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Influence of growing altitude, shade and harvest period on quality and biochemical composition of Ethiopian specialty coffee

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Abstract

BACKGROUND: Coffee quality is a key characteristic for the international market, comprising cup quality and chemical bean constituents. In Ethiopia, using total specialty cup scores, coffees are grouped into Q1 (specialty 1) ≥ 85 and Q2 (80–84.75). This classification results in market segmentation and higher prices. Although different studies have evaluated the effects of altitude and shade on bean quality, optimum shade levels along different altitudinal ranges are not clearly indicated. Information on effects of harvest periods on coffee quality is also scanty. The present study examined the influences of these factors and their interactions on Ethiopian coffee quality

RESULTS: Coffee from high altitude with open or medium shade and early to middle harvest periods had a superior bean quality. These growing conditions also favoured the production of beans with lower caffeine. An increasing altitude, from mid to high, at approximately 400 m, decreased caffeine content by 10%. At high altitude, dense shade decreased Q1 coffee by 50%. Compared to late harvesting, early harvesting increased the percentage from 27% to 73%. At mid altitude, > 80% is Q2 coffee.

CONCLUSIONS: Changes of quality scores driven by altitude, shade and harvest period are small, although they may induce dramatic switches in the fraction Q1 versus Q2 coffee. The latter affects both farmers' profits and competitiveness in international markets.

Keywords: Arabica coffee; altitude; shade; cup quality; caffeine; chlorogenic acid

INTRODUCTION

Coffee is the world's most traded commodity after oil[1] and its quality is a key characteristic for the international coffee market.[2,3] Coffee quality is determined by organoleptic characteristics (cup quality), physical appearance and chemical constituents.[4,5] The increase in demand and consumption of high-quality, single-origin, specialty coffee with specific characteristics results in market segmentation (e.g. a specialty coffee market) and creates a strong potential and new opportunities for coffee-producing countries.[6–8] Market segmentation for specialty coffee attracts new customers and results in higher coffee prices.[9] For example, specialty coffee beans receive a premium price that is approximately 20–50% higher compared to regular coffee beans.[10]

Ethiopia is one of the top ten coffee-producing countries in the world and the largest exporter in Africa.[10] The country is naturally gifted with a suitable climate and has the potential to produce single origin specialty Arabica coffee beans with a wide range of flavors.[11,12] Recently, nine single origin specialty coffees (Jimma, Nekemte, Illubabor, Limu, Tepi, Bebeka, Yirga Chefe, Sidamo and Harar) were identified and entered into trade circuits of the world coffee market. Among them, the sundried coffee beans from 'Harar', the so-called 'Mocca' and the washed beans from 'Yirga Chefe' are considered to be the finest and best quality coffee.[13]

Currently, specialty coffee accounts for approximately 20% of Ethiopia's coffee export and there is also a very high potential to boost its share in the world market.[14] Thus, an improved specialty coffee market is considered to be beneficial for Ethiopia to remain competitive in the international market. Ethiopian producers will benefit from high-quality coffee if its supply remains stable [6] and, if the global coffee chain is not changing as a result of deregulation, new consumption patterns and/or evolving corporate strategies (e.g. branding).[15] Producers, on the other hand, should implement improved, sustainable agronomic practices (e.g. shade-grown coffee) to produce beans desired by consumers.

Along the coffee supply chain, however, numerous factors affecting coffee quality have been identified. Genetic traits,[16] growing environment[17–20] and postharvest processing methods[5,21] are known to predominantly affect coffee quality. The most important

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environmental factor, most commonly linked to influence coffee quality, is the altitude where coffee is grown.[17,19] Some studies have reported that the best quality of Arabica coffee comes from a higher altitude as a result of lower daily temperatures, which results in a slower ripening of the beans and allows more time for bean filling.

Shade is also found to favour a sustainable production of high coffee quality, especially under suboptimal conditions where temperatures are higher than optimal.[19,20] Some studies have indicated that shade reduces temperature stress in the canopy and lengthens the maturation period of coffee berries. It also reduces periodic over-bearing and a subsequent die back of coffee plants.[22] In line with this, Läderach *et al.*[7] reported that coffee quality scores increase with the level of shading. Bosselmann *et al.*,[23] on the other hand, reported that, at high altitude, shade had no significant effect on cup quality attributes. At lower altitude, however, shade reduced the number of small beans with no significant effect on coffee sensorial attributes. In a study by Lara-Estrada and Vaast,[2] shade was reported not to have any significant influence on coffee organoleptic properties under any of the conditions of altitude and fertilization. These contradicting reports show that optimum shade management practices are highly site specific and also that further studies are required to clearly indicate its influence on coffee quality attributes across different altitudinal gradients.

In Ethiopia, coffee trees are grown under shade of different levels and shade management is among the dominant agronomic practices in traditional organic coffee growing systems.[22,24] Recent studies show that shade significantly affects yield[25] and cup quality attributes of Ethiopian coffee.[24,26] Although different studies have been carried out to evaluate the effect of shade on coffee yield and quality, optimum shade levels along different environmental gradients have not yet been investigated in the country. In addition, no study has been carried out aiming to investigate how altitude and shade interactively affect cup quality and the biochemical composition of Ethiopian coffee beans.

In addition to shade and growing altitude, coffee harvesting periods were also reported to affect coffee quality. Guyot *et al.*[27] indicated that late harvested coffee beans had better beverage quality and larger bean size than early harvested beans. Bertrand *et al.*,[28] on the other hand, reported that early harvested coffee beans were better with respect to beverage quality than late harvested beans. In another study, early harvesting was found to improve coffee quality compared to late harvesting.[7] This also highlights the need for further research aiming to clearly determine the effects of harvest periods on coffee bean quality.

To the best of our knowledge, no studies exist that analyse the interactive effects of growing altitude, shade levels and harvest periods on Ethiopian coffee bean quality. Hence, the main objective of the present study was to examine their interactive effects on physical characteristics, cup quality attributes and selected biochemical compounds.

MATERIALS AND METHODS

Study site

The study was carried out from October 2013 to February 2014 in the Mana district, Jimma zone, Oromia regional state, south western Ethiopia. Mana is known as one of the predominant *Coffea arabica* L. growing areas. It is located at altitudinal ranges between 1470 and 2610 m a.s.l., at 8°67′N and 37°07′E. The district has a total area of 47 898 ha of which 23% is at low (<1550 m a.s.l.), 65% is at mid (1550–1750 m a.s.l.) and the remaining 12% is at high altitude (>1750 m a.s.l.).[29] Annual average temperature and rainfall are 20.5 °C and 1523 mm, respectively. The soil type in the study area is uniform and described as Nitosol, with a pH ranging from 4.5 to 5.5.[30]

Treatments and experimental design

Two altitudinal ranges were selected: high (1950–2100 m a.s.l.) and mid altitude (1600–1680 m a.s.l.) with an average temperature over 30 years of 19.3 ± 1.9 °C and 22.5 ± 2.6 °C, respectively. Different shade levels of ten coffee farms (five from each altitudinal range) with comparable coffee ages (7–10 years) were selected taking care that agronomic management practices did not differ substantially among the farms. Shade levels above canopies of the coffee trees in each farm were quantified using the Sunscan canopy analysis system (Type SS1, BF5-RPDA1; Delta-T Devices, Cambridge, UK) and grouped into three levels: open (no shade), medium (40–55%) and dense shade (65–85%). During measurements, the reference sensor was placed in open sun (no shade) and the shade percentage was determined in relation to this reference point. In the harvest season, from October 2013 to February 2014, red ripe cherries from each altitude and shade level were harvested at three different periods (early, middle and late). Early and middle harvesting at mid altitude of open and medium shades were carried out from the first to the third week of October 2013, whereas late harvested beans were collected from the first to the third week of November 2013. Coffee samples at high altitude with open to medium shade levels were harvested early (first week of December 2013), middle (end of December 2013) and late (third week of January 2014). Under dense shade, on the other hand, early harvest was carried out in the fourth week of December 2013, middle harvest in the third week of January 2014 and late harvest in the first week of February 2014).

The experiment was arranged in a split–split plot design with two levels of altitude (mid and high) as a main plot, three levels of shade (open, medium and dense) as a subplot and three levels of harvest periods (early, middle and late) as sub-subplot with five replications (farms) at each attitude.

Coffee quality analysis

Coffee cherries were sundried immediately after harvest on raised beds in accordance with standard agronomic practices until a moisture content of approximately 11.5%. The dried coffee cherries were then dehusked using a coffee hulling machine (Coffee Huller; McKinnon, Aberdeen, UK) at Jimma University College of Agriculture and Veterinary Medicine and stored at room temperature packed in hermetic plastic bags. The 100-bean weight (g) of the dried beans was determined in three replicates using a digital sensitive balance (CTG-6H+; Citizen, Piscataway, NJ USA) and the bean diameter (mm) was measured on 45 randomly selected beans per treatment, using a digital calliper (IP 67, CD-20-PPX; Mitutoyo Technology, Kawasaki, Japan).

For quality analysis, 350 g of each coffee sample was sent to the Ethiopia Commodity Exchange (ECX), Jimma Branch, for physical and cup quality analysis, out of which 100 g was roasted at 160–200 °C for 8–12 min using a roasting machine (4 Barrel Roaster; Probat, Emmerich am Rhein, Germany) adjusted to medium roasting.[31] The roasted beans were tipped out into a cooling tray and rapidly cooled by blowing cold air through the beans for 4 min and then ground with a coffee grinding machine (K32SB2; Mahlkonig, Hamburg, Germany). Next, 13.75 g of ground coffee was diluted in 250 mL of hot water (93 °C) to prepare an infusion.

Five cups of brewed coffee of each coffee sample were prepared for analysis and a team of three professional cuppers, who operate in ECX, tasted and gave a score for each of the five cups.

The preliminary total quality score comprises physical (40/100) and preliminary cup quality (60/100) scores. The criteria commonly used to evaluate the physical quality of coffee beans include the presence of defects: primary (e.g. full black and sour beans) and secondary (e.g. partial black, insect damaged and broken beans) defects and odour.[32] Cup quality was evaluated based on acidity, body, flavour and cup cleanness scores. The sum of these four cup quality attributes gives preliminary cup quality with a score between 0 and 60. Hence, the sum of both physical and preliminary cup quality ranges between 0 and 100 and is used to classify the samples into different grades (grade 1 = 91–100; grade 2 = 81–90; grade 3 = 71–80; and grade 4 = 63–70). If the scores of all samples were higher than 70, cup quality for specialty coffees (coffees with grades ranging from 1 to 3) was further assessed based on overall cup preference, acidity, body, aroma, flavour, aftertaste, uniformity, cup cleanness, sweetness and balance, each on a scale ranging from 0 to 10. Based on the total specialty scores, coffee samples were further grouped into specialty 1 (Q1) ≥ 85, specialty 2 (Q2) (80–84.75) and a regular commercial coffee (<80).[31] The grading was carried out in accordance with an old ECX guideline. However, recently, ECX established a new grading system that reduced the old ten preliminary total quality levels to five levels: grade 1 ≥ 85; grade 2 = 75–84; grade 3 = 63–74; grade 4 = 47–62; and grade 5 = 31–46.[33] Coffee samples that fall in grade 1 and 2 qualify for specialty coffee and are further grouped into specialty 1 (Q1) ≥ 85 and specialty 2 (Q2) (80–84.75). The preliminary total quality assessment scores for grade 2 should be higher than 80 to be qualified as specialty 1 (Q1).

Caffeine and chlorogenic acid extraction

Green coffee beans were ground (<0.5 mm) prior to extraction using a grinder (M20; IKA, Staufen, Germany). The extraction was performed using 100 mg of ground coffee beans with 10 mL of methanol:water:acetic acid (30:67.5:2.5; v/v/v) containing 2 mg mL^{-1} ascorbic acid, as described by Alonso-Salces *et al.*[34] The extract was placed in an ultrasonic bath for 15 min and filtered over a 0.45-μm polytetrafluoroethylene filter prior to injection into a liquid chromatography system.

Liquid chromatography analysis

Using an established liquid chromatography-time of flight-mass spectrometry method,[35] four subclasses of chlorogenic acids were identify in the green coffee samples: caffeoylquinic acid, feruloylquinic acid, dicaffeoylquinic acid and feruloyl-caffeoylquinic acid. Quantification was performed using liquid chromatography equipped with a photodiode array detector (Surveyor; Thermo Finnigan, San Jose, CA, USA) at 280 nm for caffeine and 320 nm for chlorogenic acids. The chromatographic separation was performed using a prevail C18 (250 × 4.6 mm, 5 μm; Alltech, Klerken-Houthulst, Belgium) column, maintained at 25 °C. The chromatographic method was based on Alonso-Salces *et al.*[34] using a combination of 0.2% acetic acid in water (v/v) and high-performance liquid chromatography grade methanol. The injection volume was 50 μL at a flow rate of 1 mL min^{-1}. Calibration was achieved by injecting a concentration series of caffeine and chlorogenic acid every 30 samples, using an adapted response factor for the other chlorogenic acid types. The total chlorogenic acid concentration is reported as the sum of all above individual chlorogenic acids. Data acquisition and processing were performed using ChromQuest, version 4.1 SP2 (Thermo Fisher Scientific, Waltham, MA, USA).

Moisture content determination

To express the data on a dry weight basis instead of on a wet matter basis, the moisture content of the coffee samples was determined in accordance with AOAC procedures.[36]

Statistical analysis

Data were analysed with SAS, version 9.2 (SAS Institute Inc., Cary, NC USA) using the mixed model procedure for a split–split plot design with altitude as the main plot, shade as subplot and harvest period as sub-subplot. Significant differences between treatment means were separated using Tukey's honestly significant difference (HSD) test.

RESULTS

Coffee quality

Significant interactions between altitude and shade were observed for total specialty coffee cup quality and its specific specialty cup quality attributes (overall cup preference, acidity, body, flavour and aftertaste) ($P < 0.01$) (Table 1). Quality scores for total specialty cup quality (86.5), overall cup preference (8.2), acidity (8.3), body (8.2), flavour (8.0) and aftertaste (8.0) were higher for coffee beans grown at high altitude combined with open or medium shade level. The interaction between altitude and harvest period significantly influenced physical quality, overall cup preferences and acidity of coffee beans (Table 2). Coffee beans grown at mid altitude and collected during late harvest period had the lowest physical quality score (34.0), whereas scores for overall cup preferences and acidity were higher for coffee beans grown at high altitude and harvested in early or middle harvest periods (Table 2). Harvest period by shade interactions were not significant for any of the above variables. There were also no three-way interactions between altitude, shade and harvest period on any of the coffee bean quality attributes.

Altitude as a main effect (Table 3) indicated that preliminary cup quality, preliminary total quality, total specialty cup quality and aroma were lower at mid altitude. The scores for each of these quality attributes increased with increasing altitude (Table 3). Coffee beans grown in dense shade had a higher physical quality score (36.7). Coffee grown at open or medium shade gave a higher total specialty cup score (84.5) and overall cup preference (7.9). Beans harvested at early and middle harvest period were generally higher in preliminary cup quality, preliminary total quality, total specialty cup quality, overall cup preference and body scores compared to late harvested beans (Table 3).

100-bean weight, caffeine and total chlorogenic acid content

Three-way interactions between altitude, shade and harvest periods were significant for caffeine content (Table 4). Highest caffeine content (17.9 g kg^{-1}) was obtained from mid altitude with dense shade and early harvested beans, whereas the lowest content (14.5 g kg^{-1}) was from high altitude with medium shade and middle harvested beans (Table 4). No three-way interactions were found either for 100-bean weight or for total chlorogenic acids contents of coffee beans.

Table 1. Interactive effect of altitude (A): high (1950–2100 m a.s.l.), mid (1600–1680 m a.s.l.) and shade (S): open (0%), medium (40–55%) and dense (65–85%) on physical quality (PQ), preliminary cup quality (PCQ), preliminary total quality (PTQ), total specialty cup quality (TSCQ) and specific specialty cup quality scores

						Specific specialty cup quality attributes					
A	S	PQ	PCQ	PTQ	TSCQ	OCP	Acidity	Body	Aroma	Flavour	AT
High	Open	35.6 ± 0.4 a	49.6 ± 0.8 a	85.2 ± 0.8 a	86.5 ± 0.9 a	8.2 ± 0.2 a	8.3 ± 0.1 a	8.2 ± 0.2 a	8.1 ± 0.2 ab	8.0 ± 0.2 a	8.0 ± 0.2 a
	Medium	36.6 ± 0.4 a	49.6 ± 0.7 a	86.2 ± 0.8 a	86.5 ± 0.8 a	8.3 ± 0.1 a	8.3 ± 0.1 a	8.1 ± 0.1 ab	8.2 ± 0.1 a	7.9 ± 0.1 a	8.0 ± 0.2 a
	Dense	36.8 ± 0.6 a	47.6 ± 0.8 a	84.4 ± 0.8 a	83.7 ± 0.7 b	7.6 ± 0.1 b	7.9 ± 0.2 b	7.7 ± 0.1 bc	7.7 ± 0.1 bc	7.6 ± 0.1 b	7.5 ± 0.1 b
Mid	Open	35.4 ± 0.4 a	45.6 ± 0.4 a	81.0 ± 0.7 a	82.6 ± 0.3 b	7.6 ± 0.1 b	7.6 ± 0.1 bc	7.6 ± 0.1 c	7.5 ± < 0.1 c	7.5 ± 0.1 b	7.4 ± 0.1 b
	Medium	36.2 ± 0.4 a	46.6 ± 0.4 a	82.8 ± 0.6 a	83.2 ± 0.3 b	7.6 ± 0.1 b	7.8 ± < 0.1 bc	7.7 ± 0.1 bc	7.6 ± 0.1 c	7.5 ± 0.1 b	7.5 ± < 0.1 b
	Dense	36.6 ± 0.5 a	45.6 ± 0.4 a	82.2 ± 0.7 a	83.4 ± 0.2 b	7.6 ± < 0.1 b	7.8 ± 0.1 ab	7.7 ± 0.1 bc	7.6 ± 0.1 bc	7.6 ± < 0.1 b	7.5 ± 0.1 b
P-value		0.96	0.22	0.29	**0.006**	**<0.0001**	**0.006**	**0.008**	0.023	**0.007**	**0.0025**

OCP, overall cup preference; AT, aftertaste. Different lowercase letters in the same column indicate a significant difference according to Tukey's HSD post-hoc test ($P < 0.01$). Results are shown as the mean ± SE. The bolded values are the significant value i.e., *P*-values are < 0.01.

Table 2. Interactive effect of altitude (A): high (1950–2100 m a.s.l.), mid (1600–1680 m a.s.l.) and harvest periods (HP): early, middle and late on the physical quality (PQ), preliminary cup quality (PCQ), preliminary total quality (PTQ), total specialty cup quality (TSCQ) and specific specialty coffee cup quality scores

						Specific specialty cup quality attributes					
A	HP	PQ	PCQ	PTQ	TSCQ	OCP	Acidity	Body	Aroma	Flavour	AT
High	Early	36.8 ± 0.5 a	49.8 ± 0.7 a	85.6 ± 0.8 a	86.9 ± 0.8 a	8.3 ± 0.2 a	8.5 ± 0.1 a	8.3 ± 0.1 a	8.1 ± 0.1 a	8.1 ± 0.2 a	8.1 ± 0.2 a
	Middle	36.6 ± 0.6 a	49.2 ± 0.6 a	85.8 ± 0.6 a	85.4 ± 0.8 a	8.1 ± 0.2 a	8.2 ± 0.1 ab	7.9 ± 0.1 a	8.0 ± 0.2 a	7.8 ± 0.1 bc	7.8 ± 0.1 a
	Late	34.0 ± 0.4 b	47.8 ± 0.9 a	84.4 ± 0.9 a	84.3 ± 0.8 a	7.7 ± 0.1 b	7.9 ± 0.1 b	7.8 ± 0.1 a	7.9 ± 0.1 a	7.7 ± 0.1 bc	7.8 ± 0.2 a
Mid	Early	36.6 ± 0.5 a	46.8 ± 0.4 a	83.4 ± 0.7 a	83.1 ± 0.3 a	7.6 ± 0.1 b	7.7 ± 0.1 b	7.6 ± 0.1 a	7.6 ± < 0.1 a	7.5 ± 0.1 c	7.5 ± 0.1 a
	Middle	36.6 ± 0.2 a	46.4 ± 0.4 a	83.2 ± 0.4 a	83.3 ± 0.4 a	7.7 ± 0.2 b	7.8 ± 0.1 b	7.7 ± 0.1 a	7.5 ± 0.1 a	7.5 ± 0.1 c	7.4 ± 0.1 a
	Late	34.0 ± 0.4 b	44.6 ± 0.3 a	79.4 ± 0.4 a	82.7 ± 0.2 a	7.6 ± 0.1 b	7.7 ± < 0.1 b	7.5 ± < 0.1 a	7.5 ± < 0.1 a	7.5 ± < 0.1 c	7.5 ± 0.1 a
P-value		**0.004**	0.93	0.067	0.29	**0.007**	**0.0008**	0.09	0.86	0.012	0.35

OCP, overall cup preference; AT, aftertaste. Different lowercase letters in the same column indicate a significant difference according to Tukey's HSD post-hoc test ($P < 0.01$).
Results are shown as the mean ±SE. The bolded values are the significant value i.e., *P*-values are < 0.01.

Altitudes by shade interactions were significant for total chlorogenic acid content ($P < 0.01$) but not for 100-bean weight and caffeine contents. Total chlorogenic acid contents decreased from mid to high altitude and from dense to open shading at high altitude (Table 5). Coffee beans from mid altitude and open sun had a higher total chlorogenic acid content (46.5 g kg^{-1}), whereas beans from high altitude and open shading gave 40.5 g kg^{-1}. The interactions between altitude and harvest period were not significant for any of the 100-bean weight, caffeine or total chlorogenic acid content. The interactions between shade and harvest periods had a significant effect on 100-bean weight but not on caffeine and total chlorogenic acid content (Table 6). Coffee beans produced in dense or medium shade gave higher bean weights at any harvest period.

The main effects are given in Table 7. Altitude affected both the caffeine content and 100-bean weight. Coffee beans produced at mid altitude had a higher caffeine content (17.2 g kg^{-1}) than beans produced at high altitude (15.5 g kg^{-1}), whereas the bean weight was 16.9 and 14.1 g at high and mid altitude, respectively. Beans grown at medium or dense shade were approximately 10% heavier than beans in open sun. By contrast, the harvest period as main factor had no effect on 100-bean weight, caffeine and total chlorogenic acid content.

DISCUSSION

Several studies have reported influences of altitude[17,27] and shade[19,26,37,38] on coffee bean quality. The present study, however, expanded the scope and clarified the interaction of altitude, shade and harvest periods on physical quality, preliminary cup quality, preliminary total quality, total specialty cup quality and its specific cup quality attributes (acidity, body, aroma), 100-bean weight and the biochemical composition (caffeine and chlorogenic acid content) of Ethiopian coffee beans. Caffeine and chlorogenic acid are considered to have a direct impact on human health. In addition, these two chemicals are key biochemical components of coffee beans with respect to determining its beverage quality. The present study further discussed the effects of altitude, shade and harvest periods on both components.

Total specialty cup quality and its specific quality attributes (e.g. overall cup preference, acidity and flavour) increased with both altitude and shade level. At high altitude, however, dense shade decreased green bean cup quality and the percentage of specialty 1 (Q1) beans by approximately 50% (Table 8). Coffee beans grown in cooler environments accumulate sufficient sugars and lipids.[37,39] Both are key compounds with respect to determining coffee as a beverage and are positively correlated with bean quality.[2,20] Dense shade, however, further decreased air temperature around the coffee fruits. This effect in combination with the effect of altitude on temperature could reduce growing temperature to a

Table 3. Physical quality (PQ), preliminary cup quality (PCQ), preliminary total quality (PTQ), total specialty cup quality (TSCQ) and specific specialty cup quality scores of coffee beans as affected by altitude (A): high (1950–2100 m a.s.l.), mid (1600–1680 m a.s.l.), shade (S): open (0%), medium (40–55%) and dense (65–85%) and harvest periods (HP): early, middle and late

					Specific specialty cup quality attributes					
Factor	PQ	PCQ	PTQ	TSCQ	OCP	Acidity	Body	Aroma	Flavour	AT
A High	36.3 ± 0.3^{a}	48.9 ± 0.5^{a}	85.2 ± 0.5^{a}	85.5 ± 0.5^{a}	8.0 ± 0.1^{a}	8.2 ± 0.1^{a}	7.9 ± 0.1^{a}	7.9 ± 0.1^{a}	7.9 ± 0.1^{a}	7.9 ± 0.1^{a}
Mid	36.1 ± 0.3^{a}	45.9 ± 0.3^{b}	82.6 ± 0.4^{b}	83.0 ± 0.2^{b}	$7.6 \pm < 0.1^{b}$	7.7 ± 0.1^{b}	$7.6 \pm < 0.1^{b}$	$7.5 \pm < 0.1^{b}$	$7.5 \pm < 0.1^{b}$	7.5 ± 0.1^{b}
P-value	0.57	**<0.0016**	**<0.0025**	**<0.004**	<0.024	<0.012	**0.011**	**<0.008**	0.03	0.016
S Open	35.5 ± 0.3^{b}	47.6 ± 0.6^{ab}	83.3 ± 0.7^{a}	84.5 ± 0.6^{ab}	7.9 ± 0.1^{a}	7.9 ± 0.1^{a}	7.9 ± 0.1^{a}	7.8 ± 0.1^{a}	7.7 ± 0.1^{a}	7.7 ± 0.1^{ab}
Medium	36.4 ± 0.3^{ab}	48.1 ± 0.5^{a}	84.5 ± 0.6^{a}	85.7 ± 0.5^{a}	7.9 ± 0.1^{a}	8.0 ± 0.1^{a}	7.9 ± 0.1^{a}	7.8 ± 0.1^{a}	7.7 ± 0.1^{a}	7.8 ± 0.1^{a}
Dense	36.7 ± 0.4^{a}	46.6 ± 0.5^{b}	83.1 ± 0.6^{a}	83.6 ± 0.4^{b}	7.6 ± 0.1^{b}	7.9 ± 0.1^{a}	7.7 ± 0.1^{a}	7.7 ± 0.1^{a}	7.6 ± 0.1^{a}	7.5 ± 0.1^{b}
P-value	**0.008**	0.033	0.06	**0.009**	**0.0003**	0.37	0.055	0.148	0.17	0.04
HP Early	36.2 ± 0.4^{ab}	48.3 ± 0.5^{a}	84.5 ± 0.6^{a}	84.9 ± 0.6^{a}	7.9 ± 0.1^{a}	8.1 ± 0.1^{a}	7.9 ± 0.1^{a}	7.8 ± 0.1^{a}	7.8 ± 0.1^{a}	7.8 ± 0.1^{a}
Middle	36.7 ± 0.3^{a}	47.8 ± 0.5^{a}	84.5 ± 0.4^{a}	84.4 ± 0.5^{a}	7.9 ± 0.1^{a}	7.9 ± 0.1^{ab}	7.8 ± 0.1^{ab}	7.8 ± 0.1^{a}	7.7 ± 0.1^{ab}	7.6 ± 0.1^{a}
Late	35.7 ± 0.3^{b}	46.2 ± 0.6^{b}	81.9 ± 0.7^{b}	83.5 ± 0.4^{b}	7.6 ± 0.1^{b}	7.8 ± 0.1^{b}	7.8 ± 0.1^{b}	7.7 ± 0.1^{a}	7.6 ± 0.1^{b}	7.6 ± 0.1^{a}
P-value	0.06	**0.004**	**0.0009**	**0.003**	**0.006**	**0.005**	0.027	0.37	0.04	0.32

OCP, overall cup preference; AT, aftertaste. Different lowercase letters in the same column indicate a significant difference according to Tukey's HSD post-hoc test ($P < 0.01$).
Results are shown as the mean ±SE. The bolded values are the significant value i.e., *P*-values are < 0.01.

Table 4. Interactive effect of altitude (A): high (1950–2100 m a.s.l.), mid (1600–1680 m a.s.l.), shade (S): open (0%), medium (40–55%) and dense (65–85%) and harvest periods (HP): early, middle and late on caffeine content

Altitude	Shade	Harvest period	Caffeine (g kg^{-1})
High	Open	Early	15.9 ± 0.2 de
		Middle	15.1 ± 0.3 ef
		Late	15.1 ± 0.4 ef
	Medium	Early	17.1 ± 0.2 abc
		Middle	14.5 ± 0.3 f
		late	15.1 ± 0.5 ef
	Dense	Early	15.0 ± 0.3 ef
		Middle	16.4 ± 0.4 cd
		Late	15.2 ± 0.3 fe
Mid	Open	Early	16.7 ± 0.3 bcd
		Middle	17.2 ± 0.3 abc
		Late	16.4 ± 0.3 cd
	Medium	Early	17.1 ± 0.5 abc
		Middle	17.8 ± 0.4 a
		Late	16.9 ± 0.3 a–d
	Dense	Early	17.9 ± 0.1 a
		Middle	17.7 ± 0.5 b
		Late	17.1 ± 0.4 abc
P-value			**0.0002**

Different lowercase letters in the same column indicate a significant difference according to Tukey's HSD post-hoc test ($P < 0.01$). Results are shown as the mean ± SE; expressed as g kg^{-1} on a dry weight basis. The bolded values are the significant value i.e., *P*-values are < 0.01.

Table 5. Interactive effect of altitude (A): high (1950–2100 m a.s.l.), mid (1600–1680 m a.s.l.) and shade (S): open (0%), medium (40–55%) and dense (65–85%) on 100-bean weight, caffeine and total chlorogenic acid content

A	S	100-bean weight (g)	Caffeine (g kg^{-1})	Total chlorogenic acid (g kg^{-1})
High	Open	16.1 ± 0.3 a	15.4 ± 0.2 a	40.5 ± 0.9 c
	Medium	17.5 ± 0.3 a	15.6 ± 0.4 a	42.7 ± 0.8 bc
	Dense	17.0 ± 0.2 a	15.6 ± 0.2 a	43.2 ± 0.7 bc
Mid	Open	13.3 ± 0.2 a	16.8 ± 0.2 a	46.5 ± 0.8 a
	Medium	14.2 ± 0.2 a	17.3 ± 0.2 a	43.7 ± 0.86 b
	Dense	14.7 ± 0.2 a	17.6 ± 0.2 a	45.4 ± 1.0 ab
P-value		0.16	0.34	**0.0046**

Different lowercase letters in the same column indicate a significant difference according to Tukey's HSD post-hoc test ($P < 0.01$). Results are shown as the mean ± SE; expressed as g kg^{-1} on a dry weight for caffeine and total chlorogenic acid. The bolded values are the significant value i.e., *P*-values are < 0.01.

level below optimum (i.e. 18 °C),[40] resulting in reduced coffee bean quality. In areas where air temperature is relatively cooler, such as at high altitudes, shade levels higher than 40–50% are hence not beneficial because growing temperature may decrease below optimum.

The ideal growing temperature of Arabica coffee ranges between 18 and 21 °C,[41] and values above or below this optimum level predispose coffee beans to incomplete maturation and poor quality. Bosselmann *et al.*[23] also reported that, at high altitudes, lower temperatures as a result of excess shade from trees resulted in reduced coffee bean quality. By contrast, at mid to low altitude, higher temperature induces the accumulation of chemical compounds such as butan-1,3-diol and butan-2,3-diol in coffee beans, which reduces quality attributes (aroma, acidity).[42] At high altitude, differences between open and medium shade levels on coffee bean quality were not significant. This result was in agreement with previous findings reported by Guyot *et al.*[27] and Bosselmann *et al.*[23] This indicates that the temperature around coffee trees grown under both shade levels was still within the optimum range for coffee growth[23] or allows better exposure of the trees to sunlight. At mid altitude, on the other hand, shade was observed not to have any significant effect on coffee bean quality. This might be related to leaf fall from the shade tree during the dry season, indicating that farmers should consider the best adaptable shade tree type to grow coffee under consistent shade level.

Table 6. Interactive effect of shade (S): open (0%), medium (40–55%) and dense (65–85%) and harvest periods (HP): early, middle and late on 100-bean weight, caffeine and total chlorogenic acid content

S	HP	100-bean weight (g)	Caffeine (g kg^{-1})	Total chlorogenic acid (g kg^{-1})
Open	Early	13.7 ± 0.3 bc	16.3 ± 0.2 d	44.9 ± 1.2 a
	Middle	15.2 ± 0.3 b	16.2 ± 0.4 cd	43.1 ± 1.5 a
	Late	15.2 ± 0.6 b	15.7 ± 0.3 bcd	42.4 ± 1.4 a
Medium	Early	15.9 ± 0.5 a	17.2 ± 0.3 a	44.3 ± 1.0 a
	Middle	15.8 ± 0.5 a	16.1 ± 0.6 d	42.3 ± 0.9 a
	Late	15.8 ± 0.5 a	15.9 ± 0.4 cd	42.9 ± 0.8 a
Dense	Early	15.8 ± 0.4 a	16.5 ± 0.5 abc	45.5 ± 1.2 a
	Middle	15.8 ± 0.5 a	17.0 ± 0.4 ab	42.1 ± 1.1 a
	Late	15.9 ± 0.5 a	16.1 ± 0.4 cd	45.1 ± 0.7 a
P-value		**0.009**	0.049	0.35

Different lowercase letters in the same column indicate a significant difference according to Tukey's HSD post-hoc test ($P < 0.01$). Results are shown as the mean ± SE; expressed as g kg^{-1} on a dry weight for caffeine and total chlorogenic acid. The bolded values are the significant value i.e., *P*-values are < 0.01.

Table 7. 100-bean weight, caffeine and total chlorogenic acid as affected by altitude (A): high (1950– 2100 m a.s.l.) and mid (1600–1680 m a.s.l.), shade (S): open (0%), medium (40–55%) and dense (65–85%) and harvest periods (HP): early, middle and late

Factor	Level	100-bean weight (g)	Caffeine (g kg^{-1})	Total chlorogenic acid (g kg^{-1})
A	High	16.9 ± 0.2 a	15.5 ± 0.2 b	42.1 ± 0.5 b
	Mid	14.1 ± 0.2 b	17.2 ± 0.1 a	45.2 ± 0.5 a
P-value		**0.0009**	**<0.0001**	0.012
S	Open	14.7 ± 0.3 b	16.1 ± 0.2 a	43.5 ± 0.8 a
	Medium	15.9 ± 0.3 a	16.4 ± 0.3 a	43.2 ± 0.6 a
	Dense	15.9 ± 0.3 a	16.6 ± 0.3 a	44.3 ± 0.7 a
P-value		**<0.0001**	0.059	0.311
HP	Early	15.2 ± 0.3 a	16.7 ± 0.2 a	44.9 ± 0.7 a
	Middle	15.6 ± 0.3 a	16.5 ± 0.3 ab	42.5 ± 0.8 b
	Late	15.6 ± 0.4 a	15.9 ± 0.2 b	43.5 ± 0.6 ab
P-value		0.27	0.015	0.039

Different lowercase letters in the same column indicate a significant difference according to Tukey's HSD post-hoc test ($P < 0.01$). Results are shown as the mean ± SE; expressed as g kg^{-1} on a dry weight for caffeine and total chlorogenic acid. The bolded values are the significant value i.e., *P*-values are < 0.01.

Table 8. Percentage of coffee samples that are ranked as specialty 1 (Q1) and specialty 2 (Q2) grown at different altitude (high and mid), shade levels (open, medium and dense)[a] and harvest period (early, middle and late)[b]

Altitude			Percentage of specialty coffee: Specialty 1 (Q1)	Specialty 2 (Q2)
High	Shade level	Open	53.3	46.7
		Medium	66.7	33.3
		Dense	33.3	66.7
Mid	Shade level	Open	6.7	93.3
		Medium	13.3	86.7
		Dense	6.7	93.3
High	Harvest period	Early	73.0	27.0
		Middle	53.0	47.0
		Late	27.0	73.0
Mid	Harvest period	Early	7.0	93.0
		Middle	20.0	80.0
		Late	7.0	93.0

[a] Each value is averaged across different harvest period (early, middle and late). [b] Each value is averaged across different shade levels (open, medium and dense).

At present, air temperature is also increasing as a result of global climate change. Besides reducing coffee bean quality, this increase in air temperature is reducing the area suitable for Arabica coffee production.[43,44] Growing coffee trees at higher altitude or under tailored shading conditions could be implemented as a possible approach to adapt to the effects of climate change and to sustain the production of high quality coffee beans. However, in our study area, the high altitude area is limited. Hence, shading adaptation has to be considered in view of climate change.

Beans harvested at early and mid harvest period were generally higher in preliminary cup quality, preliminary total quality, total specialty cup quality, overall cup preference and body scores compared to late harvested beans. For example, at high altitude (Table 8), early harvesting led to 73% of specialty 1 (Q1) coffee beans compared to 27% for late harvest. Better bean quality as a result of early harvest was also reported in other studies.[28,45] This is possibly a result of the depletion of photo-assimilates because of competition among fruits, which finally leads to a shortage of photo-assimilates for the late developing fruits.[37] In addition, the production of coffee quality precursor compounds, which accumulate in coffee beans, is markedly high during endosperm expansion and dry matter accumulation stages.[46] At a later stage of bean development, however, most of these quality precursors are remobilized towards lignin biosynthesis and drop in relative content.[47] At mid altitude, the harvest period did not significantly influence coffee bean quality. This is probably a result of temperature at mid altitude being higher (approximately 4 °C) compared to temperature at high altitude. High temperature accelerates bean ripening and automatically results in shorter time intervals between the different harvest periods.

Coffee bean weight increased with increasing altitude. The result is in agreement with previous studies.[37] Coffee beans grown at higher altitudes mature slowly and are generally harvested 1–2 months later than beans from mid altitudes. A slower maturation of coffee beans allows better bean filling (e.g. more lipid accumulation).[2,19] It is also reported that environmental factors and agricultural managements modify the physiology of coffee fruit development and ripening.[48] Among environmental factors, temperature plays a significant role in regulating bean maturation and ripening processes.[27] The results of the present study support the notion that low temperature slows down the ripening process (resulting in a delayed harvesting period by approximately 50 days on average) and allows better bean filling, resulting in heavier beans.[37]

Shading also promotes bean weight. Coffee beans grown under medium or dense shade are heavier than beans grown in open sun. Beans grown under shaded conditions mature slower because shading also lowers the temperature around the coffee fruits and, in general, the beans are harvested 2–4 weeks later than beans in open sun,[23,37] In addition, shade has also been reported to reduce the number of floral initiations per plant and allow more assimilate partitioning to each developing bean.[23] Partitioning of more assimilates to individual beans increases coffee bean weight.

Caffeine contents of our coffee samples ranged from 14.5 to 17.9 g kg^{-1} (Table 4). The results are in agreement with previous studies by Silvarolla *et al.*,[49] Ky *et al.*[50] and Bekele.[51] However, these values varied by interaction effects of altitude, shade and harvest periods. Early harvested beans from mid altitude grown under dense shade contained the highest amount of caffeine, whereas the lowest caffeine content was found at high altitude, medium shading and middle harvest. In coffee bean development, biosynthesis and accumulation of most chemicals, including caffeine, is markedly higher at the early development stage and is reduced at a later stage of ripening.[46] At the later stage of bean development, the compounds are also remobilized towards lignin biosynthesis and their contents are relatively decreased compared to contents at early bean development stages.[46,47] The results of the present study corroborate these findings and indicate that early harvested beans contain more caffeine than late harvested ones.

The present study also demonstrated the influence of altitude on caffeine content. An increase in altitude by 400 m decreased the caffeine content by 10% (Table 7). Sridevi and Parvatam[52] reported similar findings, whereas Avelino *et al.*[17] and Bertrand *et al.*[53] on the other hand, reported the opposite. Bertrand *et al.*[53] showed that an increase in altitude by 500 m increased caffeine content by 15%. In the study by Bertrand *et al.*,[53] however, plots from very low altitude (e.g. 700 to 1600 m a.s.l.) were also included. This might explain such discrepancies.

Chlorogenic acids are the main phenolic compounds in coffee beans, ranging from 6% to 12% on a dry weight basis.[54] In the present study, total chlorogenic acid content (sum of four subclasses) varied from 40.5 to 45.2 g kg^{-1} on a dry weight basis (Table 5). These values are generally lower than values previously reported for coffee,[55,56] but much higher than values reported by Ky *et al.*[50] and Bekele.[51] In addition, we found that the values were affected by variation in altitude and shade. Total chlorogenic acid content decreased from mid to high altitude and from dense to open shading at high altitude. At mid altitude, however, coffee beans from mid altitude and open sun accumulated more chlorogenic acid.

Growing temperature has a direct influence on production and accumulation of chlorogenic acids.[39] The higher temperature at mid altitude probably resulted in higher chlorogenic acid production and accumulation in coffee beans grown at mid than at high altitude. A higher chlorogenic acid content indicates that coffee beans are relatively immature.[55] These differences in chlorogenic acid contents and bean maturation resulted in coffee bean quality differences (more Q1 coffees at high than at mid altitude). The result was in agreement with results reported by Avelino *et al.*[56] and Link *et al.*[55] However, studies by Bertrand *et al.*,[53] reported the opposite. In the present study, the altitude ranged from 1600 to 2010 m a.s.l. In the study by Bertrand *et al.*[53] plots from very low altitude (e.g. 700 to 1600 m a.s.l.) were also included. This might explain such discrepancies.

The present study generally indicates that coffee growing at high altitude, in open or medium shade, and harvested at an early or middle period, shows enhanced potential for producing specialty 1 (Q1) coffee (Table 8). These growing conditions also favour the production of coffee beans with lower caffeine and chlorogenic acid contents. Coffee beans with lower caffeine but higher chlorogenic acid contents are appreciated and preferred in many consumer countries because of their positive health effect. Chlorogenic acid acts as antioxidant and has radical scavenging properties. It makes coffee an acceptable beverage. It also has anti-pathogenic and allelophatic properties[54] and is described as being important for disease resistance in coffee beans.[57]

Coffee beans grown at mid altitude had lower bean quality compared to high altitude, and were grouped mostly as Q2 specialty coffees. This indicates that there is scope for mid altitude farmers, which occupy most of the area, to produce more Q1 coffee beans. For example, growing trees under medium-shaded conditions and selectively harvesting the beans at mid harvest could potentially increase the percentage of Q1 coffees (Table 8).

CONCLUSIONS

Overall, the results of the present study show that growing coffee at high altitude with open or medium shade, as well as an early or middle harvest period, increased the potential of producing beans with superior quality. In general, small changes of quality attributes, driven by altitude, shading and harvest periods, can cause substantial switches in Q1 versus Q2 classification. This quality segmentation affects coffee bean price and farmers' competitiveness in international markets. The present study, however, did not investigate year-by-year variations and soil type effects. Future studies are needed to integrate these impacts.

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REFERENCES

1 Pendergrast M, Coffee: second to oil? *TCTJ* **181**:38 (2009).
2 Lara-Estrada L and Vaast P, Effects of altitude, shade, yield and fertilization on coffee quality (Coffea arabica L. var. Caturra) produced in agroforestry systems of the Northern Central Zones of Nicaragua, in *Second International Symposium on Multi-Strata Agroforestry Systems with Perennial Crops: Making Ecosystem Services Count for Farmers, Consumers and the Environment*, Turrialba, Costa Rica, pp. 17–21 (2007).
3 Jeszka-Skowron M, Zgoła-Grześkowiak A and Grześkowiak T, Analytical methods applied for the characterization and the determination of bioactive compounds in coffee. *Eur Food Res Technol* **240**:19–31 (2015).
4 Agwanda C, Baradat P, Eskes A, Cilas C and Charrier A, Selection for bean and liquor qualities within related hybrids of Arabica coffee in multilocal field trials. *Euphytica* **131**:1–14 (2003).
5 Joët LA, Descroix F, Doulbeau S, Bertrand B and Dussert S, Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. *Food Chem* **118**:693–701 (2010).
6 Oberthür T, Läderach P, Jurgen PHA and Cock JH, eds, *Specialty Coffee: Managing Quality*. Internation Plant Nutrition Institute, Southeast Asia Program, Penang, Malaysia (2012).
7 Läderach P, Oberthür T, Cook S, Iza ME, Pohlan JA, Fisher M *et al.*, Systematic agronomic farm management for improved coffee quality. *Field Crops Res* **120**:321–329 (2011).
8 Wood L, *Research and Markets: Coffee Market in Brazil 2015–2019 – High Demand for Arabica Coffee, USA*. Technavio, Elmhurst, USA (2015).
9 Labouisse JP, B Bellachew, Kotecha S and Bertrand B, Current Status of Coffee *(Coffea arabica* L.*)* Genetic Resources in Ethiopia:

Implications for Conservation. Genetic Resources and Crop Evolution **55**:1079–1093 (2008).
10 Bart M, Seneshaw T, Tadesse K and Yaw N, Structure and performance of Ethiopia's coffee export sector. ESSP II Working Paper 66. Washington, DC and Addis Ababa, Ethiopia: International Food Policy Research Institute (IFPRI) and Ethiopian Development Research Institute (EDRI) (2014).
11 ICCO, Middle Africa briefing note, soft commodities: coffee, in *Ecobank*. The Pan African Bank, Edward George, London (2014).
12 Coste R, Cambrony H and Tindall H, *Coffee: The Plant and the Product*. Macmillan, London (1992).
13 MFA, *The 4th World Coffee Conference in Addis Ababa*, Ministry of Foreign Affairs of Ethiopia, Addis Ababa, Ethiopia (2016).
14 Robert S, *Frontlines: Foreign Aid Impact in US and Abroad, in Frontlines*. USID, Washington, DC, USA, p. 17 (2015).
15 Ponte S, The 'latte revolution'? Regulation, markets and consumption in the global coffee chain. *World Dev* **30**:1099–1122 (2002).
16 Leroy T, Ribeyre F, Bertrand B, Charmetant P, Dufour M, Montagnon C *et al.*, Genetics of coffee quality. *Braz J Plant Physiol* **18**:229–242 (2006).
17 Avelino J, Barboza B, Araya JC, Fonseca C, Davrieux F, Guyot B *et al.*, Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa María de Dota. *J Sci Food Agric* **85**:1869–1876 (2005).
18 Decazy F, Avelino J, Guyot B, Perriot JJ, Pineda C and Cilas C, Quality of different Honduran coffees in relation to several environments. *J Food Sci* **68**:2356–2361 (2003).
19 Muschler RG, Shade improves coffee quality in a sub-optimal coffee-zone of Costa Rica. *Agrofor Syst* **51**:131–139 (2001).
20 Vaast, Kanten Rv, Siles P, Dzib B, Franck N, Harmand J and Genard M, Shade: a key factor for coffee sustainability and quality, in *ASIC 2004 20th International Conference on Coffee Science, Bangalore, India*, 11–15 October 2004, Association Scientifique Internationale du Café (ASIC), Bangalore, India, pp. 887–896 (2005).
21 Selmar D, Bytof G, Knopp SE and Breitenstein B, Germination of coffee seeds and its significance for coffee quality. *Plant Biol* **8**:260–264 (2006).
22 Alemu MM, Effect of tree shade on coffee crop production. *J Sust Dev* **8**:66 (2015).
23 Bosselmann A, Dons K, Oberthur T, Olsen CS, Ræbild A and Usma H, The influence of shade trees on coffee quality in small holder coffee agroforestry systems in Southern Colombia. *Agric Ecosyst Environ* **129**:253–260 (2009).
24 Yadessa A, Burkhardt J, Denich M, Woldemariam T, Bekele E and Goldbach H, Effect of different indigenous shade trees on the quality of wild arabica coffee in the Afromontane rainforests of Ethiopia, in *22nd International Conference on Coffee Science, ASIC 2008*. Campinas, SP, Brazil, 14–19 September 2008. Association Scientifique Internationale du Café (ASIC), Campinas, Brazil, pp. 1227–1233 (2009).
25 Kufa T, Yilma A, Shimber T, Netsere A and Taye E, Yield performance of Coffea arabica cultivars under different shade trees at Jimma Research Center, Southwest Ethiopia, in *Proceedings of the Second International Symposium on Multi-strata Agroforestry Systems with Perennial Crops*, Turrialba, Costa Rica, Turrialba, Costa Rica (2007).
26 Bote AD and Struik PC, Effects of shade on growth, production and quality of coffee (Coffea arabica) in Ethiopia. *J Hortic For* **3**:336–341 (2011).
27 Guyot B, Gueule D, Manez J, Perriot J, Giron J and Villain L, Influence de l'altitude et de l'ombrage sur la qualité des cafés arabica. *Plant Rech Dév* **3**:272–280 (1996).
28 Bertrand B, Etienne H, Guyot B and Vaast P, Year of production and canopy region influence bean characteristics and beverage quality of Arabica coffee, in *ASIC 2004 20th International Conference on Coffee Science*, Bangalore, India, 11–15 October 2004. Association Scientifique Internationale du Café (ASIC), Paris, France, pp. 878–886 (2005).
29 JARC, *Recommended Production Technologies for Coffee and Associated.* Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa, Ethiopia (1996).
30 ARDO, *Annual Report of Agriculture and Rural Development Office of Manna Woreda, Yebbu, Manna.* ARDO, Addis Ababa, Ethiopia (2008).
31 John AK, *ECX Quality Operation Manual*., Ethiopia Commodity Exchange (ECX), Addis Ababa, Ethiopia (2011).
32 Santos JR, Sarraguça MC, Rangel AOSS and Lopes JA, Evaluation of green coffee beans quality using near infrared spectroscopy: A quantitative approach. *Food Chem* **135**:1828–1835 (2012).
33 Lemma B, *ECX Adopts Five Level Coffee Quality Grading System*. Ethiopia Commodity Exchange (ECX), Addis Ababa, Ethiopia (2015).
34 Alonso-Salces , Rosa M, Serra F, Reniero F and Héberger K, Botanical and geographical characterization of green coffee (Coffea arabica and Coffea canephora): chemometric evaluation of phenolic and methylxanthine contents. *J Agric Food Chem* **57**:4224–4235 (2009).
35 Alonso-Salces , Maria R, Guillou C and Berrueta LA, Liquid chromatography coupled with ultraviolet absorbance detection, electrospray ionization, collision induced dissociation and tandem mass spectrometry on a triple quadrupole for the on line characterization of polyphenols and methylxanthines in green coffee beans. *Rapid Commun Mass Spectrom* **23**:363–383 (2009).
36 AOAC, *Official Methods of Analysis of the Association of Official Analytical Chemists*, 17th edn. Association of Official Analytical, Gaithersburg, MD, USA (2000).
37 Vaast, Bertrand B, Perriot JJ, Guyot B and Génard M, Fruit thinning and shade improve bean characteristics and beverage quality of coffee (*Coffea arabica* L.) under optimal conditions. *J Sci Food Agric* **86**:197–204 (2006).
38 Geromel C, Ferreira LP, Guerreiro SMC, Cavalari AA, Pot D, Pereira LFP *et al.*, Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. *J Exp Bot* **57**:3243–3258 (2006).
39 Wintgens JN (ed.). *The Coffee Plant. Coffee: Growing, Processing, Sustainable Production: A Guidebook for Growers, Processors, Traders, and Researchers.* Wiley-VCH, Weinheim, Germany, pp. 1–24 (2004).
40 Cortez J, Aptidão climática para a qualidade da bebida nas principais regiões cafeeiras de Minas Gerais. *Informe Agropecuário* **18**:27–31 (1997).
41 Camargo A, Santinato R and Cortez J, Aptidao climática para qualidade da bebida nas principais regioes cafeeiras de café Arábica, in CONGRESSO Brasileiro de Pesquisas Cafeeiras, 18 Araxá (Brasil)*, 27–30 Outubro 1992*. Trabalhos Apresentados, Brazil (1992).
42 Bertrand B, Boulanger R, Dussert S, Laffargue A, Ribeyre F, Berthiot L *et al.*, Climatic factors directly impact the biochemical composition and the volatile organic compounds fingerprint in green Arabica coffee bean as well coffee beverage quality, in *International Conference on Coffee Science*, pp. 628–635 (2012).
43 Davis AP, Gole TW, Baena S and Moat J, The impact of climate change on indigenous arabica coffee *(Coffea arabica)*: predicting future trends and identifying priorities. *PLoS One* **7**:e47981 (2012).
44 Bunn C, Läderach P, Rivera OO and Kirschke D, A bitter cup: climate change profile of global production of Arabica and Robusta coffee. *Clim Change* **129**:89–101 (2015).
45 Oberthür T, Läderach P, Posada H, Fisher MJ, Samper LF, Illera J *et al.*, Regional relationships between inherent coffee quality and growing environment for denomination of origin labels in Nariño and Cauca, Colombia. *Food Policy* **36**:783–794 (2011).
46 Koshiro Y, Zheng XQ, Wang ML, Nagai C and Ashihara H, Changes in content and biosynthetic activity of caffeine and trigonelline during growth and ripening of *Coffea arabica and Coffea canephora* fruits. *Plant Sci* **171**:242–250 (2006).
47 Joët L, Andréina , Salmona J, Doulbeau S, Descroix F, Bertrand B *et al.*, Metabolic pathways in tropical dicotyledonous albuminous seeds: *Coffea arabica* as a case study. *New Phytol* **182**:146–162 (2009).
48 De Castro RD and Marraccini P, Cytology, biochemistry and molecular changes during coffee fruit development. *Braz J Plant Physiol* **18**:175–199 (2006).
49 Silvarolla MB, Mazzafera P, Lima MMAL, Caffeine content of Ethiopian *Coffea arabica* beans. *Genetics and Molecular Biology* **23**:213–215 (2000).
50 Ky CL, Louarn J, Dussert S, Guyot B, Hamon S and Noirot M, Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica L.* and *C. canephora* P. accessions. *Food Chem* **75**:223–230 (2001).
51 Bekele YD, Assessment of cup quality, morphological, biochemical and molecular diversity of Coffea arabica L. genotypes of Ethiopia, University of the Free State (2005).
52 Sridevi V and Parvatam G, Changes in caffeine content during fruit development in *Coffea canephora* P. ex. Fr. grown at different elevations. *J Biol Earth Sci* **4**:B168–B175 (2014).
53 Bertrand B, Vaast P, Alpizar E, Etienne H, Davrieux F and Charmetant P, 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 Physiol* **26**:1239–1248 (2006).
54 Aerts RJ and Baumann TW, Distribution and utilization of chlorogenic acid in Coffea seedlings. *J Exp Bot* **45**:497–503 (1994).
55 Link JV, Lemes ALG, Marquetti I, dos Santos Scholz MB and Bona E, Geographical and genotypic segmentation of arabica coffee using self-organizing maps. *Food Res Int* **59**:1–7 (2014).
56 Avelino J, Barboza B, Davrieux F and Guyot B, Shade effect on sensory and chemical characteristics of coffee from very high altitude plantations in Costa Rica, in *Second* International Symposium on Multi-strata Agroforestry Systems with Perennial Crops, *17–21 September 2007*, Turrialba, Costa Rica, Turrialba, Costa Rica (2007).
57 Dessalegn Y, Labuscagne M, Osthoff G and Herselman L, Variation of green bean caffeine, chlorogenic acid, sucrose and trigolline contents among Ethiopian Arabica coffee accessories. *SINET: Ethiop J Sci* **30**:77–82 (2007).

