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Simultaneous Determination of Some Biochemical Contents of Coffee Arabica (Coffea arabica L.) Varieties and Correlation with Organoleptic Cup Quality in Contrasting Altitudes in Southwest Ethiopia

Bealu Girma
Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia

Abstract
Coffee is one of the most important agricultural commodities. The objective of this study was to simultaneously determination the biochemical constituents (caffeine, chlorogenic and nicotinic acids) and correlation with organoleptic cup quality of commonly grown coffee Arabica varieties in contrasting altitude of Southwest Ethiopia. A rapid and validated HPLC Diode Array Detector method was used for the simultaneous determination of targeted analytse in green and roasted coffee beans. The moisture content and pH were recorded from 10.13±0.04 to 12.49±0.08 and 5.36±0.04 to 5.81±0.04 respectively. The results of the chemical analysis showed that caffeine content in green coffee varieties was ranged from 12.34±0.08 mg/g (high altitude) to 19.89±0.28 mg/g (low altitude), chlorogenic content was also recorded 27.17±0.38 mg/g (high altitude) to 39.18±0.24 mg/g (low altitude) and nicotinic acid was ranged from 7.13±0.52 mg/g (low altitude) to 10.16±0.75 mg/g (high altitude). The caffeine content in roasted coffee varieties was ranged from 13.93±0.29 (high altitude) to 20.88±0.14 mg/g (low altitude) and chlorogenic content was recorded 8.56±0.02 (high altitude) to 16.21±0.10 mg/g (low altitude) and nicotinic acid ranged from 8.76 ±0.11 mg/g (high altitude) to 6.34±0.031 mg/g (low altitude). A highly significant decrement was observed in chlorogenic acid content after roasting, as altitude increases the caffeine and chlorogenic acid decreased. In contrary, nicotinic acid increase as altitude increase in green coffee varieties. As can be concluded from the result, as altitude increases caffeine and chlorogenic acid decreases but nicotinic acid increase. The caffeine content in green beans was lower as compared to roasted beans; significant degradation of chlorogenic acid was observed after roasting beans of the same variety. There was a significant interaction between altitude and coffee varieties on chemical constituents of coffee. These chemical contents could be used for coffee discriminators of coffee varieties and different altitudes. A highly significant correlation was also observed between biochemical contents and cup quality evaluation with altitude.
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1. Introduction

Coffee (Coffea L.) is the world's favorite beverage and the second-most traded commodity after oil (Davis, 2012).The coffee bean is obtained from the fruit of the coffee plant, a small evergreen shrub belonging to the genus *Coffea,* family Rubiaceae (Davis et al., 2006).Only two species namely arabica (*Coffea arabica* L.) and robusta (*Coffeacanephora*Pierre) are under commercial cultivation (Lashermes et al., 1999; Anthony et al., 2002).The value of coffee for producers' country about $ 14 billion annual income generator and more than 18 countries, including Ethiopia, export coffee product to more than 165 countries providing a livelihood for an estimate of 100 million people around the world (ICO, 2001). Ethiopia is currently producing an estimated 8.1 million bags that would rank the country as Africa's largest coffee producer, fifth and tenth Arabica coffee producer and export worldwide respectively (ICO, 2013). Coffee is one of the major sources of export in the Ethiopian economy as it contributes 25-30% of Ethiopia's total export earnings (Abu Tefera, 2015).Beverage quality often referred to as drinking quality or liquor quality, is an important attribute of coffee (Muschler, 2001; Agwanda et al., 2003) and acts as a yardstick for price determination (Agwanda et al., 2003).Coffee beverage quality is based on the characterization of a large number of factors including taste and aroma. These factors are related to the biochemical content of roasted beans. A thousand compounds, appearing during roasting, are involved in coffee beverage quality. These compounds rise from a smaller number of biochemical compounds present in green beans. Their presence could have a favorable effect on the coffee beverage quality, as for trigonelline and sugars, or an unfavorable one, as for chlorogenic acids and caffeine (Clifford, 1985; Macrae, 1985). Beverage quality assessment is done organoleptically by trained coffee tasters (Van der Vossen, 1985; Agwanda, 1999) and Kathurima et al., 2010) recommended this method as sufficiently reliable for use as a basis of selection in quality improvement programs.

However, coffee quality results from interaction among many different factors including genotype (G) and environment (E) (Muschler, 2001; Sunarharum, Williams, & Smyth, 2014). Consumers of high-quality coffee may exercise a preference for genotype with the labeling of species (e.g. arabica) or environment of production (usually

country) (Ky et al., 2001; Silvarolla, Mazzafera, & Fazuoli, 2004). Environment factors such as shade and high altitude have been observed to improve coffee quality (Joet et al., 2010). Diversity of coffee quality due to genotype and environment, result from influences on the biochemical components of the coffee bean accumulated during seed development (Joet et al., 2010).

A lot of work has been done in attempting to understand the biochemical composition of green and roasted coffee beans and its effect on cup quality (Ky et al., 2001; Decazy et al., 2003; Bertrand et al., 2006; Farah et al., 2006; Kathurima et al., 2010; Tessema et al., 2011). Despite this, the specific contribution of evaluated biochemical traits to the final cup quality remains largely unknown. Kathurima et al. (2010) noted that there is a missing link between biochemical assessment studies and the genetic improvement of coffee. They noted that more progress would be expected if biochemical studies are integrated at the early stages of coffee improvement. Coffee quality is the result of complex interactions between the environment, the imposed management regime, and the plant. In coffee beans, the biochemical composition appears to be influenced by both genetic factors (Montagnon et al., 1998) and plant growth conditions (Viani, 2001; Leroy et al., 2006).

However, Southwestern Ethiopia is the natural habitat and the primary center of diversity of *Coffea arabica* (Meyer, 1965).The study was carried out to determine the beverage quality characteristics and biochemical constituents (caffeine, chlorogenic and nicotinic acid) of 12 coffee samples grown in contrasting altitudes in Southwest Ethiopia and identify discriminators of coffee quality.

2. Materials and methods

Description of the study areas

The study was carried out from November 2017 to August 2018. Coffee bean samples were collected from November to December 2017 in three different altitudes in Gera Agricultural Research Sub-Center (altitude:-1940-1960 m), Jimma Agricultural Research Center (altitude:-1754-1765 m) in Jimma Zone, Oromiya Regional State and Teppi Agricultural Research Center (altitude:-1200-1220 m) in State of Southwest Nation and Nationalities Peoples of Ethiopia (SNNPE) Region. Details of the sampling sites are presented in Table 1.

Table 1: Geographic location and biophysical data of the study areas in Southwest Ethiopia

Location	Highland (Gera)	Midland (Jimma)	Lowland (Teppi)
Region	Oromia	Oromia	SNNPE
Altitude (m.a.s.l)	1940 – 1960	1750 - 1775	1100 - 1200
Mean temperature (^{O}C)	19 - 25	20.5 - 27	25 – 30
Rain fall in (mm)	1880 - 2080	1525 - 2000	800 - 2000
Latitude/ Longitude	6º30'N and 32 °15'E	7º40'N and 36º 50' E	9º08'N and 37º13'E

m.a.s.l =meters above sea level

Moisture content

Moisture content was determined according to AOAC procedures (1995). Full matured coffee samples were pulped using single disc manual pulpier beans from the skin and pulp. It was determined by the coffee sample was weighed (g) and recorded. The green coffee sample was dried in a dry an oven to a constant weight (100 beans), at 105 ºC for 24 hrs and each sample was cooled and the weight (g) was taken. According to Adnan, et al., (2017) the percentage of moisture content was calculated. The moisture content of the parchment coffee was measured by moisture tester maintain the moisture level from 10.5-11.0% (Sultan et al., 2014).

pH

The pH was measured according to Natalina et al., 2013 at 25°C, after calibration of the electrode with pH 4.0, 7.0 and 10.0 buffer solutions. Ground green coffee (10 g ± 0.1 g) was mixed with water (200 mL) and boiled for 5 min then cooled at room temperature, and the weight was adjusted by adding water. After filtration with a Whitman No. 1 filter, the pH of the filtrate was measured at room temperature.

Roasting and sensory evaluation

Physical and coffee cup quality analysis was carried out at Jimma Agricultural Research Center. According to Navarra, et al., 2017 procedures, for the analysis 350 g of each coffee sample was used out of which 200 g roasted at 160-200°C for 8 min using a roasting machine and 150 g was used for green coffee evaluation. The roasted beans were tipped out into a cooling tray and rapidly cooled by blowing cold air through the beans for four min and then ground with coffee was grinding machine. 13.75 g of ground coffee was diluted in 250 mL hot water (93°C) to prepare an infusion (Navarra, et al., 2017).The coffee variety samples and their replicates were arranged at random and the cup quality attributes aromatic quality, aromatic intensity, acidity, body, flavor and overall cup quality was scored using scales ranging from 0 to 10 and 0 to 5. Three cups of brewed coffee of each coffee sample were prepared for analysis and a team of three professional cuppers, from Jimma Agricultural Research Center, was taste and given a score for each of the three cups. Each panelist was given independent judgment for each

sample unit of the treatment. Finally, the average results of all panelists were used for data analysis (Sultan et al., 2014).

Chemical analysis of, caffeine, chlorogenic and nicotinic acids

Sample preparation for HPLC analysis

Coffee bean sample of each variety was prepared based on the method reported by Alves, et al., 2006. Accordingly, 0.5 g of roasted or raw ground coffee sample was extracted with 30 mL of solutions (HPLC grades water/Acetonitrile (95:5 v/v) was added and allowed to boil in water bath in 80 ^{0}C for 10 min, and was filtered with Whitman No.1 (7mm diameter) in to 100 mL flask. An aliquot (5 ml) of the filtrate was transferred to a volumetric flask (25 mL), and the volume was made up with the extraction solution (HPLC grades water/Acetonitrile (95:5 v/v). The final extraction was filtered with Whitman No.1 (4.5 mm diameter) and was added to vials injected directly into the HPLC-DAD chromatography.The HPLC conditions were adopted by Alves, et al., 2006. The separation was achieved a Spherisorb ODS-1 column (150 mm × 4.6 mm; 5 µm) (England, with HPLC grade) was employed. An isocratic elution of acetic acid /H_2O (5:95 v/v) (A), and (B) acetonitrile was used until the end 10 min, injection volume 20µL, a column temperature of 30^o C at a flow rate of 1.0 mL min^{-1} was used.

Data to be collected and analysis

Raw and cup coffee quality such as moisture content, pH, shape and make, color, aromatic intensity, aromatic quality, acidity, astringency, body, bitterness, flavor and overall quality and also caffeine, chlorogenic and nicotinic acid data were collected. Various descriptive statistical procedures (mean, percent relative standard deviation, linear regression and correlation analysis) and ANOVA was utilized in this study. All the descriptive statistical procedures SAS 9.0 were used and data that were described in samples of the coffee beans were reported as the mean ± SD of measurements. Linear regression and correlation analysis were performed for the calculation of slope (m) and correlation coefficient (R) of the regression line of each sample.

3. Results and Discussions

Moisture content and pH of coffee varieties

Moisture content

Moisture content is one of the most important quality parameters of green coffee beans. Most coffee importing and exporting countries consider the moisture content of green coffee beans as one of the quality parameters. According to Adnan, et al., (2017), the safety range for moisture content of coffee is 8.0–12.5%, based on the fresh matter. In this study, the moisture contents of coffee varieties, which were cultivated in different altitudes, were evaluated (Table 2).

Table 2: **Moisture contents of coffee varieties with altitudes**

Coffee varieties	Altitude			
	Highland	**Midland**	**Lowland**	**Mean**
74110	12.49 ± 0.08	12.34 ± 0.10	11.37 ± 0.02	12.08 ± 0.07
7454	12.17 ± 0.13	12.42 ± 0.08	11.26 ± 0.09	11.97 ± 0.08
7440	12.47 ± 0.38	12.40 ± 0.05	10.56 ± 0.01	11.89 ± 0.15
74112	12.25 ± 0.08	12.15 ± 0.09	10.13 ± 0.05	11.53 ± 0.04
Mean	12.32 ± 0.19	12.40 ± 0.04	10.83 ± 0.09	11.87 ± 0.09
CV	2.89			

It was observed moisture contents of green coffee varieties were varied from 12.17±0.13 to 12.49±0.08; 12.15±0.09 to 12.42±0.08 and 10.13±0.05 to 11.37 ± 0.02 for high altitude, mid altitude,and low altitude, respectively. A statistical test, two ways ANOVA ($P < 0.05$) showed the presence of significant interaction between coffee varieties and altitudes in terms of their moister contents (Appendix Table 1). The highest moisture content was observed in 74110 coffee varieties which were collected from highaltitude. The lowest moisture content was recorded for 74112 coffee variety collected from low-altitude. Generally, the moisture contents of coffee varieties exhibited decrements with altitudes. High-altitude coffee varieties have exhibited the higher moisture contents, than the similar coffee varieties from mid-altitude and low altitude; this could be attributed to the presence of relatively high temperate and wind flow rate at mid altitude and low altitude. The moisture contents of all the studied coffee varieties were in the recommended safe range, i.e., below 12.50 %, for importing or exporting coffee beans Adnan, et al., (2017).Moisture content outside the safety range impairs the bean quality and safety. Beans with a moisture content above 12.5% are not allowed to be shipped and traded since it facilitates fungal growth and mycotoxin production (e.g., ochratoxin A) that are risks to human health Adnan, et al., (2017). On the other hand, moisture content below 8 % also causes shrunken beans and an unwanted appearance.

The pH of coffee varieties

Table 3 shows the pH of green coffee varieties. The pH of green bean coffee varieties was varied from 5.36±0.04 to 5.81±0.04. A statistical test, two-way ANOVA ($P < 0.05$) showed that there the existence of significant interaction between coffee varieties and altitude in pH contents of green beans (Appendix Table 2). The highest pH was observed green beans of variety 7440, which was collected from the high altitude. Generally, for all coffee varieties the pH of highland greatest among midland and lowland coffee.

Table 3: pH of green coffee varieties

Coffee varieties	Altitude			
	Highland	Midland	Lowland	Mean
74110	5.80 ± 0.04	5.78 ± 0.03	5.69 ± 0.01	5.75 ± 0.09
7454	5.74 ± 0.09	5.68 ± 0.09	5.36 ± 0.04	5.59 ± 0.09
7440	5.81 ± 0.04	5.58 ± 0.14	5.56 ± 0.13	5.65 ± 0.06
74112	5.66 ± 0.13	5.63 ± 0.05	5.62 ± 0.05	5.63 ± 0.19
Mean	5.73 ± 0.06	5.67 ± 0.06	5.57 ± 0.06	5.66 ± 0.14
CV	0.85			

Simultaneously determination of caffeine, chlorogenic and nicotinic acids contents of green coffee varieties

Separation and determination of caffeine, chlorogenic acid and nicotinic acid were performed using HPLC-DAD by monitoring at various wavelengths including 272 nm (for caffeine), 320 nm (for chlorogenic acid), and 260 nm (for nicotinic acid).

Table 4: Concentrations (mg/g) caffeine, chlorogenic and nicotinic acid in green coffee varieties grown in different altitudes of Southwest Ethiopia (Mean ± SD, n = 4).

Treatments		Green coffee beans sample		
Gera (High Alt.)	Varieties	Caffeine	Chlorogenic	Nicotinic
	74110	12.34±0.08	29.72±0.71	8.16±0.04
	7454	13.58±0.15	27.17±0.38	10.16±0.75
	7440	13.1±0.45	30.40±1.44	8.53±0.45
	74112	13.3±0.77	34.56±2.09	8.08±0.16
	Mean	13.08±0.63	31.50±3.05	8.73±1.12
Jimma (Mid Alt.)	74110	14.51±0.08	31.34±1.67	8.15±0.19
	7454	16.07±0.58	33.85±2.26	8.34±1.57
	7440	13.83±0.54	30.72±0.64	7.38±0.96
	74112	13.87±0.53	34.62±0.07	7.87±0.49
	Mean	14.48±1.34	33.15±2.18	7.91±0.46
Teppi (Low Alt.)	74110	15.14±0.39	32.16±2.11	7.59±0.68
	7454	19.89±0.29	39.18±0.24	8.25±0.27
	7440	15.6±0.96	32.06±1.76	7.13±0.53
	74112	15.67±0.33	33.62±0.88	7.54±0.71
	Mean	16.42±2.19	33.23±3.95	7.63±0.05

Caffeine

Table 4 shows concentrations of caffeine in green coffee beans varieties collected from different altitudes. The caffeine content of green beans varieties was varied from 15.14 – 19.89 mg/g (lowland); 13.83 - 16.07 mg/g (midland) and 12.34 – 13.58 mg/g (highland). The lowest (12.34 ± 0.076) mg/g and highest (19.89 ± 0.288) mg/g caffeine concentrations were observed in coffee varieties 47110 (at highland) and 7454 (at lowland), respectively. Two-way ANOVA results indicated caffeine content of green coffee beans is significantly affected by coffee varieties and altitude ($P < 0.005$) Appendix Table 3). (For all coffee varieties, the caffeine content of the lowland greater than midland and highland coffees. However, the obtained caffeine contents ranged from 12.34 to 19.89 mg/g. The results are in agreement with previous studies by Kassaye et al., 2016, Ky et al., 2001 and Bekele, 2005.

Chlorogenic acid

Chlorogenic acid is another important chemical for verification of coffee varieties. The influence of altitude on its concentrations in green coffee beans was investigated (Table 4). The concentrations of chlorogenic acid were ranged from 27.17±0.38 to 34.56±2.09 mg/g (in highland); 30.72±0.64 to 34.62±0.07 mg/g (in midland); and 32.06±1.755 to 39.18±0.24 mg/g (in lowland) coffee varieties. The lowest and highest concentrations of the compound were both observed in the same variety (7454) collected from highland and lowland, respectively. Coffee variety, 7454 demonstrated variations of the concentrations of chlorogenic acid with altitude, whereas the remaining three varieties exhibited almost similar concentrations in all altitudes. The obtained results were similar

to the studies reported by Kassaye et al., 2016 and Ky et al., 2001 and Bekele, 2005.

However, these values showed the effects of altitude and coffee varieties on the concentration of chlorogenic acids. That means the concentration of chlorogenic is influenced by both variations of the altitudes and coffee variety. Generally, according to the finding of this study, chlorogenic contents of green coffee beans follow similar trends as that of caffeine contents.

Nicotinic acid

The observed concentrations of nicotinic acid in green coffee varieties are presented in Table 4. The nicotinic acid contents of green coffee varieties were varied from 7.13±0.53 up to 8.25± 0.27 mg/g; 7.38±0.96 up to 8.34±1.57 mg/g; and 8.08±0.16 up to 10.16±0.75 mg/g in lowland, midland and highland coffee varieties. The smallest and highest nicotinic acid contents were observed in the same coffee variety, 7454, collected from lowland and highland, respectively. Generally, in contrast to caffeine and chlorogenic acid, lowland coffee varieties contained the lowest concentrations of nicotinic acid than coffee varieties collected from Midland and lowland. Statistical evaluation using two-way ANOVA ($p< 0.05$) revealed the presence of interaction between coffee varieties and altitude on nicotinic acid of contents green coffee beans (Appendix Table 4). Generally, in contrast to caffeine and chlorogenic acid, lowland coffee varieties contained the highest concentrations of nicotinic acid than coffee varieties collected from midland and lowland.

Simultaneously determination of caffeine, chlorogenic, and nicotinic acid contents of roasted coffee varieties

Caffeine

The observed concentrations of caffeine in roasted coffee varieties collected from different altitudes are presented in Table 5. The caffeine content of roasted beans varieties were varied from 19.21 ± 0.66 - 20.88 ± 0.14; 14.21 ± 0.71 - 18.62 ± 0.47; and 13.93 ± 0.29 -17.22 ± 0.76 mg/g in coffee varieties of lowland; midland and highland, respectively. The lowest (13.93 ± 0.29) and highest (20.88 ± 0.14) mg/g caffeine concentrations were observed in coffee varieties 74112 (from the highland) and 7454 (from the lowland), respectively. For all coffee varieties, the concentrations of caffeine were reduced when the altitude increases and thus, the highest caffeine concentration was observed for each variety of coffee collected from the lowland. Two way ANOVA results ($P < 0.05$) also confirmed the presence of interaction between coffee varieties and altitude on caffeine content in roasted beans (Appendix Table 5).

Table 5: Concentrations (mg/g) caffeine, chlorogenic and nicotinic acid in roasted coffee varieties grown in different altitudes of Southwest Ethiopia (Mean ± SD, n = 4).

Treatments		Green coffee beans sample		
Gera (High Alt.)	**Varieties**	**Caffeine**	**Chlorogenic**	**Nicotinic**
	74110	17.22 ± 0.76	11.48 ± 0.46	7.92 ± 0.18
	7454	16.92 ± 1.37	8.56 ± 0.02	8.76 ± 0.11
	7440	14.49 ± 1.69	8.77± 0.38	8.44 ± 0.08
	74112	13.93 ± 0.29	12.59 ± 0.21	7.98 ± 0.15
	Mean	15.99 ± 2.19	13.03 ± 3.36	7.84 ± 0.69
Jimma (Mid Alt.)	74110	18.62 ± 0.47	13.16 ± 0.43	7.74 ± 0.45
	7454	17.72 ± 1.46	15.25 ± 1.19	7.98 ± 0.69
	7440	16.55 ± 0.39	12.51 ± 0.21	7.23 ± 0.33
	74112	14.21 ± 0.71	13.61 ± 0.36	6.66 ± 0.03
	Mean	16.43 ± 1.58	12.02 ± 2.49	7.33 ± 0.67
Teppi (Low Alt.)	74110	20.28 ± 0.63	14.82 ± 1.50	7.74 ± 0.35
	7454	20.88 ± 0.14	15.27 ± 0.01	7.69 ± 0.46
	7440	20.54 ± 0.09	16.21 ± 0.10	7.67 ± 0.05
	74112	19.21 ± 0.66	14.19 ± 0.32	7.07 ± 0.70
	Mean	20.23 ± 0.04	14.05 ± 1.31	7.13 ± 0.66

Generally, the caffeine contents of the studied roasted coffee varieties ranged from 13.93 - 20.88 mg/g. For each variety, the observed concentration of caffeine in the roasted coffee was higher than the corresponding green coffee beans. Literature also indicated that roasted coffee has higher caffeine content than green coffee beans Kassaye et al., 2017 and Casal, et al., 2000.

Chlorogenic acid

The recorded chlorogenic acid concentrations in roasted coffee varieties are given in Table 5. The concentrations of chlorogenic acid were ranged from 8.56±0.021 to 11.48±0.46 mg/g; 12.51±0.21 to 15.25±1.19 mg/g; and 14.19±0.32 to 16.21±0.10 mg/g for coffee varieties collected from highland, midland and lowland, respectively. For every coffee variety, the lowest and highest concentrations of the analytes were observed in highland and

lowland, respectively. Two-way ANOVA ($P < 0.05$) indicated the interaction between roasted coffee varieties and altitude on chlorogenic acid contents (Appendix Table 6). The chlorogenic acid content of roasted beans of varieties 7440 higher ($P < 0.05$) (16.21±0.10) compared (mid-altitude) (12.51±0.21) mg/g and high altitude (8.77±0.38) mg/g (Table 5). The coffee variety 7454 (8.56±0.02) mg/g at high altitude has the lowest chlorogenic acid content than other varieties. Generally, the chlorogenic acid contents of the studied coffee samples ranged from 8.56 to 16.21 mg/g. As can be seen, irrespective of the variations of the altitude, roasted coffee varieties have lower chlorogenic contents than green coffee beans, indicating the possibility of transformation of the compound into other compounds Align and Sabally, 2013.It was also reported that chlorogenic acid is thermally unstable can be lost 60.9% and 96.5% after light and dark roasting Arabica coffee respectively Hiroshi et al., 2008. The presence of variation of concentrations of chlorogenic acid with the variation of altitudes as well as coffee varieties were also reported in the literature Kassaye et al., 2017 and Ky et al., 2001.

Nicotinic Acid

The observed concentrations of nicotinic acid in roasted coffee varieties are given in Table 5. The nicotinic acid contents of green coffee varieties were ranged from 6.55 ± 0.33 - 7.55 ± 0.34, 6.66 ± 0.03 - 7.98 ± 0.69 mg/g; and 7.92 ± 0.18 - 8.76 ± 0.11 mg/g in lowland, midland and highland coffee varieties. The smallest and highest nicotinic acid contents were observed in the coffee varieties, 7454, and same variety collected from lowland and highland, respectively. Unlike caffeine and chlorogenic acid, lowland coffee varieties had the lowest concentrations of nicotinic acid than coffee varieties collected from midland and lowland. Statistical analysis using two-way ANOVA ($p < 0.05$) revealed the presence of interaction between coffee varieties and altitude on nicotinic acid contents of roasted coffee beans (Appendix Table 7). Irrespective of their varieties and altitudes, roasted samples showed relatively lower concentrations of nicotinic acid than green coffee samples. But, the roasted 7440, which was collected from lowland, exhibited higher concentration nicotinic acid than its corresponding green coffee. The obtained results were agreed with previous literature findings Giovanni et al., 2014, Rafael et al., 2015.

Raw and cup quality evaluation

Shape and make of raw coffee beans

Shape and make is an important green bean physical characteristic of coffee. It affects the roasting process and subsequently reduces cup quality. The shape and make indicates the bean boldness and uniformity in a sample. Evaluated as very good, good, fairly good, mixed, and small and weighted accordingly (Abrar and Negussie, 2015).

Table 6 showed mean comparisons of coffee varieties and altitudes on the shape and make content with Mean ± SD, N=3. According to two ANOVA showed, there was no significant interaction between coffee varieties and altitude on the shape and make ($p<0.05$) (Appendix Table 8). In mean comparison, coffee variety 74110 at high altitude had higher shape and make (12.51) compare to mid (12.16) and low altitude (12.07). Coffee variety 74112 at higher had (11.00) shape and make compared to mid and high altitude (Table 6).

Table 6: Mean comparison shape and make content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	**Altitude**			
	Highland	Midland	Lowland	Mean
74110	12.51±0.51	12.16±1.258	11.52±1.20	12.07±0.58
7454	12.52±0.53	12.00±0.56	11.52±1.20	12.02±0.37
7440	11.17±1.26	11.83±0.89	12.56±1.53	11.84±0.76
74112	11.00±0.01	12.00±0.01	11.88±0.77	11.64±0.60
Mean	11.81±0.96	12.00±0.01	11.87±0.76	11.89±1.20
CV	4.80			

Scale of Shape and make: - v. good=15; Good =12; Fair good=10; Average=8; Mixed =6; Small =4"

Color

It is another physical characteristic of coffee quality. It is evaluated as bluish, grayish, greenish, coated, faded, white, and weighted (Abrar and Negussie, 2015).Table 7 showed mean comparisons of coffee varieties and altitudes on the color content with Mean ± SD, N=3. There was no significant interaction between coffee varieties and altitude on the color of roasted coffee beans ($p<0.05$) (two way ANOVA showed) (Appendix Table 9). In mean comparison, coffee variety 7454 at high altitude had higher color (13.52) compare to mid (11.50) and low altitude (11.38). Similarly coffee variety 74110 in high altitude had high (12.38) compared to mid (12.33) and low altitude (11.21). 7454 in high altitude had the highest (13.52) than the rest mid and high altitude. Coffee variety 7454 high altitudes had the lowest in color.

Table 7: Mean comparison on color content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	**Altitude**			
	Highland	Midland	Lowland	Mean
74110	12.83±1.06	12.23±1.20	11.21±1.10	12.11±0.93
7454	13.52±0.87	11.50±0.50	11.38±1.41	12.16±1.60
7440	10.33±1.15	11.0±1.01	12.00±0.89	11.10±1.31
74112	11.67±1.04	13.19±0.98	11.40±0.58	12.12±1.15
Mean	12.11±1.29	12.00±1.2	11.50±0.29	11.87±0.28
CV	8.09			

Scale of Color:-Bluish =15; Grayish =12; Greenish =10; Coated =8; Faded=6; White =4"

Color is the visual appearance of the brewed cup of coffee. Ones' aspect of visual appearance indicates color and the direct effect of caramelization power of the sugar beans based on roasting degree. The roasting degree also depends on the size and 'shape and make' of green coffee beans (Alemayehu, 2017).

Aromatic Intensity

Table 8 showed a mean comparison of coffee varieties and altitudes on Aromatic Intensity content with Mean ± SD, N=3.Two-way ANOVA shows there was no significant interaction between coffee varieties and altitude on the aromatic intensity of roasted coffee beans ($p<0.05$) (Appendix Table 10). Coffee variety 74112 at high altitude had high aromatic intensity (4.33) (strong aromatic intensity) compared to mid (4.17) and low altitude (3.67) (Table 8).Similarly, coffee variety 74110 higher altitude had higher aromatic intensity (4.00) compared to mid (4.00) and low altitude (3.83).

Table 8: Mean comparison on aromatic intensity content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	Altitude			
	Highland	Midland	Lowland	Mean
74110	4.00±1.00	4.00±0.87	3.83±1.04	3.83±0.65
7454	3.50±0.50	3.67±0.29	3.67±0.29	3.61±0.33
7440	3.83±1.04	4.00±0.87	4.00±1.00	3.94±0.85
74112	4.33±0.58	4.17±0.77	3.67±0.29	4.06±0.49
Mean	3.83±0.29	3.94±0.85	3.67±0.29	3.86±0.59
CV	7.06			

Scale of Aromatic intensity:-V. strong=5; strong=4; medium=3; light=2; V. light=1;Nil=0

Aromatic Quality

It is sensation that is hard to separate from flavor. The aroma contributes to the flavors are discern on our palates. Subtle nuance, such as 'Floral' or 'winey' characteristics, are obtained from the aroma of brewed coffee (Abrar and Negussie, 2015).

Table 9 showed Mean comparison of coffee varieties and altitudes on aromatic quality with Mean ± SD, N=3. Two-way ANOVA showed there was no significant interaction between coffee varieties and altitude on aromatic quality of roasted coffee beans ($p<0.05$) (Appendix Table 11). Coffee variety 7440 higher in high altitude (4.00) compared to mid-altitude (3.83). Coffee variety 7454 in mid-altitude had the lowest aromatic quality (Table 9).

Table 9 Mean comparison on aromatic quality content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	Altitude			
	Highland	Midland	Lowland	Mean
74110	3.83±0.29	3.67±0.29	3.83±0.29	3.78±0.26
7454	3.50±0.50	3.17±0.29	3.50±0.50	3.38±0.56
7440	4.00±1.00	3.83±1.04	4.17±0.76	4.00±0.83
74112	4.00±0.01	4.00±0.87	3.50±0.50	3.83±0.56
Mean	3.83±0.54	3.66±0.69	3.75±0.54	3.75±0.8
CV	11.84			

Scale of Aromatic quality: - excellent=5; v.good=4; good=3; regular=2; Bad=1; Nil =0"

Acidity

Table 10 showed a mean comparison of coffee varieties and altitudes on acidity content with Mean ± SD, N=3. Two-way ANOVA shows there was no significant interaction between coffee varieties and altitude on the acidity of roasted coffee beans ($p<0.05$) (Appendix Table 12).Coffee variety 74112 has higher in mid-altitude (7.50) as

compared to low (7.17) and high altitude (7.0). Coffee variety 74110 had higher acidity content in low (7.83) altitude as compared to mid (6.50) and high altitude (7.00). Coffee variety 7440 had higher in the mid-latitude (7.00) as compared to high (6.50) and low altitude (6.83). The lowest in acidity content from the varieties was 74110 in mid-altitude (Table 10).

Table 10**:** Mean comparison on acidity content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	**Altitude**			
	Highland	Midland	Lowland	Mean
74110	7.00±0.50	6.50±0.50	7.83±0.29	7.11±0.70
7454	7.00±0.50	7.00±0.50	6.67±0.58	6.89±0.49
7440	6.50±0.00	7.00±0.00	6.83±0.76	6.78±0.44
74112	7.17±0.29	7.50±0.87	7.00±0.50	7.22±0.57
Mean	6.92±0.42	7.00±0.60	7.08±0.67	7.00±0.56
CV	7.06			

Scale of Acidity Pointed=10; M. pointed=8; Medium =6; Light =4; Lacking=2;Nil=0"

Acidity indicates the bitter or acidic balance that a sweet caramelic after taste which could be affected by roast degree and phonology of coffee fruit that means shade may have indirect effect on coffee cup quality test (Alemayehu, 2017).

Astringency

Table 11 showed that mean comparison of coffee varieties and altitudes onAstringency content with Mean ± SD, N=3. Two-way ANOVA shows there was no significant interaction between coffee varieties and altitude on astringency of roasted coffee beans ($p<0.05$) (Appendix Table 13). The coffee variety of 74110 had higher antigenicity (4.33) in low altitude as compared to mid (4.01) and low altitude (4.00). The coffee variety 7440 had lower in astringency in higher altitude (4.00) as compared to mid (4.16) and low altitude (4.17). The lowest astringency was observed at low altitude in 7454.variety (Table 10).

Table 11: Mean comparison on Astringency content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	**Altitude**			
	Highland	Midland	Lowland	Mean
74110	4.00±1.00	4.01±1.00	4.33±0.58	4.11±0.60
7454	4.00±1.00	4.00±0.76	3.67±0.58	3.89±0.78
7440	4.00±1.00	4.16±0.76	4.17±0.76	4.11±0.74
74112	4.17±0.76	4.16±0.76	4.17±0.76	4.17±0.66
Mean	4.04±0.81	4.08±0.63	4.08±0.63	4.07±0.68
CV	7.98			

Scale of Astringency:-Nil =5; V.ligh t=4; Light=3; Medium=2; Strong =1; V.strong=0"

It is described complex sensation accompanied by shrinking, drawing or puckering mucosal surface in the mouth, produced by like tannins and sloe tannins (Abrar and Negussie, 2015). The higher content of astringency of the coffee beverage may be due to higher content of sucrose and chlorogenic acid in green coffee beans based on its size and ripping paired. This chlorogenic acid is reduced to organoleptic quality especially under unshaded beans than shaded ones (Morais et al., 2006).

Bitterness

Table 12 showed that mean comparison of coffee varieties and altitudes onBitterness content with Mean ± SD, N=3. Two-way ANOVA showed there was no significant interaction between coffee varieties and altitude on the bitterness of roasted coffee beans ($P<0.05$) (Appendix Table 14).Coffee variety 74110 had higher in low altitude (4.17) as compared to mid (3.50) and higher (4.00) in bitterness content. Coffee variety 7440 had higher in mid-altitude (4.33) as compared to high (3.33) and low altitude (4.17). coffee variety of 7440 in high altitude had the lowest in the high-altitude varieties (Table 12).

Table 12: Mean comparison on Bitterness content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	Altitude			
	Highland	Midland	Lowland	Mean
74110	4.00±1.00	3.50±0.50	4.17±0.76	3.89±0.74
7454	4.00±0.87	4.00±1.00	3.33±0.58	3.77±0.80
7440	3.33±0.58	4.33±0.58	4.17±0.76	3.94±0.73
74112	4.00±1.00	4.33±0.58	4.00±0.00	4.11±0.60
Mean	3.83±0.81	4.04±0.69	3.92±0.63	3.93±0.70
CV	8.97			

Scale of Bitterness:-Nil =5; V. light=4; Light=3; medium=2; Strong=1; V. strong=0"

Bitterness is the perception of coffee brew on the tongue of panelist during cup tasting. It is opposite to sweetness (Abrar and Negussie, 2015).

Body

Table 13 showed that mean comparison of coffee varieties and altitudes on Body content with Mean ± SD, N=3.Two-way ANOVA showed there was no significant interaction between coffee varieties and altitude on body roasted coffee beans (P<0.05) (Appendix Table 15). Coffee variety 7440 had higher in low altitude (7.17) as compared to mid (7.00) and high altitude (6.83) in the body. Coffee variety 74112 had higher in low altitude (7.29) as compared to mid (7.00) and high altitude (7.13) in the body. The lowest body content was observed a coffee variety of 74110 in high altitude and 7440 of high-altitude coffee (6.38)(Table 13).

Table: 13 Mean comparison on Body content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	**Altitude**			
	Highland	Midland	Lowland	Mean
74110	7.33±0.58	6.83±0.29	7.50±0.50	7.22±0.51
7454	7.33±0.76	7.00±0.50	7.17±0.29	7.17±0.50
7440	6.83±0.29	7.00±0.50	7.17±0.29	7.00±0.35
74112	7.00±0.50	7.17±0.76	7.33±0.29	7.17±0.50
Mean	7.13±0.53	7.00±0.48	7.29±0.33	7.13±0.46
CV	5.96			

Scale of Body (10%):-Full =10, Medium full =8, medium=6, Light =4, V.light=2, Nil =0"

Available of body in a cup of coffee quality test indicates viscosity or thickness of coffee brewed. It is the physical property of beverage that the result in tactile sensations perceived on the skin in the mouth during and after ingestion based on beans quality (Alemayehu, 2017).

Flavor

Table 14 showed a mean comparison of coffee varieties and altitudes on flavor content with Mean ± SD, N=3. Two-way ANOVA showed there was no significant interaction between coffee varieties and altitude on flavor roasted coffee beans (p<0.05) (Appendix Table 16). Coffee variety 74110 had higher in flavor content in low altitude (8.33) as compared to mid (7.83) and high altitude (8.00). Coffee variety 7440 had high in flavor content (8.00) in low altitude as compared to mid (7.83) and high altitude (7.50). The lowest flavor content was observed in 7440 coffee variety in high altitude (Table 14).

Table 14: Mean comparison on Flavor content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	**Altitude**			
	Highland	Midland	Lowland	Mean
74110	8.00±0.50	7.83±0.29	8.33±0.29	8.06±0.29
7454	8.00±0.50	8.00±0.50	7.67±0.29	7.89±0.47
7440	7.50±0.00	7.83±0.29	8.00±0.50	7.78±0.36
74112	8.17±0.29	8.17±0.76	7.83±0.58	8.06±0.53
Mean	7.92±0.42	7.96±0.42	7.96±0.58	7.94±0.44
CV	4.51			

Scale of Flavor (10%):-V.good=10, Good =8, Average =6, Fair =4, Bad=2, Nil=0"

Flavor is the simultaneous sensation in the test of aroma and taste. Coffee aroma is composed of the gaseous natural chemical components of roasted and brewed coffee beans, which escape as vapors after the coffee grounds are brewed. The perfume of the ground roasted coffee before water is added, it gives fragrance/aroma and one can smell the aroma, evaluate the body then perceive the taste and flavors (Muschler, 2001).

Overall cup quality

Table 15 showed a mean comparison of coffee varieties and altitudes on overall cup quality content with Mean ± SD, N=3.Two-way ANOVA showed there was no significant interaction between coffee varieties and altitude was observed in overall cup quality evaluation (P<0.05) (Appendix Table 17). Coffee variety 74110 had higher overall cup quality (8.50) as compared to mid (7.50) and low altitude. Coffee variety 74112 that grown in higher altitude had higher (8.33) overall quality as compared to mid (8.17) and low altitude (7.83). The lowest overall quality was observed in 7454 at higher altitude coffee variety. (Table 15)

Table 14 Mean comparison on overall cup quality content in green coffee beans of some coffee varieties grown across three altitudes in southwest Ethiopia (with Mean ± SD, n=3).

Coffee varieties	Altitude			
	Highland	Midland	Lowland	Mean
74110	8.50±0.00	7.50±0.00	8.17±0.29	8.06±0.46
7454	7.67±0.29	7.83±0.29	8.00±0.50	7.83±0.35
7440	7.83±0.29	7.83±0.29	7.67±0.29	7.78±0.26
74112	7.83±0.58	8.17±0.76	8.33±0.58	8.11±0.60
Mean	7.96±0.45	7.83±0.44	8.04±0.45	7.94±0.44
CV	4.18			

Scale of Overall cup quality (10%): Excellent=10, v.good =8, Good=6, Regular=4, Bad =2, unacceptable=0"

It can be recorded based on all the liquor quality attributes (intensity, aromatic quality, acidity, astringency, body, bitterness, and flavor) (Abrar and Negussie, 2015). In order to sum up all raw and cup quality evaluation were not a significant interaction between coffee varieties and altitude. But the data of physical and cup quality evaluation was considered according to in Ethiopian Commodity Exchange (ECX) grading system, the results to become more meaningful, and to quantify the quality of the coffee varieties.

Raw and cup quality evaluation according to Ethiopian Commodity Exchange grading and evaluation system.

There are two categories of coffee evaluation ways on the preliminary total quality, Physical (40/100) and preliminary cup quality (60/100) scores.

Table 16: Shows the coffee quality grading and evaluation of coffee varieties in contrasting altitude on the base of ECX was listed below.

Altitude	Coffee varieties	40%	60%	100%	Old ECX	Specialty
Highland	74110	34.5	46.3	80.8	G2	Q2
	7454	35.3	45	80.3	G2	Q2
	7440	30.5	43.8	74.3	G3	Commercial
	74112	31.5	46.7	78.2	G2	Commercial
Midland	74110	33.7	43.8	77.5	G2	Commercial
	7454	32.7	44.7	77.3	G2	Commercial
	7440	31.8	46	77.8	G2	Commercial
	74112	33.8	46.7	80.5	G2	Q2
Lowland	74110	31.3	48	79.3	G2	Commercial
	7454	31.5	43.7	75.2	G2	Commercial
	7440	32.2	46.2	79.2	G2	Commercial
	74112	32	45.8	77.8	G2	Commercial

Grade 1= 91-100, Grade 2 = 81- 90, Grade 3 = 71- 80, and grade 4= 70- 63,
Specialty1= (Q1) > 85, Specialty 2 (Q2) = 80-84.75 and Commercial = (<80)

Based on the ECX had five categories of coffee grading and evaluation , grade 1= 91-100, grade 2 = 81-90,grade 3 = 71-80, and grade 4= 70-63. If the scores of all samples were higher than 70, cup quality for specialty coffee (coffees with the grade ranging from 1 to 3). Based on the total specialty score, coffee samples further grouped into a specialty1 (Q1) > 85, specialty 2 (Q2) 80-84.75 and a regular commercial coffee (<80). (The new guideline) (ECX, 2015).

The first two of coffee varieties of 74110 and 7454 had qualified for specialty 2 (Q2),(80.8 and 80.3 respectively) from high altitude and also in old grading and evaluation (G2). New ECX grading and evaluation less than (<Q2) (<80) are a commercial purpose. Coffee variety of 74112 had passed for the qualification of 2(Q2) in 80.5 from mid-altitude, also passed G2 of old grading and evaluation of ECX. All coffee varieties qualified for old grading of G2, except coffee variety 7440 in high altitude. Therefore all the coffee varieties were high grade qualified and in raw and cup evaluation and grading.

Raw and cup quality evaluation that includes shape and make, color, odor, aromatic intensity, aromatic quality, acidity, astringency, bitterness, body, flavor, and overall quality were evaluated by three professional cuppers (Q

graders) in Jimma agriculture research center (JARC). Three measurements for each variety and all parameters was taken and quantified, analyzed and interpreted in the result part with two-way ANOVA that showed the interaction of altitude and varieties, but all were shown insignificant (p>0.05). To sum up, all the parameters were very good to interpret in Ethiopian commodity exchange evaluation that showed in Table 16. The results were supported by literature Kassaye et al., 2016.The biochemical contents and cup quality evaluation was correlation by Pearson Correlation and showed in the Table 17.

Correlation between cup quality parameters and chemical contents of green and roasted coffee beans

In this context, there was a correlation made between cup quality evaluation parameters and biochemical contents in green and roasted beans of coffee with the Pearson correlation coefficient method. From Pearson correlation, the moisture content has negatively (r = -6.10**) and a highly significant (P>0.001) correlated with caffeine in roasted coffee. The caffeine content in green coffee has positively (r = +0.78**) and highly significantly correlated with nicotinic acid of green coffee. The caffeine content in green coffee has positively (r = 0.67**) and highly significantly (P>0.001) correlated with nicotinic acid of roasted coffee. The nicotinic acid content in green coffee has positively (r = +0.77**) and highly significantly (P>0.001) correlated with nicotinic acid content of roasted coffee. Raw quality has positively (r = 0.87**) and highly significantly (P>0.001) correlated with overall quality of coffee. Cup quality has positively (r = 0.68**) and highly significantly correlated to overall quality of coffee (Table 17).

The moisture content has negatively (r = -0.48*) and significantly (>0.05) correlated with caffeine green. The caffeine green positively (r = +0.58*) and significantly (p>0.005) correlated with a chlorogenic acid content of green coffee. The moisture content has negatively (r = -0.38*) and significantly (p>0.005) correlated with nicotinic acid content of green coffee. The caffeine green content positively (r = +0.56*) and significantly (p>0.005) correlated with caffeine roasted coffee. The caffeine green positively (r = +0.59) and significantly (p>0.05) correlated with chlorogenic acid content roasted coffee. The moister content negatively (r = -0.48) and significantly (p>0.005) correlated with nicotinic acid content of roasted coffee. The chlorogenic acid content of roasted coffee negatively (r = -0.35*) and significantly (p>0.005) correlated with raw quality of the coffee (Table 17).

Table 17: The Pearson correlation coefficients among physical, cup test and chemical constituents of coffee beans

	Moisture	pH	Caffeine Green	Chlorogenic Green	Nicotinic Green	Caffeine Roast	Chlorogenic Roast	Nicotinic Roast	Quality Raw (40)	Quality Cup (60)	Quality Overall
Moisture	1.00	0.138 ns	-0.483*	-0.018 ns	-0.383*	-0.611**	-0.183 ns	-0.484*	0.154 ns	-0.048 ns	0.041 ns
pH		1.00	0.115 ns	0.295 ns	0.254 ns	0.1344 ns	0.255 ns	0.412*	-0.265 ns	0.065 ns	-0.084 ns
Caffeine Green			1.00	0.584*	0.777**	0.563*	0.343*	0.667**	-0.229 ns	-0.178 ns	0.249 ns
CGA Green				1.00	0.552 ns	0.004 ns	0.587*	0.366 ns	-0.226 ns	-0.023 ns	-0.138 ns
Nicotinic Green					1.00	0.619 ns	0.219 ns	0.773**	-0.128 ns	-0.104 ns	-0.142 ns
Caffeine Roast						1.00	0.134 ns	0.318 ns	-0.032 ns	0.087 ns	0.050 ns
Chlorogenic Roast							1.00	0.318 ns	-0.352*	0.005 ns	-0.172 ns
Nicotinic Roast								1.00	-0.141 ns	0.039 ns	-0.041 ns
Quality Raw (40)									1.00	0.239 ns	0.682**
Quality Cup (60)										1.00	0.874**
Quality Overall											1.00

*Remark: - **- Highly Significant,*- Significant and ns-non significant*

5. Summary and Conclusion

The influence of altitude on caffeine, chlorogenic acid, and nicotinic acid contents was determined on coffee varieties in high, mid and low altitudes. A rapid and validated HPLC-diode array detector method which was accurate, precise and multi-analysis (separation) technique, was used for the simultaneous determination of caffeine, chlorogenic and nicotinic acids that were applicable to four varieties in green and roasted coffee samples. The detection limit was 0.078 µg/mL for nicotinic acid, 0.067 µg/ mL for caffeine and 0.088 µg / mL of chlorogenic acid. The result of chemical constituents was chlorogenic acid in coffee variety 7454 on contains lower (27.17±0.38 mg/g), in high altitude compared to mid-altitude (33.85±2.26 mg/g) and (39.18 ± 0.24 mg/g) in low altitude green beans. Similarly coffee variety 7454 higher in nicotinic acid contents (10.16±0.75 mg/g) in high altitude compared to (8.34±1.57 mg/g) mid-altitude and (8.25±0.27 mg/g) low altitude .Coffee variety 7454 contains lower caffeine in high altitude (13.58±0.15 mg/g) compared to mid-altitude (16.07±0.58 mg/g) and (19.89±0.29 mg/g) in low altitude of green coffee beans. The caffeine content of roasted beans coffee varieties 7454 higher (20.88±0.14 mg/g) in low altitude compared to mid-altitude (17.72±1.46 mg/g) and high altitude (16.92±1.38 mg/g). Coffee variety 74110 lower in chlorogenic acid contents (11.48 ±0.46 mg/g) in low altitude compared to mid-altitude (13.16±0.44 mg/g) and high altitude (14.82±1.51 mg/g.) in roasted beans. Nicotinic acid contents of roasted beans of varieties 7454 higher in high altitude (8.76±0.11 mg/g) to compared mid-altitude

(7.97±0.67 mg/g) and low altitude (6.43±0.03 mg/g).The highly significant interaction was observed between altitude and coffee varieties in both green and roasted coffee beans on caffeine, chlorogenic and nicotinic acid.As altitude increases caffeine and chlorogenic acid decreases but nicotinic acid increase. The caffeine content in green beans was lower as compared to roasted coffee; significant degradation of chlorogenic acid was observed as goes from green to roasted coffee the same varieties. Chlorogenic acid is thermally unstable and in Arabica coffee the loss of after light roasting and after very dark roasting of beans corresponds to 60.9% and 96.5%. A strong correlation was observed between cup quality evaluation and these chemical contents and along with altitude. Thus biochemical contents could be used for discriminators coffee for organoleptic quality for coffee varieties and different altitudes.

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