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Research Article

Biochemical Components of Shaded Coffee under Different Management Levels

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Abstract: This study was conducted to evaluate the effect of management and shade levels on some biochemical components of coffee. The study was carried out at the Kenya Agricultural and Livestock Research Organization, Coffee Research Institute (KALRO-CRI) coffee farm in Bungoma County and two farmers' fields in Bungoma County, representing high, medium and low management levels. The coffee management levels were categorized depending on field operations and application of inputs. The different shade levels were based on the distances from the shade tree trunk: 0-1.5, 1.5-3, 3-4.5, 4.5-6 m, respectively and coffee trees under full sun. The shading level was estimated by measuring the Photosynthetic Photon Flux Density in μmol m^{-2}/s using a Line Quantum Sensor and expressing it as a percentage of that obtained under full sun. Fully ripe cherries were harvested, wet processed and the wet parchment dried to final moisture content of 10.5 to 11%. Caffeine, trigonelline, total chlorogenic acids, oil and sucrose were determined using specific methodologies and quantified on dry weight basis. The results showed that biochemical components were affected significantly due to shade and management levels. Most of the biochemical components were positively correlated with shade and management levels. This showed the possibility of manipulating the two parameters to enhance the quality of coffee.

Keywords: Caffeine, chlorogenic acids, management level, sucrose, shade level, trigonelline

INTRODUCTION

Coffee beans are the seeds of a perennial evergreen tropical plant, which belongs to the *Rubiceae* family and the genus *Coffea* (Davies *et al*., 2006). Green coffee beans contain a wide range of different chemical compounds which react and interact at all stages of coffee processing to produce a final product with an even greater diversity and complexity of structure (Clifford, 1985). The coffee beverage quality is based on the characterization of numerous factors including taste and aroma (Kathurima *et al*., 2009) which are related to biochemical contents of roasted coffee beans. The key biochemical compounds in coffee are caffeine, oils, trigonelline, sucrose and chlorogenic acids (Farah *et al*., 2006; Gichimu *et al*., 2014).

Caffeine has been related to the pharmacological effects of coffee (Trugo *et al*., 1985) and trigonelline has been associated with flavour formation during coffee roasting (Ky *et al*., 2001). Chemically, caffeine remains stable during coffee roasting except for minute amounts that sublime although roasting has been reported to cause a reduction in caffeine content (Franca *et al*., 2005; Hecˇimovic´ *et al*., 2011). Demethylation of trigonelline during coffee roasting generates nicotinic acid, a water-soluble B vitamin also known as niacin (Ky *et al*., 2001). N-Methylpyridinium (NMP) is a thermal degradation product of trigonelline formed upon coffee roasting and hypothesized to exert several health benefits in humans (Riedel *et al*., 2014). Other compounds formed during the degradation include pyridine, 3 methyl pyridine, nicotinic acid methyl ester which are important as they affect the overall aroma of brewed coffee (Sridevi and Giridhar, 2013).

Chlorogenic Acids (CGA) are an important group of non-volatile compounds in green coffee. They are esters of *trans*-cinnamic acids, such as caffeic, ferulic and *p*-coumaric acids, with Quinic Acid (QA) (Clifford, 2000). They play an important role in the formation of roasted coffee flavour and have an influence in determining coffee cup quality (Farah *et al*., 2006). They are known to be responsible for coffee pigmentation, aroma formation, bitterness and astringency (De Maria *et al*., 1995).

Coffee oil is composed mainly of triacylglycerols with fatty acids in proportions similar to those found in common edible vegetable oils (Folstar, 1985). During roasting the oil is expelled to the bean surface, forming a layer which may trap volatile aromas, preventing the immediate loss of these compounds (Clifford, 1985). The oil therefore, plays an important role in the overall

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presentation of coffee flavour although the oil is poorly extracted into the coffee brew. Nevertheless, considerable amount of oil may be found in coffee brew depending on the brewing method (Petracco, 2001).

Carbohydrates are the most abundant constituents in raw coffee beans accounting for more than 50% of the bean dry weight (Njoroge, 1987). Green coffee beans contain a wide range of different carbohydrates which can be grouped as simple sugars, disaccharides, oligosaccharides and polysaccharides. The main low molecular weight carbohydrate or sugar in green coffee is sucrose (Ky *et al*., 2001). It is the main contributor of reducing sugars which are implicated in Maillard reactions occurring during the roasting process (Grosch, 2001). It also acts as aroma precursors that affect both taste and aroma of the beverage (De Maria *et al*., 1994). During bean roasting sucrose is considerably degraded but remains noticeably present in roasted grains at concentrations of 0.4-2.8% dmb and is thought to contribute to beverage sweetness (Guyot *et al*., 1996).

Although coffee biochemical attributes are inherent factors, the environment which includes crop management factors can play a major role in determining their expression (Leroy *et al*., 2006). Climate, altitude and shade have a strong influence on flowering, bean expansion and ripening through their effect on temperature, availability of light and water (Harding *et al*., 1987). Shade, or conditions that imply lower air temperatures such as higher elevations, slows down the ripening process of coffee berries allowing more time for complete bean filling (Vaast *et al*., 2006), resulting in beans that are denser and far more intense in flavour than those grown under lower altitudes or under full sunlight. The slower maturation process, therefore, plays a central role in ensuring high cup quality, possibly by guaranteeing the full expression of all biochemical steps required for the development of the coffee beverage quality (Silva *et al*., 2005). Kathurima *et al*. (2012) reported significant contribution of shade to the increased premium grades (AA and AB) which are highly valued in the coffee trade in Kenya but observed no clear gain on the sensory quality parameters. Information on the effect of shade and agronomic management levels on the biochemical components in Kenyan coffee is however limited. With this understanding, this study was conducted to evaluate the effect of management and shade levels on biochemical components of coffee.

MATERIALS AND METHODS

Study site: This study was conducted at the Kenya Agricultural and Livestock Research Organization-Coffee Research Institute demonstration (KALRO-CRI) plot in Namwela and two surrounding small holder farms within the area from year 2010 to 2012. Namwela is located in Bungoma County at 0° 45'43N 34°33'42E, 1641 meters above sea level, an average rainfall of 1329 mm. The chosen small holder farms sites had similar climatic and soil conditions due to their proximity. The three farms represented high, medium and low management level treatments.

Experimental design and layout: The experimental design was a split plot, with management level as main plot treatment and shade level as the sub-plot. The management levels were categorized depending on field operations and externally applied inputs as described by Mugo (2010). Based on these criteria, a coffee plot under high management level was managed using all the recommended practices by Coffee Research Foundation (Coffee Research Foundation (CRF), 2013) for optimum production. Under medium management level, the external inputs applied included farm yard manure and inorganic fertilizers; and pesticides (insecticides and fungicides for the control of insect pests and diseases respectively). Under the low management level, there was no application of external inputs at all. The different shading levels were based on the distances from the trunk of the shade tree: 0-1.5 m (80%), 1.5-3 m (70%), 3-4.5 m (50%), 4.5-6 m (30%) and full sun (0%). The shading level was estimated by measuring the Photosynthetic Photon Flux Density (PPFD) in µmol m-2 s-1 using a Line Quantum Sensor (LI-COR Biosciences) and expressing it as a percentage of that obtained in full sun as described by Vaast *et al*. (2007).

Processing of samples: Fully ripe cherries were harvested from four trees (and bulked) in each of the five treatments in each site during the year 2010/11and 2011/12 seasons. The cherries were wet processed using standard procedures (Mburu, 2004). The parchment coffee was hulled and graded based on size, shape and density (Gichimu *et al*., 2012) and grade AB was used for the subsequent biochemical analysis. Fifty grams of dry coffee beans per treatment from each of the sites were frozen at -80^{o}C before grinding (<0.5mm particle size) in liquid nitrogen using an analytical mill (Model A10 IKA work inc. Wilmington, NC, USA).

Biochemical analysis: Caffeine, trigonelline and total Chlorogenic Acids (CGA) were extracted simultaneously from 3g of green coffee powder using ethanol and acetone (24:1v/v) and shaking in the dark for 24 hours. Caffeine, trigonelline and CGA were analyzed using HPLC system (KNEUR) equipped with a Supel Co. Discovery column and a diode array detector at three wavelengths, 278nm for caffeine and 266nm for trigonelline and 324nm for CGA. Sucrose was extracted from green coffee powder using the method of Osborne and Voogt (1978) with modifications. About 2.5g of the green coffee powder was weighed and put into a round bottomed flask.

Extraction was done for one hour in 100 mL of 96% ethanol (AR) under reflux. The extract was cooled and filtered through Whatman filter paper number 42 and evaporated to dryness. Sucrose was recovered with 10 mL de-ionized water and 2 mL of the extract mixed thoroughly with 2 mL Diethyl ether (AR) left to settle and the top layer discarded. This was repeated three times. One milliliter of the clarified extract was mixed with 1 mL of acetonitrile and filtered through a 0.45µm micro filter (Chromafil). Sucrose was analyzed using a HPLC system (KNEUR) equipped with a Eurospher 100-5 NH2 column and a refractive index detector. The mobile phase was acetonitrile HPLC grade (SCHARLAU) 75% and distilled water 25% at a flow rate 1 mL/min under ambient temperature. Caffeine, trigonelline, CGA and sucrose were identified by comparing the retention times of standards and their concentrations calculated from peak areas using calibration equations. Crude oil was analyzed following the AOAC (1995) official method 920.9. Two grams of green coffee powder was precisely weighed into an extraction thimble and the product covered with oil free cotton glass. The weight of an empty extraction flask was taken and 150 mL of petroleum ether (pb 30-60^0) dispensed into the flask. The thimbles containing the samples were placed into the extraction flasks and fixed in the soxhlet apparatus. Coffee oil extraction was completed within sixteen hours. The extraction flasks containing the oil were placed in an oven at 100±2ºC for 30 min to dry out any moisture. The extraction flasks with their contents were cooled in a desiccator and the final weight taken when the temperature reached room temperature. The increase in weight of the extraction flask was calculated as the crude oil content.

Data analysis: The biochemical data obtained were subjected to analysis of variance at 5% level of significance using Costat version 6.400 (1998-2008, Co Hort Software) statistical program. Least significant difference (LSD) was used to separate the means. Correlation analysis was done using XLSTAT 2015 Version 17.1.

RESULTS AND DISCUSSION

The caffeine content (% dry weight basis) was significantly influenced by both management and shade levels in both season 1 and 2 (Table 1). Interaction between shade and management was only significant in season 1. In both seasons, high management level resulted to significantly higher caffeine content than medium management level which in turn had higher caffeine content than the low management level. Coffee under high shade levels 80 and 70% had higher caffeine content than coffee under full sun. Shaded coffee recorded higher caffeine content than coffee under full sun in all management levels in both seasons (Table 1).

Analysis of variance showed that mean oil content (% dry weight basis) was significantly ($p<0.05$) affected by management and shade levels in both seasons (Table 2). However, significant interactions between shade and management levels were only observed in introduction. On average, coffee under high management level had higher oil content than medium and low management levels irrespective of shading level, in introduction. However, there was no significant difference ($p>0.05$) in oil content between coffee under medium and low management levels. Coffee under shade levels of 80% and 70% had higher oil content than those from lower shade levels and full sun in introduction. In materials methods, the mean oil content was also higher in coffee under high management than under medium and low management levels. Shaded coffee (80%) recorded higher mean oil content than coffee grown under full sun.

Trigonelline content was significantly ($p<0.05$) affected by management and shade levels in season 1 but only by shade level in season 2 (Table 3). There were significant ($p<0.05$) interactions between shade and management in both seasons. In season 1, management level had no effect on trigonelline content in coffee grown under high shade levels of 80 and 70%, but coffee under low management level had lower trigonelline content than high and medium management

Table 1: Mean caffeine content (% dry weight basis) analysed in green coffee under different management and shade levels

	Season 1				Season 2			
	Management intensity				Management intensity			
Distance (m)	High	Medium	Low	Mean	High	Medium	Low	Mean
0-1.5	1.25	1.26	1.15	1.22	1.44	1.35	1.17	1.32
1.5-3.0	1.21	1.17	0.91	1.10	1.47	1.39	1.17	1.34
3.0-4.5	1.26	1.11	0.61	0.99	1.34	1.35	1.17	1.29
4.5-6.0	1.20	1.02	0.62	0.95	1.35	1.30	1.15	1.27
Full sun	1.01	0.82	0.59	0.81	1.31	1.28	1.16	1.25
Mean	1.19	1.08	0.80		1.38	1.33	1.20	
LSD (ML)	0.08				0.04			
LSD (SL)	0.05				0.04			
LSD (ML x SL)	0.1				NS			
CV (%)	3.77				2.64			

ML: Management level, SL: Shade level; NS: Not significant at $p<0.05$

Table 2: Mean oil content (% dry weight basis) analysed in green coffee under different management and shade levels

	Season 1				Season 2			
	Management intensity				Management intensity			
Distance (m)	High	Medium	Low	Mean	High	Medium	Low	Mean
0-1.5	17.57	16.42	16.59	16.86	18.99	18.20	18.41	18.53
1.5-3.0	17.62	16.36	16.51	16.83	18.80	18.16	18.04	18.33
3.0-4.5	17.07	16.25	16.20	16.51	18.97	18.48	17.82	18.42
4.5-6.0	16.78	16.67	15.81	16.42	18.83	18.18	17.41	18.14
Full sun	16.71	15.94	15.79	16.15	18.27	18.30	16.94	17.84
Mean	17.15	16.33	16.18		18.77	18.26	17.72	
LSD (ML)	0.32				0.20			
LSD (SL)	0.22				0.42			
LSD (ML x SL)	0.44				NS			
CV (%)	1.05				1.85			

ML: Management level, SL: Shade level; NS: Not significant at $p<0.05$

Table 3: Mean trigonelline content (% dry weight basis) analysed in green coffee under different management and shade levels

	Season 1				Season 2			
	Management Intensity				Management Intensity			
Distance (m)	High	Medium	Low	Mean	High	Medium	Low	Mean
0-1.5	1.16	1.20	1.10	1.15	1.54	1.47	1.27	1.43
1.5-3.0	1.06	1.03	0.97	1.02	1.29	1.44	1.19	1.31
3.0-4.5	1.04	1.04	0.95	1.01	1.29	1.18	1.21	1.23
4.5-6.0	1.03	1.02	0.64	0.90	1.16	1.15	1.16	1.16
Full sun	0.87	0.75	0.56	0.73	1.14	1.08	1.11	1.11
Mean	1.03	1.01	0.84		1.28	1.26	1.19	
LSD (ML)	0.12				NS			
LSD (SL)	0.08				0.08			
LSD (ML×SL)	0.17				0.15			
CV (%)	6.85				4.94			

ML: Management level, SL: Shade level; NS: Not significant at $p<0.05$

Table 4: Mean sucrose content (% dry weight basis) analysed in green coffee under different management and shade levels

	Season 1				Season 2			
	Management Intensity				Management Intensity			
Distance (m)	High	Medium	Low	Mean	High	Medium	Low	Mean
0-1.5	8.47	8.16	7.70	8.11	7.430	9.58	8.03	8.350
1.5-3.0	8.63	8.44	8.19	8.42	8.520	9.24	8.29	8.680
3.04.5	8.39	8.19	8.05	8.21	9.220	9.94	8.05	9.070
4.5-6.0	8.89	8.09	8.57	8.52	11.16	9.40	9.17	9.910
Full sun	9.41	8.92	8.48	8.94	11.76	9.76	9.29	10.27
Mean	8.76	8.36	8.20		9.620	9.58	8.57	
LSD (ML)	0.09				0.380			
LSD (SL)	0.31				0.480			
LSD (ML x SL)	NS				0.810			
CV (%)	2.96				4.100			

ML: Management level, SL: Shade level; NS: Not significant at $p<0.05$

levels in full sun and 50% shade level. In season 2, management levels had no effect on trigonelline across the shading levels except at 80% shade. Shaded coffee had higher trigonelline content than coffee in full sun in both seasons. The trigonelline content was generally higher in the second season across all management levels (Table 3). The sucrose content was significantly affected by management and shade levels in both seasons but interaction was only significant in season 2 (Table 4). In the first season, shaded coffee had lower sucrose content than coffee in full sun. In season 2, increase in shade intensity reduced sucrose content under high management level. Coffee under high management level had significantly higher sucrose content than medium and low management levels in both seasons. The sucrose content ranged from 7.43 to 11.76% dwb and was higher in the second season than season one (Table 4).

The total Chlorogenic Acids (CGA) content was significantly ($p<0.05$) affected by management and shade level in season 1 and by shade level in season 2 (Table 5). There was significant interaction between

Table 5: Mean chlorogenic acid content (% dry weight basis) analysed in green coffee under different management and shade levels

	Season 1				Season 2			
	Management intensity				Management intensity			
Distance (m)	High	Medium	Low	Mean	High	Medium	Low	Mean
0–1.5	6.10	5.20	4.82	5.37	6.38	7.20	5.43	6.34
1.5–3.0	6.41	6.16	5.44	6.00	6.77	7.26	6.91	6.98
3.0–4.5	6.33	5.98	6.00	6.10	7.97	7.12	7.52	7.54
4.5–6.0	6.48	6.25	5.64	6.12	8.34	7.38	7.41	7.71
Full sun	6.89	6.37	6.38	6.55	9.59	7.51	7.33	8.14
Mean	6.44	5.99	5.66		7.81	7.29	6.92	
LSD (ML)	0.37				NS			
LSD (SL)	0.43				0.56			
LSD (ML×SL)	NS				1.31			
CV (%)	5.63				6.10			

ML: Management level, SL: Shade level; NS: Not significant at $p<0.05$

Table 6: Correlation coefficients of biochemical variables showing effect of shade and management levels

Variables						
Shade	Shade					
Management	0.000	Management				
Caffeine	0.016	0.555**	Caffeine			
Oil	0.048	0.409**	0.806**	Oil		
Trigonelline	0.030	0.274	0.853**	0.790**	Trigonelline	
Sucrose	-0.056	0.354	0.293	0.382	0.057	Sucrose
CGA	0.016	0.350	0.410*	0.582**	0.271	0.827**

CGA; Total chlorogenic acids, *: Correlation is significant at the 0.05 level (2-tailed), **: Correlation is significant at the 0.01 level (2-tailed)

management and shade intensity in second season. In both seasons, shaded coffee had lower chlorogenic acid content than coffee under full sun. Coffee under high management level had higher CGA content than coffee under medium and low management level except in season 2 under heavy shade (80 and 70% shade).

Correlation coefficient between biochemical variables, shade and management levels: The correlation coefficients of biochemical variables showing effect of shade and management levels are shown in Table 6. There were no significant correlations between shade and all the biochemical components. Management was positively and significantly ($p<0.05$) correlated with caffeine and oil. Caffeine was positively and significantly correlated with oil, trigonelline and chlorogenic acids. Oil was positively and significantly ($p<0.05$) correlated with trigonelline and chlorogenic acids. Sucrose and chlorogenic acids were positively and significantly ($p<0.05$) correlated.

DISCUSSION

Management and shade levels significantly influenced biochemical components attributes in this study. The higher management level had higher caffeine than the medium which in turn had higher caffeine than the low management level. Under the high management level, N fertilizers were applied as recommended and this probably led to higher caffeine content than under the lower level management where little or no inputs were applied at all. This was further confirmed by the strong and positive correlation between management level and caffeine content.

A study by Mendoza (1995) cited in Wintgens (2004), noted that Nitrogen, especially in excess, increased caffeine content. This has been supported by Gonthier *et al.* (2011) who found that caffeine concentration in phloem exudates was greater in high-Nitrogen (N) fertilized plants relative to intermediate and low-N plants. However, leaf, stem, root and total overall caffeine concentration and content did not differ across N treatments. The finding by Gonthier *et al.* (2011) suggested that the caffeine content was strongly regulated by genetic factors and the environment was not as important. Shaded coffee had higher caffeine content than coffee under full sun. The caffeine level increased with increase in shade intensity. Similar findings have been reported by Morais *et al.* (2006), Vaast *et al.* (2006: 2007) and Guyot *et al.* (1996). Coffee under high management level recorded the highest oil content and this could be explained by the positive effect of fertilizer application on bean size and weight. Studies by Wintgens (2004) and Lara-Estrada and Vaast (2007) showed that there was a higher accumulation of fat matter in green coffee beans with adequate plant nutrition. The oil content generally increased with increase in shade content intensity. Coffee under shade consistently recorded higher oil content than coffee grown in full sun. This may be explained by a slowdown in the ripening process which led to better bean filling and complete fat synthesis as postulated by Vaast *et al.* (2006). Other studies have also shown a positive correlation between shade and fat content (Morais *et al.*, 2006; Vaast *et al.*, 2007). On the other hand, Avelino *et al.* (2007) reported higher oil content in sun grown coffee. The contrast may be attributed to the use different

of coffee varieties. The oil content ranged from 15.79% to 18.99% in this study. This was within the range reported by other researchers such as Kathurima (2013) who recorded coffee oil content ranging from 10.12 to 18.75%; Speer and Kolling-Speer (2001; 2006) who reported average oil content of 15% in green Arabica coffee and Bertrand *et al*. (2006) who found levels ranging from 14.07% to 15.47% in a traditional coffee cultivar Caturra grown under different elevations in Central America.

In the present study, trigonelline content generally increased with increase in management level. This is in contrast to the findings by Lara-Estrada and Vaast (2007) who reported that fertilization increased bean size and weight but caused a reduction in trigonelline concentration. This is probably due to the dilution effect, as the volume of the bean increased the concentration of trigonelline reduced. Shaded coffee had higher trigonelline content than coffee grown in full sun. On the contrary, Vaast *et al*. (2007) recorded higher trigonelline values in full sun. Kathurima and Njoroge (2012), however, found that shade had no effect on the level of trigonelline. The levels of trigonelline ranged from a high of 1.54% dwb recorded in high management, under maximum shade, during the second season to a low of 0.56% dwb recorded in low management in full sun. Varying levels of trigonelline in arabica coffee have been reported by several authors. These include; 1.52% to 2.9% (Mazzafera, 1991), 1 to 1.94% (Martin *et al*., 1998), 0.88% to 1.77%, (Ky *et al*., 2001) and 0.50 to 1.10% (Kathurima, 2013) all in dry weight basis.

In the present study, there was an increase in the levels of total Chlorogenic Acids (CGA) as the management levels increased, though the effect was significant only in the second season. The variable effect would imply that management levels do not influence the content of chlorogenic acid in green coffee to a great extent. Other workers have also reported that agricultural practices were less important, compared to species or maturation, in determining the chlorogenic acid content (Clifford, 1985; Varnam and Sutherland, 1994; Flament, 2002; Farah *et al*., 2005). The shaded coffee had significantly lower CGA content than coffee under full sun. This is similar to the findings reported by Vaast *et al*., 2006 and Vaast *et al*., 2007. In contrast, Somporn *et al*. (2012) and Morais *et al*. (2006) observed a higher chlorogenic acid content in shade grown coffee.

In the present study, higher sucrose content was observed under high than medium and low management levels. Similar results were reported by Lara-Estrada and Vaast (2007) who found that fertilization positively influenced sucrose content. The shaded plants had lower sucrose content than those in full sun. The effect of shade on sucrose content in mature coffee remains controversial since it depends on the coffee cultivar. In this study, a traditional cultivar K7 was used. Using Catuai cultivar of *C. arabica* grown in shade, Guyot *et al*. (1996) reported an increase in sucrose content while for Catimor cultivar of *C. arabica*, Vaast *et al*., (2006) observed a negative correlation between sucrose content and shade. All biochemical components analysed showed positive correlations among each other. Caffeine, oil and trigonelline were strongly and positively correlated. Lara-Estrada and Vaast (2007) and Gichimu *et al*. (2014) also reported positive correlations among the biochemical components except for caffeine which was negatively correlated with chlorogenic acids, trigonelline and lipids. Shade was positively correlated with all biochemical components, except sucrose, though none was significant.

CONCLUSION

This study showed that biochemical components can be influenced by environment as evidenced by significant effect of shade and management levels. The significant correlations between biochemical components with shade and management show that the factors can be manipulated to enhance the biochemical components.

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