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Gastrointestinal Digestion and Absorption of Antioxidant Phenolic Compounds and Caffeine from the Coffee Pulp under Simulated Conditions †

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Abstract: Coffee is one of the most widely consumed beverages worldwide. Consequently, many by-products are generated during coffee processing, including the pulp, a source of antioxidant phytochemicals such as phenolic compounds and caffeine, reducing oxidative stress. However, phenolics' antioxidant properties are physiologically restricted to their bioaccessibility and bioavailability. This study aimed to investigate the gastrointestinal behavior of the coffee pulp's phenolic compounds under simulated conditions. The coffee pulp, obtained from the Arabica variety by the wet processing method, was milled and subsequently digested following the in vitro INFOGEST method. Phenolic compounds were analyzed using colorimetric and UPLC-MS/MS methods. The in vitro antioxidant capacity was estimated by the ABTS method. The potential bioavailability was predicted using in silico tools. The coffee pulp showed a high content of phenolic acids, especially chlorogenic (1011 ± 28 μg g^{-1}), protocatechuic (1757 ± 7 μg g^{-1}), and gallic (469 ± 20 μg g^{-1}) acids, and flavonoids, particularly quercetin derivatives. The caffeine content (5060 ± 67 μg g^{-1}) stood out among all the phenolic compounds, 4.6-fold higher than total chlorogenic acids and 1.4-fold higher than total phenolic compounds. Although the total phenolic content and antioxidant activity significantly increased ($p < 0.05$) all over the digestive process, the bioaccessibility of the individual phenolic compounds decreased ($p < 0.05$) throughout the digestive process. Hydroxybenzoic and hydroxycinnamic acids showed high intestinal bioaccessibility (79.0 ± 12.6 and 82.3 ± 11.1%, respectively), while flavonols and flavones exhibited lower values (58.7 ± 8.9 and 41.9 ± 6.8%, respectively). Caffeine (83.1 ± 5.9%) also exhibited high intestinal bioaccessibility. The potential bioavailability, expressed as human intestinal absorption, was higher for caffeine (74.0 ± 5.3%), followed by hydroxybenzoic acids (48.6 ± 7.8%) and hydroxycinnamic acids (22.8 ± 3.1%), and finally, the lowest values were obtained for flavonols (13.6 ± 2.2%) and flavones (7.8 ± 3.1%). Then, although exhibiting similar bioaccessibilities, caffeine may reach the bloodstream and target organs in a higher proportion than phenolic compounds. These results provide new knowledge into the gastrointestinal behavior of antioxidant phenolic compounds and caffeine from the coffee pulp, supporting its use as a new antioxidant food ingredient.

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1. Introduction

Coffee is one of the most widely consumed beverages worldwide. *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* (Robusta coffee), all members of the Rubiaceae family, are the most important plant species in the international coffee trade [1]. About 90% of the coffee cherry's edible sections are wasted as agricultural wastes or by-products during the coffee beverage preparation [2]. Coffee by-products have aroused considerable interest because of their abundance and interesting chemical composition. Coffee by-products, including husk, skin, pulp, coffee mucilage, coffee parchment, coffee silverskin, and spent coffee grounds, can be obtained by wet or dry processing. Coffee pulp (CP) is one of the by-products obtained during the wet processing, representing 43.2% of the fresh whole fruit. The CP can be used as food for animals or as a substrate for microbiological processes, but few studies are about its potential as a new ingredient for human food [3]. Several reports have shown that the CP is a source of bioactive compounds of high value and attractive nutritional value, such as dietary fiber and phenolic compounds [4,5]. Our group previously investigated the phenolic profile of the coffee parchment and the coffee husk [6,7]. We investigated the effects of extrusion on the release of phenolic compounds during gastrointestinal digestion and their in vitro antioxidant capacity in intestinal cells [8]. Additionally, we have proved the effects of the phenolic compounds from coffee by-products on the prevention of inflammation in macrophages [9], adipogenesis and insulin resistance in adipocytes [10], and the regulation of hepatic lipid and glucose metabolism and mitochondrial bioenergetics [11].

Although the bioactivity of phenolic compounds can be promising after in vitro studies, their low bioavailability impedes their potential efficacy in humans [12]. These bioactive compounds are just reasonably absorbed in the gastrointestinal tract and can suffer chemical modifications throughout digestion and microbial fermentation [13]. To better understand the benefits of coffee by-products, including the CP, experiments should look at how digestion and metabolism in the gastrointestinal tract affect bioaccessibility, bioavailability, and bioactivity and the role of microbiota in that process. Thus, this study aimed to investigate the gastrointestinal behavior of the phenolic compounds and caffeine from the CP under simulated in vitro and in silico conditions, associating their chemical structure properties with their distinct intestinal absorption and bioavailability.

2. Materials and Methods

2.1. Materials

The CP from the Arabica species (*Coffea arabica* L.) was obtained by wet processing and supplied by "Las Morenitas" (Nicaragua). CP was milled using a laboratory grinder, obtaining CP flour. The sample was stored in sealed flasks at −20 °C until analysis.

2.2. In Vitro Simulated Digestion

In vitro simulated gastrointestinal digestion was performed following the harmonized INFOGEST method [14] with slight modifications. In vitro simulated colonic digestion was carried out according to Papillo et al. [15].

2.3. Colorimetric Analysis of Total Phenolic Compounds and Antioxidant Capacity

2.3.1. Total Phenolic Content

The total phenolic content (TPC) was analyzed by the Folin-Ciocalteu assay [16]. The experiment was carried out in a 96-well microplate. Briefly, 10 μL of the sample, 150 μL of Folin-Ciocalteu reagent (diluted 1: 14, *v*/*v* in Milli-Q water), and 50 μL of Na_2CO_3 20%

were added to each well. The plate was incubated in the dark at room temperature for 2 h. Absorbance was measured at 750 nm in a microplate reader. A standard gallic acid curve (0.01–0.2 mg mL^{-1}) was performed, and the results were expressed as mg gallic acid equivalents per gram (mg GAE g^{-1}).

2.3.2. In Vitro Antioxidant Capacity

Antioxidant capacity was assessed by the $ABTS^{\bullet+}$ assay [17]. 2.2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid radical cations ($ABTS^{\bullet+}$) were obtained by reacting 7 mmol L^{-1} $ABTS^{\bullet+}$ solution with 2.45 mmol L^{-1} potassium persulfate and stirring it in the dark at room temperature for 16 h before use. The $ABTS^{\bullet+}$ solution obtained was diluted in 5 mmol L^{-1} PBS, pH 7.4, by adjusting the solution to an absorbance of 0.70 at 734 nm. The assay was carried out in a 96-well microplate by adding 30 µL of the sample and 270 µL of the diluted solution $ABTS^{\bullet+}$ to each well. After 10 min of incubation, the absorbance was read at 734 nm on a microplate reader. A calibration curve was made using Trolox as a standard solution (0–0.06 mg mL^{-1}). The results were expressed as mg Trolox equivalent per gram (mg TE g^{-1}).

2.4. HPLC–DAD–ESI/MS^n Qualitative and Quantitative Analyses of Bioactive Compounds

Samples were analyzed using Hewlett-Packard 1100MS (Agilent Technologies, Palo Alto, CA, USA) chromatograph equipped with a diode array detector (DAD). Solvents used were 0.1% formic acid in water (solvent A) and 100% acetonitrile (solvent B). The elution gradient established was 15% B for 5 min, 15–20% B for 5 min, 20–25% B for 10 min, 25–35% B for 10 min, 35–50% B for 10 min, and re-equilibration of the column. The separation was performed in a Spherisorb S3 ODS-2 C8 column (Waters, Milford, CT, USA) (3 µm, 150 mm × 4.6 mm) operating at 35 °C and a flow rate of 0.5 mL min^{-1}. Mass spectrometer (MS) connected to the HPLC system via the DAD cell outlet was used, and detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source, triple quadrupole-ion trap mass analyzer and controlled by the Analyst 5.1 software. The phenolic compounds and caffeine were characterized according to their UV and mass spectra and retention times and comparison with authentic standards when available. For quantitative analysis, calibration curves were prepared by injecting known concentrations of different standard compounds.

2.5. Bioaccessibility of the Bioactive Compounds in the Coffee Pulp Flour

The bioaccessibility index, expressed as a percentage for the individual compounds and the spectrophotometric measures, was determined as follows:

$$\text{Bioaccessibility (\%)} = \frac{\text{Digested Fraction}}{\text{Non-digested Fraction}} \times 100$$

where Digested Fraction corresponds to the concentration of phytochemicals in the soluble fraction obtained after in vitro digestion, and Non-digested Fraction is the concentration of phytochemicals in the sample before in vitro digestion.

2.6. In Silico Potential Bioavailability Estimation

The potential absorption of the bioactive compounds found in the CP flour was evaluated in silico. Predictions of Caco-2 and intestinal absorption, molecular surface area, and LogP were calculated using canonical SMILES sequences obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed on 17 February 2022), using pkCSM-pharmacokinetics (http://biosig.unimelb.edu.au/pkcsm/, accessed on 17 February 2022) and ADMETlab (https://admet.scbdd.com/, accessed on 17 February 2022) cheminformatics free software. The potential bioavailability of the bioactive compound was calculated as follows:

$$\text{Bioavailability (\%)} = \frac{\text{Intestinal Fraction} \times \text{Absorption}}{\text{Non-digested Fraction}} \times 100$$

where Intestinal Fraction corresponds to the concentration of phytochemicals soluble fraction obtained after in vitro digestion, Absorption corresponds to the percentage of absorption estimated in silico for each compound, and Non-digested Fraction is the concentration of phytochemicals in the sample before in vitro digestion.

2.7. Statistical Analysis

Each sample was analyzed in triplicate. Data were reported as mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA) and post hoc Tukey tests. Differences were considered significant at $p < 0.05$. Non-linear exponential regressions were calculated to associate the chemical structure of phenolic compounds and caffeine with their distinct intestinal absorption and bioavailability. The statistical analysis was performed by SPSS 23.0.

3. Results and Discussion

3.1. Main Bioactive Compounds in the Coffee Pulp Flour Were Caffeine and Chlorogenic Acids

The coffee pulp showed a high content of phenolic acids, especially chlorogenic (1011 ± 28 μg g^{-1}), protocatechuic (1757 ± 7 μg g^{-1}), and gallic (469 ± 20 μg g^{-1}) acids, and flavonoids, particularly quercetin derivatives. The caffeine content (5060 ± 67 μg g^{-1}) stood out among all the phenolic compounds, 4.6-fold higher than total chlorogenic acids and 1.4-fold higher than total phenolic compounds. Similarly, the main phenolic compounds identified in coffee parchment were chlorogenic, vanillic, protocatechuic, and *p*-coumaric acids [6]. The main phenolic compounds found in the coffee husk were also chlorogenic and protocatechuic acids, followed by kaempferol-3-*O*-galactoside and gallic acid [7]. The coffee silverskin also exhibited a remarkable concentration of caffeine and chlorogenic acid [9]. Then, independently of the coffee by-product, the main phytochemicals found seem to be caffeine and chlorogenic acids.

3.2. Gastrointestinal Digestion Reduced Phenolic Compounds and Caffeine Bioaccessibility

Although the TPC and antioxidant activity significantly increased ($p < 0.05$) all over the digestive process (Figure 1A), the bioaccessibility of the individual phenolic compounds decreased ($p < 0.05$) throughout simulated gastrointestinal digestion (Figure 1B).

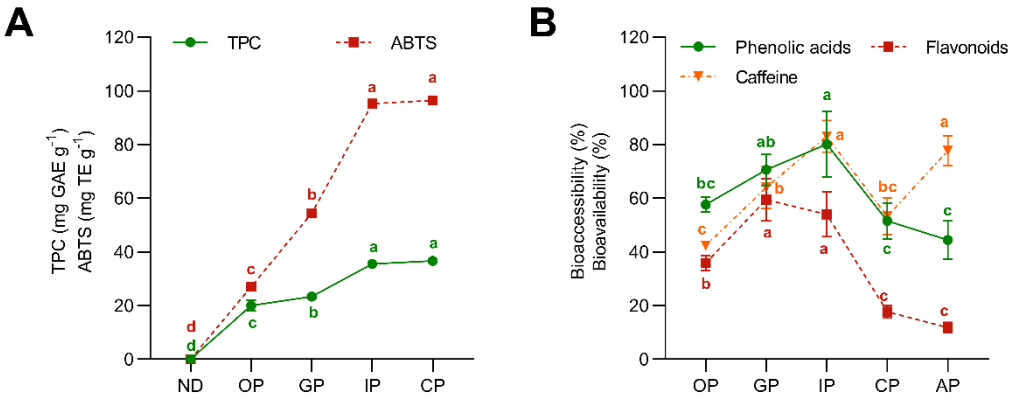

Figure 1. Effects of simulated gastrointestinal digestion on the total phenolic content (TPC) and antioxidant capacity measured by the ABTS method (**A**) of the coffee pulp flour. (**B**) Estimated bioaccessibility and bioavailability indexes calculated for each phenolic compounds' family (phenolic acids and flavonoids) and caffeine in each of the digestive phases: oral phase (OP), gastric phase (GP), intestinal phase (IP), colonic phase (CP), and absorbed phase (AP) corresponding to the potential

bioavailability. ND: non-digested coffee pulp flour. Results are expressed as the mean ± standard deviation of three independent experiments ($n = 3$). Points with different letters indicate significant differences among the digestion phases according to ANOVA and Tukey test ($p < 0.05$).

The Folin-Ciocalteau method, like other spectrophotometric methods, can be non-specific and interact with other molecules such as proteins, amino acids, nucleotides, ascorbic acid, sugars, aromatic amines, thiols, or organic acids, among others. Interaction with other molecules released during the in vitro digestive process may overestimate the content of phenolic compounds [18]. Similarly, all molecules with antioxidant capacity can also cause interference in the ABTS method. It has been demonstrated that among the phenolic acids, the hydroxycinnamic acids achieved higher values than the hydroxybenzoic acids in the Folin-Ciocalteau and ABTS assays [19]. The release of phenolic acids from the insoluble non-digestible fiber fraction may account for those higher Folin-Ciocalteau and ABTS values, even though the concentration of individual compounds suffered reduction during intestinal and colonic digestion phases. Thus, hydroxybenzoic and hydroxycinnamic acids showed high intestinal bioaccessibility (79.0 ± 12.6 and 82.3 ± 11.1%, respectively), while flavonols and flavones exhibited lower values (58.7 ± 8.9 and 41.9 ± 6.8%, respectively). Caffeine also exhibited high intestinal bioaccessibility (83.1 ± 5.9%) (Figure 1B). The high bioaccessibility of phenolic acids compared to flavonoids may be attributed to their high polarity [20]. In turn, caffeine is a stable molecule with hydrophilic and sufficiently lipophilic properties, which confers it the ability to cross biological membranes achieving high bioaccessibility and bioavailability [21].

The stability and bioaccessibility of the phenolic compounds and caffeine from the CP seemed to be dependent on the chemical class, being higher in caffeine, followed by phenolic acids, and eventually flavonoids.

3.3. Phenolic Compounds Exhibited a Lower Potential Bioavailability Than Caffeine

The potential bioavailability, based on the intestinal absorption, was higher for caffeine (74.0 ± 5.3%), followed by hydroxybenzoic acids (48.6 ± 7.8%) and hydroxycinnamic acids (22.8 ± 3.1%), and finally, the lowest values were obtained for flavonols (13.6 ± 2.2%) and flavones (7.8 ± 3.1%) (Figure 1B). Then, although exhibiting similar bioaccessibilities, caffeine may reach the bloodstream and target organs in a higher proportion than phenolic compounds. Non-linear regression demonstrated the negative association between the CP's compounds' surface area and their bioavailability ($R^2 = 0.8078$) (Figure 2A). Inversely, the intestinal absorption was correlated with LogP (hydro/lipophilicity). Lipophilic compounds (lower LogP), including flavones and flavonols, exhibited lower absorption than hydrophilic ones (phenolic acids). Hence, the non-linear regression positively correlated ($R^2 = 0.7669$) LogP with intestinal absorption (Figure 2B). Caffeine was excluded from this last regression since, although exhibiting a high lipophilicity, its intestinal absorption was high. Then, we can consider that phenolic compounds and caffeine behavior during gastrointestinal digestion and absorption are different.

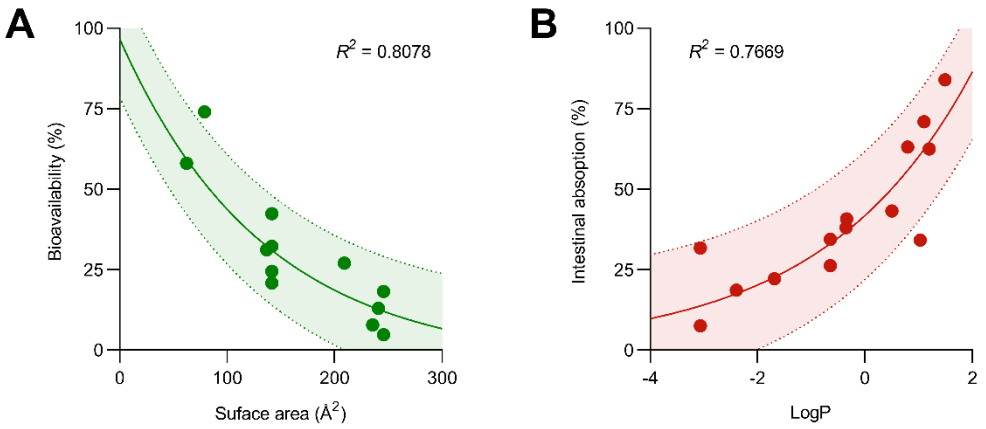

Figure 2. Associations between phytochemicals (phenolic compounds and caffeine) surface area and their bioavailability (**A**), and between phenolic compounds LogP (partition coefficient) and their intestinal absorption (**B**).

The absorption of phenolic compounds in the gastrointestinal tract is not only associated with their degradation during the digestive process. The structure and polarity of phenolic compounds play a significant role, as smaller and polar phenolic compounds are able to cross the intestinal membrane more easily [22]. Contrariwise, although caffeine is a small and apolar molecule, it is stable and highly absorbed in the intestinal phase almost entirely [23]. Consequently, the phytochemicals bioavailability from the CP depends not only on the food matrix but also on the structural properties of the different compounds. Then, the biological activity of the CP will also be influenced by digestion and absorption processes and will be mainly associated with the absorbable and bioavailable fraction. To date, the CP has been recognized as a safe and sustainable ingredient; following acute (2 g kg^{-1} day^{-1}, 1 day) and sub-chronic (1 g kg^{-1} day^{-1}, 90 days) administration in mice, no signs of toxicity were observed [24]. Nevertheless, additional animal and human studies are needed to confirm the bioaccessibility, bioavailability, and beneficial properties described in vitro and demonstrate the gastrointestinal absorption and metabolism of the phenolic compounds and caffeine from the CP.

4. Conclusions

These results provide new knowledge into the gastrointestinal behavior of antioxidant phenolic compounds and caffeine from the CP. The bioaccessibility of phenolic compounds is structure-dependent. Small compounds, hydroxybenzoic acids, are less susceptible to gastrointestinal degradation than larger ones, such as flavonoids. Likewise, caffeine is more absorbed than phenolics in the intestine due to its chemical structure and lipophilicity. This report supports the use of the CP as a new antioxidant food ingredient containing highly bioaccessible and absorbable phytochemicals.

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