Study on bioaccessibility of betacyanins from red dragon fruit (*Hylocereus polyrhizus*)

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Received: 6 September 2018 / Revised: 20 December 2018 / Accepted: 28 December 2018 / Published online: 6 February 2019
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Abstract Betacyanins are bioactive dietary phytochemicals which can be found in red dragon fruit (RDF). Therefore, the bioaccessibility of betacyanins that present in fermented red dragon fruit drink (RDFD) and pressed red dragon fruit juice (RDFJ) was accessed in simulated gastric and intestinal digestion. Results disclosed that betacyanins from RDFD and RDFJ suffered minor loss (< 25%) at gastric-like environment but greater loss was observed during the intestinal phase digestion. After subjected to intestinal digestion, RDFD retained 46.42% of

betanin while RDFJ retained 43.76%, with betanin concentration of 17.12 mM and 12.37 mM, respectively. Findings also revealed that RDFD exhibited higher antioxidant capacity compared to RDFJ after subjected to intestinal digestion, with values of 0.88 mM Trolox equivalent antioxidant capacity (TEAC) and 0.85 mM TEAC, respectively. The data suggests that betacyanins that present in RDF are bioaccessible while fermentation able to enhance the bioavailability with more betacyanins retained after intestinal digestion.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10068-018-00550-z>) contains supplementary material, which is available to authorized users.

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Keywords Fermentation · Bioaccessibility · Betacyanins · Antioxidant · Bioactive compounds

Introduction

There are a few known edible sources of betacyanins which include red beetroot, colored Swiss chard, leafy amaranth and cactus fruit. Betacyanins have been studied with more intensity nowadays due to their important role in human health with various pharmacological activities such as antioxidant, anti-cancer, anti-lipidemic and antimicrobial (Gengatharan et al., 2016). Despite having the potential benefits, the low bioavailability of betanin has to be addressed. The bioavailability of betanin is rather low with only 0.5–0.9% among human volunteers using red beet juice (Kanner et al., 2001). A study done by Tesoriere et al. (2004) showed that 0.2 µM plasma concentration is attained after single ingestion of 500 g cactus pear fruit pulp, providing 16 mg of betanin, with bioavailability around 0.68%. The findings from both studies showed that the bioavailability of betanin is lower than 1% of the administered amount.

The possible effectiveness of phytochemicals is determined by bioavailability, which is affected by a large number of factors. The low bioavailability of betanin could be attributed to food source and processing, food matrix, digestive instability, and mechanisms of absorption (Livrea and Tesoriere, 2006; Pavokovic and Krsnik-Rasol, 2011). Food matrix is among the factors that affect the bioavailability of a compound as it can alter the pharmacokinetic profiles. Findings reported by Cassidy et al. (2006) showed that isoflavones from liquid soya foods were absorbed more quickly and extensively. Moreover, results also disclosed that the liquid matrix yields a higher peak plasma concentrations than a solid matrix. This could deduce by later stomach emptying after ingestion of solid foods compared to liquid food matrices. Therefore, the complexity of the food matrix in which the bioactive compounds are contained serves as an important challenge to determine bioaccessibility of food and beverage constituents.

Food matrix is characterized by several components, including composition, pH, processing, structure, type of product and viscosity (Sensoy, 2014). Besides, it has been reported that the intestinal absorption of fruit juices bioactive compounds is even better than that coming directly from fruits (Perales et al., 2008). This showed that the type of food matrix (solid or liquid) significantly influences the bioaccessibility and bioavailability of bioactive compounds. In addition, the study showed that betanin in red beet juice and puree showed better thermal stability than betanin in a buffer solution, suggesting a protective matrix effect (Mika, 2014). On the other hand, processing such as thermal treatment and fermentation could influence on the bioaccessibility of bioactive compounds from beverages through the change in natural matrix of them (i.e. pH, viscosity, brix, etc.) or in their microstructure (i.e. cell wall rupture, release of bounded compounds, changes in their solubilisation, etc.) (Parada and Aguilera, 2007). This is possible through break down of cell walls of plant tissues or the nutrient-matrix complexes, or conversion into more active molecular structures (Guldiken et al., 2016).

A review of the literature has yielded only studies on the bio-accessibility of betacyanins from beetroot and cactus pear fruit (Guldiken et al., 2016; Tesoriere et al., 2008). To our knowledge, to date, there is no finding on the bio-accessibility of betacyanins from red dragon fruit juice or its fermented form. Juice is chosen over the fruit itself due to the advantages of liquid matrix compared to solid. In this regard, it is deemed worthwhile to study on this topic. Therefore, the present work was conducted in order to evaluate the bioaccessibility of betacyanins from pressed red dragon fruit juice and fermented red dragon fruit drink.

Materials and methods

Reagents

Chemical reagents used in this study were porcine pepsin (> 400 Units/mg solid), porcine pancreas pancreatin, porcine bile extract, sodium bicarbonate, hydrochloric acid, potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), and ($\pm$)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) all obtained from Sigma-Aldrich (St. Louis, MO, USA), betanin (TCI, Tokyo, Japan), methanol HPLC grade (Merck, Darmstadt, Germany), orthophosphoric acid (R&M Chemical, Essex, UK), Milli-Q system (Millipore, Burlington, MA, USA) deionized water and ultra-pure water were used throughout this study.

Raw materials

Red dragon fruits (RDF) with an average weight of 0.4–0.6 kg were purchased from a local market (Aeon, Kuala Lumpur, Malaysia). The selected fruits were all at commercial maturity level; without damage, insect and foreign matter. Brown sugar (Gula Prai, MSM Holdings, Kuala Lumpur, Malaysia) was purchased from local markets. RDF was rinsed with tap water to remove dirt and residues followed by pat-dry with kitchen towel paper. Then, the skin of RDF was peeled while the flesh was cut into pieces with each having an average thickness of 5 mm (Foong et al., 2012).

Preparation of samples

Fermented red dragon fruit drink

Fermentation of RDF was carried out in 2 L stainless steel fermentation tank according to Foong et al. (2012) with slight modifications. Red dragon fruit pieces were arranged layer by layer alternately with sugar. Then, fermentation tank was closed tightly and stored for 8 weeks in a clean cabinet at 25 °C. The fermented red dragon fruit drink (RDFD) from the tank was strained, pasteurized at 75 °C for 15 s and stored in individual amber bottles with lid at – 20 °C until further analysis.

Red dragon fruit juice

The red dragon fruit juice (RDFJ) was prepared by pressing the peeled fruit (MJ-70M-UB, Panasonic, Osaka, Japan) then filtered through stainless steel wire mesh (0.18 mm

pore size) to obtain purified juice and stored in individual amber bottles with lid at $-20\text{ }^{\circ}\text{C}$ until further analysis.

Simulated in vitro digestion

The simulated in vitro digestion model was performed in triplicate based on the methodology described by Tesoriere et al. (2008) and Antunes-Ricardo et al. (2017) with slight modifications. A two-step procedure was applied to stimulate the digestive processes in the stomach and small intestine. The gastric digestion started by adjusting the 20 mL sample pH to 2 with 1 M HCl and after, 2 mL of porcine pepsin solution was added (40 mg/mL in 0.1 M HCl). This mixture was blanketed with nitrogen, sealed and incubated in a shaking water bath (100 rpm) for 1 h at $37\text{ }^{\circ}\text{C}$. Then, the reaction mixture was placed on ice, and a 10 mL aliquot was stored at $-20\text{ }^{\circ}\text{C}$ (post gastric digest, PGD). Then, the pH of the remaining sample was immediately increased to 7.5 ± 0.02 with 0.5 N NaHCO_3 , and the small intestinal phase digestion was started after the addition of a mixture of bile salts and pancreatin (2 mL containing porcine pancreatin (2 mg/mL) and bile salts (12 mg/mL) dissolved in a 100 mM NaHCO_3 solution. Concentrations of pancreatin and bile extract in the final solution were 0.4 and 2.4 mg/mL, respectively. The mixture was blanketed with nitrogen, sealed and incubated in the shaking water bath as above, for 2 h at $37\text{ }^{\circ}\text{C}$. At the end of the incubation, aliquots of the reaction mixture (post intestinal digest, PID) were stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Preparation of bioaccessible fraction

The PGD and PID digest of both samples were centrifuged (X-22R, Beckman Coulter, Brea, CA, USA) at 10,000 rpm at $4\text{ }^{\circ}\text{C}$ for 5 min to separate the aqueous fraction from particulate material. The recovery percentage of betacyanins was calculated based on the concentration of the supernatant recovered after each phase (gastric and intestinal) of the in vitro digestion. The bioaccessible fractions were subjected to betacyanin analysis and antioxidant scavenging capacity analysis (Tesoriere et al., 2008).

Betacyanins analysis

Betacyanins quantification was done according to Foong et al. (2012) with slight modifications. A Agilent 1200 series HPLC system (Agilent, Santa Clara, CA, USA) fitted with G1322A degasser, C1325B injector, G1329A column oven and G1315D diode array detector (DAD) was employed. Working solutions of betanin standard which consists of betanin and isobetanin (2 g/L, 4 g/L, 6 g/L, 8 g/L

and 10 g/L) was prepared. Each sample was analyzed in triplicate.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging assay was carried out according to Rebecca et al. (2010) with minor modifications. 0.1 mL of sample was reacted with 3.9 mL of 80% ethanolic 60 μM DPPH solution in a test tube. The test tube was vortexed for 15 s and solution was allowed to stand in dark at a controlled temperature ($25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) for 30 min. Absorbance was measured at 515 nm. Antioxidant activity was expressed by (i) calculating the radical scavenging activity: Median effective concentration (EC_{50}) = concentration of sample required to decrease 50% in absorbance of DPPH radicals and (ii) inhibition (%) of DPPH $\text{absorbance} = (A_{\text{control}} - A_{\text{test}}) \times 100/A_{\text{control}}$. Ethanol (80%) was used as blank and DPPH solution without test sample was used as control. A dose–response curve (% inhibition of DPPH versus concentration of sample (v/v) %) was established and the EC_{50} was determined using Trolox (0.1–0.8 mM) as a standard with calibration equation of $y = 112.94x + 2.0021$ ($R^2 = 0.998$). Results were expressed as Trolox equivalents antioxidant capacity (TEAC).

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging capacity

The ABTS radical scavenging assay was carried out according to Foong et al. (2012). ABTS radical solution was prepared by mixing 10 mL of 7 mM ABTS solution with 10 mL of 2.45 mM potassium persulfate solution. The mixture was stored in a cabinet at room temperature for 12–16 h before use. The ABTS radical solution was adjusted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm before use. The sample (25 μL) was added to 975 μL of ABTS radical solution and allowed to react for 6 min. The absorbance of the sample was measured at 734 nm against blank. The percentage of ABTS radical scavenging capacity was calculated as $[1 - (A_e/A_c)] \times 100\%$ ($A_e = A_{734}$ in the presence of sample; $A_c = A_{734}$ of negative control solution). The ABTS was expressed as millimoles of Trolox equivalents antioxidant capacity (TEAC) using an equation ($y = 103.97x + 4.489$; $R^2 = 0.996$) obtained from standard curve of Trolox (0.1–0.9 mM).

Statistical analysis

All experiment results were analyzed using the Statistical Package for Social Science software (SPSS version 23.0).

All numerical data were expressed as mean $\pm$ standard deviations of triplicate measurements. One-way analysis of variance (ANOVA) with Tukey's test and *T* test were used to determine significant differences ($p < 0.05$) between means.

Results and discussion

Bioaccessibility study

As shown in Table 1, the fermentation process has significantly increased the betacyanins content in red dragon fruits, from 28.27 to 36.88 mM, up to 1.3 fold increment. This finding was in line with previous findings that suggested fermentation as an alternative promising technique to improve the bioactive compounds profile in fruits and vegetables (Cerrillo et al., 2014; Sravan Kumar et al., 2014; Svensson et al., 2012). In the present study, fermented red dragon fruit drink and fresh red dragon fruit juice were submitted to the in vitro simulated gastrointestinal digestion. Table 1 shows that betanin from fermented drink underwent a 13.56% loss after simulated gastric digestion, with a total 53.58% loss following simulated intestinal digestion. On the other hand, a decrease of 22.32% of betanin content was observed after simulated gastric digestion with a total loss of 56.24% following simulated intestinal digestion in red dragon fruit juice. The loss of betanin in red dragon fruit juice was significantly higher ($p < 0.05$) than that in fermented red dragon fruit drink. This was believed to be due to the significant higher betanin content in fermented drink before subjected to in vitro digestion. On the other hand, the lower water activity value of fermented red dragon fruit drink (0.954) compared to red dragon fruit juice (0.974) was suggested to enhance the stability of betacyanins during the in vitro digestion process (Khan, 2016).

The findings in the present study were in line with a study done by Tesoriere et al. (2008) on bioaccessibility of raw and manufactured beetroot products. Both studies showed that betacyanins (betanin and isobetanin) were decreased by about 50% after subjected to simulated

intestinal digestion while there were not many variations at the end of the simulated gastric digestion. Nevertheless, it was worthy to note that the bioaccessible fraction of betanin retained in fermented red dragon fruit drink (17 mM) is higher than that in raw red beet (64.5 μ M) after submitted to simulated digestive process, which increases the chance of absorption of betanin into cellular level. In another study done by Tesoriere et al. (2013), results revealed that betanin is well absorbed through the simulated model of the intestinal lining, mostly in their un-metabolized form via paracellular transport. In addition, an in vivo study done by Allegra et al. (2007) showed that betanin in 0.5–10.0 μ M range inhibited the myeloperoxidase/nitrite-induced low-density lipoprotein oxidation, in a dose-dependent manner. This deduced that betacyanins are able to maintain its molecular structure through several phases of digestion to be absorbed into the systemic circulation, allowing them to exert potential biological activity within cells. In present study, the amount of bio-accessible fraction of betanin retained in fermented red dragon fruit drink (17 mM) is possibly sufficient to be absorbed into systemic circulation and able to exert potential health benefits to human body.

Apart from betanin, isobetanin is one of the betacyanins present in red dragon fruit. Following gastrointestinal digestion, it was observed that isobetanin in fermented drink suffered a loss of 23.67% while a loss of 35.33% was observed in fresh juice after subjected to simulated gastric digestion. Overall, it was observed that the total loss of isobetanin following simulated intestinal digestion was higher in fermented drink compared to red dragon fruit juice, 43.26%, and 42.29%, respectively as shown in Table 2. The present study showed that there was no significant difference in the loss of isobetanin in both fermented red dragon fruit drink and red dragon fruit juice. This was probably due to the heat applied that lead to spontaneous isomerization of betanin to isobetanin because of an unstable resonance of the coloring structure during the digestion process (Castellar et al., 2003).

Table 1 Betanin content in fermented red dragon fruit drink and red dragon fruit juice after subjected to simulated in vitro digestion

	RDFD	Remained (%)	RDFJ	Remained (%)
Betanin (mM)				
Original	36.88 $\pm$ 1.79 ^{aA}	0.00	28.27 $\pm$ 2.30 ^{aB}	0.00
PGD	31.88 $\pm$ 2.32 ^{bA}	86.44	21.96 $\pm$ 3.63 ^{bB}	77.68
PID	17.12 $\pm$ 3.18 ^{cA}	46.42	12.37 $\pm$ 3.02 ^{cB}	43.76

Each value in table represents the mean value $\pm$ standard deviation (n = 3)

^{abc}Mean value with different superscript in each column differs significantly ($p < 0.05$)

^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$)

Table 2 Isobetanin content in fermented red dragon fruit drink and red dragon fruit juice after subjected to simulated in vitro digestion

	RDFD	Remained (%)	RDFJ	Remained (%)
Isobetanin (mM)				
Original	11.28 ± 1.17 ^{aA}	0.00	9.34 ± 1.64 ^{aA}	0.00
PGD	8.61 ± 1.72 ^{bA}	76.33	6.04 ± 1.80 ^{bA}	64.67
PID	6.40 ± 2.36 ^{cA}	56.74	5.39 ± 1.16 ^{bA}	57.71

Each value in table represents the mean value ± standard deviation (n = 3)

^{abc}Mean value with different superscript in each column differs significantly ($p < 0.05$)

^AMean value with different superscript in each row differs significantly ($p < 0.05$)

Radical scavenging capacity study

It was observed that both RDFD and RDFJ exhibited similar radical scavenging capacity (0.77 mM TEAC) before subjected to in vitro digestion as shown in Table 3. This was most probably due to the high amount of betacyanins, predominantly betanin and phyllocactin that present in RDFD and RDFJ, respectively (Naderi et al., 2010). However, phyllocactin could not be quantified in this study due to unavailability of commercial standard. It was notable to mention that the radical scavenging capacity in the present study (0.77 mM TEAC) was higher compared to a previous study (0.59 mM TEAC) done by Foong et al. (2012), most certainly due to the higher betacyanins amount present in RDFD used in this study. Besides, the radical scavenging of RDFD (0.77 mM TEAC) was notably higher than that in red dragon fruit flesh (around 0.20 mM TEAC) in a study done by Suh et al. (2014). This showed that consumption of red dragon fruit in fermented drink form is more favourable. On the other hand, it was found that the results of the present study were comparable to findings of Wootton-Beard and Ryan (2011), with a total antioxidant capacity of more than 80% accessed using ABTS focusing on juices with betacyanins as the main compound present. The fold change was higher in RDFD compared to other juices accessed in study done by Wootton-Beard and Ryan (2011) as shown in Table 4. A higher fold change indicates higher total antioxidant

capacity measured in digested sample compared to that in original sample.

Then again, Table 3 shows that radical scavenging capacity of both RDFD and RDFJ significantly increased after subjected to simulated gastric and intestinal digestion. The findings were similar to a study done by Wootton-Beard and Ryan (2011) which showed that the ferric reducing antioxidant power of beetroot juice increases after simulated digestion. One of the possible phenomena was several compounds are being structurally altered to secondary metabolites that possess antioxidant functions (Wootton-Beard and Ryan, 2011). Besides, it was observed that radical scavenging capacity of betacyanins are pH dependent in a study done by Gliszczynska-Swiglo et al. (2006) whereby betanin was 1.5–2 times more efficient as a free radical scavenger than anthocyanins at neutral or basic pH. This could be explained by the degradation of betanin into betalamic acid and cyclo-DOPA-5-*O*- β -D-glucoside upon subjected to heating and basic pH environment. It was found that anionic forms of cyclo-DOPA-5-*O*- β -D-glucoside are expected to be very good hydrogen and electron donors, therefore contributed to the higher antioxidant activity observed after simulated digestion (Gliszczynska-Swiglo et al., 2006; Khan, 2016). This also could explain by the assay used as ABTS assay is an electron transfer based reaction mechanisms to measure the antioxidant capacity of a sample (Zulueta et al., 2009).

Table 3 Antioxidant scavenging capacity of fermented red dragon fruit drink and red dragon fruit juice before and after subjected to simulated in vitro digestion

	RDFD (mM TEAC)	ABTS (% inhibition)	RDFJ (mM TEAC)	ABTS (% inhibition)
Original	0.77 ± 0.01 ^{cA}	84.96 ± 1.19	0.77 ± 0.01 ^{cA}	84.83 ± 0.67
PGD	0.85 ± 0.01 ^{bA}	92.48 ± 0.87	0.83 ± 0.01 ^{bA}	91.14 ± 0.36
PID	0.88 ± 0.01 ^{aA}	96.42 ± 1.09	0.85 ± 0.01 ^{aA}	93.00 ± 0.94

Each value in table represents the mean value ± standard deviation (n = 3)

TEAC Trolox equivalent antioxidant capacity

^{abc}Mean value with different superscript in each column differs significantly ($p < 0.05$)

^AMean value with different superscript in each row differs significantly ($p < 0.05$)

Table 4 ABTS inhibition of samples before and after the gastric and intestinal phases of in vitro digestion

Samples	ABTS (% inhibition)	ABTS gastric (fold change)	ABTS intestinal (fold change)
Fermented red dragon fruit drink	84.96 ± 1.19	1.09	1.13
Red dragon fruit juice	84.83 ± 0.67	1.07	1.10

Each value in table represents the mean value ± standard deviation (n = 3)

Fold change refers to the fold increase or decrease in total antioxidant capacity of digested samples compared to the original juice prior to digestion

Table 5 EC₅₀ of fermented red dragon fruit drink and red dragon fruit juice using ABTS and DPPH assay

	EC ₅₀ (μg/mL)	
	RDFD	RDFJ
ABTS	16.54 ^b	27.57 ^a
DPPH	11.03 ^b	22.06 ^a

^{ab}Mean value with different superscript in each column differs significantly ($p < 0.05$)

In the present study, both ABTS and DPPH analysis were used to access the EC₅₀ of RDFD and RDFJ. The commonly used DPPH assay could only use to access antioxidant capacity of diluted samples due to the absorbance interruption at 515 nm by the similar purple color of RDFD and RDFJ (Shalaby and Shanab, 2013). Lower EC₅₀ indicates that the antioxidant activity is high. From Table 5, it shows that the EC₅₀ values for RDFD and RDFJ using ABTS assay are 16.54 g/mL and 27.57 μg/mL, respectively. However, the values are 11.03 μg/mL and 22.06 μg/mL for RDFD and RDFJ, respectively using DPPH assay. In comparison to other studies, the EC₅₀ accessed using DPPH for Moroccan prickly pears ranged from 52.48 to 135.96 μg/mL and 2.69 μg/mL in red beet powder (Cai et al., 2003; Dehbi et al., 2013). The results obtained in the present study showed that the antioxidant activity exhibited by both RDFD and RDFJ are comparable to Moroccan prickly pears and red beet which proved with prominent health effects in various studies. Notably, RDFD possessed a higher antioxidant activity and lower EC₅₀ than those of RDFJ. It was worthy to note that both ABTS and DPPH are in vitro models that incapable to access all of the antioxidant activities in the samples (Floegel et al., 2011). However, ABTS assay was more preferable than DPPH in the present study to access the antioxidant capacity of hydrophilic betacyanins compound that present in red dragon fruit.

To conclude, the results of the present study indicate that both RDFD and RDFJ are highly bioaccessible with 46.42% and 43.72% bioaccessible fraction that remained after subjected to in vitro simulated digestion, respectively.

RDFD is performing better than RDFJ with a higher amount of betacyanins present after digestion (17.12 mM in RDFD compared to 12.37 mM in RDFJ). In addition, antioxidant scavenging capacity of RDFD is 0.88 TEAC with EC₅₀ of 16.54 μg/mL compared to RDFJ with scavenging capacity of 0.85 TEAC with EC₅₀ of 27.57 μg/mL accessed using ABTS assay. The results obtained demonstrate the need for further both in vitro and in vivo studies of RDFD to determine the potential secondary metabolites that confer higher antioxidant capacity.

Acknowledgements Financial support is given by the university: CERVIE research grant scheme (Proj-In-FAS 054) is gratefully acknowledged. The authors would like to thank UCSI University for the laboratory facilities.

Compliance with ethical standards

Conflict of interest None of the authors has any financial interest or conflict with industries or parties.

References

- Allegra M, Tesoriere L, Livrea MA. Betanin inhibits the myeloperoxidase/nitrite-induced oxidation of human low-density lipoproteins. *Free. Radic. Res.* 41: 335–341 (2007)
- Antunes-Ricardo M, Rodriguez-Rodriguez C, Gutierrez-Urbe JA, Cepeda-Canedo E, Serna-Saldivar SO. Bioaccessibility, intestinal permeability and plasma stability of isorhamnetin glycosides from *Opuntia ficus-indica* (L.). *Int. J. Mol. Sci.* 18: 1816–1831 (2017)
- Cai YZ, Sun M, Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae. *J. Agric. Food Chem.* 51: 2288–2294 (2003)
- Cassidy A, Brown JE, Hawdon A, Faughnan MS, King LJ, Millward J, Zimmer-Nechemias L, Wolfe B, Setchell KDR. Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *J. Nutr.* 45–51 (2006)
- Castellar MR, Obon JM, Alacid M, Fernandez-Lopez A. Fermentation of *Opuntia stricta* (Haw.) fruits for betalains concentration. *J. Agric. Food Chem.* 56: 4253–4257 (2008)
- Cerrillo I, Escudero-Lopez B, Hernero-Mendez D, Martin F, Fernandez-Pachon M. Effect of alcoholic fermentation on the carotenoid composition and provitamin A content of orange juice. *J. Agric. Food Chem.* 62: 842–849 (2014)

- Dehbi F, Hasib A, Bouaziz M, Ouattmane A, Elbatal H, Jaouad A, Sayadi S. Effect of phenolic compounds and betalain pigments on the antioxidant capacity of Moroccan prickly pear juices. *J. B Agric. Biol. Sci.* 9: 2–7 (2013)
- Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Compos. Anal.* 24: 1043–1048 (2011)
- Foong JH, Hon WM, Ho CW. Bioactive compounds determination in fermented liquid dragon fruit (*Hylocereus polyrhizus*). *Borneo Sci.* 31: 31–48 (2012)
- Gengatharan A, Dykes GA, Choo WS. Stability of betacyanin from red pitahaya (*Hylocereus polyrhizus*) and its potential application as a natural colourant in milk. *Int. J. Food Sci. Technol.* 51: 427–434 (2016)
- Gliszczynska-Swiglo A, Szymusiak H, Malinowska P. Betanin, the main pigment of red beet—molecular origin of its exceptionally high free radical scavenging activity. *Food Addit. Contam.* 23: 1079–1087 (2006)
- Guldiken B, Toydemir G, Memis KN, Okur S, Boyacioglu D, Capanoglu E. Home-processed red beetroot (*Beta vulgaris* L.) products: changes in antioxidant properties and bio accessibility. *Int. J. Mol. Sci.* 17: 858–870 (2016)
- Kanner J, Harel S, Granit R. Betalains: A new class of dietary cationized antioxidants. *J. Agric. Food Chem.* 49: 5178–5185 (2001)
- Khan MI. Plant betalains: Safety, antioxidant activity, clinical efficacy and bioavailability. *Compr. Rev. Food Sci. F.* 15: 316–330 (2016)
- Livrea MA, Tesoriere L. Health benefits and bioactive components of the fruits from *Opuntia ficus-indica* [L]. *Mill. J. Prof. Assoc. Cactus.* 8: 73–90 (2006)
- Mika K. Stability of natural colorants of plant origins [dissertation]. [Finland (Turku)]: University of Turku (2014)
- Naderi N, Stintzing FC, Ghazali HM, Manap YA, Jazayeri SD. Betalain extraction from *Hylocereus polyrhizus* for natural food coloring purposes. *J. Prof. Assoc. Cactus.* 12: 143–154 (2010)
- Parada J, Aguilera JM. Food microstructure affects the bioavailability of several nutrients. *J. Food Sci.* 72: 21–32 (2007)
- Pavokovic D, Krsnik-Rasol M. Complex biochemistry and biotechnological production of betalains. *Food Technol. Biotech.* 49: 145–155 (2011)
- Perales S, Barberá R, Lagarda MJ, Farré R. Antioxidant capacity of infant fruit beverages; influence of storage and in vitro gastrointestinal digestion. *Nutr. Hosp.* 23: 547–553 (2008)
- Rebecca OPS, Boyce AN, Chandran S. Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*). *Afr. J. Biotechnol.* 9: 1450–1454 (2010)
- Sensoy I. A review on the relationship between food structure, processing and bioavailability. *Crit. Rev. Food Sci. Nutr.* 54: 902–909 (2014)
- Shalaby EA, Shanab, SMM. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. *Indian J. Geomarine Sci.* 42: 556–564 (2013)
- Sravan Kumar S, Manoj P, Giridhar P. A method for red-violet pigments extraction from fruits of Malabar spinach (*Basella rubra*) with enhanced antioxidant potential under fermentation. *J. Food Sci. Technol.* 52: 3037–3043 (2014)
- Suh DH, Lee S, Heo DY, Kim YS, Cho SK, Lee S, Lee CH. Metabolite profiling of red and white pitayas (*Hylocereus polyrhizus* and *Hylocereus undatus*) for comparing betalain biosynthesis and antioxidant activity. *J. Agric. Food Chem.* 62: 8764–8771 (2014)
- Svensson L, Sekwati-Monang B, Lutz DL, Schieber A, Ganzle MG. Phenolic acids and flavonoids in nonfermented and fermented red sorghum (*Sorghum bicolor* (L.) Moench). *J. Agric. Food Chem.* 58: 9214–9220 (2012)
- Tesoriere L, Allegra M, Butera D, Livrea MA. Absorption, excretion, and distribution of dietary antioxidant betalains in LDLs: potential health effects of betalains in humans. *Am. J. Clin. Nutr.* 80: 941–945 (2004)
- Tesoriere L, Fazzari M, Angileri F, Gentile C, Livrea, MA. In vitro digestion of betalainic foods Stability and bio accessibility of betaxanthins and betacyanins and antioxidative potential of food digesta. *J. Agric. Food Chem.* 56: 10487–10492 (2008)
- Tesoriere L, Gentile C, Angileri F, Attanzio A, Tutone M, Allegra M, Livrea, MA. Trans-epithelial transport of the betalain pigments indicaxanthin and betanin across Caco-2 cell monolayers and influence of food matrix. *Eur. J. Nutr.* 52: 1077–1087 (2013)
- Wootton-Beard P, Ryan L. A beetroot juice shot is a significant and convenient source of bio accessible antioxidants. *J. Funct. Foods.* 3: 329–334 (2011)
- Zulueta A, Esteve MJ, Frigola A. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chem.* 114: 310–316 (2009)

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