Hindawi Publishing Corporation
Journal of Chemistry
Volume 2016, Article ID 5194901, 7 pages
http://dx.doi.org/10.1155/2016/5194901

Research Article

Phenolic Composition, Antioxidant Activity, and *In Vitro* Availability of Four Different Berries

Javier Marhuenda,[1] María Dolores Alemán,[1] Amadeo Gironés-Vilaplana,[2] Alfonso Pérez,[1] Gabriel Caravaca,[1] Fernando Figueroa,[1] Juana Mulero,[1] and Pilar Zafrilla[1]

[1]*Department of Food Technology and Nutrition, Catholic University of San Antonio, 30107 Murcia, Spain*
[2]*Department of Food Technology, EPSO, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Orihuela, Alicante, Spain*

Correspondence should be addressed to Pilar Zafrilla; mpzafrilla@ucam.edu

Received 21 October 2015; Revised 11 January 2016; Accepted 21 January 2016

Academic Editor: Angela Cardinali

Polyphenols from berries have proved healthy effects after *"in vitro"* and *"in vivo"* studies, such as preventing tumor growing and neurodegenerative and cardiovascular diseases. We compared four different kinds of berries—strawberry, raspberry, blackberry, and blueberry—with the aim to distinguish their phenolic composition, concerning their antioxidant capacity along with their *"in vitro"* availability. Folin-Ciocalteu method was used for the determination of phenolic compounds, and the antioxidant capacity was measured by ORAC method. Moreover, the determination of anthocyanins was accomplished with an HPLC-DAD. Finally, we carried out an *"in vitro"* digestion to simulate the gastrointestinal digestion. All berries showed good antioxidant capacity with significant differences, besides high total phenolic compounds. Content of anthocyanins measured by HPLC-DAD varied between the different berries, namely, blackberries and strawberries which showed higher anthocyanin concentration. After *"in vitro"* digestion, berries showed poor bioavailability of the analysis of anthocyanins (9.9%–31.7%). Availability of total phenolic compounds was higher than anthocyanins (33%–73%). Moreover, strawberries and blackberries presented the less availability grade. Decrease in antioxidant activity measured by ORAC method was about 90% in all berries studied. Therefore, bioavailability of phenolic compounds remains unclear and more correlation between *"in vitro"* and *"in vivo"* studies seems to be necessary.

1. Introduction

Reactive oxygen species (ROS) produce oxidative damage on cells structures, being responsible for high variety of diseases. Oxidative stress (OS) is a consequence of ROS proliferation, owing to the disproportion between oxidant and antioxidant species [1]. Antioxidants are known to influence a change in this proportion, leading to a decrease of prooxidant species and preventing the damage on the organism. They are recognized to act by three different methods: (1) according to the hydrogen atom transfer (OH hemolytic rupture), (2) according to the single electron transfer (electron abstraction from the radical), and (3) according to the transition metal chelation (metals ligation, leading to stable complexed compounds) [2].

Antioxidants as polyphenols are very common in fruits and vegetables, being a target for researchers over the years. Their beneficial effects on human health include preventing tumor growing or decreasing neurodegenerative and cardiovascular diseases progression [3, 4]. Furthermore, polyphenols are able to modulate enzymatic activity of cyclooxygenase [5], lipoxygenase, and cellular receptors [6].

Berry fruits, small fruits, or berries generally refer to any small fruit that lacks seeds and can be eaten in one piece, being promoted for dietary consumption regarding their content in bioactive nutrients and nonnutrients. These kinds of fruits are widely distributed, including blackberry (*Rubus* spp.), blueberry (*Vaccinium corymbosum*), red raspberry (*Rubus idaeus*), and strawberry (*Fragaria* spp.). Berries, as the majority of fruits, are known to be a good source of polyphenols, especially anthocyanins [7]. Previous studies target anthocyanins as cardio- and neuroprotective compounds [8, 9], inhibiting cancer proliferation and tumor growing [10]. Moreover, polyphenols increase insulin sensitivity

by upregulating the expression of adiponectin from mice adipocytes [11]. Anthocyanins also showed desirable *"in vitro"* benefits for glucose metabolism, causing the inhibition of α-amylase [12] and α-glucosidase [13].

Despite their biological activities, bioavailability of phenolic compounds in general and especially of anthocyanins is very low, ranging from 1.7% to 3.3% [14]. Therefore, knowing gastrointestinal absorption of phenolic compounds would lead to a better understanding of their biological characteristics. *"In vitro"* studies are an efficient, rapid, and innocuous method to simulate gastrointestinal tract working without the same restrictions as the *"in vivo"* methods, being proved in different food matrices [15]. Hence, advances in characterization of polyphenols would lead to a better estimation of their dietary intake, due to their wide variance in foods [14].

Therefore, supporting relevant assays which allow evidence of the beneficial effect of berries and anthocyanins seem to be desirable. The aim of this paper was determining the different phenolic and anthocyanin composition and bioavailability of strawberry, blackberry, raspberry, and blueberry, comparing their polyphenolic composition to their antioxidant capacity.

2. Material and Methods

2.1. Sample Pretreatment. Commercial berries were used for the assay. For the measurement of antioxidant capacity and the total phenol content, they were homogenized with ultraturrax T-18 basic (24.000 rpm) for 1 minute. Secondly, 10 g of each fruit was weighed and was centrifuged in a centrifuge Heraeus Biofuge stratos for 10 minutes (5000 g). Finally, the supernatant was measured and used for the development of the study. Any extraction of the phenolic compounds was done because of the importance of simulating as real as possible the physiological conditions for the *"in vitro"* digestion.

Finally, for the development of the *"in vitro"* digestion berries were previously homogenized with ultraturrax T-18 basic (24.000 rpm) for 1 minute and 40 mL of each sample was procured for the assay.

2.2. Antioxidant Capacity. Method used for the current study was ORAC assay, a common and contrasted analysis used in the scientist literature [22]. The assay consists of the oxidation of fluorescein by mixture with APPH radical. Fluorescein was diluted in phosphate buffer 75 mM (pH 7.4) and was preserved at −20°C for four weeks at most. Final dilution employed was 6 nM. Trolox C 0.25 mM was used for the calibration curve. APPH (127 nM) and fluorescein were prepared by dilution in phosphate buffer 75 mM (pH 7.4). Determination was realized in a microplate reader Synergy HT multidetec microplate reader from Biotek Instruments, Inc. (Winooski, VT, USA). Fluorescein was measured with wavelength of 485/20 nm. The absorption capacity of radical was determined as previously published, with some minor modifications [23]. All measures were performed in triplicate.

2.3. Total Phenolic Compounds by Folin-Ciocalteu Method. A mixture of phosphowolframic acid and phosphomolybdic acid in basic dissolution was employed for the quantification of total phenolic compounds by Folin-Ciocalteu method. Absorbance of blue color originated was measured at 765 nm at 20°C in a spectrophotometer (Varian Cary 50-Bio, Victoria, Australia). For the analysis, 40 μL of each berry was employed, followed by 500 μL of Folin-Ciocalteu reagent. Then, 2 mL of sodium carbonate (20%) was added and completed to 10 mL of miliQ water. Finally absorbance was measured at 765 nm after shaking.

2.4. HPLC-DAD Analysis of Phenolic Compounds. Berries were filtered through a 0.45 μm filter (type Millex HV13, Millipore Corp., Bedford, MA) before HPLC analysis. Twenty microliters of every sample was injected for HPLC analysis on equipment using a Merck-Hitachi pump L-6200 (Merck-Hitachi, Darmstadt, Germany) and a diode array detector Shimadzu SPD-M6A UV (Shimadzu, Kyoto, Japan) using a reversed-phase column Lichrochart RP-18 column (Merck, Darmstadt, Germany) (25 0.4 cm, 5 μm particle size), using as solvents water plus 5% formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 mL min^{-1}. Elution was performed with a gradient starting at 2% B to reach 32% B at 30 min, 40% B at 40 min, and 95% B at 50 min and became isocratic for 5 min. Chromatograms were recorded at 510, 320, and 360 nm. The total phenolic compounds were calculated by addition of the amounts of the anthocyanins, flavonols, and hydroxycinnamic acids detected in each chromatogram, as previously reported [16].

2.5. Phenolic Compounds Identification and Quantification. The phenolic compounds in berries were identified by their UV-Vis spectra, recorded with a DAD, by comparison with previous bibliography and, wherever possible, by chromatographic comparison with commercial markers. Individual anthocyanins were quantified by comparisons with an external standard of cyanidin 3-rutinoside at 510 nm. Flavonols were quantified as rutin at 360 nm and stilbenes at 320 nm as trans-resveratrol. All analyses were repeated three times, and the results were expressed as mean values in milligrams per 100 gr of sample ± SD. The reproducibility of the HPLC analyses was 5%.

2.6. "In Vitro" Digestion. To represent the digestion as realistic as possible, any extraction from berries was developed, simulating gastrointestinal environment. Procedure was adapted from the previous work of Gil-Izquierdo et al. [24], which allows knowing the grade of liberation and stability of phenolic compounds.

The pepsin solution is prepared with 4 gr of pepsin (Sigma-Aldrich) which was added to 25 mL of distilled water and brought to stirring. The pancreatin solution was prepared with 0.42 gr of $NaHCO_3$, with 1.25 g of bile salts (Sigma-Aldrich), and with 0.2 gr of pancreatin (Sigma-Aldrich); the mixture was dissolved in 50 mL of distilled water. First, the pH was measured and the sample was titrated with 0.6 N HCL to pH 2. Then 6 mL of the solution of pepsin and

Table 1: Anthocyanins measured by HPLC in berries. Results are expressed as mg/100 g FW ± SD.

	Strawberry	Blackberry	Blueberry	Raspberry
Delphinidin 3-glucoside	NI	516.5 ± 9.3	27.3 ± 3.1	NI
Cyanidin 3-glucoside	NI	57.2 ± 2.5	5.1 ± 0.9	57.5 ± 3.4
Petunidin 3-glucoside	NI	NI	28.1 ± 4.1	57.5 ± 3.4
Peonidin 3-glucoside	NI	NI	15.1 ± 2.4	NI
Malvidin 3-glucoside	NI	NI	1.9 ± 0.8	NI
Cyanidin 3-sophoroside	NI	NI	NI	0.4 ± 0.1
Cyanidin 3-glucosylrutinoside	NI	NI	NI	56.4 ± 3.8
Cyanidin 3-rutinoside	0.7 ± 0.1	25.0 ± 2.8	NI	19.6 ± 1.2
Pelargonidin 3-glucoside	347.8 ± 10.5	NI	NI	NI
Pelargonidin 3-rutinoside	52.4 ± 4.8	NI	NI	NI
Peonidin 3-rutinoside	7.6 ± 1.4	NI	NI	NI
Cyanidin 3-xyloside	NI	48.3 ± 5.6	NI	NI
TAC	407.8 ± 16.8	647.0 ± 19.2	77.5 ± 11.3	133.9 ± 8.4

NI: not identified. Anthocyanins were tentatively identified according to [16–21]. Individual anthocyanins were quantified by comparisons with an external standard of cyanidin 3-rutinoside at 510 nm.

acid digestion was performed for 2 h at 37°C, in a bath with constant mild agitation, mimicking the peristalsis and human body temperature. During this time, it was observed every half hour maintaining the pH = 2. Secondly, an aliquot of 20 mL (aliquot 1) of the sample was added to 5 mL of solution of bile salts and pancreatin and titrated with NaOH to pH 7. Another aliquot (aliquot 2) of 20 mL remained in an ice bath since the acid digestion maintained stopped.

Third, aliquot 2 is subjected to a second digestion and dialysis, at 37°C for 2 h in a water bath with constant stirring moderately, simulating human conditions. Membranes were filled with 25 mL of water and known amounts of $NaHCO_3$ equivalent to the previous valuate acidity ($NaHCO_3$ equivalents necessary for dialyzed mixture of pepsin and biliary-pancreatic extracts at pH 7.5 mL of mixture of biliary-pancreatic extracts) were added, and enzyme was allowed to act for 2 h at physiological temperature to obtain a balance between the dialyzed fraction (bioavailable) and the nondialyzed fraction (not bioavailable). Finally, dialysate was collected, filtered through a membrane filter 0.45 μm Millex-HV13 (Millipore, USA), and stored at −80°C until analysis. Compounds present in both fractions were then analyzed, quantifying the volume of the dissolution.

2.7. Statistical Analysis. Statistical analysis was performed using SPSS software. Duncan's multiple range tests were applied to determine differences between group means. A Pearson correlation test (r) was conducted to determine correlations between variables.

3. Results and Discussion

3.1. Chemical Composition: Total Phenolic Content by Folin-Ciocalteu Method and Anthocyanin Content by HPLC. The total phenolic content (TPC) measured by Folin-Ciocalteu method showed a statistical variability between berries ($p <$ 0.05, Figure 1). This TPC was higher in all berries analyzed than previously observed by Wang and Lin [25].

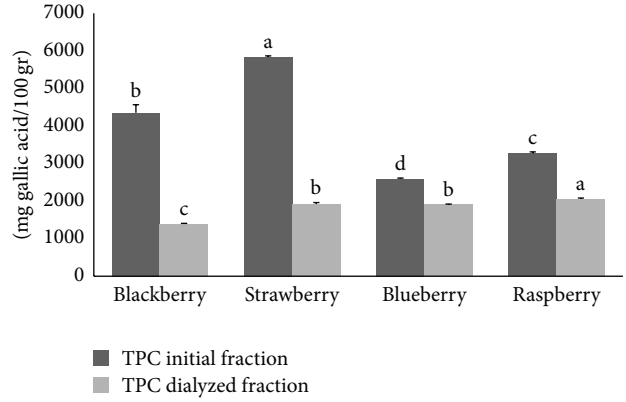

Figure 1: Total phenolic content. Results are expressed as mg gallic acid/100 gr. "a," "b," "c," and "d" indicate the statistical differences between samples.

Individual anthocyanins were tentatively identified according to [16–21] and are represented in Table 1. All samples showed four different anthocyanins except blueberry, which showed five different anthocyanins. Pelargonidin 3-glucoside was the principal anthocyanin identified in strawberry (347.8 ± 10.5 mg/100 gr), followed by pelargonidin 3-rutinoside (52.4 ± 4.8 mg/100 gr), peonidin 3-rutinoside (7.6 ± 1.4 mg/100 gr), and cyanidin 3-rutinoside (0.7 ± 0.1 mg/100 gr). Kelebek and Selli [17] found cyanidin 3-glucoside in strawberry extracts, and two more esterified forms of pelargonidin. Nevertheless, they did not report any evidence of peonidin 3-rutinoside. Moreover, strawberry total anthocyanin content (TAC) found in their research was extremely lower compared to the TAC reported in the present study.

Blackberry showed the greater TAC of four berries, showing delphinidin 3-glucoside as the most abundant anthocyanin (516.5 ± 9.3 mg/100 gr). In turn, three cyanidin esterified forms (cyanidin 3-glucoside (57.2 ± 2.5 mg/100 gr), cyanidin 3-xyloside (47.3 ± 5.6 mg/100 gr), and cyanidin 3-rutinoside (25.0 ± 2.8 mg/100 gr)) were found in minor quantity. Lugasi et al. [18] analyzed different varieties of blackberries, reporting less TAC (between 50 and 233 mg/100 gr) than berries of the present survey.

Contrary to Huang et al. [19], blueberry exhibited the lower TAC of four berries. In the present study, blueberry showed similar content of delphinidin 3-glucoside (27.3 ± 3.1 mg/100 gr) and petunidin 3-glucoside (28.1 ± 4.1 mg/100 gr) but slightly lower peonidin 3-glucoside concentration (15.1 ± 2.4 mg/100 gr). However, our data are in agreement with the results obtained by Gavrilova et al. [20] in different varieties of blueberries, showing similar TAC (from 41.99 to 83.64 mg/100 g) and HPLC-elution conditions (formic acid (5%, v:v) in water (phase A) and methanol (phase B)) but still different anthocyanin glucosides profile.

Results from the present study revealed that raspberry TAC (133.9 ± 8.4 mg/100 gr) was slightly higher than blueberry's TAC (77.5 ± 11.3 mg/100 g). Cyanidin 3-glucoside (57.5 ± 3.4 mg/100 gr) and cyanidin 3-glucosylrutinoside (56.4 ± 3.8 mg/100 gr) were identified as major anthocyanins in raspberry, followed by cyanidin 3-rutinoside (19.6 ± 1.2 mg/100 gr) and cyanidin 3-sophoroside at very low concentration (0.4 ± 0.1 mg/100 gr). The results of the present study were higher than those reported by McDougall et al. [26] who reported lower TAC (from 14.5 to 78.4 mg/100 gr). However, our results are in the range observed in different commercial varieties of raspberries [21].

3.2. Antioxidant Activity of Berries by ORAC Method. The different methods available for the measurement of the antioxidant capacity offer different information because of their different reagents and chemical mechanism of action during each procedure. Therefore, approaching the study of the antioxidant activity of foods is advisable to use diverse methods [22].

Differences in values between different samples were minor, reporting similar antioxidant activity. Results are highlighted in Table 2. Strawberry exerts the highest antioxidant capacity when measured by DDPH, since raspberry antioxidant activity measured by ORAC is higher than the other berries. Wang and Lin [25] also analyzed the antioxidant activity of blackberry, strawberry, and raspberry by the ORAC method, showing minor results compared to those obtained in our study in all cases. Moreover, DPPH method showed higher concentration than other food matrices as wine or grapes [27].

It is important to note that the structure of the polyphenols determines the chemical absorption and effectiveness in the organism. Therefore, a high antioxidant capacity does not always mean to be more effective *"in vivo."* Finally, measuring bioavailability of the different food matrices allows determining the effectiveness of their phenolic composition.

Table 2: Antioxidant activity measured by ORAC. Values expressed as TE.

	ORAC (mM TE)	SD
Blackberry	39.16	0.251
Strawberry	37.23	0.321
Blueberry	33.16	0.450
Raspberry	32.77	0.172

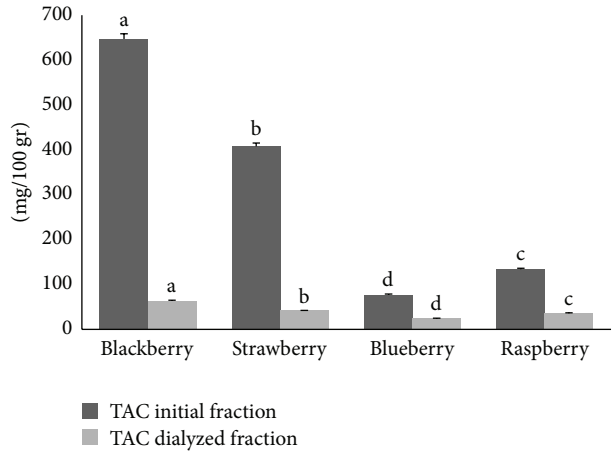

Figure 2: Total anthocyanin content. Results are expressed as mg anthocyanins/100 gr. "a," "b," "c," and "d" indicate the statistical differences between samples.

3.3. "In Vitro" Digestion Consequences on Total Phenolic Content. Depending on the berry analyzed, decreases between 27 and 67% were observed after *"in vitro"* digestion. A reduction of 67% was found after the digestion of strawberries, followed by a decrease of 32% for blackberry and a decrease of 63% for raspberry; finally a decrease of 27% was observed after the digestion of blueberries (Figure 1). In other food matrices, *"in vitro"* bioavailability studies have been carried out observing results that are in agreement with those obtained in the analyzed berries. Fazzari et al. [28] found a decrease between 70 and 74% of total phenols measured in frozen cherries. Other authors observed a bioavailability of 10.3% of total phenols in raspberry, lower values than those observed in our study (63% in case raspberries and 33% for strawberries) [29, 30]. Moreover, McDougall et al. [26] observed a bioavailability of 7.2% in red wine, resulting in a poor bioavailability compared with the results found in our study with different berries.

3.4. "In Vitro" Digestion Consequences on TAC. TAC shows great variability in all different berries analyzed. Values decreased from 68.3% for blueberries to 90.1% in case of blackberries, comprising 89.8% for strawberries and 73% for raspberries. Results are expressed and compared with the initial fraction in Figure 2. Interestingly, blackberry and strawberry, which showed the greater TAC, lead to lowest bioavailability of the four analyzed berries. More simulated digestion studies with berries would clarify the bioavailability potential of these fruits. In turn, comparison of *"in vitro"* and

"in vivo" studies would lead to a better comprehension of the berries matrices.

Results of McDougall et al. [29] differ from our reports. They reported only a 5.5% of anthocyanin availability after a simulated gastrointestinal digestion of raspberry. McDougall et al. [30] evaluated various soft fruits, including strawberries and blueberries. They found about 1% of anthocyanins in the dialyzed fraction, which represents a very low range of availability compared with our results.

Some authors have studied the simulated bioavailability of different food matrices. Higher values were found by Fazzari et al. [28], who reported 15–21% of anthocyanin availability after *"in vitro"* digestion of frozen sweet cherries. Chokeberries were quantified by Bermúdez-Soto et al. [31], presenting an increase in the availability fraction, showing more than 57% of anthocyanins after *"in vitro"* digestion.

Anthocyanins are very reactive compounds and susceptible to multiple factors such as temperature, light, pH, and enzymes/oxygen action [32]. During *"in vitro"* and *"in vivo"* digestion, pH varies from 2 to 7, depending on the location of digestion (stomach or intestine). Therefore, chemical structure of anthocyanins would vary along the broad digestion and pH-dependent balance between the five species (flavylium cation, carbinol base, chalcone, quinonoidal base, and anionic quinonoidal base) would lead to a disproportion on the initial structures of anthocyanins [32].

A significant decrease in anthocyanins of all berries was observed after pancreatic digestion (from 1265.4 to 166.3 mg/100 gr) (Figure 2). This decrease could be explained by the partial transformation of anthocyanins to colorless chalcones at pH 7.3, and the degradation of these substances along the intestinal tract [24]. Therefore, the fact that the availability of TPC is greater than the availability of TAC makes it evident that pH variations are in part responsible for the poor bioavailability of anthocyanins.

The health impact related to anthocyanins in epidemiologic studies refutes the apparent low bioavailability observed in the study. Taking into account the susceptibility of anthocyanins to pH modifications, changes in pH during *"in vitro"* digestion would affect their bioavailability and posterior identification on the dialyzed fraction in DAD-HPLC.

3.5. "In Vitro" Digestion Consequences on Antioxidant Activity by ORAC. After *"in vitro"* digestion, all analyzed berries presented a decrease in the antioxidant activity of about 90% (Figure 3). This decrease was higher for blackberry and lower in case of raspberry. Antioxidant activity of the berries after *"in vitro"* digestion measured by ORAC was 3.46 mM TE for blackberry (availability of 8.8%), 3.51 mM TE for strawberry (availability of 9.4%), 3.95 mM TE for blueberry (availability of 11.9%), and 4.73 mM for raspberry (availability of 14.4%). Notably, the matrices with the highest initial antioxidant activity did not lead to the highest antioxidant activity after the *"in vitro"* digestion. In fact, raspberry, which presented the minor initial antioxidant activity (32.77 mM TE), showed the higher ORAC values (4.73 mM TE) after *"in vitro"* digestion. On the contrary, blackberry (39.16 mM TE ORAC values) did not present de mayor values before *"in vitro"* digestion. Consequently, antioxidant activity after *"in vitro"* digestion is dependent on the food matrix and the method used for the analysis, more than the initial values.

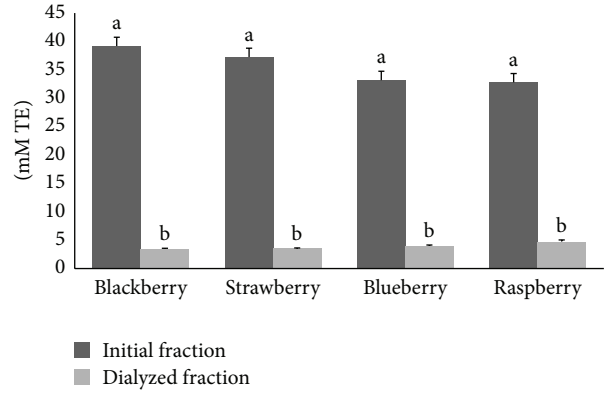

Figure 3: Antioxidant activity measured by ORAC. Results are expressed as mM TE. "a" and "b" indicate the statistical differences between samples.

Many published studies have observed different results. Cerezo et al. [33] subjected strawberries to an *"in vitro"* digestion, observing decrease of 50% on the antioxidant activity, supposing a higher availability values than those observed in our study. Tavares et al. [34] observed an 84% decrease in the antioxidant activity of blueberries, slightly higher availability than values reported in our study. Finally, Record and Lane [35] found moderate losses on the antioxidant activity of green and black tea extracts after *"in vitro"* digestion (about 25%).

3.6. Correlation between Antioxidant Capacity, Anthocyanin Content, and Phytochemical Content. To elucidate if phenolic compounds were responsible for the antioxidant activity of berries analyzed, statistical analyses were carried out comparing the different data obtained. The comparison of the antioxidant capacity measured by ORAC and the TPC measured by Folin-Ciocalteu method resulted in a direct and statistical correlation ($r = 0.755$; $p < 0.01$). That fact was previously reported by the scientific literature, showing that TPC contributed to the antioxidant activity of different berry fruits [10]. Correlation between TAC and ORAC or TPC measured by Folin-Ciocalteu method was also performed. In both cases, TAC-ORAC ($r = 0.978$; $p < 0.01$) and TAC-TPC measured by Folin-Ciocalteu method ($r = 0.703$; $p < 0.05$), the correlation found was direct and statistically significant. Judging by the results, anthocyanins seem to be the most abundant phenolic compounds in berries.

The correlation between the antioxidant activities measured by ORAC and TAC or TPC by Folin-Ciocalteu method was also performed after *"in vitro"* digestion. The results ($r = 0.620$; $p < 0.05$) highlight phenolic compounds as the principal antioxidant compounds in berry matrix after *"in vitro"* digestion. Similarly, the correlation between TAC-TPC by Folin-Ciocalteu method of dialyzed fraction showed a statistical direct correlation ($r = 0.835$; $p < 0.01$) after *"in vitro"* digestion.

These outcomes suggest that anthocyanins and TPC, still decreasing after *"in vitro"* digestion, remain in high enough concentrations to exert beneficial effects on the organism. Moreover, results strengthen and reinforce the previous knowledge about anthocyanins in berries [10].

4. Conclusions

Disproportion between oxidant and antioxidant species could lead to a disruption on the correct working of the organism. Polyphenols from foods exert multiple benefits on the organism, mainly due to their antiradical scavenging activity. Berries studied contain high concentrations of phenolic compounds, showing noticeable concentration of anthocyanins, as contrasted by the scientific literature. Blackberries and strawberries exert higher concentrations of anthocyanins than observed previously by other authors.

Despite their bioactivity, the bioavailability of the anthocyanins is shown to be low from all berries matrices. Blackberry and strawberry especially showed the lower availability range. It should be noted that the availability of total phenolic content is higher than that observed for anthocyanins. That fact could be explained by the pH fluctuation during digestion, which leads to changes in conformational form of anthocyanins, leading to the formation of uncolored chalcones which are not detected by fluorescence at the same range of anthocyanins.

When analyzing antioxidant activity by ORAC, all berries showed good scavenging activity, being similar both before and after *"in vitro"* digestion. It shows that concentration of polyphenols is not enough to determine the final antioxidant capacity of the different berries. However, bioavailability studies are necessary to conclude the antioxidant capacity once digested and absorbed.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank UCAM for the support bridged during the development of the assay for the study. Special thanks are due to Connor McMullen for the expert English reviewing of the paper.

References

[1] B. Halliwell and J. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, UK, 4th edition, 2007.

[2] M. Leopoldini, N. Russo, and M. Toscano, "The molecular basis of working mechanism of natural polyphenolic antioxidants," *Food Chemistry*, vol. 125, no. 2, pp. 288–306, 2011.

[3] A. Scalbert, C. Manach, C. Morand, C. Rémésy, and L. Jiménez, "Dietary polyphenols and the prevention of diseases," *Critical Reviews in Food Science and Nutrition*, vol. 45, no. 4, pp. 287–306, 2005.

[4] F. Visioli and A. Davalos, "Polyphenols and Cardiovascular disease: a critical summary of the evidence," *Mini-Reviews in Medicinal Chemistry*, vol. 11, no. 14, pp. 1186–1190, 2011.

[5] K. A. O'Leary, S. de Pascual-Teresa, P. W. Needs, Y. P. Bao, N. M. O'Brien, and G. Williamson, "Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription," *Mutation Research—Fundamental and Molecular Mechanisms of Mutagenesis*, vol. 551, no. 1-2, pp. 245–254, 2004.

[6] C. D. Sadik, H. Sies, and T. Schewe, "Inhibition of 15-lipoxygenases by flavonoids: structure-activity relations and mode of action," *Biochemical Pharmacology*, vol. 65, no. 5, pp. 773–781, 2003.

[7] S. de Pascual-Teresa, D. A. Moreno, and C. García-Viguera, "Flavanols and anthocyanins in cardiovascular health: a review of current evidence," *International Journal of Molecular Sciences*, vol. 11, no. 4, pp. 1679–1703, 2010.

[8] M. F.-F. Chong, R. MacDonald, and J. A. Lovegrove, "Fruit polyphenols and CVD risk: a review of human intervention studies," *British Journal of Nutrition*, vol. 104, no. 3, pp. S28–S39, 2010.

[9] A. Tarozzi, F. Morroni, A. Merlicco et al., "Neuroprotective effects of cyanidin 3-O-glucopyranoside on amyloid beta (25–35) oligomer-induced toxicity," *Neuroscience Letters*, vol. 473, no. 2, pp. 72–76, 2010.

[10] M. Mushtaq and S. M. Wani, "Polyphenols and human health—a review," *International Journal of Pharma and Bio Sciences*, vol. 4, no. 2, pp. B338–B360, 2013.

[11] T. Tsuda, Y. Ueno, T. Yoshikawa, H. Kojo, and T. Osawa, "Microarray profiling of gene expression in human adipocytes in response to anthocyanins," *Biochemical Pharmacology*, vol. 71, no. 8, pp. 1184–1197, 2006.

[12] K. Iwai, M.-Y. Kim, A. Onodera, and H. Matsue, "α-Glucosidase inhibitory and antihyperglycemic effects of polyphenols in the fruit of *Viburnum dilatatum* thunb," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 13, pp. 4588–4592, 2006.

[13] S. Adisakwattana, P. Charoenlertkul, and S. Yibchok-Anun, "α-Glucosidase inhibitory activity of cyanidin-3-galactoside and synergistic effect with acarbose," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 24, no. 1, pp. 65–69, 2009.

[14] C. Manach, A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez, "Polyphenols: food sources and bioavailability," *American Journal of Clinical Nutrition*, vol. 79, no. 5, pp. 727–747, 2004.

[15] L. Liang, X. Wu, T. Zhao et al., "*In vitro* bioaccessibility and antioxidant activity of anthocyanins from mulberry (*Morus atropurpurea* Roxb.) following simulated gastrointestinal digestion," *Food Research International*, vol. 46, no. 1, pp. 76–82, 2012.

[16] P. Zafrilla, A. Valero, and C. García-Viguera, "Stabilization of strawberry jam colour with natural colourants," *Food Science and Technology International*, vol. 4, no. 2, pp. 99–105, 1998.

[17] H. Kelebek and S. Selli, "Characterization of phenolic compounds in strawberry fruits by RP-HPLC-DAD and investigation of their antioxidant capacity," *Journal of Liquid Chromatography and Related Technologies*, vol. 34, no. 20, pp. 2495–2504, 2011.

[18] A. Lugasi, J. Hóvári, G. Kádár, and F. Dénes, "Phenolics in raspberry, blackberry and currant cultivars grown in Hungary," *Acta Alimentaria*, vol. 40, no. 1, pp. 52–64, 2011.

[19] W.-Y. Huang, H.-C. Zhang, W.-X. Liu, and C.-Y. Li, "Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing," *Journal of Zhejiang University: Science B*, vol. 13, no. 2, pp. 94–102, 2012.

[20] V. Gavrilova, M. Kajdžanoska, V. Gjamovski, and M. Stefova, "Separation, characterization and quantification of phenolic compounds in blueberries and red and black currants by HPLC-DAD-ESI-MS^n," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 8, pp. 4009–4018, 2011.

[21] L. Chen, X. Xin, H. Zhang, and Q. Yuan, "Phytochemical properties and antioxidant capacities of commercial raspberry varieties," *Journal of Functional Foods*, vol. 5, no. 1, pp. 508–515, 2013.

[22] K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, and D. Hawkins Byrne, "Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts," *Journal of Food Composition and Analysis*, vol. 19, no. 6-7, pp. 669–675, 2006.

[23] A. Dávalos, C. Gómez-Cordovés, and B. Bartolomé, "Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 1, pp. 48–54, 2004.

[24] A. Gil-Izquierdo, P. Zafrilla, and F. A. Tomás-Barberán, "An in vitro method to simulate phenolic compound release from the food matrix in the gastrointestinal tract," *European Food Research and Technology*, vol. 214, no. 2, pp. 155–159, 2002.

[25] S. Y. Wang and H.-S. Lin, "Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 2, pp. 140–146, 2000.

[26] G. J. McDougall, S. Fyffe, P. Dobson, and D. Stewart, "Anthocyanins from red wine—their stability under simulated gastrointestinal digestion," *Phytochemistry*, vol. 66, no. 21, pp. 2540–2548, 2005.

[27] J. Mulero, P. Zafrilla, J. M. Cayuela, A. Martínez-Cachá, and F. Pardo, "Antioxidant activity and phenolic compounds in organic red wine using different winemaking techniques," *Journal of Food Science*, vol. 76, no. 3, pp. 436–440, 2011.

[28] M. Fazzari, L. Fukumoto, G. Mazza, M. A. Livrea, L. Tesoriere, and L. Di Marco, "In vitro bioavailability of phenolic compounds from five cultivars of frozen sweet cherries (*Prunus avium* L.)," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 10, pp. 3561–3568, 2008.

[29] G. J. McDougall, P. Dobson, P. Smith, A. Blake, and D. Stewart, "Assessing potential bioavailability of raspberry anthocyanins using an in vitro digestion system," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 15, pp. 5896–5904, 2005.

[30] G. McDougall, P. Dobson, F. Shpiro, P. Smith, D. Stewart, and S. Fyffe, "Assessing bioavailability of soft fruit polyphenols in vitro," *Acta Horticulturae*, vol. 744, pp. 135–148, 2007.

[31] M.-J. Bermúdez-Soto, F.-A. Tomás-Barberán, and M.-T. García-Conesa, "Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to in vitro gastric and pancreatic digestion," *Food Chemistry*, vol. 102, no. 3, pp. 865–874, 2007.

[32] D. Del Rio, A. Rodriguez-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges, and A. Crozier, "Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases," *Antioxidants and Redox Signaling*, vol. 18, no. 14, pp. 1818–1892, 2013.

[33] A. B. Cerezo, E. Cuevas, P. Winterhalter, M. C. Garcia-Parrilla, and A. M. Troncoso, "Isolation, identification, and antioxidant activity of anthocyanin compounds in *Camarosa* strawberry," *Food Chemistry*, vol. 123, no. 3, pp. 574–582, 2010.

[34] L. Tavares, I. Figueira, D. MacEdo et al., "Neuroprotective effect of blackberry (*Rubus* sp.) polyphenols is potentiated after simulated gastrointestinal digestion," *Food Chemistry*, vol. 131, no. 4, pp. 1443–1452, 2012.

[35] I. R. Record and J. M. Lane, "Simulated intestinal digestion of green and black teas," *Food Chemistry*, vol. 73, no. 4, pp. 481–486, 2001.