THE EFFECT OF BREWING METHOD AND DIFFERENT ROASTING DEGREE ON PHYSICOCHEMICAL CHARACTERISTICS, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF AMAZONIAN ROBUSTA COFFEE (COFFEA CANEPHORA)

Luciana de Oliveira Silva¹ Carolyne Pimentel Rosado² Fernanda dos Santos Ferreira³ Julliana Melengati⁴ Sofia Terra⁵ Anderson Junger Teodoro⁶

ABSTRACT

Purpose: This work aimed to conduct chemical characterization, assess antioxidant activity, and quantify phenolic compounds in Amazonian Robusta Coffee beverages prepared using diverse roasting degrees and extraction methods.

Theoretical Framework: Coffee is globally consumed and holds substantial importance as a commodity. Its chemical composition and biological activity of coffee are influenced by several factors, such as cultivar, roasting degree and brewing method.

Methods: Amazonian Robusta Coffee beverages were prepared using diverse roasting degrees and extraction methods (Hario V60, French press and Cold Brew). Characterization was conducted to physicochemical analysis (color parameters, brewing index, pH, Brix and total dissolved solids), total phenolic compounds (Folin-Ciocalteu method) and antioxidant activity (FRAP, DPPH, ABTS+ and ORAC assays).

Results and Conclusions: Hario V60 beverage demonstrated the highest levels of total phenolic compounds (+50%) and antioxidant activity (+40%) across all roast levels (p<0.01), with significantly increased concentrations of total dissolved solids and phenolic compounds compared to French Press and Cold Brew. This enhanced extraction efficiency is likely due to greater surface area exposure and the percolation method. In contrast, Cold Brew exhibited higher a*, b* and Browning Index values (p<0.05), indicating more intense colors from prolonged extraction and greater melanoidin solubilization.

Research Implications: This work showed the chemical composition and antioxidant activity of Amazonian Robusta coffee beverages and their potential application for sustainable production.

Originality/Value: No previous study has assessed the antioxidant potential, phenolic content and physicochemical characterization of Amazonian Robusta coffee beverages.

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The Effect of Brewing Method and Different Roasting Degree on Physicochemical Characteristics, Total Phenolic Content and Antioxidant Activity of Amazonian Robusta Coffee (Coffea canephora)

Keywords: Beverages, Amazonian Robusta Coffee, Geographic Indication, Phenolic Compounds, Antioxidant potential, Sustainable Agriculture.

EFEITO DO MÉTODO DE PREPARO E DIFERENTES GRAUS DE TORMAGEM NAS CARACTERÍSTICAS FÍSICO-QUÍMICAS, CONTEÚDO FENÓLICO TOTAL E ATIVIDADE ANTIOXIDANTE DO CAFÉ ROBUSTA AMAZÔNICO (COFFEA CANEPHORA)

RESUMO

Objetivo: Este trabalho teve como objetivo realizar a caracterização química, avaliar a atividade antioxidante e quantificar compostos fenólicos em bebidas de Café Robusta Amazônico preparadas com diversos graus de torra e métodos de extração.

Enquadramento Teórico: O café é consumido globalmente e tem uma importância substancial como mercadoria. A composição química e a atividade biológica do café são influenciadas por diversos fatores, como cultivar, grau de torra e método de preparo.

Métodos: As bebidas de Café Robusta Amazônico foram preparadas utilizando diversos graus de torra e métodos de extração (Hario V60, prensa francesa e Cold Brew). A caracterização foi realizada para análise físico-química (parâmetros de cor, índice de escurecimento, pH, Brix e sólidos totais dissolvidos), compostos fenólicos totais (método Folin-Ciocalteu) e atividade antioxidante (ensaios FRAP, DPPH, ABTS+ e ORAC).

Resultados e Conclusões: A bebida Hario V60 demonstrou os mais altos níveis de compostos fenólicos totais (+50%) e atividade antioxidante (+40%) em todos os níveis de torra (p<0,01), com concentrações significativamente aumentadas de sólidos totais dissolvidos e compostos fenólicos em comparação para a French Press e Cold Brew. Esta maior eficiência de extração é provavelmente devido à maior exposição da área superficial e ao método de percolação. Em contrapartida, Cold Brew apresentou maiores valores de a*, b* e Índice de Browning (p<0,05), indicando cores mais intensas provenientes de extração prolongada e maior solubilização de melanoidina.

Implicações de Pesquisa: Este trabalho mostrou a composição química e a atividade antioxidante das bebidas de café Robusta da Amazônia e sua potencial aplicação para produção sustentável.

Originalidade/Valor: Nenhum estudo anterior avaliou o potencial antioxidante, o conteúdo fenólico e a caracterização físico-química de bebidas de café Robusta Amazônico.


EL EFECTO DEL MÉTODO DE ELABORACIÓN Y LOS DIFERENTES GRADOS DE TOSTADO SOBRE LAS CARACTERÍSTICAS FISICOQUÍMICAS, EL CONTENIDO FENÓLICO TOTAL Y LA ACTIVIDAD ANTIOXIDANTE DEL CAFÉ ROBUSTA AMAZÓNICO (COFFEA CANEPHORA)

RESUMEN

Objetivo: Este trabajo tuvo como objetivo realizar la caracterización química, evaluar la actividad antioxidante y cuantificar compuestos fenólicos en bebidas de Café Robusta Amazónico preparadas utilizando diversos grados de tostado y métodos de extracción.

Marco Teórico: El café se consume en todo el mundo y tiene una importancia sustancial como producto básico. Su composición química y actividad biológica del café están influenciadas por varios factores, como el cultivo, el grado de tostado y el método de elaboración.

Métodos: Se prepararon bebidas de Café Robusta Amazónico utilizando diversos grados de tostado y métodos de extracción (Hario V60, prensa francesa y Cold Brew). La caracterización se realizó mediante análisis fisicoquímicos (parámetros de color, índice de pardeamiento, pH, Brix y sólidos disueltos totales), compuestos fenólicos totales (método Folin-Ciocalteu) y actividad antioxidante (ensayos FRAP, DPPH, ABTS+ y ORAC).

Resultados y Conclusiones: La bebida Hario V60 demostró los niveles más altos de compuestos fenólicos totales (+50%) y actividad antioxidante (+40%) en todos los niveles de tueste (p<0,01), con concentraciones
The Effect of Brewing Method and Different Roasting Degree on Physicochemical Characteristics, Total Phenolic Content and Antioxidant Activity of Amazonian Robusta Coffee (*Coffea canephora*)

significativamente mayores de sólidos disueltos totales y compuestos fenólicos en comparación, a prensa francesa y cerveza fría. Esta mayor eficiencia de extracción probablemente se deba a una mayor exposición de la superficie y al método de percolación. Por el contrario, Cold Brew exhibió valores más altos de a*, b* e índice de pardeamiento (p<0.05), lo que indica colores más intensos debido a una extracción prolongada y una mayor solubilización de melanoidina.

**Implicaciones de la Investigación:** Este trabajo mostró la composición química y la actividad antioxidante de las bebidas de café Robusta Amazónico y su potencial aplicación para la producción sustentable.

**Originalidad/Valor:** Ningún estudio previo ha evaluado el potencial antioxidante, el contenido fenólico y la caracterización fisicoquímica de las bebidas de café Robusta Amazónico.

**Palabras clave:** Bebidas, Café Robusta Amazónico, Indicación Geográfica, Compuestos Fenólicos, Potencial antioxidante, Agricultura Sostenible.

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1 INTRODUCTION

Coffee is a significant global commodity (Oktaviani, 2020; Shahbandeh, 2022). Brazil is a major player in the coffee economy, contributing 30% of global production (CONAB, 2023). Coffee production in the Amazon began in the 1970s with inefficient practices. Over time, particularly in Rondônia, coffee cultivation improved, adopting new technologies for higher yields (Volski et al., 2019; Teixeira et al., 2020). Rondônia dominates Amazonian coffee farming, contributing 97% of production, ranking fifth nationally, and second in Coffea canephora output (Volksi et al., 2019). The "Matas de Rondônia" region was the first to receive Geographical Indication (GI) status for sustainable robusta coffee, with biodiversity and cultural richness shaping its flavor profile (INPI, 2021; Mancini et al., 2022; Agnoletti et al., 2024).

It is essential to note that coffee, one of the most consumed beverages globally, undergoes compositional changes influenced by management, processing, roasting, and preparation methods (SCAA, 2013). Roasting transforms green coffee beans according to established guidelines, which enhances the flavor, aroma and antioxidant properties of the final beverage (Sacchetti et al., 2009; Tarigan et al., 2022). Infusion techniques in production modify coffee beverage composition, elevating caffeine and chlorogenic acid levels with prolonged exposure (Zapata, Arango & Rojano, 2019; Angeloni et al., 2019; Olechno et al., 2020; Tarigan et al., 2022).

In this context, understanding the chemical composition and bioactivity of beverages derived from Amazonian Robusta coffee, originating from the Rondônia Forests region, is...
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crucial. This comprehension offers strategic pathways for the Amazonian coffee sector, highlighting sustainable production practices involving family producers and contributing to community development. It underscores the significance of Amazonian Robusta coffee, presenting substantial economic potential for the state.

Aligned with this perspective, this study aims to conduct chemical characterization, assess antioxidant activity, and quantify phenolic compounds in Amazonian Robusta Coffee beverages prepared using diverse roasting degrees and extraction methods.

2 THEORETICAL FRAMEWORK

Coffee is a major commodity in international and national markets, vital to many economies with growing global demand (Oktaviani, 2020; Shahbandeh, 2022; CONAB, 2023). Recent shifts in the international coffee market involve new marketing and consumption patterns driven by research and the introduction of health, environmental, and sensory-friendly products. These trends make coffee essential in modern lifestyles.

Brazil is the largest producer of Coffea arabica and Coffea canephora (Teles, C. R. A., & Behrens, J. H., 2020), contributing 30% of global production (CONAB, 2023) and ranks second in global coffee consumption due to internal quality enhancement programs (Vegro & De Almeida, 2020). According to the Brazilian Household Budget Survey (POF 2017-2018), coffee ranked second in mentions among Brazilians, trailing only water, demonstrating an increase compared to the previous survey conducted in 2008-2009 (Bezerra et al., 2022). Presently, Brazil exhibits heightened innovation across agricultural, industrial, and consumer services sectors, while maintaining a growing demand for high-quality coffees, particularly in the specialty or gourmet category, which accounts for approximately 10% of market share and is poised for further expansion (Teles & Behrens, 2020).

Within this context, coffee production in the Amazon biome emerged, gaining economic significance and characterized by extractive agricultural activities and inefficient land use. Rondônia now leads Amazonian coffee production, accounting for 97% of it, ranking fifth nationally and second in Coffea canephora production (Volsi et al., 2019; Teixeira et al., 2020). Amid evolving coffee cultivation, Matas de Rondônia became the first region to obtain a Geographical Indication (GI) for sustainable robusta coffees, influenced by geographical attributes, promoting regional development, environmental preservation, sustainable practices and quality coffee production (INPI, 2021; Mancini et al., 2022; Sgroi, 2021; Agnoletti et al.,
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2024). This GI elevates Rondônia as a specialty coffee region, enhancing credibility and opportunities for family agriculture (Da Silva et al., 2022).

**Figure 1**

*Geographical indication of the “matas de Rondônia” region, denomination of origin for Amazonian Robusta coffee*

![Geographical indication of the “matas de Rondônia” region, denomination of origin for Amazonian Robusta coffee](image)

Source: Authors

However, it is pertinent to consider that conventional coffee processing, which includes roasting and brewing methods, affects the quality of coffee and consequently the final beverage in the cup (Herawati et al., 2018; Fadri et al., 2019; Król et al., 2019; Olechno et al., 2020). Roasting green coffee beans induces various chemical reactions such as the Maillard reaction, caramelization and Strecker degradation, affecting color, stability of bioactive compounds and their antioxidant activity (Bobková et al., 2020; Tarigan et al., 2022).

In terms of beverage production methods, infusion techniques have been found to modify the composition of coffee beverages, resulting in elevated levels of caffeine and chlorogenic acid, which escalate with prolonged exposure (Zapata, Arango & Rojano, 2019; Angeloni et al., 2019; Rao et al., 2022). Coffee is rich in bioactive compounds, including caffeine, hydroxycinnamic acid family phenolic acids with chemoprotective, antioxidant, anti-inflammatory, and anticancer properties (Angeloni et al., 2019; Rao et al., 2022).

In conclusion, the comprehensive understanding of the chemical composition of beverages from Amazonian Robusta coffee, highlights sustainable production practices, quality and the role of family-based producers.
3 MATERIALS AND METHODS

3.1 PLANT MATERIAL

Green coffee beans of Amazonian Robusta Coffee (*C. canephora*), submitted to the pulped natural process, from the geographical indication "Matas de Rondônia", were sourced from Cacoal in Rondônia, Brazil, at an altitude of 200m. The roasting process of the coffee beans occurred in Rio de Janeiro in May 2022, at three distinct roast levels: light (192°C), medium (198°C), and dark (211°C). Roast profiles were classified using the Agrtron(SCAA) Roast Classification Color Disk system (AGTRON INC., 1997). The roasted coffee beans were stored in an ultrafreezer at -80°C.

3.2 COFFEE BREWING METHODS

The extraction methods employed included filtration and infusion. Preparation was tailored for each of the 3 methods - Cold Brew (CB), Hario V60 (HV60) and French Press (FP) - as specified by the SCAA (2013) and method manufacturers. Standardized procedures were established for each preparation method, considering variables such as particle size, temperature and brewing time. Extraction parameters are summarized in Table 1. Three replicates were conducted for each method. For comparative purposes, all methods were prepared according to the Golden Cup ratio recommended by the SCAA (2015), with a recommended coffee/water ratio of 55 g/L ± 10% (1:20). All beverages were prepared with filtered water.

**Table 1**

*Extraction parameters for coffee beverage preparation methods*

<table>
<thead>
<tr>
<th>Brewing Methods</th>
<th>Particle Size / Grind setting*</th>
<th>Water temperature (°C)</th>
<th>Extraction Time (h:m:s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold Brew</td>
<td>Coarse/ 28</td>
<td>12</td>
<td>18:00:00</td>
</tr>
<tr>
<td>French Press</td>
<td>Coarse/ 28</td>
<td>93</td>
<td>00:04:00</td>
</tr>
<tr>
<td>Hario V60</td>
<td>Medium/ 15</td>
<td>93</td>
<td>00:03:00</td>
</tr>
</tbody>
</table>

* Grinder Baratza Encore (Bellevue, WA 98005, USA).
3.3 PHYSICOCHEMICAL CHARACTERIZATION AND EVALUATION THE ANTIOXIDANT ACTIVITY OF COFFEE BEVERAGES

The steps for conducting the physicochemical characterization and antioxidant activity of coffee beverages are described in Figure 2, serving as the foundation for comprehending the procedures employed in this study. This characterization involved determining colorimetric parameters, such as Browning Index (BI), °Brix, Total Dissolved Solids (TDS) and pH. Concurrently, total phenolic content (TPC) and antioxidant activity was evaluated through various spectrophotometric assays, including FRAP, DPPH, ABTS+ and ORAC. For all assays, two dilutions were prepared from the beverages, which were initially at 55 g/L ± 10% (1:20), at ratios of 1/40 and 1/60.

Figure 2
Experimental design of Physicochemical characterization and antioxidante assays of Amazonian Robusta Coffee beverages.

3.3.1 Colorimetric parameters and Browning Index (BI)

The colorimetric analysis of coffee beverages employed a Delta Vista digital colorimeter and the CIE Lab* color space system. This system utilizes L* to represent brightness (0-100), a* for red/green intensity (+/-), and b* for yellow/blue intensity (+/-)
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(Gonnet, 1998). Parameters such as Chroma (C*) for color saturation and Browning Index (BI) were derived from L*, a*, and b*, providing an indirect measure of darkened compounds formed during caramelization and the Maillard reaction (Maskan, 2001). The formula to calculate the Browning Index (BI) is BI = 100 \[\frac{x - 0.31}{0.17}\], where x represents x = ([a' + 1.75L'] \times a') / ((5.645L' + a') - [0.30121 \times b']).

3.3.2 °Brix, pH and Total Dissolved Solids (TDS)

The determination of soluble solids, °Brix, was performed using a portable digital refractometer (Akso Brand). pH measurements were conducted utilizing a benchtop pH meter (Mylabor Brand). Additionally, the quantification of total dissolved solids (TDS) content was carried out by applying Equation \(\text{TDS\%}=0.85 \times ^{\circ}\text{Brix}\), formulated based on a correlation with soluble solids content (Gómez, 2019).

3.3.3 Total phenolic content (TPC)

The total phenolic content (TPC) was quantified using the Folin-Ciocalteu method as described by Singleton and Rossi (1999). A 10% Folin-Ciocalteu reagent was added to aliquots of each sample extract, followed by the addition of a 4% sodium carbonate solution. Absorbance was measured at 750 nm utilizing spectrophotometry (Turner® 340, Haverhill, MA, USA). TPC was expressed in milligrams of gallic acid equivalents (GAE) per milliliter (mL) of coffee beverages.

3.3.4 In vitro antioxidant activity

Antioxidant activity was evaluated through various methods. The FRAP assay, following Benzie & Devaki (2018), determined absorbance at 595 nm, expressing results as \(\text{Fe}_2\text{SO}_4 \mu\text{mol/mL}\). DPPH radical scavenging was conducted according to Brand-Williams, Cuvelier & Berset (1995), with absorbance measured at 515 nm and results quantified in Trolox equivalent \(\mu\text{mol/mL}\) using a commercial Trolox standard (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid). ABTS radical (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging capacity was assessed as per Miller et al. (1993), with absorbance recorded at 734 nm, and antioxidant capacity quantified in Trolox equivalent \(\mu\text{mol/mL}\). The ORAC assay, adapted from Huang et al. (2002) and Ou, Hampsch-Woodill, & Prior (2001),
captured peroxyl radicals during a 3-hour incubation at 37°C. Readings, collected every 5 minutes using a fluorimeter (SpectraMax i3, Molecular Devices, USA) with maximum fluorescence emission at 575 nm and 578 nm, were compared to a Trolox standard curve and expressed in Trolox equivalent μmol/mL.

3.4 STATISTICAL ANALYSIS

Data were expressed as mean ± standard deviation, with analyses performed in triplicate (n=3). Experimental data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's post hoc test at a significance level of 5%, using GraphPad Prism 9 software.

4 RESULTS AND DISCUSSION

4.1 COLORIMETRIC PROFILE AND BROWNING INDEX

Coffee beverage color, a quality indicator, is influenced by roast level, brewing temperature, and preparation methods (Shan et al., 2016; Rao et al., 2022). It reflects brewing intensity and the extraction of compounds from beans, affecting physicochemical composition and antioxidant potential (Janda et al., 2020; Wu et al., 2022; Yeager et al., 2022). Table 2 shows the colorimetric profile values and Browning Index (BI) for Amazonian Robusta Coffee at different roast levels.

Table 2

<table>
<thead>
<tr>
<th>Robusta Coffee Beverages</th>
<th>Colorimetric variables</th>
<th>Browning Index (BI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Luminosity (L*)</td>
<td>Chroma a (a*)</td>
</tr>
<tr>
<td>Cold Brew</td>
<td>Light</td>
<td>38.81 ± 1.43a</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>37.84 ± 2.76a</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>31.25 ± 1.45b</td>
</tr>
<tr>
<td>French Press</td>
<td>Light</td>
<td>29.19 ± 0.21c</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>30.41 ± 0.35d</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>26.87 ± 1.57e</td>
</tr>
</tbody>
</table>

The Effect of Brewing Method and Different Roasting Degree on Physicochemical Characteristics, Total Phenolic Content and Antioxidant Activity of Amazonian Robusta Coffee (*Coffea canephora*)

**Table 1**

<table>
<thead>
<tr>
<th>Hario V60</th>
<th>Light</th>
<th>Medium</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>29.15 ± 1.34f</td>
<td>27.55 ± 1.38f</td>
<td>27.46 ± 0.48g</td>
</tr>
<tr>
<td>Medium</td>
<td>8.31 ± 0.91c</td>
<td>7.50 ± 0.588d</td>
<td>3.59 ± 0.40e</td>
</tr>
<tr>
<td>Dark</td>
<td>6.76 ± 0.42d</td>
<td>4.88 ± 0.30a</td>
<td>5.90 ± 0.41b</td>
</tr>
<tr>
<td></td>
<td>10.71 ± 1.16</td>
<td>8.98 ± 1.43</td>
<td>4.49 ± 0.79</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. Means with different letters in the same column denote significant differences according Tukey's test at the 5% level. Abbreviations: *L*. lightness; *a*. Chroma a; *b*. Chroma b; *C*. Chroma c.

Lab* values reflect color differences based on brewing methods and roast profiles (Table 2), with significant differences (p<0.05) observed in color parameters among the beverages. Luminosity (L*) indicates the whiteness of roasted coffee (Yeager et al., 2022), and it was observed in this study that the L* value tends to decrease significantly (p < 0.05) with increased roasting time, possibly due to brown compounds produced by non-enzymatic browning (Wang et al., 2016; Shan, Zzaman & Yang., 2016; Yeager et al., 2022). In French Press (FP) and Hario V60 (V60) beverages, as the roasting profile increases, the beverage color approaches black, thus the a* and b* values tend to approach 0, making color differences harder to detect (Yeager et al., 2022). On the other hand, Cold Brew (CB) exhibits a different behavior, with higher a* and b* values, suggesting more intense colors due to prolonged extraction. This result may be attributed to the "cold" brewing temperatures, which produce a more reddish beverage compared to the typical black-brown color of hot brewed coffee (p<0.05). Additionally, Gökmen & Şenyuva (2006) found that the redness of coffee is inversely proportional to its acrylamide content, suggesting that Cold Brew (CB) may have a lower level of this compound. Our findings are in agreement with other authors, who identified that the beverage color varies significantly based on the brewing temperature (Yeager et al., 2022).

Browning Index (BI) serves as an indirect indicator of darkened compounds, such as melanoidins, formed during coffee roasting through caramelization and the Maillard reaction (Herawati et al., 2018; Fadri et al., 2019). Elevated BI levels suggest a higher concentration of these melanoidins in the heated product (Brzezińska et al., 2023). Our findings align with recent studies, indicating an increase in BI as roasting progresses. However, it is worth noting that beverages with higher BI do not always exhibit higher antioxidant activity because of the degradation of phenolic compounds during color formation (Shan, Zzaman & Yang., 2016). Among the three preparation methods analyzed, CB exhibited the highest BI value in dark roast compared to the other methods (12638; p < 0.05). However, the light and medium roasts of CB, as well as the medium roast of FP, showed the second-highest BI values. This dark roast BI index of CB suggests efficient extraction of melanoidins (Sacchetti et al., 2009). Increased contact time of coffee with water (18h), even at cold temperature, enhanced melanoidin solubilization, resulting in antioxidant activity, despite the absence of hot water...
usage, which typically extracts compounds with antioxidant potential, such as caffeine (Yeager et al., 2022; Brzezińska et al., 2023).

The physicochemical characterization of coffee beverages, including pH, Brix (%), and TDS (%), is presented in Table 3. Simple measurements of pH and °Brix (Table 3) were conducted, but no significant differences were identified between the beverages in pH and soluble solids, indicating no correlation between changes in chemical composition and these parameters. In terms of TDS values, V60 exhibits higher levels among the three brewing methods (p < 0.01). Wang et al. (2016) and Nguyen et al. (2024) observed a significant difference in coffee TDS prepared using different grind sizes. This finding is consistent with our results, wherein brewing coffee with medium grinds (V60) resulted in higher TDS content (1.36 to 1.45%) compared to coarser grinds (FP and CB) with TDS content (0.85 to 1.11%), likely due to increased surface area exposure to water, enhancing extraction efficiency.

### Table 3

**Physicochemical characteristics of Amazon Robusta coffee beverage obtained by different methods and roast degrees.**

<table>
<thead>
<tr>
<th>Robusta Coffee Beverages</th>
<th>Methods</th>
<th>Roast degree</th>
<th>pH</th>
<th>Brix (%)</th>
<th>TDS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Light</td>
<td>5.17 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.10 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium</td>
<td>5.17 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.10 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark</td>
<td>5.39 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.30 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.11 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cold Brew</td>
<td></td>
<td>Light</td>
<td>4.96 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.85 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium</td>
<td>5.02 ± 0.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.10 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.94 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark</td>
<td>5.10 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.20 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.02 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>French Press</td>
<td></td>
<td>Light</td>
<td>5.02 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.60 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.36 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium</td>
<td>5.07 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.60 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.36 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark</td>
<td>5.20 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.70 ± 0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.45 ± 0.02&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hario V60</td>
<td></td>
<td>Light</td>
<td>5.17 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.10 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.94 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium</td>
<td>5.17 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.10 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.94 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark</td>
<td>5.17 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.10 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.94 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD or absolute value when obtained through calculation. Different letters in the same column indicate significant difference. p≤0.05 (Tukey test).

### 4.2 TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY (FRAP, DPPH, ABTS+ AND ORAC ASSAYS)

The extraction of coffee significantly influences the characteristics of the final beverage. The polyphasic nature and concentration of non-volatile and volatile compounds define the physicochemical, antioxidant, and sensory properties of different coffee beverages (Angeloni et al., 2019; Chavez et al., 2022).

TPC and the antioxidant activities of coffee beverages were affected by the brewing methods to varying degrees of roast (Table 4) and are consistent with the findings of previous
The Effect of Brewing Method and Different Roasting Degree on Physicochemical Characteristics, Total Phenolic Content and Antioxidant Activity of Amazonian Robusta Coffee (*Coffea canephora*)

Studies (Zapata, Arango & Rojano, 2019; Janda et al., 2020; Bobková et al., 2020; Olechno et al., 2020; Chavez et al., 2022; Rao et al., 2022; Wu et al., 2022).

Table 4

*Total phenolic content (TPC) and antioxidant activity of Amazon Robusta coffee beverage, in varied roast levels, obtained by different brewing methods.*

<table>
<thead>
<tr>
<th>Robusta Coffee Beverages</th>
<th>TCP (mg GAE/mL)</th>
<th>FRAP (µmol Fe₂SO₄/mL)</th>
<th>DPPH (µmol TE/mL)</th>
<th>ABTS⁺ (µmol TE/mL)</th>
<th>ORAC (µmol TE/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Methods</strong></td>
<td><strong>Roast degree</strong></td>
<td><strong>Light</strong></td>
<td><strong>Medium</strong></td>
<td><strong>Dark</strong></td>
<td></td>
</tr>
<tr>
<td>Cold Brew</td>
<td>Light</td>
<td>1.29 ± 0.02</td>
<td>1.37 ± 0.00</td>
<td>1.49 ± 0.02</td>
<td>24.30 ± 3.46</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.27 ± 0.01</td>
<td>1.36 ± 0.13</td>
<td>3.98 ± 0.11</td>
<td>16.92 ± 1.48</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>0.68 ± 0.01</td>
<td>6.06 ± 0.17</td>
<td>5.09 ± 0.07</td>
<td>16.86 ± 2.20</td>
</tr>
<tr>
<td>French Press</td>
<td>Light</td>
<td>1.06 ± 0.04</td>
<td>5.47 ± 0.23</td>
<td>6.85 ± 0.15</td>
<td>22.42 ± 1.32</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.16 ± 0.01</td>
<td>6.23 ± 0.18</td>
<td>6.36 ± 0.34</td>
<td>15.18 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>1.27 ± 0.04</td>
<td>4.63 ± 0.07</td>
<td>4.37 ± 0.12</td>
<td>15.44 ± 2.09</td>
</tr>
<tr>
<td>Hario V60</td>
<td>Light</td>
<td>1.70 ± 0.06</td>
<td>2.13 ± 0.04</td>
<td>7.33 ± 0.1</td>
<td>24.78 ± 2.96</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>2.57 ± 0.02</td>
<td>1.99 ± 0.04</td>
<td>6.94 ± 0.68</td>
<td>29.16 ± 2.49</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>2.32 ± 0.08</td>
<td>6.48 ± 0.62</td>
<td>5.35 ± 0.12</td>
<td>23.08 ± 3.64</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation; values were compared using One-way ANOVA, followed by Tukey’s test (p <0.005). Different letters in the same column indicate significant differences.

Abbreviations: GAE – Gallic Acid Equivalent; TE – Trolox Equivalent.

Coffee brewed using the V60 method exhibited the highest TPC content across light, medium, and dark roasts (p<0.01). This method showed higher TPC values (ranging from 1.70±0.06 to 2.57±0.02 mg GAE/mL) and greater antioxidant activity, as measured by FRAP (ranging from 1.99±0.04 to 2.13±0.04 µmol Fe₂SO₄/mL) and DPPH assays (ranging from 6.48±0.62 to 7.63±0.18 µmol TE/mL). For the ABTS⁺ and ORAC assays, increased roasting temperature was found to influence antioxidant activity, with darker roasts exhibiting lower antioxidant activity compared to lighter ones (p<0.01). Medium roasts generally displayed higher antioxidant activity across all preparation methods, suggesting that intermediate temperatures favor the formation and/or release of bioactive compounds. However, continuous elevation of temperature may lead to the degradation of these compounds (Herawati et al., 2018; Wu et al., 2022).

Brewing time and water temperature had less influence on TPC and antioxidant activity, as also observed by Klótz et al. (2020). This suggests that the main factor influencing extraction was the kinetics of compound extraction, with the percolation method being more effective than the infusion method (Derossi et al., 2018; Bobková et al., 2020; Janda et al., 2020; Olechno et al., 2020). Researches demonstrated that coarser grinds, like those used in FP and CB, result in lower TPC and antioxidant activity beverages compared to finer grinds used in V60, which
result in higher TPC and antioxidant capacity due to increased surface area contact with water (Wang et al, 2016; Derossi et al, 2018; Nguyen et al. 2024).

CB showed higher TPC than FP in both light and medium roasts. Specifically, CB had approximately 22% higher TPC in light roast and 9% higher in medium roast compared to FP (p<0.01). Both methods used the same grind size and infusion technique, indicating that the differences were water temperature and extraction time. Research indicates that hot water enhances extraction, especially for compounds like caffeine, leading to higher antioxidant activity (Yeager et al., 2022; Brzezińska et al., 2023). However, the higher TPC in CB suggests that contact time may play a more significant role in extraction than water temperature (Derossi et al, 2018; Da Silva Portela et al., 2021; Rao et al., 2022).

This pattern is also observed in the antioxidant assays, with CB showing higher FRAP values compared to FP. Specifically, CB had approximately 12% higher FRAP in light roast, 6% higher in medium roast, and 21% higher in dark roast compared to FP (µmol Fe$_2$SO$_4$/mL). In ORAC assay, there were no significant differences between CB and FP within each roast profile (p>0.05). The trend of higher antioxidant activity in FP observed in DPPH and ABTS assays highlights the complex interplay between brewing parameters and bioactive compound extraction.

Da Silva Portela et al. (2021) found that Robusta coffee beverages have higher concentrations of bioactive compounds, such as total phenolics and caffeine, resulting in greater antioxidant activity compared to Arabica. These findings support our results, indicating that Robusta coffee brewed using percolation methods is a potent source of bioactive compounds, underscoring its health benefits and economic value, especially for GI-certified, sustainably produced varieties.

5 CONCLUSION

Coffee brewing methods significantly influence the physicochemical characteristics, total phenolic content and antioxidant activity of the final beverage. Hario V60 consistently showed the highest total phenolic compounds and antioxidant activity across all roasts, attributed to its finer grind size and effective percolation, enhancing bioactive compound extraction. These findings emphasize the importance of brewing methods in optimizing bioactive compound extraction, improving coffee quality and health benefits to enhance coffee's physicochemical, sensory properties and socio-economic development.
REFERENCES


