



Relationship between physicochemical properties of roasted cocoa beans and climate patterns: Quality and safety implications

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ABSTRACT

The characteristics of *Theobroma cacao* beans are strongly influenced by factors such as climate, origin, processing methods, and plant genotype. This study aimed to explore the relationship between the physicochemical properties of roasted cacao beans specifically, their quality and chemical safety, and the environmental factors of their origin, using multivariate analysis techniques. Samples from various cocoa roasters were analyzed for water activity, moisture, fat content, pH, color, total phenols, antioxidant capacity, and levels of theobromine, caffeine, and neo-formed contaminants (NFCs) like acrylamide, hydroxymethylfurfural, furfuryl alcohol, and 2-furoic acid. Additionally, mid-infrared spectroscopy was employed to assess the chemical composition of the beans. Multivariate methods were then used to identify correlations between the physicochemical characteristics of the analyzed samples and the climatic conditions of their origin. The results showed that NFCs, fat content, and bioactive compounds (phenols and caffeine) were key differentiators. Beans from regions with high rainfall (2000–2500 mm/year) and mild temperatures (15–18 °C) exhibited higher levels of color, a higher theobromine-to-caffeine ratio, fats, acidity, and antioxidant properties, while beans from drier regions (1000–1500 mm/year) had lower levels of these characteristics. Finally, this study identified patterns showing how physicochemical properties, bioactive compounds, and NFCs influence the safety and quality of roasted cacao beans.

1. Introduction

Colombia's geographical location and varied microclimates contribute to the diverse chemical composition of *Theobroma cacao* beans. Factors such as climate, origin, processing methods, and plant genotype are closely linked to the sensory attributes of the final product (Carrilo, Londoño, & Gil, 2014). Most of the cacao produced in Colombia is for the domestic market. Historically, cacao consumption has been 1 kg/year per capita, which is relatively low compared with industrialized countries, where consumption exceeds 8 kg/year (Ministerio de Agricultura de Colombia, 2018).

T. cacao is traditionally divided into three main genetic groups of commercial interest, often referred to as cacao varieties or genotypes:

Forastero, Criollo, and Trinitario (Scollo, Neville, Oruna, Trotin & Cramer, 2020). These groups are rich in polyphenols, particularly flavonoids and catechins (37%), anthocyanins (4%), and proanthocyanidins (58%) (Hii et al., 2009), compounds with strong antioxidant properties shown to protect against disease in both animal and human studies (Elwers, Zambrano, Rohsius, & Lieberei, 2009). Cacao beans also contain caffeine and theobromine, two methylxanthines with physiological effects such as stimulating the central nervous system, heart muscle, relaxing smooth muscle, and acting as diuretics (Franco, Oñatibia, & Martínez, 2013). Methylxanthine levels can vary based on genotype, phenotype, and climate conditions (e.g., extreme rainfall) (Mihai et al., 2022), as well as average storage temperatures (~25 °C), which can degrade these compounds (Scollo, Neville, Oruna-Concha,

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Trotin, & Cramer, 2020). The genotype can also affect the methylxanthine content. For example, Forastero beans typically contain more theobromine than caffeine, Criollo beans have higher caffeine, and Trinitario beans fall between (Carrillo, Londoño, & Gil, 2014). Criollo and Trinitario varieties are known for producing “high quality fine cacao”, while Forastero, which makes up 95% of global production due to its high yield and disease resistance, is mainly used for bulk cacao (Devy, Susilo, Wachjar, & Sobir, 2019).

Although the cacao production chain in Colombia has great economic potential (Ramírez, Abaunza, Rodríguez, Varon, & Barragán, 2020), it faces several challenges: limited technological advances in farm management, lack of standardized harvesting methods, and scarce knowledge about the physicochemical characteristics of the beans after processing. For example, roasting is still done traditionally, with little technological innovation compared to industries like coffee, which uses specialized roasters that carefully control time, temperature, and moisture for consistent quality. Proper roasting is key to developing desirable flavors and aromas, as it triggers chemical reactions that produce sensory properties of beans (Masi, Dinnella, Barnabà, Navarini, & Monteleone, 2013). However, this process can also generate harmful contaminants such as: acrylamide (AA), furfuryl alcohol (FA), hydroxymethylfurfural (HMF), furan and 2-furoic acid (2-FA) (Gutiérrez, 2017), which have been associated to potential neoplastic effects and the development of peripheral neuropathy (Chaves, Irias, & Arias, 2015). Therefore, it is crucial to monitor their baseline concentrations to ensure food safety.

Thus, to support the development of the cacao production chain in regions like Huila, the 5th largest producer in Colombia (3518 tons/year) (Fedecacao, 2022), it is essential to understand the physicochemical properties of the cultivated cacao and explore how these factors influence its quality and safety. Compared to the more established coffee and banana production chains in both national and international markets, cacao production in Huila is still in its developmental stage (Rojas Martinez & Martinez, 2013). Some studies link cacao bean quality to its chemical components, such as amino acids, sugars, phenols, and methylxanthines (Carrillo, Londoño, & Gil, 2014; Collazo, Barrios, Bahamon, & Gutiérrez, 2023). However, most of these studies do not consider the influence of environmental conditions in the bean's region of origin. Multivariate statistical methods, such as principal component analysis (PCA) and fuzzy clustering, are valuable tools for analyzing data on cacao beans roasted under diverse climatic conditions, helping to identify safety and quality patterns linked to their origin. These methods reveal hidden patterns and relationships across multiple variables simultaneously. For instance, PCA reduces the dimensionality of large datasets by identifying new uncorrelated variables, making it easier to visualize associations between different samples. Fuzzy clustering, on the other hand, can identify overlapping groups, which is particularly useful when samples share characteristics influenced by different environmental factors. In cacao research, multivariate analysis has been used to classify beans based on origin and properties, uncovering relationships between spectral data and sensory attributes to predict the overall quality of roasted cacao (Collazos, Barrios, Bahamon, & Gutiérrez, 2024). Therefore, this study used multivariate analysis to examine how physicochemical properties, bioactive compounds, and NFCs such as AA, HMF, and FFA influence the quality and safety of roasted *T. cacao* beans, identifying patterns related to their origin and environmental factors in Huila, Colombia.

2. Material and methods

2.1. Chemicals and reagents

Hexane ($\geq 96\%$), petroleum ether (99.9%), Folin-Ciocalteu (2N), and sodium carbonate ($\geq 99.9\%$) were purchased from Merck, Germany. The 5-hydroxymethylfurfural $\geq 99.8\%$, gallic acid $\geq 98\%$, 1,1-diphenyl-2-picrylhydrazyl (DPPH) $\geq 97\%$, 2,2-Azobis 2-methylpropanimidine (ABTS)

$\geq 97\%$, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) $\geq 97\%$, caffeine $\geq 99.8\%$, theobromine $\geq 98\%$, Carrez reagent clarification kit, and acetic acid $\geq 99.7\%$ were purchased from Merck, USA. Acetonitrile ($\geq 99.9\%$), methanol ($\geq 99.9\%$), furfuryl alcohol $\geq 99.8\%$, and furoic acid $\geq 99.9\%$ were supplied by Scharlau, Spain. Potassium persulfate $\geq 99\%$ was obtained by ABC, Colombia. Ethanol ($\geq 97\%$) was supplied by CIACOMEC, Colombia. All experiments used ultra-pure distilled water (dH₂O), obtained through the Purelab Water Purification System (Elga Veolia, UK).

2.2. Sample collection

Cacao roasters (n = 12) from the municipalities with the highest cacao production in Huila, Colombia -Campaalegre, Íquira, Nátaga, Neiva, Paicol, Rivera, Tello and Yaguará-were visited to collect 24 samples of ~250 g of roasted beans. In this region, cacao roasting is a small-to medium-scale process, varying by tradition and available equipment, ranging from pan or skillet methods to drum roasters. The samples were vacuum-packed, labeled by geographical location and stored at -20 °C. Before conducting the analytical determinations, the beans were ground using an A11 mill (IKA, Germany) and sieved to achieve a particle size <1000 µm.

2.3. Collection and analysis of climate information

Climate data was obtained from the Hydrology, Meteorology, and Environmental Studies Institute of Colombia. Data was collected from 98 stations for precipitation and 12 stations for temperature in the sampling areas, with averages calculated for the period 1990–2020. Precipitation fields were generated using the Inverse Distance Weighting method, which interpolates values based on proximity, while temperature fields were estimated using least squares regression to analyze the relationship between variables.

2.4. Physicochemical characterization

2.4.1. Color and pH measurement

Color was measured on ~30 g of roasted, ground beans using a CR-410 Chroma Meter (Konica Minolta, Japan), calibrated with a standard white plate ($Y = 87$, $x = 0.316$, $y = 0.3231$), and results were expressed in the CIELAB system (L^* : white/black, a^* : red/green, b^* : yellow/blue). The pH was measured in a 1:20 (w/v) suspension of roasted, ground cacao (without defatting) in dH₂O at 90 °C, with values recorded at room temperature (25 °C) using a calibrated Starter 5000 pH meter (OHAUS, USA). At least three measurements were taken per sample for both analyses.

2.4.2. Water activity and moisture determination

These analyses were performed on 5 g of each roasted, ground cacao sample. Water activity (a_w) was measured using a Vapor Sorption Analyzer (Aqualab, USA), and moisture content was determined by dehydrating the sample in a UF 55 drying oven (Mettler, Germany) at 105 °C for 24 h until a constant weight was reached.

2.4.3. Fat content determination

Fat content was quantified using the Soxhlet extraction method with a TE-044 system (Tecnal, Brazil). About 2 g of dried sample were placed in a 25 × 80 mm cellulose thimble, and fat was extracted with 100 mL of petroleum ether for 1 h at 60 °C. The solvent was then evaporated, and the fat residue was weighed to determine the fat content, expressed as g/100g.

2.4.4. Fourier transform infrared spectroscopy

Mid-infrared spectra were obtained using a Cary 630 FTIR spectrometer (Agilent, USA) with a DLATGS detector and ATR sampling accessory. Approximately 80 mg of ground sample was placed directly

on the spectrometer for readings in the 4000–650 cm^{-1} range, with a resolution of 8 cm^{-1} and 32 scans. Each sample's spectrum was averaged from at least five readings. Spectra were pretreated with baseline correction and multiplicative scatter correction (MSC) for better quality and functional group identification using R ChemoSpec software (Hanson, Bostock, Keinsley, & Gupta, 2024).

2.5. Total phenolic content and antioxidant capacity

2.5.1. Sample extraction

Approximately 4 g of sample was placed in a 50 mL Erlenmeyer flask with 10 mL of hexane. The mixture was shaken for 5 min and repeated twice. It was then filtered through a 0.45 μm membrane filter (Merck, Germany), and the retained solids were dried at 30 °C for 24 h. The defatted sample was stored under vacuum until analysis. To determine total phenols and antioxidant capacity, 100 mg of defatted cacao was dissolved in 10 mL of an 80:20 methanol- dH_2O solution, sonicated for 10 min in a S30H ultrasonic bath (Elma, Germany), and centrifuged for 20 min at 2287 g in a Bioprocen 22R centrifuge (Ortoalresa, Spain). The supernatant was filtered through Whatman paper No. 1 (11 μm) and stored at 4 °C until analysis.

2.5.2. Total phenols determination

Total phenols (TP) were determined using the Folin-Ciocalteu method, as described by Caporaso, Whitworth, Fowler, and Fisk (2018). A 100 μL of the defatted extract and 4.9 mL of dH_2O were added to a volumetric flask. Afterward, 0.5 mL of Folin-Ciocalteu reagent was added, followed by 1.7 mL of sodium carbonate at 20 g/100 g after 3 min. The mixture was incubated in the dark for 30 min, and absorbance was measured at 765 nm using a UV/Vis GENESYS™ 180 spectrophotometer (Thermo Scientific, USA). A gallic acid calibration curve (50–650 $\mu\text{g}/\text{mL}$) was used, and results were expressed as mg of Gallic Acid Equivalents (GAE) per gram of defatted roasted cacao, on a dry weight basis (DW).

2.5.3. Antioxidant capacity by ABTS

An ABTS solution was prepared by dissolving 36.02 mg of ABTS reagent in 10 mL of dH_2O to achieve an absorbance of 0.7. Then, a 2.45 mmol/L potassium persulfate solution was made by dissolving 66.23 mg in 10 mL of dH_2O . The ABTS•+ solution was prepared by adding the ABTS solution to a 20 mL flask and oxidizing it with potassium persulfate, followed by refrigeration for ~14 h. For the assay, 1400 μL of ABTS•+ solution and 14 μL of each sample dilution or Trolox standard (in duplicate) were added to test tubes, with a blank prepared using 14 μL of methanol (80 mL/100 mL H_2O) and 1400 μL of ABTS•+. After shaking and a 6-min reaction time, absorbance was measured at 734 nm using a UV–vis spectrophotometer. Results were expressed as μmol of Trolox equivalents (TE) per gram of roasted, defatted cacao on DW.

2.5.4. Antioxidant capacity by DPPH

A 29 mg/L methanolic DPPH solution was prepared by dissolving 14.5 mg of DPPH in a 500 mL volumetric flask and diluting it with methanol. A Trolox calibration curve was created using a 10 $\mu\text{mol}/\text{mL}$ stock solution, with concentrations ranging from 50 to 800 $\mu\text{mol}/\text{mL}$. To each test tube, 100 μL of sample extract or standard was added, followed by 1.95 mL of the DPPH solution. The tubes were shaken and incubated in the dark for 30 min. A control was prepared using 1000 μL of methanol (80 mL/100 mL dH_2O) and 1.95 mL of DPPH solution. After incubation, absorbance was measured at 517 nm using a spectrophotometer. Results were expressed as μmol of Trolox equivalents (TE) per gram of roasted, defatted cacao on DW.

2.6. Bioactive and neo-formed contaminants determination

2.6.1. Caffeine, theobromine and their ratios

About 200 mg of defatted cacao was placed in a test tube, and 5 mL of

dH_2O , along with 0.75 mL each of Carrez I ($\text{K}_4[\text{Fe}(\text{CN})_6]$) and Carrez II (ZnSO_4) solutions, were added. The mixtures were shaken for 30 s using a vortex (Thermo Scientific™, USA) and allowed to rest for 10 min. The samples were centrifuged at 7000 g for 10 min (20 °C), and the supernatant was extracted and filtered through 0.22 μm nylon filters for analysis. Theobromine (TB) and caffeine (CF) were determined using a HPLC-DAD 1260 Infinity II system (Agilent Technologies, USA). The mobile phase consisted of acetic acid (1 mL/100 mL dH_2O) adjusted to pH 3.1, and acetonitrile, with a 5 μL injection volume and a C18 column (5 μm $\times$ 4.6 mm id $\times$ 150 mm). Retention times were 5.38 min for caffeine and 2.55 min for theobromine. Method validation yielded an R^2 of 0.9998 for both compounds, with accuracies of 91.6% for caffeine and 86.9% for theobromine. The limits of detection (LOD) were 0.0403–0.1345 $\mu\text{g}/\text{L}$ for theobromine and 0.1181–0.3938 $\mu\text{g}/\text{L}$ for caffeine. Reproducibility was estimated with a Horwitz Ratio (HorRat) of 0.05 for theobromine and 0.4 for caffeine (Medina-Orjuela, Barrios-Rodríguez, Carranza Gutierrez, Amorcho-Cruz, & Girón-Hernández, 2024). The theobromine/caffeine (TB/CF) ratio Eq. (1) was used to approximate *T. cacao* genotype (Carrillo, Londoño-Londoño, & Gil, 2014). Forastero beans typically have higher theobromine and lower caffeine levels, while Trinitario beans show intermediate concentrations. Criollo beans usually contain more caffeine and less theobromine (Borja Fajardo, Horta Tellez, Peñaloza Atuesta, Sandoval Aldana, & Mendez Arteaga, 2022).

$$\frac{TB}{CF} = \frac{\text{Theobromine concentration} \left[\frac{\text{mg}}{\text{g DW}} \right]}{\text{Caffeine} \left[\frac{\text{mg}}{\text{g sample DW}} \right]} \quad [\text{Eq. 1}]$$

2.6.2. HMF, FFA and 2-FA determination

Extract preparation involved adding 200 mg of defatted sample to 5 mL of water, followed by the addition of 0.75 mL each of Carrez I ($\text{K}_4[\text{Fe}(\text{CN})_6] \times 3\text{H}_2\text{O}$) and Carrez II ($\text{ZnSO}_4 \times 7\text{H}_2\text{O}$). Each addition was shaken for 30 s at 2500 rpm using a vortex. The mixture was then centrifuged at 4500 g for 20 min, and the supernatant was filtered through 0.2 μm nylon filters into a vial. The analysis was performed using HPLC-DAD with a mobile phase of 80% acetic acid (1 $\mu\text{L}/100$ mL dH_2O) and 20% methanol, using a PROTECOL C8 H 125 column (150 mm $\times$ 4.6 mm) (SGE Analytical Science, Australia). The injection volume was 20 μL , with retention times of 3.66 min (HMF), 4.99 min (FFA), and 5.47 min (2-FA). Absorbance was measured at 217 nm, 250 nm, and 284 nm for HMF, FFA, and 2-FA, respectively. Results were reported in mg/kg of roasted cacao DW, with at least three replicates per sample. The method showed an $R^2 > 0.997$ and accuracy ranging from 89.94% to 100.5%. The LOD and LOQ were 0.249–0.832 $\mu\text{g}/\text{L}$ for HMF, 0.081–0.272 $\mu\text{g}/\text{L}$ for FFA, and 0.1181–0.3938 $\mu\text{g}/\text{L}$ for 2-FA. Maximum intraday precision was 2.85% Relative Standard Deviation (RSD), and interday precision was 13.43% RSD (Medina, Barrios, Carranza, Amorcho, & Girón, 2024).

2.7. Acrylamide determination

Acrylamide extraction involved 0.5 g of ground cacao in 4 mL of ultra-pure water, shaken in a vortex at 2500 rpm for 5 min, with repeated solvent additions. After enriching with 1 mL of acrylamide d3 (1000 $\mu\text{g}/\text{L}$), 5 mL of dichloromethane was added, and the mixture was centrifuged at 7000 g for 5 min. A 4 mL aliquot of the aqueous phase was transferred to a test tube containing 5 mL of acetonitrile and 2 g NaCl. The organic phase (4 mL) was added to QuEChERS AOAC 2007 kit 15 mL tubes with 1200 g MgSO_4 and 400 mg PSA (Thermo Scientific, USA), then filtered through a 0.2 μm nylon filter into vials.

Determination was performed using a UPLC system H-Class series coupled with a Xevo TQ-S triple quadrupole micro mass spectrometer (Waters Corporation, USA). The mobile phase included formic acid (1 $\mu\text{L}/100$ mL dH_2O) (A) and formic acid (1 $\mu\text{L}/100$ mL acetonitrile) (B),

following a gradient: 90% An until 1.5 min, reducing to 50% An until 1.8 min and remaining constant until 2.1 min, then decreasing to 5% until 2.5 min, and increasing to 10% at 2.8 min, 50% at 3.2 min, and back to 90% at 3.5 min, held until 5 min. The ACQUITY UPLC HSS T3 1.8 μ m, 2.1 mm $\times$ 100 mm column (Waters Corporation, USA) was used with a 3 μ L injection volume. Capillary and cone voltages were set to 0.5 kV and 50 V, respectively, with ESI + ionization, a source temperature of 150 °C, desolvation temperature of 500 °C, and desolvation gas flow of 1000 L/h. Quantification was conducted via linear regression in the 5 μ g/L to 400 μ g/L range, achieving an $R^2 > 0.998$. The LOD and LOQ were between 0.003 and 0.011 μ g/mL, with recovery accuracy of 86.9%–90.7%. Intraday and interday precision were 8.91% and 15.8% RSD, respectively, while reproducibility was estimated at a HorRat of 0.98% (Medina-Orjuela et al., 2024).

2.8. Statistical analysis

The physicochemical variables are presented as mean $\pm$ standard error, and Pearson correlation was used to examine relationships between them. A matrix was constructed from physicochemical data (24 samples $\times$ 11 variables) and the infrared spectrum with MSC (24 samples $\times$ 900) for PCA, which reduced dimensionality and identified sample grouping patterns. Block scaling was applied, treating the spectrum variables as the first block and physicochemical variables as the second. Each block was scaled to unit variance, with variables divided by the square root of the number of variables in each block to ensure equal variance and prevent any variable from dominating the analysis. PCA loadings (24 $\times$ 911) were obtained to explain variability in the score space. Fuzzy clustering was performed using latent variables PC1 to PC4 to detect patterns and subgroups, assigning samples to clusters with partial memberships. Finally, the importance of predictor variables (PC1:PC4) was assessed using the Mean Decrease Accuracy (variable importance based on reduced model accuracy when values are permuted) and the Mean Decrease Gini index (contribution of variables to node homogeneity), both of which contribute to creating the most homogeneous nodes in the prediction and enhancing the model's predictive power in the Random Forest model. All analyses were conducted using the R statistical computing software (R Core Team, 2023).

3. Results and discussion

3.1. Physicochemical characterization of roasted cacao beans

Roasting is a crucial stage in cacao production, significantly affecting the physicochemical and sensory characteristics of the beans. In Huila, cacao production is still developing and requires additional technical and scientific knowledge. This will facilitate strategies to ensure high-quality roasted cacao beans, maximizing their nutritional value and sensory appeal. Results of the physicochemical characterization of cacao

samples from various regions of Huila, Colombia, are presented in Table 1. The cocoa samples exhibited low lightness (25.63–35.56) and a noticeable red hue in color coordinate a, typical of processed cocoa. In color coordinate b, the samples tended towards yellowish tones (5.32–13.52). These color results may be linked to the low moisture content, with a_w ranging from 0.34 to 0.59, similar to the reported moisture values of 2.45 g/100 g (Agus, Mohamad, & Hussain, 2018). The samples also showed a moderately acidic pH, typical of slightly acidic cocoa products, which may influence taste. The average fat content of the samples (37–56 g/100 g) ranged close to other reported ranges (46–64 g/100 g) (Álvarez et al., 2012). Roasted cacao fat typically ranges from 50 to 56 g/100 g, depending on the variety (Forastero has higher fat) and the roasting process, which causes fat to migrate from the nib to the shell (Oracz, Nebesny, & Zyzelewicz, 2014). Higher fat content also contributes to darker cocoa colors (Ramos-Escudero et al., 2021), which aligns with the observed color ranges.

The ABTS and DPPH values revealed significant variability in antioxidant activity across the roasted cocoa samples, while phenol content was relatively consistent. This variability may be due to differences in roasting processes or cocoa's chemical composition. These ranges differ from other values reported in literature for ABTS (482–1406.7 μ mol TE/g DW) and DPPH (323.8–1370.1 μ mol TE/g DW) (Oracz et al., 2014), and phenol levels (19–33 mg GAE/g) (Ramos-Escudero et al., 2021). Such discrepancies can be attributed to extraction, quantification methods, and cocoa origin or processing variations. Climate, particularly precipitation and temperature, plays a key role in antioxidant variations (Di Mattia et al., 2013). This study found that cocoa beans from Paicol and Íquira, which have higher rainfall (1500–2000 mm/year) and cooler temperatures (11–15 °C), exhibited higher antioxidant capacities: DPPH (123–168 μ mol TE/g DW) and ABTS (89–123 μ mol TE/g DW) (Fig. 1).

In contrast, samples from areas with lower rainfall (1000–1500 mm/year) and higher temperatures (15–18 °C), such as Nátaga and Yaguará, had lower ABTS (33–49 μ mol TE/g DW) and DPPH (33–48 μ mol TE/g DW) values. Prolonged heavy rainfall creates a wetter environment in cocoa plantations, increasing susceptibility to fungal growth, which can trigger the synthesis of protective compounds against pests and diseases (Bernays, Oppenheim, Chapman, Kwon, & Gould, 2000). Methylxanthine levels for caffeine (2.01–6.28 mg/g) and theobromine (25.03–37.39 mg/g) were comparable to previously reported levels in the region: caffeine (1.7–2.9 mg/g) and theobromine (18.7–20.3 mg/g) (Collazos, Barrios, Bahamon, & Gutiérrez, 2023). The TB/CF ratio (Fig. 2) indicated that beans from Campoalegre, Neiva, and Rivera had values of 9.3–13.8, indicating a Forastero-type group, consistent with other studies (Borja, Horta, Peñaloza, Sandoval, & Mendez, 2022). In contrast, samples from Colombia, Íquira, Nátaga, Paicol, Tello, and Yaguará revealed ratios of 5.1–9.1 and 5.7–7.7, suggesting Trinitario-type cacao. Regions such as Íquira, Colombia, Nátaga, and Paicol which receive similar precipitation levels of 1500–2500 mm/year, may experience increased humidity that affects bean development and alters theobromine and caffeine concentrations, thus influencing the TB/CF ratio. A rainfall rate of 1400–2000 mm/year is sufficient for profitable cacao yields (Galanakis & Charis, 2022).

The infrared spectrum analysis revealed functional groups linked to the chemical composition of roasted cacao beans, showing differences in absorbance across both the functional region (4000–1500 cm^{-1}) and fingerprint region (1500–650 cm^{-1}) (Fig. 3). These variations may be due to different genetic groups (Forastero and Trinitario) or origin of the beans. A peak at 3280 cm^{-1} , related to hydroxy groups (–OH) and water (Domínguez-Pérez et al., 2019), is commonly found in food. Peaks at 2922 and 2855 cm^{-1} indicate CH_2 and CH_3 group stretching, likely from cacao fatty acids (Reis, Franca, & Oliveira, 2013), while wavenumbers near 1700 cm^{-1} suggest carbonyl group vibrations in lipids (Oliveira et al., 2016). Differences in absorbance here indicate distinct chemical compositions. Finally, aldehydes (CHO) appear between 1500 and 764 cm^{-1} , associated to carbohydrates and sucrose (Reis et al., 2013).

Table 1
Physicochemical characterization of roasted cacao beans.

Determination	Range		Average	Standard deviation
	Min	Max		
L*	25.63	35.56	30.72	2.91
a	7.51	14.64	11.29	2.24
b	5.32	13.52	9.96	2.57
pH	4.85	5.99	5.46	0.37
Fat (g/100g)	37.64	56.45	47.30	4.90
ABTS	33.41	123.64	65.74	25.24
DPPH	33.19	168.52	83.40	44.20
Total phenols	2.54	11.96	6.07	2.68
Theobromine (mg/g)	25.03	37.39	31.51	3.22
Caffeine (mg/g)	2.01	6.28	3.91	1.14

ABTS and DPPH values are reported in μ mol TE/g DW, Total phenols in mg GAE/g DW.

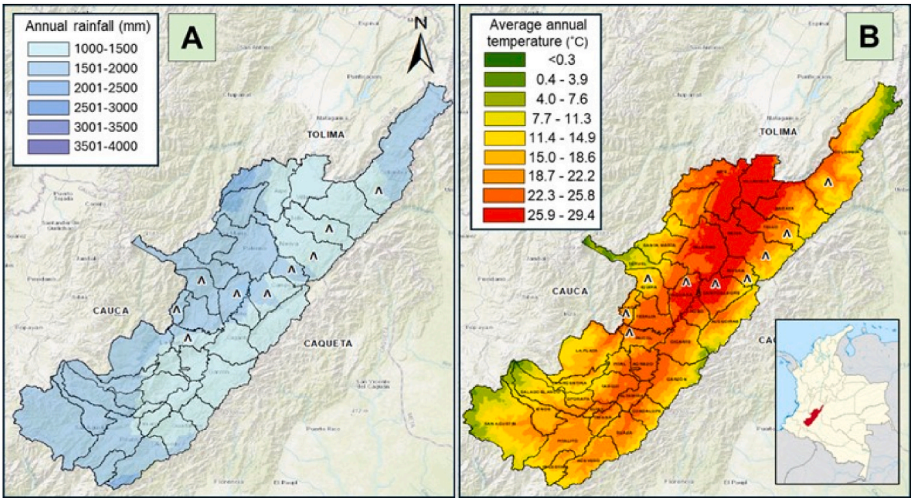


Fig. 1. Precipitation (A) and temperature (B) fields on the evaluated area based on the climate normal period from 1990 to 2020.

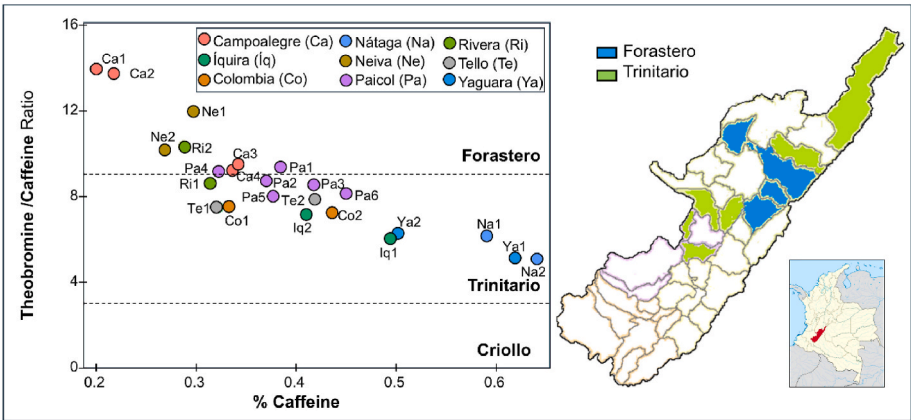


Fig. 2. TB/CF ratio used to differentiate the genotypes of roasted cacao beans from various regions in Huila, Colombia.

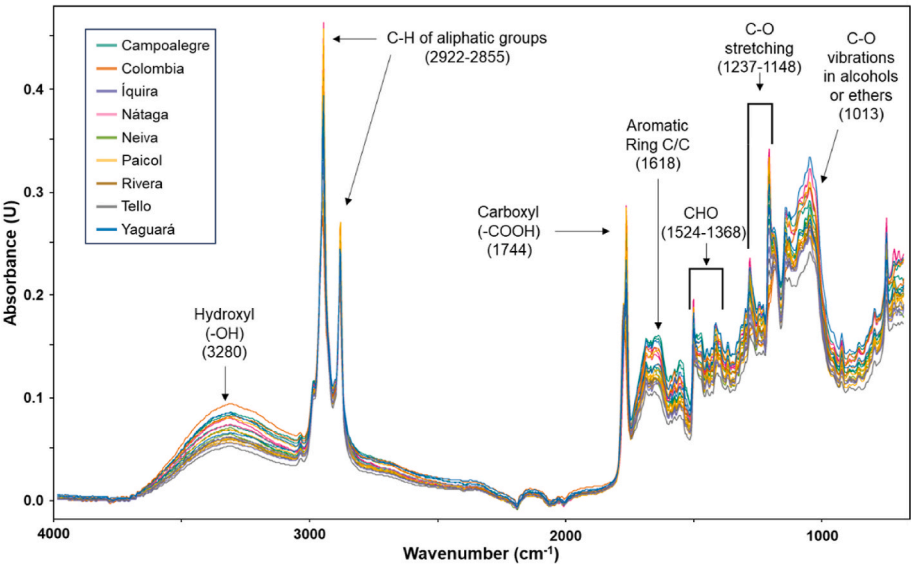


Fig. 3. ATR- FTIR analysis of functional groups in roasted cacao beans.

3.2. Neo-formed contaminants in roasted cacao beans

The analysis of the samples revealed acrylamide levels ranging from acrylamide values between 4.25 ± 47.37 to 1015.02 ± 119.01 $\mu\text{g/kg}$, comparable to other values of 62.8 – 922.2 $\mu\text{g/kg}$ reported in literature (Granvogl & Schieberle, 2007). Acrylamide, a significant NFC, is associated to consumer health risks, including neoplasm development (Chaves et al., 2015). Although no reference values exist for cacao, levels exceeding 500 $\mu\text{g/kg}$ in this study suggest a high concentration, like the threshold set for coffee (400 $\mu\text{g/kg}$) by the European Food Safety Authority (EFSA, 2015), the values over 500 $\mu\text{g/kg}$ reported in this study can be considered to indicate a high concentration of these NFCs. These elevated levels may result from roasting conditions, pH, precursor availability (amino acids and reducing sugars), and water content (Maan et al., 2022).

HMF levels ranged from 1406.14 ± 57.91 to 2443.093 ± 122.16 mg/kg , significantly higher than the 260 mg/kg reported in roasted cacao beans (Urbańska & Kowalska, 2019). These elevated levels may result from roasting intensity, growing conditions, and genotype, which affect carbohydrate content, a key precursor for HMF formation. Cacao, along with coffee and bread, is a major contributor to dietary HMF intake (Morehouse, Nyman, McNeal, DiNovi, & Perfetti, 2008). Although HMF is only cytotoxic at high levels, it has genotoxic potential (Capuano & Fogliano, 2011). FFA values ranged from 202.36 ± 56.63 to 910.52 ± 89.00 mg/kg , much higher than the 0.02 mg/kg found in powdered cocoa (Okaru & Lachenmeier, 2018). Despite its contribution to food aroma, FFA has been linked to toxicity in animals and humans, particularly affecting the olfactory and respiratory epithelium (Arts et al., 2004; Okaru & Lachenmeier, 2018). 2-FA values between 22.77 ± 1.18 and 52.83 ± 2.62 mg/kg were observed. As a furan precursor, 2-FA is potentially carcinogenic at high concentrations (Becalski et al., 2005; Crews & Castle, 2007).

3.3. Exploring correlations among variables in cacao samples across regions

The correlation analysis clarified the relationships between bioactive compounds and NFCs in cacao samples from different regions in Huila, Colombia (Fig. 4). A notable correlation of 0.7^{***} was found between FFA and ABTS, likely due to the hydroxyl group ($-\text{OH}$) in FFA, which enhances polarity and electron donation, improving the retention of $\text{ABTS}^{\bullet+}$ free radical cations (Miller et al., 2006). Also, a significant correlation of 0.7^{***} was observed between the TB/CF ratio and the b color coordinate. Previous studies indicate that prolonged roasting at high temperatures can decrease methylxanthine content, directly impacting color parameters (Urbańska, Derewiaka, Lenart, & Kowalska, 2019). A correlation of 0.6^{**} between moisture and L values suggests that higher water content in beans limits the Maillard reaction, leading to reduced browning and higher L values. The correlation between pH and moisture (0.7^*) is associated with hydroxide ion content, indicating that increased moisture levels may reduce hydroxide ion (OH^-) concentration (Zhao, Patange, Sun, & Tiwari, 2020). Furthermore, a correlation of -0.6^{**} between 2-FA and the b color coordinate suggests that reducing sugar degradation affects 2-FA levels, with 2-FA formation decreasing as sugars are depleted and browning increasing as sugars degrade (Crews & Castle, 2007). Finally, the correlation between AA and aw was -0.5 , indicating that a moderate-to-low aw range may promote increased acrylamide formation.

3.4. Multivariate analysis to identify trends in cacao characteristics

The multivariate analysis visualized the combined effects of physicochemical components, bioactive compounds and NFCs, with PC1 and PC2 accounting for 44.8% of the total variation. The score graph indicates that samples from Íquira, Paicol, Tello, and Yaguará cluster in the upper parts of quadrants I and II, while those from Campoalegre,

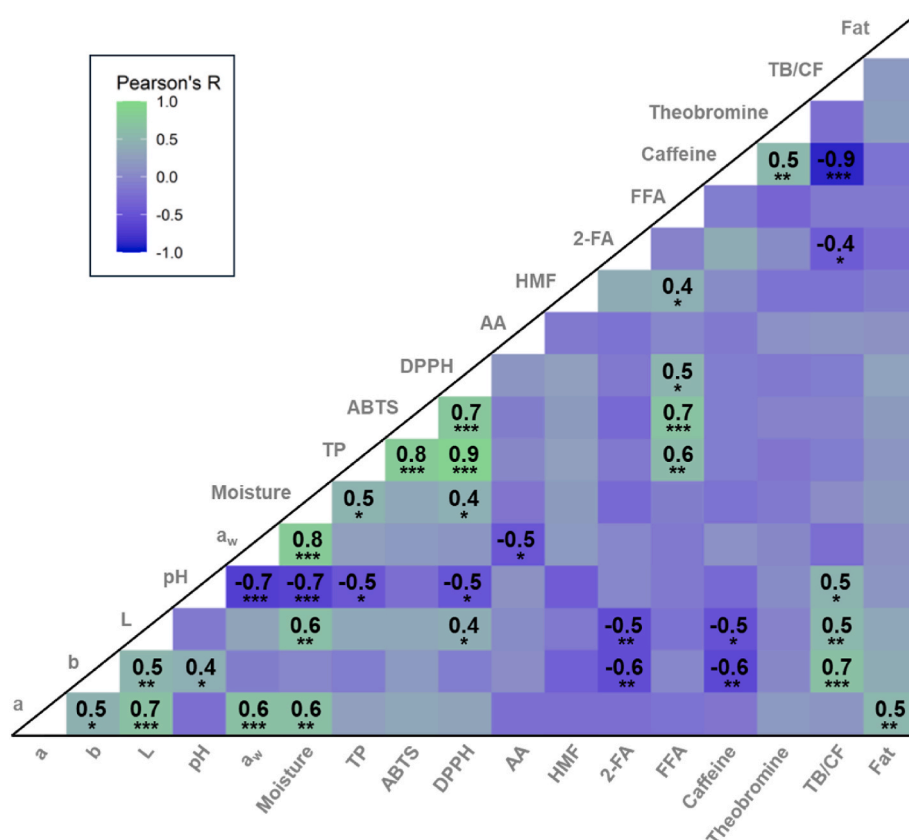


Fig. 4. Correlation of physicochemical variables in roasted cacao beans.

Nátaga, Neiva, Rivera, and some samples Paicol are found in the lower parts of quadrants III and IV (Fig. 5A). This analysis proposes that the region of origin influences the physicochemical characteristics of the cacao. Fuzzy clustering of the first four components PC1:PC4 (~70% of variance), revealed three distinct clusters based on the partitioning index (Table S1). Table S2 outlines each sample's membership grade to the clusters, where Cluster 1 includes samples from Campoalegre, Colombia, and Neiva while Cluster 2 contains samples from Paicol, Nátaga, and Rivera and Cluster 3 groups samples from Íquira, Tello, and Yaguará (Fig. 5B). This indicates that the physicochemical characteristics and quality of cacao beans depend on their region of origin.

To explain the resulting clusters, a Random Forest model was applied to assess the contributions of latent variables PC1 to PC4 in sample classification. The analysis indicated that, based on the Accuracy and Gini index, PC1, PC2, and PC4 were the most significant contributors, while PC3 was less important (Fig. 5C). Subsequently, variable loadings for each component and their relationship with sample classification were established. To better understand each variable's contribution to sample distribution, the percentage contribution per component was calculated (Fig. 6), where the dashed line indicates the significance of each contribution. Latent variable PC1 showed a high contribution from variables such as 2-FA, b, AA, FFA, and HMF (Fig. 6A), indicating that this component effectively describes brown-colored roasted cacao bean samples with a high NFC contribution (Fig. 5A). Additionally, PC1 significantly contributes to wavelengths between 876 and 1800 cm^{-1} (Fig. 6A), which are associated with carboxyl groups in carbohydrates. PC1 also influences the red cluster, where samples from Campoalegre, Neiva, and Colombia are located (Fig. 5B), suggesting these samples may have higher levels of NFCs (2-FA, HMF, AA, and FA).

Unlike PC1, latent variable PC2 contributed significantly to the TB/CF ratio, caffeine, color coordinate a, DPPH, total phenols, fats, and pH (Fig. 6B), indicating that samples associated with this component are linked to brown-colored beans, high fat content, and low pH. The contribution of wavenumbers between 794 and 824 cm^{-1} may reflect out-of-plane C-H bond vibrations in aromatic compounds or heterocyclic rings. Also, caffeine and total phenols in PC2 are responsible for bitterness, astringency, and a fruity, green aroma (Vázquez, Ovando, Adriano, Betancur, & Salvador, 2016). This component influences the separation of samples in the green and blue clusters (Fig. 5B). Samples from Íquira, Tello, and Yaguará, grouped in cluster 2 (green), are associated with high values in physicochemical properties like color, TB/CF ratio, fats, and acidity-related traits such as pH, total phenols, and DPPH. These samples originate from areas with 2000–2500 mm/year precipitation (Fig. 1A) and average temperatures of 15–18 °C (Fig. 1B), characteristic of temperate climates, which may influence the beans' chemical composition. In contrast, samples in Cluster 3 (blue), from Nátaga, Paicol, and Rivera, are associated with lower values in TB/CF

ratio, fats, pH, total phenols, and DPPH. These areas receive 1500–2000 mm/year of rainfall and have similar temperatures of 15–18 °C. This suggests that annual rainfall may affect cocoa composition, impacting fat, caffeine, theobromine, and antioxidant content.

The multivariate analysis revealed behavioral patterns in cacao samples based on their origin, reflecting interactions between the analyzed variables. These differences are influenced by factors affecting the physicochemical properties of the beans. Key factors include roasting temperature (110–150 °C) and time (30–120 min), which impact the degradation of phenols and antioxidant capacity due to the thermolabile nature of their molecules (Barišić et al., 2019). In Huila, Colombia, the roasting process can be artisanal (in pans) or done with drum roasters, depending on the cocoa farmers' resources. The lack of technology limits control over key parameters, such as temperature, affecting the physico-chemical and sensory quality of the final product. Finally, cocoa genotype also plays a significant role, with the Forastero variety losing more aldehyde groups associated with carbohydrates during roasting than the Trinitario variety (Oracz & Nebesny, 2019).

4. Conclusion

Based on the physicochemical variables evaluated, this study establishes a clear relationship between the properties of roasted cacao beans and the climatic conditions of their region of origin. Samples from Campoalegre and Neiva were characterized by high neo-formed contaminants content, influenced by the presence of precursors such as reducing sugars and amino acids, as well as artisanal roasting methods. In contrast, beans from Íquira, Paicol, and Yaguará, regions with prolonged rainfall (2000–2500 mm/year) and moderate temperatures (15–18 °C), exhibited higher concentrations of bioactive compounds such as phenols and caffeine, along with increased fat content. These attributes may enhance the value of cacao-based products by catering to consumer demand for both flavor and health benefits. Optimizing processing techniques and understanding the impact of climatic variability could refine these findings further. By identifying and characterizing cacao-producing regions, targeted strategies can be developed to improve the quality and safety of the final product.

CRedit authorship contribution statement

Federman Betancourt-Sambony: Writing – original draft, Methodology, Investigation, Formal analysis. **Yeison Fernando Barrios-Rodríguez:** Writing – original draft, Methodology, Investigation, Formal analysis. **Maria Elena Medina-Orjuela:** Writing – original draft, Supervision, Data curation. **Nelson Gutiérrez-Guzmán:** Writing – review & editing, Data curation. **Claudia Milena Amorcho-Cruz:** Writing – review & editing, Supervision, Data curation. **Carlos**

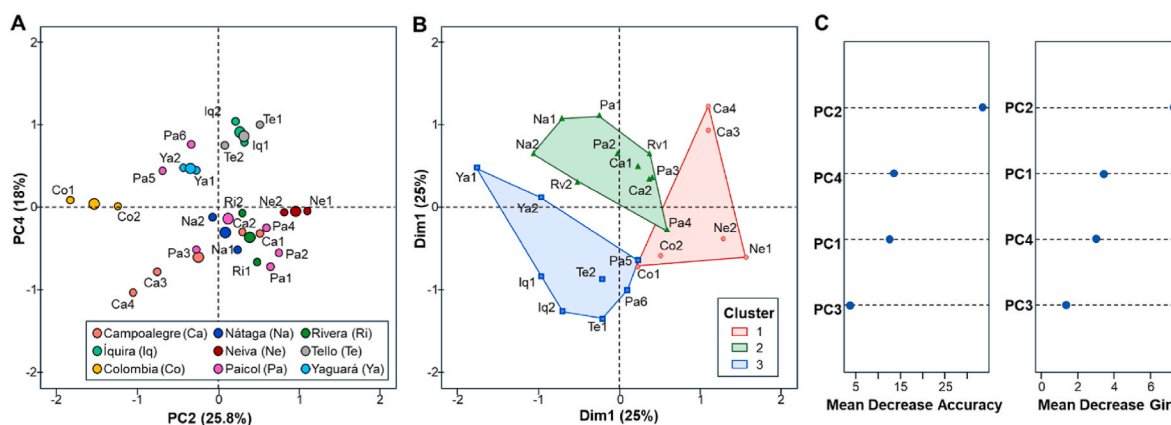


Fig. 5. Sample groups obtained from (A) PCA using PC2 vs. PC4, (B) fuzzy clustering based on latent variables PC1 to PC4, and (C) their importance in sample grouping via the Random Forest model.

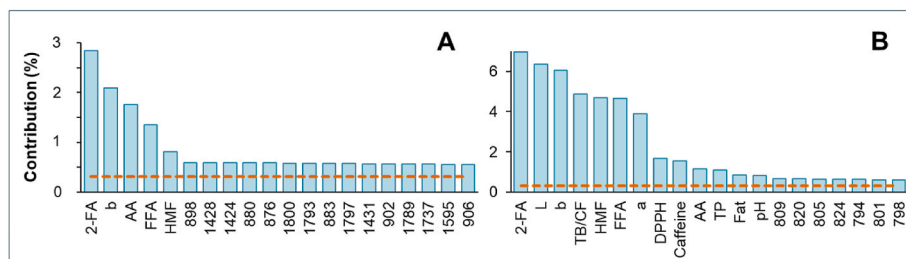


Fig. 6. Variables contribution to the (A) PC1 (B) PC2 in the principal components analysis.

Carranza: Writing – review & editing, Funding acquisition, Data curation. **Joel Girón-Hernández:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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During the preparation of this work, the author(s) used ChatGPT for proofreading purposes. After utilizing this tool/service, the author(s) reviewed and edited the content as needed, taking full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2025.117320>.

Data availability

All data created during this research are openly available at 10.25398/rd.northumbria.28173434.

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