Avelino *et al.* (2005) in Costa Rica, Salvador at 1200 and 1300 meters above sea level also showed similar results where non-significant differences were observed between the varieties in either overall preference or body.

Effect of altitude: The general trend of attributes indicated that most highland samples scored better values than lowland and midland samples (Figure 1). As a result, high altitude contributed high number of first grade and Q1 grade point. Avelino *et al* (2005) also found a positive relation between altitude and taster preferences in Orosi and Santa Mar´ıa de Dota. Similar studies by Bertrand *et al.* (2006) found that samples originating from higher elevations (1350, 1400 and 1450 m) had higher beverage preference scores than samples from lower elevations (900, 1100, 1200 and 1300 m). The highest score values for highland coffees may be due to regulated temperature. The findings of Bertrand, *et al.* (2006), Guyot *et al.* (1996) and Rojas, (1985) are in agreement with this result in which elevation was explained by temperature. It is generally accepted that, in the tropical climate, an increase in elevation of 100 m decreases the mean daily temperature by 0.65–1^{0}C (Guyot *et al.*, 1996). It is understood that temperature plays an important role in the phenologic cycle of coffee, particularly on berry development and physiological maturity. Coffee berries grown at high altitude experience lower temperatures which lengthen the maturation period leading to higher accumulation of aroma precursors (Avelino *et al*, 2005).

Furthermore, at low altitudes where the climate is warmer than optimal for coffee, shade was found to improve physical quality and organoleptic attributes most likely owing to a reduction in heat-induced stress in the plant and a lengthening of the maturation period of coffee berries (Vaast and Bertrand, 2006).

This study showed that the mean value for 3,4-DCQA, 4,5-DCQA, CFQA, TCGA, sucrose, and trigonelline decreased in the order of lowland, midland and highland samples probably due to better physiological maturity at lower temperature which gives the beans longer time to complete physiological maturity (Avelino *et al.*, 2005)**.** Trigonelline is an important precursor of the volatile compounds that contribute to the aroma and taste of roasted coffee (Malta and Chagas, 2009). However, for caffeine, 4-CQA, and 5-CQA, the values increased in the order of middle altitude, high altitude, and low altitude coffees whereas for 3-CQA and FQA the increase was in the order of middle altitude, low latitude, and high altitude samples. Factors such as variety, soil, microclimate management practices may result in slight variations (Vaast and Bertrand, 2006). It is well documented that leaf to fruit ratios are higher at high elevations than at low elevations because leaf life span is longer and result in an increased carbohydrate supply to berries and higher bean fat synthesis. Furthermore, berry flesh ripening is delayed at the lower temperatures encountered at higher elevations, allowing longer and better bean filling (Vaast and Bertrand, 2006). In another study by Bertrand *et al.* (2006) elevation was not found to be a determining factor in explaining variation in caffeine and trigonelline concentrations of beans. However, an increase in chlorogenic acid concentration with increasing elevations was reported by Decazy *et al.* (2003) who also reported a trend of more chlorogenic acid accumulation at lower elevations and sharp increments in fat concentration with increasing elevation.

This study did not reveal any association between coffee quality attributes considered and biochemical compounds including caffeine, 3-CQA, 4-CQA, 5-CQA, and TCGA composition of green coffee beans. Studies by Bertrand *et al.* (2006) also discovered the same trend where caffeine, trigonelline, fat, sucrose and chlorogenic acid contents did not show good correlations with the sensory characteristics.

Most highland samples scored better and contributed high number of first grade and Q1 grades. As the final coffee grade was most affected by increased level of FQA and 3,4-DCQA biochemical compositions of green coffee beans, which in turn increased with altitude, these compounds could help as an alternate and complementary means to predict coffee quality. Thus, the findings imply that coffee cultivation in Ethiopia needs to be gradually shifted towards higher altitudes particularly in response to the climate change and accompanying rising temperatures for sustainable production and for meeting the growing demand for quality coffee in the international market to increase farmers' income and foreign exchange earnings of the country.

Effect of shade: Most attributes were favoured by absence of shade as compared to shading most probably due to less competition from shade trees and prevailing optimal conditions for the coffee trees to grow and develop. Several researchers discussed on both negative and positive influences of shade on coffee quality depending on the ecological variables. For instance, DaMatta (2004) found positive effects in sub-optimal locations whereas Soto-Pinto *et al.* (2000) found negative effects when shade density is above 50%. In other studies, Bosselmann, *et al.* (2009) found negative effects of shade on sensory attributes and positive effects of altitude on physical attributes.

Similarly, Vaast *et al.* (2005) reported that shade reduces yield by 20–30% under optimal conditions although that is compensated by higher quality beans and coffee prices. Unshaded and unwashed coffee grade was better most likely due to favourable climatic conditions prevailing in south-western Ethiopia and most likely due to better physiological development and uniform maturity of the fruit under such optimal weather conditions. Consistent with this finding, Bosselmann *et al.* (2009) revealed that fragrance, acidity, body, sweetness and preference were negatively influenced by shade cover. Previous studies (DaMatta, F.M., 2004) have shown that shade has a positive impact on coffee quality at lower elevations, implying that optimal agronomic shade management for coffee quality needs site-specific considering climatic and other environmental conditions. Under optimal growing conditions, the need for shade declines and this could be an opportunity to maintain high density coffee trees to increase yield per hectare (Bosselmann *et al.* 2009). Thus, agronomic shade management for coffee quality should be site-specific considering climatic and other environmental conditions.

Effect of processing methods: Two types of processing methods were applied including washed coffee (green coffee prepared by wet processing of the fruit) and unwashed coffee (green coffee prepared by dry processing of the fruit). Unwashed coffee had superior values for primary defect, secondary defect, odour, total-point and preliminary grade while washed coffee showed better score for acidity, body, and flavour attributes which have a distinguishing influence on the ultimate taste profile of coffee origins. The results are in agreement with that of of Boot (2011) which summarized that washed coffee tended to have clarity of flavour and aroma that is often lacking in natural coffees. Accordingly, Acidity comes through more clearly, and the cup is generally cleaner that can have an intensely refreshing character. Some studies indicated that processing is determinant of quality recording higher scores for wet-processed coffee than for dry-processed coffee (Selmar *et al.,* 2006). This was assumed to be due to the fact that wet-processed coffee had a higher percentage of ripe fruit harvested, while dry-processed had a mixed range of ripe fruits. However, taking samples of similar ripeness showed significant differences in the beans between washed and unwashed lots (Selmar *et al.*, 2006). Clarke and Macrae (1985) also noted that dry processed coffee has a better body due to the fact that the bean was in contact with its mucilage through a greater part of the processing phase, but wet processed Arabica tends to be aromatic with a fine acidity due to the formation of acids in under water fermentation, but some astringency. In line with this Gonzales Rios *et al.* (2006) found the importance of mucilage removal in water to obtain coffees with better aroma quality. The wet processed coffees were found pleasant with fruity aroma characteristics whereas those obtained by mechanical removal of mucilage were characterized by less pleasant aromatic notes. However, unwashed coffee showed higher value for 3-CQA and CFQA, washed had higher values for 5-CQA, 4,5-DCQA, TCGA and Trigonilline composition of green coffee beans. Furthermore, it was reported that the washed and unwashed methods played a role in complex metabolic processes of the beans during processing and drying due to effects on the sugars and flavor precursors present. Varying forms of variability in biochemical composition (lipid, chlorogenic acid, sugar and caffeine content) of green Arabica beans was found by Joet *et al.* (2010) where they reported a close correlation between the type of post harvest processing and the content of fructose and glucose in the coffee bean. Thus, a small amount of either hexos was present in washed coffee while in unwashed coffees they were significantly higher. It has been revealed that low levels of both fructose and glucose were associated with decrease in the wet process while dry processing did not influence much of Fructose and glucose levels. These metabolic processes may be related to germination, which starts to occur even when the period between harvest and final drying is short, and drying may cause stress metabolism that can also play a role in the chemical compounds present (Bytof *et al.*, 2005).

5. Conclusion

This study has demonstrated that both coffee quality attributes and contents of the associated chlorogenic acids, which markedly influence coffee quality and play an important role in the formation of coffee acidity and bitterness, generally improved in response to increased altitude under both shaded and unshaded conditions regardless of washing or not washing the beans. This implies that shifting the area of coffee production from lower to higher altitudes contributes to enhanced coffee quality attributes. This would enable better exploitation of the rising demands for quality coffees in the international market for enhancing foreign exchange earnings and income of coffee farmers in the country. Further research needs to be done to optimize the altitude at which to grow the plant for attaining the best quality coffee attributes desired by the international market.

6. Acknowledgements

The authors are grateful to the IUC-JU Soil Fertility Project for funding the research. We are especially thankful for the laboratory facilities of Ghent University, Belgium through IUC-JU Soil Fertility Project for providing all facilities required to analyse the coffee bean samples. We are also indebted to Ethiopian Commodity Exchange for testing the coffee quality.

7. References

Adugnaw Mintesnot, 2014. Association of Arabica Coffee Quality with Geographic Origins in Ethiopia. PhD Dissertation, Haramaya University, Ethiopia, 129 pp.

Adugnaw Mintesnot, Nigussie Dechassa and Ali Mohammed, 2015a. Association of Arabica Coffee Quality Attributes With Selected Soil Chemical Properties. *East African Journal of Sciences,* 9 (2): 73-84.

Adugnaw Mintesnot, Nigussie Dechassa and Ali Mohammed, 2015b. Bean Quality Attributes of Arabica Coffee Grown in Ethiopia and the Potential for Discovering New Specialty Coffees. *East African Journal of Sciences,* 9 (2): 121-130.

Aerts, R., Gezahegn Berecha, Gijbels, P., Kitessa Hundera, Glabeke, S. V., Vandepitte, K., Muys, B. Rolda´ n-Ruiz, I., and Olivier, H. 2012. Genetic variation and risks of introgression in the wild *Coffea arabica* gene pool in south-western Ethiopian montane rainforests. *Evolutionary Applications,* ISSN 1752-4571. doi:10.1111/j.1752-4571.2012.00285.x. Blackwell Publishing Ltd.

Alonso-Salces, R. M., Serra, F., Reniero, F., Berger, and K. ´ R. H. 2009. Botanical and Geographical Characterization of Green Coffee (Coffea arabica and Coffea canephora): Chemometric Evaluation of Phenolic and Methylxanthine Contents. *J. Agric. Food Chem*., 2009 (57): 4224–4235.

Avelino, J., Barboza, B. Araya, J. C., Fonseca, C., Davrieux, F., Guyot, B., and Cilas, C. 2005. Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa Maria de Dota. *Journal of the Science of Food and Agriculture*, 85: 1869 - 1876.

Balyaya, K. J. and Clifford, M. N. 1995. Chlorogenic acids and caffeine contents of monsooned Indian Arabica and robusta coffees compared with wet and dry processed coffees from the same geographic area. In: Proc 16th Int. Sci. Coll. Coffee. ASIC, Paris, pp. 316-325.

Bertrand, B., Vaast, P., Alpizar, E., Etienne, H., Davrieux F. and Charmetant, P. 2006. Comparison of bean biochemical composition and beverage quality of Arabica hybrids involving Sudanese-Ethiopian origins with traditional varieties at various elevations in Central America. *Tree Physiology,* 26: 1239–1248. http://treephys.oxfordjournals.org/ at Ghent University on March 15, 2013

Bosselmann, A. S., Dons, K., Oberthur, T., Olse, C. S., Rabild, A. and Usma, H. 2009.The influence of shade trees on coffee quality in small holder coffee agroforestry systems in Southern Colombia. *Agriculture, Ecosystems and Environment*, 129: 253–260.

Bytof, G., Knopp, S. E., Schieberle, P., Teutsch, I. and Selmar D. 2005. Influence of processing on the generation of γ-aminobutyric acid in green coffee beans. *European Food Research and Technology*, 220: 245–250.

Clarke, R., and Macrae, R. 1985. Coffee chemistry (Vol. 1, p. 306). Elsevier Applied Science Publishers: London and New York.

Clifford, M. N. 2003. Hierarchical Scheme for LC-MS n Identification of Chlorogenic acids. *J. Agric. Food. Chem.,* 51: 2900-2911.

DaMatta, F. M. 2004. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. *Field Crops Res*., 86: 99–114.

Decazy, F., Avelino, J., Guyot, B., Perriot, J. J., Pineda, C. and Cilas, C. 2003. Quality of different Honduran coffees in relation to several environments. *J. Food Sci*., 68: 2356–2361.

ECX (Ethiopia Commodity Exchange) (2010). ECX Coffee contracts. Addis Ababa, Ethiopia.

Farah, A; de Paulis, T., Moreira, D. P., Trugo, L. C. and Martin, P. R. 2006. Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffees. *J. Agric. Food Chem.,* 54: 374-381.

Gichimu, B. M., Gichuru, E. K., Mamati, G .E. and Nyende, A. B. 2014. Biochemical Composition Within Coffea arabica cv. Ruiru 11 and Its Relationship With Cup Quality. *Journal of Food Research*, 3 (3): 31-44.

Gonzalez-Rios, O., Suarez-Quiroz, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J. P. and Schorr- Galindo, S. 2006. Impact of "ecological" post-harvest processing on coffee aroma: II. Roasted coffee. *Journal of Food Composition and Analysis,* 20 (3-4): 297-307.

Guyot, B., Manez, J.C., Perriot, J.J., Giron, J. and Villain, L. 1996. Influence de l'altitude et de l'ombrage sur la qualité des cafés arabica. Plant. *Rech. Dév.* 3: 272–280.

ISO (International Standard) (2004). International Standard ISO 10470:2004.

Joët, T., Laffargue, A., Descroix, F., Doulbeau, S., Bertrand, B., kochko, A. d. and Dussert, S. 2010. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. *Food Chemistry,* 118 (3): 693-701.

Knopp, S., Bytof, G. and Selmar, D. 2006. Influence of processing on the content of sugars in green Arabica coffee beans. *European Food Research and Technology*, 223 (2): 195-201.

Ky, C. L., Louarn, J., Dussert, S., Guyot, B., Hamon, S. and Noirot, M. 2001. 2001. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica L.* and *C. canephora* P. accessions. *Food Chem.,* 75: 223-230

Leloup, V., Gancel, C., Liardon, R., Rytz, A. and Pithon, A. 2005. Impact of wet and dry process on green coffee composition and sensory characteristics. 20ème Colloque Scientifique International sur le Café, Bangalore, India, pp. 93–101

Malta M. R. and Chagas, S. J. R. 2009. Avaliação de compostos não-voláteis em diferentes cultivares de cafeeiro produzidas na região Sul de Minas Gerais. *Acta Scientiarum Agronomy,* 31: 57-61.

Mazzafera, P. and Robinson, S. P. 2000. Characterization of polyphenol oxidase in coffee. *Phytochemistry,* 55: 285-296.

Pohlan, Hermann A. Jurgen and Janssens, Marc J. J., nd. Growth and Production of Coffee. http://W.W.W.eolss.net/Eolss-Sample Chapter. aspx: Accessed on 20 September 2016.

Rojas, O. 1985. Estudio Agroclimatico de Costa Rica. Serie Publicaciones Miscelaneas no. 617. IICA, San José, Costa Rica, 65 p.

Scott, B. 2015. How Does Elevation Affect the Taste of Coffee? Coffeecademy, Tasting. <https://driftaway.coffee/elevation/> accessed on 05/23/2018.

Selmar, D. Kleinwächter, M. and Bytof, G. 2000. Metabolic Responses of Coffee Beans during Processing and Their Impact on Coffee Flavor. In: Cocoa and Coffee fermentations. P 432 – 462. https://www.researchgate.net/publication/287273355_Coffee [accessed May 23 2018].

Silva, E. B. 1999. Fontes e doses de potássio na produção e qualidade de café provenientes de plantas cultivadas em condições edafoclimáticas. Universidade Federal de Lavras, Lavras, MG, Brasil. Doctorate thesis.

Soto -Pinto, L., Perfecto, Y., Castilio-Hernandez, J. and Caballero-Nieto, J. 2000. Shade effect on coffee production at the northern Tzeltal zone of the State of Chiapas, Mexico. *Agric. Ecosyst. Environ*, 80: 61-69.

Stanculescu, D., Scholer, M. and Kotecha, S. 2011. Ethiopia Coffee Quality Improvement Project: Aid For Trade Global Review: Case Story. The International Trade Centre.

Tadesse Woldemariam Gole. 2015. Coffee Production Systems in Ethiopia. Environment and Coffee Forest Forum (ECFF), Addis Abeba, Ethiopia.

Vaast, P. and B. Bertrand. 2006. Date of harvest and altitude influence bean characteristics and beverage quality of Coffea arabica in intensive management conditions. Hortscience.

Vaast, P., Bertrand, B., Perriot, J.P., Guyot, B. and Génard, M. 2005. Fruit thinning and shade influence bean characteristics and beverage quality of C. arabica in optimal conditions. *J. Sci. Food Agric.,* 86: 197–204.

Yigzaw Desalegn, Labuschagne, M. T., Osthoff, G. and Herselman, L. 2007. Variation for green bean caffeine, Chlorogenic acid, Sucrose, and Trigonelline contents among Ethiopian Arabica coffee accessions. Short communication. *SINET: Ethiopia, J. Sc.,* 30(1): 7-82.

Hilina, A. 2010. Specialty Coffee: The New Thing at ECX, Addis Fortune, Volume 10, Number 10.

SCAA. 2004. Coffee Cuppers Handbook. SCAA CUPPING FORM. P. 9.

Boot, W. J. 2011. Ethiopian Coffee Buying Manual: Practical Guidelines for Purchasing and Importing Ethiopian Specialty Coffee Beans. U.S. contractor implementing USAID/Ethiopia's Agribusiness and Trade Expansion Program. Fintrac Inc. www.fintrac.com, info@fintrac.com.