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Bioactive compounds in golden coffee, roasted beans, effect of geographic zones and ecological floors

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Abstract

This study aimed to assess the levels of total polyphenols (PPT), anthocyanins (AA), reducing sugars (AR), antioxidant capacity (CA) against DPPH and ABTS radicals, caffeic acid (AC), caffeine (CF), and chlorogenic acid (ACG) in green coffee from four different zones and three altitudes, as well as in seven commercial blends of green coffee and roasted-ground coffee (TE, QA2C, Q90, Q80, Q85, Misty, and Mountain). The highest PPT content was discovered in green coffee from Rupa Rupa and Padre Abad at 1200 m a.s.l. (3.70 ± 0.02 and 3.91 ± 0.01 g GAE/100 g) ($p \leq 0.05$), as well as in the green coffee blends TE and Mountain (3.44 ± 0.07 and 3.57 ± 0.17 g GAE/100 g coffee). The AA content ranged from 7,521 ± 0.001 to 61.4230 ± 0.011 mg of cyanidin 3 glycoside/L. Higher RA was observed in Rupa Rupa at 1200 m a.s.l. (0.66 ± 02%) ($p \leq 0.05$), as well as in Misty and Mountain green coffee (0.59 ± 0.03% and 0.59 ± 0.02%). Monzón at 1800 and 1200 m a.s.l. exhibited higher CA against DPPH and ABTS (IC50 = 3.5670 ± 0.43 and 4.7425 ± 0.37 µg/g coffee), while Monzón at 1800 m a.s.l. showed higher CA against DPPH (IC50 = 2.0570 ± 0.15 µg/g coffee). Monson at 1200 m a.s.l. had a higher AC content (6.8712 ± 0.0068 g of AC/100 g of coffee), and Rupa Rupa at 1800 and 1200 m a.s.l. exhibited higher CF content (3.7065 ± 0.18 and 3.5718 ± 0.05 g of caffeine/100 g of coffee). Padre Abad at 1200 m a.s.l. displayed a higher ACG content (8.8013 ± 0.49 g of ACG/100 g sample).

Keywords: Total polyphenols; anthocyanins; reducing sugars; chlorogenic acid; caffeic acid

INTRODUCTION

Coffee is a shrub of tropical regions of the genus Coffea, from the family of Rubiaceae, with the two main crop species, Arabica (Coffea arabica) and Robusta Coffee (*C. canephora*) (Hamon et al., 2017), its dry, roasted and ground seed is widely used, in the preparation of various beverages, generally called coffee, as the sensory attributes become more complex and exotic, the value of coffee increases (Ribeiro et al., 2011), the popularity of coffee has made it one of the most important products in the world; coffee is the most consumed beverage after water: 255 kg per second or eight million tons per year (Castanheira, 2020; S/f, 2023).

The quality of the coffee considers the physical attributes, organoleptic characteristics and chemical components of the grain, each of these attributes depends on climatic factors, edaphic, geographical area, genotype, crop management (Ahmed et al., 2021; Bote & Vos, 2017), processing parameters (Abreu et al., 2019). Within the chemical components, Chlorogenic acids (CGA) are naturally occurring esters formed between (-) - quinic acid and different transcinamic acids (such as caffeic, ferulic and p-coumaric acid) and since quinic acid has four hydroxyl groups, they can be formed a wide variety of isomers of chlorogenic acid (Gutiérrez, 2017), more than 60 different AGCs and their derivatives have been identified in coffee, although with a limited characterization for each of them. 5-CQA is the most abundant in coffee, hence its common name chlorogenic acid (Sinisi et al., 2016). These polyphenols have recently attracted the attention of researchers, due to their biological activity that includes antioxidant, anti-inflammatory and antiviral properties (Gutiérrez Ortiz et al., 2019).

Simple carbohydrates, such as sucrose, glucose, and fructose in coffee, participate in the Maillard reactions during the roasting process, affecting the aroma and color characteristics. Proteins also participate in these reactions, which form melanoidins and low molecular weight volatile compounds (Barbosa et al., 2019).The metabolites trigonelline, caffeine, caffeoylquinic acid and sugars revealed higher concentrations in new genotypes. According to sensory analysis, one genotype showed the highest final score (82), which was even higher than Arabica coffees (Lemos et al., 2020).Coffee infusions with lower quality scores correlate with high levels of caffeine, protein, chlorogenic acids, and total titratable acidity (TTA) in green coffee beans. High proportions of sucrose / TTA and cafestol / kahweol in green coffee beans were associated with

higher scores for infusions (Barbosa et al., 2019). Green coffee bean metabolites related to coffee bean quality were identified using a 1H NMR based metabolomic analysis of special grade green coffee beans. Highest levels of sucrose and lowest levels of γ-aminobutyric acid (GABA), quinic acid, choline, acetic acid, and fatty acids seen in specialty or high-grade green coffee beans, compared to commercial grade beans, suggested to be multiple markers to characterize the specialty green coffee bean (Kwon et al., 2015).

Higher geographic zones and shading at lower altitudes improve the physical and quality attributes of the coffee cup, however; this may depend on the variety and growing environment (e.g. soil fertility, temperature, precipitation) (Worku et al., 2018).There are contradictory reports considering the effect of shade and altitude, indicating that it has and does not have a significant influence on the organoleptic properties of coffee in any of the altitude and fertilization conditions (Tolessa et al., 2017).

An investigation conducted on unblended ground coffee from the Korean market found the highest total chlorogenic acid (CGA) content, while the group with the most caffeine was American coffee sold in coffee shops. According to the caffeine / CGA ratio, the best quality coffee was the unmixed roast and ground coffee on the market, which had the lowest ratio (Jeon et al., 2019). CGA demonstrated therapeutic functions, such as antioxidant, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, antiviral, antimicrobial, antihypertensive, free radical scavenger, and a stimulator of the central nervous system (CNS) (Naveed et al., 2018).

In distinct parts of the world, until now, the effect of geographical areas and ecological floors on the content of bioactive compounds and their antioxidant capacity in green coffee beans has not been investigated, nor is it known how they vary after roasting and grinding the beans. This also occurs in Peru. Therefore, the objective of the research was to evaluate the content of total polyphenols, anthocyanins, reducing sugars, caffeic acid, caffeine, chlorogenic acid and antioxidant capacity against DPPH and ABTS radicals, in gold coffee extracts from different geographical areas, ecological floors, in golden coffee blends and roast-ground coffees.

MATERIALS AND METHODS

Samples. The 19 coffee samples were obtained from the Cooperativa Agraria Cafetalera "La Divisoria", which were: golden coffee from the geographical areas of Rupa Rupa and Monzón (department of Huánuco), Tocache (department of San Martin) and Padre Abad (department of Ucayali), considering 3 ecological floors with heights of 1200, 1500 and 1800 m a.s.l.; Similarly, 7 commercial mixtures of gold coffee for export and roasted-ground coffee for national sale (TE, Misti, Mountain, A2C, Q80, Q85, Q90). The coffee sample for each treatment consisted of one kilogram (Horwitz, s. f.). The coffee bean samples were packed in high-density polyethylene bags, stored in the laboratory at room temperature, to then proceed to the extraction of bioactive compounds and corresponding analysis.

Quantification total polyphenols. The quantification of total polyphenols was conducted according to the Folin-Ciocalteu method (Tripetch & Borompichaichartkul, 2019) Starting from the 100 mg / mL aqueous extract (filtered and centrifuged 10,000 rpm / 10 min at 4 ºC) the working dilution was 15 mg / mL, with 3 repetitions per treatment. 1580 µL of distilled water, 20 µL of diluted extract (15 mg / mL), 100 µL of phenol Folin-Ciocalteu and finally 300 µL Na2CO3 at 20% were added to the tubes for each treatment and it was incubated for 2 hours, then it was read absorbance at 700 nm, these results were quantified with a standard curve made with gallic acid expressed in mg GAE / 100 g.

Quantification of anthocyanins. The quantification of anthocyanins was conducted by the differential pH method (Symonowicz et al., 2012). Two buffer solutions were prepared: Buffer pH = 1: 125 mL of 0.2 M KCl and 375 mL of 0.2 M HCl and volumetric to 1 L with deionized water. Buffer pH = 4.5: 200 mL of 1 M CH3COONa, 120 mL of 1 M HCl and 180 mL of deionized H2O and volumetric at 1 L. For the quantification of anthocyanins, the aqueous extract 100 mg / mL was used, in a cuvette of polystyrene, different sample volumes were added according to the treatments. The absorbances obtained were replaced in the equation and expressed in mg cyanidin-3-glucoside / g sample.

$$C(mg/g) = \left(A_{pH=1,0} - A_{pH=4,5}\right) * 482{,}82(1000/24825) * DF$$

Where: = mg of cyanidin-3-glucoside per g of dry sample.$C(mg/g)$

MW = The molecular mass of cyanidin-3-glucoside is 484.82.

AbM = Molar absorptivity at 510 nm, at pH = 1.0; pH = 4.5 is the correction for the formation of degradation products is 24825

DF = Dilution factor.

Determination reducing sugars. The Dinitro salicylic Acid Reagent, developed for the determination of reducing sugars, is composed of dinitro salicylic acid, Rochelle's salt, phenol, sodium bisulfite and sodium hydroxide. Rochelle's salt is introduced to prevent the reagent from dissolving oxygen; phenol, to increase the amount of color produced; and bisulfite, to stabilize the color obtained in the presence of phenol. Alkali is necessary for the reducing action of glucose on dinitro salicylic acid (Gusakov et al., 2011; Miller, 1959).

Capacity of Inhibition of 1,1 diphenyl-2-picryl-hydrazyl radical (DPPH). According to the method described by Brand-Williams modified (Scherer & Godoy, 2009), The reagent 1,1 diphenyl-2-picryl-hydrazyl (DPPH) dissolved in 96% methanol was used, this free radical (DPPH) is stable and was used as an indicator to measure the sequestration capacity of any compound that has antioxidant activity. The percentage of inhibition of the radical DPPH was calculated with the following equation:

% DPPH inhibition = [(Ac - Am) / Ac] x 100

Where: Ac: Absorbance of the controls.

Am: Absorbance of the sample as a function of time (10 minutes).

Antioxidant activity was expressed in IC50For this, concentration versus percentage of inhibition was plotted, with which a linear type of fit curve was obtained, on which the IC50 value was determined. The results were analyzed using the DCA with factorial arrangement, 4A3B with three repetitions, and at the levels where there was statistical significance, the Tukey test was applied ($p \leq 0.05$).

Ability to inhibit 2,2-azinobis (3-ethylbenzothiazoline-6 sulfonic acid) cation (ABTSº +). The ABTS test was used (Re et al., 1999). The method consists of measuring the capacity of the sample to stabilize the respective chemical radical in aqueous medium. According to the methodology, the ABTSº + radical is formed after the reaction of ABTS (7mM) with potassium persulfate (140 mM, final concentration) incubated at room temperature and in the dark for 16 h. Once the ABTSº + radical has been formed, it is diluted with methanol until an absorbance value between 0.7 to 1.2 is obtained. The mother extract was prepared using 2 g of coffee in 10 mL of water, from which concentrations of the aqueous extract of the coffee beans were taken (0.25, 0.75, 2.5, 5.0, 6.5 ug / g coffee). The reaction was developed in a polystyrene cuvette (1 cm x 1

cm x 4.5 cm), with a volume of 10 µl of sample and 990 µl of the ABTSº + radical. The decrease in absorbance was recorded at 734 nm for 5 minutes.

% Inhibition ABTSº + = [(Ac - Am) / Ac] x 100

Where: Ac: Absorbance of the controls.

Am: Absorbance of the sample as a function of time (5 minutes).

Antioxidant activity was expressed in IC50 for this, concentration versus percentage of inhibition was plotted with which a linear type of fit curve was obtained on which the IC50 value will be determined.

HPLC analysis. Chlorogenic, ellagic, caffeic and caffeine acids were determined by the HPLC-FR chromatography method (Leucuta et al., 2005). Chromatographic analysis was performed using an LC-10 AT VP Shimadzu Scientific, MD, USA. Reversed phase liquid chromatograph, consisting of a Shimadzu degasser, DGU-14A; a Rheodyne 7725i manual injector; a solvent delivery module including a quaternary pump, Shimadzu LC-10 AT; a Shimadzu CTO-10AS column oven; a Shimadzu UV-Vis detector (272 nm), SPD_10AV, used to determine the purity of the chromatographic peak; SCL 10AV interface. Peak identification and integration were performed through Shimadzu, Class - VP software, version 6.13 SP2. The mobile phase was double distilled water at a flow rate of 0.5 ml / min. The quantification was by comparison with the standards of the areas of caffeine and chlorogenic, ellagic, caffeic acids.

Statistical analysis. The statistical analysis was conducted using a complete randomized design (CRD), specifically a factorial arrangement of 4A (geographical areas) x 3B (altitudes), resulting in 12 treatment combinations. Each treatment combination was replicated three times to ensure the reliability and robustness of the results. Prior to analysis, it was confirmed that the experimental data met the assumption of normality through appropriate statistical tests. The Tukey test was employed as a post-hoc analysis to determine significant differences between treatment means (L'Hocine & Pitre, 2016).

RESULTS AND DISCUSSION

Total polyphenols. The total polyphenol content (Table 1) of coffee is of significant interest due to its potential health benefits. Polyphenols are bioactive compounds known for their antioxidant properties, which play a crucial role in scavenging free radicals and reducing oxidative stress in the body. In this study, the highest total phenol content was observed in the gold coffee samples from the Padre Abad and Rupa Rupa areas, situated at 1200 and 1800 meters above sea level, respectively. This finding suggests that altitude may influence the polyphenol content of coffee beans, as higher elevations often result in slower bean maturation and increased polyphenol synthesis, these results are contrary to those reported by (Girma et al., 2020), the findings of the study showed that the contents of caffeine and 5-CQA in both raw and roasted coffee beans decrease as the growing altitude increases and, thus, for all varieties, their highest concentrations were recorded in lowland coffee beans.

The observed total phenol content ranged from 3.42 ± 0.07 to 3.91 ± 0.01 g GAE / 100 g, which is consistent with previous studies ((Khelil et al., 2016; Vignoli et al., 2011). Khelil et al. (2016) reported a similar total phenol content of 3.24 g GAE / 100g in their study on coffee samples from a different geographical region, highlighting the reproducibility of these findings across diverse coffee varieties and origins. Moreover, Vignoli et al. (2011) emphasized the importance of total phenols in coffee's antioxidant activity, underscoring their potential role in reducing the risk of chronic diseases such as cardiovascular diseases and certain types of cancer.

Table 1. Total phenol content in golden coffee, in golden coffee mixtures and in ground roasted coffee*.

Zone	**Height (m)**	**Total phenols (g GAE/100 g)**			
		Gold coffee	**Mixtures**	**Gold coffee**	**Roasted ground coffee**
Padre Abad	1800	3.62 ± 0.08 aa	TEA	3.44 ± 0.07 a	0.25 ± 0.11 a
	1500	2.80 ± 0.12 ab	QA2C	2.72 ± 0.11 c	0.39 ± 0.13 a
	1200	3.91 ± 0.10 aa	Q90	3.10 ± 0.03 b	0.31 ± 0.12 a
Monson	1800	2.68 ± 0.19 ba	Q80	1.72 ± 0.14 e	0.52 ± 0.14 a
	1500	2.90 ± 0.08 bb	Q85	2.37 ± 0.05 d	0.27 ± 0.11 a
	1200	3.36 ± 0.28 ba	MISTY	2.93 ± 0.05 b	0.57 ± 0.03 a
Tocache	1800	3.67 ± 0.05 ca	MOUNTEIN	3.57 ± 0.17 a	0.54 ± 0.17 a

	1500	2.38 ± 0.12 cb
	1200	2.25 ± 0.11 ca
Rupa Rupa	1800	3.42 ± 0.07 aa
	1500	2.72 ± 0.08 ab
	1200	3.70 ± 0.02 aa

* Values represent the mean ± SEM, n = 3, p ≤ 0.05.
Equal scripts indicate that there is no statistical difference.
GAE: Gallic Acid Equivalents.
Q90, Q80, Q85: Blends of coffees for national sale.
TE, QA2C, MISTY, MOUNTEIN: Blends of export coffees.

Furthermore, the highest phenol content was found in the TE and Mountain blends, with values of 3.44 ± 0.07 g GAE / 100 g and 3.57 ± 0.17 g GAE / 100 g, respectively. These results were statistically significant (Tukey, p ≤ 0.05) and exceeded those of other samples. (Tripetch & Borompichaichartkul, 2019), reported a similar total phenol content of 4.01 g GAE / 100 g in their study, further supporting the notion that certain coffee blends or processing methods may enhance polyphenol retention.

In conclusion, the findings of this study underscore the variability in polyphenol content among different coffee samples, influenced by factors such as altitude, blend composition, and processing techniques. These polyphenols contribute to the antioxidant capacity of coffee and may confer potential health benefits to consumers.

Anthocyanins. the anthocyanin content exhibited no significant differences when considering planting zones and altitudes (p ≤ 0.05). The content ranged from 7.521 ± 0.001 mg/mL to 61.4230 ± 0.011 mg of cyanidin 3 glucoside per liter. These findings align with previous research conducted on arabica coffee during the ripening process, where the anthocyanin content ranged from 12.88 ± 0.18 to 63.14 ± 0.28 cyanidin 3 glucoside per liter, depending on the degree of maturation from green to mature (Barbosa et al., 2019). Thus, the color of yellow-peeled varieties is due to carotenoids, while that of orange and red-peeled varieties is due to both carotenoids as well as low and high levels of anthocyanins, respectively (Esquivel Rodríguez et al., 2020).

Table 2. Anthocyanin content in golden coffee samples, in golden coffee mixtures and in ground roasted coffee.

Zone	**Height (m)**	**Anthocyanins (mg of cyanidin 3 glucoside/L)**			
		Gold coffee	**Names of mixtures**	**Gold coffee**	**Roasted ground coffee**

Padre Abad	1800	23.81 ± 0.005a	TEA	66.43 ± 0.016a	ND
	1500	20.05 ± 0.007a	QA2C	62.67 ± 0.007a	ND
	1200	25.07 ± 0.004a	Q90	70.19 ± 0.001a	ND
Monson	1800	28.83 ± 0.002a	Q80	21.31 ± 0.002a	ND
	1500	36.35 ± 0.002a	Q85	56.40 ± 0.001a	ND
	1200	7.52 ± 0.001a	MISTY	18.80 ± 0.025a	ND
Tocache	1800	25.07 ± 0.004a	MOUNTEIN	32.59 ± 0.006a	ND
	1500	43.87 ± 0.002a			
	1200	61.42 ± 0.011a			
Rupa Rupa	1800	12.53 ± 0.010a			
	1500	23.81 ± 0.006a			
	1200	47.63 ± 0.008a			

Values represent the mean ± SEM, n = 3, $p \leq 0.05$.
Equal scripts indicate that there is no statistical difference.
Q90, Q80, Q85: Blends of coffees for national sale.
TE, QA2C, MISTY, MOUNTEIN: Blends of export coffees.

Moreover, Table 2 illustrates that the highest anthocyanin content was observed in the Q90 green coffee blends, with a concentration of 70.198 ± 0.001 mg of cyanidin 3 glucoside per liter. These values were statistically similar ($p \leq 0.05$) to those reported by Barbosa et al. (2019). The presence of anthocyanins, although not significantly influenced by planting zones and altitudes in this study, underscores the potential health-promoting properties of coffee, as anthocyanins are known for their antioxidant and anti-inflammatory effects (Zheng et al., 2011).

Reducing Sugars. Table 3 illustrates the variations in reducing sugar content among the coffee samples, with the highest concentration observed in the Rupa Rupa area (1200 m a.s.l.), measuring at 0.66 ± 0.02%. Interestingly, no significant differences were found in reducing sugar content across different altitudes (Tukey, $p \leq 0.05$), suggesting that altitude may not have a pronounced effect on this aspect of coffee composition. However, it is noted that planting altitude has a significant impact on the content of reducing sugars in coffee beans, with glucose content positively affected by altitude (Joët et al., 2010). While specific references regarding reducing sugar content in coffee were not identified, it is established that fresh coffee typically contains approximately 6-7% sucrose, 0.11% fructose, and 0.05% glucose (Lemos et al., 2020). The levels of reducing sugars could be attributed to the degradation of sucrose during fermentation processes inherent to coffee production. Additionally, a discernible trend is noted wherein reducing sugar content tends to decrease with increasing altitude across all ecological floors,

coinciding with reports indicating that this content varies during coffee processing. For instance, the fermentation process leads to a decrease in lactic acid bacteria in pulped coffee at lower altitudes, and an increase at higher altitudes (Martins et al., 2020).

Furthermore, Table 3 outlines the reducing sugar content in both green coffee blends and ground roasted coffee. Notably, the Misty and Mountain coffee blends demonstrated the highest reducing sugar content, with concentrations of 0.59 ± 0.03% and 0.59 ± 0.02%, respectively, for green coffee. In contrast, the A2C sample of roasted ground coffee exhibited a remarkable content of 0.94 ± 0.01%. Interestingly, the reducing sugar contents in the green coffee blends were statistically similar (Tukey, $p \leq 0.05$), suggesting that the processing method may have a more significant influence on reducing sugar content than the blend composition itself.

This observation prompts further inquiry into the mechanisms underlying reducing sugar formation during coffee processing and its implications for flavor development and quality. Understanding the dynamics of reducing sugar content in coffee blends and its interaction with other chemical constituents could provide valuable insights for optimizing processing techniques and enhancing coffee flavor profiles.

Table 3. Reducing sugar content in golden coffee samples, in golden coffee mixtures, and in ground roasted coffee.

Zone	**Height (m)**	**Reducing sugars (%)**			
		Gold coffee	**Mix names**	**Gold coffee**	**Roasted ground coffee**
Padre Abad	1800	0.36 ± 0.02bc	TEA	0.50 ± 0.02cd	0.93 ± 0.02^{ab}
	1500	0.47 ± 0.01bb	QA2C	0.45 ± 0.03d	0.94 ± 0.01^{a}
	1200	0.54 ± 0.02ba	Q90	0.58 ± 0.01ab	0.84 ± 0.04abc
Monsoon	1800	0.36 ± 0.01bc	Q80	0.52 ± 0.01bc	0.81 ± 0.04c
	1500	0.58 ± 0.01bb	Q85	0.52 ± 0.01bc	0.87 ± 0.05abc
	1200	0.47 ± 0.00ba	MISTY	0.59 ± 0.03a	0.82 ± 0.04bc
Tocache	1800	0.41 ± 0.01bc	MOUNTEIN	0.59 ± 0.02a	0.92 ± 0.05abc
	1500	0.44 ± 0.01bb			

	1200	0.56 ± 0.01ba
Rupa Rupa	1800	0.38 ± 0.01ac
	1500	0.46 ± 0.01ab
	1200	0.66 ± 0.02aa

Values represent the mean ± SEM, n = 3, $p \leq 0.05$.
Equal scripts indicate that there is no statistical difference.
Q90, Q80, Q85: Blends of coffees for national sale.
TE, QA2C, MISTY, MOUNTEIN: Blends of export coffees.

Antioxidant capacity with radical's DPPH and ABTS. Table 4 presents the antioxidant capacity (measured by radical DPPH) of the coffee samples, indicating the highest values in the Monzón area at altitudes of 1800 and 1200 meters above sea level. The IC50 values for these samples were 3.5670 ± 0.43 and 4.7425 ± 0.37 µg/g coffee, respectively. Despite being statistically similar (Tukey, $p \leq 0.05$), these values surpassed those observed in other areas and planting altitudes. The study suggests that this remarkable antioxidant activity may be attributed to the presence of polyphenols and methylxanthines in green coffee, as evidenced by previous research (Barbosa et al., 2019). A range of studies have explored the antioxidant capacity of coffee samples, with varying results. Jiménez Monreal et al., (2012) found that the antioxidant activity of coffee from different origins is similar, with differences only in the type of procedure used. Brezová et al. (2009) identified caffeine and caffeic acid as key antioxidants in coffee, while Samsonowicz et al. (2019) reported that coffee substitutes, particularly those made from acorns, have high radical scavenging capacities. Górnaś et al. (2016) and Cämmerer & Kroh, (2006) both highlighted the role of phenolic acids and melanoidins in coffee's antioxidant capacity, with the latter noting changes in this capacity during storage.

Table 4. Antioxidant capacity with the radical DPPH.

Zone	Height (m)	DPPH IC50 (µg/g coffee)			
		Gold coffee	Mix names	Gold coffee	Roasted ground coffee
Padre Abad	1800	5.25 ± 0.02ba	TEA	3.9291 ± 0.12bcd	5.01 ± 0.14ab
	1500	5.02 ± 0.12bb	QA2C	2.9562 ± 0.05th	4.37 ± 0.35a
	1200	4.48 ± 0.35ba	Q90	3.9920 ± 0.11cd	4.87 ± 0.16ab
Monson	1800	3.56 ± 0.43aa	Q80	3.8327 ± 0.00bc	5.06 ± 0.21ab
	1500	4.89 ± 0.28ab	Q85	3.7668 ± 0.05b	5.43 ± 0.03b
	1200	4.74 ± 0.37aa	MISTY	3.9526 ± 0.04bcd	5.24 ± 0.34b
Tocache	1800	3.86 ± 0.55aba	MOUNTEIN	4.1071 ± 0.01d	5.08 ± 0.52ab
	1500	5.57 ± 0.34abb			
	1200	4.05 ± 0.41aba			
Rupa Rupa	1800	4.43 ± 0.33aba			
	1500	4.52 ± 0.29abb			
	1200	4.74 ± 0.16aba			

Values represent the mean ± SEM, n = 3, $p \leq 0.05$.
Equal scripts indicate that there is no statistical difference.
Q90, Q80, Q85: Blends of coffees for national sale.
TE, QA2C, MISTY, MOUNTEIN: Blends of export coffees.

In Table 5, the results of the antioxidant capacity (radical ABTS) of the green coffee blends and ground roasted coffee samples are presented. The A2C blends showed the highest antioxidant capacity, with values of 2.9562 ± 0.05 µg/g coffee for green coffee and 4.37 ± 0.35 µg/g coffee for ground roasted coffee. These values statistically exceeded (Tukey, $p \leq 0.05$) those of the other samples. The decrease in antioxidant capacity can be attributed to the degradation of antioxidant compounds during the roasting process, which is consistent with findings from previous studies on coffee and the effect of roasting (Kwon et al., 2015). The Q85 sample ranked second in terms of antioxidant capacity.

Additionally, in Table 5, it can be observed that the highest antioxidant capacity was found in coffees from the Monzón, Tocache, and Padre Abad areas, specifically at an altitude of 1800 m a.s.l. Among these, the Padre Abad area at 1800 m a.s.l. exhibited the highest antioxidant capacity, with an IC50 value of 2.0570 ± 0.15 µg/g coffee. This value was statistically similar

(Tukey, $p \leq 0.05$) but exceeded the antioxidant capacity of the other areas and planting altitudes. It is well-known that green coffee, regardless of species and origin, represents an important source of polyphenols and methylxanthines, which contribute to its high antioxidant capacity (Barbosa et al., 2019).

The antioxidant capacity of green coffee blends and ground roasted coffee samples is influenced by various factors. Brezová et al., (2009) found that ground coffees have a lower antioxidant capacity than instant coffees, with a linear relationship between antioxidant capacity and phenolic content. van der Werf et al., (2014) and Górnaś et al., (2016) both noted a decrease in antioxidant capacity during the roasting process, with the latter attributing this to a reduction in phenolic acids. Acidri et al., (2020) reported that green coffee beans have the highest antioxidant capacity, which decreases by 66% after roasting. del Castillo et al. (2002) observed a decrease in antioxidant activity with roasting, while Daglia et al. (2000) found that roasted coffee has higher antioxidant activity than green coffee.

Tables 4 and 5 present the results of the antioxidant capacity of the green coffee blends and ground roasted coffee samples. The Q80 green coffee blend exhibited the highest antioxidant capacity, with an IC50 value of 2.7921 ± 0.42 µg/g coffee. Additionally, the blends A2C, Mountain, TE, and Q90, as ground roasted coffee, showed high antioxidant capacity with values of 9.8825 ± 0.19 and 9.9700 ± 0.18 µg/g coffee, respectively. These values statistically surpassed (Tukey, $p \leq 0.05$) the antioxidant capacity of the other samples. Roasting process significantly affects the oxidative activity and polyphenolic content, with light roasted coffees showing the highest antioxidant capacity (Bobková et al., 2020)

Table 5. Antioxidant capacity with the radical ABTS.

Zone	**Height (m)**	**ABTS IC50 µg / g coffee**			
		Gold coffee	**Mix names**	**Gold coffee**	**Roasted ground coffee**
Padre	1800	2.05 ± 0.15aa	TEA	5.2541 ± 0.15b	9.9423 ± 0.54a

Abad	1500	3.40 ± 0.60ab	QA2C	4.7819 ± 0.06b	9.8825 ± 0.19a
	1200	4.24 ± 0.54ab	Q90	4.1835 ± 1.45ab	14.9563 ± 0.97b
Monson	1800	2.58 ± 0.16aa	Q80	2.7921 ± 0.42a	14.1097 ± 0.30b
	1500	2.98 ± 0.30ab	Q85	4.7334 ± 0.11b	15.0277 ± 0.12b
	1200	3.27 ± 0.30ab	MISTY	4.6826 ± 0.19b	9.9700 ± 0.18a
Tocache	1800	2.59 ± 0.26aa	MOUNTEIN	4.5294 ± 0.09b	9.9009 ± 0.29a
	1500	3.49 ± 0.30ab			
	1200	3.28 ± 0.44ab			
Rupa Rupa	1800	3.82 ± 0.39ba			
	1500	3.94 ± 0.91bb			
	1200	4.11 ± 0.61bb			

Values represent the mean ± SEM, n = 3, p ≤ 0.05.
Equal scripts indicate that there is no statistical difference.
Q90, Q80, Q85: Blends of coffees for national sale.
TE, QA2C, MISTY, MOUNTEIN: Blends of export coffees.

HP

LC analysis. The compounds analyzed through HPLC analysis in this study were caffeic acid (AC), caffeine (CF), and chlorogenic acid (ACG). Coffee is known to be rich in bioactive substances, including nicotinic acid, trigonelline, quinolinic acid, tannic acid, pyrogallic acid, and particularly caffeine (Jeon et al., 2019). Chlorogenic acids (CGAs) are the primary phenolic compounds found in coffee (Awwad et al., 2021). They are esters of trans-cinnamic acids, such as caffeic, ferulic, and p-coumaric acids, with (-) quinic acid (QA). The main classes of CGAs in green coffee are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), and feruloylquinic acids (FQA), each with at least three isomers. In Coffea arabica and Coffea canephora (Robusta coffee) significant variations were observed for feruloyl quinic acids, dicaffeoyl quinic acids and 5-sinapoylquinic acid while the mono-caffeoyl quinic acids showed no variation when the two coffee varieties were compared (Badmos et al., 2019).

In Table 6, the Rupa Rupa area at an altitude of 1200 meters above sea level stands out, boasting a caffeic acid content of 6.8712 ± 0.0068 g per 100 g of sample (Tukey, p ≤ 0.05). This caffeic acid content notably exceeds that reported in previous studies, wherein Arabica coffee typically contains 0.8-1.4% caffeic acid, while Robusta coffee contains 1.7-4.0% caffeic acid (Jeon et al., 2019).

Table 6 presents the results of the caffeic acid content in the samples of green coffee and roasted and ground coffee. Among the green coffee blends, the Misti blend exhibited the highest content of caffeic acid with 6.9380 ± 0.0031 g of caffeic acid per 100 g. The Q80 blend also showed a significant content of 3.44 ± 0.07 g of caffeic acid per 100 g. In the roasted and ground coffee samples, the A2C, Mountein, Misti, and TE blends had values ranging from 5.9546 ± 0.0112 to 5.7005 ± 0.0125 g of caffeic acid per 100 g. These values were statistically similar (Tukey, $p \leq 0.05$) but higher than the other blends. The observed caffeic acid contents in these samples are higher than what has been reported in other investigations (Jeon et al., 2019).

Table 6. Content of caffeic acid, in golden coffee, golden coffee blends and ground roasted coffee.

Zone	**Height (m)**	**Caffeic acid (g/100 g)**			
		Gold coffee	**Mix names**	**Gold coffee**	**Roasted ground coffee**
Pade Abad	1800	5.7510 ± 0.0007bb	TEA	5.6577 ± 0.0155c	5.7332 ± 0.0056abc
	1500	5.3084 ± 0.0015bc	QA2C	5.7482 ± 0.0042c	5.9546 ± 0.0112a
	1200	5.5328 ± 0.0005ba	Q90	6.0243 ± 0.0103bc	5.5643 ± 0.0023bc
Monson	1800	5.3962 ± 0.0009bb	Q80	6.4569 ± 0.0255ab	5.5419 ± 0.0049c
	1500	5.6752 ± 0.0011bc	Q85	5.9512 ± 0.0220bc	5.5305 ± 0.0040bc
	1200	5.5518 ± 0.0032ba	MISTY	6.9380 ± 0.0031a	5.8613 ± 0.0111ab
Tocache	1800	5.2578 ± 0.0009cb	MOUNTEIN	5.9660 ± 0.0009bc	5.7005 ± 0.0125abc
	1500	5.1378 ± 0.0002cc			
	1200	5.2275 ± 0.0047ca			
Rupa Rupa	1800	6.1318 ± 0.0058ab			
	1500	5.5799 ± 0.0052ac			
	1200	6.8712 ± 0.0068aa			

Values represent the mean ± SEM, n = 3, $p \leq 0.05$.
Equal scripts indicate that there is no statistical difference.
Q90, Q80, Q85: Blends of coffees for national sale.
TE, QA2C, MISTY, MOUNTEIN: Blends of export coffees.

In Table 7, the highest caffeine content was found in the samples from the Rupa Rupa area at altitudes of 1500 and 1800 m a.s.l., with values of 3.7065 ± 0.18 and 3.5718 ± 0.05 g of caffeine per 100 g of sample, respectively (Tukey, $p \leq 0.05$). These caffeine contents are higher than what

has been reported in other studies, where Arabica coffee typically contains 0.8-1.4% caffeine and Robusta coffee contains 1.7-4.0% caffeine (Naveed et al., 2018). Among the green coffee blends, Q80, Q85, and Q90 exhibited higher caffeine contents, ranging from 3.6969 ± 0.06 to 4.0288 ± 0.27 g of caffeine per 100 g. As for the roasted and ground coffee, the Q90 blend showed the highest caffeine content with 5.3566 ± 0.01 g of caffeine per 100 g. These values were statistically similar ($p \leq 0.05$) but higher than the other blends. These caffeine contents are like what has been reported in another investigation with values ranging from 2.84 ± 0.00 to 5.82 ± 0.11 g of caffeine per 100 g (Gutiérrez Ortiz et al., 2019).

Table 7. Caffeine content in golden coffee, golden coffee blends and roasted ground coffee.

Zone	**Height (m)**	**Caffeine (g/100 g)**			
		Gold coffee	**Mix names**	**Gold coffee**	**Roasted ground coffee**
Padre Abad	1800	3.1393 ± 0.02ba	TEA	3.1832 ± 0.00b	4.76 ± 0.01c
	1500	3.5224 ± 0.03ba	QA2C	2.7716 ± 0.06bc	4.48 ± 0.00d
	1200	3.2509 ± 0.08bb	Q90	3.6969 ± 0.06a	5.36 ± 0.01a
Monson	1800	3.3315 ± 0.00ba	Q80	3.8884 ± 0.01a	4.47 ± 0.02d
	1500	3.2385 ± 0.03ba	Q85	4.0288 ± 0.27a	5.06 ± 0.03b
	1200	3.1749 ± 0.06bb	MISTY	2.5422 ± 0.07c	4.96 ± 0.17bc
Tocache	1800	3.3688 ± 0.01ca	MOUNTEIN	3.9267 ± 0.02a	5.15 ± 0.01ab
	1500	3.1449 ± 0.09ca			
	1200	2.6119 ± 0.04cb			
Rupa Rupa	1800	3.5718 ± 0.05aa			
	1500	3.7065 ± 0.18aa			
	1200	3.2956 ± 0.02ab			

Values represent the mean ± SEM, n = 3, $p \leq 0.05$.
Equal scripts indicate that there is no statistical difference.
Q90, Q80, Q85: Blends of coffees for national sale.
TE, QA2C, MISTY, MOUNTEIN: Blends of export coffees.

In Table 8, the highest content of ACG was observed in Rupa Rupa at an altitude of 1200 m a.s.l., with a value of 9.0937 ± 0.9128 g of ACG per 100 g of sample (Tukey, $p \leq 0.05$). This ACG content surpassed that of another study where values ranged from 6.1 ± 0.7 to 6.6 ± 1.1% for various Arabica coffee cultivars (Naveed et al., 2018). Similarly, the highest ACG content (as shown in Table 2) was identified in the AC2 and Mountain green coffee blends, with 10.8055 ± 0.32 and 10.7588 ± 0.40 g of ACG per 100 g respectively. These values were statistically equivalent (Tukey, $p \leq 0.05$). In ground roasted

coffee, the TE blend stood out with 9.6171 ± 0.10 g of ACG per 100 g. Notably, these ACG contents were lower than those found in green coffee blends, attributed to the roasting process, which decreases ACG stability (Tripetch & Borompichaichartkul, 2019). It has been demonstrated that Maillard reaction products (MRPs) are the primary antioxidants in roasted coffees, with this process reducing free CGA content by over 90%. Antioxidant mechanisms associated with coffee MRPs include hydrogen atom transfer and single electron transfer mechanisms (Tripetch & Borompichaichartkul, 2019).

Table 8. Chlorogenic acid content in golden coffee, golden coffee blends and ground roasted coffee.

Zone	**Height (m)**	**Chlorogenic acid (g acid/100 g)**			
		Gold coffee	**Mix names**	**Gold coffee**	**Roasted ground coffee**
Padre Abad	1800	7.0439 ± 0.3313a	TEA	10.4525 ± 0.6157a	9.62 ± 0.10a
	1500	7.3788 ± 0.0840a	QA2C	10.8055 ± 0.3231a	7.84 ± 0.03b
	1200	8.8013 ± 0.490aa	Q90	9.3627 ± 0.0032ab	3.80 ± 0.04d
Monson	1800	6.5094 ± 0.4131ab	Q80	8.0336 ± 0.1917b	4.55 ± 0.15d
	1500	6.9843 ± 0.6251ab	Q85	9.0328 ± 0.2818ab	4.38 ± 0.42d
	1200	8.4718 ± 0.4226aba	MISTY	9.6169 ± 0.8621ab	6.74 ± 0.24c
Tocache	1800	8.0984 ± 0.5297b	MOUNTEIN	10.7588 ± 0.4053a	7.34 ± 0.11bc
	1500	6.4232 ± 0.5425b			
	1200	6.1228 ± 0.4886ba			
Rupa rupa	1800	7.4728 ± 0.2513ab			
	1500	5.8099 ± 0.2716ab			
	1200	9.0937 ± 0.9127aba			

Values represent the mean ± SEM, n = 3, $p \leq 0.05$.
Equal scripts indicate that there is no statistical difference.
Q90, Q80, Q85: Blends of coffees for national sale.
TE, QA2C, MISTY, MOUNTEIN: Blends of export coffees.

The chlorogenic acid content in coffee is influenced by various factors, including roasting conditions (Moon et al., 2009), processing (Mills et al., 2013), and post-harvest processing methods (Zanin et al., 2016). Studies have shown that the highest chlorogenic acid content is found in green coffee, with levels decreasing as the beans are roasted to different degrees (Awwad et al., 2021).

CONCLUSION

The study revealed significant variations in total polyphenol content across different geographical areas and altitudes. The highest levels were observed in green coffee from Padre Abad at 1200 m a.s.l. (3.91 ± 0.01 g GAE / 100 g) and Rupa Rupa at 1200 m a.s.l. (3.70 ± 0.02 g GAE / 100 g). However, no significant differences were found in anthocyanin content among the planting zones and altitudes studied.

Regarding reducing sugars, higher levels were detected in coffee from Rupa Rupa at 1200 m a.s.l. (0.66 ± 0.02%). The antioxidant capacity against the DPPH radical was notably higher in Monzón coffees at 1800 and 1200 m a.s.l. (IC50 = 3.5670 ± 0.43 and 4.7425 ± 0.37 µg/g coffee, respectively). Monzón coffee at 1800 m a.s.l. exhibited greater antioxidant capacity against the ABTS radical (IC50 = 2.0570 ± 0.15 µg/g coffee).

Concerning specific compounds, higher levels of caffeic acid were found in coffee from Rupa Rupa at 1200 m a.s.l. (6.8712 ± 0.0068 g of acid / 100 g sample), while higher caffeine content was observed in coffee from Rupa Rupa at 1500 and 1800 m a.s.l. (3.7065 ± 0.18 and 3.5718 ± 0.05 g of caffeine / 100 g of coffee, respectively). Additionally, green coffee from Rupa Rupa at 1200 m a.s.l. exhibited elevated chlorogenic acid (ACG) content (9.0937 ± 0.9127 g of ACG / 100 g sample).

Among the blends, the TE and MOUNTAIN coffee blends showed the highest total polyphenol content, with values of 3.44 ± 0.07 and 3.57 ± 0.17 g GAE / 100 g coffee, respectively. The Q90 green coffee blend displayed higher anthocyanin content, with a concentration of 70.198 ± 0.001 mg of cyanidin 3 glucoside per liter.

Regarding reducing sugars, Misty and Mountain green coffee blends had higher levels, with concentrations of 0.59 ± 0.03% and 0.59 ± 0.02%, respectively. Notably, the A2C sample for ground roasted coffee exhibited the highest content of reducing sugars, with a concentration of 0.94 ± 0.01%.

The A2C blend demonstrated greater antioxidant capacity against the DPPH radical, both as green coffee (2.9562 ± 0.05 µg/g coffee) and as ground roasted coffee (4.37 ± 0.35 µg/g coffee). Higher antioxidant capacity against the ABTS radical was observed in the Q80 green coffee blend (2.7921 ± 0.42 µg/g coffee) and in the mixtures of roasted ground coffee, including A2C (9.8825 ± 0.19 µg/g coffee), Mountain (9.9009 ± 0.29 µg/g coffee), and TE (99.423 ± 0.54 µg/g coffee).

For caffeic acid content, the Misti and Q80 green coffee blends exhibited higher levels, with concentrations of 3.44 ± 0.07 and 6.9380 ± 0.0031 g caffeic acid per 100 g of coffee, respectively. Similarly, the roasted and ground coffee blends of A2C, Mountain, Misti, and TE showed higher caffeic acid content, ranging from 5.9546 ± 0.0112 to 5.7005 ± 0.0125 g per 100 g of coffee.

Regarding caffeine content, the Q80, Q85, and Q90 green coffee blends had higher concentrations, ranging from 3.6969 ± 0.06 to 4.0288 ± 0.27 g caffeine per 100 g of coffee. Additionally, the Q90 blend as ground roasted coffee exhibited a higher caffeine content of 5.3566 ± 0.01 g per 100 g of coffee.

Furthermore, the AC2 and Mountain green coffee blends showed higher chlorogenic acid (ACG) content, with concentrations of 10.8055 ± 0.32 and 10.7588 ± 0.40 g ACG per 100 g of coffee, respectively. The roasted ground coffee blend of TE also exhibited higher ACG content, with a concentration of 9.6171 ± 0.10 g per 100 g of coffee.

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