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Proximate and mineral composition of venezuelan cocoa beans from Cata and Cuyagua

Composición proximal y mineral de almendras de cacao venezolano de Cata y Cuyagua

Composição proximal e mineral de amêndoas de cacau venezuelano de Cata e Cuyagua

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Abstract

Venezuelan cocoa is renowned for its high sensory quality; however, the presence of heavy metals may restrict its access to highly regulated international markets such as the European Union. This study aimed to evaluate the proximate composition and mineral content of cocoa beans (*Theobroma cacao* L.) collected from Cata and Cuyagua (Aragua, Venezuela) during the main harvest seasons of 2013 and 2014. Thirty samples (15 per year) were analyzed. Proximate composition was determined using official AOAC methods, while mineral elements were quantified following microwave-assisted acid digestion and ICP-OES analysis. Results showed that moisture content was significantly higher in 2013 compared to 2014 ($p<0.001$), whereas fat, protein, ash, and carbohydrate contents did not differ between years. Significant interannual differences were observed in mineral composition, with higher concentrations of Ca, Cu, Fe, Mg, and Mn in 2013, and increased levels of K and Na in 2014. Cadmium exhibited a non-significant decrease between years (1.62-1.04 $mg.kg^{-1}$), while chromium remained low and stable. Arsenic and mercury were detected only at trace levels, with no significant differences between years. These results provide reference values for cocoa produced in Cata and Cuyagua, confirm interannual variability in proximate and mineral composition, and emphasize the importance of continuous monitoring of cadmium and other metals to ensure compliance with international regulations.

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Resumen

El cacao venezolano es reconocido por su alta calidad sensorial; sin embargo, la presencia de metales pesados puede limitar su acceso a mercados internacionales exigentes, como la Unión Europea. El objetivo de este estudio fue evaluar la composición proximal y mineral de almendras de cacao (*Theobroma cacao* L.) provenientes de las localidades de Cata y Cuyagua (Aragua, Venezuela), correspondientes a las cosechas principales de 2013 y 2014. Se analizaron 30 muestras (15 por año), determinándose la composición proximal mediante métodos oficiales AOAC, mientras que los elementos minerales se cuantificaron tras digestión ácida asistida por microondas y análisis por ICP-OES. Los resultados evidenciaron una mayor humedad en las muestras de 2013 respecto a 2014 ($p<0,001$), sin diferencias significativas en grasa, proteína, cenizas ni carbohidratos. En la composición mineral se observaron variaciones interanuales significativas, destacando mayores concentraciones de Ca, Cu, Fe, Mg y Mn en 2013, y valores superiores de K y Na en 2014. El cadmio mostró una disminución no significativa entre años (1,62-1,04 $mg.kg^{-1}$), mientras que el cromo se mantuvo en niveles bajos y estables. Arsénico y mercurio se detectaron únicamente en trazas, sin diferencias relevantes. Estos resultados aportan valores de referencia para el cacao de Cata y Cuyagua, confirman la variabilidad interanual de su composición proximal y mineral, y refuerzan la necesidad de un monitoreo continuo de cadmio y otros metales para asegurar el cumplimiento de las regulaciones internacionales.

Palabras clave: cacao venezolano, análisis proximal, minerales, cadmio, ICP-OES, Cata, Cuyagua.

Resumo

O cacau venezuelano é reconhecido por sua elevada qualidade sensorial; entretanto, a presença de metais pesados pode restringir seu acesso a mercados internacionais altamente regulados, como o da União Europeia. Este estudo teve como objetivo avaliar a composição proximal e mineral de amêndoas de cacau (*Theobroma cacao* L.) provenientes das localidades de Cata e Cuyagua (Aragua, Venezuela), correspondentes às principais colheitas de 2013 e 2014. Foram analisadas 30 amostras (15 por ano). A composição proximal foi determinada por métodos oficiais da AOAC, enquanto os elementos minerais foram quantificados após digestão ácida assistida por micro-ondas e análise por ICP-OES. Os resultados indicaram que o teor de umidade foi significativamente maior em 2013 em comparação a 2014 ($p<0,001$), enquanto os teores de gordura, proteína, cinzas e carboidratos não diferiram entre os anos. Diferenças interanuais significativas foram observadas na composição mineral, com maiores concentrações de Ca, Cu, Fe, Mg e Mn em 2013, e níveis mais elevados de K e Na em 2014. O cádmio apresentou redução não significativa entre os anos (1,62-1,04 $mg.kg^{-1}$), enquanto o cromo manteve-se em níveis baixos e estáveis. Arsênio e mercúrio foram detectados apenas em níveis traço, sem diferenças significativas. Esses resultados fornecem valores de referência para o cacau produzido em Cata e Cuyagua, confirmam a variabilidade interanual da composição proximal e mineral e reforçam a necessidade de monitoramento contínuo do cádmio e de outros metais, visando ao atendimento das regulamentações internacionais.

Palavras-chave: cacau venezuelano, composição proximal, minerais, cádmio, ICP-OES, Cata, Cuyagua.

Introduction

Venezuelan cacao (*Theobroma cacao* L.) is internationally recognized for its genetic and sensory quality, being considered one of the most appreciated origins for the production of fine and aromatic chocolates. Coastal regions such as Cata and Cuyagua, in the state of Aragua, are part of the corridor of Creole and Trinidadian cocoas, characterized by distinctive aromatic attributes and artisanal production of high commercial value (Portillo *et al*., 2012; Katz *et al*., 2011).

In recent years, the presence of heavy metals in cocoa has become very relevant due to the regulatory requirements of international markets. The European Union established maximum limits for cadmium (Cd) in cocoa products by Regulation (EU) No. 488/2014, which were subsequently consolidated in Regulation (EU) 2023/915. These thresholds, which vary between 0.60 $mg.kg^{-1}$ in cocoa powder and 0.80 $mg.kg^{-1}$ in chocolates with $\geq$ 50 % cocoa, have prompted the study of the content of Cd and other toxic elements in cocoa beans produced in Latin America, a region that concentrates more than 70 % of the world's production (Gramlich *et al*., 2018).

The mineral composition of cocoa not only has regulatory implications, but also nutritional and technological ones. Elements such as K, Mg, Ca, Fe, and Mn are relevant for nutritional quality and for processes such as fermentation and drying, while the presence of Cd, Pb, Hg, and As represents a risk to food safety and can affect the export of grains (Gramlich *et al*., 2018; García-Porras *et al*., 2025). In Venezuela, the first systematic studies on metals in cocoa included the analyses of Portillo *et al*. (2012) in cocoa porcelain from the South of the Lake. Subsequently, Lanza *et al*. (2016) reported levels of Cd, Cu, Ni, Cr and Fe in hybrid cocoas and porcelain from Santa Bárbara del Zulia, establishing initial reference values for the country. More recently, Padilla *et al*. (2025) confirmed the presence of Cd in Venezuelan cocoa beans, with concentrations that in some cases exceeded the limits established by the European Union, providing current evidence of the magnitude of the problem.

At the regional level, research in Ecuador, Peru, and Colombia has documented significant variations in Cd levels in soils and cocoa beans, mainly associated with edaphic factors, agronomic management, and cultivar genetics (Arévalo-Gardini *et al*., 2017; Barraza *et al*., 2017; Meter *et al.*, 2019). These findings reinforce the need to generate local information to understand the interannual and regional variability of mineral content in Venezuelan cocoa.

In this context, the present study aimed to evaluate the proximal and mineral composition of roasted cocoa beans collected in Cata and Cuyagua during the 2013 and 2014 harvests, with emphasis on the levels of Cd and other minerals of nutritional and regulatory interest, comparing the results with international reference values.

Materials and methods

Study Location

Samples of cocoa beans were collected in the towns of Cata and Cuyagua, Aragua state (Venezuela), during the months of September to December 2013 and 2014, corresponding to the main harvest.

Sample Preparation

The cocoa beans were roasted at 120 °C for 30 minutes, hulled manually and ground into a homogeneous flour. Subsequently, they were preserved in airtight containers until analysis.

It is important to note that for this study, roasted beans were used as the basis for the analyses, in order to guarantee the homogeneity of the matrix and the elimination of residual moisture, avoiding variations during milling and acid digestion. This approach remains consistent with previous research conducted by Lanza *et al*. (2016), in which the grains were subjected to a similar heat treatment (100 °C, 30 min). The inclusion of roasting also allows for improved comparability with studies of proximal and mineral composition in derived products, where cocoa is usually processed before consumption.

In total, 30 samples were analyzed (15 samples per year), each consisting of 1 kg of roasted almonds. Each sample was analyzed in triplicate, so that the values reported correspond to averages and standard deviations calculated from three independent determinations.

Proximate analysis

The proximate composition was determined by applying official AOAC methods: moisture (925.10), ash (923.03), protein by Kjeldahl method (984.13), fat (963.15), and carbohydrate calculated by difference. Each determination was made in triplicate, including random duplicates as an internal precision check.

Determination of minerals

The elements As, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na and Ca were quantified after microwave-assisted acid digestion, using a Milestone Start D digester (Milestone, Italy), with a mixture of nitric acid (65 %) and hydrogen peroxide (30 %). The analysis was performed by optical emission spectrometry with inductively coupled plasma (ICP-OES, Perkin Elmer Optima 5300 DV), following the official AOAC 999.11 method. To ensure traceability and accuracy, certified reference materials were used: NIST SRM 2384 (Baking Chocolate, 100% cocoa beans) and ERM-BD512 (Dark Chocolate), as well as certified multi-elemental standards (Certipur®, Merck). Detection limits (LOD) ranged from 0.01 to 0.05 $mg.kg^{-1}$, depending on the element.

Table 1 summarizes the methods used for the determination of the proximal and mineral composition in cocoa beans below.

Quality control

In the proximate analyses (moisture, fat, protein, ash and carbohydrates), each sample was processed in triplicate. Additionally, random duplicates were included in each batch of analyses, which allowed us to verify the accuracy (≥ 90 %). Recovery standards were applied where appropriate, especially in the determinations of fats (AOAC 920.39, 2012) and proteins (AOAC 960.52, 2012), by fortifying samples with reference standards.

In the mineral analyses, quality control included the preparation of multi-elemental calibration curves from certified standards, ensuring a minimum linearity of $R^2 \geq 0.99$ in the range of interest. Certified Recovery Standards (MRCs), such as NIST SRM 2384 (Baking Chocolate, 100 % Cocoa Beans) and ERM-BD512 (Dark Chocolate), were also used to verify accuracy and ensure comparability in similar matrices (table 2). With each batch, reagent targets were analyzed to rule out possible contaminations during sample preparation and digestion, sample duplicates were included in each run to evaluate analytical repeatability.

Statistical analysis

Results were expressed as mean ± standard deviation of three independent determinations (triplicates). Descriptive statistics were applied for each proximal and mineral component. Differences between years (2013 - 2014) were assessed using a one-factor ANOVA, followed by the Tukey HSD multiple comparison test. In cases where the averages between years for each mineral or proximal component were directly compared, a Welch's t-test was also applied for independent samples (Welch's t-test). Statistical significance was established at $p \leq 0.05$, considering stricter levels of evidence ($p \leq 0.01$ and $p \leq 0.001$) to highlight highly significant differences. All analyses were performed with SPSS v.22.0 software (IBM Corp., Armonk, NY, USA), previously verifying the assumptions of normality and homogeneity of variances.

Table 1. Summary of the methods used for the determination of the proximate and mineral composition in cocoa beans.

Parameter	Method / Standard	Technique	Instrument / Conditions	Observations
Humidity	AOAC 925.10	Oven drying	105 °C to constant weight	Triplicate; Random duplicates
Ashes	AOAC 923.03	Muffle incineration	550 °C to constant weight	-
Protein	AOAC 984.13	Kjeldahl (N × 6.25)	Automatic digestion and distillation	Applied Recovery Standard
Fat	AOAC 963.15	Soxhlet	Petroleum ether as a solvent	Recovery 80 -120 %
Carbohydrates	Calculated	By difference	100 - (moisture + fat + protein ashes)	-
As, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ca	AOAC 999.11	ICP-OES After microwave-assisted acid digestion	Digestor Milestone Start D; ICP-OES Perkin Elmer Optima 5300 DV; mezcla HNO_3 65 % + H_2O_2 30 %	MRC NIST SRM 2384, ERM-BD512; LOD: 0.01 - 0.05 $mg.kg^{-1}$

Table 2. Summary of Acceptance Criteria for Quality Controls.

Quality Control	Application	Standard/Reference	Acceptance criteria
Triplicate	Proximal and minerals	All Samples	CV <10 %
Random duplicates	Proximal	Analysis Batches	Accuracy ≥90 %
Recovery Standard	Proximal (fats, proteins)	AOAC 920.39 (2012), AOAC 960.52 (2012)	80 - 120 %
Calibration curve	Minerals	Certified multi-elementary standards	$R^2 \geq 0,99$
Certified Standard (MRC)	Minerals	NIST SRM 2384, ERM-BD512	80 - 120 %
Reagent Target	Minerals	Non-sample reagents	≤ Detection limit
Repeatability (duplicates)	Minerals	Sample Lots	CV <10 %

Results and discussion

Proximate composition

Proximal analyses (table 3) confirmed values consistent with those reported for Venezuelan cocoa.

Table 3. Proximate composition of cocoa beans from Cata and Cuyagua (2013 - 2014).

Parameter	2013	2014
Humidity**	3.79 ± 0.42[a]	2.65 ± 0.68[b]
Fat**	49.69 ± 1.91[a]	45.27 ± 3.90[b]
Protein*	13.80 ± 0.79[a]	13.56 ± 0.98[a]
Ashes*	3.28 ± 0.33[a]	3.27 ± 0.26[a]
Carbohydrates*	29.44 ± 1.85[a]	29.25 ± 3.92[a]

Values expressed as mean ± SD, (*p < 0.05; **p < 0.01); equal letters indicate that there are no statistically significant differences

Humidity was significantly higher in 2013 (3.79 %) compared to 2014 (2.65 %) ($p < 0.0001$). Likewise, fat had a significantly higher value in 2013 (49.69 %) compared to 2014 (45.27 %) ($p < 0.001$). In contrast, protein, ashes and carbohydrates did not show statistically significant differences between years ($p > 0.05$). These results suggest that, although the proximate composition of cocoa remained relatively stable between both harvest years, moisture and fat showed relevant variations, possibly associated with climatic or post-harvest conditions, indicating that they influenced both water retention and lipid content. In contrast, protein, ash, and carbohydrates remained stable between 2013 and 2014, which coincides with what was previously reported, indicating that the main determinants of these fractions are genotype and postharvest practices (fermentation and drying), rather than interannual climatic variation (Arévalo-Gardini *et al*., 2017; Gramlich *et al*., 2018). Recent studies confirm that fermentation and drying dynamics influence not only the proximate composition, but also the bioavailability of nutrients and metals (García-Porras *et al*., 2025).

Mineral profile

The mineral profile showed notable differences between the harvest years (table 4).

Table 4. Mineral content of cocoa beans harvested in Cata and Cuyagua (2013–2014).

Mineral	2013 (media ± DE)	2014 (media ± DE)
As*	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Cd*	1.62 ± 1.08[a]	1.04 ± 0.59[a]
Cr*	0.22 ± 0.16[a]	0.23 ± 0.08[a]
Cu*	24.14 ± 6.83[a]	19.19 ± 2.40[b]
Fe**	83.18 ± 61.54[a]	34.57 ± 7.88[b]
Hg**	0.19 ± 0.15[a]	0.04 ± 0.08[b]
K**	4.959.60 ± 632.60[b]	7.447.73 ± 227.04[a]
Mg**	4.473.60 ± 887.85[a]	3.566.20 ± 418.38[b]
Mn**	29.4 ± 12.00[a]	19.40 ± 8.39[b]
Na*	60.84 ± 36.60[b]	96.32 ± 20.30[a]

Values expressed as mean SD ± (*p < 0.05; **p < 0.01); equal letters indicate that there are no statistically significant differences.

Calcium (Ca) and potassium (K) showed highly significant differences ($p<0.0001$), with higher concentrations of Ca in 2013 and K in 2014. Similarly, iron (Fe) and magnesium (Mg) levels were significantly higher in 2013. while in 2014 they were reduced, Manganese (Mn) was also higher in 2013, while sodium (Na) reached higher values in 2014.

Copper (Cu) showed a significant decrease in 2014 compared to 2013, while mercury (Hg), although present in low concentrations, was higher in 2013 and practically undetectable in 2014 ($p<0.001$). On the other hand, arsenic (As) was not detected at quantifiable levels (values below the detection limit in both campaigns), and both cadmium (Cd) and chromium (Cr) remained at low levels with no significant differences between years ($p>0.05$).

Although several of the elements analysed correspond to essential minerals, only cadmium has maximum levels specifically established for cocoa products in the European Union. For other potentially toxic elements, such as As, Hg and Cr, there are currently no specific regulatory limits for cocoa beans, so their interpretation must be carried out within the framework of general toxicological assessments, such as those established by the Codex Alimentarius and the World Health Organization (Codex Alimentarius Commission. 2019; World Health Organization. 2011).

Year-on-year change in essential minerals

The increase in K in 2014 and the decrease in Ca, Mg, Fe and Mn compared to 2013 can be explained in the framework of soil and climatic factors. Properties such as pH, organic matter, texture and cation exchange capacity influence the mobility and bioavailability of nutrients and metals in the soil (Meter *et al*., 2019; Barraza *et al*., 2017). In addition, rainfall and water balance modify the composition of the soil solution and therefore, the absorption of cations (Meter *et al*., 2019). These patterns are comparable with reports of cocoa in Peru and Ecuador, in which K is reported as the most abundant macroelement, while Fe and Mn vary according to local conditions (Arévalo-Gardini *et al*., 2017; Gramlich *et al*., 2018).

Cadmium (Cd)

Although the Cd showed a decrease between 2013 and 2014 (1.62 to 1.04 $mg.kg^{-1}$), the difference was not statistically significant. These values are in the high range of the values reported in other producing countries such as Ecuador, Peru and Colombia, in which concentrations usually vary between 0.5 and >1.0 $mg.kg^{-1}$, depending on the soil characteristics and genotype (Arévalo-Gardini *et al*., 2017; Barraza *et al*., 2017; Meter *et al*., 2019).

During the study period, the European Union had already established maximum limits of Cd in cocoa products by Regulation (EU) 488/2014, subsequently consolidated in Regulation (EU) 2023/915. These thresholds correspond to 0.60 $mg.kg^{-1}$ for cocoa powder and 0.80 $mg.kg^{-1}$ for chocolates with a high cocoa content (European Union 2014; 2023). Although these limits apply to the final product and not to the grain, the concentrations observed in this study confirm the need for systematic on-farm monitoring and the application of mitigation strategies, such as liming, phosphorus and zinc management, or agroforestry practices, which have already been shown to be effective in other countries (Meter *et al*., 2019; García-Porras *et al*., 2025).

It should also, be noted that the analyses were carried out on roasted almonds. in line with a previous study (Lanza *et al*., 2016). Although most, research on metals in cocoa uses raw beans. the heat treatment applied in this study sought to simulate conditions closer to the real processing of cocoa. This methodological difference must be considered when comparing directly with published values for raw grains. Consequently, the Cd levels reported in this study should be interpreted as an indicator of potential risk for export, rather than as a direct exceedance of regulatory limits, given that the final concentration in chocolate depends on multiple industrial processes: fermentation, drying, roasting, grinding and above all, on the dilution associated with the mixture with other ingredients (sugar, powdered milk, cocoa butter).

Mercury (Hg). arsenic (As) and chromium (Cr)

In this study, Hg and As were detected in traces and Cr remained stable, with no differences between years. These findings are consistent with regional research in which Cd emerges as the main contaminant of interest in Latin American cocoa, while other toxic metals are usually at low levels (Padilla *et al*., 2025).

The changes observed between 2013 and 2014 can be attributed to different factors that influence the absorption and accumulation of minerals in the grain. These assumptions are summarized in table 5.

Table 5. Climatic. edaphic and postharvest factors associated with the year-on-year variation of minerals in cocoa beans.

Hypothesis	Description	References
Weather conditions	Rainfall and water balance affect the availability of K, Ca and Mg in the soil solution. modifying their absorption by the plant.	Arévalo-Gardini *et al*. (2017)
Soil factors	Variations in pH, organic matter and phosphorus regulate the bioavailability of Cd and its interaction with bivalent cations (Ca^{2+}, Mg^{2+}).	Barraza *et al*. (2017); Meter *et al*. (2019)
Post-harvest practices	Fermentation and drying processes modify the apparent concentration of minerals and their bioaccessibility in the bean.	García-Porras *et al*. (2025)

In summary, the interannual differences observed in this study can be explained mainly by the combination of climatic conditions, soil factors and postharvest practices (table 5). These three elements constitute the most documented mechanisms in the literature for mineral variation in cocoa; however, other possible determinants, such as cultivar genetics, plant-microbiota interactions, or agricultural management practices, should not be ruled out. The findings presented here reinforce the need to address these factors in an integrated manner in future studies that directly correlate soil, climate and genotype parameters with the mineral composition of the grain.

Nutritional implications

The high content of K and Mg confirms cocoa as an important source of minerals, in agreement with composition data of other Latin American cocoas (Arévalo-Gardini *et al*., 2017; Gramlich *et al*., 2018). These minerals contribute to its nutritional value and potential as a functional food.

Overall, the stability of the proximate composition and the interannual differences in minerals observed in this study are consistent with the Latin American literature. The downward trend in Cd, although not significant, reinforces the importance of monitoring by origin to comply with international regulations and maintain the competitiveness of Venezuelan cocoa in demanding markets.

Conclusions

The proximate composition of Cata and Cuyagua cocoa beans remained relatively stable between 2013 and 2014, except for moisture, which was significantly lower in 2014, which can be attributed to climatic or post-harvest conditions.

The mineral profile showed significant year-on-year variations: Ca, Mg, Fe, Mn and Cu were higher in 2013, while K and Na increased in 2014. These results suggest the combined influence of climatic, edaphic and postharvest factors on the mineral composition of the grain.

Although the decrease in cadmium (Cd) was not statistically significant, the levels found are relevant in the face of European regulation (Regulation (EU) 488/2014), which underscores the need for systematic monitoring and on-farm mitigation strategies.

The high content of K and Mg confirms the importance of cocoa as a nutritional source of essential minerals, in accordance with reports in the literature for Latin American cocoa.

These results constitute a baseline for future studies that integrate edaphic, climatic and genetic variables, in order to design specific mitigation strategies by origin.

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