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In Vitro Digestion of Betalainic Foods. Stability and Bioaccessibility of Betaxanthins and Betacyanins and Antioxidative Potential of Food Digesta

LUISA TESORIERE, MARCO FAZZARI, FRANCESA ANGILERI, CARLA GENTILE, AND MARIA A. LIVREA*

Dipartimento Farmacochimico Tossicologico e Biologico, Università di Palermo, Via M. Cipolla 74, 90128 Palermo, Italy

Betalains are considered to be bioactive dietary phytochemicals. The stability of betacyanins and betaxanthins from either fresh foods or manufactured products of cactus pear fruit (*Opuntia ficus indica* L. Mill. cv. Gialla and Rossa) and red beet (*Beta vulgaris* L. ssp. *vulgaris*) was assessed in a simulated oral, gastric, and small intestinal digestion and compared with the digestive stability of purified pigments. A minor loss of indicaxanthin, at the gastric-like environment only, and a decrease of vulgaxanthin I through all digestion steps were observed, which was not affected by food matrix. In contrast, food matrix prevented decay of betanin and isobetanin at the gastric-like environment. Loss of betacyanins, either purified or food-derived, was observed during the small intestinal phase of digestion. Betalamic acid accumulated after digestive degradation of purified pigments, but not of food betalains. Betaxanthins were wholly soluble in the aqueous (bioaccessible) fraction after ultracentrifugation of the postintestinal (PI) digesta, whereas release of betacyanins from the matrix was incomplete. PI digesta inhibited dose-dependently the oxidation of methyl linoleate in methanol, an effect not correlated with the betalain content. The data suggest that digestive stability controls bioaccessibility of dietary betaxanthins, whereas additional factors, relevant to the food matrix and style of processing, affect betacyanin bioaccessibility.

KEYWORDS: Betalainic foods; simulated digestion; betaxanthins; betacyanins

INTRODUCTION

A substantial amount of epidemiological data points to the consumption of a plant-based diet as a factor essential for human health. Apart from nutrients and vitamins, fruits and vegetables provide a vast number of polyphenol phytochemicals, the activity of which is now considered to be important to maintain the redox conditions necessary to control cell functions. Beetroot (*Beta vulgaris* L. ssp. *vulgaris*) and cactus pear fruit (*Opuntia ficus indica* L. Mill.) are pigmented foods; their intensive color is due to the presence of characteristic betalain pigments. These compounds are immonium derivatives of betalamic acid and include a quite modest number of structures. The violet-red betacyanins are conjugates of betalamic acid with either *cyclo*-DOPA or *cyclo*-DOPA glycosyl and acyl derivatives at the C-5 or C-6 positions, whereas the orange-yellow betaxanthins are conjugates with amino acids or amines (*1*) (**Figure 1**). A number of in vitro and ex vivo studies have shown that purified betaxanthins or betacyanins possess antioxidant activity in biological environments from human low-density lipoproteins to cell membranes and whole cells (*2*−*4*), can modulate redox-mediated signal transduction pathways involved in inflammation in cultured endothelium (*5*), and have antiproliferative effects in human tumor cell lines (*6, 7*). Although these studies may

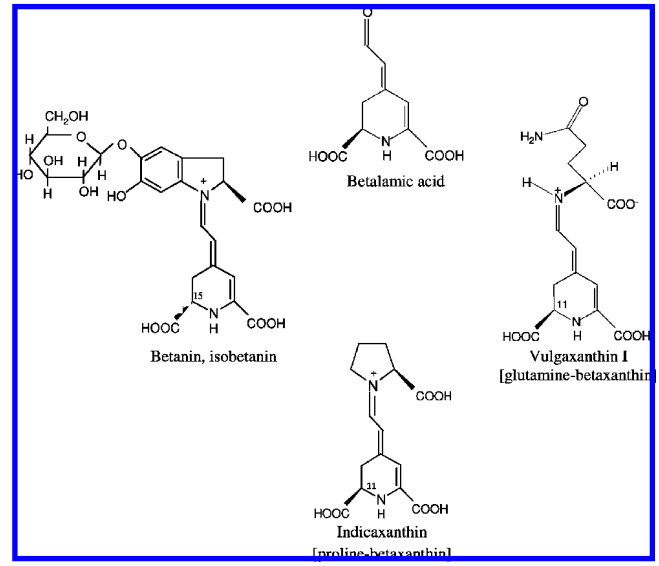

Figure 1. Betalamic acid and predominant betalains in cactus pear fruit and red beet.

* Corresponding author (e-mail mal96@unipa.it).

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suggest potential usefulness for human health, the real effectiveness of betalains as dietary compounds, and their bioactivity, can be assessed only after proving how much of the active molecule is absorbed and the eventual digestive transformations.

Studies on the bioavailability of betalains in humans are relevant to indicaxanthin and betanin from cactus pear fruits (*8*) and betanin from red beet juice (*9, 10*) and seem to indicate that indicaxanthin is much more bioavailable than betanin. However, various other exogenous, such as food source, or individual factors may affect the bioavailability of food components, so that in vivo findings from different study groups can hardly be compared.

The in vitro measurement of bioaccessibility is now considered to be a reliable tool to approach the bioavailability of a dietary compound, taking into account eventual variations due to food source, digestive stability of the molecule, and maximum solubility in the gastrointestinal (GI) medium, as an index of availability for eventual processes of apical uptake by absorptive epithelial cells. In light of the potential bioactivity of betalains, the present study was carried out to investigate digestive stability and bioaccessibility of a number of betacyanins and betaxanthins from betalain-rich foods. Cactus pear fresh fruit and juice, raw red beet, commercial either steamed red beet or red beet juice, and homemade red beet jam were processed through a simulated oral, gastric, and small intestinal digestion, mimicking the physicochemical and biochemical changes that occur in the upper GI tract, and the fate of the most representative betalains in these foods was monitored. Finally, because antioxidative properties of food may be an important means to protect the GI tract itself and avoid the eventual absorption of food-derived deleterious products (*11, 12*), the peroxyl radical scavenging activity of the postintestinal digest from the betalainic foods was also assessed.

MATERIALS AND METHODS

2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) was from Polyscience, Inc. (Warrington, PA). Unless stated otherwise, all reagents and materials were purchased from Sigma Chemical Co. (St. Louis, MO), and solvents were of the highest purity or HPLC grade.

Food Material. Fresh cactus pear fruits (*O. ficus indica* L. Mill.) from the yellow and red Sicilian cultivars, red beet roots (*B. vulgaris* L. ssp *vulgaris*) from Tuscany (Italy) cultivations, and packed steamed red beet (*B. vulgaris* L. ssp *vulgaris*) were obtained from a local market. Cactus pear fruits and raw red beet were selected at full maturity without being overripe. Red beet juice (Biotta AG, CH-8274, Tagerwilen, Switzerland) was purchased in a health food store in Palermo (Italy) and used without any manipulation. Cactus pear fruit juice was prepared as follows. After manual separation of the peel, the fruit pulp was weighed and briefly homogenized in a kitchen-type blender. The seeds were strained with a colander (0.2 mm mesh size), and the volume of the resulting juice was determined. The volume/weight conversion factors for the red and yellow cultivars were quite comparable (0.80 mL of juice per gram of pulp). Jam from red beet was freshly prepared by processing raw beet and white sugar (1:1, w/w) at boiling temperature, for 25 min, to jell. The preparation required the addition of water (1:4, v/w).

Processing of Food. Four cactus pear fruits were peeled, and pulp was gathered, chopped, and separated from seeds. Four raw red beets were peeled and cut. Juices and jam underwent processing without any preventive manipulation. Each betalainic food, portioned in 20 g samples, was submitted simultaneously either to in vitro digestion, as below described, or to homogeneization with 80 mL of saline, in a laboratory blender (Waring, New Hartford, CT) for 2 min. After the sample had been homogenized, the dispersing probe was submerged in clean saline (10 mL) and engaged for 5 s to collect the residual food sample, which was added to the initial homogenate. Aliquots of food homogenates were stored at −80 °C until measurement of betalain content.

In Vitro Digestion. The simulated in vitro digestion procedure was performed three times with different lots of each food.

Oral Phase. The oral phase relevant to all simulated digestion procedures was carried out by a single investigator, after an overnight fasting, to rule out potential interindividual and minimize intraindividual variations in the saliva composition. After the oral cavity had been rinsed with deionized water, 20 g of food sample, combined with 10 mL of physiological saline solution (saline, pH 7.4, 37 °C), was chewed 10 times and subsequently expelled into a tared beaker. The oral cavity was rinsed twice with 10 mL of saline, and the contents were expelled after each rinse into the beaker containing the chewed food. Saline (50 mL) was added, and the sample stirred for 2 min at room temperature. The sample was homogenized as described before and diluted to a final volume of 110 mL (postoral digest, PO). The final pH of the preparations ranged between 4.0 and 4.5. An aliquot of 20 mL was stored at −80 °C until analysis.

Gastric and Small Intestinal Phase. The sample from the oral phase was transferred to an amber bottle and acidified at pH 2.0 with HCl, and 8 mg/mL porcine pepsin (3200−4500 units/mg) was added. The bottle was then blanketed with nitrogen, sealed, and incubated in a shaking (100 rpm) water bath (type M 428-BD, Instruments s.r.l., Bernareggio, Mi, Italy) at 37 °C, for 2 h. Then the reaction mixture was placed on ice, and a 10 mL aliquot was stored at −80 °C (postgastric digest, PG). The pH of the remaining sample was immediately increased to 7.5 ± 0.2 with 0.5 N $NaHCO_3$, and the small intestinal phase of digestion was started after the addition of 2.4 mg/mL porcine bile extract and 0.4 mg/mL of pancreatin, an enzyme mixture of amylase, trypsin, lipase, ribonuclease, and protease, from hog pancreas (amylase activity > 100 units/mg). The amber bottle was blanketed with nitrogen, sealed, and incubated in the shaking water bath as above, for 2 h at 37 °C. At the end of the incubation, aliquots of the reaction mixture (postintestinal digest, PI) were stored at −80 °C until analysis. When required, purified betanin, indicaxanthin I, and vulgaxanthin I were submitted to all phases of the simulated digestion as above. Betanin underwent the intestinal-like digestion step either in the presence or in the absence of pancreatin.

Preparation of the Bioaccessible Fraction. The PI digest was centrifuged at 167000*g*, for 35 min at 4 °C in a Beckman Optima TLX ultracentrifuge, equipped with an MLA-55 rotor (Beckman Instruments, Inc., Palo Alto, CA), to separate the aqueous fraction (bioaccessible fraction) from particulate material. Aliquots of the supernatant were stored at −80 °C until analysis.

Extraction of Betalains. Frozen samples were thawed at room temperature and vortexed for 1 min. Aliquots (2 mL) were transferred to 25 mL centrifuge tubes and mixed with 1 volume of methanol. The mixtures were vortexed for 5 min and centrifuged at 14000*g*, for 10 min, at 4 °C. The supernatants were then recovered, and the extraction was repeated as above, until the absorbance of the methanol phase at either 482 or 536 nm was negligible. The extracts were combined and immediately filtered (0.2 μm syringe filter, Alltech Associates Inc., Deerfield, IL) and analyzed for betalain content.

HPLC. Analysis of betalains was carried out using a Gilson modular liquid chromatography system (Gilson Inc., Middleton, WI) equipped with M 302 and 305 pumps, and injector model 77-25 (Rheodyne, Berkeley, CA) with a 100 μL injector loop and a M 802 manometric module. The chromatographic column was a monolithic RP-18e Performance column (100 × 4.6 mm; Merck, Darmstadt, Germany) and an RP-18e Chromolith guard cartridge (5 × 4.6 mm, Merck). Detection was by an M 118 UV−vis detector, used along with the Gilson 712 HPLC system controller software. Sensitivity was 0.05% absorbance unit (AUFS). Elution was with a 20 min linear gradient elution from solvent A (1% acetic acid in water) to 20% solvent B (1% acetic acid in acetonitrile), at a flow rate of 1 mL/min. Vulgaxanthin I (3.8 min) and indicaxanthin (9.3 min) were spectrophotometrically detected at 482 nm, betalamic acid (8.2 min) was detected at 425 nm, and betanin (12.5 min), isobetanin (13.8 min), or betanidin (16.5 min) was detected at 536 nm. Suitable automatic wavelength changes allowed the detection of the compounds relevant to the different samples in the same run. With the exception of isobetanin, quantitation was by reference to standard curves constructed with 5−100 ng of purified betalains and by relating the amount of the compound under analysis

to the peak area. Betanin and indicaxanthin were extracted from fruit of *O. ficus indica* (red cultivar) as reported (*13*) and then purified according to Stintzing et al. (*14*) and spectrophotometrically quantified using molar absorbances of 65000 at 536 nm and 42800 at 482 nm, for betanin and indicaxanthin, respectively. Vulgaxanthin I was isolated from aqueous extracts of red beet by liquid chromatography on a Sephadex G-25 column (80 × 2.2 cm) (*15*), and spectrophometrically quantified using a molar absorbance 25500 (*16*). Betanidin was obtained from 4.0 μM betanin by enzyme hydrolysis with 14 units/mL β-glycosidase in 50 mM acetate buffer (37 °C, 30 min) and spectrophotometrically quantified at 536 nm, with a molar absorbance 54000 (*17*). Betalamic acid was prepared by alkaline hydrolysis of purified indicaxanthin and spectrophotometrically quantified at 425 nm using a molar absorbance 30000 (*18*) The C-15 betanin epimer (isobetanin) peak was identified by the ratio R_t betanin/R_t isobetanin = 0.80 (*19*), and isobetanin was quantified by assuming the same betanin absorbance (*17*). The assignment of peak was initially made by spectral analysis.

Peroxyl Radical Scavenging Activity of PI Digest. Peroxidation of methyl linoleate was performed by incubating 300 mM linoleic acid methyl ester (LAME) and 2.0 mM AMVN, in a final methanol volume of 1.0 mL, in a water bath at 37 °C, under air (control), followed by HPLC analysis of the products using the instrument described above. Portions of the mixture (10 μL) were taken at intervals and injected onto a Supelco Supelcosil (Bellafonte, PA) LC-18 colum (250 × 4.6 mm, i.d. = 5 μm), equilibrated and then eluted with methanol at a flow rate of 1.0 mL/min. LAME hydroperoxides were detected at 234 nm, and quantitation was by reference to a standard curve constructed with known amounts of linoleic acid hydroperoxide. Methanol extracts of PI digesta, relevant to 0.1−0.4 mg of fresh food, were added to the solution of methyl linoleate and allowed to equilibrate at 37 °C for 60 s. The azo-initiator was added, and the incubation was carried out as above. When required, the antioxidant activity of either individual purified betalains or α-tocopherol, in the range from 1 to 10 μM, was assayed in the same system.

Statistical Analysis of Data. Three independent in vitro digestion procedures were performed with three different samples of each food. From each procedure multiple samples from PO (n = 3), PG (n = 3), PI (n = 4), and bioaccessible fraction (n = 4) were analyzed. All data are expressed as means ± SD. Calculations and graphs were obtained by Instat-3 statistical software (GraphPad Software Inc., San Diego, CA), using repeated-measures ANOVA test, with Bonferroni's correction for multiple comparisons. In all cases, significance was accepted if the null hypothesis was rejected at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Betalains, recently investigated as bioavailable phytochemicals (*8*−*10*), occur only in a few plant foods, including beetroot and cactus pear fruits. In the present study fresh beetroot and cactus pear fruits, and manufactured products from these sources, have been submitted to a simulated GI digestion, and the stability and bioaccessibility of a number of betalains was evaluated. The amount of individual betalains chosen for the purpose of the study was first determined in our dietary sources, and data are reported in **Table 1** on a weight basis. Although *O. ficus indica* fruits grown in California show substantial amounts of vulgaxanthin I (*19*), only traces were detected in the Sicilian fruits in other studies (*14*), and no vulgaxanthin I was found in the fruits used in the present work. All manufactured foods from red beet show a total loss of vulgaxanthin I and substantially lower amounts of betanin than the fresh vegetal, as well as an increased isobetanin/betanin ratio. In accordance with other data, heat-processing of beetroot or acidification of juice may account for this evidence (*17, 20*). On the other hand, when the juice volume/pulp weight ratio was corrected for, the freshly prepared cactus pear juice did not show variation of total betalains with respect to fruits, indicating that betalains were not susceptible to the procedure used. Finally, although moderate amounts of betanidin have been reported in other commercial beetroot juice (*20*), this compound was not found in our red beet juice or in any of our either raw or heated foods (not shown). No food contained betalamic acid.

Table 1. Betalains in Various Foods under Study

	mg/100 g[a]			
	betanin	isobetanin	indicaxanthin	vulgaxanthin I
cactus pear				
yellow fruit	0.9 ± 0.09	nd	12.32 ± 1.01	nd
red fruit	14.1 ± 1.0	0.96 ± 0.08	4.70 ± 0.35	nd
yellow fruit juice	1.12 ± 0.09	nd	15.40 ± 1.21	nd
red fruit juice	17.5 ± 1.25	1.15 ± 0.05	5.87 ± 0.45	nd
red beet				
raw	71.4 ± 4.2	5.42 ± 0.45		81.6 ± 6.2
steamed	15.9 ± 1.1	4.77 ± 0.35		nd
jam	20.3 ± 1.5	15.62 ± 1.00		nd
juice	15.9 ± 1.2	13.35 ± 1.10		nd

[a] Values are the mean ± SD of three determinations carried out on different lots of food (n = 3). nd, not detectable.

The betalainic foods were submitted to the in vitro simulated GI digestion, and the stability of individual betalains was checked by measuring the amount of compounds remaining after each digestion step (**Figure 2**). Indicaxanthin from fruits or juice of either red or yellow cactus pear cultivars underwent a 20% loss during the gastric-like digestion, with no further loss in the PI digest (**Figure 2A**). Vulgaxanthin I, shown to be a poorly stable betalain under a number of conditions (*21*), underwent a progressive decay through all digestion phases, with a total 70% loss (**Figure 2B**). Betanin and isobetanin did not show substantial variations at the end of the gastric-like digestion, whether raw or manufactured food source. A decrease of both betacyanins by about 50% was observed in the PI digest (**Figure 2C**,**D**), with the exception of isobetanin from the red beet juice and jam, the decay of which was higher (85 and 70%, respectively, **Figure 2D**). This finding suggests that components of the mucillagenous pulp material and/or heat-sensitive beetroot compounds may substantially affect isobetanin loss.

Both betacyanins and betaxanthins have a broad pH stability in the range pH 3−7 (*21*), with molecular cleavage, decarboxylation, and formation of other products at very acidic or basic pH (*21*). In the applied digestion model, at 37 °C, decay of betaxanthins and betacyanins at the gastric-like environment may be expected as the result of the strongly acidic pH. In this light, the observed stability of betanin and isobetanin in the gastric-like milieu was noticeable and suggested some food matrix influence. Attack by the amylase activity of pancreatin could account for the quite high decay of both betalains observed in the moderately basic intestinal-like milieu. Unfortunately, possibly because of the high instability of betanidin at pH >6.0 (*22*), we failed to isolate this reaction product. Betalamic acid (BA), as the product of the hydrolytic cleavage of the immine C−N bond of betalains was also researched; however, it was not observed in any of the PI digesta (not shown).

For comparative reasons, amounts of purified betanin, vulgaxanthin I, and indicaxanthin, comparable with the the molar amounts measured in raw red beet and cactus pear fruit, respectively, were individually submitted to the simulated digestion. Decay of either indicaxanthin or vulgaxanthin I was quite comparable with that observed when the molecules underwent digestion in their matrix (**Figure 3A**). In contrast, whereas betanin had appeared to be stable during the gastric-like digestion when in its food matrix, the purified compound underwent a remarkable loss in the gastric-like milieu, with an additional decrease in the intestinal-like milieu (**Figure 3A**).

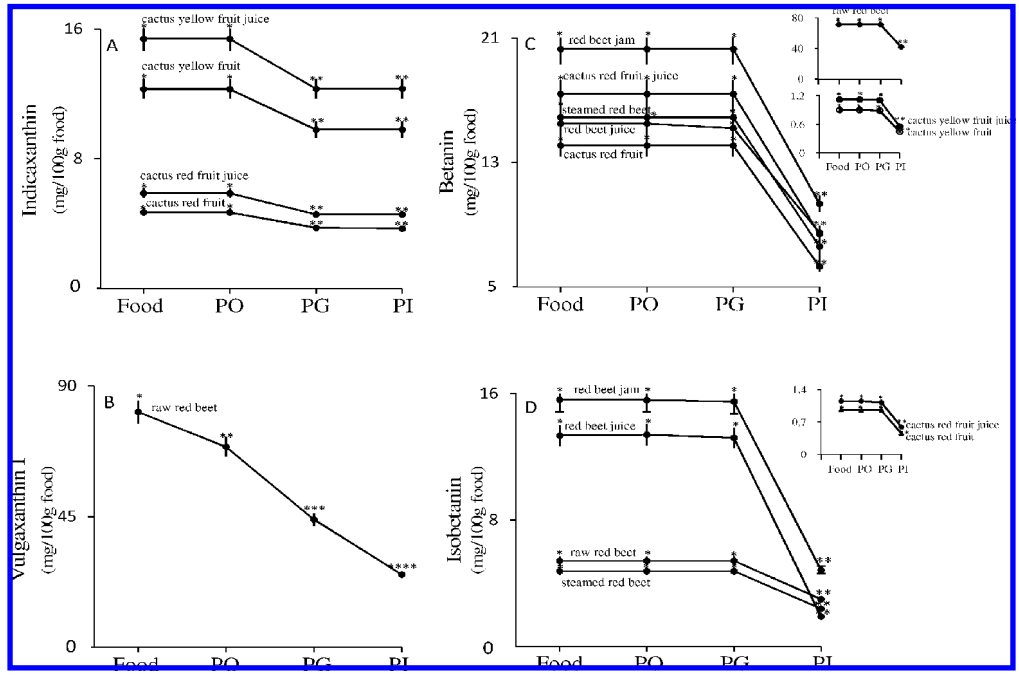

Figure 2. Decay of betalains during simulated gastrointestinal digestion of betalainic foods. Means in a line with different symbols are significantly different, $P < 0.05$ (repeated-measures ANOVA followed by a Bonferroni corrected *t* test).

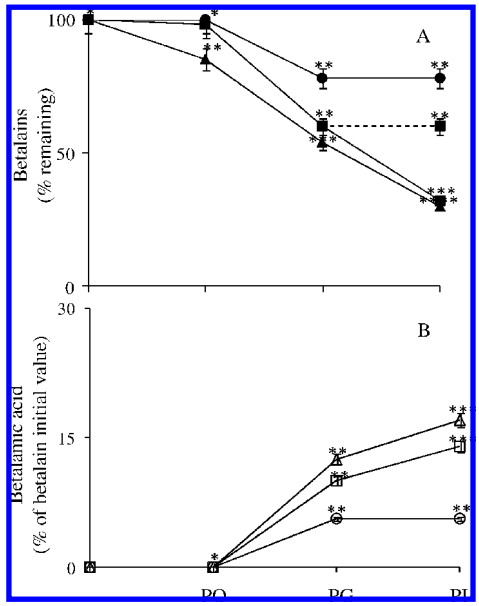

Figure 3. Decay of purified indicaxanthin (●), vulgaxanthin I (▲), and betanin (■) (**A**) and relevant formation of betalamic acid (open symbols) (**B**) during simulated gastrointestinal digestion. Dashed line refers to small intestinal-like digestion in the absence of pancreatin. Means in a line with different symbols are significantly different, $P < 0.05$ (repeated-measures ANOVA followed by a Bonferroni corrected *t* test).

The intestinal step of the simulated digestion was also carried out in the absence of pancreatin to assess the relevance of pancreatic enzyme activities on the betanin decay. No loss of compound was observed (**Figure 3A**), which suggested a role for the pancreatic amylase in the betanin degradation. Quite interestingly, the simulated digestion of all purified pigments resulted in accumulation of BA (**Figure 3B**). Betanidin, instead, was not found (not shown).

The extent of pigment loss, and also the degradation path, would be determined by the presence and nature of the accompanying food matrix (*23*). Taken together, our results show that the matrix of foods investigated while making betacyanins stable at the acidic environment can also affect the degradation path of betanin, indicaxanthin, and vulgaxanthin I. Betalamic acid, which is poorly stable at acidic pH but quite stable at basic pH (*24*), was observed only after simulated digestion of purified betalains. Remarkable reducing activity of BA has been reported (*25*), which may suggest oxidation by food components and/or products from digestion. Other authors (*26*) have reported decay of betacyanins and betaxanthins in ethanol extracts from *B. vulgaris* hairy root culture submitted to a simulated digestion process. Although the pattern of stability of betaxanthins has appeared to be comparable with our findings, degradation of betacyanins at the gastric-like environment was evident, which may further highlight the importance of food matrix in affecting betacyanin stability to the acidic pH.

The PI digesta were submitted to ultracentrifugation to estimate betalain bioaccessibility, which is the fraction of soluble betalains potentially available for uptake by absorptive epithelial cells. The molar amount of individual betalains recovered in the supernatant (bioaccessible fraction) is reported in **Table 2**, in comparison with the amount of betalains in the PI digesta before centrifugation. The partition of either betaxanthins or betacyanins in the soluble fraction appeared to differ remarkably. Indicaxanthin and vulgaxanthin I were totally recovered in the bioaccessible fraction, whereas the recovery of betanin and isobetanin ranged between 90 and 37% according to the food matrix (**Table 2**). Then, besides the stability to the digestive environment, the bioaccessibility of betacyanins may depend on additional factors. Eventual precipitation of betacyanins with cell particulate was considered in the light that previous studies have shown that betanin, but not indicaxanthin, can locate at the lipid core of a phospholipid bilayer (*27*). However additional experiments, where the simulated small intestinal digestion was carried out in the absence of bile extract, showed that the partition of betacyanins in the aqueous fraction of the digesta was comparable with that observed in its presence (data not shown), indicating that betalain solubilization did not depend on micellarization. Binding of betacyanins to insoluble material, including protein aggregates possibly formed during digestion,

Table 2. Recovery of Individual Betalains in the Postintestinal (PI) Digest and in the Bioaccessible Fraction from Foods Submitted to a Simulated Digestive Process

betalain	food source	PI digest (μM)	bioaccessible fraction[a]	
			μM	% of food content
indicaxanthin	cactus yellow fruit	31.2 ± 2.5	31.0 ± 3.1	77 ± 4.5
	cactus red fruit	11.7 ± 1.2	11.9 ± 1.1	78 ± 5.0
	cactus yellow fruit juice	39.1 ± 3.9	38.5 ± 3.2	77 ± 4.3
	cactus red fruit juice	15.3 ± 1.2	15.4 ± 1.1	77 ± 5.0
vulgaxanthin I	raw red beet	74.0 ± 6.5	73.6 ± 6.9	30 ± 1.8
betanin	raw red beet	78.5 ± 6.8	64.5 ± 5.9a	50 ± 3.0
	steamed red beet	16.7 ± 1.5	7.8 ± 0.9a	27 ± 2.5
	red beet jam	22.2 ± 1.9	16.9 ± 1.8a	46 ± 2.8
	red beet juice	14.2 ± 1.3	10.9 ± 1.0a	38 ± 2.9
	cactus yellow fruit	0.8 ± 0.1	0.7 ± 0.1b	45 ± 3.1
	cactus red fruit	11.5 ± 1.0	10.2 ± 0.9a	40 ± 2.2
	cactus yellow fruit juice	1.0 ± 0.1	0.9 ± 0.1b	44 ± 3.3
	cactus red fruit juice	16.7 ± 1.1	13.9 ± 1.2a	44 ± 2.2
isobetanin	raw red beet	5.5 ± 0.4	4.8 ± 0.4a	49 ± 3.4
	steamed red beet	5.6 ± 0.4	2.1 ± 0.2a	25 ± 3.0
	red beet jam	8.5 ± 0.7	6.3 ± 0.6a	22 ± 1.7
	red beet juice	3.9 ± 0.4	2.6 ± 0.2a	11 ± 1.8
	cactus red fruit	0.8 ± 0.1	0.7 ± 0.1b	43 ± 2.2
	cactus red fruit juice	1.1 ± 0.1	0.9 ± 0.1a	44 ± 3.4

[a] Values are the mean ± SD of four determinations carried out on different food samples ($n = 3$): with respect to the relevant PI digest, values were significant with a, $P < 0.001$, and b, $P < 0.05$ (Student's t test).

could be hypothesized to explain the loss of betanin and isobetanin with the PI digest precipitate. When heat and pH sensitivity of the protein material are taken into consideration, the lower bioaccessibility of betanin and isobetanin from red beet jam and steamed red beet, as well as red beet juice, could support this hypothesis.

Bioaccessibility of betalains, expressed as the percent of the food content, is also shown in **Table 2**. Because of stability to the simulated GI digestion and solubility in the postintestinal digest, indicaxanthin from the cactus pear fruits appears to be by far the most bioaccessible among the betalains studied, with a release and solubilization in the PI milieu of 77% of the amount in the fruits, whereas the bioaccessibility of betanin and isobetanin varies between 11 and 50%, according to the food considered. Vulgaxanthin I from raw red beet showed a bioaccessibility of 30%.

Bioavailability of indicaxanthin and betanin from the Sicilian cactus pear fruits has been reported in humans (*8*). The present study offers the opportunity to compare and validate the in vitro bioaccessibility data with the bioavailability of these betalains under in vivo conditions. Although showing that indicaxanthin is more extensively preserved than betanin during the in vitro digestion, is entirely soluble in the GI digest, and is bioaccessible to the extent of 77% of the amount in fruits, our findings appear in accordance with the in vivo observations that the bioavailability of indicaxanthin (76 ± 3%) is much higher than that of betanin (3.7 ± 0.3%) and suggest that the digestive stability of the molecule may play the most important role in the in vivo absorption. On the other hand, other reasons including the presence of glucosidases at the intestinal level and/or mechanisms of intestinal uptake, could explain why the presently observed bioaccessibility of betanin from cactus pear fruits (40−45% of the fruit content) is quite higher than the bioavailability measured in vivo.

For the purpose of providing readily accessible information to the consumer, **Table 3** lists the bioaccessibility of betalains in terms of total amount of pigments per serving of commercial foods.

Betalainic fruits and vegetables possess antioxidant capacities (*8, 13, 19, 28*); however, the potential activity of these foods should be evaluated after simulated gastrointestinal digestion.

Table 3. Bioaccessibility of Betalains per Serving Size[a] of Commercial Foods

	serving description	serving size (g)	bioaccessibility[b] (mg of betalain/serving)
cactus yellow fruit	1 medium fruit	149	14.7
cactus red fruit	1 medium fruit	149	14.4
raw red beet	0.5 cup	68	42.4
steamed red beet	0.5 cup slices	85	4.6
red beet juice	8 oz (240 mL)	250	18.7

[a] USDA National Nutrient Database for Standard Reference. [b] Reported amounts are calculated from data in **Tables 1** and **2** and refer to the global amounts of betalains in each food source.

Then the antioxidative activity of hydrophilic extracts from the postintestinal digesta can be evaluated in an oxidation model of LAME, in the presence of a lipid-soluble azo-initiator. Only the commercial betalain-bearing foods were considered for these experiments. When assayed on a comparable weight basis, all postintestinal digesta showed a remarkable dose-dependent inhibitory effect ($0.98 < r^2 < 1.0$). When the amount of lipid hydroperoxides formed after a 30 min incubation in the absence of any extract is taken as a reference end-point, the inhibition can be expressed in terms of IC_{50}, that is, the amount of betalainic food required for a 50% inhibition (**Table 4**). The calculated IC_{50} values provide evidence that the antioxidant effectiveness of the raw red beet is quite higher than that of the other betalainic foods, at least under the applied in vitro conditions. Which food component(s) is/are relevant to the observed activity may be a matter of speculation. In the same LAME oxidation model, individual betalains such as indicaxanthin, vulgaxanthin I, and betanin exhibited IC_{50} values of a micromolar order comparable with vitamin E (**Table 4**), indicating high peroxyl radical scavenging effectiveness. However, no significant correlation was found between the IC_{50} values calculated for the PI digest extracts and their content of either individual or total betalains ($0.15 < r^2 < 0.25$), suggesting that the antioxidative activity of food extracts may depend on interactions between betalains and other food components.

In light of the fact that a number of dangerous products, including

Table 4. Inhibitory Effect on Methyl Linoleate Peroxidation of PI Digest from Foods Submitted to a Simulated Digestive Process and Individual Betalains

	IC_{50}[a] (μg of fresh wt)	IC_{50}[b] (μM)
cactus red fruit	650 ± 50	
cactus yellow fruit	600 ± 50	
raw red beet	30 ± 2	
steamed red beet	370 ± 40	
red beet juice	240 ± 30	
betanin		1.0 ± 0.10
indicaxanthin		0.7 ± 0.06
vulgaxanthin I		0.75 ± 0.07

[a] Values are the mean ± SD of two determinations carried out in duplicate with different food samples. [b] Values are the mean ± SD of three determinations carried out in duplicate.

lipoperoxides, can arise from food digestion with potential damage to the GI cells, the GI tract is now considered to be an important site for antioxidant action (*13, 29*). The antioxidant potential of codigested betalain-rich food could be of valuable protection. In this context, nutritional studies in healthy volunteers showed that cactus pear fruits may provide protection against oxidative stress as part of the regular diet (*30*).

LITERATURE CITED

(1) Strack, D.; Vogt, T.; Schliemann, W. Recent advances in betalain research. *Phytochemistry* **2003**, *62*, 247–269.

(2) Tesoriere, L.; Butera, D.; D'Arpa, D.; Di Gaudio, F.; Allegra, M.; Gentile, C.; Livrea, M. A. Increased resistance to oxidation of betalain-enriched human low density lipoproteins. *Free Radical Res.* **2003**, *37*, 689–696.

(3) Tesoriere, L.; Butera, D.; Allegra, M.; Fazzari, M.; Livrea, M. A. Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to ex vivo-induced oxidative hemolysis in humans. *J. Agric. Food Chem.* **2005**, *53*, 1266–1270.

(4) Tesoriere, L.; Allegra, M.; Butera, D.; Gentile, C.; Livrea, M. A. Cytoprotective effects of the antioxidant phytochemical indicaxanthin in β-thalassemia red blood cells. *Free Radical Res.* **2006**, *40*, 753–761.

(5) Gentile, C.; Tesoriere, L.; Allegra, M.; Livrea, M. A.; D'Alessio, P. Antioxidant betalains from cactus pear (*Opuntia ficus indica*) inhibit endothelial ICAM-1 expression. *Ann. N.Y. Acad. Sci.* **2004**, *1028*, 481–486.

(6) Sreekantah, D. S.; Arunasree, M. K.; Roy, K. R.; Reddy, T. C.; Reddy, G. V.; Reddanna, P. Betanin a betacyanin pigment purified from fruits of *Opuntia ficus indica* induces apoptosis in human chronic myeloid leukemia cell line-K562. *Phytomedicine* **2007**, *14*, 739–746.

(7) Reddy, M. K.; Alexander-Lindo, R. L.; Nair, M. G. Relative inhibition of lipid peroxidation, cycloxygenase enzymes, and human tumor cell proliferation by natural food colors. *J. Agric. Food Chem.* **2005**, *53*, 9268–9273.

(8) Tesoriere, L.; Allegra, M.; Butera, D.; Livrea, M. A. Absorption, excretion, and distribution in low density lipoproteins of dietary antioxidant betalains. Potential health effects of betalains in humans. *Am. J. Clin. Nutr.* **2004**, *80*, 941–945.

(9) Kanner, J.; Harel, S.; Granit, R. Betalains—a new class of dietary cationized antioxidants. *J. Agric. Food Chem.* **2001**, *49*, 5178–5185.

(10) Frank, T.; Stintzing, F. C.; Carle, R.; Bitsch, I.; Quaas, D.; Strass, G.; Bitsch, R.; Netzel, M. Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. *Pharmacol. Res.* **2005**, *52*, 290–297.

(11) Kanner, J. Dietary advanced lipid oxidation end-products are risk factors to human health. *Mol. Nutr. Food Res.* **2007**, *51*, 1094–1101.

(12) Halliwell, B.; Zhao, K.; Whiteman, M. The gastrointestinal tract: a major site of antioxidant action. *Free Radical Res.* **2000**, *33*, 819–830.

(13) Butera, D.; Tesoriere, L.; Di Gaudio, F.; Bongiorno, A.; Allegra, M.; Pintaudi, A. M.; Kohen, R.; Livrea, M. A. Antioxidant activities of Sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. *J. Agric. Food Chem.* **2002**, *50*, 6895–6901.

(14) Stintzing, F. C.; Schieber, A.; Carle, R. Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2002**, *50*, 2302–2307.

(15) Escribano, J.; Pedreno, M. A.; Garcia-Carmona, F.; Munoz, R. Characterization of the antiradical activity of betalains from *Beta vulgaris*, L. roots. *Phytochem. Anal.* **1998**, *9*, 124–127.

(16) Nilsson, T. Studies into the pigments in beetroot. *Lantbrukshoegsk. Ann.* **1970**, *36*, 179–219.

(17) Kugler, F.; Stintzing, F. C.; Carle, R. Identification of betalains from petioles of differently colored Swiss chard (*Beta vulgaris* L. ssp. *cicla* [L.] Alef. Cv. Bright Lights) by high-performance liquid chromatography−electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 2975–2981.

(18) Stintzing, F. C.; Herbach, K. M.; Mosshammer, M. R.; Carle, R.; Weiguang, Y.; Sellappan, S.; Akoh, C. C.; Bunch, R.; Felker, P. Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. *J. Agric. Food Chem.* **2005**, *53*, 442–451.

(19) Schwartz, S. J.; von Elbe, J. H. Quantitative determination of individual betacyanin pigments by high-perforance liquid chromatography. *J. Agric. Food Chem.* **1980**, *28*, 540–543.

(20) Herbach, K. M.; Stintzing, F. C.; Carle, R. Impact of thermal treatment on color and pigment pattern of red beet (*Beta vulgaris*, L.) preparations. *J. Food Sci.* **2004**, *69*, C491–C498.

(21) Herbach, K. M.; Stintzing, F. C.; Carle, R. Betalain stability and degradation. Structural and chromatic aspects. *J. Food Sci.* **2006**, *71*, R41–R50.

(22) Gandia-Herrero, F.; Escribano, J.; Garcia-Carmona, F. Characterization of the activity of tyrosinase on betanidin. *J. Agric. Food Chem.* **2007**, *55*, 1546–1551.

(23) Herbach, K. M.; Rohe, M.; Stintzing, F.; Carle, R. Structural and chromatic stability of purple pitaya (*Hylocereus polyrhizus* [Weber] Britton & Rose) betacyanins as affected by the juice matrix and selected additives. *Food Res. Int.* **2006**, *39*, 667–677.

(24) Huang, A. S.; Von Elbe, J. H. Effect of pH on the degradation and regeneration of betanine. *J Food Sci.* **1987**, *52*, 1689–1693.

(25) Livrea, M. A.; Tesoriere, L. Antioxidant activities of prickly pear (*Opuntia ficus indica*) fruit and its betalains. In *Herbal and Traditional Medicines*; Packer, L., Ong, C. N., Halliwell, B., Eds.; Dekker: New York, 2004; pp 537−556.

(26) Pavlov, A.; Kovatcheva, P.; Tuneva, D.; Ilieva, M.; Bley, T. Radical scavenging activity and stability of betalains from *Beta vulgaris* hairy root culture in simulated conditions of human gastrointestinal tract. *Plant Foods Hum. Nutr.* **2005**, *60*, 43–47.

(27) Turco-Liveri, M. L.; Sciascia, L.; Lombardo, R.; Tesoriere, L.; Passante, E.; Livrea, M. A. Spectrophotometric evidence for the solubilization site of betalain pigments in membrane biomimetic systems. *J. Agric. Food Chem.* **2007**, *55*, 2836–2840.

(28) Kugler, F.; Stintzing, F.; Carle, R. Evaluation of the antioxidant capacity of betalainic fruits and vegetables. *J. Appl. Bot. Food Qual.* **2007**, *81*, 69–76.

(29) Cohn, J. S. Oxidised fat in the diet, postprandial lipaemia and cardiovascular disease. *Curr. Opinion, Lipidol.* **2002**, *13*, 19–24.

(30) Tesoriere, L.; Butera, D.; Pintaudi, A. M.; Allegra, M.; Livrea, M. A. Supplementation with cactus pear (*Opuntia ficus indica*) fruits decreases oxidative stress in healthy humans. A comparative study with vitamin C. *Am. J. Clin. Nutr.* **2004**, *80*, 391–395.

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