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Influence of cultivation region on color, volatile compounds, phenolics, and antioxidant activity of Arabica coffee (*Coffea arabica*) Catuaí cultivar in Brazil

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Abstract

Coffee is one of the world's main commodities, with Brazil being the largest producer. When it meets production and sensory quality requirements, coffee can be classified as a specialty by the Brazilian Coffee Industry Association or Specialty Coffee Association, being recognized for its aroma and taste, defined by volatile and non-volatile organic compounds, as well as the benefits such as antioxidant activity, which can vary according to the cultivation region. This study evaluates the influence of different planting locations on volatiles, color, phenolics, and antioxidant activity in Arabica coffee (Catuaí cultivar) from five Brazilian regions. The coffees were roasted uniformly, analyzed by gas chromatography-mass spectrometry for volatiles, colorimeter for color, and liquid-solid extraction for phenolics (Folin-Ciocalteu) and antioxidant activity [2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid), 2,2-diphenyl-1-picrylhydrazyl, and ferric reducing antioxidant power]. The data was treated using Analysis of variance and Tukey's test, as well as boxplot, principal component analysis, and heatmap. The colors varied significantly between the regions. Twenty-seven volatiles were common to the 5 regions, with distinct sensory contributions such as pyridine and acetic acid in coffee from the Rio Paranaíba region, and 1-methylpyrrole for the other regions. For phenolic and antioxidant content, coffee from the Rio Paranaíba region had the highest values. It was possible to see a distinction between the coffees by planting location.

Keywords: specialty coffee; brazilian regions; organic compounds; antioxidant activity.

Practical Application: Insights on regional effects help to optimize specialty coffee quality, flavor, and health benefits.

1 INTRODUCTION

Coffee is one of the most consumed beverages in the world, second only to water, with an estimated 2.2 billion cups a day (Surma & Oparil, 2021). World production is dominated by 2 of the 80 species of the Coffea genus, namely *Coffea* arabica and *Coffea canephora*, which accounted for 56 and 43%, respectively, of the total production in the 2021/2022 harvest (Internacional Coffee Organization [ICO], 2022).

Brazil is the largest producer and exporter of coffee worldwide (Volsi et al., 2019), with an output of 54.2 million 60 kg bags in 2024. The Brazilian state of Minas Gerais leads national production, accounting for 52%, followed by the states of Espírito Santo, with 26%, São Paulo, with 10%, and Bahia with 6% (Companhia Nacional de Abastecimento [CONAB], 2025; Empresa Brasileira de Pesquisa Agropecuária [Embrapa], 2023).

However, the chemical composition of coffee is influenced by several factors, including species (Freitas et al., 2024); variety (Kitzberger et al., 2014); planting location and climatic conditions (Ahmed et al., 2021; Bertrand et al., 2012; Cassamo et al., 2022; Tieghi et al., 2024); post-harvest processing (Borém et al., 2023; Cao et al., 2023); and roasting degree (Mestanza et al., 2023; Odžaković et al., 2016).

According to the Brazilian Ministry of Agriculture and Livestock (*Ministério da Agricultura e Pecuária – MAPA*) and the Brazilian Coffee Industry Association (*Associação Brasileira da Indústria de Café – ABIC*), roasted coffee is classified into five quality categories: extra strong (lower quality); traditional; superior; gourmet; and specialty (higher quality) (ABIC, 2021; Brasil, 2022). At the international level, the Specialty Coffee Association (SCA) classifies coffee based on sensory analysis performed by trained tasters, known as Q-Graders. Specialty coffee is defined as that which obtains a score of 80 or above on a 100-point scale (SCA, 2024; Tieghi et al., 2024). It is characterized by high sensory quality (aroma and taste) and minimal defects, resulting from complex production and processing practices that distinguish it from traditional coffees (Chang et al., 2021; Ramírez-Correa et al., 2020; Raveendran & Murthy, 2022).

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Coffee quality is attributed to both volatile and non-volatile organic compounds. Shen et al. (2025) found, in arabica coffee fermented by *Lactipçantibacillus plantarum*, a total of 2,275 non-volatile organic compounds and 1,139 volatile organic compounds, which enhanced the taste and aroma of the coffee, contributing to the differentiation between specialty and non-specialty (traditional) coffee. Similarly, Alcantara et al. (2021) reported that specialty coffees have a higher concentration of compounds such as caffeic acid, hydroxymethylfurfural (HMF), and 3-caffeoylquinic acid (3-CQA), which contribute to superior sensory quality.

In addition to its sensory attributes, coffee is associated with numerous health benefits due to its antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. Its bioactive compounds, including alkaloids (caffeine and trigonelline) and phenolics (chlorogenic acids) (Tieghi et al., 2024), have been linked to the prevention of conditions such as obesity, metabolic syndrome, type 2 diabetes, and Parkinson's disease (Koníčková et al., 2024; Santos et al., 2021; Shen et al., 2025).

Given this, the present study aimed to emphasize how variations in planting location influence the composition of volatile organic compounds, color, total phenolic content, and antioxidant activity in specialty coffee of the species *Coffea arabica*, Catuaí cultivar (cv), from five different locations in Brazil.

1.1 Relevance of the work

The production of specialty coffee holds significant economic and sensory importance, especially in Brazil, the world's largest producer. Volatile and phenolic compounds, responsible for aroma, taste, and health benefits, like antioxidant activity, vary according to the cultivation region, directly impacting final quality. This study provides essential insights into how different Brazilian regions affect these chemical and physical properties, contributing to regional coffee valorization and offering relevant data for producers, exporters, and consumers.

2 MATERIAL AND METHODS

2.1 Coffee

The coffees were donated by Muy Café ® (Goiânia, Goiás, Brazil). Specialty coffees from five different Brazilian regions were provided: Cerrado Mineiro – Rio Paranaíba, Minas Gerais (C1); Chapada Diamantina – Piatã, Bahia (C2); Serra do Caparaó – Ibatiba, Espírito Santo (C3); Serra do Caparaó – Espera Feliz, Minas Gerais (C4); and Serra da Mantiqueira – Natércia, Minas Gerais (C5), as shown in Figure 1. All samples are of the species *Coffea arabica* and Catuaí cv.

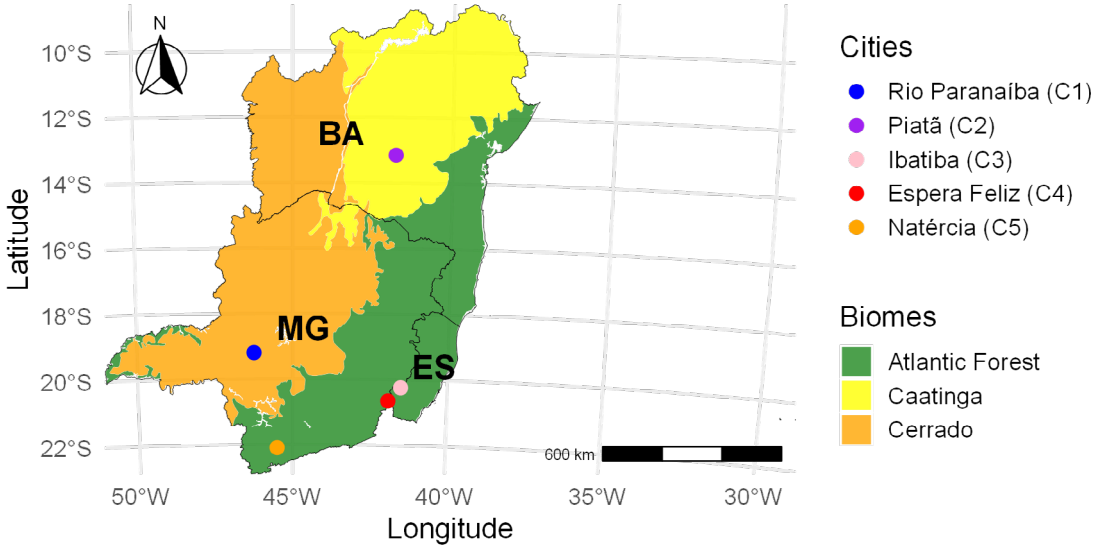
A Kaleido roaster (Sniper M2, Wuhan, China) was used to roast the samples for an average of 8 minutes and 45 seconds, with an input temperature of 139°C and an output temperature of 203°C. The roasting process continued until the "first crack," followed by a cooling stage to halt the process. Once cooled, an electric coffee grinder (Oster OMDR 100-220, Newell Brands, Brazil) was used to grind the samples. At the end of the process, 100 g of each ground coffee sample was stored in amber glass containers and kept frozen at –30°C until further analysis.

2.2 Color analysis

The ground samples were analyzed using a ColorQuest XE spectroophotometer (Hunter Associates Laboratory, Virginia, USA). The results were expressed on the International Commission on Illumination (CIE) LAB scale, with L* (lightness), a* (red-green), and b* (yellow-blue). Equations 1 and 2 were used to determine C* (saturation) and H° (hue angle), respectively.

$$C^* = \sqrt{a^{*\,2} + b^{*\,2}} \quad (1)$$

$$H^\circ = \arctan\left(b^* / a^*\right) \quad (2)$$



Map of the Brazilian states of Minas Gerais (MG), Bahia (BA), and Espírito Santo (ES), with representation of the Caatinga, Cerrado and Atlantic Forest biomes in yellow, orange, and green, respectively. City of Rio Paranaíba (MG), in blue. Piatã (BA) in purple. Ibatiba (ES) in pink. Espera Feliz (MG) in red. Natércia (MG) in orange. Source: Brazilian Institute of Geography and Statistics (*Instituto Brasileiro de Geografia e Estatística* – IBGE), 2024.

Figure 1. Map of the producing regions of the analyzed specialty Arabica coffees.

2.3 Volatile organic compounds

For the analysis of volatile organic compounds, 3 grams of each ground coffee sample were incubated in vials for gas chromatography-mass spectrometry (GC-MS) analysis using a Nexis GC 2030 (Shimadzu, Kyoto, Japan) coupled to a Shimadzu QP2020 Nexis (Shimadzu, Kyoto, Japan), operating in Scan mode, with an SH-Stabilwax-MS column (30 m x 0.25 mm x 0.25 µm). The sample was heated using the headspace method, with an incubation temperature of 60°C and a syringe temperature of 70°C. The stirrer speed was set to 300 rpm, the pre-purge time to 5 seconds, the injection flow rate to 10 mL/min, and the total analysis time to 60 minutes. Chromatographic conditions included an oven temperature of 40°C and an injection temperature of 250°C in split mode. Flow control was maintained at a linear speed, with a pressure of 45.1 kPa, a total flow of 13.4 mL/min, and a column flow of 0.94 mL/min. The linear velocity was 35 cm/s, and the purge flow was 3.0 mL/min.

2.4 Determination of total phenolic content and antioxidant activity

Previously, the roasted and ground coffee samples were subjected to liquid-solid extraction. The total phenolic content was determined using the Folin-Ciocalteu method, while antioxidant activity was assessed using the 2,2-azinobis-(3-ethylbenzthiazolin-6-sulfonicacid) (ABTS), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods.

2.4.1 Extraction

Two grams of each sample were placed in beakers containing 50 mL of 70% (v/v) methanol. The samples were then subjected to an ultrasonic bath (Digital Ultrasonic Cleaner, D409X, CTA, Brazil) at 25°C for 15 minutes to extract bioactive compounds from the coffee powder. The mixture was subsequently filtered using filter paper and a glass funnel.

2.4.2 Total phenolic content

To determine the total phenolic content of each sample, the Association of Official Analytical Collaboration (AOAC) 2017.13 methodology was followed, using the Folin-Ciocalteu method. Standard calibration solutions ranging from 40 to 200 mg/L were prepared from a 1.1 g/L gallic acid solution, transferred to test tubes, and combined with 0.75 mL of 20% sodium carbonate. The standard curve was constructed using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) at an absorbance of 765 nm. Measurements were made in triplicate, and the results were expressed as mg gallic acid equivalent (GAE)/g of sample on a dry basis (db.) (AOAC, 2016).

2.4.3 ABTS$^{\bullet+}$

Antioxidant activity was determined using the 2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) radical scavenging method, following the methodology proposed by Rufino et al. (2007). The ABTS$^{\bullet+}$ radical was prepared by mixing a 7 mM ABTS solution with potassium persulfate (K2S2O8). Then, 1mL of this solution was diluted in methanol until an absorbance of 0.700 nm was obtained in a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 734 nm. From the previously prepared solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) at 2 mM, the standard solutions of Trolox and methanol were prepared in a 10 mL volumetric flask, obtaining final concentrations 100-2,000 µM of Trolox. The standard curve was then obtained by mixing 3.0 mL of ABTS$^{\bullet+}$ and 30 µL of Trolox. Finally, the extracts were analyzed. Measurements were performed in triplicate, and the results were expressed as mg of Trolox equivalent (TE)/g of sample on db.

2.4.4 2,2-diphenyl-1-picrylhydrazyl

The antioxidant activity was also determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, following the AOAC 2012.04 methodology. A 40 mg/L DPPH radical solution and a 0.5 mg/mL Trolox solution were prepared. Standard solutions were prepared by pipetting 0.2–0.8 mL of Trolox and topping up with the DPPH solution and methanol. The samples were analyzed using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 517 nm. Each sample was analyzed in triplicate, and the results were expressed as mg of TE/g of sample on db (AOAC, 2016).

2.4.5 Ferric reducing antioxidant power

Antioxidant activity was determined using the iron ion (Fe^{3+}) reduction method, ferric reducing antioxidant power (FRAP), using the methodology proposed by Rufino et al. (2006). Starting with a solution of FRAP and Trolox µM and making standard solutions of Trolox and methanol 160.0–800.0 µmol/L, the standard curve was constructed by mixing 90 µL of Trolox with 270 µL of milli-Q water and 2.7 mL of FRAP reagent. Absorbance was measured at 595 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). All samples were analyzed in triplicate, and the results were expressed as mg of TE/g of sample on db.

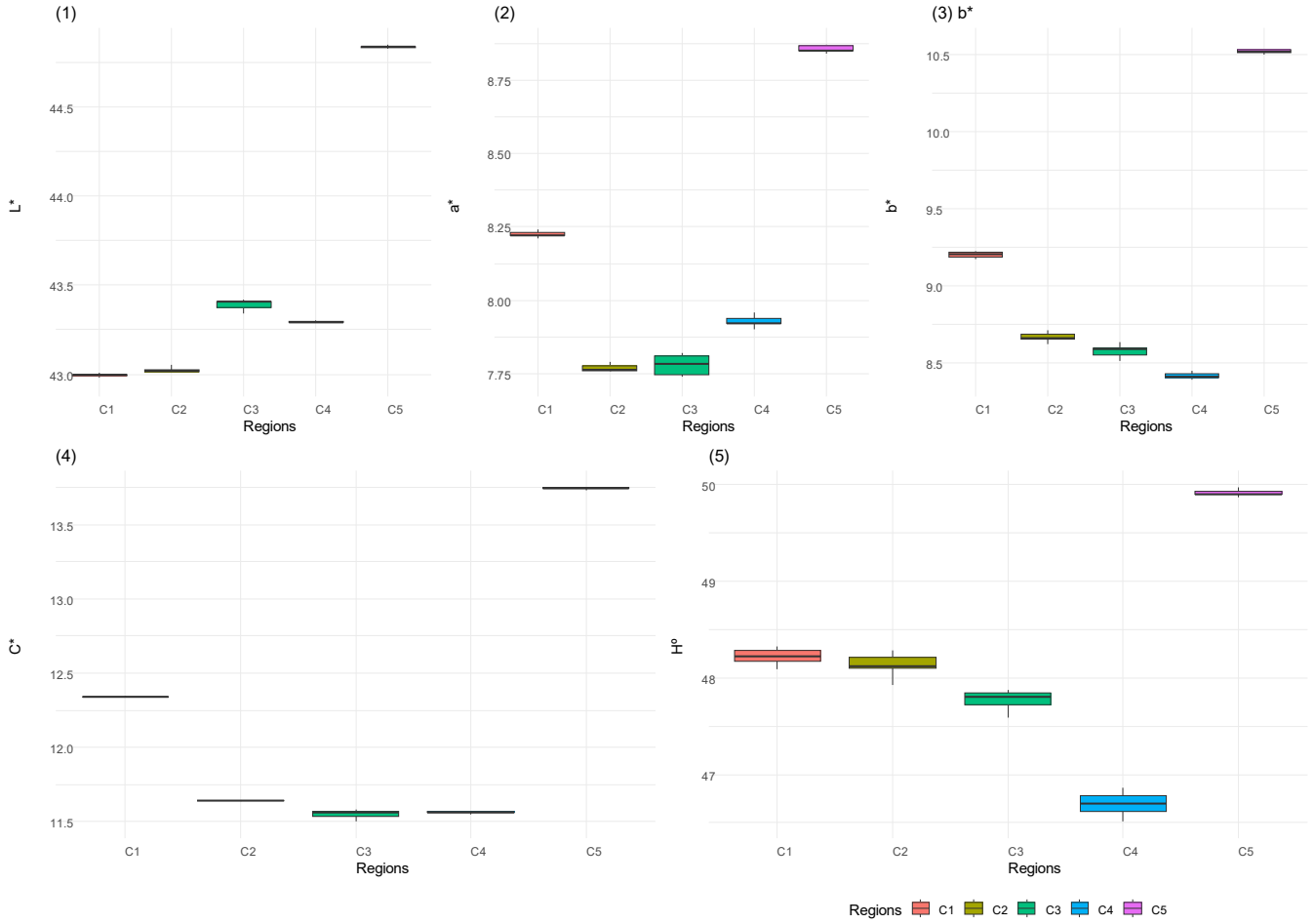
2.5 Statistical analysis

Experimental data were presented as mean and standard deviation. Statistical analysis was performed using TIBCO Statistica software (Version 14.0), with Analysis of variance (ANOVA) and Tukey's applied at a 95% significance level to determine statistically significant differences among mean values. In addition, using RStudio software (Version 2024.09.0 + 375), a principal component analysis (PCA), boxplots, and heatmaps were prepared to evaluate the similarities and differences between the characteristics obtained from the five coffee regions.

3 RESULTS

3.1 Color

Using the ColorQuest XE colorimeter (Hunter Associates Laboratory, Virginia, USA) the L*, a*, and b* coordinates values were obtained. Additionally, using Equations 1 and 2, the C* and H° values were calculated for the samples from the five regions, as shown in Figure 2. For the L* parameter,



1) L* parameter (luminosity) by region. 2) a* parameter (red-green) by region. 3) b* parameter (yellow-blue) by region. 4) C* parameter (saturation) by region. 5) H° parameter (hue angle) by region.

Figure 2. Parameters L*, a*, b*, C*, and H° for the five coffee regions.

which ranges from 100 (completely white) to 0 (completely black) (Advancing Standards Transforming Markets [ASTM], 2023), all regions exhibited significantly different values, according to the Tukey test with $p > .05$. This indicates that the coffee beans from the C1 region, after the roasting process, had darker tones (43.00 ± 0.02), while those from the C5 region had lighter tones (44.84 ± 0.01). Regarding a* values, which represent shades of red for positive values and shades of green for negative values (Broadbent, 2017), the parameters for the C2 and C3 regions were statistically the same, at 7.77 ± 0.02 and 7.78 ± 0.03, respectively. However, for b*, which indicates shades of yellow for positive values and blue for negative values (Broadbent, 2017), a significant difference was observed among all regions.

For saturation values (chroma), regions C3 and C4 had the lowest values and were significantly equal. C1 had an intermediate result, while C5 exhibited the highest saturation (13.75 ± 0.02). Overall, the data obtained pointed to a low saturation (low brightness) of the samples, since values close to zero are related to dull and grayish colors (Tuberoso et al., 2014). As for H° values, which range from 0° (red) to 90° (yellow), 180° (green), and 270° (blue) to 360° (Broadbent, 2017), region C4 had the lowest value (48.13 ± 0.11), while C1 and C2 had significantly equal results. The highest values was observed in C5 (49.89 ± 0.07), indicating orange tones in all samples, intermediate between red and yellow.

Finally, for all parameters, the C5 region exhibited the highest L*, a*, b*, C*, and H° values. Although all samples underwent the same roasting process, the grains acquired significantly different parameters.

3.2 Volatile organic compounds

In total, 57 volatile organic compounds were identified in the samples by GC-MS. In C1, 43 compounds were identified, while 37 were identified in C2 and C3, 33 in C4, and 39 in C5. Of the 57 total compounds, 27 were present in all samples. Table 1 lists these compounds along with their retention time, percentage area of the chromatographic peaks, chemical formula, functional group, and sensory contribution.

Using bibliographic data, the sensory contributions of each volatile organic compound were categorized individually, as shown in the "Sensory Contribution" column of Table 1.

Table 1. 27 volatile organic compounds found in the five regions.

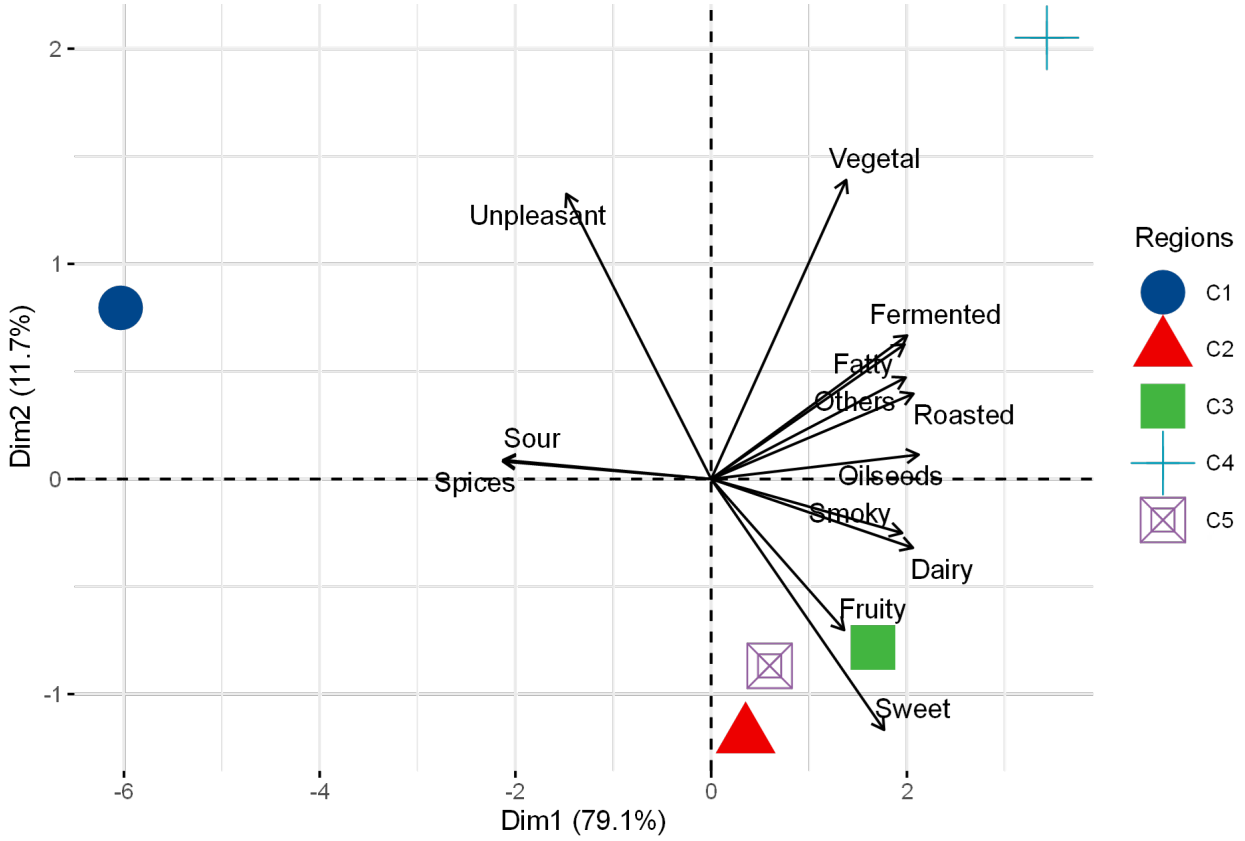
Volatile organic compounds	C1		C2		C3		C4		C5		Chemical formula	Functional group	Sensory contribution
	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %			
2-Methylbutanal	3.122	4.37	3.119	5.85	3.111	5.13	3.113	8.01	3.111	6.17	$C_5H_{10}O$	Aldehyde	Malty, buttery, green
2,3-Butanedione	4.198	5.32	4.192	8.11	4.182	10.29	4.189	11.6	4.18	10.08	$C_4H_6O_2$	Ketone	Buttery, extravagant
2,3-Pentanedione	6.355	3.53	6.348	4.86	6.334	6.31	6.344	7.03	6.336	5.69	$C_5H_8O_2$	Ketone	Oily, buttery
1-Methylpyrrole	8.683	0.19	8.680	0.17	8.646	0.26	8.679	0.34	8.665	0.19	C_5H_7N	Heterocyclic N	Smoky, woody, herbal
Pyridine	10.192	2.08	10.219	1.22	10.233	1.40	10.208	1.36	10.218	1.05	C_5H_5N	Heterocyclic N	Sour, bitter, toast, putrid
Pyrazine	11.092	0.40	11.101	0.52	11.092	0.67	11.085	0.82	11.085	0.60	$C_4H_4N_2$	Pyrazine	Cooked spinach, peanut, rancid, strong
Dihydro-2-methyl-3(2H)-furanone	12.620	1.38	12.619	1.65	12.610	2.13	12.614	2.17	12.608	1.88	$C_5H_8O_2$	Furan	Sweet, baked, bread
Methylpyrazine	12.752	2.19	12.752	3.12	12.738	3.98	12.740	4.82	12.735	3.82	$C_5H_6N_2$	Pyrazine	Nut
Acetoin	13.281	1.13	13.283	1.26	13.279	1.62	13.275	1.31	13.272	1.47	$C_4H_8O_2$	Ketone	Sweet, buttery, creamy
1-Hydroxy-2-propanone	13.752	8.13	13.750	13.49	13.749	13.76	13.742	10.87	13.742	14.91	$C_3H_6O_2$	Ketone	Creamy, buttery, caramel
2,5-Dimethylpyrazine	14.485	0.44	14.477	0.81	14.460	1.00	14.468	1.13	14.461	0.95	$C_6H_8N_2$	Pyrazine	Nut, toast, fatty, peanut, moldy
2,6-Dimethylpyrazine	14.642	0.44	14.638	0.68	14.624	0.84	14.628	1.00	14.622	0.83	$C_6H_8N_2$	Pyrazine	Chocolate, cocoa, toast nuts
Ethylpyrazine	14.730	0.42	14.727	0.56	14.715	0.73	14.721	0.85	14.714	0.67	$C_6H_8N_2$	Pyrazine	Nut, peanut, butter
2-Hydroxy-3-pentanone	15.439	0.14	15.435	0.20	15.429	0.24	15.434	0.23	15.425	0.22	$C_5H_{10}O_2$	Ketone	Truffle
1-Hydroxy-2-butanone	15.903	0.39	15.903	0.65	15.892	0.68	15.900	0.52	15.888	0.66	$C_4H_8O_2$	Ketone	Sweet, coffee
2-Ethyl-6-methylpyrazine	16.216	0.15	16.207	0.21	16.193	0.29	16.199	0.34	16.190	0.29	$C_7H_{10}N_2$	Pyrazine	Floral, fruity, hazelnut-like
2-Ethyl-5-methylpyrazine	16.381	0.09	16.380	0.14	16.363	0.20	16.370	0.20	16.361	0.18	$C_7H_{10}N_2$	Pyrazine	Coffee
Acetic acid	17.881	39.31	17.972	28.39	17.958	26.69	18.014	21.62	17.962	27.44	$C_2H_4O_2$	Organic Acid	Sour, spicy, vinegar
Furfural	18.426	2.13	18.418	4.69	18.404	5.37	18.417	4.95	18.404	5.33	$C_5H_4O_2$	Aldehyde	Bread, almond, sweet
1-Acetoxy-2-propanone	18.506	1.83	18.501	2.01	18.495	2.58	18.499	2.36	18.490	2.43	$C_5H_8O_3$	Ketone	No aroma
Furfuryl formate	19.245	0.18	19.242	0.18	19.226	0.28	19.240	0.28	19.230	0.25	$C_6H_6O_3$	Furan	Ethereal, volatile
1-(2-Furyl)ethanone	19.491	0.28	19.487	0.47	19.471	0.58	19.486	0.46	19.474	0.51	$C_6H_6O_2$	Ketone	Sweet, balsam, almond, cocoa
Propionic acid	20.122	0.17	20.122	0.21	20.110	0.27	20.117	0.25	20.111	0.22	$C_3H_6O_2$	Organic Acid	Pungent, sour, rancid
Furfuryl acetate	20.239	1.11	20.239	0.72	20.224	0.85	20.238	0.94	20.227	0.73	$C_7H_8O_3$	Furan	Ethereal floral, spicy herbs, garlic, onion
5-Methyl-2-furancarboxaldehyde	21.241	0.71	21.241	1.37	21.225	2.01	21.240	2.14	21.229	1.92	$C_6H_6O_2$	Furan	Spices, caramel, maple
2-Furanmethanol	23.267	5.42	23.267	7.12	23.259	8.17	23.263	7.55	23.257	7.24	$C_5H_6O_2$	Furan	Sweet, banana fruit, ethereal aroma, caramel, burnt, smoky
3-Methylbutanoic acid	23.469	0.44	23.465	0.65	23.456	0.68	23.464	0.67	23.457	0.63	$C_5H_{10}O_2$	Organic acid	Cheese, dairy, creamy, fermented

RT: Retention time, in minutes; Area %: percentage area of the chromatographic peaks of the samples.

Many terms are repeated, meaning multiple compounds contribute to the same sensory perception. For example, 2-methylbutanal, 2,3-butanedione, 2,3-pentanedione, and others contribute to a "buttery" sensory note, while methylpyrazine, 2,5-dimethylpyrazine, ethylpyrazine, and other compounds contribute to a "nutty" perception. Since several sensory attributes are closely related (*e.g.*, buttery, cheesy, and creamy correspond to dairy; putrid and musty indicate unpleasant odors), these contributions were grouped into 13 similarity categories (sweet, sour, smoky, unpleasant, spicy, fermented, fruit, fatty, dairy, oilseeds, other, roasted, and vegetal). By analyzing the peak areas of the volatile compounds, a PCA was performed to compare the predominant sensory contributions of the volatile organic compounds across the five regions, as illustrated in Figure 3.

3.2.1 Principal component analysis

The PCA graphic shows that the two dimensions (Dim1 and Dim2) together explain 90.8% of the sensory variations in the volatile organic compounds across the regions. Dim1 (79.1%) represents a balance between sensory contributions, with the variables roasted (9.13%), sour (9.64%), oilseeds (9.60%), spices (9.67%), fatty (8.34%), smoky (8.13%), and other (8.43%) standing out. The variables exert both positive (roasted, oilseeds, smoky, and other) and negative (spices and sour) influences along the horizontal axis. Dim2 (11.7%), which represents variations along the vertical axis, is primarily influenced by a few variables, being strongly characterized by vegetal (27.79%), unpleasant (25.27%), and sweet (19.56%) sensory contributions. As a result, based on the PCA, C1 and C4 were the most distinct regions, while regions C2, C3, and C5 exhibited greater



Regions represented by blue circle (C1), red triangle (C2), green square (C3), blue "+" symbol (C4), square with purple edges (C5). In black are the sensory contributions.

Figure 3. Principal component analysis of sensory contributions in the five regions.

similarity in the sensory contributions of the analyzed volatile organic compounds.

3.2.2 Heatmap

A heatmap (Figure 4) was generated to illustrate variations in the main volatile organic compounds, *i.e.* those with a chromatographic peak area percentage greater than 1%, across the regions, providing a clearer visualization of regional similarities.

Compounds such as acetic acid, pyridine, and furfuryl acetate were found in higher proportions in region C1. However, for the remaining compounds, this region exhibited the lowest percentage in area. In region C2, no compound had a higher percentage of area than in the other regions, whereas C3 (acetoin), C4 (2-methylbutanal, methylpyrazine, 2,3-pentanedione), and C5 (1-hydroxy-2-propanone) each had specific compounds with the highest values. The dendrogram at the top indicates similarity between regions C3 and C5, with C1 showing the least similarity to the other regions. The dendrogram on the left highlights similarities among certain compounds across regions, such as 2,3-butanedione and 2,6-dimethylpyrazine, which reached maximum values in C4 and minimum values in C1.

3.3 Total phenolic content and antioxidant activity

Data on the total phenolic content and antioxidant activity of specialty coffees from the five regions, analyzed using the Folin-Ciocalteu, ABTS, DPPH, and FRAP methods, are presented in Table 2.

Regions C3 and C5 exhibited the lowest phenolic compound content, while C1 had the highest value. Regions C2 and C4 had intermediate values. Comparing the maximum (C1) and minimum (C3) values, the percentage difference in phenolic content was 18.86%.

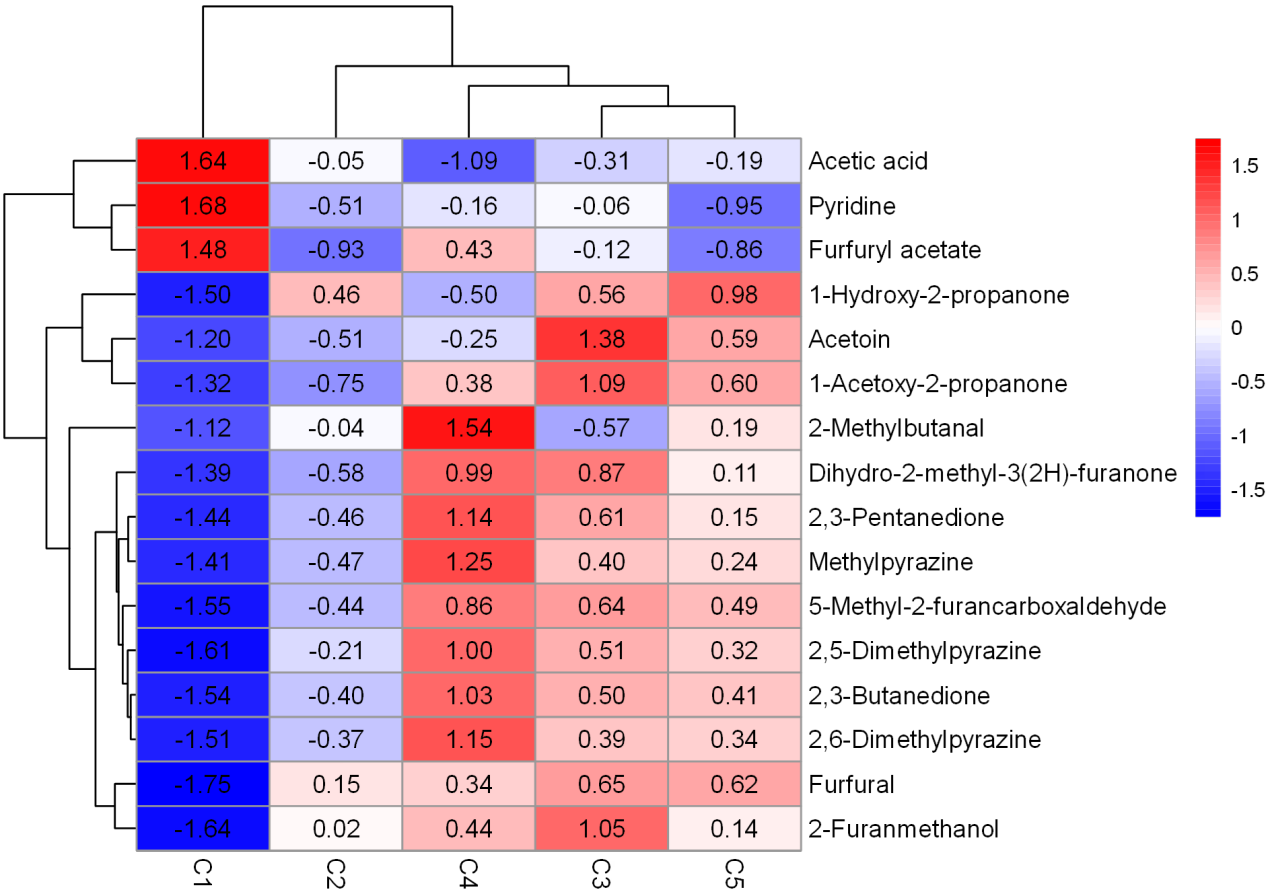
In the ABTS analysis, all regions showed statistically equal averages, unlike the other methods. In the DPPH analysis, regions C3 and C5 had statistically equal averages, C2 and C4 exhibited intermediate values, and region C1 had the highest experimentally obtained value. Similarly, the FRAP results followed the same trend as the DPPH method, with C3 and C5 showing statistically equal averages, C2 and C4 intermediate values, and C1 the highest result.

A consistent pattern was observed when comparing phenolic content with antioxidant across all methods (ABTS, DPPH, or FRAP). Regions C3 and C5 consistently showed the lowest statistical values in all analyses, C2 and C4 had intermediate values, and C1 exhibited the highest statistical values.

4 DISCUSSION

4.1 Color

Roasting is one of the final stages of coffee processing and directly impacts the drink's final quality (Freitas et al., 2024). It leads



C1, C2, C3, C4, and C5 represent the five coffee regions. The dendrograms are shown at the top and left-hand side of the heatmap. On the right-hand side, the name of each compound is listed alongside a correlation color scale between the compounds and the regions. A higher shade of red indicates a stronger correlation, while a higher shade of blue indicates a weaker correlation.

Figure 4. Heatmap of volatile compound correlations by region.

Table 2. Phenolic compounds, 2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid), 2,2-diphenyl-1 picrylhydrazyl, and ferric reducing antioxidant power of the five regions.

Regions	Phenolic compounds (mg G.A.E. g $^{-1}$)	ABTS (mg T.E. g $^{-1}$)	DPPH (mg T.E. g $^{-1}$)	FRAP (mg T.E. g $^{-1}$)
C1	45.43 ± 0.28 ^{c}	62.80 ± 8.83 ^{a}	71.59 ± 3.39 ^{b}	72.72 ± 3.77 ^{c}
C2	42.50 ± 1.14 ^{b}	66.05 ± 4.52 ^{a}	65.84 ± 2.45 ^{ab}	62.90 ± 0.69 ^{b}
C3	38.22 ± 0.97 ^{a}	66.80 ± 5.86 ^{a}	60.28 ± 0.50 ^{a}	50.72 ± 3.36 ^{a}
C4	42.19 ± 0.64 ^{b}	61.14 ± 4.91 ^{a}	65.69 ± 3.14 ^{ab}	61.67 ± 0.89 ^{b}
C5	39.55 ± 0.54 ^{a}	57.70 ± 11.30 ^{a}	61.87 ± 1.50 ^{a}	53.76 ± 2.39 ^{a}

Averages in the column with the same letter are not significantly different (Tukey test; $p > .05$). Phenolic content is expressed in milligrams of gallic acid equivalent per gram of sample on a dry basis. ABTS [2,2'-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid)], DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power) values are expressed in milligrams of Trolox equivalent per gram of sample on a dry basis.

to the formation of various compounds responsible for sensory characteristics such as aroma and taste, while also causing physical changes in the beans, including alterations in color, density, size, and relative humidity (Freitas et al., 2023; Ramanda et al., 2024). One of the most significant changes is the transition in color, from greenish to yellowish, ultimately reaching brown due to the Maillard Reaction and caramelization (Al-Shemmeri et al., 2024).

By adjusting time and temperature parameters, three different roast levels can be achieved: light, medium, and dark. In this experiment, the average values obtained for L*, a*, b*, C*, and H° were 43.51, 8.11, 9.08, 12.17, and 48.14, respectively, consistent with the data reported by Bicho et al. (2012). These values can be attributed to variations in the roasting method, specifically differences in time and temperature. Somporn et al. (2011), when analyzing three reast levels for Arabica coffee Catimor cv., found approximate L*, a*, and b* values of 40, 20, and 5, respectively, for light roasting. L* values decreased with roasting, indicating darker grains, while b* values also decreased, reducing the intensity of the yellow hue. Conversely, a* values increased slightly with roasting intensity, reflecting an enhancement of red tones. Pramudita et al. (2017) examined the influence of roasting temperature and time on bean color, reporting that temperatures below 220°C significantly affected melanoidin accumulation, resulting in higher L* values than those observed at higher temperatures. Bicho et al. (2012) applied three different roasting levels — varying time and temperature — to Arabica and Robusta coffee beans, as well as ground coffee from these species They obtained the following values for Arabica coffee powder: L* = 49.8 to 37.4; a* = 10.1 to 4.63; b* = 17.2 to 3.74; C* = 19.9 to 5.95; and H° = 59.5 to 38.9, highlighting the significant influence of roasting parameters on lightness (L*), red intensity

(a*), yellow intensity (b*), saturation (C*), and hue angle (H°). These findings confirm that different roasting temperatures and exposure times result in distinct color parameters.

As all coffee samples were subjected to the same roasting conditions, the beans showed significant differences across all parameters (Figure 1), except for a* in the C3 and C4 regions and the H° in C1 and C2. Although all samples belonged to the arabica species and Catuaí cv., factors such as climate conditions, altitude, and processing methods may also influence the final color of the coffee (Cheng et al., 2016). Cwiková et al. (2022) analyzed the effects of dry and wet processing and roasting degree on coffee bean coloration, finding that processing type accounted for 43% of the variation in luminosity (L*). Differences in dry or wet processing can lead to varying concentrations of melanoidins and Maillard Reaction-derived polymeric compounds, which in turn affect L* values. Cassamo et al. (2022) studied 95 Arabica coffee samples in Costa Rica and found that higher planting altitudes extended the fruit ripening period, moderately influencing b*, C* (saturation), and color index (CI) parameters. This resulted in beans with lower yellow intensity and brightness, as high-altitude regions typically have lower temperatures and greater water availability. These findings reinforce the significant impact of geological location on coffee color, both in its green or roasted states.

4.2 Volatile organic compounds

The 27 compounds common to all five regions are classified into six functional groups: ketone (8); pyrazine (7); furan (5); organic acid (3); aldehyde (2); and nitrogenous heterocyclic compounds (2), as shown in Table 1. These compounds are formed through various reactions during coffee processing, including the Maillard reaction and caramelization for ketones and furans, Strecker degradation for aldehydes, and interactions between sugars and amino acids that generate pyrazines (Cao et al., 2023).

2-methylbutanal and 2,3-butanedione, which had the largest chromatographic area in C4, contribute to malty and buttery, and buttery and extravagant notes, respectively (Borém et al., 2023; Cao et al., 2023; Yang et al., 2016). In contrast, 1-hydroxy-2-propanone, which had the highest concentration in C5, imparts creamy, buttery, and caramel-like sensations (Cao et al., 2023). Acetic acid, more abundant in C1, is associated with sour, spicy, and vinegary sensory characteristics (Cao et al., 2023; Dryahina et al., 2018; Yang et al., 2016). Meanwhile, 2-furanmethanol, which was more prevalent in C4, contributes sweetness along with banana, caramel, and smoky notes (Borém et al., 2023; Cao et al., 2023; Caporaso et al., 2018).

The PCA (Figure 3) reveals that the C1 region, located in the left quadrant, is primarily characterized by spice and sour notes, associated with compounds such as pyridine, acetic acid, and 5-methyl-2-furancarboxyaldehyde (Marek et al., 2020; Rusinek et al., 2022) This explains the greater distinction between C1 and C4 along the horizontal axis. In contrast, the sensory contributions of C2, C3, C4, and C5, analyzed from Dim1 — such as smoky, dairy, roasted, and fruity — are more prominent, driven by compounds including 1-methylpyrrole, 2,3-butanedione, 3-methylbutanoic acid, 2-methylbutanal, pyridine, dihydro-2-methyl-3(2H)-furanone (Borém et al., 2023; Cai et al., 2024; Cao et al., 2023; Rusinek et al., 2022; Yang et al., 2016). To differentiate C4 from other regions (except C1), the contributions of Dim2 must be considered. C4 is characterized by compounds such as 2-methylbutanal, 1-methylpyrrole, pyrazine and 2,5-dimethylpyrazine, which are associated with vegetal sensory notes (green, herbal, spinach, grassy, and hay) (Cai et al., 2024; Chen et al., 2021; Frost et al., 2022). These compounds show a positive correlation along the vertical axis, positioning C4 higher than C2, C3, and C5, which are characterized by sweet and fruity descriptors, exhibiting negative correlations and lower positions on the vertical axis.

These distinctions in volatile organic compounds are further illustrated in the Heatmap (Figure 4). The heatmap reinforces the dominant sour and spicy characteristics of the C1 region, driven by the higher correlation of acetic acid, pyridine, and furfuryl acetate. In the C3 region, the high correlation of acetoin and 2-furanmethanol influences its fruity and sweet sensory profile. Similar variations in volatile organic compounds were reported by Mourão et al. (2023) in analysis of Arabica coffee from various Brazilian production regions, demonstrating that these variations are strongly linked to soil microbiota, which is influenced by altitude. Given that the coffee samples in this study originate from different regions with distinct biomes, climates, humidity levels, and latitudes, variations in volatile organic compound concentrations are expected (Ahmed et al., 2021; Bertrand et al., 2012).

4.3 Total phenolic content and antioxidant activity

Phenolic compounds are secondary metabolites found in various plant products, exhibiting high antioxidant activity that is directly associated with human health benefits (Bondam et al., 2022; Wu, Liu et al., 2022). The primary group of phenolic compounds in coffee consists of hydroxycinnamic acids, which, when esterified with quinic acid, form chlorogenic acids (Strocchi et al., 2023; Liao et al., 2022).

Ali et al. (2022) evaluated the total phenolic content (TPC) of medium-roast coffee from four different locations (Australia, Colombia, Ethiopia, and Peru), finding that Colombian coffee had the highest TPC (17.74 mg GAE/g), while Peruvian coffee had the lowest (10.24 mg GAE/g). This variation was attributed to both geographical differences and the Arabica coffee cultivar. Similarly, Alnsour et al. (2022) investigated the influence of both location and roast degree on TPC, reporting that green beans from Kenyan had the highest TPC (17.25 mg GAE/g), while Colombian coffee exhibited the highest values for light, medium, and dark roast, with values of 19.05, 24.28, and 21.41 mg GAE/g, respectively. However, no clear correlation between TPC and geographical origin was established, as variations may be attributed to different coffee cultivars.

The values obtained in this study were higher than those reported by previous authors, likely due to differences in extraction method used for TPC analysis (Ali et al., 2022; Alnsour et al., 2022). Significant differences were observed among all regions, except between C3 and C5 (Table 2). These results

align with those of Liao et al. (2022) for medium-roasted Brazilian coffee, medium- and dark-roasted Mexican coffee, and dark-roasted Ethiopian coffee. Similar values were also reported by Mestanza et al. (2023) for three coffee cultivars (Bourbon, Caturra, and Catimor) roasted for 17 to 21 minutes. Since all coffees in this study underwent the same roasting process, factors such as climatic conditions, temperature, and light exposure likely influenced TPC levels (Mullen et al., 2013). Wu, Gu et al. (2022) further emphasized that environmental factors, along with processing methods such as dry or wet processing, fermentation, decaffeination, and roasting, significantly impact bioactive compounds in coffee, including phenolic compounds.

The determination of antioxidant activity by the ABTS method involves an oxidation reaction in which the nitrogen atom of the ABTS molecule transfers electrons, generating the $ABTS^{\bullet+}$ radical. This leads to a loss of color in the solution, which can be quantified using a spectrophotometric method (Pisoschi & Negulescu, 2011; Wolfenden & Willson, 1982).

The antioxidant activity measured by the ABTS method in this experiment was comparable to values reported by Odžaković et al. (2016), who examined the impact of roasting degrees on Arabica coffee beans of varying quality. Their findings indicated antioxidant activity values of 211.03–215.09 μmol TE/g for light roast, 223.66–228.79 μmol TE/g for medium roast, and 179.28–204.61 μmol TE/g for dark roast, suggesting a relationship between coffee quality, roasting method, and antioxidant activity. Similar results were reported by Kitzberger et al. (2014), who analyzed the antioxidant activity of different cultivars (Bourbon, Catuaí, and Icatu for the arabica species, and Iapar 59, IPR 98, and IPR 103 for the canephora species), achieving values between 3.75 and 5.42 g TE/100 g. These findings highlight the influence of cultivar variation on antioxidant activity. Although this study focused exclusively on the Catuaí variety, extrinsic factors such as climate, light exposure, and processing likely contributed to the observed variations in antioxidant activity measured by the ABTS method. Bressani et al. (2021) demonstrated that Arabica coffee Catuaí cv. fermented with different yeast strains achieved higher antioxidant activity than the samples analyzed in this study, reaching values between 3,4338.93 and 5,835.66 μM TE/g. This suggests that fermentation can enhance antioxidant activity by breaking down coffee fibers and releasing phenolic compounds.

The determination of antioxidant activity by the DPPH method is based on the reduction of the nitrogen atom in the DPPH molecule, resulting in a loss of color, which is measured at 515–520 nm (Musa et al., 2016; Rana et al., 2024).

The results obtained in this study were lower but comparable to those reported by Alamri et al. (2022), who evaluated the impact of roasting degree on Arabica coffee Kholani cv. They observed a decline in DPPH radical elimination from 88.72 to 78.76 mg TE/g with increasing roasting time, as antioxidant compounds degrade at high temperatures. Liao et al. (2022) also found similar but higher values when analyzing the effects of roast degree and coffee origin. Despite roasting-induced losses in antioxidant compounds, Maillard Reaction products such as melanoidins may compensate for these reductions. However, post-harvest processing and storage conditions can further influence antioxidant capacity.

Bilge (2020) reported that geographical origin had no impact on antioxidant activity in brewed coffee but found that brewing methods (French press, paper filter, and cold extraction) significantly influenced antioxidant levels. However, variations in antioxidant activity in coffee beans determined by the DPPH method remain underexplored. As with TPC and antioxidant activity measured by the ABTS method, bioactive compound content is influenced by planting location, climate, and processing methods.

Like the ABTS method, the FRAP method is based on the electron transfer reaction, where antioxidant compounds are oxidized, in this case, by the Fe^{3+}ion (Jiménez-Morales & Cañizares-Macias, 2024).

As previously explored by other authors, the roasting process has a significant influence on antioxidant activity. Tripetch and Borompichaichartkul (2019), when evaluating the storage of green coffee in different packaging, reported concentrations ranging from approximately 350 to 400 mmol TE/g, confirming that green beans contain higher concentrations of bioactive compounds responsible for antioxidant activity than roasted beans (Mestanza et al., 2023). Cheong et al. (2013) studied variations in volatile compounds and antioxidant activity in four different Arabica coffee cultivars from various planting locations, obtaining values that were higher than but comparable to those found in this study, ranging from 78.92 to 109.02 mg TE/g in Thailand and Indonesia, respectively, due to regional differences in phenolic content.

It should be noted that the bioactive compounds analyzed in this section, particularly phenolic compounds and antioxidant activity, varied significantly depending on the coffee-growing region. Tieghi et al. (2024) corroborated the findings of this study by analyzing specialty coffees from different regions of the state of Minas Gerais. Their results indicated that in the Cerrado Mineiro (referring to region C1 in this study) and Sul de Minas (region C5) regions, naturally processed coffees, *i.e.* dried with the husk, showed the most distinct sample variations due to factors such as biome differences (Cerrado and Atlantic Forest, respectively) and variations in latitude, which directly affect the local climate. Geographical variations influence the composition of secondary metabolites, including phenolic compounds, alkaloids, and volatile organic compounds (Cwiková et al., 2022).

5 CONCLUSION

The analyses carried out in this study demonstrated that coffees from the five regions exhibited significant differences in color, volatile organic compound content, phenolic compound content, and antioxidant activity, indicating that the planting location was a determining factor. Each region presents unique climatic conditions, including variations in sunlight exposure, rainfall, and topography. However, planting location is only one of many variables influencing the biochemical composition of coffee. Pre- and post-harvest methods, such as soil management, fertilizer use, dry or wet processing, fermentation, bean storage, and roasting techniques, also significantly impact coffee

composition. Further studies are necessary to quantify the specific and unique effects of each variable on coffee compounds.

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