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Bioaccessibility Performance of Phenolic Compounds from Red Fruits During Simulated Gastrointestinal Digestion and Colonic Fermentation

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ABSTRACT: Red fruits are rich in phenolic compounds, particularly anthocyanins, which contribute to their vibrant colors and health benefits. However, anthocyanins are chemically sensitive during digestion, which affects their bioaccessibility. This study evaluated the impact of simulated gastrointestinal digestion on whole red fruits (oral, gastric, intestinal, and colonic phases). Total phenolic content (TPC) ranged from 8.60−50.41 mg GAE/g DW, total flavonoid content (TFC) from 1.66−10.67 mg CAT/g DW, and total monomeric anthocyanins (TMA) from 0.54−1.28 mg CYA/g DW. Antioxidant activity strongly correlated with TPC ($r \geq 0.91$). High-phenolic fruits showed lower bioaccessibility compared with low-phenolic ones. HPLC-DAD-ESI/MS^n identified glycosylated anthocyanins, which were highly sensitive; jaboticaba anthocyanins were nonbioaccessible, while 51.83% remained bioaccessible in raspberries. Colonic fermentation further reduced TPC and TFC, and anthocyanins were entirely degraded. These results highlight the need for innovative delivery systems to improve anthocyanin bioaccessibility and functional benefits.

KEYWORDS: *anthocyanins, phenolic compounds, antioxidant activity, red fruits, bioaccessibility, colonic fermentation*

1. INTRODUCTION

Red fruits, also called berries, make up a diverse group of small fruits characterized by their reddish hue, which is often due to the presence of natural pigments. These fruits may present significant contents of phenolic compounds, including a broad range of phenolic acids, such as gallic, vanillic, chlorogenic, ferulic, ellagic, caffeic, and *p*-coumaric acids, and flavonoids in the form of quercetins, myricetins, kaempferols, and anthocyanins.[1] The vibrant hues of red fruits are mainly related to the accumulation of anthocyanins, which work as a defensive mechanism and natural biomarkers during fruit ripening.[2] Anthocyanins are flavonoid compounds responsible for the intense reddish, orangish, bluish, and purplish hues. The main anthocyanins found in nature are cyanidins, delphinidins, pelargonidins, peonidins, petunidins, and malvidins, which can be in aglycone forms or glycosylated.[1] The diverse phenolic profile of red fruits has been strongly related to biological properties, including potential anti-inflammatory,[3] antidiabetic,[4] antiobesity,[5] and anticancer[6−8] properties.

Although they are important biological properties, phenolic compounds are susceptible to modification during human digestion because of changes in pH and the action of enzymes and biliary salts, resulting in the modification of such compounds into different molecules with different bioactive properties, thereby modifying their bioaccessibility.[9,10] Furthermore, after the digestive process in the small intestine, bioactive compounds pass to the large intestine, where the gut microbiota metabolizes the remaining compounds.[11,12]

Most studies have investigated the effects of simulated gastrointestinal digestion on red fruit.[13−15] However, these experiments are limited to the gastric and intestinal phases. This study aimed to address these gaps by evaluating the effects of simulated gastrointestinal digestion across all phases—oral, gastric, intestinal, and colonic fermentation—using whole red fruits, providing a more realistic representation of how digestion impacts fruits consumed in their natural state.

2. MATERIAL AND METHODS

2.1. Fruit Materials. *Euterpe oleracea* Mart. (acai) frozen pulp, *Vaccinium myrtillus* L. (blueberry), *Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier (strawberry), *Rubus idaeus* L. (raspberry), and *Rubus fruticosus* G. N. Jones (blackberry) frozen fruits were purchased from a local commercial store of Curitiba, Paraná, Brazil. In addition, *Plinia cauliflora* (Mart.) Kausel (jaboticaba fruits) were collected in Colonia Dona Luiza (Lapa, Paraná, Brazil). All fruits were mixed to obtain a smoother puree, lyophilized for better storage, and kept at −18 ± 1 °C.

2.2. *In Vitro* Gastrointestinal Digestion. The *in vitro* digestion assays were carried out applying the methodology described by Gawlik-Dziki et al.[16] in whole lyophilized fruits. Briefly, 5 g of each lyophilized red fruit was used. Simulated digestion was performed in 4 steps: oral, gastric, small

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Table 1. Bioaccessibility of Bioactive Compounds and Antioxidant Activity of Red Fruits[a,b]

Sample	Treatment	TPC[c] (Bioaccessibility)[d]	TFC[e] (Bioaccessibility)[d]	TMA[f] (Bioaccessibility)[d]	ABTS[g]	DPPH[g]
Acai	Undigested	22.28^{a} ± 1.62 (100)	10.67^{a} ± 0.01 (100)	1.28^{a} ± 0.03 (100)	179.44^{a} ± 4.83	30.10^{a} ± 0.05
	Oral digestion	18.09^{b} ± 1.56 (81.19)	5.89^{b} ± 0.18 (55.20)	0.42^{b} ± 0.05 (32.81)	160.54^{b} ± 2.42	24.10^{b} ± 0.07
	Gastric digestion	10.53^{c} ± 0.85 (47.26)	4.34^{c} ± 0.27 (40.67)	0.08^{c} ± 0.02 (6.25)	116.88^{c} ± 6.25	22.50^{c} ± 0.02
	Intestinal digestion	6.78^{d} ± 0.59 (30.43)	4.46^{c} ± 0.13 (41.80)	0.05^{c} ± 0.02 (3.91)	76.55^{d} ± 5.65	20.37^{d} ± 0.19
	Colonic fermentation	6.45^{d} ± 0.19	2.58^{d} ± 0.17	-	64.72^{e} ± 2.11	24.05^{b} ± 0.27
Blackberry	Undigested	11.60^{a} ± 1.05 (100)	3.24^{ab} ± 0.01 (100)	1.27^{a} ± 0.04 (100)	102.82^{b} ± 2.88	32.84^{a} ± 0.07
	Oral digestion	6.30^{c} ± 0.06 (54.31)	1.92^{c} ± 0.08 (59.25)	0.85^{c} ± 0.02 (66.92)	122.61^{a} ± 2.56	26.51^{b} ± 0.31
	Gastric digestion	10.01^{b} ± 0.80 (86.29)	3.01^{ab} ± 0.36 (92.90)	1.13^{b} ± 0.03 (88.98)	74.75^{d} ± 0.51	32.58^{a} ± 0.03
	Intestinal digestion	10.94^{ab} ± 0.74 (94.31)	3.39^{a} ± 0.21 (104.63)	1.22^{a} ± 0.05 (96.06)	84.98^{c} ± 2.18	25.46^{c} ± 1.30
	Colonic fermentation	3.32^{d} ± 0.30	3.21^{ab} ± 0.02	-	42.02^{e} ± 1.39	8.75^{d} ± 0.23
Blueberry	Undigested	10.13^{a} ± 0.45 (100)	2.77^{a} ± 0.19 (100)	1.25^{a} ± 0.06 (100)	88.71^{a} ± 5.83	29.85^{a} ± 0.04
	Oral digestion	6.00^{b} ± 0.62 (59.23)	2.56^{b} ± 0.17 (92.42)	0.91^{b} ± 0.02 (72.8)	70.61^{c} ± 2.09	20.81^{d} ± 0.56
	Gastric digestion	5.90^{b} ± 0.93 (58.24)	2.51^{b} ± 0.01 (90.61)	0.85^{c} ± 0.01 (68)	75.89^{b} ± 0.40	25.49^{b} ± 0.54
	Intestinal digestion	4.56^{c} ± 0.20 (45.01)	1.80^{c} ± 0.04 (64.98)	0.58^{d} ± 0.02 (46.4)	41.37^{d} ± 3.95	22.93^{c} ± 0.03
	Colonic fermentation	2.25^{d} ± 0.52	1.29^{d} ± 0.07	-	18.08^{e} ± 2.39	29.20^{a} ± 0.01
Jaboticaba	Undigested	50.41^{a} ± 9.93 (100)	6.97^{a} ± 0.23 (100)	0.69^{a} ± 0.02 (100)	642.72^{a} ± 21.96	168.86^{b} ± 0.13
	Oral digestion	43.14^{ab} ± 3.08 (85.57)	6.60^{b} ± 0.42 (94.69)	0.68^{b} ± 0.01 (98.55)	631.05^{a} ± 24.51	140.26^{c} ± 0.33
	Gastric digestion	36.76^{ab} ± 0.39 (72.92)	5.48^{c} ± 0.18 (78.62)	0.49^{a} ± 0.02 (71.01)	408.94^{b} ± 13.93	170.48^{a} ± 0.68
	Intestinal digestion	36.01^{ab} ± 3.09 (71.43)	5.32^{c} ± 0.20 (76.32)	0.42^{b} ± 0.02 (60.87)	232.87^{c} ± 75.44	167.96^{b} ± 0.46
	Colonic fermentation	13.28^{c} ± 1.48	2.09^{d} ± 0.17	-	128.72^{d} ± 0.81	26.92^{d} ± 0.07
Raspberry	Undigested	8.60^{a} ± 0.71 (100)	1.66^{b} ± 0.12 (100)	0.92^{a} ± 0.08 (100)	78.90^{b} ± 0.19	33.44^{a} ± 0.01
	Oral digestion	7.55^{b} ± 0.20 (87.79)	1.61^{b} ± 0.10 (96.99)	0.86^{a} ± 0.06 (93.47)	74.20^{c} ± 3.11 (94.04)	26.87^{d} ± 0.71 (79.40)
	Gastric digestion	7.50^{b} ± 0.72 (87.21)	2.06^{a} ± 0.04 (124.1)	0.85^{a} ± 0.02 (92.39)	89.40^{a} ± 6.02	32.60^{b} ± 0.50
	Intestinal digestion	7.69^{b} ± 0.34 (89.41)	2.09^{a} ± 0.07 (125.9)	0.79^{a} ± 0.03 (85.87)	58.83^{d} ± 0.86	32.98^{ab} ± 0.17
	Colonic fermentation	4.95^{c} ± 0.35	-	-	43.73^{e} ± 2.88	28.64^{c} ± 0.73
Strawberry	Undigested	15.26^{a} ± 1.37 (100)	3.43^{a} ± 0.28 (100)	0.54^{a} ± 0.06 (100)	123.45^{b} ± 2.21	33.71^{a} ± 0.05
	Oral digestion	12.81^{b} ± 0.04 (83.94)	2.62^{b} ± 0.35 (76.38)	0.53^{a} ± 0.05 (98.14)	129.92^{a} ± 3.46	27.45^{e} ± 0.26
	Gastric digestion	13.75^{ab} ± 0.80 (90.10)	1.33^{d} ± 0.23 (38.77)	0.57^{a} ± 0.04 (105.55)	134.33^{a} ± 3.74	33.22^{b} ± 0.02
	Intestinal digestion	10.44^{c} ± 0.83 (68.41)	2.28^{c} ± 0.17 (66.47)	0.43^{b} ± 0.02 (79.63)	86.56^{c} ± 7.07	32.72^{c} ± 0.31
	Colonic fermentation	2.18^{d} ± 0.54	-	-	24.68^{d} ± 3.14	30.15^{d} ± 0.06

[a]Note: -: not determined; TPC: total phenolic compounds; TFC: total flavonoid compounds; TMA: total monomeric anthocyanins. [b]a,b,c: Different letters in the same columns mean significant differences ($p < 0.05$) among digestive phases of the same fruit. [c]mg GAE/g DW: GAE is gallic acid equivalent and DW is dry weight. [d]%. [e]mg CAT/g DW: CAT is catechin equivalent. [f]mg CYA/g DW: CYA is cyanidin 3-glucoside equivalent. [g]μmol TE/g DW: TE is trolox equivalent.

intestinal digestions, and colonic fermentation (large intestine digestion). Each fruit sample was digested in 4 separate flasks; thus, at the end of each digestion step, one flask was removed for analysis. The digestion was immediately stopped in the removed flasks by cooling, followed by filtration, freezing, lyophilization, and storage until further analysis, while the others went on to the following digestion steps.

Oral digestion was simulated using a salivary fluid composed of 200 U/L α-amylase, 2.38 g/L $NaHPO_4$, 0.19 g/L KH_2PO_4, and 8 g/L NaCl. The pH of the salivary solution was adjusted to 6.75. Red fruit samples (5 g) were added to 10 mL of salivary solution and kept for 2 min at 120 rpm, 37 °C.

Fluid gastric was prepared using 0.03 mol/L NaCl to dilute 3.2 g/L pepsin. The pH 2 was achieved using concentrated HCl. Samples from oral digestion were added to 15 mL of gastric fluid and incubated at 150 rpm, 37 °C, for 2 h. Intestinal fluid was prepared using 1.42 g/L pancreatin and 8.57 g/L biliary salts prepared with 0.1 mol/L $NaCO_3$. The solution was adjusted to pH 6.0 by using 1 mol/L $NaHCO_3$. The remaining part of the gastric phase was added to 15 mL of intestinal fluid. The reaction occurred in a 2 h, 37 °C, 150 rpm shaker.

2.3. *In Vitro* Colonic Fermentation. Colonic fermentation was performed according to the test reported by Gonçalves et al.[12] with modifications proposed by Correa et al.[11] The basal culture medium was prepared using peptone water (2 g/L), yeast extract (2 g/L), NaCl (0.1 g/L), K_2HPO_4 (0.04 g/L), $MgSO_4{\cdot}7H_2O$ (0.01 g/L), $CaCl_2{\cdot}2H_2O$ (0.01 g/L), NH_2CO_3 (2 g/L), cysteine HCl (0.5 g/L), bile salts (0.5 g/L), 2 mL of Tween 80 (80 g/L), and 0.2 g of hematin diluted in 5 mL of NaOH. Sterile tubes (15 mL) containing 9 mL of basal culture medium were used, and the headspace was maintained in $H_2-CO_2-N_2$ (10:10:80) for 24 h. Fresh feces of two healthy women were collected in sterile flasks for laboratory analysis containing an anaerobic generator. The fecal suspension was prepared through 2 g of fresh feces mixed

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with 18 mL of sodium phosphate buffer (0.1 M, pH 7.0). Fecal suspension (1 mL) was added to tubes containing the basal culture medium and 100 mL of the nonpermeabilized extract fraction from the previous digestion step. The samples were vortexed and kept at 37 °C for 24 h. Samples were filtered using filter paper, and the fermentation was stopped by fast freezing (−85 °C), followed by lyophilization. After the digestion process, the whole fruit samples and the digested fruit samples were extracted using a methanol:water solution (1:1 *v*/*v*) at 10 mg/mL.[12]

2.4. Bioactive Compounds Through Colorimetric Analysis. Total phenolic compounds (TPC) were determined by the Folin-Ciocalteau method, according to Singleton and Rossi.[17] Total flavonoid compounds (TFC) were determined by Zishen et al.[18] Total monomeric anthocyanins (TMA) were determined according to the method described by Giusti and Wrolstad.[19] These methods were used as parameters to determine bioaccessibility.

Antioxidant activity was determined by DPPH radical scavenging[20] and the formation of the $ABTS^{\bullet+}$[21] assays.

2.5. Bioaccessibility of Bioactive Compounds. Bioaccessibility was determined according to the initial and final concentrations of bioactive parameters, as per eq 1.[22]

$$B = \left(\frac{C}{C_0}\right) \times 100 \quad (1)$$

where B is the bioaccessibility, C is the final concentration, and C_0 is the initial concentration.

2.6. Anthocyanins Analysis by HPLC-DAD-ESI/MS^n. Samples were submitted to the cleaning step. A solution of methanol:acetonitrile was prepared in a proportion of 1:1 (*v*/*v*), and 1 mL was transferred to a test tube containing 1 mL of sample extract. Thus, the liquids were mixed and centrifuged for 10 min at 5000 rpm. Supernatants were collected using a 1 mL micropipette, filtered in a 0.22 μm filter, and put in vials for liquid chromatographic analysis.[9]

For anthocyanin analysis, the extracts were dissolved in water to a final concentration of 5 mg/mL, filtered (0.2 μm), and injected into HPLC equipment (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) coupled to a diode-array detector (280, 330, 370, and 520 nm wavelengths) and an electrospray ionization mass spectrometer (Linear Ion Trap LTQ XL, Thermo Scientific) working in positive mode, as previously described by Guimarães et al.[23] For compound separation, an AQUA reverse-phase C18 column (5 μm, 150 mm × 4.6 mm, Phenomenex) was used at 35°C, using previously described gradients. The anthocyanin determination was performed according to their retention time and UV−vis and mass spectra, in comparison with authentic standards and using literature data. The quantification was achieved using a seven-level calibration curve obtained for different standard compounds. The results were expressed in mg per gram of fruit and used as parameters to determine bioaccessibility.

2.7. Statistical Analysis. Results were determined in triplicate and expressed as the average ± standard deviation. The Shapiro-Wilk test was applied to verify the distribution of data. Thus, ANOVA followed by the Tukey test ($p < 0.05$) was used to estimate the data set variance. Statistical analyses were performed in Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

3.1. Phenolic Compounds and Antioxidant Activity of Red Fruits. Bioactive compounds are compounds that have important biological activities. For example, phenolic compounds, the main bioactives produced as secondary metabolites from plants, are divided into flavonoid and nonflavonoid compounds. They have the ability to stabilize free radicals, inferring antioxidant activity. Thus, some researchers have related the regular consumption of phenolic-rich foods to the reduction of the risks of metabolic diseases,[24] leukemia,[8] and other types of cancer.[25]

The concentration of total phenolic compounds (TPCs) ranged from 8.60 (raspberry) to 50.41 mg GAE/g DW (jaboticaba) (Table 1). The TPC determined in red fruits (average of 19.71 mg GAE/g DW) was superior to the results reported by Stafussa et al.[13] (up to 13.89 mg GAE/g DW) in frozen fruit purees.

Most phenolic compounds are grouped into phenolic acids and flavonoid classes. Total flavonoids were determined in red fruits. Raspberry had the lowest concentration (1.66 mg of CAT/g of DW), whereas acai showed the highest concentration (10.67 mg of CAT/g of DW). Flavonoids are divided into flavanols, flavonols, flavones, flavanones, and anthocyanins. Anthocyanins are natural red-purple pigments present in red fruits. Total monomeric anthocyanins ranged from 0.54 mg (strawberry) to 1.28 mg of CYA/g of DW (acai).

Antioxidant activity was assessed using ABTS and DPPH assays, both of which are colorimetric methods based on the radical-scavenging mechanism. Among the red fruits analyzed, antioxidant activity ranged from 78.9 to 642.72 μmol of TE/g of DW for ABTS and 30.1 to 186.86 μmol of TE/g of DW for DPPH. Notably, jaboticaba exhibited the highest antioxidant activity, whereas raspberry and strawberry showed the lowest values across both assays. The differences observed among the red fruits highlight the variability in phenolic composition, which can be influenced by factors such as fruit maturity, environmental conditions, and genetic characteristics.

A strong positive correlation was observed between antioxidant activity and total phenolic content (TPC), as indicated by Pearson's correlation coefficients ($r_{\text{TPC x ABTS}} = 0.9466$; $r_{\text{TPC x DPPH}} = 0.9148$). Thus, the high antioxidant activity of jaboticaba may be attributed to its high phenolic content, since these compounds are well-known for their potent radical-scavenging abilities. The strong correlation between TPC and antioxidant activity supports the hypothesis that phenolics are key contributors to the antioxidant capacity measured by these assays.

3.1.1. Bioaccessibility of Phenolic Compounds. Bioaccessibility was expressed as the percentage of the remaining concentration after each step of simulated digestion, available for absorption in the small intestine.[22] Because of the literature definition, colonic fermentation was not considered for bioaccessibility determination.

Different from previous studies in which phenolic extracts were added to digestive fluids, in this study, red fruits were added as a lyophilized powder directly to gastrointestinal fluids in this experiment. A simulated digestion process assay was initiated by adding *in vitro* salivary fluid containing α-amylase at pH 6.75. Although raspberry had the lowest initial concentrations of TPC and TFC, this fruit was less affected by digestion in the oral phase, showing the highest remaining percentage for these colorimetric parameters. In contrast, acai,

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the second most phenolic-rich red fruit, had the lowest remaining percentages of TFC and TMA after oral digestion. These variations in the oral phase can be related to the presence of dietary fiber from fruits.[26,27] The losses of bioactive compounds can be associated with the interaction between polyphenols and α-amylase enzymes present in the simulated salivary fluid.[28] Furthermore, the interaction of phenolic compounds, especially anthocyanins abundantly found in red fruits, with α-amylase can result in inhibitory activity, resulting in an antidiabetic activity.[29]

Following the simulated oral phase digestion, the next step consists of simulated gastric digestion, which occurs in a strongly acidic environment (pH $\approx$ 2) due to hydrochloric acid in the gastric fluid. The reactions also occur because of the catalytic effects of pepsin, which is the primary enzyme present in the stomach. After the simulated gastric digestion step, acai had the lowest remaining concentrations for all phenolic assays (TPC, TFC, and TMA). Considering all red fruits evaluated, raspberry and strawberry showed the first and second lowest concentrations of phenolic compounds, respectively. Surprisingly, raspberry was highlighted in the remaining concentration of TFC, whereas strawberry had the highest results for TPC and TMA parameters after the gastric phase, meaning that the raspberry and strawberry phenolic profiles had slight modifications during gastric digestion. The remaining concentrations above 100% found in some parameters may be related to the possible extraction of phenolic compounds from fruit matrices once the whole fruit, lyophilized and powdered, was used in this experiment. The observed effects may be related to the way phenolic compounds are bound to other components of the matrix. Some classes of phenolic compounds, such as anthocyanins and procyanidins, are found in cell vacuoles, while other classes, such as phenolic acids, are predominantly covalently bound to polysaccharides in plant cell walls.[26,27] Therefore, the release of such compounds during simulated digestion may be related to the way in which such compounds interact with cellular components of the matrix.[30] Also, the low pH of the medium may have contributed to the phenolic compounds' extraction in the gastric phase, as observed by Ferarsa et al.[31] in the extraction of bioactive compounds from purple eggplant peel.

After gastric digestion, intestinal digestion was carried out simulating the small intestine portion, which has a predominantly neutral pH environment (pH $\approx$ 6.8) and the presence of biliary salts and enzymes such as lipases. The concentration of phenolic compounds quantified in the intestinal phase was used to calculate bioaccessibility.

The concentration of phenolic compounds in the intestinal phase maintained the trend already observed in the oral and gastric phases. Acai, the second fruit richest in phenolic compounds, presented the lowest bioaccessibility, ranging from 30.43% for the content of total phenolic compounds (TPC) to 41.8% for the content of total flavonoids (TFC), in addition to exhibiting the lowest bioaccessibility for total anthocyanins (TMA), with a value of 3.91%. In contrast, blackberries were the red fruit that presented the highest bioaccessibility for TPC (94.31%) and TMA (96.6%), while raspberries stood out with the highest bioaccessibility for TFC (125.9%). Bioaccessibility levels above 100% for TFC can be attributed to chemical modifications caused by gastrointestinal digestion, in which polymeric phenolic compounds can be broken down into monomeric forms, increasing the concentration of available TFC.[32] The differences observed between the initial concentration of phenolic compounds and their bioaccessibility may be associated with the different chemical structures of these compounds.[9] It is possible that raspberries and blackberries contain polymeric compounds or classes that are more resistant to digestion compared to acai. This characteristic contributes to maintaining the integrity of the compounds throughout the digestive process, resulting in higher bioaccessibility.

3.1.2. Effect of In Vitro Digestion on Antioxidant Activity. Phenolic compounds are mainly responsible for antioxidant activity.[33] As explored in Section 3.1, correlation matrices analyzed through Pearson's method resulted in a strong positive correlation ($r_{\text{TPC x ANTIOXIDANT ACTIVITY}} \geq 0.9148$), emphasizing the role of phenolic compounds in this bioactive property. Thus, considering that gastrointestinal digestion affected the phenolic composition of red fruits, antioxidant activity was consequently altered. For this reason, this study investigated the effects of simulated gastrointestinal digestion on the antioxidant activity of red fruits.

The changes in the antioxidant activity of red fruits after simulated gastrointestinal digestion did not follow the same trend observed in their phenolic composition. For instance, after the digestive process, strawberries retained only 19.99% of their initial antioxidant activity according to the ABTS method, while the highest remaining percentage for this method was found in raspberries, at 55.42%. Using the DPPH method, blackberries exhibited the highest remaining activity at 97.82%, whereas jaboticaba showed the lowest activity with only 15.94%.

The variations observed between the two methods used to evaluate the antioxidant activity of fruits can be explained by several factors, such as the specific mechanisms of the assays employed, the diverse phenolic composition of red fruits, and the chemical transformations that these compounds undergo during gastrointestinal digestion. Although both methods are based on the mechanism of radical scavenging, they exhibit significant differences. For instance, the ABTS method encompasses a broader spectrum of phenolic classes, as it operates in both hydrophilic and lipophilic media. In contrast, the DPPH method is more limited, predominantly functioning in hydrophobic media, which often results in lower antioxidant activity measurements.[33] Additionally, antioxidant activity is directly influenced by the chemical structure of the compounds, which determines how they interact with the DPPH and ABTS reagents.[34,35] These characteristics highlight the importance of combining different methods for a more comprehensive evaluation of the antioxidant activity.

3.1.3. Effect of Colonic Fermentation on Phenolic Compounds. Part of the bioaccessible fraction is absorbed in the small intestine, while the nonabsorbed fraction moves to the large intestine, where it is anaerobically fermented by the microbiota present in this environment. In this study, only the bioaccessibility of phenolic compounds from red fruits was evaluated, without including tests related to the intestinal barrier. However, the effects of microbial fermentation, simulating large intestine conditions, were analyzed in the bioaccessible fraction to identify possible modifications in the remaining compounds.

Gut microbiota is composed of several types of microorganisms (bacteria, archaea, bacteriophages, eukaryotic viruses, and fungi) that may vary among human hosts because of many factors such as genetics, feeding, mode of birth, habits, and lifestyle.[36] Colonic fermentation was simulated using a

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Table 2. Anthocyanins Profile of Before and After *In Vitro* Digestion Model as Determined by HPLC-DAD-ESI/MS[n][a,b]

	Before Digestion	Digestion phases			
Anthocyanins	Whole fruit	Oral	Gastric	Intestinal	Colonic Fermentation
Açai					
Cyanidin-*O*-hexoside	3.75[a] ± 0.06	1.74[b] ± 0.03	1.69[b] ± 0.03	1.52[c] ± 0.01	0[d] ± 0
B (%)	100	46.4	45.07	40.53	
Total	3.75	1.74	1.69	1.52	
B (%)	100	46.4	45.07	40.53	
Blackberry					
Cyanidin-*O*-hexoside	5.77[a] ± 0.01	4.7[b] ± 0.01	3.22[c] ± 0.01	2.6[d] ± 0.01	0[e] ± 0
B (%)	100	81.45	55.81	45.06	
Peonidin-3-*O*-glucoside	3.05[a] ± 0.01	1.48[b] ± 0.01	0.96[c] ± 0.01	0[d] ±0	0[d] ±0
B (%)	100	48.52	31.47	0	
Total	8.82	6.18	4.18	2.6	
B (%)	100	70.07	47.39	29.48	
Blueberry					
Peonidin-3-*O*-galactoside	1.36[a] ± 0.04	0.5[b] ± 0.01	0[c] ± 0	0[c] ± 0	0[c] ± 0
B (%)	100	36.76	0	0	
Cyanidin-*O*-hexoside	3.95[a] ± 0.03	1.83[b] ± 0.04	0.72[c] ± 0.02	0.0[c] ± 0.01	0[d] ± 0
B (%)	100	46.33	18.23	17.72	
Peonidin-3-*O*-rutinoside	2.91[a] ± 0.01	2.41[b] ± 0.05	2.16 [c] ± 0.07	1.74[d] ± 0.03	0[e] ± 0
B (%)	100	82.82	74.23	59.79	
Peonidin 3-*O*-glucoside	6.5[a] ± 0.07	1.42[b] ± 0.04	1.09[c] ± 0.01	0.71[d] ± 0.01	0[e] ± 0
B (%)	100	21.85	16.77	10.92	
Total	14.72	6.16	3.97	3.15	
B (%)	100	41.84	26.97	21.40	
Jaboticaba					
Delphinidin-3-*O*-glucoside	0.83[a] ± 0.01	0.41[b] ± 0.01	0.28[c] ± 0.01	0[d] ± 0	0[d] ± 0
B (%)	100	49.4	33.73	0	
Cyanidin-3-*O*-glucoside	2.16[a] ± 0.11	1.35[b] ± 0.05	1.28[b] ± 0.04	0[c] ± 0	0[c] ± 0
B (%)	100	62.5	59.26	0	
Total	2.99	1.76	1.56	0	
B (%)	100	58.86	52.17	0	
Raspberry					
Peonidin-3-*O*-galactoside	2.93[a] ± 0.01	2.14[b] ± 0.06	1.95[c] ± 0.06	1.03[d] ± 0	0[e] ± 0
B (%)	100	73.04%	66.55%	35.15%	
Peonidin-*O*-deoxyhexoside-hexoside	3.47[a] ± 0.08	3.41[a] ± 0.06	2.81[b] ± 0.02	1.85[c] ± 0.03	0[d] ± 0
B (%)	100	98.27	80.98%	53.31%	
Cyanidin-*O*-hexoside	1.24[a] ± 0.06	1.21[a] ± 0.02	1.11[b] ± 0.04	1.08[b] ± 0.02	0[c] ± 0
B (%)	100	97.58	89.52	87.10	
Total	7.64	6.76	5.87	3.96	
B (%)	100	88.48	76.83	51.83	
Strawberry					
Pelargonidin-*O*-3,5-diglucoside	0.2[b] ± 0.01	0.33[a] ± 0.01	0[c] ± 0	0[c] ± 0	0[c] ± 0
B (%)	100	165	0	0	
Pelargonidin-3-*O*-glucoside	1.49[a] ± 0.02	0.87[b] ± 0.03	0.76[c] ± 0.02	0.56[d] ± 0.03	0[e] ± 0
B (%)	100	58.39	56.01	37.58	
Total	1.51	0.93	0.76	0.56	
B (%)	100	61.59	50.33	37.09	

[a]Note: Results are expressed in mg/g DW. The total was obtained from the sum of the individual anthocyanins' concentration averages. B (%): Recovery index of anthocyanin in the following phase vs. the initial (before digestion). [b]abc: Different letters in the same line mean significant differences between treatments. Results are expressed as mean ± SD of three different experiments at $p < 0.05$.

fecal solution containing essential nutrients for the microbiota to grow and fresh feces obtained from 2 healthy donors. A control using water instead of fruit samples was carried out to reduce interferences from the feces components.

In general, significant reductions in the concentrations of phenolic compounds were observed after colonic fermentation of the bioaccessible fraction of berries. However, colonic fermentation affected the phenolic profile of the berries differently. For example, after colonic fermentation, 57.55% of the TPC of raspberries was quantified. However, TFC and TMA were not identified after colonic fermentation in this fruit. TFC was also not identified in strawberries after colonic fermentation, indicating that the bioactives belonging to this class of phenolic compounds present in these fruits are more sensitive to the intestinal microbiota. Unfortunately, TMA was not identified after colonic fermentation of the six berries analyzed. These findings corroborate the study by Brown et al.,[37] who observed the total disappearance of anthocyanins

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from strawberry, blackberry, and blackcurrant after colonic fermentation.

These results were explored by HPLC-DAD-ESI/MS^n in order to determine the effect of simulated gastrointestinal digestion and colonic fermentation on the individual anthocyanins.

3.2. Identification of Anthocyanins Through HPLC-DAD-ESI/MS^n. Anthocyanins are phenolic compounds belonging to the flavonoids group, responsible for the intense color of red fruits,[38] composed of a C6−C3−C6 chain resulting from a C ring (3-carbon ring) in the middle of A and B rings (6-carbon rings),[39] as shown in Figure S1. Glycosylated forms of cyanidins, delphinidins, peonidins, and pelargonidins were found in the studied red fruits through high-performance liquid chromatography coupled to diode array and mass spectrometry detectors (HPLC-DA-ESI/MS^n) (Figure S1 and Table S1).

The attempt to identify anthocyanins by HPLC-DAD-ESI/MS^n analysis in red fruits was based on retention times (rt), maximum absorption wavelengths of the UV−vis region (max), pseudomolecular ion ($[M]^+$), and molecular ion fragmentation (MS^2, with the identification performed by comparison with available standards and/or literature data. For all samples, it was possible to identify ten classes of anthocyanin compounds. Figure S1 presents the possible chemical structure of the anthocyanin compounds detected, and their respective substituents are presented in Table S1. In the acai pulp, one anthocyanin was identified; in blackberry, jaboticaba, and strawberry, two anthocyanins were identified; raspberry and blueberry were identified with three and four anthocyanins, respectively (Table S1). **Peak 9** (cyanidin-3-*O*-glucoside) was positively identified in comparison with the chromatographic and MS characteristics of the commercial standard. **Peaks 1, 2, 5,** and **12** ($[M]^+$ at m/z 449) presented the same pseudomolecular ion as **peak 9**, with a loss of one hexose (−162 u). In this case, it was not possible to identify the position and nature of the hexoside portion because the peak retention times do not correspond to any of the available standards. Then, these compounds were positively quantified in comparison with the chromatographic and MS characteristics of the commercial standard (Table S1). **Peaks 4** and **10** ($[M]^+$ at m/z 463) presented the same pseudomolecular ion as **peaks 3** and **7**, with a loss of one hexoside (−162 u), with the tentative identification of the linked sugar type as a galactoside due to its retention time and the elution order. **Peak 6** of blueberry and **peak 11** of raspberry were assigned as peonidin-3-*O*-rutinoside and peonidin-*O*-deoxyhexoside-hexoside, respectively, based on their mass spectra, which showed an MS^2 signal at m/z 301 (peonidin; $[M\text{-}308]^+$, loss of a deoxyhexoside-hexosyl moiety), while peak 6 was compared to a commercial standard, no possible direct identification was obtained for peak 11. **Peak 8** ($[M]^+$ at m/z 465) showed mass spectra with an MS^2 signal at m/z 303 (delphinidin; $[M\text{-}162]^+$, loss of a hexosyl moiety) and was identified as delphinidin-*O*-glucoside. Finally, **peak 14** (pelargonidin-3-*O*-glucoside) was positively identified in comparison with the chromatographic and MS characteristics of the commercial standard, and **peak 13** ($[M]^+$ at m/z 595) presented two MS^2 fragments, revealing two losses of glucose units (m/z at 303; −162 u and −162 u); therefore, it was identified as pelargonidin-3,5-*O*-diglucoside. These anthocyanins were exclusively found in strawberries, including pelargonidin-3-*O*-glucoside and pelargonidin-3,5-*O*-diglucoside. Delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside were identified only in jaboticaba fruit.

The presence and variety of these anthocyanins are related to several health-promoting properties. Therefore, the differences in the anthocyanin groups present in each fruit may directly impact the stability of such compounds. These anthocyanins, especially the former, are very characteristic of being present in high quantities in these types of fruits. The highest anthocyanin content was found in blueberry pulp.

3.2.1. Bioaccessibility of Individual Anthocyanins. The monitoring of anthocyanins was carried out during a simulated digestion process (Table 2). The anthocyanin compound clearly identified to be present at the highest concentration in the gastric phase for blackberry was cyanidin (cyanidin-*O*-hexoside; m/z 449), which was found at concentrations several-fold higher than any other anthocyanin, accounting for 77% of the total anthocyanin content. For raspberry, the concentration of most anthocyanin species was found to be highest in the gastric phase, which contained 76.86% of the total anthocyanins found throughout the digestion model. The blueberry contrasted greatly with the other fruits in terms of the distribution of anthocyanin type in the digestion model, as only 26.97% of the total anthocyanins were present in the gastric phase. The remaining anthocyanins were present primarily in the intestinal phase, which comprised 21.4% of the total anthocyanins. For all fruits, a biphasic pattern in anthocyanin concentrations was observed in terms of no anthocyanin concentrations after colonic fermentation, which has been previously seen in TMA analysis (Table 1). Anthocyanins are likely to be unstable in the intestinal phase due to their chemical decomposition at neutral pH, which is known to occur before subsequent exposure to colonic microbial metabolism. Degradation of anthocyanins that has ranged from 40 to 90% has been reported in pancreatic *in vitro* digestion studies.[40] Significant anthocyanin losses after intestinal digestion have been observed previously with simulated digestion model studies.[41] Interestingly, despite the overall several-fold higher total anthocyanin concentrations in the digestion model for blueberry, a 2-fold higher anthocyanin content in the raspberry intestinal phase was observed compared to intestinal phase anthocyanin concentrations for blueberry. The findings presented above signify major differences in anthocyanin breakdown and release among the various digestive compartments among the six fruits. In contrast, no anthocyanin content in the jaboticaba intestinal phase was observed.

The divergences in the bioaccessibility of total anthocyanins in the 6 red fruits studied may be related to the initial concentrations and different classes found in each fruit. Furthermore, the gastrointestinal digestion process may cause bioconversions between anthocyanins because of the presence of available glucosidases.[9]

The monitoring of the anthocyanin profile during simulated digestion showed that anthocyanins from red fruits were degraded during colonic fermentation (Table 2), which corroborates the colorimetric analysis results (Table 1). Some factors have been discussed to explain the low bioaccessibility of anthocyanins after colonic fermentation. According to Aura et al.,[42] the adsorption of anthocyanins into the cell wall of gut microorganisms and proteins from fecal material, the partial and total deglycosylation of anthocyanins caused by the presence of β-glycosidases, and the breaking of anthocyanins into other classes of phenolic compounds are

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possible reasons for the losses of anthocyanins. Furthermore, the transformation of phenolic precursors into metabolites may occur. Dall'asta et al.[43] show that red fruits, such as strawberries, blueberries, blackberries, and raspberries, present several classes of glycosylated anthocyanins and some phenolic acids as phenolic precursors, while the main metabolites generated after colonic fermentation of such red fruits are phenolic acids, e.g., protocatechuic, benzoic, gallic, coumaric, quinic, dihydrocaffeic, and hydroxybenzoic acids.

This study focused on anthocyanins; however, other classes of phenolic compounds not determined by HPLC analysis have greater bioaccessibility than anthocyanins (Table 1). Therefore, an in-depth study of the bioaccessibility of general classes of phenolic compounds from red fruits may be complementary for future work. Thus, anthocyanins from red fruits can be wasted together with fecal matter or may have been chemically converted into more minor compounds unidentified in this study. The low bioaccessibility of anthocyanins during digestion highlights the potential for innovative delivery systems,[32,44,45] as demonstrated by prior studies that used coating technologies or biosorption methods to improve anthocyanin stability and bioavailability in specific digestive phases. Thus, this investigation can encourage researchers to develop a new anthocyanin delivery system to protect such compounds during digestive conditions.

The present research highlights that red fruits are sources of bioactive compounds, including phenolic acids, flavonoids, and anthocyanins. In this study, the colorimetric analysis of red fruits showed significant amounts of total phenolic compounds, flavonoids, and anthocyanins, which were correlated with antioxidant activity through ABTS and DPPH methods. The bioaccessibility of the phenolic compounds varied among red fruits. In general, red fruits with higher phenolic concentrations tended to have lower bioaccessibility, likely due to the specific classes of phenolic compounds present in their matrices. Individual anthocyanins were analyzed through liquid chromatography and showed different classes of these compounds among red fruits. Jaboticaba's anthocyanins were not bioaccessible, while raspberry had the highest anthocyanin bioaccessibility in red fruits. Colonic fermentation expressively reduced the levels of TPC and TFC in bioaccessible fractions of red fruits. Anthocyanins were completely degraded during colonic fermentation, as determined by both colorimetric and chromatographic methods. These findings highlight the need for innovative technologies to preserve anthocyanins throughout gastrointestinal digestion and colonic fermentation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.4c00707.

Table S1 provides the retention times, UV−vis maximum absorption wavelengths, pseudomolecular ions, MS^2 fragment ions, and the tentative identification of anthocyanins in six fruit extracts. Figure S1 illustrates the general molecular skeleton of anthocyanins. (PDF)

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