Free Radical Research, October 2008; 42(10): 885–891

informa
healthcare

Singlet oxygen quenching by anthocyanin's flavylium cations

VERIDIANA V. DE ROSSO[1], FAUSTINO E. MORÁN VIEYRA[2], ADRIANA Z. MERCADANTE[1], & CLAUDIO D. BORSARELLI[2]

[1]*Department of Food Science, Faculty of Food Engineering, University of Campinas, UNICAMP, PO Box 6121, Campinas, São Paulo, 13083-862, Brazil, and* [2]*Instituto de Química del Noroeste Argentino (INQUINOA-CONICET), Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero, Av. Belgrano (S) 1912, G4200ABT, Santiago del Estero. Argentina*

Accepted by Professor J. Cadet

(Received 6 August 2008; revised 30 August 2008)

Abstract
The quenching of singlet molecular oxygen ($^1O_2$) by the flavylium cation form of six widespread anthocyanin derivatives: cyanidin 3-glucoside (CG), cyanidin 3-rutinoside (CR), cyanidin 3-galactoside (CGL), malvidin (M), malvidin 3-glucoside (MG) and malvidin 3,5-diglucoside (MDG) was studied in 1% HCl methanol solution by time-resolved phosphorescence detection (TRPD) of $^1O_2$ and photostationary actinometry using perinaphthenone and methylene blue as sensitizers, respectively. The average value of the total (k_0) and chemical (k_c) quenching rate constants were $\sim 4 \times 10^8$ M^{-1} s^{-1} and 3×10^6 M^{-1} s^{-1}, respectively, indicating the good performance of the studied anthocyanins as catalytic quenchers of $^1O_2$. The quenching efficiency was larger for malvidin than for cyanidin derivatives, probably by the extra electron-donating methoxy group in ring B of the malvidin derivatives; and it was also dependent on the number and type of glycosylated substitution. As observed for other phenolic-like derivatives, the quenching of $^1O_2$ by anthocyanins was mediated by a charge-transfer mechanism, which was modulated by the total number of –OR substituents that increases the electron-donating ability of these compounds.

Keywords: *Anthocyanins, singlet oxygen, flavylium cation, pigments, antioxidant capacity*

Introduction

Anthocyanins are naturally occurring flavonoid pigments, widely distributed in vegetables, fruits, grains and flowers, with colour varying from red to blue, depending on the structure and environmental conditions, such as pH and interaction with other pigments [1,2]. Traditionally known as pigments present in all types of berries and grapes [3,4], anthocyanins are also found in tropical exotic fruits, such as camu-camu [5], dovyalis and tamarillo [6], açai and acerola [7].

Epidemiological studies showed that increased ingestion of vegetables and fruits containing natural polyphenolic antioxidants has been correlated to decreased prevalence of several chronic-degenerative diseases, such as cancer, inflammation, cardiovascular disease, cataract, age-related macular degeneration, etc. [8–11]. The mechanisms of antioxidant action can include suppression of reactive species formation either by inhibition of enzymes or by chelation of trace elements involved in free radical production; scavenging of reactive oxygen species

Correspondence: Claudio D. Borsarelli, Instituto de Química del Noroeste Argentino (INQUINOA-CONICET), Facultad de Agronomía y Agroindustrias, Av. Belgrano (S) 1912, G4200ABT, Santiago del Estero, Argentina. Tel/Fax: +54-385-4509585. Email: cborsa@unse.edu.ar; cborsarelli@yahoo.com.ar

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760802506349

(ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals, etc.; quenching of singlet oxygen and up-regulating or protecting antioxidant defense [12].

Anthocyanins have shown good performance as scavengers of several radical and neutral oxidizing species, e.g. superoxide [13], peroxyl radical [14] and hydrogen peroxide [15]. Besides free radicals, singlet oxygen ($^1O_2$) is an excited state species harmful to several biological components, causing DNA damage and lipid oxidation [16,17]. Although carotenoids are well-known as the best natural quenchers of $^1O_2$ with total quenching rate constant k_0 values closer to the diffusion-controlled limit, e.g. 10^9–10^{10} M^{-1} s^{-1} [18–20], flavonoids also showed some interesting quenching ability of $^1O_2$ with k_0 values in the range of 10^5–10^8 $M^{-1}s^{-1}$ [21–23]. However, the interaction of $^1O_2$ with anthocyanins, which are colourful flavonoids, has not received much attention. Recently, Jang et al. [24] verified that anthocyanins from bilberry extracts showed 30–70% protection against self-photooxidation of the pyridinium bisretinoid A2E in retinal epithelial cells. Nevertheless, since this study was carried out at physiological pH, the antioxidant activity was mainly assigned to the blue quinonoidal anhydrobase form due to its pH-dependent equilibrium with the red flavylium cation [24]. Regardless of the fact that most anthocyanins are found in food and vegetables in its colourful flavylium cation, their reactivity towards $^1O_2$ is not yet fully available. Therefore, the goal of this study is to determine the bimolecular rate constants for the quenching of $^1O_2$ by six widespread anthocyanins in their red flavylium cation form that is stable in acidic media.

Experimental

Materials

High performance liquid chromatography (HPLC) grade standards of cyanidin 3-glucoside (CG), cyanidin 3-rutinoside (CR), cyanidin 3-galactoside (CGL), malvidin (M), malvidin 3-glucoside (MG) and malvidin 3,5-diglucoside (MDG) were obtained from Extrasynthèse (Genay, France) and used as received. Methanol and hydrochloric acid were obtained from Merck (Darmstadt, Germany). The other reagents used were all of analytical grade, sodium azide, 9, 10-dimethylanthracene (DMA), 1,4-diazabicyclo[2.2.2]octane (DABCO), methylene blue (MB) and perinaphthenone (PN) from Sigma (St. Louis, US).

HPLC-MS and UV-Vis

The purity of the standards ranged from 95–100%, measured using a Shimadzu (Kyoto, Japan) HPLC equipment with quaternary pumps (model LC-20AD), with PDA detector (Shimadzu, model SPD-M20A) and a MS with an ion-trap analyser (MS/MS), Esquire 4000 model, from Bruker Daltonics (Bremen, Germany) as described before [6]. UV-Vis spectra were monitored with a OceanOptics USB2000 diode array spectrophotometer (Dunedin, FL).

Singlet oxygen quenching measurements

All experiments were done in air-saturated 1% HCl methanol solutions at room temperature in duplicate. Stationary and pulsed photosensitized generation of $^1O_2$ was performed as described before [23,25]. The bimolecular rate constant for the total quenching (physical + chemical) of $^1O_2$ by the anthocyanins, namely k_0, was determined by time-resolved phosphorescence detection (TRPD) using a Peltier cooled Ge photodiode Judson J16TE2-66G (Judson Technol., Montgomeryville, PA) placed at right angle of the excitation laser pulse. A Continuum Minilite II Nd:YAG Q-switched laser (Santa Clara, CA), operating at 355 nm (10 ns FWHM, ca. 3 mJ/pulse) was used to excite the sensitizer perinaphthenone (PN). Spurious light was obstructed using a 1270 nm band pass filter (Spectrogon BP-1260, Taeby, Sweden) and the output of the detector was fed via amplifier stages ($\times 10$) to a Tektronix TDS3032B (Beaverton, OR) digital oscilloscope linked to an on-line PC for data transfer and analysis. The concentration of anthocyanins was varied up to ca. 30 μm and under this condition the initial absorbance at 355 nm of the sensitizer ($A_{355} = 0.8$) remained nearly unchanged, since the anthocyanins are almost transparent at that wavelength. The observed first-order decay rate constant of $^1O_2$ in the absence and presence of anthocyanins, i.e. k_Δ^0 and k_Δ, respectively, were obtained by exponential fitting of the decay portion of phosphorescence signals and the k_0 value was obtained by linear fitting of equation (1), where [A] represents the anthocyanin concentration:

$$k_\Delta = k_\Delta^0 + k_Q[A] \quad (1)$$

The bimolecular rate constant for the chemical reaction between $^1O_2$ and the anthocyanins, namely k_c, was determined by a comparative method using DMA as reference compound and MB as sensitizer under identical experimental conditions, using as excitation source a 150 W filament lamp coupled with a red cut-off filter (Schott RG645, Mainz Germany) to avoid direct excitation of the anthocyanins. The excitation light (> 650 nm) was focused into the sample cell at right angle of the analysing beam light of the OceanOptics USB2000 diode array spectrophotometer. The photosensitized consumption of both anthocyanins and the reference DMA were monitored at ca. 530 nm and 375 nm and up to 10% of reaction to avoid possible interference of photooxidation products. The k_c value was calculated

with equation (2), where $k_{c,ob}$ is the observed pseudo-first rate constant for the bleaching of both sample and reference compounds by $^1O_2$, considering $k_c^{DMA} = 4.7 \times 10^7$ M^{-1} s^{-1} as the reactive rate constant quenching of $^1O_2$ by the reference DMA [26].

$$k_c = k_c^{DMA}(k_{c,ob}/k_{c,ob}^{DMA}) \quad (2)$$

Results and discussion

In protic solvents and aqueous environments, anthocyanins may exist in a variety of protonated, deprotonated, hydrated and isomeric forms and the relative proportion of these molecules is dependent on pH [1,2]. The red flavylium cation is dominant at very acidic pH (<3) and it can alternatively be transformed to quinonoidal bases through proton transfer reactions and at pH 6–7 be further converted to the blue-purple quinonoid anions. Additionally, in aqueous media at pH 4–5 hydration reactions generate the colourless carbinol pseudo-base, which can further undergo ring opening to the light yellow chalcone [1,2]. In the present case, the UV-Vis absorption spectra of the anthocyanins in 1% HCl methanol solution were characteristic of the flavylium cation form (deep red colour), with two absorption maxima at ca. 285 and 530 nm. The stationary photolysis at $\lambda > 645$ nm of 10 μm methylene blue (MB) air-saturated solutions in the presence of DMA produced a large degradation of the reference compound. However, under the same experimental conditions, only a small degradation of the flavylium cations was observed, as is shown for the reference compound DMA and cyanidine-3-glucoside (CG) in Figure 1.

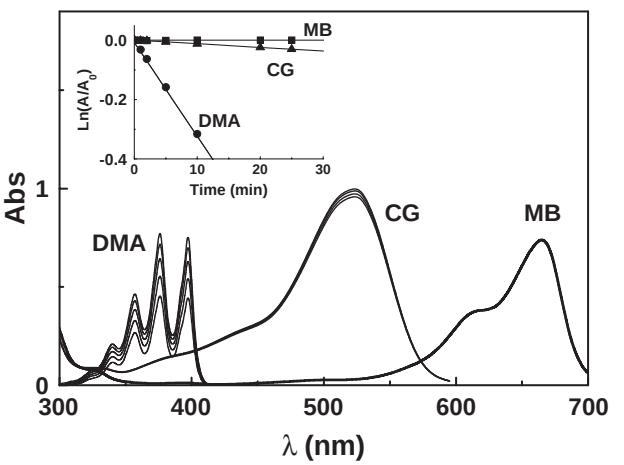


Figure 1. UV-Vis spectral changes of 9,10-dimethyantracene (DMA), cyanidin 3-glucoside (CG) and methylene blue (MB) observed by steady state excitation at >645 nm of MB in air-saturated 1% HCl methanol solutions. Inset: First-order plots for the consumption of the compounds.

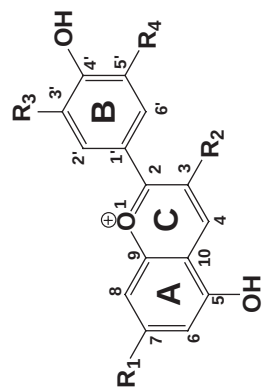
For both compounds, the photosensitization degradation followed first-order kinetic. On the other hand, the addition of DABCO or sodium azide, which both are efficient physical quenchers of $^1O_2$ [26], diminished the degradation of the anthocyanin or DMA (data not shown). Furthermore, the sensitizer MB was not consumed either in aerobic or anaerobic conditions (e.g. by N_2-bubbling), indicating the lack of reaction between the anthocyanins and the excited states of the sensitizer, suggesting that the photo-oxidation of the anthocyanins takes place mainly by type-II mechanism. From the observed slopes of the first-order plots and using equation (2), the chemical quenching rate constants k_c for the anthocyanins were calculated (Table I). The photo-oxidation rate of DMA was the same in methanol and in 1% HCl methanol solutions, allowing the use of $k_c^{DMA} = 4.7 \times 10^7$ M^{-1} s^{-1}, which is the recommended average value among several organic solvents [26].

The inset of Figure 2 shows the phosphorescence signal of $^1O_2$ observed after 355 nm laser excitation of 50 μm perinaphthenone (PN) in air-saturated solution. Under these conditions, the $^1O_2$ lifetime ($1/k_\Delta^0$) was 8.7 μs, as expected for methanol mixtures [27]. The addition of anthocyanins reduced the observed lifetime of $^1O_2$. However, the initial phosphorescence signal intensity remained unchanged indicating that the pigment did not affect the formation quantum yield of $^1O_2$ by the sensitizer. The variation of the observed first order decay constant of $^1O_2$ (k_Δ) was linear with the pigment concentration (Figure 2), allowing the calculation of the total quenching rate constant, i.e. $k_0 = k_c + k_p$, with equation (1) (Table I).

The above results are summarized in Scheme 1, which represents the photosensitized generation and deactivation pathways of $^1O_2$ in the absence and presence of anthocyanins (A) by type II mechanism. As compared with other flavonoid compounds [21–23], anthocyanins are very efficient $^1O_2$ quenchers with k_0 values $> 2 \times 10^8$ M^{-1} s^{-1}, only comparable with those observed for highly hydroxylated flavonols, such as quercetin and myricetin [22]. Moreover, the extent of the chemical reaction of the flavylium cation with $^1O_2$ was quite low, as noted by the reaction efficiency $k_c/k_0 < 8 \times 10^{-3}$ (Table I), indicating that approximately one anthocyanin molecule was degraded by every 125 molecules of $^1O_2$ quenched. This result indicates that the physical interaction between $^1O_2$ and the flavylium cations (k_p) is the principal quenching pathway (Scheme I).

The biological relevance of a predominant physical quenching pathway is that the quencher molecule is not consumed during the process, thus allowing its reutilization in consecutive interactions with $^1O_2$ or other ROS. Therefore, the flavylium cation shows very good catalytic $^1O_2$ quencher ability, closer to that observed for C_{40} carotenoids ($k_c/k_0 \approx 1 \times 10^{-4}$

Table I. Structure of flavylium cation of anthocyanins indicating the classical classification of flavonoids compounds defined by the ($C_6C_3C_6$) structure consisting of two benzene rings (A and B) flanking the pyran ring (C). Glu = glucose; Rut = rutinose; Gal = galactose. Total (k_0) and reactive (k_c) quenching rate constants of $^1O_2$ by several anthocyanins and wavelength maximum of the absorption band I of the anthocyanins in 1% HCl methanol solutions at 25°C.



Anthocyanin	R_1	R_2	R_3	R_4	λ_{max} (nm)	$K_0/10^8$ (M^{-1} s^{-1})	$k_c/10^6$ (M^{-1} s^{-1})	k_c/k_0 ($\times 10^3$)
Malvidin (M)	OH	OH	OCH_3	OCH_3	547	5.6±0.6	4.5±0.5	8.0
Malvidin 3-glucoside (MG)	OH	OGlu	OCH_3	OCH_3	540	5.5±0.2	4.0±0.5	7.2
Malvidin 3,5-diglucoside (MDG)	OGlu	OGlu	OCH_3	OCH_3	537	4.3±0.2	3.0±0.5	7.0
Cyanidin 3-glucoside (CG)	OH	OGlu	OH	H	528	3.3±0.2	1.9±0.2	5.8
Cyanidin 3-rutinoside (CR)	OH	ORut	OH	H	531	3.2±0.2	1.2±0.2	3.8
Cyanidin 3-galactoside (CGL)	OH	OGal	OH	H	529	2.8±0.2	1.3±0.3	4.6

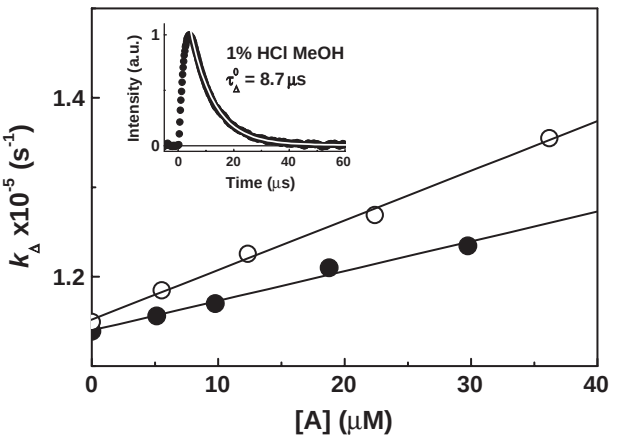
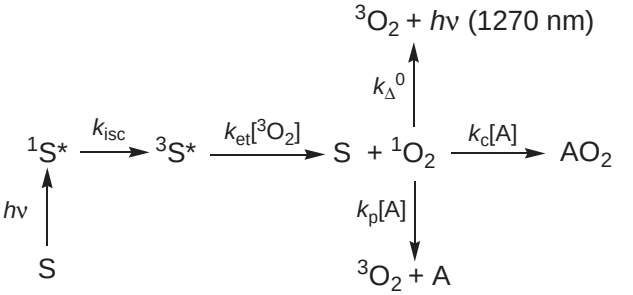


Figure 2. Singlet oxygen ($^1O_2$) quenching by anthocyanins in 1% HCl methanol solutions. (○) malvidin (M); (●) cyanidin 3-rutinoside (CR). Inset: Phosphorescence signal of $^1O_2$ at 1270 nm in the absence of anthocyanins obtained by 355 nm laser excitation of 50 µm perinaphthenone (PN).

[20]) and much better than for other natural occurring quenchers, such as naringenin ($k_c/k_0 \approx 0.05$ [23]), crocetin, bilirubin and tryptophan ($k_c/k_0 \approx 0.16$, 0.30 and 0.5, respectively, [28]) and β-ionone ($k_c/k_0 \approx 0.83$ [25]).

From Table I it is observed that both k_0 and k_c values for malvidin derivatives are larger than for cyanidin derivatives. These results were comparable with the trend observed in the photoprotection of the retinal AE2 by the quinonoidal anhydrobase form of the same anthocyanins at neutral pH in aqueous media, where it was suggested that the presence of a 1,3-diene system in the ring C of the quinonoidal anhydrobase enabled the $^1O_2$ quenching [24]. In the case of the flavylium cation form, AM1 semi-empirical molecular orbital calculations indicated that 4'-OH substitution in the ring B causes resonance increment with C-ring allowing strong delocalization of the π electrons and the positive charge producing also a 1,3-diene system in the ring



Scheme 1. Singlet oxygen ($^1O_2$) photosensitization generation and deactivation pathways, with k_{isc} as the unimolecular rate constant for intersystem crossing of the sensitizer molecule and k_{et} as the bimolecular energy-transfer quenching rate constant of the triplet state of the sensitizer $^3S^\star$ by ground state triplet oxygen molecule $^3O_2$. k_Δ^0 represents the unimolecular decay of $^1O_2$ to $^3O_2$ and k_c and k_p are the bimolecular chemical and physical quenching rate constants of $^1O_2$ by the anthocyanins (A), respectively, with the total quenching rate constant defined as $k_0 = k_c + k_p$.

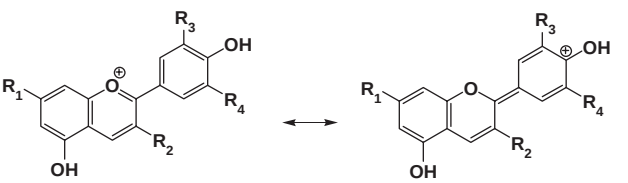
C [29] (Scheme 2). Therefore, it can be expected that increasing the number of electron-donating groups in the anthocyanin skeleton stabilizes the resonance structure of the diene system form in the flavylium cation favouring a [4+2] type interaction with the electrophilic species $^1O_2$. This effect can explain the larger efficiency of malvidin compared to cyanidin derivatives because of the extra methoxy group in ring B.

In this framework, it is shown that the k_0 values systematically increase as the number of electron-donating group increases in several phenolic compound series, as denoted by linear correlations of k_0 with the Hammett σ, σ^+ and σ^- parameter [30]. Figure 3 shows the linear dependence of log k_0 vs the total number of –OR groups (with R = H, CH_3 or glycoside) attached to the aromatic rings for the studied anthocyanins together with available literature data for flavonol [22] and hydroxycinnamic acid [31] series. Regardless of the different experimental conditions in each study, both flavonoid (flavonols + anthocyanins) and hydroxycinnamic acid series showed similar slope value, indicating that the same quenching mechanism was operating for all phenol-like quenchers. It is accepted that the quenching mechanism of $^1O_2$ by phenol derivatives (Q) involves an excited state encounter complex (exciplex) with a partial charge-transfer character, in which $^1O_2$ is the acceptor electron species [30,32], equation (3).

$$^1O_2 + Q \rightleftharpoons {}^3(O_2^{\delta-} \cdots Q^{\delta+}) \rightarrow {}^3O_2 + Q + QO_2 + heat \quad (3)$$

In the present case, the physical quenching is the main deactivation pathway, since $k_c/k_0 < 8 \times 10^{-3}$ (see above). Therefore, the observed increment of k_0 with the number of –OR groups can be considered as a consequence of the enhancement on the electron-donor ability by the progressive attaching of electron-donating groups to the aromatic skeleton.

The increment of the number of electron-donating groups is also accompanied by a progressive red shift of the absorption maximum of band I of the anthocyanins (Table I). The upper limiting k_0 value for malvidin (the more red-shifted pigment of the series) is within the range of bimolecular quenching rate constants involved in charge-transfer quenching of $^1O_2$ [27], as expected for molecules with higher triplet energies than $^1O_2$ ($E_T > 94$ kJ mol^{-1}) and



Scheme 2. Resonance structures proposed for the flavylium cation [29].

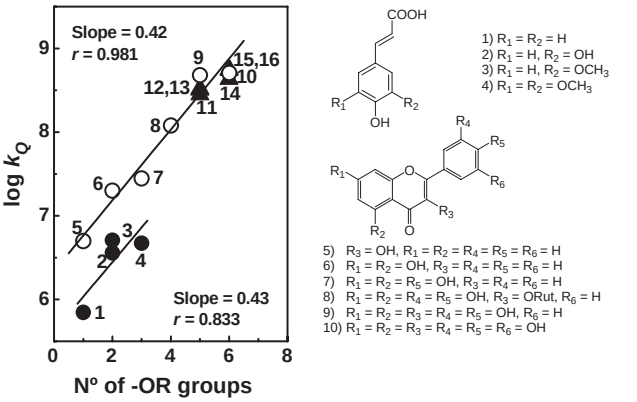


Figure 3. Plot of log k_0 vs the total number of –OR groups (with R = H, CH_3 or glycoside) attached to the aromatic structure of: (●) hydroxycinnamic acid series: (1) p-coumaric acid, (2) ferulic acid, (3) caffeic acid, (4) sinapic acid; (○) flavonol series: (5) flavonol, (6) chrysin, (7), apigenin (8) rutin, (9) quercetin, (10) myricetin; (▲) anthocyanin series: (11) cyanidin 3-galactoside, (12) cyanidin 3-rutinoside, (13) cyanidin 3-glucoside, (14) malvidin 3,5-diglucoside, (15) malvidin 3-glucoside, (16) malvidin. The k_0 values for hydroxycinnamic acid and flavonol series were in acetonitrile [31] and in ethanol [22] solutions.

lower oxidation potentials $E_{ox} \leq 1.9$ V (vs SCE) [27], as the case of malvidin and cyanidin derivatives with $E_{ox} < 1$ V [33].

Table I also indicates that the nature and number of glycoside groups attached to the flavylium cation may modulate the $^1O_2$ quenching capacity. For instance, the k_0 value of malvidin 3,5-diglucoside (MDG) was almost 25% lesser than that for the aglycone malvidin. This effect is probably due to steric hindrance effects by the bulky glycoside groups, since both anthocyanins contain the same number of substituent groups (-OR) and therefore similar electron-donating stabilization degree of the flavylium cation. Furthermore, the k_0 value of cyanidin derivatives with different glycoside substitution at the C3 decreases in the order glucose $\approx$ rutinose > galactose. This finding also agreed with the reduction of photoprotection of A2E self-oxidation by cyanidin 3-galactoside (CGL) as compared with cyanidin 3-glucoside (CG) [24]. This effect is particularly interesting since the only difference between glucose and galactose moiety is the orientation of one hydroxyl group at their pyran ring. It is remarkable to note that the same effect of glycosylation on anthocyanins has been observed in several free-radical scavenging tests. For instance, the oxygen radical absorbance capacity (ORAC) [14], Trolox equivalent antioxidant activity (TEAC) [34] and scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) [35,36] also showed the same antioxidant trends by changing the glycoside substitution as those observed in this study for the quenching of $^1O_2$, i.e. antioxidant capacities were in the order CG > CGL and M $\approx$ MG > MDG. Thus, it is conceivable that different sugar molecules had

differential effects on the antioxidant potency of anthocyanins. Since the radical scavenging efficiency of an antioxidant depends on its ability to form a stable radical itself [37] and that charge-transfer quenching of $^1O_2$ depends on the electron-donor capacity of the quencher [27], it can be assumed that in both cases different sugar molecules and *ortho* substitution to the functional 4'-OH group in the B ring with electron-donating groups may provide different molecular structures that modulates both the hydrogen and electron donor ability of the anthocyanins affecting their antioxidant capacity.

In summary, the present results indicate that widespread anthocyanins in their coloured flavylium form (acid pH) are promising quenchers of $^1O_2$ ($k_0 \approx 3\text{–}6 \times 10^8$ M^{-1} s^{-1}) with very good catalytic quencher performance as given by the low k_c/k_0 ratio ($< 8 \times 10^{-3}$). As observed for other phenolic-like derivatives, the quenching of $^1O_2$ by anthocyanins is mediated by a charge-transfer mechanism, which is modulated by the total number of –OR substituents that increases the electron-donating ability of the anthocyanins. Considering that C_{40} carotenoids, which are the most efficient quenchers of $^1O_2$ with $k_0 \approx 1 \times 10^{10}$ M^{-1} s^{-1} [17–20,27], are also very labile molecules in strongly acid media [38]. Therefore, the flavylium cation form of anthocyanins become suitable antioxidant molecules in those media, such as is found in some food and beverages preparations. In addition, intact anthocyanin glycosides appear to be distributed into the circulatory systems [39] and directly interacting with cells, since anthocyanins do indeed cross the cell membrane and can be detected in the interior of cells and protect them against reactive oxygen species [15,40].

Acknowledgements

We thank CICyT-UNSE and CONICET (Argentina) and the Brazilian Foundation FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) for financial support. C. D. B. is a member of the Research Career of CONICET. F. E. M. V. thanks CONICET for a graduate fellowship. Dedicated to Professor Florinda Bobbio (UNICAMP, Brazil), who introduced us to the anthocyanin world, on occasion of her retirement.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Brouillard R. Chemical structure of anthocyanins. In: Markasis P, editor. Anthocyanins as food colors. London: Academic Press; 1982. p 1–40.

[2] Heredia FJ, Franchia-Aricha EM, Rivas-Gonzalo JC, Vicario IM, Santos-Buelga C. Chromatic characterization of anthocyanins from red grapes—I. pH effect. Food Chem 1998;63:491–498.

[3] Wu H, Prior RL. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. J Agric Food Chem 2005;53:2589–2599.

[4] Mercadante AZ, Bobbio FO. Anthocyanins in foods: occurrence and physicochemical properties. In: Socaciu C, editor. Food colorants: chemical and functional properties. Boca Raton: CRC Press; 2008. p 241–276.

[5] Zanatta CF, Cuevas E, Bobbio FO, Winterhalter P, Mercadante AZ. Determination of anthocyanins from camu-camu (*Myrciaria dubia*) by HPLC-PDA, HPLC-MS and NMR. J Agric Food Chem 2005;53:9531–9535.

[6] De Rosso VV, Mercadante AZ. HPLC-PDA-MS/MS of anthocyanins and carotenoids from dovyalis and tamarillo fruits. J Agric Food Chem 2007;55:9135–9141.

[7] De Rosso VV, Hillebrand S, Montilla EC, Bobbio FO, Winterhalter P, Mercadante AZ. Determination of anthocyanins from acerola (*Malpighia emarginata* DC.) and açai (*Euterpe oleracea* MART.) by HPLC-PDA-MS/MS. J Food Compos Anal 2008;21:291–299.

[8] Seeram NP. Berry fruits for cancer prevention: current status and future prospects. J Agric Food Chem 2008;56:630–635.

[9] Cooke D, Steward WP, Gescher AJ, Marczilo T. Anthocyanins from fruits and vegetables—does bright colour signal cancer chemopreventive activity? Eur J Cancer 2005;41:1931–1940.

[10] Mertens-Talcott SU, Rios JM, Jilma-Stohlawetz P, Talcott ST, Derendorf H. Absorption and antioxidant effects of polyphenolics from açai (*Euterpe oleracea* Mart.) in healthy human volunteers. FASEB 2006;21:103–107.

[11] Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. J Agric Food Chem 1995;43:2800–2802.

[12] Halliwell B, Gutteridge JMC, editors. Free radicals in biology and medicine. Oxford: Oxford University Press; 1998.

[13] Yamasaki H, Uefuji H, Sakihama Y. Bleaching of the red anthocyanin induced by superoxide radical. Arch Biochem Biophys 1996;332:183–186.

[14] Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem 1997;45:304–309.

[15] Youdim AK, Martin A, Joseph JA. Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. Free Radic Biol Med 2000;29:51–60.

[16] Geoffroy M, Lambelet P, Richert P. Role of hydroxyl radicals and singlet oxygen in the formation of primary radicals in unsaturated lipids: a solid state electron paramagnetic resonance study. J Agric Food Chem 2000;48:974–978.

[17] Ravanat JL, Martinez GR, Medeiros MHG, Di Mascio P, Cadet J. Mechanistic aspects of the oxidation of DNA constituents mediated by singlet molecular oxygen. Arch Biochem Biophys 2004;423:23–30.

[18] Edge R, McGarvey DJ, Truscott TG. The carotenoids as anti-oxidants—a review. J Photochem Photobiol B Biol 1997;41:189–200.

[19] Baltschun D, Beutner S, Briviba K, Martin H-D, Paust J, Peters M, Röver S, Sies H, Stahl W, Steigel A, Stenhorst F. Singlet oxygen quenching abilities of carotenoids. Liebigs Ann Recueil 1997;9:1887–1893.

[20] Montenegro MA, Nazareno MA, Durantini EN, Borsarelli CD. Singlet oxygen quenching ability of carotenoids in a reverse micelle membrane mimetic system. Photochem Photobiol 2002;75:353–362.

[21] Tounaire C, Croux S, Maurette MT, Beck I, Hocquaux M, Braun AM, Oliveros E. Antioxidant activity of flavonoids: efficiency of singlet oxygen ($^1\Delta_g$) quenching. J Photochem Photobiol B Biol 1993;19:205–215.

[22] Nagai S, Ohara K, Mukai K. Kinetic study of the quenching reaction of singlet oxygen by flavonoids in ethanol solutions. J Phys Chem B 2005;109:4234–4240.

[23] Montenegro MA, Nazareno MA, Borsarelli CD. Kinetic of the photosensitized oxygenation of the flavanone naringin and its chalcone. J Photochem Photobiol A Chem 2007;186: 47–56.

[24] Jang YP, Zhou S, Nakanishi K, Sparrow JR. Anthocyanins protect against A2E photooxidation and membrane permeabilization in retinal pigment epithelial cells. Photochem Photobiol 2005;81:529–536.

[25] Borsarelli CD, Michne M, La Venia A, Morán Vieyra FE. UVA self-photosensitized oxygenation of β-ionone. Photochem Photobiol 2007;83:1313–1318.

[26] Wilkinson F, Brummer JG. Rate constants for the decay and reactions of the lowest electronically excited singlet state of molecular oxygen in solution. J Phys Chem Ref Data 1981; 10:809–999.

[27] Schweitzer C, Schmidt R. Physical mechanisms of generation and deactivation of singlet oxygen. Chem Rev 2003;103: 1685–1757.

[28] Lissi EA, Encinas MV, Lemp E, Rubio, MA. Singlet oxygen O_2 ($^1\Delta_g$) bimolecular processes. Solvent and compartmentalization effects. Chem Rev 1993;93:699–723.

[29] Pereira GK, Donate PM, Galembeck SE. Effects of substitution for hydroxyl in the B-ring of the flavylium cation. J Mol Struct 1997;392:169–179.

[30] Thomas MJ, Foote CS. Chemistry of singlet oxygen. XXVI. Photooxygenation of phenols. Photochem Photobiol 1978;27: 683–693.

[31] Foley S, Navaratnam S, MacGarvey DJM, Land EJL, Trusccott TG, Rice-Evans CA. Singlet oxygen quenching and the redox properties of hydroxycinnamic acids. Free Radic Biol Med 1999;26:1202–1208.

[32] Gorman AA, Gould IR, Hamblett I, Standen MC. Reversible exciplex formation between singlet oxygen, $^1\Delta_g$, and vitamin E. Solvent and temperature effect. J Am Chem Soc 1984;106:6956–6959.

[33] Janeiro P, Brett AMO. Redox behavior of anthocyanins present in *Vitis vinifera L.* Electroanalysis 2007;19: 1779–1786.

[34] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996;20:933–956.

[35] Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. J Agric Food Chem 2000;48:3597–3604.

[36] Kähkönen M, Heinonen M. Antioxidant activity of anthocyanins and their aglycons. J Agric Food Chem 2003;51: 628–633.

[37] Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Methods Enzymol 1990;186:343–355.

[38] Mercadante AZ. Analysis of carotenoids. In: Socaciu C, editor. Food colorants: chemical and functional properties. Boca Raton: CRC Press; 2008. p 447–478.

[39] McGhie TK, Walton MC. The bioavailability and absorption of anthocyanins: towards a better understanding. Mol Nutr Food Res 2007;51:702–713.

[40] Ghosh D, McGhie TK, Zhang J, Adaim A, Skinner M. Effects of anthocyanins and other phenolics of Boysenberry and blackcurrant as inhibitors of oxidative stress and damage to cellular DNA in SH-SY5Y and HL-60 cells. J Sci Food Agric 2006;86:678–686.