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Microstructural characterization and evaluation of the physical and chemical quality of specialty roasted *Coffea arabica* L. with Pluma designation of origin

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ORIGINAL ARTICLE

Abstract

The state of Oaxaca, Mexico obtained the designation of origin "Pluma." Roasting is one of the stages contributing to the coffee bean's physical, chemical, and sensory quality. Therefore, this study aimed to analyze the microstructure, physical parameters, and chemical composition in special grains of *Coffea arabica* L. with designation of origin "Pluma." Scanning electron microscopy (SEM) and dispersive X-ray spectroscopy (EDX) analysis provided elemental analysis and chemical composition of coffee beans' external surface and internal macrostructure, respectively. Therefore, the nitrogen concentration in coffee beans was PLU 8.65%, AGL 10.06%, TEO 12.01%, MAP 13.26, and PTO 15.04%. In addition, trace elements of tellurium, iodine, calcium, and titanium were identified in the coffee bean samples. The porosity by Imagine J and the physical analysis were carried out according to regulations. The *C. arabica* L. beans from MAP and HUA are the roughest and both are located at a lower altitude than the rest. The quantification of total polyphenols is higher for coffee beans of PLU with 56.04 and AGL with 56.72 mg EAG g-1 bs and the antioxidant capacity was PLU 320.54 and AGL 330.10 μmol Eq. Trolox g-1 bs, these values correspond to coffee beans grown at higher altitudes from 1343 to 1820 masl, respectively. The total polyphenol content and antioxidant capacity increased in coffee beans as the altitude at which they were grown increased. The control sample showed similar characteristics in terms of the attributes analyzed. C (66.48–81.14%) and O (18.86–28.69%) were found on the outside of all grains, except in AGL where Ca (4.5%) was detected, which can be a discriminating element to determine its authenticity.

Keywords: *Coffea arabica* L.; physical and chemical analysis; microstructure; quality

Introduction

Coffee is one of the world's most popular and consumed beverages, which has stimulant effects determined by its composition in the green bean and post-harvest stages (Ceoromila *et al*., 2020; Klingel *et al*., 2020). *Coffea arabica* (*C. arabica*) and *Coffea canephora* (*C. canephora*) are the two most widely cultivated commercial species in the world (Lu *et al.* 2023). However, arabica accounts for more than 60% of world coffee production (Mihailova *et al.* 2022). In the Mexican Republic, the states with the highest production are Chiapas, Veracruz, Puebla, and Oaxaca, the latter obtained the designation of origin "Pluma" (NOM 2022), benefiting the producers of this state who also have specialty coffee (Giacalone *et al.* 2019; Juárez González *et al.* 2021), whose attributes have been sensory evaluated after roasting, and according to the Specialty Coffee Association of America these are appreciated by consumers (Adhikari *et al*., 2019).

This coffee type is influenced by geographical origin, climate, species, harvesting, processing, and storage methods (Dippong *et al*., 2022). The designation of the origin of coffee is relevant in some regions such as Brazil, Colombia, Honduras, and Mexico. Existing studies have focused on the authenticity of roasted coffee beans. In Colombia, they have studied the production level of the plantations in the geographical area with their designation of origin (Trujillo *et al*., 2021). In Oaxaca, Mexico studies have been carried out based on the economic and social impact of the "Pluma" designation of origin (Dávila-Aragón and Rivas-Aceves, 2021). Roasting has been studied to evaluate its effect on its quality (Jung *et al*. 2021; Suárez *et al*., 2021) on the physical and chemical composition of the grain (Bolka and Emire, 2020; Jung *et al*., 2021; Król *et al*., 2020; Nakilcioğlu-Taş and Ötleş, 2019; Ormaza and Rojano, 2018; Sarghini *et al*., 2019; Toci and Farah, 2020), to know their effect on grain microstructure (Borém *et al*. 2013; Raba *et al*. 2018) and porosity (Schenker *et al*., 2000) to analyze the properties of ground beans for other uses (Chen *et al*., 2024), and for the analysis of green beans, characterization of metabolites, and synthesis of nanoparticles (Gao *et al*., 2022) and residues from coffee processing by scanning electron microscopy (Dong *et al*., 2018; Mariem *et al*., 2024; Rodrigues *et al*., 2022; Vilas *et al*., 2020), a technique widely used for morphological, food quality, and various materials analysis (Afonso *et al*., 2020), in which, by using software such as Imagine J, the number of pores in the samples can be known (AlMarzooqi *et al*., 2016; Altenbach *et al*., 2023; Mathers *et al*., 2018). The objective of the study is to analyze specialty *C. arabica* beans from municipalities belonging to the "Pluma" denomination of origin with medium roast, which are commonly used by producers in the state of Oaxaca, Mexico, and which have not been studied to obtain information on the microstructure, surface porosity, as well as the physical and chemical characteristics. This will allow the establishment of preliminary reference values for specialty *C. arabica* beans with medium roast and with the "Pluma" denomination of origin to evaluate the authenticity and contribute to the knowledge of the recent denomination.

Materials and Methods

Materials and instruments

NaOH 0.1 N, H_2S0_4, and H_3BO_3 with a purity of ≥ 99.5% (Meyer) and Folin-Ciocalteu reagent, gallic acid, DPPH, and Trolox with a purity of ≥ 99.8% (Sigma-Aldrich) were used. Scanning Electron Microscope (Phenom Pro X Thermo Scientific) equipped with a solid state EDS electron detector used to detect chemical elements and a BSD Full detector, potentiometer (HANNA Instruments HI 2215, Rhode, Island, USA), Soxtec extraction unit (TM 8000), Micro Kjeldahl digester-distiller (FOSS), and UV vis spectrophotometer (Thermoscientific model Genesys 150) were used.

Samples of specialty *C. arabica*

Six samples of 100 g each of *C. arabica* specialty coffee beans were obtained from batches previously sensorially evaluated by a Q Grader based on the protocols of the Specialty Coffee Association from San Mateo Piñas (1000 masl), Huatulco (1058 masl), San Miguel del Puerto (1149 masl), Pluma Hidalgo (1343 masl), San Agustín Loxicha (1820 masl), and Teojomulco (1200 masl), which was the witness because it did not belong to the Pluma designation of origin (Figure 1).

Roasting of *C. arabica* samples

The roasting conditions used by special coffee producers with designation of origin in Oaxaca are light roast (165–168°C/10–12 min), medium roast (170–180°C/12 to 15 min) which is the degree of roasting to which the *C. arabica* beans were subjected due to the greater acceptance of consumers in the state of Oaxaca and the dark (200–212°C/ 7 to 10 min) demanded by the foreign market. The *C. arabica* beans were roasted in a rotary equipment brand 100 MEX model MON-110V until obtaining a medium roast (Figure 2B).

Microstructural and elemental analysis of roasted *C. arabica* beans

The grains were spread out on a clean white surface to select a representative grain from each sample. For the

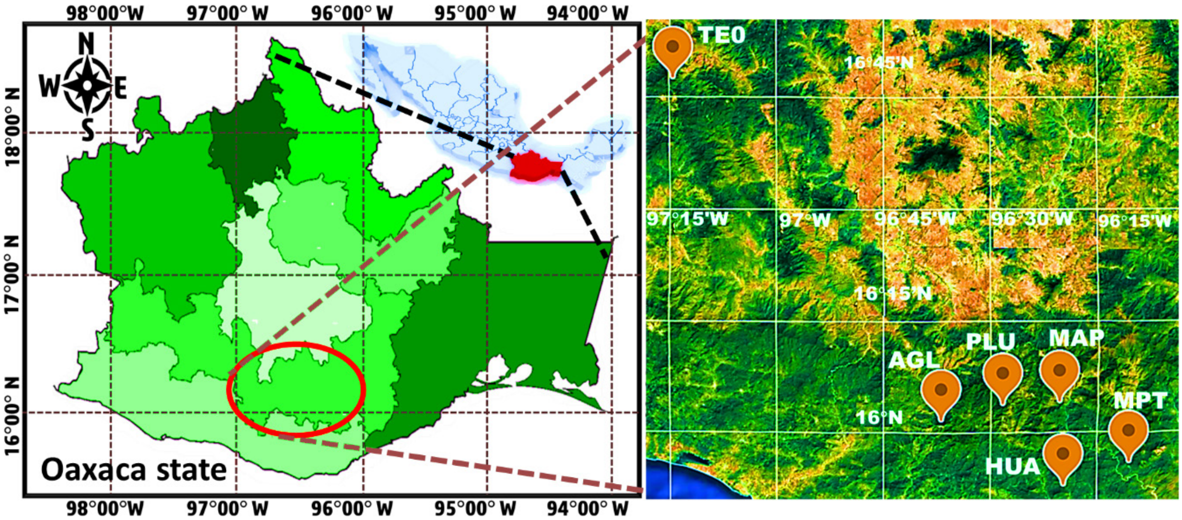

Figure 1. Location of study area for the selection coffee beans.

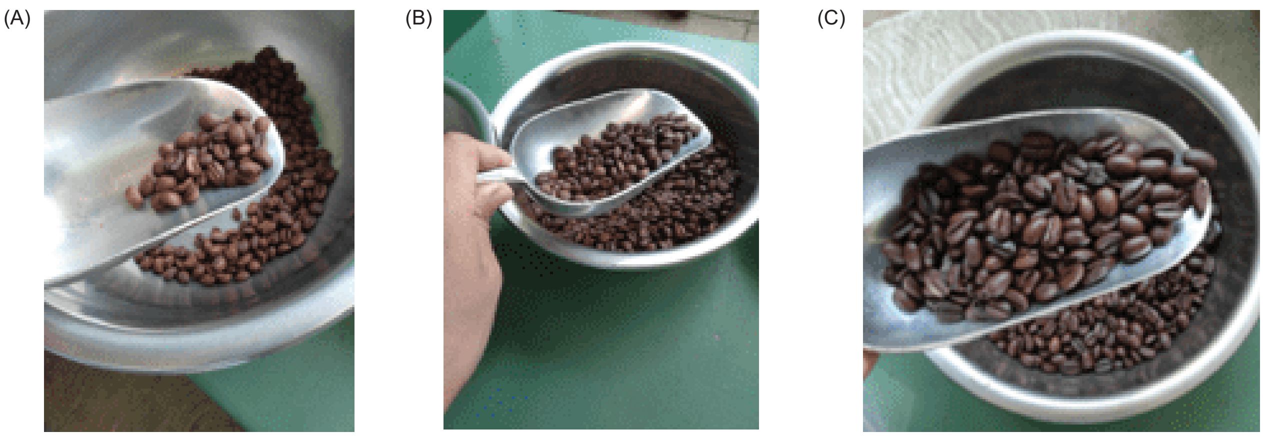

Figure 2. Degrees of roasting of specialty *C. arabica* commonly used by producers in the area with designation of origin "Pluma" (A) light roasted (B) medium and (C) dark.

external analysis (surface), images were taken of the expanded side of the grain and for the analysis of the inner part, the grain was cut in half longitudinally. They were placed under a Scanning Electron Microscope (Phenom Pro X Thermo Scientific) equipped with a solid-state EDS electron detector used to detect chemical elements and a BSD Full detector at 10 kV acceleration (Feria-Reyes *et al.*, 2023). The analyses were performed at 400 and 2000 magnifications to obtain the three-dimensional profilometry, morphology, and elemental chemical composition in these grains.

Surface porosity analysis of roasted *C. arabica* beans

The micrographs obtained from the interior and exterior of the *C. arabica* L. grain samples were used for analyses of particle and pore measurement tools of the Image J software, with which the statistics of average pore area and percentage of surface porosity were obtained. This study was carried out three times on each sample to guarantee its reproducibility and repeatability. Since it is an image, the same values were obtained in each repetition.

Physical and chemical analysis of roasted *C. arabica* grains

For the physical analysis, 100 g of *C. arabica* medium roasted beans were weighed, and the physical parameters (expansion, color, roasting lightness, cracking, and texture) were evaluated according to the methodology established in the Mexican Official Standard 255. For the chemical analysis, the beans were crushed, and 2 g of each sample was weighed for each test. For pH analysis, method 981.12b (AOAC, 2005) was followed, and for titratable acidity by procedure 942.15a (AOAC, 2005) using a potentiometer (HANNA Instruments HI 2215, Rhode, Island, USA). In the case of Moisture and Ash analyses, the methodology of the Mexican Standard for pure roasted coffee beans or ground coffee (NMX-F-013-SCFI-2010) was followed, for the determination of ethereal extract, AOAC method 945.16 (Horwitz *et al.*, 2010) was followed in a Soxhlet extraction unit (TM 8000); and for proteins, method 2001.11c (AOAC, 2005) was used using a Micro Kjeldahl digester-distiller (FOSS). All analyses were carried out in triplicate.

Total phenolic content and antioxidant capacity of roasted *C. arabica* beans

First, the extract was prepared, for which 0.5 g of medium roasted ground coffee was weighed and diluted with distilled water, boiled for 5 min, and then filtered. For the analysis of total polyphenols, a dilution was performed and 400 μL of the dilution obtained was taken. The Folin–Ciocalteu method (Singleton, 1965) was used for the calibration curve. The readings were performed in a UV vis spectrophotometer (Thermoscientific model Genesys 150) at a λ = 750 nm. The results obtained were expressed as mg of gallic acid per gram of coffee for dry basis (mg EAG g-1 bs). For the determination of total antioxidant capacity, 100 μL of the extract was taken and 2.9 mL of diluted DPPH reagent was added. For the calibration curve, Trolox was used, and shaken and incubated for 30 min. The readings were performed in a UV vis spectrophotometer (Thermoscientific model Genesys 150) at λ = 517 nm, and the results obtained were expressed as mg of Trolox per gram of coffee to dry basis (μmol Eq. Trolox g-1 bs) (Pérez-Ochoa *et al.*, 2016). In both trials, the analysis was performed in triplicate.

Statistical analysis

The data obtained were processed in Excel (Microsoft Mondo 2016) for analysis of variance and Tukey HSD test to know the significant difference between means with a significance level of α = 0.05 and IBM SPSS v statistics. 25 (International Business Machines Corp.). Principal Component Analysis (PCA) software was used in which only attributes with values greater than 1, a Pearson correlation and a k-means analysis were included.

Results

Microstructural and elemental analysis of roasted *C. arabica* beans

Table 1 shows that at 400x and 2000x the roughness values Rz and Ra (μm) are higher in the samples from San Mateo Piñas and at 2000x it is the second after Huatulco, both with the lowest altitude (masl) to rest. The Tukey test showed that the MAP grains at 400x are statistically different with a 95% confidence interval in roughness to the others; however, at 2000 x differences in roughness were observed in the TEO and AGL samples. Figure 3 shows the SEM image of the medium-roasted *C. arabica* L for the MAP coffee bean with the external surface pore size was 150–200 μm. Elemental analysis and chemical composition by X-ray identified carbon (75.54%), oxygen (23.58%), and tellurium (0.88). The internal analysis of the coffee bean showed roughness and pore sizes of 135 to 154 μm; the elemental analysis and chemical composition quantified carbon (54.17%), oxygen (32.57%), and nitrogen (13.68%).

In the analysis of the HUA coffee bean, the micrograph of the external surface shows roughness with pore sizes of 130–170 μm with 78.97% carbon and 21.03% oxygen. In the internal analysis of the coffee bean, 58.27% carbon and 41.73% oxygen were quantified, identifying pore sizes of 120–165 μm and roughness inside the bean.

In the analysis of the external surface of the PTO coffee bean, pore sizes of 160–200 μm were identified and in the elemental analysis it presents carbon (77.17%), oxygen (22.63%), and tellurium (0.19%). The internal

Table 1. Altitude (masl) and values of roughness Rz and Ra in *C. arabica* beans.

Samples	Rz (μm)		Ra (μm)	
	400 x	2000 x	400 x	2000 x
MAP	34.18 ± 1.15[a]	19.86 ± 2.48[a]	8.27 ± 0.82[a]	8.38 ± 1.68[a]
HUA	26.62 ± 2.31[b]	21.85 ± 6.63[a]	5.75 ± 0.46[b]	11.53 ± 1.65[a]
PTO	25.93 ± 2.69[b]	16.33 ± 2.33[a]	5.20 ± 0.47[b]	6.55 ± 0.94[ab]
TEO	27.54 ± 5.31[b]	13.85 ± 1.66[ac]	6.16 ± 1.83[b]	7.22 ± 2.17[ab]
PLU	26.43 ± 0.37[b]	26.43 ± 0.37[ab]	5.55 ± 0.48[b]	5.54 ± 0.48[ab]
AGL	26.53 ± 3.13[b]	18.26 ± 3.47[a]	5.73 ± 0.28[b]	7.58 ± 0.87[ab]

MAP: San Mateo Piñas, HUA: Huatulco, MPT: San Miguel del Puerto, TEO: Teojomulco, PLU: Pluma Hidalgo and AGL: San Agustín Loxicha.

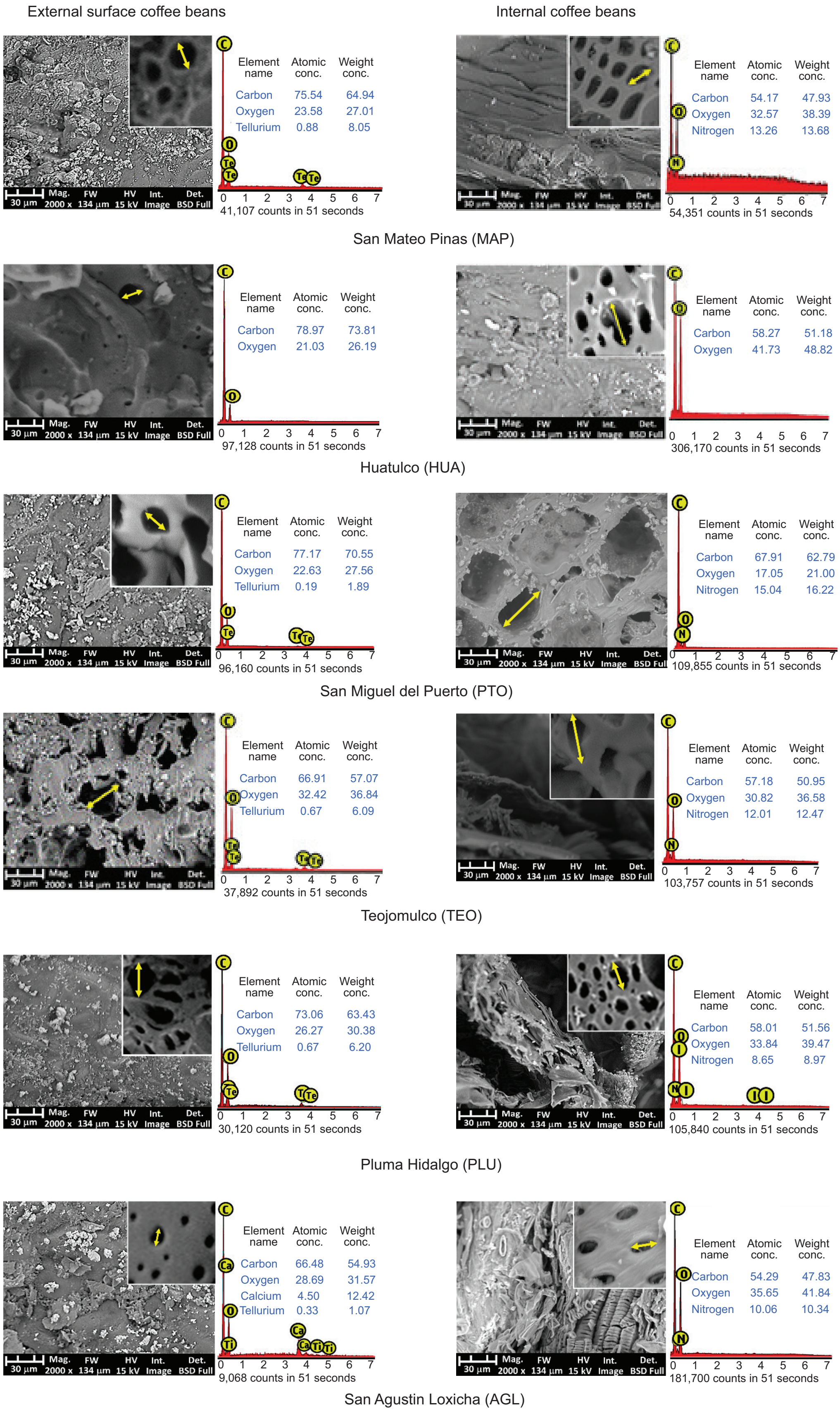

Figure 3. Scanning electron microscopy (SEM) micrograph of the external and internal surface of coffee beans and elemental analysis by EDS. Field area analyzed was 30 µm and thickness was 134 µm and with magnification of 2000 X.

analysis of the coffee bean shows well-defined pore sizes from 150 to 180 μm, quantifying carbon (67.91%), oxygen (17.05%), and nitrogen (15.04%).

In the case of the TEO coffee beans, pore sizes of 200–250 μm were identified on the external surface, the elemental analysis identified carbon (66.91%), oxygen (32.42%), and tellurium (0.67%). The internal analysis of the coffee bean shows roughness and pore sizes of 150–180 μm, quantifying carbon (57.18%), oxygen (30.82%), and nitrogen (12.01%).

In the analysis of the PLU coffee beans, pore sizes of 170–220 μm were identified on the external surface, the EDS analysis identified carbon (73.06%), oxygen (26.27%), and tellurium (0.67%). The internal analysis of the coffee bean shows roughness and pore size from 120 to 200 μm, quantifying carbon (58.01%), oxygen (33.34%), nitrogen (8.65%), and traces of iodine that cannot be quantified were also identified. For the analysis of the AGL coffee beans, pore sizes from 100 to 200 μm were identified on the external surface, the elemental analysis by EDS identified carbon (66.48%), oxygen (28.69%), calcium (4.50%), and titanium (0.33%). The internal analysis of the coffee bean shows roughness and spiral-type formations with pore size from 60 to 120 μm, quantifying carbon (54.29%), oxygen (35.65%), and nitrogen (10.06%). Figure 3 shows the elemental chemical composition of the exterior and interior of *C. arabica* beans, with C, O, and N standing out in most of the beans; however, in the exterior of the bean, Ca was found in the sample from AGL, an element which has been considered as a discriminant for coffee producing regions (Vezzulli *et al*., 2023).

Percentage of surface porosity

Figure 4 shows the external and internal porosities (%) of the medium-roasted *C. arabica* beans**,** the porosity obtained from the medium-roasted *C. arabica* samples ranged from 8.8 to 17.13% externally and from 2.1 to 8.5% internally. For the analysis, ImageJ 1.52 A software was used which determined only the surface area (Altenbach *et al*., 2023; Haeri and Haeri, 2015), being different from the porosity reported by (Pittia *et al*., 2011; Schenker *et al*., 2000) where the porosity analyzed was volumetric; however in this work, only the surface porosity was determined because there is access to the scanning electron microscope and there is no transmission electron microscope. It is important to highlight that the average porosity of the medium roasted *C. arabica* beans in the inner part (analysis of surface) was 4.44% with a standard deviation of 2.47% and the exterior was 15.34% with a standard deviation of 3.23%.

Physical and chemical analysis of roasted *C. arabica* grains

Table 2 shows the physical and chemical analysis values. According to the Tukey test with a 95% confidence interval in the physical attributes of expansion and roasting lightness in all samples, there is a significant difference, while in color only the samples from Huatulco and Pluma Hidalgo showed similarity. Regarding the crack attribute, the samples from San Mateo Piñas and Pluma Hidalgo are statistically similar, and in the last physical attribute of texture, the sample from Teojomulco is the only one that did not show statistical similarity to the others.

The first chemical parameter evaluated was acidity, which is related to grain quality and is influenced by factors such as the level of maturation of the grain, its subsequent processing, and roasting (Wang and Lim, 2012). In this study, the results were expressed, with the samples coming from San Mateo Piñas and San Agustín Loxicha, those that presented the highest acidity, and that from Huatulco the one with the lowest. The pH is influenced by acidity, since, except in the sample from San Mateo Piñas, there is a tendency that indicates that increasing pH increases titratable acidity.

The samples with the highest percentage of moisture were those from Huatulco and Pluma Hidalgo, due to the climatic conditions of the farms where the samples were obtained (1058 and 1343 meters above sea level), with the highest moisture content in Pluma Hidalgo (4.40%). The ash content was not influenced by the altitude (masl) of the farms where the samples were obtained. The analyzed samples were within the allowable limits for moisture (<6%) and ash (maximum 6.5%) of NMX-F-013-SCFI-2010. Regarding fat and protein analysis, all samples presented values similar to those reported for pure roasted coffee (NMX-F-013-SCFI-2010). The sample from San Agustín Loxicha presented the highest content in these two macromolecules. Additionally, the samples of San Miguel del Puerto with Pluma Hidalgo and San Mateo Piñas with Teojomulco, according to Tukey's test with a 95% confidence interval, do not present statistical differences.

Total polyphenol analysis and antioxidant capacity of roasted *C. arabica* beans

Polyphenols are micronutrients found in some foods, and they are related to the prevention of cardiovascular degeneration, depending on the amount consumed and their bioavailability (Manach *et al*., 2004). Table 2 shows the values of total polyphenols (mg EAG g-1 bs) present in medium-roasted *C. arabica* beans of the Pluma specialty denomination of origin.

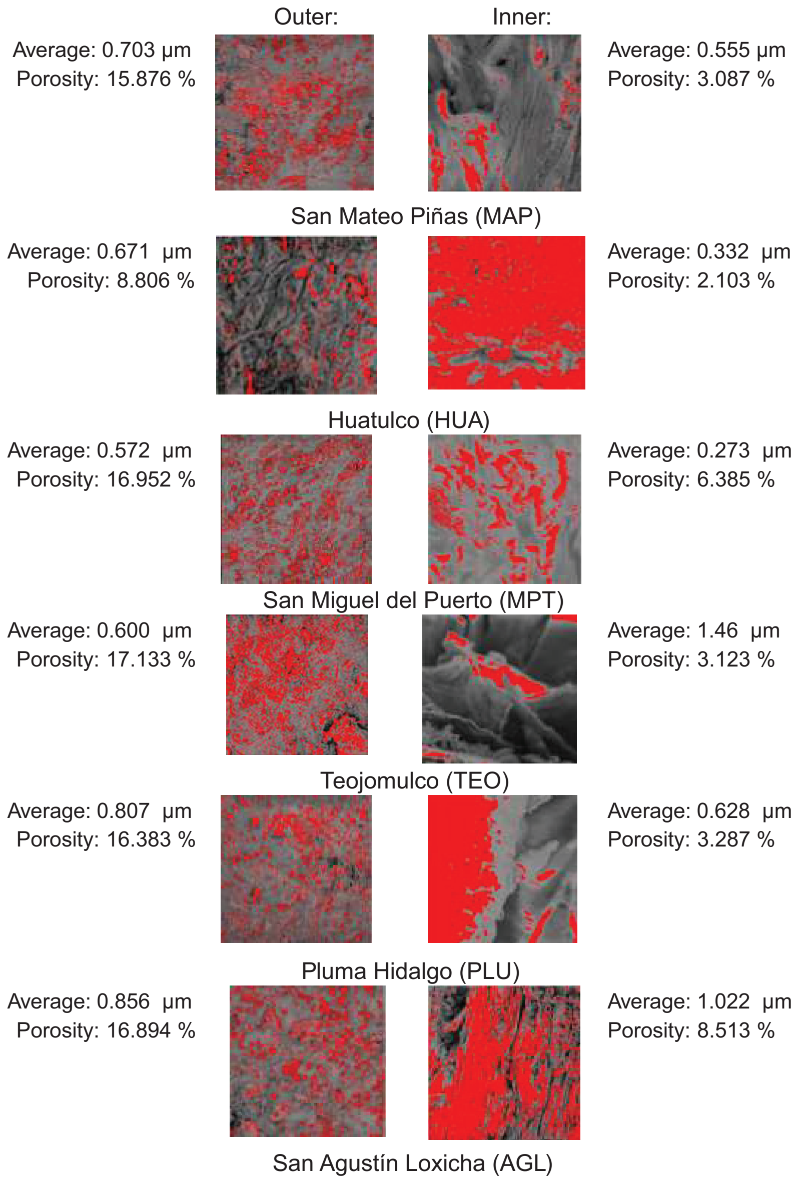

Figure 4. External and internal porosity (%) of the medium roasted *C. arabica* beans MAP: San Mateo Piñas, HUA: Huatulco, MPT: San Miguel del Puerto, TEO: Teojomulco, PLU: Pluma Hidalgo and AGL: San Agustín Loxicha.

The grains with the highest polyphenol content belonged to the Loxicha farm and a lesser extent to the San Mateo Piñas farm. There is a tendency of an increase in the values obtained for total polyphenols to the altitude of cultivation of the localities from which the samples were obtained (Table 1). The Tukey analysis with a 95% confidence interval indicates that there is a significant difference between the samples except for the values obtained for the samples from Huatulco and San Mateo Piñas.

Table 2 shows the values obtained for total antioxidant activity in *C. arabica* beans, which coincide with those reported in the literature for roasted coffee beans from the southeast of the Mexican Republic (Pérez-Ochoa *et al.*, 2016) also showing the same trend as with total polyphenols since values increase as the altitude of origin of the samples analyzed increases.

Principal component analysis, Pearson correlation, and k-means

The value of the determinant of the correlation matrix resulting from the factor analysis (Table 3) is zero, which indicates that the variables are linearly related and that the factor analysis used was a relevant technique for the analysis of all the variables.

Table 2. Values obtained from the physical and chemical analysis of medium roasted specialty *C. arabica* beans.

Sample	Expansion (%)	Color (%)	Roasting light (%)	Crack (%)	Texture (%)	pH	T.A	Humidity (%)	Ash (%)	Fat (%)	Protein (%)	T.P	T.C.A.
MAP	86.33±0.01^{c}	87.66±0.01^{c}	84.33±0.03^{c}	82.66±0.01^{c}	97.67±0.06^{b}	5.32±0.01^{c}	25.04±0.82^{c}	2.43±0.06^{c}	3.89±0.03^{bc}	14.78±0.06^{c}	8.46±0.02^{a}	48.88±0.21^{a}	300.51±0.01^{c}
HUA	95.33±0.04^{a}	90.33±0.01^{a}	95.33±0.01^{a}	77.67±0.01^{a}	98.67±0.01^{a}	5.56±0.01^{a}	19.37±0.82^{a}	3.73±0.14^{a}	4.08±0.01^{a}	14.39±0.58^{a}	8.50±0.04^{a}	49.23±0.17^{a}	301.20±0.01^{a}
PTO	91.67±0.02^{b}	87.33±0.01^{b}	92.67±0.01^{b}	94.67±0.01^{b}	98.67±0.01^{a}	5.41±0.01^{b}	22.20±0.82^{b}	2.87±0.10^{b}	3.82±0.02^{b}	13.20±0.38^{b}	8.68±0.04^{b}	53.37±0.02^{b}	305.45±0.03^{b}
TEO	93.67±0.01^{d}	90.67±0.01^{d}	96.67±0.06^{d}	81.67±0.06^{d}	98.33±0.06^{c}	5.63±0.06^{d}	25.03±0.82^{c}	2.40±0.03^{c}	4.18±0.07^{a}	14.82±0.30^{a}	8.70±0.01^{b}	55.60±0.16^{c}	303.91±0.02^{d}
PLU	92.67±0.01^{e}	90.33±0.01^{a}	98.64±0.04^{e}	82.67±0.01^{c}	97.67±0.01^{b}	5.53±0.01^{a}	23.14±0.82^{bc}	4.40±0.14^{d}	4.13±0.10^{a}	13.10±0.28^{b}	8.85±0.04^{c}	56.04±0.02^{d}	320.54±0.02^{e}
AGL	92.00±1.00^{f}	86.37±0.58^{e}	94.33±0.03^{f}	79.37±0.06^{e}	97.65±0.05^{b}	5.38±0.01^{c}	25.03±0.82^{cd}	2.04±0.08^{e}	3.67±0.06^{d}	16.43±0.23^{d}	9.04±0.04^{d}	56.72±0.17^{e}	330.10±0.01^{f}

MAP: San Mateo Piñas, HUA: Huatulco, MPT: San Miguel del Puerto, TEO: Teojomulco, PLU: Pluma Hidalgo and AGL: San Agustín Loxicha.
T.A: Titratable acidity expressed in (mg of chlorogenic acid -1 g of coffee), T.P: Total polyphenols expressed in (mg EAG g-1 bs) and T.C.A: Total Antioxidant capacity expressed in (μmol Eq. Trolox g-1 bs).

Table 3. Correlation matrix based on altitude and physical and chemical attributes.

	Expansion	Color	R.L	Crack	Texture	pH	T.A	H	Ashes	Lipids	Proteins	T.P	T.A.C	Rz	Ra
Expansion	1.000	0.567	0.920	−0.207	0.595	0.774	−0.525	0.384	0.369	−0.229	0.337	0.402	0.215	−0.060	0.311
Color	0.567	1.000	0.527	−0.359	0.624	0.934	−0.396	0.506	0.953	−0.592	−0.345	−0.081	−0.437	−0.014	0.495
R.L	0.920	0.527	1.000	−0.148	0.398	0.759	−0.323	0.502	0.429	−0.134	0.528	0.637	0.414	−0.063	0.319
Crack	−0.207	−0.359	−0.148	1.000	0.288	−0.305	−0.024	−0.048	−0.282	−0.222	−0.030	0.090	−0.166	−0.402	−0.560
Texture	0.595	0.624	0.398	0.288	1.000	0.659	−0.578	0.184	0.469	−0.644	−0.355	−0.117	−0.571	−0.380	−0.046
pH	0.774	0.934	0.759	−0.305	0.659	1.000	−0.325	0.425	0.844	−0.406	−0.026	0.237	−0.182	−0.191	0.349
T.A	−0.525	−0.396	−0.323	−0.024	−0.578	−0.325	1.000	−0.646	−0.259	0.798	0.392	0.450	0.332	−0.504	−0.607
H	0.384	0.506	0.502	−0.048	0.184	0.425	−0.646	1.000	0.579	−0.706	−0.133	−0.083	−0.025	0.619	0.817
Ashes	0.369	0.953	0.429	−0.282	0.469	0.844	−0.259	0.579	1.000	−0.595	−0.366	−0.069	−0.432	0.024	0.502
Lipids	−0.229	−0.592	−0.134	−0.222	−0.644	−0.406	0.798	−0.706	−0.595	1.000	0.692	0.547	0.682	−0.317	−0.509
Proteins	0.337	−0.345	0.528	−0.030	−0.355	−0.026	0.392	−0.133	−0.366	0.692	1.000	0.913	0.946	−0.208	−0.226
T.P	0.402	−0.081	0.637	0.090	−0.117	0.237	0.450	−0.083	−0.069	0.547	0.913	1.000	0.760	−0.464	−0.317
T.A.C	0.215	−0.437	0.414	−0.166	−0.571	−0.182	0.332	−0.025	−0.432	0.682	0.946	0.760	1.000	0.095	−0.016
Rz	−0.060	−0.014	−0.063	−0.402	−0.380	−0.191	−0.504	0.619	0.024	−0.317	−0.208	−0.464	0.095	1.000	0.851
Ra	0.311	0.495	0.319	−0.560	−0.046	0.349	−0.607	0.817	0.502	−0.509	−0.226	−0.317	−0.016	0.851	1.000

a. Determinant=0.00.
R.L: Roasting Light, T.A: Titratable Acidity, T.P: Total Polyphenols and C.A: Total Antioxidant Capacity.

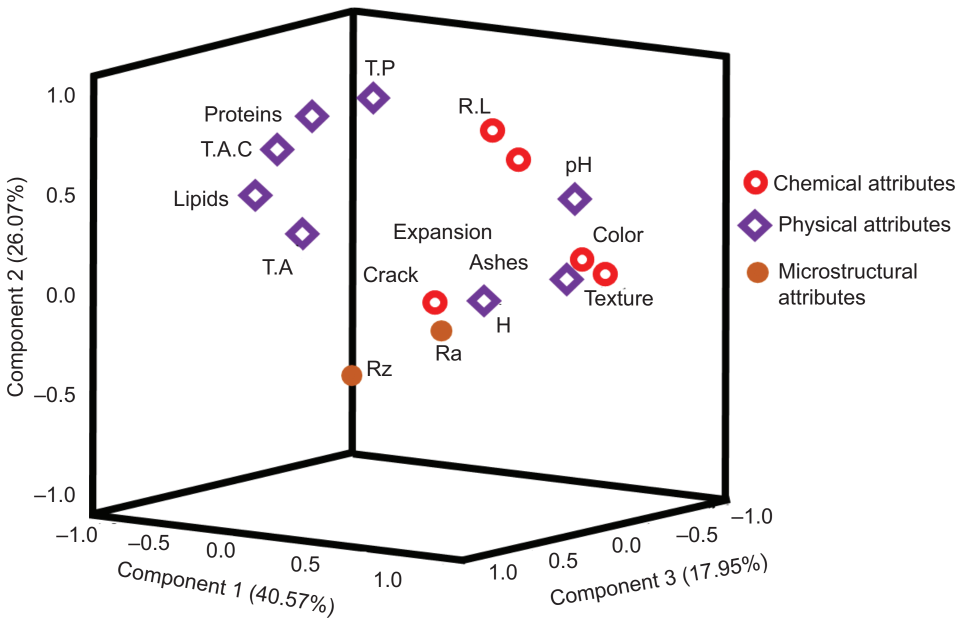

Figure 5. Simple scatter plot with 3 components in 3D.

All physical and chemical variables were reduced to three principal components (Figure 5). It can be seen that the highly correlated variables are texture, pH, titratable acidity, moisture, ash, expansion, and color, which were grouped in component 1 with a total explained variance of 40.54%, while in component 2 with 26.07% and in which were grouped the variables related to the chemical composition of the grain, such as proteins, total polyphenols, which were grouped the variables related to the chemical composition of the bean, such as proteins, total polyphenols, and antioxidant capacity, the latter two in which a relationship has been seen to exist in plants and beans (Dong *et al.*, 2018), finally, in component 3 are the lipids and coffee bean crack with a variance of 17.95%. The results and PCA analysis indicate that the content of fat, protein, total polyphenols, and antioxidant capacity is influenced by the altitude at which the *C. arabica* beans were harvested, so these chemical parameters could be used as markers of quality for these grains with designation of origin "Pluma." Based on the analysis of the six coffee samples from the different places of origin, an inversely proportional dependence was obtained between the porosity and the roughness parameter Ra, with a determination coefficient of 62.79%.

Starting from the hypothesis that the altitude of origin of the grains with the "Pluma" designation of origin influences the physical, chemical, and microstructural characteristics, once the Pearson correlation analysis has been carried out, it is found that it provides significant empirical evidence on the aforementioned variables.

Table 4. Physicochemical characterization of coffee beans and their significant, considerable and non-significant correlation variables.

Variable	r	P
Expansion-Roasting Light	0.92**	0.009
Color-pH	0.934**	0.006
Color-Ashes	0.953**	0.003
Humidity-Ra	0.817*	0.047
Lipids-Titratable Acidity	0.798	0.057
Proteins-Total Polyphenols	0.913*	0.011
Proteins-Total Antioxidant Capacity	0.946**	0.004
Rz-Ra	0.851*	0.032

**The correlation is significant at the 0.01 level (bilateral).
*The correlation is significant at the 0.05 level (bilateral).

Table 4 shows the strongest and most significant correlations as well as considerable and nonsignificant correlations, such as ash color and proteins with antioxidant capacity, which coincide with the results obtained by PCA (Figure 5). According to the similarity in the values obtained from each sample to the parameters that make up the physical, chemical, and microstructural attributes, three clusters were obtained through a k-means analysis (Figure 6).

Discussion

The coffee bean analyzed according to the harvested production area presents a unique morphology, and so the

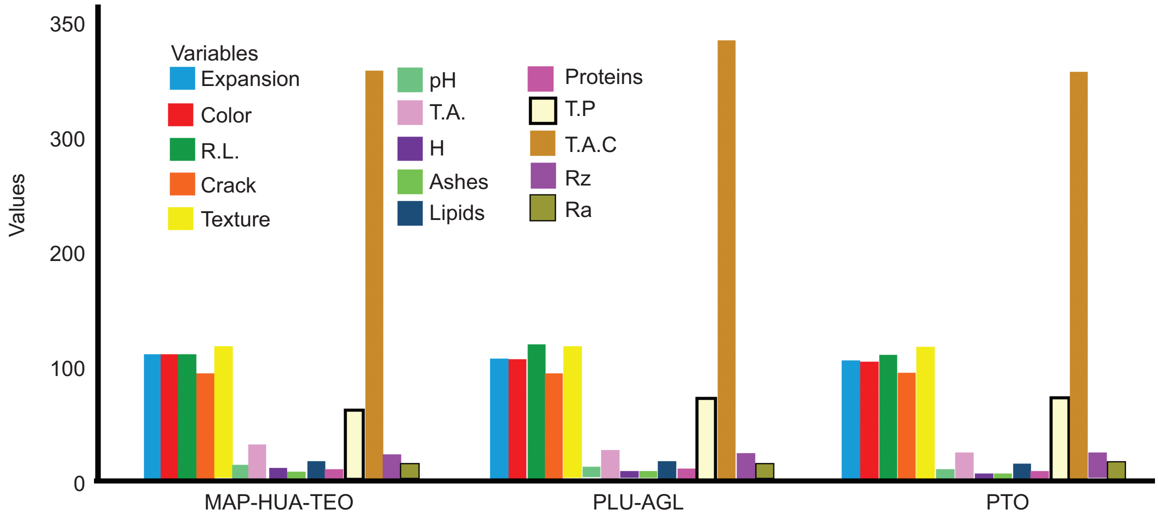

Figure 6. Grouping of the samples of origin of the *C. arabica* grains according to their similarity with respect to the analyzed variables. MAP: San Mateo Piñas, HUA: Huatulco, MPT: San Miguel del Puerto, TEO: Teojomulco, PLU: Pluma Hidalgo and AGL: San Agustín Loxicha.

energy pattern is distinctive, generating unique electromagnetic emission spectra depending on the atoms present in the sample.

Where the major elements of its organic structure are carbon and oxygen, other chemical elements found on the external surface of coffee beans are calcium, tellurium, and titanium. However, when the internal part of the coffee bean is analyzed, in addition to carbon and oxygen as major elements, nitrogen was identified in 8–15% of concentrations, except for the bean from the HUA zone. The nitrogen compounds present in coffee beans such as peptides, oligopeptides of short chains of amino acids (2–20) covalently linked to each other, polypeptides (chains of amino acids considered the basic units that makeup proteins), proteins of covalently linked amino acid chains (>50) and free amino acids are responsible for 9–16% of the chemical composition of the raw coffee bean. Coffee contains other nitrogen compounds such as caffeine, trigonelline, theobromine, and theophylline; the structures of these compounds are shown in Figure 7. In general, only 50% of coffee bean proteins are water-soluble (85% globulins and 15% albumins) since only 33% of the bean proteins are solubilized with hot water. In addition, some authors claim that coffee bean proteins are nutritionally deficient due to their lack of essential amino acids such as lysine and hydrophobic amino acids such as methionine and tryptophan.

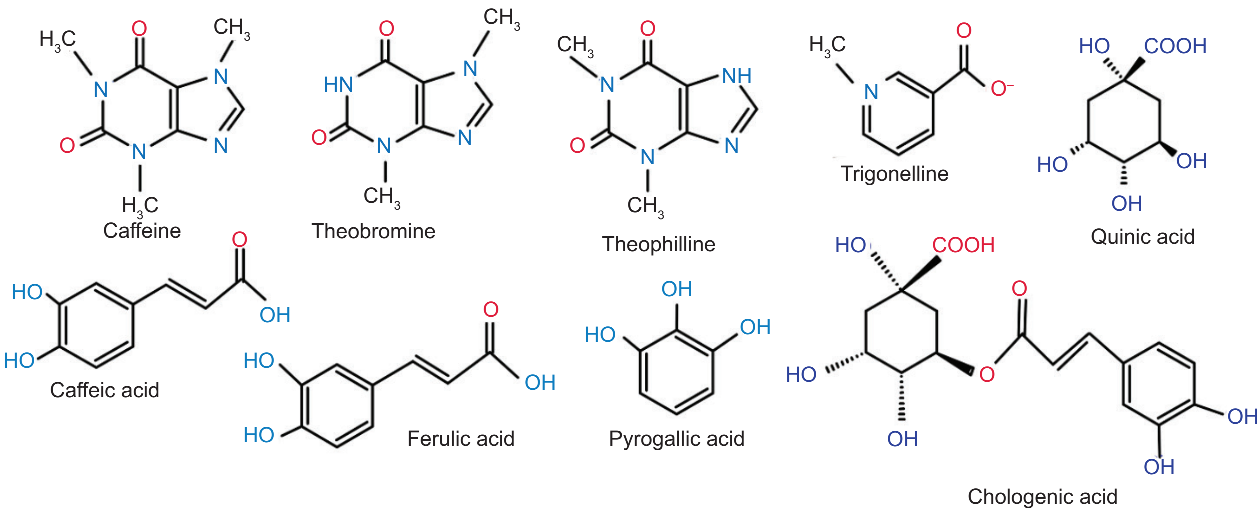

Figure 7. Chemical structures of aminoacids and polyphenols.

Whereas, tryptophan is present in meagre quantities in Arabica coffee beans.

A considerable portion of minerals, particularly nitrogen, sulfur, and calcium, is present as part of its organic structure. However, other minerals may be present simply because they are absorbed in the form of ions together with water from the soil and tend to accumulate as dissolved or stored ionic substances or as precipitates in the coffee beans. Therefore, various investigations have begun into the chemical composition, antioxidant properties and molecular coupling at different stages of roasting coffee beans (Aytar and Aydın, 2024; Liao *et al.*, 2022) roasting effect on antioxidant activity remains inconclusive, and changes of phenolic composition in coffee beans occurring upon roasting could provide valuable information about the health attributes of phenolic compounds in coffee. This study aims to determine antioxidant activity, total phenolic content, purpurogallin and other phenolic compounds in coffees from different geographic origins with different roasting degrees. In coffee extracts, antioxidant activity and total phenolic content ranged from 63.9 to 92.0 mg Trolox equivalents per gram dry weight of coffee, and 36.0–57.7 mg gallic acid equivalents per gram dry weight of coffee, respectively. However, both antioxidant activity and total phenolic content did not correlate with roasting degree ($p > 0.05$).

The roughness is due to the geographical origin and climatic conditions and to the influence of roasting on the porosity of the structure of the beans (Farah, 2004), since both samples come from locations with higher environmental humidity. Previous studies indicate that the physical characteristics of coffee beans depend on certain factors, such as the geographical location of the coffee growing areas, geographical characteristics and the chemical composition of the bean (Cordoba *et al.*, 2020; Figueiredo *et al.*, 2019; Oestreich-Janzen, 2013; Torres *et al.*, 2022), as well as the roasting to which the beans are subjected, which influences the integrity of their cell membranes (Raba *et al.*, 2018).

The results obtained from Figure 3 agree that the analysis of the cellular ultrastructures of coffee beans can be related to the loss of quality during bean processing (Figueiredo *et al.*, 2019) since roasting water gradually evaporates as heat is transferred to the bean and the internal structure of the cell is dislocated and irreversibly damaged, exposing certain chemical components and when these are added in the coffee plant they influence the final quality of *C. arabica* beans as well as the beverage (Clemente *et al.*, 2018).

The microstructural analysis of roasted coffee beans contributes to the "Pluma" designation of origin by providing information to establish the operating parameters of the roasting stage. During the roasting of the grain at temperatures higher than 150–200°C, a glass transition occurs since the water, volatiles, and gases produced by the degradation of organic matter evaporate (Schenker *et al.*, 2000). Previous 3D analysis studies carried out on coffee beans have shown that at this stage there is a volume expansion caused by water vapor and CO_2, resulting in an ordered microstructure due to the resistance of the green coffee bean to the applied heat, the effects of heat and mass transfer also affect the distribution and characteristics of the pores (Pittia *et al.*, 2011). Therefore, the roasting analysis is critical to determine the chemical composition of these grains, although storage and grinding also influence (Dippong *et al.*, 2022). Additionally, it is also important because it helps to evaluate the sensory quality specifically regarding the texture and differentiate the grains. *C. arabica* grains from *C. canephora*, this is because it has been described that they should be strictly soft, barely white, hard and from the river zone (Craig *et al.*, 2018; Tugnolo *et al.*, 2019).

In addition to this, the porosity percentages provide us with information about the effect of the roasting effect on the surface of the grain, in such a way that if it becomes too swollen due to evaporation, the pore size increases and consequently the percentage of porosity too (Clemente *et al.*, 2018; Pittia *et al.*, 2011). On the other hand, said porosity can be increased or decreased depending on the roasting time and the temperature at which it is carried out (Oliveros *et al.*, 2017).

Regarding the elemental chemical composition, this may be due to the differences in altitude (1000–1820 masl) of the *C. arabica* coffee plantations where the samples were obtained (Sim *et al.*, 2023), as well as the flowering, harvesting periods and climatic conditions of the coffee plantations (Árvay *et al.*, 2018). The designation of origin of coffee is relevant in some regions such as Brazil, Colombia, Honduras, and Mexico. Existing studies have focused on the authenticity of roasted coffee beans. In Colombia they have studied the level of production and plantations in the geographical area with their designation of origin (Trujillo *et al.*, 2021). In Oaxaca, studies have been carried out related to the economic and social impact of the "Pluma" designation of origin (Dávila-Aragón and Rivas-Aceves, 2021). In a study carried out to analyze the antioxidant activity in roasted grains in Nigeria (Agunbiade *et al.*, 2022), values in chemical composition were found to be higher in ash, lower in lipids, and higher in crude protein than those reported in Table 2. In the physical analysis, all samples presented a non-homogeneous coloration, which makes them categorized as good or ordinary quality in addition to the fact that the physical parameters evaluated are subjective since they depend on the human eye, so it is essential to contribute to the denomination of origin of "Pluma" coffee through chemical analysis of those components involved in the organoleptic attributes evaluated during the coffee cupping (Martins *et al*., 2019; Specialty Coffee Association 2021).

In chemical analysis, the acids in coffee are divided into organic and chlorogenic acids (Yeager *et al*., 2023). The latter compounds found in coffee are formed by caffeic and quinic acid, which are precursors of flavor and confer bitterness, astringency, and acidity to coffee. Chlorogenic acids are esters of quinic acid and the most abundant is 5- caffeoylquinic acid (Solís and Herrera, 2005). On the other hand, pH is associated with acidity and this is associated with the presence of chlorogenic acid, which is found in 7% of green coffee and in roasted coffee in approximately 4%, by partial decomposition of minor components such as malic and citric acids (Solis and Herrera, 2005). Now, considering that the acidity is inversely proportional to the roasting treatment, being lower for the beans that have been mostly roasted, we can deduce that the beans from Huatulco had a lower roasting treatment given that it was the sample with the lowest acid content chlorogenic. Concerning the other components, such as humidity, ash, and proteins, we could not deduce that there is an influence of altitude on these components, however in the case of lipids, total polyphenols and antioxidant capacity there is. Previous data have reported that lipids contribute to aroma formation during the roasting process due to decomposition and autooxidation reactions as well as provide body to the coffee beverage (Barbosa *et al*., 2019). The results obtained influence the sensory quality of coffee (Hu *et al*., 2020; Münchow *et al*., 2020), since roasting is known to modify the biochemical composition (Bastian *et al*., 2021; Munyendo *et al*., 2021) has an impact on the porosity of the grains and this is reflected in the texture, a parameter that has served to differentiate *C. arabica* grains from *C. canephora*. (Craig *et al*., 2018; Tugnolo *et al*., 2019).

The values obtained in the analysis of total polyphenols coincided with those reported (Pérez-Ochoa *et al*., 2016; Lazcano *et al*., 2016), which indicate that they are dependent on the degree of roasting and variety of coffee used (Gotteland and Saturnino, 2007). However, due to the degradation of phenolic compounds and the generation of others such as those from the Maillard Reaction (Shaheen *et al*., 2022), which have antioxidant activity such as metal chelating capacity and radical scavenging, antioxidant activity can increase, decrease or remain unchanged (Wang *et al*., 2011), as well as the use of high temperatures at a shorter roasting time helps to retain the content of phenolic compounds and antioxidant capacity (Lu *et al*., 2023).

These results are significant considering that among the compounds that provide antioxidant capacity to coffee, in addition to phenolic compounds and the products of the Maillard Reaction, there are phenolic compounds, chlorogenic acids and other compounds such as caffeine and tocopherols (Liang and Kitts, 2014; Murcia *et al*., 2007), which through consumption have been shown to confer beneficial effects to humans mainly in decreasing the risk of Parkinson's, Alzheimer's, cardiovascular and type 2 diabetes mellitus and cirrhosis of the liver (Janda and Baranowska-bosiacka, 2020).

Coffee producers in the state of Oaxaca are recommended to carry out a medium roast since it has been seen that due to excessive exposure time of the beans to heat, oxidized compounds are obtained (Williamson and Hatzakis, 2018). Therefore, it is important to have control of temperature and time to damage the physicochemical and sensory properties (Freitas *et al*., 2023) as well as their porosity (Schenker *et al*., 2000). Therefore, images of medium roasted beans treated under the conditions presented in this study similar to the color charts issued by the Specialty Coffee Association could be used.

Finally, appellations of origin have contributed to the growth of coffee production (Trujillo *et al*., 2021) and impacted the economic and social development of a locality with special characteristics. In the case of the state of Oaxaca, as a factor of innovation in the protected geographic region as new marketing methods (Dávila-Aragón and Rivas-Aceves, 2021), increasing the demand for *C. arabica* and encouraging its production and positively impacting the economic and socio-cultural development of Oaxaca, Mexico.

Conclusions

The roasted *C. arabica* beans from MAP and HUA were the roughest and both were at lower altitudes than the rest. PLU and PTO showed the greatest physical and structural integrity. In all the beans C (66.48–81.14%) and O (18.86–28.69%) were found on the exterior, except AGL where Ca (4.5%) was detected, which could be a discriminating element in determining the authenticity of the coffee. While in the interior, in addition to C and O, N was found in the samples from MAP (13.26%), PTO (15.04%), TEO (12.01%), and AGL (10.06%).

According to physical attributes, the grains from the Pluma Hidalgo sample showed greater integrity than other samples. However, the analysis of the chemical composition indicates that there is a relationship between the altitude of origin of the samples with respect to the percentages of pH, titratable acidity, humidity, ash, lipids, and proteins. All ground coffee samples analyzed were within the permissible limits of moisture, ash, lipids, and proteins established for pure roasted coffee (NMX-F-013-SCFI-2010).

The total polyphenol content was 48.88 ± 0.21–56.72 ± 0.17 (mg EAG g-1 bs), for the samples of beans from the Loxicha farms with the highest content, in addition to being the one with the highest altitude of the crop. Surface roughness and porosity, and physical and chemical values obtained could be used as preliminary quality parameters for the identification of *C. arabica* beans medium and specialty roasts geographically located in the "Pluma" appellation of origin area. The sample from Teojomulco (control) showed similar characteristics regarding the attributes analyzed, therefore, it is important to carry out similar studies with the rest of the municipalities that make up the state of Oaxaca. This study contributes to the knowledge of the quality of special *C. arabica* beans with Pluma designation of origin to establish preliminary parameters that determine their authenticity.

Data Availability Statement

Data will be made available on request.

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Author Contributions

Jesica Jiménez, Luis Robledo, and Rossy Feria contributed to the conceptualization of the study. Jesica Jiménez and Luis Robledo contributed to the study methodology. The software part was handled by Jesica Jiménez and Magdaleno Caballero. Luis Robledo and Marco Sánchez were involved in the study validation. Alma Dolores, Iván García, and Carlos Varapizuela performed the formal analysis. Jesica Jiménez, Luis Robledo, Rossy Feria, and Moisés Martínez helped with the investigation, and review and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Appendix A

The following table shows the 30 municipalities that make up the designation of origin established in the NOM-255-SE (2022) and the altitude of each of them. Those that, according to data provided by the "Green Gold Cluster," which is the representative of the producers of Oaxaca with the "Pluma" designation of origin, are marked with (S), are those that have specialty coffee and their respective tasting results carried out by a Q Grader. Additionally, this manuscript is the product of doctoral research where from the beginning the selection criteria for the samples were the following: they must be *C. arabica* beans, greater than 1000 masl, with a designation of origin and specialty. Therefore, samples from producers who had their sensory evaluation results to demonstrate that they are specialty beans were selected. Regarding the sample from Teojomulco (1200 masl), it was considered a control since it is not located within the area with the designation of origin, however, its sensory evaluation results are available, in such a way as to provide us with information about whether other municipalities could in the future be integrated into said designation if they coincide in the attributes analyzed.

Municipality	Altitude (masl)	Municipality	Altitude (masl)
San Pedro Mixtepec	147	Pluma Hidalgo (S)	1343
Tataltepec de Valdés	385	San Francisco Ozolotepec	1356
Candelaria Loxicha	445	San Gabriel Mixtepec	1380
San Juan Lachao	600	San Pedro Pochutla	1400
Santos Reyes Nopala	732	Santa Catarina Juquila	1432
Putla Villa de Guerrero	744	San Pablo Coatlán	1474
San Baltazar Loxicha	767	Santiago Yaitepec	1515
Santiago Xanica	822	Villa de Tututepec de Melchor Ocampo	1600
San Bartolomé Loxicha	872	San Juan Ozolotepec	1625
San Miguel Panixtlahuaca	934	San Jerónimo Coatlán	1713
San Mateo Piñas (S)	1000	Santa María Ozolotepec	1786
Santa María Huatulco (S)	1058	San Agustín Loxicha (S)	1820
San Miguel del Puerto (S)	1149	San Sebastián Coatlán	2016
Santa Catarina Loxicha	1285	San Pedro El Alto	2074
Santa María Temaxcaltepec	1320	San Marcial Ozolotepec	2365