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Light and heat sensitivity of red cabbage extract in soft drink model systems

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Abstract

Anthocyanins of red cabbage (*Brassica oleracea* L.) display colour over a larger range of pH-values than the majority of anthocyanins from other natural sources, being pink at pH 3, violet at pH 5 and blue at pH 7. Using reversed phase high-performance liquid chromatography with diode array detection, cyanidin was found to be the only anthocyanidin of red cabbage extract and, tentatively, identified as cyanidin-3,5-diglucoside and cyanidin-3-sophoroside-5-glucoside, the latter found in various acylated forms. The apparent quantum yield for photobleaching has been determined for red cabbage extract at 25°C in air-saturated McIlvaine buffer, using monochromatic light at each of the irradiation wavelengths, 313, 366, and 436 nm, in continuous photolysis experiments, in order to provide an objective measure for the sensitivity of this food colorant to ultraviolet and visible light. The quantum yield was found to depend on both pH and irradiation wavelength, ranging from 0.2×10^{-4} mol einstein^{-1} for 436 nm light at pH 7.0 to 3.7×10^{-4} mol einstein^{-1} for 366 nm light at pH 5.0. The thermal stability at pH 3.0 in McIlvaine buffer of four different anthocyanin extracts was compared and, for the temperature range investigated (25–80°C) the degree of stability was red cabbage > blackcurrant > grape skin > elderberry. The thermal stability of the same anthocyanin extracts was also compared for a non-carbonated soft drink medium of pH 3.0 yielding the same order of stability but with rates of degradation approximately twice as high as in buffer, which may indicate a detrimental effect of sugar and ascorbic acid. Due to the high thermal stability of red cabbage extract in solution, photobleaching will be the primary destabilising factor for red cabbage anthocyanin-coloured products in display.

1. Introduction

Anthocyanins are glycosylated polyhydroxy and polymethoxy derivates of 2-phenylbenzopyrylium (flavylium) salts (Brouillard, 1982), which are natural colorants widely spread in nature, accounting for the colour in several fruits, vegetables and flowers (Koswig & Hofsommer, 1995; Mazza & Miniati, 1993). The colour of anthocyanins is dependent on pH due to the existence of four different structures in aqueous solutions (Brouillard & Delaporte, 1977; Mazza & Brouillard, 1987b). The four structures have different colours and, since each of them vary in concentration throughout the pH-scale with the red flavylium cation as the dominant structure in acidic environment, the exact colour of the solution depends on the pH-value (Mazza & Brouillard, 1987a). Red cabbage (*Brassica oleracea* L.) is a promising source of anthocyanins for coloration of foods since its anthocyanins are unique in being coloured over a very broad pH-range compared to anthocyanins from, e.g. grape skin, black currant and elderberry, which only possess a reasonable degree of colour at pH < 4 (Brouillard & Delaporte; Mazza & Brouillard, 1987b). The colours of anthocyanins from red cabbage vary from red at low pH to blue and green at high pH (Mazza & Miniati, 1993), and use of anthocyanins from red cabbage is therefore not limited to acidic foodstuffs but can be extended to neutral products, for which they may provide a natural alternative to synthetic blue colorants. The anthocyanin composition of red cabbage is very complex, due to glucosylation of the anthocyanidin (cyanidin) with two different sugars and acylation with several aromatic acids. However, the dominant structures are cyanidin-3,5-diglucoside

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and cyanidin-3-sophoroside-5-glycoside acylated with sinapic acid, ferulic acid, *p*-coumaric acid, caffeic acid or malonic acid (Hrazdina, Iredale & Mattick, 1977; Tanchev & Timberlake, 1969).

Several physical and chemical factors have a negative effect on the stability of anthocyanins, two of the most important being heat and light (Cemeroglu, Velioglu & Isik, 1994; Maccarone, Ferrigno, Longo & Rapisarda, 1987). The degradation pathway of anthocyanins is well described in the literature (Furtado, Figueiredo, Chaves das Neves & Pina, 1993; Maccarone et al., 1987) and it is generally agreed that the degradation can be modelled as a first-order rate reaction (Cemeroglu et al., 1994; Sapers, Taffer & Ross, 1981). Some studies (Sapers et al., 1981; Shi, Francis & Daun, 1992) have shown that anthocyanins from red cabbage are very stable but knowledge of the exact stability under exposure to heat and light has not been fully described. The aims of this study were: (1) to identify the specific anthocyanins of the extract; (2) to determine the apparent quantum yield for photodegradation of red cabbage anthocyanins at different pH and varying wavelengths of irradiation; and (3) to compare the heat stability of red cabbage extract with extracts from grape skin, black currant and elderberry, three widely used anthocyanin colorants.

2. Materials and methods

2.1. Materials

The extracts ''Red Cabbage extract'', ''Grape skin extract'', ''Blackcurrant pomace extract'' and ''Elderberry juice concentrate'' were obtained from Société Sefcal s.a. (30760 Saint Julien de Peyrolas, France). The anthocyanin standards, cyanidin-3,5-diglucoside and cyanidin chloride, were obtained from Carl Roth GmbH (D-76185 Karlsruhe, Germany) and Apin Chemicals Ltd. (Oxon, OX14 4RT, United Kingdom), respectively. All chemicals used were of analytical grade and water was purified through a MilliPore (Bedford, MA01730, USA) MilliQ-unit prior to use. Solutions of extracts were made in McIlvaine (citric acid-Na_2HPO_4) buffers (McKenzie & Dawson, 1974) at pH 3.0, 5.0 and 7.0, respectively. For the study of thermal stability, extracts were also dissolved in a non-carbonated aqueous soft drink medium of pH 3.0, containing per 1000 g: sucrose 86.0 g, sodium benzoate 0.14 g, potassium sorbate 0.18 g, ascorbic acid 0.02 g and citric acid 1.52 g.

2.2. Quantification of total anthocyanin content

The total content of anthocyanin in the red cabbage extract was measured spectrophotometrically using the pH-differential method of Fuleki and Francis (1968). The molar extinction coefficient of cyanidin-3,5-diglucoside, the predominant anthocyanin of red cabbage, was determined to be 26,300 M^{-1} cm^{-1}. Using Lambert-Beer's law, and expressing the molar content of anthocyanin in the extract as cyanidin-3,5-diglucoside, the content was accordingly determined to be 11.2% (w/w).

2.3. Analysis of anthocyanins of Red Cabbage extract

The reversed-phase high-performance liquid chromatography (HPLC) method of Koswig and Hofsommer (1995), with a slight modification of the gradient programme, was used. Eluents A and B were water/formic acid (90:10, v/v) and water/acetonitrile/formic acid (40:50:10, v/v/v), respectively, and the binary elution mixture was changed linearly using the following time/composition set points: 0 min: 88% A + 12% B, 26 min: 70% A + 30% B, 40–43 min: 0% A + 100% B, 48–50 min: 88% A + 12% B. For the separation, a sample volume of 30 μl was injected into a 250×4.6 mm 5 μm HiRPB column from Hichrom Ltd (RG7 4PE Reading, UK) used with an HPLC system from Merck Hitachi (D-64293, Darmstadt, Germany) consisting of an L-5025 column thermostat, an L-4500 diode array detector, a D6000A pump and an AS-4000A autosampler.

2.4. Hydrolyses of red cabbage extract

For acidic hydrolysis, 10 mg extract was dissolved in 20 ml of 5.1 M HCl, subsequently boiled for 1 h, and finally dried using a rotary evaporator before redissolving in a small aliquot of methanol. For alkaline hydrolysis, 0.5 mg extract was weighed in to a vial, treated with a drop of demineralised water and flushed with N_2 for 10 min before closing with a septum through which 0.5 ml 1.78 M KOH was added. A hydrolysis time of 10 min in complete darkness was used, after which 2 M HCl was added to acidic reaction.

2.5. Photolysis experiments

Solutions of red cabbage extract were made in McIlvaine buffers at pH 3.0, 5.0 and 7.0, respectively, and diluted to obtain an appropriate absorbance of the solutions, typically $Abs(\lambda_{irr}) = 0.6–1.4$, corresponding to 7.4–17.2 mg anthocyanin/l solution (using the extinction coefficient and molecular weight of cyanidin-3,5-diglucoside). The solutions, which needed no readjustment of pH, were air-saturated and thermostatted to 25°C for 30 min. Then 5.0 ml of solution was transferred to a quartz cell with a 2 cm light path and exposed to monochromatic light (wavelengths 313, 366 or 436 nm) selected from an OSRAM (D-81543 München, Germany) HBO 200/2 high pressure Hg lamp (line spectrum) mounted as part of a Spindler und Hoyer (D-37070 Göttingen, Germany) optical train. The optical train also included a light condenser, a heat filter, an interference filter, a

shutter connected to an electronic timer, and lenses focusing the light into a thermostatted (25.0±0.5°C) cell-holder. Light intensities were determined by ferrioxalate actinometry (Hatchard & Parker, 1956). The extent of photodegradation was monitored at regular intervals by spectrophotometric measurements using a Shimadzu UV-2101 PC spectrophotometer (Kyoto 604-8511, Japan).

The apparent photodegradation quantum yield

$$\Phi_{app} = \frac{\text{number of anthocyanins degraded}}{\text{number of photons absorbed by anthocyanins}} \quad (1)$$

was calculated from the degree of colour bleaching of the solution monitored at the absorption maximum in the visible range for up to 12% bleaching in a typical experiment lasting 1–1½ h, in combination with the light flux I_0 as determined by actinometry and expressed in quanta·s^{-1}.

The number of photons absorbed by the anthocyanins, Q_A, was calculated by adding the light absorbed in small, but finite, time intervals, $t_i - t_{i-1}$, for a solution with a total anthocyanin concentration, c_0.

$$Q_A = \frac{I_0}{N_A V c_0} \sum_i \left(1 - 10^{-\overline{A_{irr}}}\right)(t_i - t_{i-1}) \quad (2)$$

where N_A is Avogadro's number, V is the volume (in litres), and $\overline{A_{irr}}$ is the average absorbance at the wavelength of irradiation at the time $\frac{1}{2}(t_i + t_{i-1})$. The quantum yield Φ_{app}, which is reported as the mean of three determinations, was then calculated from

$$\Phi_{app} = \frac{(A(t_0) - A(t_i))/A(t_0)}{Q_A(t_i)} \quad (3)$$

where $A(t_0)$ is the initial absorbance, and $A(t_i)$ is the absorbance at time i.

2.6. Thermal experiments

Solutions of the four anthocyanin extracts were made in McIlvaine buffer or non-carbonated aqueous soft drink medium, both at pH 3.0 and, in order to obtain visually equal colour strength, all solutions were made to have a tristimulus L-value of approximately 75 as determined with a Minolta CT-310 spectrometer (Osaka 564-8556, Japan). From each solution, 5 ml was transferred to each of six plastic tubes that were well capped to avoid evaporation. The tubes were placed in thermostatic water baths operating at 25, 40, 60 and 80°C. One sample of each anthocyanin extract in each medium was removed after 15 min, 30 min, 1 h, 2 h, 4 h and 6 h, cooled rapidly in ice-water and the thermal degradation was measured spectrophotometrically at 520 nm with a Hewlett-Packard (CA 94303, Palo Alto, USA) HP 8453 diode array spectrophotometer. Only singular determinations were made.

2.7. Statistical analysis

Data from the photolysis experiments were evaluated by analysis of variance using PROC GLM (General Linear Model) in SAS Windows v. 6.12 software (SAS Institute, Cary, NC 27513, USA).

3. Results and discussion

In order to determine the nature of the aglycone and its glucosylations, a comparison of the acid and alkaline hydrolysates of red cabbage extract with the two standards cyanidin and cyanidin-3,5-diglucoside was made. HPLC retention times and spectral characteristics of peaks, revealed by diode array detection (results not shown), confirmed that cyanidin was the only aglycone and that only two different anthocyanins were present in the extract. These were, according to results of Idaka (1987), Idaka, Suzuki, Yamakita, Ogawa, Kondo and Goto (1987) and Giusti, Rodríguez-Saona, Griffin and Wrolstad (1999) assigned as cyanidin-3-sophoroside-5-glucoside (80%) and cyanidin-3,5-diglucoside (20%). A representative chromatogram of the unhydrolysed extract is shown in Fig. 1 and, as may be seen, the extract contained at least 15 different compounds.

Using peak spectral characteristics λ_{vis}, λ_{uv}, and λ_{acyl}, and their corresponding absorptivities (Table 1), it was possible to identify the anthocyanins as either mono- or biosides (E_{440}/E_{vis} ratio of 29–35% indicating a monoside and a ratio of 15–24% indicating a bioside) and to determine the degree of aromatic acid acylation (E_{acyl}/E_{vis} ratio of 53–69% indicating monoacylation and ratio of 98–128% indicating diacylation;Hong & Wrol-

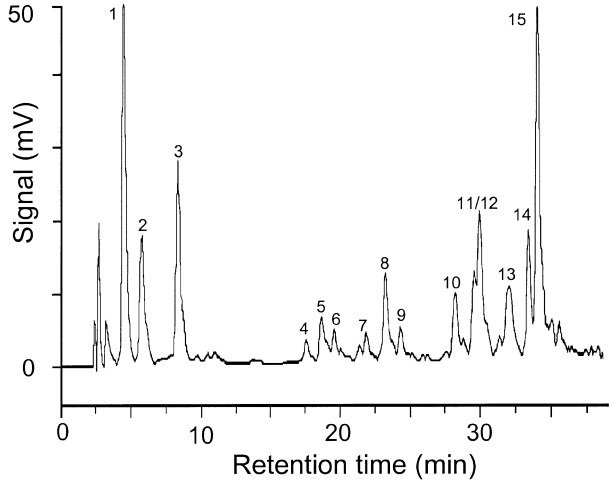

Fig. 1. Representative chromatogram for red cabbage extract using HPLC as described in Section 2.

Table 1
Retention times (R_t) of peaks of red cabbage extract resolved in the chromatogram (Fig. 1) and the tentative identification of anthocyanins (derived from cyanidin) revealed by spectroscopic characteristics (Harborne, 1958) and measured by diode array detection (Hong & Wrolstad 1990a, b)[a]

Peak No.	R_t (min)	λ_{vis} (nm)	λ_{uv} (nm)	λ_{acyl} (nm)	E_{440}/E_{vis} (%)	E_{acyl}/E_{vis} (%)	E_{440}/E_{vis} indicating	E_{acyl}/E_{syn} indicating	λ_{acyl} indicating	Tentative identification
1	4.47	511	279		22		Bioside	Non-acylated		3-soph-5-glu
2	5.78	511	280		22		Bioside	Non-acylated		3-glu-5-glu
3	8.35	523	284	333	21	91	Bioside	Monoacylated	Sinapic or ferulic acid	
4	17.57	517	280	*316	29	138	Monoside ?	Diacylated	*p*-Coumaric acid	
5	18.66	518	282	320	22	108	Bioside	Diacylated	*p*-Coumaric acid	
6	19.58	517	284	*319	22	130	Bioside	Diacylated	*p*-Coumaric acid	
7	21.86	528	288	*320	22	140	Bioside	Diacylated	*p*-Coumaric acid	
8	23.24	528	287	327	20	115	Bioside	Diacylated	Sinapic or ferulic acid	
9	24.34	529	295	318	23	150	Bioside	Diacylated	*p*-Coumaric acid	
10	28.21	518	282	319	22	115	Bioside	Diacylated	*p*-Coumaric acid	
11	29.56	518	282	328	22	85	Bioside	Monoacylated	Sinapic or ferulic acid	3-ferulylsoph-5-glu
12	29.95	518	282	330	21	79	Bioside	Monoacylated	Sinapic or ferulic acid	3-sinapylsoph-5-glu
13	32.03	524	286	324	23	114	Bioside	Diacylated	Mix of *p*-Coumaric and Ferulic/sinapic acid	3-*p*-coumarylsinapyl-soph-5-glu
14	33.43	528	295	332	22	128	Bioside	Diacylated	Sinapic or ferulic acid	3-ferulylsinapyl-soph-5-glu
15	34.02	528	299	333	22	133	Bioside	Diacylated	Sinapic or ferulic acid	3-disinapylsoph-5-glu

[a] The absorption maxima λ_{vis} and λ_{uv} are due to the presence of the anthocyanidin chromophore while λ_{acyl} is due to the chromophore of the acylating acid, and E_{vis} and E_{acyl} are the absorptivities at the corresponding maxima while E_{440} is the absorptivity at 440 nm. Peak numbering refers to the peaks of Fig. 1, and abbreviations 'soph' and 'glu' to sophoroside and glucoside, respectively. An asterisk indicates the presence of a 'shoulder' in the spectrum.

stad, 1990a, b). Furthermore, λ_{acyl} gives information about the acylating aromatic acid as λ_{acyl} in the 320–333 nm range indicates sinapic or ferulic acid whereas λ_{acyl} around 310–315 nm indicates *p*-coumaric acid (Hong & Wrolstad, 1990a). We attempted to identify the 15 compounds found in red cabbage extract using the spectral characteristics shown in Table 1. Seven peaks were identified, all in accordance with Idaka (1987), Idaka et al. (1987) and Giusti et al. (1999). The identification of the peaks is, however, complicated, not only by absorption spectra, depending on solvent polarity, but also by the complex acylation pattern of red cabbage anthocyanins, including acylations with glucosylated aromatic acids (Giusti et al., 1999; Idaka et al., 1987). Thus, compounds of peaks 4–10 are all diacylated, but shorter elution times than peaks identified as simple diacylated anthocyanins (peaks 13–15) indicate increased polarity due to additional glucosylation.

For the wavelengths of interest for coloured foods in display, absorption of visible light is assigned to the cyanidin moiety. However, the pH-region of coloration of Red Cabbage extract is largely extended and the complex acylation pattern of the extract is the likely cause for the almost 20 nm span of the visible absorption spectrum maximum (λ_{vis}) of the different peaks at pH of separation (Osawa, 1982).

In order to quantify the role of different wavelengths of irradiation on the bleaching rate of red cabbage extract, the apparent photodegradation quantum yields (Φ_{app}) were determined at 313, 366 and 436 nm at each of the pH-values 3, 5 and 7, respectively. To our knowledge,

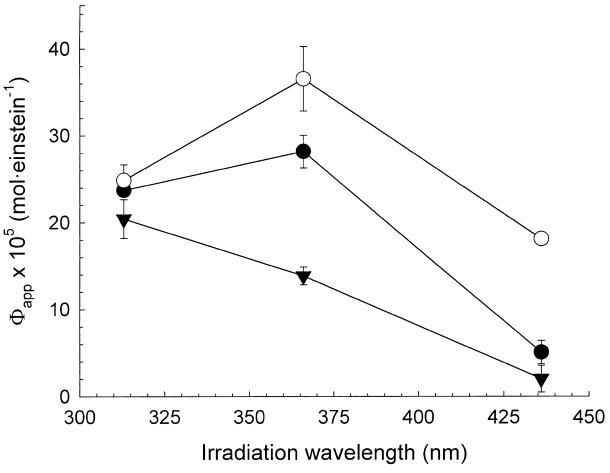

Fig. 2. Apparent photodegradation quantum yields (Φ_{app}), at 25°C for red cabbage extract in McIlvaine buffer at pH 3.0 (●), pH 5.0 (○) and pH 7.0 (▼), respectively. Results shown are means of three determinations ± standard deviation.

the results presented in Fig. 2 represent the first quantification of the light stability of anthocyanins from red cabbage measured as Φ_{app} and, as may be seen in Fig. 2, a rather complex relationship with strongly significant ($P < 0.001$) interaction between pH and irradiation wavelength was found.

The photodegradation quantum yield is seen to vary by more than one order of magnitude, from 0.2×10^{-4} mol einstein^{-1} for 436 nm light at pH 7.0 to 3.7×10^{-4} mol einstein^{-1} for 366 nm light at pH 5.0. For all three wavelengths of irradiation, the photodegradation quan-

tum yield was found to be highest at pH 5 and lowest at pH 7, indicating a higher stability to photobleaching in the region which may be the most promising for red cabbage extract, i.e. pH-neutral foods where a natural blue colour is desirable. However tempting, light sensitivity of colorants are only comparable when photon fluxes at specific wavelengths (i.e. lamp spectrum and intensity) and absorbances are taken into account. This is a consequence of the Stark-Einstein relationship law stating that only absorbed photons can lead to photochemical reactions, and the relationship between the rate of photodegradation of colorant ($-d$[colorant]$/dt$), the absorbed intensity (I_{abs}) and the photodegradation quantum yield (Φ_{app}) is $-d[\text{colorant}]/dt = \Phi_{app} \times I_{abs}$ (Wayne, 1988). Light stability of red cabbage extract is comparable to elderberry extract (Carlsen & Stapelfeldt, 1997), the only other anthocyanin-based food colorant for which Φ_{app} has been measured. However the pH-dependence of the photodegradation quantum yield of red cabbage extract is clearly different from elderberry extract for which no significant pH-dependence was observed in the range pH 3.0–3.8. Maccarone et al. (1987) proposed that photochemical degradation should proceed through the colourless forms, i.e. from flavylium cation via carbinol pseudobase to the chalcone, whereas Furtado et al. (1993) proposed a mechanism from which direct photochemical degradation of the flavylium cation also occurs. The results of Fig. 2 are likely to be in accordance with both mechanisms, but the large number of compounds in the extract together with the lack of knowledge of equilibrium constants for the acylated forms render it impossible to verify the photodegradation mechanism of the current extract. Comparing the results of Fig. 2 with the wavelength dependence of other food colorants, it may at first hand seem surprising that the photodegradation quantum yield does not decrease with increasing wavelength from 313 to 436 nm, as observed for lutein, xanthophyll and β-carotene (Jørgensen & Skibsted, 1990), cochineal (Jørgensen & Skibsted, 1991), elderberry extract (Carlsen & Stapelfeldt), and annatto (Petersen, Wiking & Stapelfeldt, 1999). In contrast to these, red cabbage anthocyanins contain at least two interacting chromophore systems, the anthocyanidin and the aromatic acids which both absorb 313 nm light whereas the anthocyanidin is expected to be the major absorber at higher wavelengths. It therefore seems likely that the aromatic acids may provide a filter-like property to the anthocyanins, in effect decreasing their UV-sensitivity.

We performed some preliminary experiments in order to compare the heat stability of red cabbage extract with anthocyanins from other sources (grape skin, black currant and elderberry) in McIlvaine buffer and non-carbonated soft drink medium, both at pH 3.0. Thermal degradation at four temperatures, representing from uncooled storage (25°C) to pasteurisation (80°C) was measured spectrophotometrically and results were, according to results of Sapers et al. (1981), Shi et al. (1992) and Cemeroglu et al. (1994), fitted to first-order rate law. Within the rather short time period applied (6 h) it was, for red cabbage extract, only possible to quantify the thermal degradation at 80°C, showing that thermal stability was far higher for this anthocyanin source compared to the others (Table 2).

Inspecting the apparent first-order rate constants for McIlvaine buffer pH 3.0, the order of stability was red cabbage > blackcurrant > grape skin > elderberry. The excellent thermal stability of red cabbage extract is likely due to protection of the flavylium system towards nucleophilic addition of water caused by complexes between this and the π-electron rich copigment moieties (Brouillard, 1983), in effect protecting the flavylium system against hydrolysis leading to the colourless hemiacetal adduct, and may be further stabilized by formation of 'sandwich'-like stacking complexes due to high degree of glucosylation (Table 1; Dangles, Saito & Brouillard, 1993). In contrast, anthocyanins from blackcurrant and elderberry are not acylated by aromatic acids and are mainly monosides, while grape skin anthocyanins are all monosides and less acylated than anthocyanins from red cabbage (Hong & Wrolstad, 1990a). As copigmentation is enthalpy-driven with usually unfavourable entropy changes (Brouillard, Mazza, Saad, Albrecht-Gary & Cheminat, 1989), the protection of red cabbage

Table 2
Apparent first-order rate constants (h^{-1}) for thermal degradation of anthocyanin-based colorants at pH 3.0 in McIlvaine buffer (MI) and non-carbonated soft drink (SD) medium, respectively (energy of activation is calculated according to the Arrhenius-equation)

	Temperature (°C)								Energy of activation (kJ mol^{-1}) (r^2)	
	25		40		60		80			
	MI	SD	MI	SD	MI	SD	MI	SD	MI	SD
Red cabbage	n.d.[a]	n.d.	n.d.	n.d.	n.d.	n.d.	9.0×10^{-3}	3.6×10^{-3}	–	–
Blackcurrant	2.4×10^{-3}	4.5×10^{-3}	2.1×10^{-3}	8.3×10^{-3}	9.8×10^{-3}	4.4×10^{-3}	4.3×10^{-2}	8.7×10^{-2}	69 (0.999)	50 (0973)
Grape skin	3.6×10^{-3}	2.4×10^{-3}	6.7×10^{-3}	1.2×10^{-2}	1.8×10^{-2}	5.0×10^{-2}	1.5×10^{-2}	3.2×10^{-1}	58 (0.930)	77 (0.995)
Elderberry	3.1×10^{-4}	5.5×10^{-3}	3.2×10^{-3}	1.6×10^{-2}	2.1×10^{-2}	6.7×10^{-2}	9.0×10^{-2}	1.8×10^{-1}	89 (0.986)	56 (0.998)

[a] n.d. = Non-determinable within 6 h.

anthocyanins is expected to decrease further at temperatures >80°C. The same order of stability was found for the non-carbonated soft drink medium pH 3.0, but rates of degradation were approximately twice as high as for buffer, indicating the detrimental effects of sugar and ascorbic acid on thermal stability of these colorants (Markakis, 1982). However, further investigations, preferably involving chromatographic separation, are needed in order to draw safer conclusions about the kinetics of thermal degradation and for prediction of heat induced changes of hue.

4. Conclusions

Red cabbage has been shown to be a very interesting anthocyanin source for coloration of soft drinks due to: (1) extended pH-region of coloration compared to other anthocyanin sources, providing natural blue colour in neutral solution; (2) low sensitivity to photodegradation from pH 3–7; and (3) excellent heat stability allowing heat treatment of coloured products with only limited loss of colour. These properties are ascribed to the complex nature of red cabbage anthocyanins, most of them being diacylated with a number of aromatic acids. Due to the very high thermal stability of red cabbage extract in solution, photobleaching is accordingly expected to be the primary destabilising factor for red cabbage anthocyanin-coloured products in display. Red cabbage has, however, not found widespread use as a colorant of soft drinks or foodstuffs due to a slight cabbage-like off-flavour of the extract and effort should be made to remove this after which its use appears very promising.

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