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BIOACTIVE COMPOUNDS AND HEAVY METALS CONTENT IN CASCARA *COFFEA ARABICA*

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ABSTRACT

The primary aim of the scientific article is to comprehensively analyze the chemical composition of Cascara. This study investigates the physicochemical properties of Cascara (100% *Coffea arabica*) sourced from America (Colombia, Panama, Costa Rica, Brazil, Guatemala). The cascara samples were analyzed for dry matter content, pH, water activity, polyphenol content, antioxidant capacity, chlorogenic acids profile, caffeine content and heavy metals (copper, zinc, manganese, iron, nickel, cobalt, lead, cadmium) concentrations. Our results revealed significant variations among the samples. Dry matter content ranged from 85.46% to 88.49%, with the highest levels found in the Guatemala sample (5C). pH values varied significantly, from 3.58 in the Colombian sample (1C) to 4.50 in the Brazilian sample (4C). Water activity was lowest in the Colombian sample (0.49) and highest in the Guatemala sample (0.55). In terms of bioactive compounds, chlorogenic acids content was highest in the Guatemala sample (0.97 mg.100 g^{-1}), while the highest caffeine concentration was detected in the Costa Rican sample (2.22 mg.100 g^{-1}). The total antioxidant capacity was highest in the Brazilian sample using the DPPH method (84.635%) and in the Panamanian sample using the ABTS method (92.30%). Heavy metal analysis indicated significant differences, with the highest concentrations of copper (10.13 mg.kg^{-1}) and iron (112.09 mg.kg^{-1}) in the Costa Rican sample, and the highest zinc (5.17 mg.kg^{-1}) in the Panamanian sample. The lowest concentrations of nickel (0.07 mg.kg^{-1}) and cobalt (0.24 mg.kg^{-1}) were found in the Colombian sample. The presence of lead and cadmium, particularly in American samples, highlights potential health risks, necessitating stringent monitoring. A strong positive correlation was found between neochlorogenic acid concentration and total phenolic content (TPC) (r = 0.84) and between neochlorogenic acid concentration and manganese (Mn) presence (r = 0.83). Concentrations of nickel (Ni) and cobalt (Co) (r = 0.920) and Pb and Cd (r = 0.74) also demonstrated strong correlations. Detailed knowledge of Cascara's chemical composition can aid in the development of new food and beverage products. This can expand the market for Cascara, promoting sustainability by utilizing coffee by-products more effectively.

Keywords: Cascara, coffee co- product, heavy metals, antioxidant and polyphenol content, caffeine

INTRODUCTION

Cascara, the dried husk of the coffee cherry (*Coffea arabica*), has gained attention for its potential health benefits due to its rich composition of bioactive compounds, including antioxidants, polyphenols, and caffeine. Traditionally considered a by-product of coffee production, Cascara is now being used for its nutritional and therapeutic properties (**Esquivel and Jiménez, 2012**).

Cascara is rich in bioactive compounds, including polyphenols, antioxidants, and caffeine, which contribute to its potential health benefits. Polyphenols, such as chlorogenic acids, are known for their antioxidant properties, which can mitigate oxidative stress and reduce the risk of chronic diseases **(Gulcin, 2020).** The high antioxidant capacity of Cascara is a key factor in its nutritional profile, offering potential health benefits such as improved cardiovascular health and reduced inflammation **(Ranheim and Halvorsen, 2005).**

The antioxidant capacity of Cascara is primarily attributed to its high polyphenol content. Polyphenols, including chlorogenic acids, are known for their potent antioxidant properties, which help in neutralizing free radicals and reducing oxidative stress **(Gulcin, 2020).** Studies have shown that the antioxidant activity of Cascara can vary based on factors such as geographical origin, processing methods, and environmental conditions **(Esquivel and Jiménez, 2012).**

Caffeine, a well-known stimulant, is another significant component of Cascara. Its concentration in Cascara can influence its overall antioxidant capacity and potential health benefits (**Ranheim and Halvorsen, 2005**).

The caffeine content in Cascara, while lower than in coffee beans, still provides stimulatory effects, enhancing cognitive function and alertness. However, the presence of caffeine necessitates careful consumption to avoid potential adverse effects, particularly in sensitive individuals **(Nawrot *et al.*, 2003; Nehlig, 2006).**

Caffeine's role in enhancing alertness and cognitive function is well documented, but its presence also necessitates careful evaluation of intake levels to avoid potential adverse effects **(Nawrot *et al.*, 2003).**

Understanding the chemical composition of Cascara, particularly its heavy metal content, is crucial for ensuring its safety and efficacy as a consumable product. Heavy metals such as cadmium (Cd), lead (Pb), nickel (Ni), and mercury (Hg) pose significant health risks due to their toxicity and bioaccumulation in human tissues. The contamination of food products by these metals can occur through various pathways, including soil composition, agricultural practices, and industrial pollution **(Jaishankar *et al.*, 2014; Abdullahi *et al.*, 2021).**

Regular monitoring and analysis of heavy metal content in food products, including Cascara, are essential to safeguard consumer health **(Scutarașu and Trincă, 2023).**

The contamination of food by heavy metals can occur at multiple stages of the food production process. Soil contamination is a primary route, especially in areas near industrial sites, where metals like lead and cadmium can be absorbed by crops **(Rai *et al.*, 2019).**

Water sources contaminated with mercury can affect aquatic life, leading to the accumulation of methylmercury in fish, a significant source of mercury exposure for humans **(Al-Sulaiti *et al.*, 2022).**

Additionally, food processing and packaging materials can contribute to contamination, as heavy metals can leach from equipment and containers into food products **(Eti *et al.*, 2023).**

Lead exposure is particularly harmful to the nervous system. Chronic exposure to lead, even at low levels, can lead to cognitive deficits, particularly in children. Studies have shown that elevated blood lead levels are associated with reduced IQ and attention span in children, as well as increased behavioral issues. In adults, prolonged lead exposure can cause hypertension, kidney damage, and reproductive problems **(Sanders *et al.*, 2009)**.

Cadmium is known for its nephrotoxic effects. Long-term exposure to cadmium through contaminated food, particularly in populations consuming large amounts of shellfish or rice grown in contaminated areas, can lead to kidney damage and bone demineralization. Cadmium is also classified as a human carcinogen, with evidence linking it to lung, prostate, and kidney cancers **(Genchi *et al.*,2020).**

The most common adverse health effects of cobalt include cardiomyopathy and damage to vision or hearing **(Paustenbach, 2014).**

Most metals have toxic effects on human health and accumulate in the different organs such as skeleton, liver, spleen, and kidney. Metals have negative effects on plant and animal production **(Abdel-Rahman, 2021).**

The chemical composition of cascara is thus a critical factor in determining its suitability as a functional food. The presence of beneficial compounds like antioxidants and polyphenols needs to be balanced against potential contaminants like heavy metals. This study aims to provide a comprehensive analysis of the chemical composition of cascara, focusing on its heavy metal content, antioxidant capacity, polyphenols, and caffeine. By doing so, it seeks to contribute valuable insights into the safety and health benefits of this emerging product.

MATERIAL AND METHODS

Material

For this study, 100% Cascara Coffea arabica samples from different regions of the Americas, including Colombia, Panama, Costa Rica, Brazil, and Guatemala, were used. A comprehensive description of the samples is presented in Table 1.

Table 1 List of analyzed samples

Samples	Country	Variety	Processing	Altitude (m)
1C	Colombia	G	D	1700 - 1800
2C	Panama	G	D	1100 - 1400
3C	**Costa Rica**	VS	D	1500 -1800
4C	Brazil	C	D	1250 - 1400
5C	Guatemala	B	D	1100 - 2000

Note: Samples: Variety: B – Bourbon, C – Catuai, G – Geisha, VS – Villa Sarchi; D – dry.

Methodology

Extract preparation

The Cascara samples were first homogenized by grinding using an electric grinder, Grindomix GM 200 (Retsch, Haan, Germany), operating at 10,000 rpm for 60 seconds. A 7 g portion of each sample was then extracted with 120 mL of deionized water at 95°C for 5 minutes, with intermittent stirring. After extraction, the samples were filtered using Sartorius filter paper (Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany). The resulting extracts were used for subsequent individual analyses.

Determination of dry matter

The dry matter content of the Cascara coffee powder samples was determined using the KERNDAB 100 - 3 laboratory instrument (KERN & SOHN GmbH, Balingen, Germany). A specific drying program was applied with a set temperature of 110°C. The dry matter content was expressed as a percentage (%).

Determination of pH

The pH of the Cascara extracts was determined at 20°C using a portable pH meter, model pH 70 (XS Instruments, Italy).

Determination of water activity

Water activity in the Cascara samples was assessed using the Meter Fast-Lab instrument (Germany). Each sample was measured individually in triplicate.

Determination of total antioxidant capacity using the DPPH radical

The total antioxidant capacity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay, as outlined by Brand-Williams et al. (1995). To begin, 0.025 g of DPPH radical was weighed and dissolved in 96% ethanol (Centralchem, Bratislava, Slovakia) to create a 100 mL stock solution. This solution was then diluted with ethanol. Next, 3.9 mL of the diluted DPPH solution was placed in glass cuvettes, and the initial absorbance (A0) at 515.6 nm was recorded using a T80 UV/VIS Spectrometer (PG Instruments, Ltd.; Lutterworth, UK). Following this, 100 µL of the coffee sample extract was added to the cuvette, and the mixture was stirred with a glass rod. After 10 minutes, the final absorbance (At) was measured at 515.6 nm. The percentage inhibition of DPPH radicals by the extract samples was then calculated using the following equation:

$$\% \text{ inhibition DPPH } = \frac{(A_0 - A_s) - (A_t - A_s)}{(A_0 - A_s)} \text{ x } 100$$

This parameter indicates the antioxidant activity of the Cascara extract samples by measuring the percentage of DPPH radicals inhibited.

Where:
- A_0 is the initial absorbance of DPPH solution,
- A_s is the absorbance of ethanol (blank), and
- A_t is the absorbance after 10 min.

Determination of total antioxidant capacity using the ABTS radical

Preparation of Reagents and Measurement of Antioxidant Activity Using ABTS Radical Cation: To prepare the ABTS solution, 0.192 g of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) was weighed and dissolved in distilled water to a final volume of 100 mL. For the potassium persulfate ($K_2S_2O_8$) solution, 1.622 g was weighed and dissolved in distilled water to make 100 mL. Next, 800 µL of the ABTS solution was combined with 400 µL of the $K_2S_2O_8$ solution and allowed to react for 5 minutes at room temperature with occasional stirring. The resulting dark blue solution was then diluted with 50 mL of fresh acetate buffer (pH 4.3). Preparation of acetate buffer (pH 4.3): To prepare 0.5 L of acetate buffer, 200 mL of 0.1M acetic acid solution was mixed with 200 mL of 0.1M sodium acetate solution. Measurement of Antioxidant Activity: In a cuvette, 3 mL of the prepared ABTS radical cation solution was mixed with 50 µL of the sample extract. The contents were mixed, covered, and allowed to stand for 20 minutes. Absorbance was then measured at 734 nm before (A_0) and after incubation (A_t). The results were expressed as a percentage of ABTS radical inhibition.

Determination of polyphenols content

Before measurement, the samples were prepared as follows: 50 µL of the extracts were transferred into 50 mL volumetric flasks. The Folin–Ciocalteu reagent was diluted with distilled water, and 2.5 mL of this diluted reagent was added to each flask containing the extract. Next, 5 mL of a 20% aqueous Na_2CO_3 solution was added to the flasks. The flasks were then filled with distilled water up to the 50 mL mark and left at room temperature for 2 hours to allow the blue-colored complex to form. The absorbance of the samples was then measured at 765 nm (Fu et al., 2011).

Determination of caffeine and chlorogenic acids

The HPLC analysis for chlorogenic acids and caffeine content was performed according to the procedure described by Bobková et al. (2021). Separation was achieved using a C-18 Poroshell 12 column (150 mm × 3 mm × 2.7 µm; Agilent Technologies, Waldbronn, Germany), with a mobile phase composed of acetonitrile (A) and 0.1% H_3PO_4 in distilled water (B) (v/v). The gradient elution program began with isocratic elution of 20% A + 80% B for 1 minute, followed by a linear gradient from 25% A + 75% B to 40% A + 60% B over 1 to 25 minutes. The equilibration time between injections was 3 minutes. The flow rate was maintained at 1 mL/min, with an injection volume of 10 µL and a separation temperature of 30°C. Detection was carried out at wavelengths of 276 nm for caffeine and 320 nm for chlorogenic acids, with data collected over a wavelength range of 210 to 400 nm. Standards used included caffeine (purity 98%, Sigma-Aldrich GmbH, Steinheim, Germany), neochlorogenic acid (purity 99%, Sigma-Aldrich GmbH, Steinheim, Germany), chlorogenic acid (purity 99%, Sigma-Aldrich GmbH, Steinheim, Germany), cryptochlorogenic acid (purity 98%, Sigma-Aldrich GmbH, Steinheim, Germany), 4,5-dicaffeoylquinic acid (purity 99%, Sigma-Aldrich GmbH, Steinheim, Germany), and 3,5-dicaffeoylquinic acid (purity 99%, Sigma-Aldrich GmbH, Steinheim, Germany). The solvents used were deionized water (18.2 MΩ·cm at 25°C) and HPLC-grade acetonitrile (purity ≥ 99.8%, Sigma-Aldrich GmbH, Steinheim, Germany).

Determination of heavy metals

Exactly 1 g of the homogenized Cascara sample was weighed using an analytical balance with a precision of ±0.0001 g. The sample was then placed in a mineralization cartridge, to which 5 cm³ of redistilled water and 5 cm³ of concentrated nitric acid were added. The sealed cartridge underwent mineralization in a MARS X-press microwave digestion system (USA). After mineralization, the sample was filtered through MUNKTELL grade 390.84 g/m² quantitative filter paper into a 50 mL volumetric flask, and the volume was adjusted with distilled water. The sample was subsequently analyzed using a VARIAN AA 240FS (Australia) according to the specified mineralization conditions (Table 2). A multi-element standard for GF AAS (16 elements) from Merck (Germany) was employed, ensuring a maximum analysis deviation of 3%. The gas flow rates were set at 13.5 L/min for air and 2.0 L/min for acetylene. Mercury was directly measured in the sample without additional treatment using an AMA 254 instrument (Czech Republic), employing atomic absorption spectrometry with mercury vapor generation. The wavelength for mercury detection was set to 253.65 nm, with a detection limit of 1.5 ng/kg dry matter. The AAS conditions are provided in Table 3.

Table 2 Parameters of the mineralization process

Phase	Power (W)	Power (%)	Building Up Time (min)	Temperature (°C)	Hold-Off (min)
Initialization (Achieving the specified conditions)	800	90	15	160	0
Mineralization (Maintaining the specified conditions)	800	90	0	160	20
Cooling	–	–	–	–	20

Table 3 Condition of Atomic Absorption Spectroscopy measurements of heavy metals

Heavy Metal	Detection Limit ($mg.L^{-1}$)	Sensitivity ($mg.L^{-1}$)	Wavelength (nm)
Cd	0.001	0.01	228.8
Pb	0.02	0.1	217.0
Cu	0.002	0.03	324.8
Zn	0.006	0.008	213.9
Co	0.005	0.05	240.7
Cr	0.003	0.04	357.9
Ni	0.008	0.06	232.0
Mn	0.003	0.02	279.5
Fe	0.005	0.04	241.8

Notes: Cu - copper, Zn - zinc, Mn - manganese, Fe - iron, Ni - nickel, Co - cobalt, Pb - lead, Cd - cadmium

Statistical analysis

To summarize and interpret our findings, descriptive analysis was employed, which included calculating arithmetic means, minimum and maximum values, and standard deviations for the measured parameters. Statistical tests such as ANOVA with Duncan's test and REGWQ were conducted to assess potential differences among samples across the determined parameters (bioactive compounds TAC, TPC and heavy metals in Cascara samples). All statistical analyses were performed using Microsoft Office Excel 365 for Windows with the XLSTAT Addinsoft (2021) and Microsoft Office Excel 365 for iOS with the XLSTAT (2022)**.**

RESULTS AND DISCUSSION

The differences in parameters regarding on geographical origin water activity and dry matter are shown in **Table 4.**

Table 4 Average value of dry matter (*DM %*), pH and water activity (a_w) of Cascara samples.

ID	*DM %*	pH	a_w
1C	85.92 ^{a}	3.57 ^{a}	0.49 ^{a}
2C	85.46 ^{a}	4.05 ^{c}	0.51 ^{c}
3C	87.73 ^{b}	3.81 ^{b}	0.49 ^{b}
4C	87.49 ^{b}	4.50 ^{e}	0.50 ^{c}
5C	88.49 ^{b}	4.22 ^{d}	0.55 ^{d}

Notes: a, b, c, d, e = groups within a column with different superscripts differ significantly at $p \leq 0.05$; ANOVA Duncan test

In our study, various Cascara samples were analyzed to determine differences in dry matter content (% dry matter), pH, and water activity (a_w). The results showed significant differences among the samples in each of the analyzed parameters. The dry matter content exhibited statistically significant differences with values ranging from 85.46% in sample 2C to 88.49% in sample 5C. The samples were divided into two groups, with samples 1C and 2C having a significantly lower dry matter content than samples 3C, 4C, and 5C, which were comparable to each other. Similar research by **Lachenmeier *et al.*, (2021)** reported dry matter content in Cascara ranging from 84.5% to 89.2%, aligning closely with our findings. Authors also noted that the variation in dry matter content could be attributed to differences in drying methods and environmental conditions during the Cascara's processing.
The pH values ranged from 3.57 in sample 1C to 4.498 in sample 4C, with statistical analysis showing highly significant differences among the samples. Samples 1C and 2C had the lowest pH values, while sample 4C exhibited the highest pH value, with all differences being statistically significant. This is consistent with the findings of **Esquivel and Jiménez (2012),** who reported pH values for Cascara between 3.6 and 4.5. The acidity of Cascara can be influenced by the coffee cherry's ripeness and the fermentation process, factors that vary across different studies. Authors also explored the effect of Cascara fermentation on pH levels, indicating that longer fermentation times typically result in higher acidity. This could explain the variation in pH observed in our samples.
Water activity (a_w) also significantly differed among the samples. The a_w values ranged from 0.485 in sample 1C to 0.548 in sample 5C. Sample 1C had the lowest a_w value, while sample 5C exhibited the highest a_w value. The differences among the samples were statistically significant, with samples 2C and 4C having comparable a_w values, which were higher than observed in sample 1C but lower than in sample 5C. In a study by **Murthy and Naidu (2012),** a_w values for Cascara ranged from 0.47 to 0.55, which is in line with our results. Water activity is critical for understanding the shelf life and microbial stability of Cascara, and these findings suggest that our samples are within the expected range for safe storage. The authors of the study emphasized the importance of controlling water activity to prevent mold growth and ensure the safety of Cascara as a food product. They also suggested that optimal aw levels should be maintained below 0.6 to avoid microbial spoilage **(Murthy and Naidu, 2012)**. In conclusion, there are significant differences in dry matter content, pH, and water activity among the analyzed samples, indicating variability in their physicochemical properties. These differences may have important implications for their storage, processing, and final use.

Table 5 ANOVA of water-soluble chemical compounds

ID	Neochlorogenic acid	Chlorogenic acid	Cryptochlorogenic acid	Caffeine	TAC-DPPH	TAC-ABTS	TPC
1C	0.99 ^{b}	0.24 ^{a}	0.00 ^{a}	1.89 ^{b}	64.57 ^{a}	91.64 ^{b}	26.69 ^{e}
2C	0.07 ^{a}	0.88 ^{d}	0.24 ^{c}	1.12 ^{a}	75.62 ^{b}	92.30 ^{b}	11.67 ^{b}
3C	0.07 ^{a}	0.53 ^{c}	0.16 ^{b}	2.22 ^{e}	63.54 ^{a}	87.10 ^{a}	14.41 ^{d}
4C	0.00 ^{a}	0.36 ^{b}	0.00 ^{a}	2.01 ^{c}	84.64 ^{b}	92.16 ^{b}	10.92 ^{a}
5C	0.08 ^{a}	0.97 ^{e}	0.22 ^{c}	2.13 ^{d}	59.54 ^{a}	91.33 ^{b}	13.13 ^{c}

Notes: chlorogenic acids and caffeine mg. ($100g^{-1}$), TAC DPPH and TAC ABTS total antioxidant capacity (%), TPC total polyphenol content g GAE. ($100g^{-1}$)

The phenolic compounds analyzed included neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 4.5- dicaffeoylquinic acid and 3.5-dicaffeoylquinic acid. 4.5- dicaffeoylquinic acid and 3.5-dicaffeoylquinic acid was not identified in our samples. Our findings revealed significant differences among samples, with the highest content observed for chlorogenic acid in the 5C sample 0.97 mg. ($100g^{-1}$), followed by neochlorogenic acid in the 1C sample 0.99 mg. ($100g^{-1}$). Previous studies by **Martinez-Saez *et al*. (2017)**, have also confirmed that cascara is rich in chlorogenic acids, which contribute to its antioxidant capacity. Similar results were reported in a study by **Subiria-Cueto *et al*. (2017)**, where cascara exhibited high TPC, correlating with its strong antioxidant activities. This suggests that Cascara could be a valuable addition to diets aimed at increasing antioxidant intake. Total antioxidant capacity in our study (TAC) was highest in the 4C sample for the DPPH method (84.64 %) and in the 2C sample for the ABTS method (92.30). Total phenolic content (TPC) was highest in the 1C sample (26.69 %).
Studies by **Martinez-Saez *et al*. (2017)** have shown that the high levels of phenolic compounds in Cascara contribute to its potent antioxidant activities, which can be beneficial in reducing oxidative stress in the body.
Caffeine reached its highest concentration in the 3C sample 2.22 mg. ($100g^{-1}$). For instance, a study by **Iriondo-DeHond *et al.* (2020)** found similar caffeine concentrations in Cascara, which are significant enough to consider its potential stimulant effects. In their research authors investigated the chemical composition and health benefits of Cascara, highlighting the presence of chlorogenic acids and caffeine as significant contributors to its health-promoting properties. These compounds are known for their potential to modulate glucose metabolism and exhibit anti-inflammatory effects.

Our study highlights the potential of these substances as important constituents in cascara extracts, potentially offering beneficial health effects.
Cascara is rich in bioactive compounds, including polyphenols and antioxidants, which can contribute to its health benefits. Understanding its antioxidant capacity helps in promoting it as a functional food with potential health advantages **(Van *et al.*, 2023)**.

Table 6 ANOVA of heavy metals (mg.kg $^{-1}$)

ID	Cu	Zn	Mn	Fe	Ni	Co	Pb	Cd
1C	8.69 ^{a}	2.15 ^{c}	65.88 ^{e}	33.50 ^{b}	0.073 ^{a}	0.24 ^{a}	1.96 ^{b}	0.70 ^{b}
2C	10.09 ^{d}	5.17 ^{d}	10.16 ^{b}	54.68 ^{c}	0.37 ^{c}	0.25 ^{a}	0.60 ^{a}	0.40 ^{a}
3C	10.13 ^{e}	2.19 ^{c}	23.98 ^{d}	112.09 ^{e}	0.31 ^{b}	0.28 ^{a}	2.84 ^{d}	0.67 ^{b}
4C	10.00 ^{c}	1.35 ^{a}	12.56 ^{c}	64.09 ^{d}	0.95 ^{e}	0.49 ^{c}	2.48 ^{c}	0.95 ^{c}
5C	9.07 ^{b}	1.70 ^{b}	9.67 ^{a}	18.67 ^{a}	0.54 ^{d}	0.40 ^{b}	2.50 ^{c}	0.68 ^{b}

Notes: Cu - copper, Zn - zinc, Mn - manganese, Fe - iron, Ni - nickel, Co - cobalt, Pb - lead, Cd - cadmium

Ensuring Cascara does not contain harmful levels of heavy metals is vital for consumer safety. Heavy metals can accumulate in the body over time, leading to serious health issues such as kidney damage, neurological disorders, and cancer **(Van *et al.*, 2023).**
Analysis of heavy metals confirmed the presence of 8 (copper, zinc, manganese, iron, nickel, cobalt, lead, cadmium) out of 9 specific compounds. Chromium was not detected in our samples. Our results show that sample 3C exhibited the highest concentration of Cu at 10.13 mg.kg $^{-1}$), while the lowest was found in sample 1C at 8.69 mg.kg^{-1}. Zinc (Zn) concentrations were highest in sample 2C (5.17 mg.kg^{-1}) and lowest in sample 4C (1.35 mg.kg^{-1}). Manganese (Mn) was particularly high in sample 1C (65.88 mg.kg^{-1}) compared to the lowest in sample 5C (9.67 mg.kg^{-1}). Iron (Fe) content was highest in sample 3C (112.087 mg.kg^{-1}) and lowest in sample 5C (18.67 mg.kg^{-1}). Nickel (Ni) concentration peaked in sample 4C (0.95 mg.kg^{-1}) and was lowest in sample 1C (0.07 mg.kg^{-1}). Cobalt (Co) was found in highest amounts in sample 4C (0.488 mg.kg^{-1}) and lowest in samples 2C and 1C (0.25 mg.kg^{-1} and 0.24 mg.kg^{-1}, respectively). Lead (Pb) was highest in sample 3C (2.84 mg.kg^{-1}) and lowest in sample 2C (0.60 mg.kg^{-1}). Cadmium (Cd) showed the highest concentration in sample 4C (0.95 mg.kg $^{-1}$) and the lowest in sample 2C (0.40 mg.kg^{-1}). Our results indicate that there are significant differences in heavy metal content among the Cascara samples. These variations could be attributed to different geographical origins, agricultural practices, or processing methods. High concentrations of heavy metals, particularly lead and cadmium, are concerning due to their potential health risks. The findings are consistent with previous studies indicating that plant-based products can accumulate heavy metals from contaminated soils and water. For instance, a review on heavy metal contamination in natural foods highlighted similar risks, emphasizing the need for stringent monitoring of metal concentrations to protect human health **(Hlihor *et al.*, 2022; Munir *et al.*, 2021).**
Another study focused on medicinal plants found that heavy metals such as lead, and cadmium pose significant health risks due to their toxicity and tendency to bioaccumulate in human tissues. These studies underscore the widespread nature of heavy metal contamination in various plant-derived products, reinforcing the importance of monitoring and regulating these contaminants **(Vinogradova *et al.*, 2023).**
Based on our results we could argue that the geographic origin of the Cascara samples plays a crucial role in determining their heavy metal content. The observed differences in metal concentrations among individual countries can be attributed to variations in soil composition, industrial and agricultural activities, and environmental regulations.
The study of heavy metals in Cascara highlights a critical food safety issue. The significant presence of metals like lead, cadmium, and nickel in various samples suggests a need for comprehensive strategies to monitor and mitigate contamination. This will help ensure the safety and health of consumers while maintaining the nutritional and medicinal benefits of plant-based products **(Munir *et al.*, 2021; Vinogradova *et al.*, 2023).**

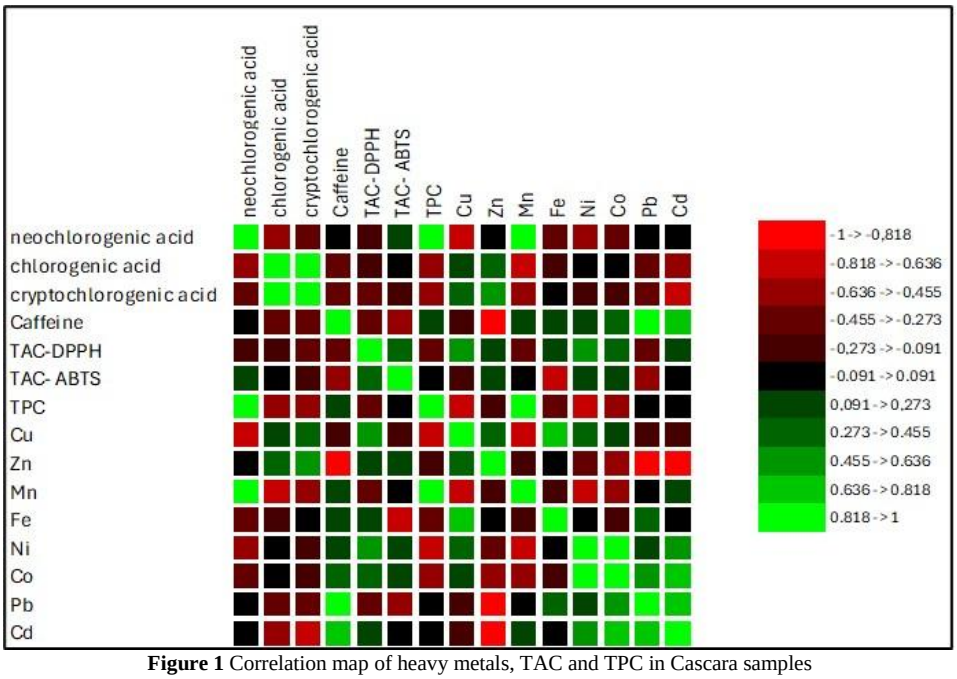

Figure 1 Correlation map of heavy metals, TAC and TPC in Cascara samples

Our results were subjected to correlation analysis to better understanding of potential ties between measured parameters. Our results showed that there is a strong positive correlation between neochlorogenic acid content and TPC (0.84). These findings align with the findings of **Górnáš *et al.*, (2015)** who noted that phenolic compounds, including neochlorogenic acid, contribute significantly to the antioxidant capacity of coffee. This relationship underscores the role of neochlorogenic acid as a major phenolic component enhancing the overall phenolic content. Strong positive correlation during our analysis was also observed between neochlorogenic acid content and Mn concentration (0.83) that indicating that higher neochlorogenic acid is associated with higher manganese content. Similarly, the strong correlation between TPC and Mn concentration was observed suggesting that phenolic compounds may influence the uptake or accumulation of manganese. This could be related to the role of manganese as a cofactor in enzymes involved in phenolic biosynthesis, as noted by **de Oliveira Rocha *et al.* (2022).** Very strong positive correlation was noted between chlorogenic acid and cryptochlorogenic acid presence (0.92), meaning these two acids often increase together is consistent with the findings of **Wang *et al.*, (2022)** who reported that these isomers often co-occur and are synthesized through similar metabolic pathways in coffee beans. Extremely high positive correlation was indicated in our research between caffeine and Pb (0.99), suggesting caffeine and lead

concentrations are closely linked. Previous studies, such as those by **Berego *et al.* (2023)** have indicated that heavy metals can co-occur with caffeine in coffee plants, possibly due to environmental factors or agricultural practices. Caffeine content also correlates with Cd (0.71), Ni and Co (0.92) concentrations, suggesting these elements often occur together. The same correlation was indicated between elements Pb and Cd (0.74). On the other hand, was observed negative correlations between Caffeine and Zn (-0.37), indicating higher caffeine levels are associated with lower zinc levels. General correlation map show that Antioxidant capacities (TAC-DPPH and TAC-ABTS) show different correlations with other variables, indicating their complex interactions with different compounds and elements.

Overall, this matrix reveals complex relationships between these variables, highlighting both strong positive and negative correlations, which could be crucial for understanding their interactions. Comparing our findings with existing literature highlights similar trends. **Quadra *et al.* (2020)** reported significant correlations between heavy metal content and caffeine levels in coffee products, suggesting common pathways or sources of contamination. Their study emphasizes the need for stringent quality control measures to minimize heavy metal contamination in coffee and related products. Moreover, research by **Alengebawy *et al.* (2021)** supports the hypothesis of environmental contamination, noting that areas with high industrial activity and use of certain fertilizers show elevated levels of heavy metals in crops, including coffee. These results collectively highlight the importance of chlorogenic acids, caffeine, and phenolic compounds in determining the antioxidant capacity and health benefits of coffee-related products, including Cascara. The correlation between these compounds and their health benefits underscores the potential of Cascara as a functional food.

CONCLUSION

This study underscores the considerable variability in the chemical composition of Cascara sourced from different geographical regions, emphasizing the influence of origin on its physicochemical properties, bioactive compound profiles, and heavy metal content The correlations observed between various bioactive compounds and heavy metals provide insights into the complex interactions within Cascara's chemical matrix. The detection of significant levels of heavy metals, particularly lead and cadmium, in samples from the Americas raises concerns regarding potential health risks, emphasizing the importance of stringent quality control and monitoring in the production and commercialization of cascara-based products. Understanding the chemical composition of cascara is essential not only for ensuring consumer safety but also for maximizing its potential as a functional food ingredient, thereby contributing to the sustainable use of coffee by-products.

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