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Bioactives in Berry Fruits with Emphasis on In Vitro Bioaccessibility for Human Nutrition

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Abstract: This study aimed to investigate the bioaccessibility and biostability of carotenoids, vitamin E isomers, and individual polyphenolic compounds after the in vitro gastrointestinal digestion of two types of berries (raspberry and blackberry fruits). The results of the polyphenols analysis showed that raspberry fruits contained higher concentrations of hydroxybenzoic acids, hydroxycinnamic acids, flavanols, and flavonols compared to blackberry fruits, but exhibited the lowest bioaccessibility values for all the studied polyphenol classes. Ellagic acid represented 13.63% and 2.65% of the hydroxybenzoic acids in raspberry and blackberry fruits. The hydroxybenzoic acids exhibited the highest bioaccessibility index in the intestinal phase of both types of berries, and gallic acid emerged as one of the most bioaccessible phenolic compounds. The bioaccessibility of carotenoids ranged between 15.7 and 17.30% for lutein, 5.52 and 7.56% for astaxanthin, and 7.85 and 9.93% for canthaxanthin, with elevated values being observed in raspberry fruits. Although vitamin E and carotenoids follow a similar path for absorption, the bioaccessibility of vitamin E isomers was higher than that of carotenoids, with γ-tocopherol being the most bioaccessible isomer in both raspberries and blackberries. Knowing the bioaccessibility of food constituents during digestion is crucial, as the potential effectiveness of bioactives for human health largely depends on the bioavailability of these molecules.

Keywords: raspberries; blackberries; in vitro digestion; antioxidants; bioaccessibility; biostability

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1. Introduction

Fruits are important parts of a healthy diet. Dietary guidelines worldwide encourage an increased consumption of fruits and vegetables, with special attention paid to fruits like berries. These fruits are abundant in essential nutrients, including vitamins, minerals, and phytochemicals, which may contribute to disease prevention [1]. Moreover, foods characterized by a high concentration of bioactive compounds represent a significant advancement in the domains of health and nutrition. Through the utilization of raw materials derived from plants and vegetables, it becomes feasible to develop innovative functional foods that provide nutritional advantages and enhance well-being by delivering bioactive molecules such as vitamins, carotenoids, and polyphenols [2].

Berry fruits have received significant attention because of their potential positive impacts on human health. Some of the most common types include blueberries, bilberries, cranberries, blackberries, and raspberries, as well as black, white, and red currants and strawberries. These fruits are well-known for their rich contents of bioactive compounds with antioxidant properties, such as polyphenols—including flavonoids (anthocyanins, flavonols, and flavonols), condensed tannins (proanthocyanidins), hydrolyzable tannins (ellagitannins and gallotannins), phenolic acids (hydroxybenzoic acids, hydroxycinnamic acids, and chlorogenic acid), and stilbenes and lignans [3,4]. These compounds are believed

to play a significant role in lowering the risk of various lifestyle-related diseases through their consumption [5].

Numerous bioactive compounds are frequently found within specific anatomical structures in the natural matrices of these fruits, leading to a low bioavailability. The investigation of bioactive compounds' bioavailability in foods is a crucial process, enabling the accurate assessment of their nutritional contributions. In vitro screening methods have been developed and refined to determine the nutrient bioaccessibility and bioavailability from foods. These methods provide valuable insights, especially considering the numerous factors that can influence nutrient absorption. Bioavailability refers to the proportion of an ingested nutrient that is absorbed and made available for physiological functions [6]. It is influenced by factors such as digestion, release from the food matrix, absorption by intestinal cells, and transport to body cells. On the other hand, bioaccessibility, defined as the amount of an ingested nutrient that is potentially available for absorption, depends solely on digestion and release from the food matrix [7].

Apart from variations in the antioxidant contents found in raw fruits, berry species also differ in several other characteristics, such as their dietary fiber content [8]. Studies have demonstrated a direct interaction between dietary fiber content and food antioxidants, hindering the proper assimilation of these compounds [9]. Therefore, the digestibility of food matrices may vary among different types of berries. When assessing the nutritional value for human consumption, merely quantifying the nutrient composition within food is insufficient.

This study aims to assess the bioaccessibility and biostability of carotenoids, vitamin E isomers, and individual polyphenolic compounds after the in vitro gastrointestinal digestion of two types of berries (raspberry and blackberry fruits).

2. Materials and Methods

2.1. Chemicals and Reagents

Methanol, petroleum ether, chloroform, ethanol, acetone, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Acetic acid and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). The following phenolic standards were purchased from Sigma-Aldrich (St. Louis, MO, USA): ellagic acid (95%), syringic acid (98%), epicatechin (96%), 4-hydroxy-3-methoxycinnamic acid (95%), rutin (95%), vanillic acid (95%), 3-hydroxybenzoic acid (95%), protocatechuic acid (96%), caffeic acid (95%), coumaric acid (98%), epigallocatechin (97%), catechin (95%), quercetin (95%), and resveratrol (99%). Ferulic acid (97%) and chlorogenic acid (95%) were purchased from European Pharmacopoeia (EP). The following standards for the carotenoids and vitamin E isomers were purchased from Sigma-Aldrich (St. Louis, MO, USA): lutein (95%), astaxanthin (97%), canthaxanthin (95%), α-tocopherol (96%), γ-tocopherol (96%), and δ-tocopherol (95%).

Stock solutions for individual polyphenols were prepared in methanol and kept in the refrigerator, maintained as stable for at least 1 month. The stock solutions for carotenoids were prepared in chloroform, and for vitamin E isomers, in methanol; the stock solutions of liposoluble compounds were kept in the freezer, maintained as stable for at least 3 months. The working standards were prepared freshly from the stock solutions for each new measurement.

2.2. Plant Material

The material used for the study consisted of fruits from raspberry (*Rubus idaeus* L.) and blackberry (*Rubus fruticosus* L.), which were harvested from the wild flora of Olt County (44°26′00″ N, 24°22′00″ E), Romania. The collected material was dried at a temperature of 65 °C for 48 h, and finely powdered using a Grindomix GM 200 mill (Retsch, Haan, Germany). Average samples were subsequently formed and carefully stored in a dark environment until the analyses were performed.

2.3. Polyphenols Analysis

The weighted plant material (0.5 g) was added to a 50 mL centrifuge tube along with 10 mL of a 69:30:1 H_2O/MeOH/acetic acid mixture. Extraction was performed at 50 °C for 1 h. The hydromethanolic extracts were centrifuged at 4500 rpm for 15 min. An aliquot part of the supernatant (1 mL) was passed through an SPE cartridge with silica (1000 mg/6 mL, particle size 40–75 µm, pore size 70 Å), which was previously conditioned with 2 mL of methanol followed by 2 mL of distilled water. After passing the sample, the cartridge was rinsed with 5 mL of a 69:30:1 H_2O/MeOH/acetic acid mixture. The sample was filtered with a nylon syringe filter (0.2 µm, 25 mm diameter), and a 500 µL aliquot of the sample was transferred into a vial and mixed with 500 µL of the mobile phase (90:5:5 1% acetic acid in distilled water /MeOH/H_2O). For each digestive phase (oral, gastric, and intestinal phase), a 1 mL aliquot part was passed through an SPE cartridge and prepared in the same conditions as those described for solid samples.

The profile of the polyphenols was assessed using a previously described liquid chromatographic method [10], a Vanquish Core HPLC system equipped with a DAD manufactured by Thermo Fisher Scientific (Bremen, Germany) and a BDS HyperSil C18 column (250 × 4 mm, 5 µm particle size) from Thermo Fisher Scientific (Bremen, Germany). The chromatographic method employed a binary gradient consisting of 1% acetic acid in distilled water (*v*/*v*) as solvent A, methanol as solvent B, and acetonitrile as solvent C, with a flow rate set at 0.5 mL/min. The elution program was as follows: 0–15 min: 5% solvent B, 5% solvent C; 15–20 min: 4% solvent B, 15% solvent C; 20–25 min: 3% solvent B, 25% solvent C; 25–40 min: 2% solvent B, 38% solvent C; and 40–50 min: 5% solvent B, 5% solvent C. The injection volume was 40 µL. The chromatographic column was maintained at 25 °C. Chromatograms were recorded at 254, 270, 280, 310, and 320 nm. Individual standards of polyphenols were used for the identification and quantification of polyphenolic compounds.

2.4. Carotenoids Analysis

The preparation of berry extracts for liposoluble compounds analysis involved a saponification phase with an ethanolic potassium hydroxide solution in a water bath for 30 min at 80 °C. The extraction was performed with petroleum ether. The extract was passed through a filter with anhydrous sodium sulfate to remove any suspended water and evaporated under vacuum until dry. The residue was dissolved in 10 mL of methanol and analyzed. An aliquot part of each digestive phase (oral, gastric, and intestinal phases) (500 µL) was mixed in a vial with the same volume of methanol and analyzed.

Xanthophylls (lutein, astaxanthin, and canthaxanthin) were analyzed according to the method described by [11] with slight modifications, using a Surveyor Plus HPLC system (Thermo-Electron Corporation, Waltham, MA, USA), a PDA-UV detector (λ = 450 nm), and a C18 reversed-phase column (250 × 4.60 mm, 5 µm) (Nucleodur, Macherey-Nagel, Duren, Germany). The chromatographic method involved isocratic working conditions, a flow rate of 0.5 mL/min at 25 °C, and a mobile phase of 10% water, 15% methanol, and 75% acetone. The injection volume was 25 µL The compounds were identified and quantified using individual analytical standards. The results are expressed as µg/g.

2.5. Vitamin E Isomers Analysis

The vitamin E isomers were analyzed from the same extract prepared for the carotenoids analysis. The isomers of vitamin E were assessed as previously described by [12,13], with a Vanquish Core HPLC System (Thermo-Electron Corporation, Waltham, MA, USA), a PDA-UV detector (λ = 292 nm), and an Accucore C18 column (150 mm × 4.6 mm, 4 µm particle size) (Thermo-Electron Corporation, Waltham, MA, USA). The method employed isocratic conditions, a mobile phase of methanol (96%) and water (4%), and a flow rate of 0.5 mL/min. The injection volume was 40 µL. The results are expressed as µg/g.

2.6. Simulated In Vitro Gastrointestinal Digestion

The method for in vitro gastrointestinal digestion consisted of three steps—oral, gastric, and intestinal digestion, and was previously described by [14]. Three saline solutions were prepared to simulate salivary (SSF), gastric (SGF), and intestinal (SIF) fluids, with specific enzymes added at each phase.

The simulated salivary fluid (SSF) contained 15.1 mM KCl, 3.7 mM KH_2PO_4, 13.6 mM $NaHCO_3$, 0.15 mM $MgCl_2$ $(H_2O)_6$, 0.06 mM $(NH_4)_2CO_3$, and 1.5 mM $CaCl_2$. The simulated gastric fluid (SGF) was formulated with 6.9 mM KCl, 0.9 mM KH_2PO_4, 25 mM $NaHCO_3$, 47.2 mM NaCl, 0.10 mM $MgCl_2$ $(H_2O)_6$, 0.50 mM $(NH_4)_2CO_3$, and 0.15 mM $CaCl_2$. The simulated intestinal fluid (SIF) consisted of 6.8 mM KCl, 0.8 mM KH_2PO_4, 85 mM $NaHCO_3$, 38.4 mM NaCl, 0.33 mM $MgCl_2$ $(H_2O)_6$, and 0.6 mM $CaCl_2$.

A total of 5 g of the samples was mixed with 3.5 mL of simulated salivary fluids (SSFs) and 0.5 mL of α-amylase (prepared in SSF; final concentration 75 U/mL) preheated at 37 °C. Then, 25 µL of 0.3 M calcium chloride solution and 975 µL of distilled water were added. The mixture was incubated at 37 °C for 2 min.

To simulate the gastric phase, 7.5 mL of preheated simulated gastric fluid (SGF) at 37 °C and 1.6 mL of a pepsin solution (prepared in SGF at a final concentration of 2000 U/mL) were added to the oral bolus obtained from the simulated oral phase. Next, 5 µL of 0.3 M calcium chloride solution was added, and the pH was adjusted to 3 using 6 M HCl. Distilled water was then added to bring the total volume to 10 mL. The mixture was incubated at 37 °C for 2 h.

To simulate the intestinal phase, 11 mL of simulated intestinal fluid (SIF), 5 mL of pancreatin at 800 U/mL (prepared in SIF; final concentration 100 U/mL), 2.5 mL of bile salts at 160 mM (final concentration 10 mM), and 40 µL of 0.3 M $CaCl_2$ were added to the gastric chyme. The pH was then adjusted to 7 using 1 M NaOH, and water was added to reach a 1:1 (*v*:*v*) ratio with the gastric chyme. The mixture was incubated at 37 °C for 2 h. After each stage of the in vitro digestion simulation, the samples were centrifuged at 4500 rpm for 15 min at 4 °C using a refrigerated centrifuge (2-16KL, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The resulting supernatants were stored at 4 °C until analysis. The remaining residue was dried and stored in a dark environment until the analyses were performed.

The bioaccessibility index (BI) is defined as the ratio between the concentration of bioactive compound released in the simulated digestion compared to the concentration of the bioactive compound in the undigested plant, and was calculated using the following equation [15]:

$$BI = (DC \times 100)/PC, \quad (1)$$

where DC represents the concentration of the bioactive compound released during digestion, and PC refers to the concentration of the bioactive compound present in the plant matrix before digestion.

The biostability (BS) indicates the percentage of the bioactive compound that remained in the digested residue and was not released into the digestive tract. The following formula was used to calculate the biostability [16]:

$$BS = (RC \times 100)/PC, \quad (2)$$

where RC represents the concentration of the bioactive compound in the residue after digestion, and PC refers to the concentration of the bioactive compound present in the plant matrix before digestion.

2.7. Statistical Analysis

All measurements were performed in triplicate. The data obtained were statistically evaluated using analysis of variance (ANOVA), followed by Tukey's test ($p < 0.05$). The Prism GraphPad software v. 9.03 (San Diego, CA, USA) was used to present the data regarding the bioaccessibility and biostability of the studied bioactive compounds.

3. Results

3.1. Bioaccessibility of Polyphenols

After the HPLC analysis, 18 individual polyphenols, namely gallic acid, epigallocatechin, catechin, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, epicatechin, 3-hydroxybenzoic acid, rutin, coumaric acid, ellagic acid, methoxycinnamic acid, ferulic acid, protocatechuic acid, resveratrol, quercetin, and cinnamic acid, were detected in the raspberry and blackberry fruit samples (Figure 1).

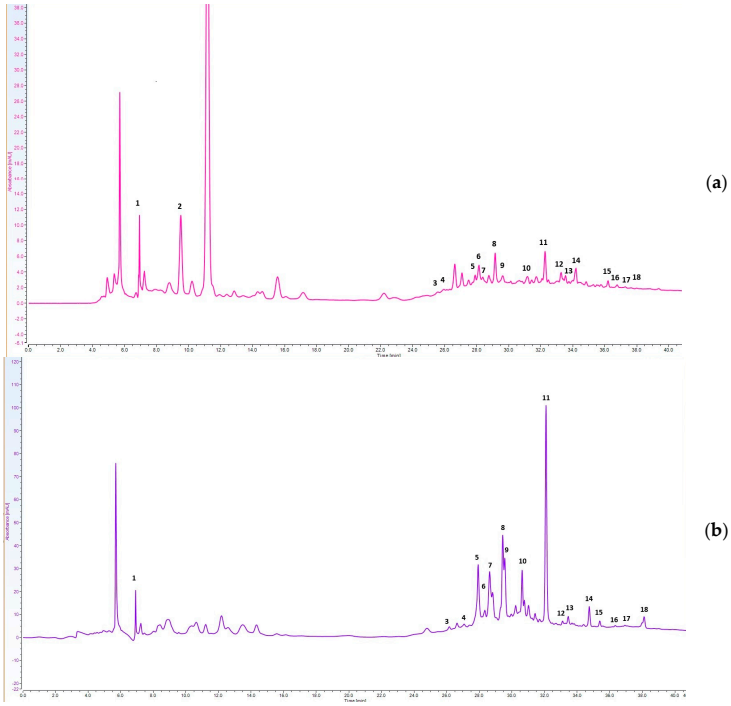

Figure 1. Chromatograms of the phenolic compounds in raspberry fruits (**a**) and blackberry fruits before digestion. (**b**) Peaks identification: 1—gallic acid, 2—epigallocatechin, 3—catechin, 4—chlorogenic acid, 5—vanillic acid, 6—caffeic acid, 7—syringic acid, 8—epicatechin, 9—3-hydroxybenzoic acid, 10—rutin, 11—coumaric acid, 12—ellagic acid, 13—methoxycinnamic acid, 14—ferulic acid, 15—protocatechuic acid, 16—resveratrol, 17—quercetin, and 18—cinnamic acid.

The phenolic compositions of the blackberry and raspberry fruits before and after simulated digestion are presented in Tables 1 and 2. The chromatograms of the polyphenols analysis from digested samples are presented in Figure S1. Epigallocatechin was the most abundant polyphenol in the raspberry fruits, while in the blackberry fruits, it was not detected. Chlorogenic acid registered the highest concentration among the polyphenols analyzed in the blackberry fruits.

The results of the polyphenols analysis showed that the raspberry fruits contained higher concentrations of hydroxybenzoic acids, hydroxycinnamic acids, flavanols, and flavonols compared to the blackberry fruits. Ellagic acid represented 13.63% and 2.65% of the hydroxybenzoic acids in the raspberry and blackberry fruits. Moreover, the quercetin concentrations appeared to be higher in the raspberry fruits compared to the blackberry fruits.

Important increased concentrations were observed during digestion, mainly in the intestinal phase. In the raspberry fruits, ferulic acid registered the highest bioaccessibility, followed by gallic acid. The blackberry fruits were characterized by elevated bioaccessibility values for almost all the analyzed hydroxybenzoic acids. In blackberries, the amount of resveratrol in the intestinal phase was very close to the one found in fruits before digestion, with an intestinal bioaccessibility of 99.05%. In raspberries, the bioaccessibility of resveratrol was approximately half of that observed in blackberries.

Table 1. Polyphenols profile (mg/g) of blackberry fruits after simulated in vitro gastrointestinal digestion.

Specification	Blackberry Fruits						
	BD	**OP**	**BI (%)**	**GP**	**BI (%)**	**IP**	**BI (%)**
				Phenolic acids			
				Hydroxybenzoic acids			
Gallic acid	0.194	0.037	18.85	0.077	39.57	0.298	153.78
Vanillic acid	0.025	0.006	22.67	0.009	35.82	0.025	98.70
Syringic acid	0.012	0.003	27.14	0.005	44.10	0.006	49.14
3-Hydroxybenzoic acid	0.019	0.011	57.80	0.017	92.88	0.030	158.33
Ellagic acid	0.007	0.002	31.22	0.007	96.19	0.008	111.49
Protocatechuic acid	0.008	0.003	32.10	0.004	49.26	0.013	161.78
				Hydroxycinnamic acids			
Chlorogenic acid	0.391	0.084	21.56	0.232	59.22	0.408	104.27
Caffeic acid	0.011	0.002	21.89	0.005	49.29	0.009	84.87
Methoxycinnamic acid	0.023	0.006	25.57	0.015	67.32	0.021	91.29
Ferulic acid	0.033	0.013	40.34	0.014	42.63	0.006	19.43
Coumaric acid	0.007	0.002	21.71	0.003	37.38	0.004	61.10
Cinnamic acid	0.027	0.003	9.23	0.000	0.00	0.027	99.36
				Flavonoids			
				Flavanols			
Epigallocatechin	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Catechin	0.316	0.069	21.84	0.168	53.07	0.195	61.85
Epicatechin	0.011	0.002	19.56	0.009	88.15	0.013	121.69
				Flavonols			
Rutin	0.012	0.002	15.87	0.007	63.74	0.017	142.91
Quercetin	0.007	0.002	27.88	0.003	45.27	0.010	139.34
				Stilbenes			
Resveratrol	0.002	0.000	11.57	0.001	40.75	0.002	99.05

BD = before digestion, OP = oral phase, GP = gastric phase, IP = intestinal phase, and BI = bioaccessibility index.

Table 2. Polyphenols profile (mg/g) of raspberry fruits after simulated in vitro gastrointestinal digestion.

Specification	Raspberry Fruits						
	BD	**OP**	**BI (%)**	**GP**	**BI (%)**	**IP**	**BI (%)**
				Phenolic acids			
				Hydroxybenzoic acids			
Gallic acid	0.368	0.021	5.68	0.085	23.03	0.449	122.13
Vanillic acid	0.019	0.004	22.76	0.006	33.35	0.012	60.80
Syringic acid	0.064	0.003	4.68	0.016	25.70	0.040	62.35
3-Hydroxybenzoic acid	0.125	0.014	11.15	0.061	49.04	0.055	43.68
Ellagic acid	0.122	0.003	2.32	0.018	14.58	0.076	62.76
Protocatechuic acid	0.198	0.009	4.79	0.020	10.14	0.080	40.42
				Hydroxycinnamic acids			
Chlorogenic acid	0.455	0.006	1.28	0.054	11.78	0.081	17.90
Caffeic acid	0.030	0.003	8.99	0.010	32.53	0.011	37.61
Methoxycinnamic acid	0.012	0.001	10.61	0.005	43.67	0.011	93.99
Ferulic acid	0.027	0.004	13.12	0.007	26.42	0.043	160.59
Coumaric acid	0.037	0.001	2.39	0.001	3.73	0.021	55.02
Cinnamic acid	0.015	0.001	4.03	0.001	8.28	0.005	30.26
				Flavonoids			
				Flavanols			
Epigallocatechin	1.179	0.052	4.38	0.112	9.49	0.298	25.29
Catechin	0.501	0.019	3.85	0.075	14.99	0.068	13.60
Epicatechin	0.300	0.007	2.40	0.026	8.66	0.109	36.33
				Flavonols			
Rutin	0.070	0.003	4.13	0.006	9.18	0.020	28.29
Quercetin	0.049	0.001	1.78	0.002	3.65	0.006	11.48
				Stilbenes			
Resveratrol	0.031	0.001	2.52	0.002	7.16	0.014	45.13

BD = before digestion, OP = oral phase, GP = gastric phase, IP = intestinal phase, and BI = bioaccessibility index.

The impacts of gastrointestinal digestion on different classes of polyphenols are shown in Figure 2.

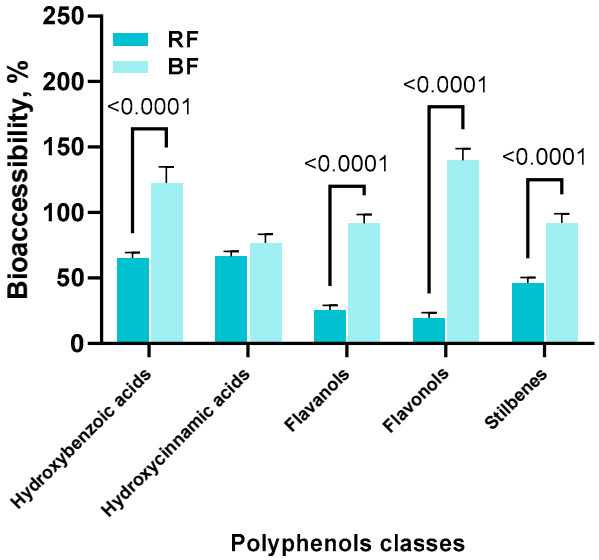

Figure 2. Bioaccessibility of the polyphenols classes in the intestinal phase of the analyzed berries. RF represents raspberry fruits; BF represents blackberry fruits. The results are expressed as mean ± standard deviation (n = 3). Significant differences were considered at $p < 0.05$.

The results showed significantly ($p < 0.0001$) lower levels of bioaccessible hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols, and stilbenes in the raspberry fruits compared to the blackberry fruits.

3.2. Bioaccessibility of Carotenoids

The concentrations of the analyzed carotenoids in the raspberries and blackberries before and after digestion are presented in Table 3.

Table 3. Carotenoids (μg/g) of raspberry and blackberry fruits after simulated in vitro gastrointestinal digestion.

Specification	Raspberry Fruits			Blackberry Fruits		
	BD	IP	IR	BD	IP	IR
Lutein	46.09	7.612	28.77	110.4	18.76	21.44
Astaxanthin	27.93	2.061	11.20	10.87	0.642	6.433
Canthaxanthin	1.47	0.138	0.953	5.76	0.479	1.222

BD = before digestion, IP = intestinal phase, and IR = intestinal residue.

The most abundant carotenoid was lutein in both types of fruits. Canthaxanthin and lutein were presented in higher amounts in the blackberry fruits, while astaxanthin registered a higher concentration in the raspberry fruits. Reduced carotenoid concentrations were found in the intestinal phase of the digested berries. The bioaccessibility of carotenoids, assessed in the intestinal phase of the raspberry and blackberry fruits, is presented in Figure 3.

The bioaccessibility of carotenoids ranged between 15.7 and 17.30% for lutein, 5.52 and 7.56% for astaxanthin, and 7.85 and 9.93% for canthaxanthin, with elevated values being observed in the raspberry fruits. Nevertheless, a significantly higher bioaccessibility ($p < 0.05$) was found only for astaxanthin. Part of the carotenoids remained in the digested berries' residue, suggesting that they were not bioaccessible. Further, the biostability of these carotenoids was studied (Figure 4). Lutein and canthaxanthin exhibited a significantly higher biostability in the raspberry fruits, while astaxanthin displayed an elevated biostability ($p < 0.05$) in the blackberry fruits.

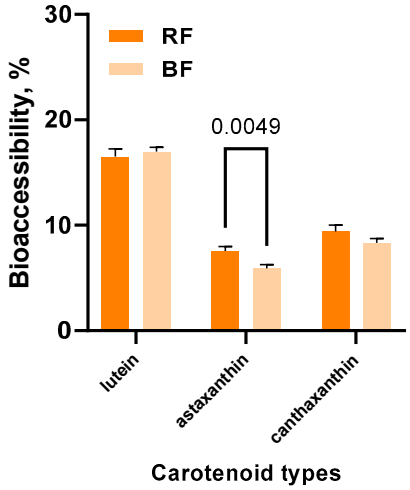

Figure 3. Bioaccessibility of carotenoids in the intestinal phase of the analyzed berries. RF represents raspberry fruits; BF represents blackberry fruits. The results are expressed as mean ± standard deviation (n = 3). Significant differences were considered at $p < 0.05$.

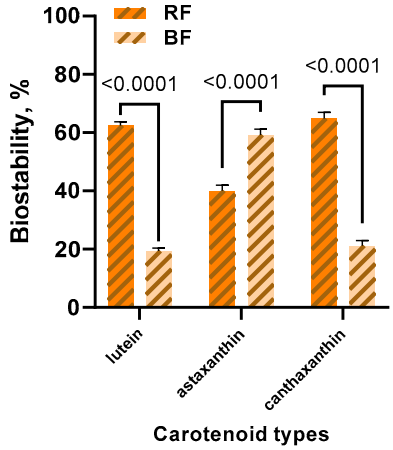

Figure 4. Biostability of carotenoids in the intestinal phase of the analyzed berries. RF represents raspberry fruits; BF represents blackberry fruits. The results are expressed as mean ± standard deviation (n = 3). Significant differences were considered at $p < 0.05$.

3.3. Bioaccessibility of Vitamin E Isomers

The results regarding the tocopherol analysis of the raspberry and blackberry fruits before and after digestion are presented in Table 4.

Table 4. Vitamin E isomers (μg/g) of raspberry and blackberry fruits after simulated in vitro gastrointestinal digestion.

Specification	Raspberry Fruits			Blackberry Fruits		
	BD	IP	IR	BD	IP	IR
δ-tocopherol	56.60	15.54	30.91	145.1	44.20	97.73
γ-tocopherol	139.3	50.52	79.91	299.6	140.4	135.4
α-tocopherol	229.7	67.97	158.0	253.4	90.95	145.8

BD = before digestion, IP = intestinal phase, and IR = intestinal residue.

Both types of fruits have been shown to contain α, δ, and γ-tocopherol, with higher levels being found in blackberry fruits. The raspberries were characterized by an elevated content of α-tocopherol from the analyzed isomers, while in the blackberries, the most abundant was γ-tocopherol, followed closely by α-tocopherol. After in vitro simulated

digestion, the concentrations found in the intestinal phase of the studied berries were drastically reduced. The bioaccessibility of vitamin E isomers in the intestinal phase is presented in Figure 5. Higher concentrations of vitamin E isomers were found in the blackberries before and after digestion, which also exhibited greater values for tocopherols' bioaccessibility, statistically significant ($p < 0.05$) for α and γ-tocopherol. The assessment of tocopherol biostability (Figure 6) showed that α and γ-tocopherol had an elevated biostability in the raspberries compared to the blackberries.

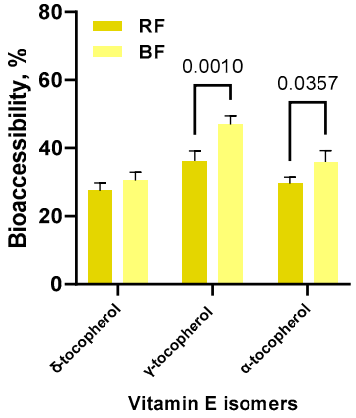

Figure 5. Bioaccessibility of vitamin E isomers in the intestinal phase of the analyzed berries. RF represents raspberry fruits; BF represents blackberry fruits. The results are expressed as mean ± standard deviation (n = 3). Significant differences were considered at $p < 0.05$.

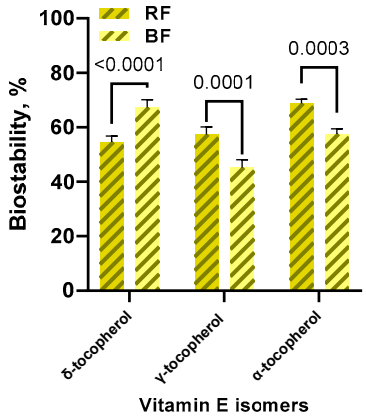

Figure 6. Biostability of vitamin E isomers in the intestinal phase of the analyzed berries. RF represents raspberry fruits; BF represents blackberry fruits. The results are expressed as mean ± standard deviation (n = 3). Significant differences were considered at $p < 0.05$.

4. Discussion

4.1. Bioaccessibility of Polyphenols

The profiling of the individual polyphenols contained in the raspberry and blackberry fruits showed that the raspberries registered the highest concentrations in the undigested samples. In a study on the chemical compositions of different raspberry cultivars, [17], high concentrations of quercetin and rutin were found (5.51–57.47 mg/kg and 7.26–88.57 mg/kg, respectively), with the results obtained in the current study being in the reported concentrations range (49 mg/kg quercetin and 70 mg/kg rutin). Moreover, the content of syringic acid in the raspberry fruits was in line with the results found by the same authors (64 mg/kg vs. 29.02 to 88.51 mg/kg). In this study, raspberry fruits registered higher concentrations of quercetin compared to blackberry fruits. It was shown that the types and quantities of different quercetin glycosides may have large variations between berries from different families and genera [18].

The concentration of ellagic acid analyzed in the raspberry fruits (0.7 mg/100 g DW) was close to the one reported by [19] (1.052 mg/100 g DW), which also observed that lower concentrations could be found in wild Rubus species compared to cultivated ones. The intake of ellagic acid has been linked to health benefits, being known to have anticancer properties [20] and anti-inflammatory activity [21]. Ellagic acid can be found in a limited number of commonly consumed plant foods, including certain fruits like raspberries, blackberries (*Rubus* sp.), cloudberries (*Rubus chamaemorus*), strawberries (*Fragaria* × *ananassa* D), pomegranates (*Punica granatum* L.), and muscadine grapes (*Vitis rotundifolia*), as well as some nuts like walnuts and pecans, as shown by [22]. The proportion of ellagic acid from the analyzed hydroxybenzoic acids was below the values reported by [8]. These differences could be caused by the degradation of the ellagic acid during storage time, when it can interact with free radicals due to its metal chelating ability. Moreover, polyphenol oxidase can be released during storage time and can oxidize polyphenols into quinones [23].

Anthocyanins are considered to be the second major group of polyphenols present in raspberry fruits, being responsible for over 25% of their total antioxidant activity [24]. Phenolic acids are known as the main metabolites of anthocyanins, tannins, and flavanols before their total degradation or absorption by the gut epithelium [25]. The bioaccessibility of polyphenols is influenced by several factors, such as the chemical structure of the aglycone and the main type of glycoside present. In plants, polyphenols are rarely found in their basic structure (aglycone). In fruits, they are usually bound to different sugars.

Although gallic acid is unstable in alkaline conditions, higher concentrations were found in the intestinal phase of both types of fruits. Similar results were observed by [26] in wild and commercial blackberries, being explained by the fact that gallic acid could result from the hydrolytic degradation of tannins, the fragmentation of the cyanidin aglycone, or through singular hydration coupled with the carboxylation of ferulic acid. Moreover, in this study, ferulic acid had a low bioaccessibility in the intestinal phase of blackberries, which can be linked with the elevated bioaccessibility of gallic acid. On the other hand, the raspberries were characterized by a lower intestinal bioaccessibility of caffeic acid and a higher intestinal bioaccessibility of ferulic acid, which could be caused by the fact that ferulic acid is an intermediate product of the spontaneous carboxylation of caffeic acid in the gastrointestinal tract [27].

Similar values were found for the bioaccessibility of coumaric acid (61% in blackberries vs. 55% in raspberries). In [26], a large variation in coumaric acid bioaccessibility was demonstrated, reporting results of a 143% bioaccessibility in commercial blackberries vs. a 1371% bioaccessibility in wild blackberries. Interestingly, [28] could not detect coumaric acid after the in vivo gastrointestinal digestion of blueberries, but found it in the bloodstream after blueberry consumption, possibly due to the metabolism of the phenolic acids, like caffeic or ferulic acids, or flavonoid degradation.

Flavanols exhibited a significantly higher bioaccessibility in the intestinal phase of the blackberries when compared to the raspberries, despite the raspberries having a higher total flavanols content due to the presence of epigallocatechin, which is absent in blackberries. Studies have shown that flavanols have a low bioavailability in the human body, but with the pancreatic enzymes added in the intestinal phase, glycosylated flavanols are degraded in more stable compounds such as phenolic acids, including gallic, ferulic, vanillic, and hydroxybenzoic acids [29].

The bioaccessibility of polyphenols from raspberry and blackberry leaves was reported to be similar to the results of the current study, with hydroxybenzoic acids exhibiting the highest bioaccessibility index during the intestinal phase and gallic acid emerging as one of the most bioaccessible phenolic compounds [30]. Polyphenols with a high molecular weight, such as anthocyanins, are mostly degraded during gastrointestinal digestion. This degradation is linked to an increase in the concentration of protocatechuic acid, known as the primary metabolite of cyanidin-3-glucoside, with positive health effects associated with anthocyanin consumption [25]. In this study, the concentration of protocatechuic acid in the intestinal phase of the blackberries was higher than that analyzed in the undigested

fruits, with a bioaccessibility of 161%. Furthermore, [28] highlighted that anthocyanin metabolites are often found in body fluids at concentrations that cannot be correlated with their concentrations in fruits.

4.2. Bioaccessibility of Carotenoids

Carotenoids have garnered significant interest in the scientific community, not only because some can be converted into retinoid forms, providing pro-vitamin A activity, but also because of their antioxidant properties. More than 600 carotenoids are found in nature, causing the red, orange, and yellow hues in many fruits and vegetables. The main six carotenoids commonly found in nature are beta-carotene, α-carotene, and β-cryptoxanthin, which have pro-vitamin A activity, but also lycopene, lutein, and zeaxanthin, which do not possess pro-vitamin A activity. The dietary intake of carotenoids is linked with a reduced risk of cardiovascular diseases, cancer, cataracts, and age-related macular degeneration, likely due to their antioxidant effects [31].

For carotenoids to achieve their biological roles, they must be released from foods and made accessible for absorption by the human body. However, their availability from natural sources is relatively low, ranging from 5 to 30%, compared to other phytochemicals in food [32]. Consequently, additional strategies, including digestion protocols, are being employed to develop new delivery systems and functional foods that enhance carotenoid availability and create technological processes for improving their bioavailability from plant-based foods [33]. The in vitro methodology employed to study carotenoid stability and partitioning during digestion encompasses simulated small intestinal digestion, isolated intestinal segments, brush-border and basolateral membrane vesicles, isolated enterocytes, and transformed intestinal cell lines, with a particular focus on the Caco-2 human cell line [34].

In this study, the bioaccessibility of carotenoids was assessed only in the intestinal phase, since carotenoids are bioactive compounds that present a low oral bioavailability [33], and most carotenoid absorption is believed to occur in the small intestine. Due to their lipophilic properties requiring micellization, absorption in the stomach is considered to be unlikely. Additionally, the absorption of carotenoids from the colon remains uncertain and is still under investigation [35]. Furthermore, in the current study, the bioaccessibility of lutein, zeaxanthin, astaxanthin, and canthaxanthin was assessed, since previous research has shown that these are readily bioaccessible and bioavailable xanthophylls, compared to other carotenoids that are considered as not absorbable [36]. Lutein and zeaxanthin possess hydroxyl groups that confer molecular polarity and also enable the formation of hydrogen bonds with the aqueous environment surrounding the micelles. Even though are unable to form hydrogen bonds, astaxanthin and canthaxanthin have a ketone group that makes them bioaccessible for absorption.

The most abundant carotenoid in both types of berries was lutein, with significantly higher concentrations observed in the blackberry fruits. Raspberry exhibited a superior bioaccessibility of astaxanthin and canthaxanthin, but this was statistically significant only for astaxanthin. Although the level of lutein was 2.3 fold higher in the blackberry fruits, the lutein bioaccessibility was similar in the two studied berries. A study conducted on different dietary sources for human nutrition, [37], showed that the bioaccessibility of lutein ranged between 37.6 and 59.4%. Moreover, independent of the food matrix, lutein had a higher bioaccessibility than the other studied carotenoids, which is in line with the results of the present study. Carotenoids are lipophilic compounds, and the presence of other food constituents such as fat may influence their bioaccessibility and biostability. The dietary intake of fat produces hydrophobic conditions which favor the release of carotenoids, which are further dissolved into small lipid droplets. Carotenoids are absorbed in the intestine from bile salt micelles, which are formed with the addition of bile salts [38].

Despite the fact that the raspberries and blackberries had similar values for lutein bioaccessibility, the results obtained for lutein biostability were significantly different. The raspberries registered increased ($p < 0.05$) values of biostability for lutein and canthaxanthin

compared to the blackberries. In [33], it was reported that, during in vitro digestion, carotenoid losses are between 8 and 40%, with greater losses being observed in colonic fermentation processes. Carotenoids with an elevated intestinal biostability, which are not absorbed or degraded in the small intestine, reach the colon within micelles or are precipitated, with small amounts of carotenoids being reported in human feces [39].

4.3. Bioaccessibility of Vitamin E Isomers

The analysis of vitamin E isomers from raspberry and blackberry fruits showed that, in raspberries, the main isomer was α-tocopherol, while in blackberries, it was γ-tocopherol. Similar results were reported by [40], who showed that raspberries can contain over 300 mg/kg (dry weight) of tocopherols, predominantly α, δ, and γ-tocopherol, with γ-tocopherol being the most abundant. α-tocopherol is the isomer with the highest vitamin E activity, being found in elevated concentrations in leaves, while higher amounts of γ-tocopherol are found in the seeds of fruits [41]. The leaves of raspberry have been shown to have a lower concentration of vitamin E compared with the results found for the fruits in the present study, but an important antioxidant activity of leaves was demonstrated through DPPH, superoxide anion, hydroxyl radical, hydrogen peroxide, and lipid peroxidation assays, and it was suggested that their usage in animal feeds can increase their shelf life by inhibiting and delaying the oxidation of meat [42].

The evaluation of the bioaccessibility of the vitamin E isomers showed that γ-tocopherol had the highest bioaccessibility in both types of berries. In this study, the bioaccessibility of tocopherols ranged between 24.98 and 49.23%. In a study regarding the bioaccessibility of vitamin E in foods, [37] highlighted that it can be very variable, with values ranging from 0.47% in apples to almost 100% in banana or white bread. This highly variable bioaccessibility supports the fact that the food matrix strongly influences tocopherol bioavailability [43].

The literature contains only limited data on the vitamin E bioavailability from different food matrices, with it being assumed that the bioavailability from seeds is reduced due to the fact that these sources are not readily digested in the GI tract. It is believed that vitamin E and carotenoids follow a similar path in the duodenum. Additionally, in this study, it was shown that the bioaccessibility of vitamin E isomers was higher than that of carotenoids, which is in agreement with the results of [37], who showed that vitamin E is more readily absorbed than carotenoids.

The limitations of the study are related to the fact that the research relies on in vitro gastrointestinal digestion to assess the bioaccessibility of the studied antioxidants, and it may not fully replicate the complex processes occurring in the human digestive system. Nevertheless, it was shown that in vivo models have a high interindividual variability in the response of the liposoluble antioxidants, since populations are made up of both low and high absorbers [37]. Another limitation of the study is the influence of the food matrix, which was not explored in the present paper. This study reports varying bioaccessibility values for different compounds, such as polyphenols, carotenoids, and vitamin E isomers. However, the presence of other food constituents, such as fat, may influence their bioaccessibility and biostability, favoring the release of liposoluble antioxidants from the food matrix. On the contrary, the dietary fiber content may decrease the bioaccessibility of polyphenols. Other limitations include environmental factors. The study did not account for environmental factors that may influence the bioactive contents of berries, such as soil quality, climate, and agricultural practices. These factors can significantly affect the nutritional profiles of fruits.

The novelty of this study lies in its detailed examination of the bioaccessibility of the various bioactive compounds in berries, while its significance is reflected in the implications for dietary practices, public health, and food product development. The findings of this paper could have significant implications for the development of innovative food products, including combinations of different components or the development of new food processing methods, taking into consideration the bioaccessibility and biostability of the studied antioxidants.

It is vital to comprehend the bioaccessibility and behavior of antioxidant compounds during digestion, as the effectiveness of berries' metabolites for human health heavily relies on the bioavailability of these substances. These findings underscore the potential for developing functional foods that maximize the bioavailability of the beneficial compounds found in berries. This is significant for the food industry, as it opens up avenues for creating products that enhance health benefits through improved nutrient absorption.

5. Conclusions

In this study, an in vitro digestion model was used to measure the bioaccessibility of individual polyphenols, carotenoids, and vitamin E isomers in two types of berry fruits, raspberries and blackberries. The results showed that hydroxybenzoic acids exhibited the highest bioaccessibility index in the intestinal phase of both types of berries, and gallic acid emerged as one of the most bioaccessible phenolic compounds. Even though the raspberries registered the highest concentrations of polyphenols in the undigested samples, they exhibited the lowest bioaccessibility values for all the studied polyphenol classes. The most abundant carotenoid was lutein in both types of fruits, which also had a higher bioaccessibility than the other studied carotenoids. Although vitamin E and carotenoids follow a similar path for absorption, the bioaccessibility of vitamin E isomers was higher than that of carotenoids, with γ-tocopherol being the most bioaccessible isomer in both the raspberries and blackberries. Knowing the bioaccessibility of food constituents during digestion is crucial, as the potential effectiveness of bioactives for human health largely depends on the bioavailability of these molecules.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations11100286/s1, Figure S1. Chromatograms of the phenolic compounds in the intestinal phase of raspberry fruits (a), and in the intestinal phase of blackberry fruits (b). Peaks identification: 1—gallic acid, 2—epigallocatechin, 3—catechin, 4—chlorogenic acid, 5—vanillic acid, 6—caffeic acid, 7- syringic acid, 8—3-hydroxybenzoic acid, 9—epicatechin, 10—rutin, 11—coumaric acid, 12—ellagic acid, 13—methoxycinnamic acid, 14—ferulic acid, 15—protocatechuic acid, 16—resveratrol, 17—quercetin, and 18—cinnamic acid.

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