

Impact of Altitude and Fermentation Temperature on the Quality of Panamanian Geisha Coffee

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ABSTRACT

Premium Panamanian Geisha coffee is renowned for its exceptional sensory quality, yet the environmental and processing factors underlying these attributes are not fully understood. This study evaluated the effects of altitude and fermentation temperature on the sensory and physicochemical properties of *Coffea arabica* var. Geisha. Coffee cherries from four farms at different altitudes in Chiriquí, Panamá, were fermented under three temperature conditions (ambient farm temperature ~19 °C, 24 °C, and 30 °C). Sensory evaluation was conducted by professional cuppers, while brewed samples were analyzed for chlorogenic acids, caffeine, pH, soluble solids, total acidity, polyphenols, and antioxidant activity. Coffees grown above 1700 masl achieved significantly higher cupping scores (87–90 points) compared to those from lower altitudes (86–87 points, $p < 0.01$). High elevation was associated with increased chlorogenic acids and reduced caffeine, Total soluble solids, and pH. Fermentation at the cooler ambient temperature yielded superior sensory scores relative to 24 °C and 30 °C treatments ($p < 0.05$). Principal component analysis confirmed altitude as the dominant factor shaping sensory quality, while classification tree analysis identified brew pH as the most influential physicochemical predictor. Altitude alone explained ~72% of the variance in sensory quality, and models integrating key chemical variables increased explanatory power to 75–78%. Results demonstrate that high-altitude cultivation combined with cooler fermentation enhances Geisha coffee quality. Future research should integrate soil properties, shade management, and microbial profiling to strengthen predictive models and optimize production strategies for this globally prized variety.

Key words: Cup quality; decision tree; multivariate analysis; specialty coffee.

1 INTRODUCTION

Coffee is the second most consumed beverage in the world, and the flavor and aroma of the brew are its most important quality attributes (Fassio et al., 2017). Specialty coffee producers differentiate their products by cultivating unique sensory characteristics that command premium prices in the international market (Laukaleja & Kruma, 2018; Samoggia & Riedel, 2019; Sepúlveda et al., 2016). The Geisha variety spearheaded the rise of the specialty coffee industry in Panama (*Coffea arabica* var. Geisha). The exceptional performance of Geisha coffee at the 2005 “Best of Panama” competition marked the beginning of this trend, and its popularity is still increasing. In 2025, a washed-process Panama Geisha achieved a record cupping score of 98 and sold for US\$30,204 per kg at an auction (Specialty Coffee Association of Panamá - SCAP, 2025). This extraordinary value highlights the rarity and quality of Geisha coffee. However, such success is not widespread; only a few growers attain these elite quality levels. Identifying the factors contributing to the exceptional cup quality of Geisha and sharing this knowledge is necessary for enabling other producers in similar agroecological zones to replicate Panama’s success.

The quality of coffee in the cup is influenced by many factors, from seed to bean (Bastian et al., 2021; Silva Oliveira et al., 2022; Kim et al., 2018; Laukaleja & Kruma, 2018; Sepúlveda et al., 2016). Key determinants include the coffee genotype (Gumecindo-Alejo et al., 2021; Krohling et al., 2018), agroecological conditions during cultivation (Krohling et al., 2018; Martins et al., 2020), post-harvest processing methods (Carvalho-Ferreira et al., 2023), the biochemical composition of the beans (Bastian et al., 2021), storage conditions (Salvio et al., 2023), and the composition and dynamics of the coffee microbiome during fermentation, which play critical role in flavor development and sensory quality (Todhanakasem et al., 2024). These factors have been extensively studied in different varieties of *C. arabica*. The altitude of a coffee farm positively affects coffee quality for many cultivars (Silva Oliveira et al., 2022; Martins et al., 2020; Peñuela-Martínez, Moreno-Riascos, & Medina-Rivera, 2023). Higher elevations are characterized by lower temperatures, which slow the maturation of coffee cherries, facilitating greater accumulation of flavor and aroma precursors (Joët et al., 2010). Consequently, beans grown at high altitudes produce greater cup quality compared to those from lower elevations. While this effect of altitude has been observed in several Arabica varieties, it has not been

quantitatively evaluated in the Geisha variety. Geisha develops its best characteristics above 1600 masl, but empirical data on how altitude influences the sensory scores, and chemistry of Geisha are scarce (World Coffee Research, 2024).

Another major factor in specialty coffee production is the fermentation process of harvested coffee cherries. Traditionally, fermentation is performed to remove mucilage from beans, but modern specialty coffee processing uses fermentation to enhance desirable aromas and flavors (Carvalho-Ferreira et al., 2023; Resende, Borém, & Cirillo, 2023). During fermentation, microorganisms (bacteria, yeasts, and fungi) break down the complex sugars in mucilage into simpler compounds, producing various metabolites and volatile substances that can affect them (Haile & Kang, 2019; Jitjaroen, Chaisri, & Panjai, 2023). The outcome of fermentation depends on parameters such as duration (Ahmad, Sa'diyah, & Subramaniam, 2023; Santos-Silva et al., 2022), temperature, and microbial community present. Lower fermentation temperatures and specific microbial consortia are associated with improved flavor development, as they favor specific metabolic pathways and slower, more controlled fermentation. Despite growing interest in fermentation techniques among Geisha producers, studies on how fermentation temperature influences the cup quality and chemical profile of Geisha coffee are limited (Cruz-O'byrne, Piraneque-Gambasica, & Aguirre-Forero, 2020; Girma & Sualeh, 2022; Haile & Kang, 2019).

The quality assessment of coffee is generally performed by cupping according to the Specialty Coffee Association (SCA) protocol. By conducting this sensory evaluation, attributes such as fragrance/aroma, flavor, aftertaste, acidity, body, balance, sweetness, clean cup, uniformity, and overall impression are scored, and the total score is calculated. Coffees with scores above 80 are considered "specialty," with scores of 85–90 labeled excellent and those above 90 labeled outstanding. Cupping is a subjective, skill-intensive, and time-

consuming process (Domingues et al., 2020). To complement sensory tests, researchers have applied instrumental techniques (electronic noses, NMR and NIR spectroscopy) combined with chemometrics to relate the physicochemical properties of coffee to its sensory quality. Several studies have correlated the chemical composition of coffee, such as the volatile profile, chlorogenic acid content, caffeine content, Total soluble solids, pH, and other parameters, with sensory attributes across various Arabica coffees (Santamaría et al., 2023; Silva et al., 2024; Vega et al., 2021; Vega, Reyes & Troestch, 2025; Yeager et al., 2023). Although the interest in Geisha coffee is increasing, research on the physicochemical factors underlying its sensory quality is scarce.

This study aimed to evaluate the effect of farm altitude and fermentation temperature on the sensory quality and chemical composition of Geisha coffee. A nested experimental design and multivariate analyses were combined to determine how these two factors contribute to variations in the cup score and identify key chemical quality predictors. The findings may help growers and processors optimize agroecological and post-harvest conditions for the Geisha variety, thereby helping transform the "art" of producing exceptional Geisha coffee into a scientific process accessible to more producers.

2 MATERIAL AND METHODS

2.1 Sample collection

Four specialty coffee farms in Chiriquí Province, Panama, which are known for cultivating *C. arabica* var. Geisha under different agroecological conditions, were surveyed. The location, altitude, climate, and shade tree species of each farm are summarized in Table 1. The elevation of the farms ranged from 1375 to 1850 masl, covering the typical range where Geisha is grown in Panama (Figure 1). Ripe coffee cherries

Table 1: Locations and general characteristics of Geisha coffee farms sampled in Chiriquí, Panama.

Location	Geographical coordinates	Altitude (masl) (cultivation site)	Average temperature (°C)	Altitude (masl) Fermentation site	Average temperature (°C)	Tree species for shade
Jurutungo, Renacimiento	8.901 N, 82.733 W	1850	15	1850	16.8	<i>Allium ampeloprasum</i> var. Porru, <i>Citrus sinensis</i> , <i>Citrus limonum</i> , <i>Citrus × aurantiifolia</i> , <i>Citrus × paradisi</i> , <i>Persea americana</i> , <i>Inga edulis</i> .
Santa Clara, Renacimiento	8.868 N, 82.739 W	1800	16	1300	22	<i>Ricinus communis</i> , <i>Pterocarpus orbiculatus</i>
Los Naranjos, Boquete	08.810406° N 82.483238° W	1725	16	1700	17.4	<i>Solanum betaceum</i> , <i>Ricinus communis</i> , <i>Brugmansia</i> spp.
Coordillera arriba, Tierras Altas	8°45'22"N 82°36'45"W	1375	19	1375	20.6	<i>Inga edulis</i>

from the 2021–2022 harvest season were collected from each farm. To ensure comparability, only fully ripe, defect-free cherries were selected at each farm and initially separated by flotation to remove under-ripe or damaged cherries. To minimize variability, all cherries were harvested and processed on the same day at each farm. The picked cherries were stored in clean, sealed containers before fermentation treatment.

2.2 Fermentation process

In each farm, lots of Geisha coffee cherries were collected (15 samples per farm). To initiate fermentation, freshly harvested cherries were washed by immersion in water to remove floaters and debris, thereby ensuring the exclusive use of fully ripe, high-quality cherries. The selected cherries were then vacuum-sealed in plastic bags to create an anaerobic environment and subjected to three fermentation treatments: (a) ambient farm temperature (16.8–22.0 °C), (b) controlled fermentation at 24 °C, and (c) controlled fermentation at 30 °C. For each treatment, 20 samples of 1 kg were used. Fermentation continued for five days until the mucilage was visibly broken down. Subsequently, all cherries were dried in a controlled environment at 24 °C and 55% relative humidity for 2 months. After drying, the coffee was mechanically hulled to remove parchment and then visually sorted to remove defective samples. After screening, the green beans were vacuum-packed and stored for subsequent sensory and chemical analyses.

2.3 Sensory analysis (Cupping)

The roasting profile was set with an initial temperature of 160°C, a development time ratio, of 11%, a total time of 7 minutes and 45 seconds, and a final temperature of 180 °C. The sensory quality of each coffee sample was evaluated by a panel of eight professional cuppers (two women and six men) following the cupping protocol provided by the SCA. For each sample, the standard attributes of the cuppers were scored: fragrance/aroma, flavor, aftertaste, acidity, body, balance, sweetness, clean cup, uniformity, and overall impression. The scores for each attribute (out of 10) were summed to calculate the final cupping score (out of 100). According to

the SCA standards, a score of 80–85 is considered *very good*, 85–90 is *excellent*, and a score of greater than 90 is considered outstanding for specialty coffee. Each sample was cupped in duplicate to ensure reliability. The cuppers were blinded to sample identities to reduce bias.

2.4 Physicochemical analyses

To associate chemical attributes with sensory quality, a portion of each brewed coffee sample used in cupping was collected for laboratory analysis. The brews were prepared using the SCA brewing ratio (13 g of ground coffee per 140 g of deionized and demineralized water at 96 °C) during the cupping sessions. Triplicate composite samples were prepared from each brewed coffee for chemical analysis: brews from three cups were combined to make a 100 mL sample, which was done at three different cupping tables (randomly selected), yielding three independent replicates per treatment. Each 100 mL composite mixture was immediately placed in an amber bottle, stored on ice, and then refrigerated at 10–15 °C. All chemical measurements described below were performed in triplicate for each stored brew sample.

Several physicochemical parameters that influence coffee flavor and quality were analyzed, using standard methods and instruments as follows:

Total titratable acidity: The acidity was measured by titrating a 10 mL aliquot of the coffee brew with 0.1 M NaOH to the phenolphthalein endpoint (first persistent color change). The results are expressed as mEq of acid per gram of sample. This procedure followed AOAC official methods (AOAC, 2010).

pH: The pH of the coffee brew was measured directly at room temperature using a calibrated pH meter (OAKTON pH 1100, Illinois, USA) (AOAC, 2010).

Total soluble solids determination: Total soluble solids values were determined using a refractometer (Thermo Fisher Scientific, AGON model, 0–32% Total soluble solids range) at 20 °C. A brew drop was placed on the refractometer prism, and the results were reported as the percentage of soluble solids.

Protein content: Protein content was estimated using the Kjeldahl method. A 5.0 g sample of the coffee brew was

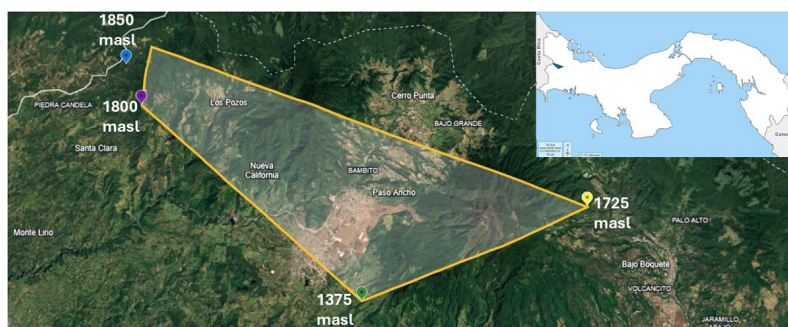


Figure 1: Distribution of sampling sites located in Chiriquí Province, Panamá.

digested to determine the total nitrogen content, which was then multiplied by 6.25 to obtain the percentage of protein.

Chlorogenic acids (3-CQA, 4-CQA, and 5-CQA) and caffeine: These were identified and quantified by high-performance liquid chromatography (HPLC). Brew samples were diluted (1:10 in water) and filtered (0.45 µm) before injection. The method described by Wen *et al.* (2005) was used with an HPLC system (Agilent 1260 Infinity, California, USA), which was equipped with a ZORBAX SB-C18 column (4.6 × 150 mm, 5 µm). The mobile phase consisted of water with 0.02% trifluoroacetic acid (solvent A) and methanol with 0.02% TFA (solvent B) at a flow rate of 0.4 mL/min. A gradient elution mixture was used: 98%A:2%B at 0 min, linearly increased to 68%A:32%B at 30 min, then 60%A:40%B at 45 min, followed by a return to 98%A:2%B by 50 min, with a total run time of 60 min. A diode-array spectrometer was used for detection at 280 nm and 325 nm for caffeine and chlorogenic acids, respectively. The compounds were identified by comparing their retention times with those of the respective standards. Calibration curves were prepared for 5-caffeoylquinic acid (5-CQA), 3-CQA (0.5–100 mg/L), and caffeine (10–250 mg/L). The concentration of 4-CQA was calculated using the 5-CQA standard curve, as the chlorogenic acid isomers have similar molar extinction coefficients (Naegele, 2013). The results were expressed in milligrams per liter (mg/L) of the brew.

Total polyphenols: Total polyphenols were assessed by the Folin-Ciocalteu spectrophotometric method following the procedure described by Vega, De León, and Reyes (2017). Aliquots of the coffee brew were reacted with Folin-Ciocalteu reagent and sodium carbonate, and the absorbance was measured at 765 nm after incubation. Gallic acid was used as the calibration standard. The total polyphenol content was expressed as milligrams of gallic acid equivalents per liter of coffee (mg GAE/L).

Antioxidant activity (DPPH assay): This activity was measured using the DPPH radical scavenging method suggested by Tan *et al.* (2023). A volume of each coffee sample was mixed with a DPPH radical solution, and the decrease in absorbance at 517 nm was recorded. Using a Trolox calibration curve, the results were expressed in millimoles of Trolox equivalents per liter (mmol Trolox/L).

Antioxidant activity (ABTS assay): This activity was measured using the ABTS radical cation decolorization method proposed by Vega, De León and Reyes (2017). An ABTS working solution was reacted with the coffee sample, and the reduction in absorbance at 734 nm was measured after a fixed time. The results were expressed in mmol Trolox/L and calibrated against Trolox.

The chemical measurements for each sample were performed in triplicate, and the mean values, along with their standard errors, were reported.

2.5 Experimental design and data analysis

A nested experimental design was used because the fermentation treatments were not independent of the farm (altitude) factor. The ambient fermentation temperature was related to the specific conditions of each farm. Altitude (farm) was treated as the main factor, with four levels corresponding to the four farms, and fermentation temperature as a secondary factor, with three levels nested within each farm (farm ambient, 24 °C, and 30 °C). A linear model was constructed with altitude and fermentation (nested within altitude) as fixed effects. In the analysis of variance (ANOVA), the appropriate error term for testing the effect of altitude was the mean square of fermentation temperature with respect to altitude. When the ANOVA indicated a significant effect ($p < 0.05$), post hoc pairwise comparisons were made using Tukey's honestly significant difference (HSD) test.

Multivariate analysis of variance (MANOVA) was performed to assess overall differences, considering the interrelated response variables, including sensory scores and multiple chemical parameters. Prior to conducting ANOVA and MANOVA, the assumptions of homogeneity of variances (Levene's test) and normality (Shapiro-Wilk test) were verified.

Principal component analysis (PCA) was performed to further establish the relationships among variables. A PCA biplot was used to visualize how samples cluster according to altitude and fermentation treatment and identify which physicochemical variables are associated with sensory quality.

A classification and regression tree approach were used to identify the most influential chemical predictors of cup quality. A classification model was constructed to distinguish between high-scoring and lower-scoring coffees using the scikit-learn decision tree algorithm (Jijo & Abdulazeez, 2021). For this analysis, the sensory scores were divided into two categories based on the observed distribution: Level 1 (<87.5 points) and Level 2 (≥87.5 points). The altitude factor was excluded as an input to isolate the effect of the measured physicochemical variables. The decision tree was trained on 70% of the dataset (randomly selected) and tested on the remaining 30%. The tree classification was repeated 500 times with different random training and test splits to ensure that the results were robust. Then, the frequency with which each variable was used as a decision node in the trees was recorded. This frequency served as an indicator of the relative importance of the variable in classifying the quality of coffee. The outcome was summarized as a frequency bar graph in a variable importance plot.

Finally, regression models were constructed using measured variables to predict the sensory quality score. One approach adopted was knowledge-driven selection,

where altitude, along with the top-ranked physicochemical predictors identified by the classification tree, was included. All statistical analyses were conducted using R and Python (for the machine learning component), and the results were statistically significant at $p < 0.05$.

3 RESULTS

The effects of farm environmental conditions, specifically altitude and post-harvest fermentation temperature, on the cup quality and chemical properties of Geisha coffee were investigated. Four representative farms from the specialty coffee region of Panama were sampled, and their coffees were processed under three fermentation temperature regimes. Expert cuppers were used to evaluate the sensory qualities of the coffees, and the key chemical constituents and antioxidant activities of the brews were analyzed. The results of the sensory evaluation and physicochemical analyses of the coffee samples are presented in Tables 2 and 4.

3.1 Effect of altitude on the sensory quality of Geisha coffee

All samples in this study were rated as excellent specialty coffees by the cupping panel, with final scores ranging from 86 to 90 points (out of 100) (SCAP, 2023). Although all samples were of high quality, the ANOVA results confirmed that the farm altitude significantly affected the cupping score ($p < 0.01$). Coffee grown at altitudes above 1700 masl achieved significantly higher scores on average than those from lower altitudes ($p < 0.01$; see Table 2). The coffees from 1725, 1800, and 1850 masl scored 88.7–89.0 points, whereas the coffee from 1375 masl scored about 86.6 points. This altitude effect is visualized in Figure 2, which shows the distribution of scores (box plot) for the high-altitude (>1700 masl) and middle-altitude (<1700 masl) groups. The high-altitude group consistently had higher scores (88.6–89.5) compared to the lower-altitude group (86.3–87.1).

Table 2: Mean values (with standard errors) of the sensory score and physicochemical variables for Geisha coffee as a function of altitude. Different superscript letters indicate significant differences between means in the same column (Tukey's HSD, $p < 0.05$).

Altitude (masl)	Final score**	pH**	Total soluble solids (%)**	Total acidity (meq/g)*	Caffeine (mg/L)**	3-CQA (mg/L)**	5-CQA (mg/L)**	4-CQA (mg/L)**	Polyphenols (mg GAE/L)**	DPPH (mmol trolox/L)**	ABTS (mmol trolox/L)**
1850	88.82 ^a	4.79 ^b	1.45 ^c	0.0142 ^{b,c}	587.89 ^c	664.76 ^a	1358.92 ^a	679.81 ^a	2252.62 ^b	6.88 ^b	9.23 ^b
1800	88.78 ^a	5.12 ^a	1.55 ^{b,c}	0.0141 ^{b,c}	631.78 ^b	348.48 ^c	801.96 ^b	396.04 ^c	2334.26 ^{a,b}	7.20 ^b	10.58 ^a
1725	88.97 ^a	5.13 ^a	1.74 ^a	0.0151 ^a	684.96 ^a	394.60 ^c	893.70 ^b	449.62 ^{b,c}	2481.52 ^a	8.14 ^a	10.48 ^a
1375	86.58 ^b	4.99 ^b	1.60 ^b	0.0138 ^c	636.09 ^b	504.90 ^b	829.72 ^b	489.93 ^b	2462.81 ^a	8.00 ^a	9.27 ^b
S.E.M	0.12	0.01	0.03	0.0003	11.02	27.63	40.55	21.22	41.45	0.17	0.18

CQA: Caffeoylquinic acids, **DPPH:** 2,2-diphenyl-1-picrylhydrazyl, **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), **S.E.M:** Standard

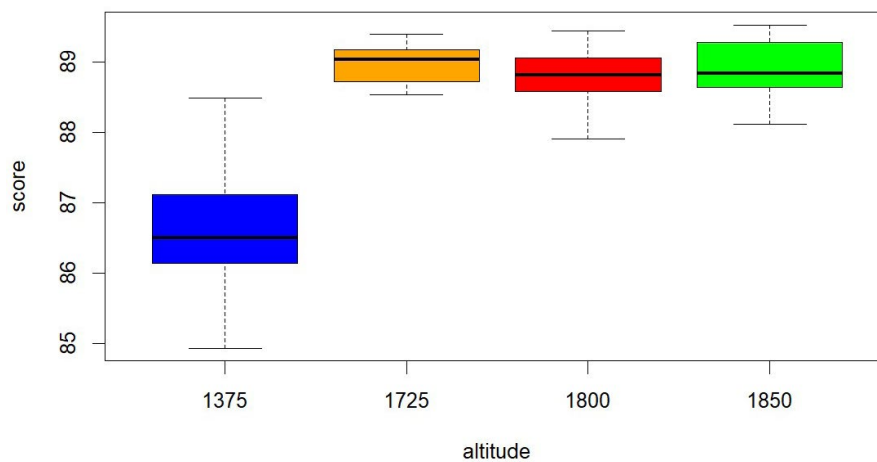


Figure 2: Box plot of the sensory quality (cupping score) of *C. arabica* var. Geisha coffees grown at different altitudes. High-altitude farms (>1700 masl) yielded significantly higher scores than mid-altitude farms (<1700 masl).

3.2 Effect of altitude on physicochemical properties of the coffee brews

The altitude significantly influenced the sensory score and affected several physicochemical parameters of the coffee brews (Table 2). The most notable trend was observed for chlorogenic acids: the highest-altitude coffee (1850 masl) had the highest total chlorogenic acid content (sum of 3-CQA, 4-CQA, and 5-CQA) among the samples ($p < 0.01$). At 1850 masl, the total chlorogenic acid concentration was 2703 mg/L, whereas at 1375 masl, the total chlorogenic acid concentration was lower (about 1824.55 mg/L). In contrast to chlorogenic acids, other measured components presented an inverse relationship with altitude. As the altitude increased, significantly lower values of pH, Total soluble solids, caffeine, and total polyphenols were found in the brews (all $p < 0.01$ for the overall altitude effect; see Table 2 for pairwise differences).

For example, the lowest-altitude coffee (1375 masl) had the highest measured pH (least acidic, pH =4.99) and the highest total soluble solids (1.60% Total soluble solids). In contrast, the highest-altitude coffee (1850 masl) had the lowest pH (4.79) and lowest Total soluble solids (1.45%). The caffeine concentration decreased from 636 mg/L at 1375 masl to 588 mg/L at 1850 masl in the analyzed samples.

The lowest polyphenol concentration was found in the coffee grown 1850 masl, whereas the highest polyphenol concentration was found in the coffee grown at 1375 masl (Table 2). Similarly, the antioxidant activity (by ABTS and DPPH radical scavenging) was lower in the highest-altitude samples than in the other samples, although the relationship was not linear.

Despite these overall altitude-associated trends, some differences and overlaps were found among specific altitudes. A few key comparisons are highlighted below (letters in Table 2 indicate groups not significantly different, Tukey $p > 0.05$):

- Coffee samples from 1725 masl had a significantly greater soluble solids content (Total soluble solids 1.74%) and total titratable acidity (0.0151 mEq/g) than coffee samples from all other altitudes ($p < 0.01$ for Total soluble solids; $p < 0.05$ for acidity). Samples from 1725 masl were statistically similar to samples from 1800 masl in several aspects (pH, total chlorogenic acids, total polyphenols, and ABTS antioxidant activity).
- The specific components of samples from 1375 masl and 1725 masl did not differ significantly: for example, the 5-CQA content, total polyphenol content, and DPPH antioxidant activity were similar between these two altitudes ($p > 0.05$). This is notable because 1375 masl and 1725 masl represent the extremes of the “below 1700” and “above 1700” categories; however, for those variables, lower-altitude coffee was similar to higher-altitude coffee.
- Samples from 1800 masl and 1850 masl (both in the high category) were very similar in terms of soluble solids,

total polyphenol content, and DPPH activity ($p > 0.05$). However, samples from 1850 masl had a higher total chlorogenic acid concentration than those from 1800 masl.

- When coffee from the middle altitude was compared to that from the lowest altitude, i.e., 1375 masl vs. 1800 masl, significant differences in pH or ABTS antioxidant activity were found. Additionally, caffeine and polyphenol levels did not differ significantly between 1375 masl and 1800 masl.

3.3 Effect of fermentation temperature on sensory quality and chemistry

The second factor under study was the fermentation temperature applied to the harvested cherries. Coffees fermented at each farm’s ambient temperature, which varied from 16.8°C at the highest-altitude farm to 22°C at the lowest-altitude farm, were compared to those fermented at 24°C and 30°C. The fermentation temperature had a noticeable effect on the cup quality (Table 3). Across all farms, coffees fermented at ambient farm temperatures achieved higher average cupping scores than those fermented at 24°C or 30°C. In practical terms, allowing fermentation to proceed at naturally cooler temperatures (especially for high-altitude farms with an ambient temperature of ~17°C) resulted in a better flavor profile than forcing fermentation at 30°C. Scores for samples fermented at ambient temperatures were about 0.5–1.0 points higher than those for their counterparts fermented at 30°C (e.g., at 1850 masl, ambient-fermented samples scored 88.8, whereas samples fermented at 30 °C scored ~88.3; at 1375 masl, ambient-fermented samples scored 86.6 , whereas the samples fermented at 30 °C scored 86.0). Although the observed differences may seem modest in magnitude, in the context of SCA cupping even variations of 0.25 points are relevant, as they represent perceptible changes in the sensory profile and can distinguish coffees with different quality levels.

Table 3: Mean values of the sensory score for Geisha coffee as a function of temperature.

	Altitude (masl)			
	1375	1725	1800	1850
Temperature (°C)				
Farm (19°C)	86.62	89.05	88.96	88.79
24	86.45	88.82	88.96	88.61
30	86.04	88.75	88.41	88.29

Fermentation temperature significantly affected a few chemical constituents of the coffee brews (Table 4). Significant differences were found in 4-CQA, 5-CQA (chlorogenic acids), and ABTS antioxidant activity, attributable to fermentation

temperature ($p < 0.05$ or $p < 0.01$). The highest values were found in the coffees fermented at the farm's ambient temperature (average 19.2°C). For example, concentration of 5-CQA in brews was the highest when fermentation was conducted at ambient temperature (1073 mg/L on average across farms), and it decreased as fermentation temperature increased (to 949 mg/L at 24°C and 892 mg/L at 30°C; see Table 4).

A similar pattern was observed for concentration of 4-CQA and antioxidant activity ABTS, indicating that cooler, slower fermentations retained more of these compounds. Fermentation at 24°C often resulted in intermediate values statistically similar to those of ambient fermentation for some variables (e.g., 4-CQA and antioxidant activity ABTS were not significantly different between the ambient and 24°C

treatments). In contrast, fermentation at 30°C resulted in a notable decrease in these parameters, which were significantly lower than those under ambient conditions in most cases.

3.4 Multivariate analysis of sensory quality and chemical properties

A principal component analysis was performed to synthesize the information from multiple variables. A PCA biplot was obtained based on the first two principal components (PC1 and PC2), which together explained about 58% of the total variance in the dataset (Figure 3). Each point in the biplot represents a coffee sample (identified by its farm), and points are grouped according to farm altitude with 95% confidence ellipses. Vectors indicate the loadings of the original variables on the principal components.

Table 4: Mean values (with standard errors) of the physicochemical variables for Geisha coffee as a function of fermentation temperature. Different superscript letters indicate significant differences between means in the same column (Tukey's HSD, $p < 0.05$).

Temperature (°C)	Final score	pH	Total Soluble Solid (%)	Total acidity (meq/g)	Caffeine (mg/L)	3-CQA (mg/L)	5-CQA (mg/L)**	4-CQA (mg/L)*	Polyphenols (mg GAE/L)	DPPH (mmol trolox/L)	ABTS (mmol trolox/L)**
Farm (19°C)	88.36 ^a	5.01 ^a	1.55 ^a	0.0146 ^a	642.77 ^a	506.35 ^a	1073.12 ^a	546.58 ^a	2429.51 ^a	7.70 ^a	10.34 ^a
24	88.21 ^a	5.01 ^a	1.63 ^a	0.0141 ^a	634.58 ^a	477.86 ^a	948.58 ^b	492.07 ^{a,b}	2386.44 ^a	7.59 ^a	9.93 ^a
30	87.87 ^a	5.00 ^a	1.57 ^a	0.0142 ^a	635.31 ^a	450.33 ^a	891.53 ^b	472.90 ^b	2332.35 ^a	7.36 ^a	9.35 ^b
S.E.M	0.33	0.04	0.08	0.0004	16.62	57.77	35.12	18.37	62.38	0.25	0.15

CQA: Caffeoylquinic acids, **DPPH:** 2,2-diphenyl-1-picrylhydrazyl, **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), **S.E.M:** Standard Error of Mean.

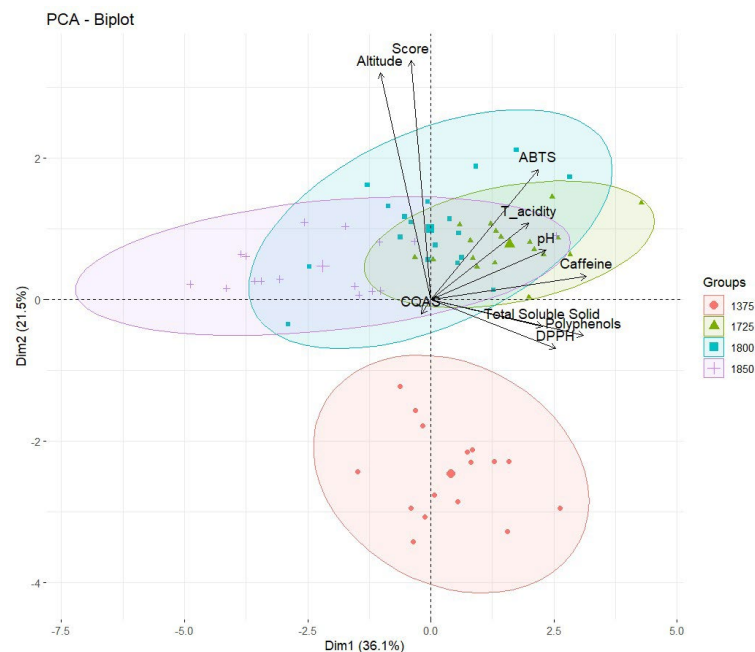


Figure 3: The biplot of the first two principal components shows the relationships between physicochemical variables and the sensory quality of Geisha coffee grown in Panama at different altitudes.

Several prominent patterns emerged from the PCA (Figure 3). First, samples were clustered primarily by altitude. The ellipses for the high-altitude farms (>1700 masl) were well-separated from the ellipse for the lowest-altitude farm (1375 masl) along the PC2 axis. These findings confirmed that overall physicochemical profile of high-altitude Geisha coffees was distinct from that of mid-altitude coffees, supporting the univariate findings. High-altitude samples (1725, 1800, and 1850 masl) clustered relatively closely, indicating that these coffees had similar physicochemical characteristics, although they were from different farms. This suggested a convergent “high-altitude Geisha” profile. In contrast, samples from 1375 masl were offset, driven by their higher Total soluble solids, caffeine, and other factors.

The variable vectors in Figure 3 showed that the altitude vector and the sensory score (cupping score) vector were oriented in nearly the same direction and were roughly aligned with PC2. They were also of relatively large magnitude. This finding indicated a strong positive correlation between altitude and cupping score in this data, suggesting that altitude is a reliable predictor of sensory quality. The near-parallel alignment of the altitude and score on PC2 confirmed earlier observations that a higher altitude was associated with higher scores.

Several other vectors were important. Along the horizontal axis (PC1), a large vector for ABTS antioxidant activity, total acidity, pH was found (in the opposite direction, as higher acidity corresponds to lower pH), caffeine, Total soluble solids, polyphenols, and DPPH. These vectors reflected the significant sources of variance among samples that were not strictly aligned with altitude or score. Many of these vectors were clustered or pointed in similar directions, implying that they were correlated. For example, the vectors for caffeine, Total soluble solids, and pH pointed in roughly the same direction on the biplot, which matched the observed trend lower-altitude samples presented higher caffeine contents, higher Total soluble solids values, and higher pH. These traits occurred together and opposed high-altitude traits. In contrast, the ABTS and total acidity vectors clustered, corresponding to high-altitude samples that had higher acidity and slightly higher ABTS activity than other samples. The chlorogenic acid (CQA) vectors (when plotted individually, 3-CQA, 4-CQA, and 5-CQA) were short in length and pointed in opposite direction of the score vector, toward lower quality on PC2. This suggested that chlorogenic acids content varied and contributed to some variance, variables did not strongly influence the differentiation between high-scoring and low-scoring samples in the presence of the other factors. A higher total CQA(3-CQA+4-CQA+5-CQA), coincided with slightly lower sensory scores in the samples. Thus, the PCA suggested that extremely high chlorogenic acid levels may not necessarily imply better flavor, probably due to the associated bitterness; however, within the range studied, this was a minor effect.

3.5 Predictive modeling of sensory quality

Regression models were constructed to further quantify the relationships and predict the sensory quality of the data (cupping score). The modeling efforts yielded two equations of interest:

Altitude-only model: Using the farm altitude (h , in masl) as the only predictor of cupping sensory quality (SQ), a simple linear model was obtained 1:

$$SQ = 79.83 + 0.005 h \quad (1)$$

Here, h was in meters. According to this model, every 100 m increase in altitude could increase the predicted score by 0.5 points. The altitude alone explained 72% of the variation in the score ($R^2 = 0.72$), and the model had an Akaike information criterion (AIC) of -70.36 . The slope was highly significant ($p < 0.01$). This finding reinforced that altitude was a dominant factor, as about three-quarters of the observed quality differences could be attributed to altitude differences.

Tree-selected variables model: Based on the results of the classification tree analysis, pH, Total soluble solids, DPPH antioxidant, and total chlorogenic acids were identified as the most influential chemical predictors of cup quality. The decision tree importance ranking (aggregated over 500 runs) indicated that pH was the top contributor (relative importance 0.778), followed by Total soluble solids (0.070), DPPH (0.045), and total CQA (0.022). Incorporating altitude along with these key variables in a multiple regression yielded the following model 2:

$$SQ = 72.954 + 0.0051 * h + 1.470 * pH - 0.393 * \text{Total soluble solids} \quad (2)$$

Here, h indicated the altitude (masl), pH indicated the brew pH, and Total soluble solids indicated the percentage of soluble solids. DPPH and total CQA were excluded from the final equation, probably because the included variables already captured their effects. For example, DPPH was correlated with polyphenols, which in turn was correlated with Total soluble solids to some extent, and total CQA was inversely correlated with Total soluble solids and pH in the dataset. In this model, R^2 improved to 0.75, and the AIC improved to about 0.78, with a residual standard error of 0.56 points. The coefficients for altitude and pH were significant ($p < 0.01$), whereas the Total soluble solids coefficient was not significantly different from zero at $p < 0.05$. The positive coefficient for pH initially appeared to be counterintuitive, as higher pH values indicate lower acidity, and high-quality coffees are expected to be more acidic. However, pH served as an indirect marker: the highest-scoring coffees (from high altitudes) had moderately low pH (about 5.0). In contrast, the lowest-scoring coffee had a

slightly higher pH (~5.12 at 1800 masl). The model suggested that when altitude is kept constant, a one-unit increase in pH increases the score by ~1.47 points. This magnitude was large, but in practice, the pH range is narrow (4.8–5.2); therefore, the pH differed by around 0.5 points across samples. Total soluble solids had a negative coefficient, which indicated that an overly high sugar content in the cup might suggest less thorough fermentation or a lower altitude, which in turn is correlated with less complexity. This model highlighted brew pH as a critical quality indicator, potentially related to perceived acidity and brightness in flavor, and confirmed the independent contribution of altitude.

4 DISCUSSION

4.1 Effect of altitude on the sensory quality of Geisha coffee

The substantial positive effect of altitude on Geisha coffee cup quality observed here was like findings reported for other Arabica coffee varieties (de Souza Rolim et al., 2020). The only study published on Geisha by Vega et al. (2021) noted that Geisha samples grown above 1700 masl in Panama had cupping scores between 87% and 92%, which is similar to the results of this study. Several studies on coffee genotypes have reported higher sensory scores at relatively high elevations (Barbosa et al., 2019; Ferreira et al., 2021, 2022). The enhancement of flavor at higher altitudes is generally attributed to distinct environmental factors: lower temperatures slow bean maturation, enabling the accumulation of more complex flavor precursors (Avelino et al., 2005; Leite et al., 2021), whereas greater variation in diurnal temperatures and greater exposure to UV radiation promote the synthesis of desirable metabolites (Ferreira et al., 2022). Higher-altitude beans often have greater acidity (Avelino et al., 2005; Martinez et al., 2022) and lower bitterness (Bertrand et al., 2006). These factors are associated with modifications in the biochemical composition of beans and the activity of microbes on the fruit, resulting in slower ripening and the accumulation of more desirable aroma and flavor compounds (Martinez et al., 2022). Similar altitude-related quality enhancements have even been noted in robusta coffee (*C. canephora*) (Pereira et al., 2021).

4.2 Effect of altitude on physicochemical properties of the coffee brews

This study revealed that higher-altitude coffees contained high levels of chlorogenic acids. This finding were similar to those reported by Martins et al. (2020) and Cassamo et al. (2022). Chlorogenic acids are essential for their antioxidant properties and contribute to bitterness and

astringency in the cup (Cassamo et al., 2022; Martinez et al., 2021). An increase in levels of chlorogenic acids at higher altitudes can thus influence flavor perception, adding desirable complexity up to a point, although excessive levels can be harmful. Total polyphenol content and antioxidant capacity (by ABTS and DPPH assays) also differed with altitude. Polyphenols (which include chlorogenics and other phenolics) are major contributors to the antioxidant activity of coffee, and many studies have reported a decrease in total polyphenols as altitude increases (Martinez et al., 2021; Sik et al., 2023). Consistent with previous findings in fermented coffees, the sample from 1375 masl, characterized by high polyphenol content, exhibited higher ABTS antioxidant activity.

The cooler climates at higher altitudes prolong the maturation and fermentation of trees. Slower ripening allows organic acids to accumulate in the cherry, resulting in a more acidic brew (lower pH). The prolonged presence of active microbiota in cherry during extended maturation can lead to more complete consumption of sugars and lower soluble solids in brewed cups (Martinez et al., 2021, 2022). Caffeine biosynthesis may also decrease slightly in beans grown under cooler, higher-altitude conditions (Cassamo et al., 2022; Girma, Gure, & Wedajo, 2020), which is supported by observations in other studies that caffeine concentrations in Arabica coffee beans decrease with altitude, although not all studies agree, possibly due to differences in varieties (Avelino et al., 2005). Robusta coffees have higher caffeine and chlorogenic acid levels than Arabica coffees (Bertrand et al., 2006), which contribute to a harsher taste (Bastian et al., 2021). Therefore, the relatively lower caffeine and moderate chlorogenic acid contents in high-altitude Geisha coffees partly explain their increased smoothness and complexity.

While altitude affects overall trends, other site-specific factors can influence the chemical profile of coffee (Worku et al., 2018). Factors such as soil fertility, shade cover, species, microclimate (including humidity and sun exposure), and on-farm processing practices may influence the results. For example, the farm at 1375 masl might have had richer soil or a different shade that promoted higher polyphenol retention, like a higher-altitude sample in some respects. The variability among the high-altitude farms (1725, 1800, and 1850 masl) suggested that altitude alone does not account for all differences and reinforced the idea that the terroir of each farm—the combination of altitude and other environmental and management factors—shapes the characteristics of coffee beans. Overall, results highlighted a complex interplay: altitude sets a broad range of conditions, but farm-specific factors and microbial ecology can lead to variability in bean composition within those conditions. This complexity emphasizes the need for further research under controlled conditions to isolate the effects of altitude while keeping other variables constant.

4.3 Effect of fermentation temperature on sensory quality and chemistry

Findings concerning the effects of fermentation temperature on sensory quality and chemistry agreed with those of previous studies on other Arabica coffees. For example, one study noted that beans fermented at 15°C had higher cup scores than those fermented at 30°C (Krohling *et al.*, 2018; Peñuela-Martínez, Moreno-Riascos, & Medina-Rivera, 2023). The improvement at lower temperatures is attributed to the activity of certain microorganisms (such as strains of lactic acid bacteria and some yeasts) that thrive at cooler temperatures and produce desirable metabolites (such as lactic acid, esters, and higher alcohols) that enhance the flavor of coffee. At higher fermentation temperatures, a different microbial community might dominate, leading to over fermentation or the production of off-flavors if not carefully managed (Tan *et al.*, 2023; Várady *et al.*, 2022). Although the microbial populations in this study were not monitored, results indirectly suggested that the microbiota present under ambient cool conditions contributed to the improvement in the quality of Geisha coffee. Future studies should identify and quantify these microbial players during fermentation, as has been performed for other varieties, to better understand how fermentation temperature and microbiology influence the quality of Geisha coffee.

The findings concerning the effects of fermentation temperature on physicochemical parameters aligned with emerging studies on coffee processing. Cooler fermentation might help preserve certain phenolic compounds or slow down enzymatic degradation pathways. During fermentation, chlorogenic acids can be degraded by microbial enzymes or oxidative processes. At higher temperatures, microbial metabolism is faster and probably more exhaustive, potentially breaking down more chlorogenic acids or binding them into insoluble compounds. The lower antioxidant activity in the brews from the 30°C fermentations suggested a loss of some antioxidant constituents, likely probably polyphenols, compared to those in the cooler ferments. Higher-temperature fermentations may produce or leave behind fewer antioxidant compounds in beans. Controlled fermentation conditions can significantly alter the chemical composition of coffee beans. Tan *et al.* (2023) reported that fermented coffee beans generally have higher levels of phenolics and antioxidants than unfermented beans, highlighting the role of fermentation in modulating these compounds. Várady *et al.* (2022) reported that different processing methods (including natural and anaerobic fermentation) could lead to different levels of bioactive and volatile compounds, with some methods preserving more chlorogenics. Roasting substantially reduces the chlorogenic acid content (through conversion into melanoidins and other products). That possibility was ruled out in this case since all

samples were roasted similarly, and the differences occurred due to pre-roast differences in the green beans. These findings indicated that a carefully controlled fermentation process, in this case, naturally cooler ferments, was advantageous for retaining certain quality-related chemicals in Geisha coffee. These chemical differences may contribute to sensory discrepancies; for example, the higher retention of chlorogenic acids and related polyphenols in cooler-fermented coffees correlated with higher complexity or brightness in the cup, up to the threshold where bitterness becomes a factor. Overall, these results reinforced that fermentation is a critical step that requires optimization; small temperature changes can significantly alter the balance of microbial activity and chemical outcomes, thereby affecting the final cup quality.

The fermentation results suggested that maintaining a cooler temperature benefits sensory and chemical quality. This raises an important question for producers: should they attempt to ferment at even lower temperatures than the ambient temperature, for example, using cooling techniques to approach ~15°C, as some studies have reported, or should the natural ambient temperature of a high-altitude farm be sufficient? The findings indicate that ambient temperatures, averaging ~19°C, yielded better results than 24°C or 30°C. Lowering the temperature may further enhance specific profiles, but extremely low temperatures might also prolong fermentation excessively or result in the selection of different microbes. Controlling fermentation conditions is a key parameter for quality modulation, and its importance was second only to altitude in this study.

4.4 Multivariate analysis of sensory quality and chemical properties

The PCA results confirmed that altitude is associated with many changes in bean chemistry that are correlated with cup quality. It also showed that some variables were redundant. For example, polyphenol content and DPPH antioxidant activity vectors overlapped considerably, indicating that these measures provided overlapping information. Similarly, Total soluble solids and caffeine levels were positively correlated, suggesting that coffee cherry ripening and metabolic activity are related. Therefore, when constructing predictive models, including all correlated variables may not improve the predictive power beyond the use of one or two representative variables.

4.5 Predictive modeling of sensory quality

Two prediction models were constructed: the altitude-only model, which explained 72% of the variation in the score ($R^2 = 0.72$), and the tree-selected variables model, with an R^2 of 0.78, in which altitude, pH, and Total soluble solids explained 78% of the variation in the score. Even the “best” model left 22% of the sensory variation unexplained. This finding

indicated that factors outside the measured set contributed to differences in cup quality. The model had the greatest difficulty with the sample that had the highest observed score (88.97 at 1725 masl); the uncertainty for that prediction was the greatest. Unmeasured variables, such as soil nutrients, micronutrients, specific microbial metabolites, or other post-harvest handling nuances, might explain the remaining variation. The best predictor, altitude, is a proxy for several environmental conditions. To increase the predictive accuracy beyond 78%, additional agroecological and processing factors need to be incorporated.

In future studies, variables such as soil chemical properties (pH and mineral content) and the type and density of shade trees, which can affect the microclimate and bean development, as well as detailed fermentation microbiology, can help reduce the unexplained variance (Tsegay et al., 2020). For example, soil nutrient availability can influence the composition of green beans and, consequently, their flavor. Shading influences the temperature and humidity around coffee and might alter the development of berries (Leite et al., 2021). Additionally, even within different lineages of Geisha or their adaptations to local conditions, genetic factors might play a role, although all samples from this study were of the same variety. Developing a comprehensive model for coffee quality may require a combination of environmental data, controlled experiments, and sensory panel differences. This study took a step in that direction for the Geisha variety, demonstrating that a significant portion of its famed quality can be explained scientifically (altitude and chemistry) and also highlighting that some of the “magic” of the Geisha variety is attributable to subtle and complex influences.

5 CONCLUSIONS

Altitude was the dominant driver of Geisha coffee quality in Panama, explaining 70–75% of sensory variation, with higher elevations (≥ 1700 masl) associated with favorable chemical profiles and superior cup quality. Cooler fermentation temperatures further enhanced quality by preserving key flavor-related compounds and antioxidant capacity. Multivariate models integrating altitude and selected chemical indicators accounted for up to 78% of the variation in quality, indicating that while altitude is critical, additional environmental, management, and microbial factors warrant further investigation.

6 AUTHORS' CONTRIBUTION

Conceptual Idea: Vega, A.; Gallardo, J.M.; Methodology design: Vega, A.; Quiroz, R.; González Bermann, P.; Data collection: Reyes, S.M.; Quiroz, R.; Gallardo, J.M.; Ruiz, M.E.; Data analysis and interpretation: Vega, A.; Reyes, S.M.

González Bermann, P., and Writing and editing: Vega, A.; Reyes, S.M.; Quiroz, R.; González Bermann, P.

7 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

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