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Light sensitivity of elderberry extract. Quantum yields for photodegradation in aqueous solution

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The quantum yield for photobleaching of a commercial elderberry (*Sambucus nigra* L.) extract, mainly composed of anthocyanins, has been determined at 25°C for air-saturated 10 mM citrate buffer solutions, using monochromatic light at each of the wavelengths 313 nm, 366 nm, and 436 nm, respectively, in continuous photolysis experiments, in order to provide an objective measure for the sensitivity of this food colorant to ultraviolet and visible light. In the pH region investigated, pH 3.0–3.8, typical of fruit-based products, the quantum yield for photodegradation was found to depend only on the wavelength of irradiation, being $2.1 \pm 0.2 \cdot 10^{-4}$ mol·einstein^{-1} at 313 nm, $1.6 \pm 0.2 \cdot 10^{-4}$ mol·einstein^{-1} at 366 nm, and $2.8 \pm 0.6 \cdot 10^{-5}$ mol·einstein^{-1} at 436 nm, whereas solution pH only affects the colour intensity. Results show that light bleaching will be the major destabilising factor for anthocyanin-coloured products in display and that exclusion of uv-light will greatly improve their colour stabilty. © 1997 Elsevier Science Ltd

INTRODUCTION

Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium (flavylium) salts (Brouillard, 1982), which are widely spread in nature, being the major water-soluble colorant of fruits, vegetables, flowers and leaves (Strack & Wray, 1993). Elderberry (*Sambucus nigra* L.) is one of the richest sources of these pigments, having contents of 200-1000 mg/100 g fresh weight (Brønnum-Hansen *et al.*, 1985), which is far higher than that found in grapes. In comparison with the complex anthocyanin composition of other sources, elderberry only contains four different anthocyanins of which cyanidin-3-sambubioside and cyanidin-3-glucoside are quantitatively the most important, accounting for more than 85% of the anthocyanin content, whereas cyanidin-3-sambubioside-5-glucoside and cyanidin-3,5-diglucoside are only present in minor amounts (Brønnum-Hansen & Hansen, 1983; Drdák & Daucik, 1990). Anthocyanins are accepted as stable and safe colorants for acidic foodstuffs such as marmalades and jellies since these colorants only possess reasonable degrees of colour at pH < 4 due to the existence of four anthocyanin species in equilibrium in aqueous solution (Brouillard & Delaporte, 1977; Brouillard & Dubois, 1977), as shown in Fig. 1.

Since the molecular structures of the species displayed in Fig. 1 each bring about a different colour, the position in the equilibrium-system is evident for the colour of the anthocyanin solution, with the flavylium cation as the major contributor in acidic solution. In this equilibrium-system, where the equilibriums K_a and K_h are pH-dependent, the relationship between pH and colour is well documented (*cf.* Brouillard, 1982). Light has a negative effect on the stability of anthocyanins (Palmidis & Markakis, 1975; Stringheta *et al.*, 1992) resulting in cleavage of the molecule yielding 2,4,6-trihydroxy benzaldehyde from the benzopyrylium part of the anthocyanin and 3,4-dihydroxy benzoic acid from the phenyl ring bonded to C-2 of the former (Maccarone *et al.*, 1987; Furtado *et al.*, 1993). The mechanism of photochemical breakdown is, however, not fully understood and knowledge of the overall light stability of the anthocyanins is likewise limited. The purpose of this study has therefore been to explore the relationship between pH and wavelength of irradition in order to provide a quantitative measure of the light stability of anthocyanin extracts to provide a safe basis for their use in products employing transparent packaging materials.

MATERIALS AND METHODS

Materials

Elderberry extract, Elderberry Red WS, was obtained from Chr. Hansen's Laboratorium A/S (Hørsholm, Denmark). All chemicals used were of analytical grade

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and water was purified through a MilliPore MilliQ-unit prior to use.

Photolysis experiments

All extract solutions were made in 10 mM citric acid-sodium citrate buffer and diluted 2000-fold to obtain the needed absorbance of solution, typically $A(\lambda_{irr}) = 0.8$-1.0, corresponding to 8.7 mg/l solution (using the molecular weight of cyanidin-3-glucoside). All solutions were sterilfiltered through a 0.22 μm filter in order to exclude the risk of microbial growth. Solution pH was measured with a Metrohm 654 pH meter fitted with a Metrohm combined electrode (type 6.0234.110). The buffered solutions used for pH meter standardization were pH 4.01 and 6.86 NBS standards.

The solutions were air-saturated and 5.0 ml of solution was transferred to a quartz cell with a 2 cm light path and exposed to monochromatic light (wavelengths 313 nm, 366 nm or 436 nm) selected from an OSRAM HBO 200/2 high pressure Hg lamp (line spectrum), mounted as part of a Spindler and Hoyer (Göttingen, Germany) optical train, which 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 thermostated (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 (Kyotu, Japan).

The apparent photodegradation quantum yield

$$\Phi_{app} = \frac{\textit{number of anthocyanins degraded}}{\textit{number of photons absorbed by anthocyanin}} = \frac{(A(t_0) - A(t_i))/A(t_0)}{Q_A(t_i)} \quad (1)$$

was calculated from the degree of colour bleaching of solution monitored at the absorption spectrum in the visible range for up to 20% photodegradation in a typical experiment, in combination with the light intensity, 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.

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

Where N_A is Avogadro's number, V is the volume (in litres), and $\bar{A}_{irr}$ is the average absorbance at the wavelength of irradiation at the time $^1/_2(t_i + t_{i-1})$. For all quantum yields the means of at least two determinations are reported.

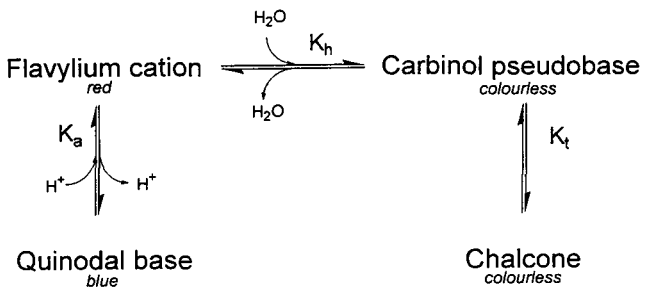

Fig. 1. Interconversion of anthocyanin forms in which K_a, K_h and K_t are the equilibrium constants for, respectively, the acid-base equilibrium, the hydration, and the ring chain tautomerism (Brouillard & Delaporte, 1977).

Quantification of anthocyanin content

The content of anthocyanin was measured spectrophotometrically using the pH-differential method of Fuleki and Francis (1968). Results were expressed as content of cyanidin-3-glucoside, the predominant anthocyanin of elderberry, using a molar extinction coefficient of 29 600 $M^{-1}\cdot cm^{-1}$ (Wrolstad, 1976) for the calculation of molar concentration.

Statistical analysis

Data were evaluated by analysis of variance using proc GLM (general linear model) in SAS 6.04 software (SAS Institute, Cary, NC). For linear modelling of the quantum yield data, a Box-Cox data tranformation was carried out to ensure homogeneity of variance (Box *et al.*, 1978).

Thermal reactions

Thermal reactions during the time span of a photolysis experiment were monitored spectrophotometrically using the same solutions as for the photolysis experiments, but keeping these solutions rigorously excluded from light.

RESULTS AND DISCUSSION

The anthocyanin pigments of the elderberry extract were found to degrade as a result of light absorption as seen from the spectral changes during irradiation displayed in Fig. 2.

As may be seen from the differential spectrum for 120 min of irradiation, light absorption leads to colour bleaching and degradation of the anthocyanin molecules, as evidenced from the decrease of absorption at the maxima at 517 nm and 278 nm assigned to the flavylium cation and the carbinol pseudobase, respectively (Mazza & Brouillard, 1987). Figure 2 was typical for the irradiation at 313 nm and 436 nm. Irradiation at 366 nm caused an initial increase in absorbance through the first

40 min of irradiation before a degradation pattern comparable to that of Fig. 2 appeared. This initial increase was probably due to photochemical processes, and further investigations using pure anthocyanins are needed to elucidate this phenomenon. The quantum yields for the degradation at 366 nm were therefore calculated from the point at which the absorbance was highest, i.e. from where a degradation comparable to the one displayed in Fig. 2 began. Thermal degradation (dark reaction) accounted for up to ten per cent of the photodegradation at the wavelength yielding lowest photodegradation quantum yield, and all photodegradation data are consequently corrected for dark reaction. Variations in the degree of thermal degradation is most likely due to different times required to reach the equilibria, shown in Fig. 1, since rather long times (in the order of hours) are required to reach equilibrium between the colourless carbinol base and chalcone form in contrast to the fast transformation of the carbinol base to the coloured cationic form (Brouillard & Delaporte, 1977; Brouillard & Dubois, 1977), as observed by Havliková & Miková (1985).

The apparent quantum yields for the photodegradation of the elderberry extract showed a significant ($P < 0.001$) dependence of the irradiation wavelength while no statistical significance between the different pH values appeared. Consequently, the photodegradation quantum yields, shown in Fig. 3, are means for pH 3.0–3.8.

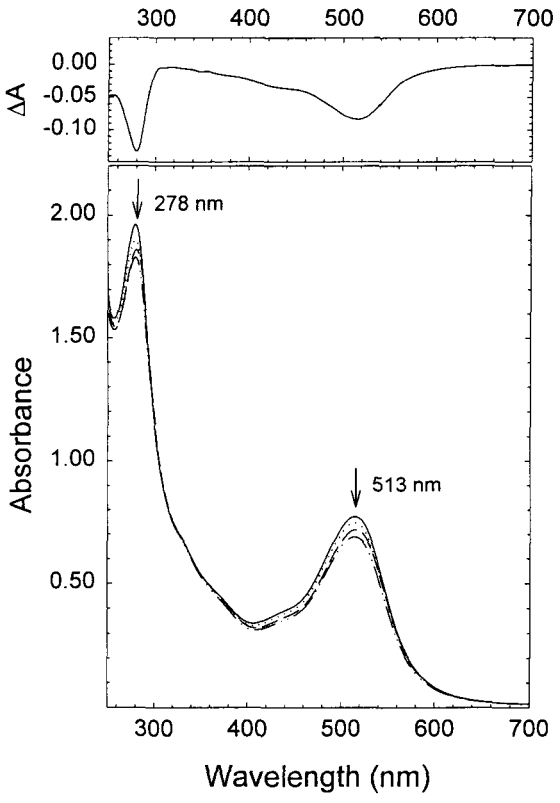

Fig. 2. Absorption spectrum for light degradation at 25°C of Elderberry Red WS at pH 3.0 upon irradiation with 436 nm monochromatic light. Time interval between spectra is 40 min. Shown above is the differential spectrum between the initial absorption spectrum and the absorption spectrum after 120 min of irradiation.

As may be seen from Fig. 3, the quantum yield for photodegradation decreases almost one order when going from 313 nm to 436 nm irradiation wavelength. Several statistical models were tested in order to provide a continuous form for the wavelength dependence of the photodegradation quantum yield which could be helpful from a practical point of view. Simple linear regression yields $r^2 = 0.93$ but neither transformation of wavelength to wavenumber scale, fitting to square root forms or logarithmic forms, nor combinations of these, resulted in significant improvement with respect to the linear model.

The lack of dependence of photodegradation quantum yield on pH is somewhat surprising since the mechanism for photochemical degradation has been proposed to proceed from the flavylium cation through the colourless forms (cf. Fig. 1) thus indicating the process to be pH-dependent (Maccarone *et al.*, 1987). However, results of Furtado *et al.* (1993) support a mechanism from which direct photochemical degradation of the flavylium cation also occur.

To our knowledge, only one photodegradation quantum yield of an anthocyanin solution has been reported, this being $1.0 \cdot 10^{-3}$ mol·einstein^{-1} for malvidin-3-glucoside at pH 1.0 upon 313 nm irradiation at 25°C (Furtado *et al.*, 1993). This higher quantum yield is likely due to the different pH of solution since the flavylium cation is the major species present at pH 1.0 (pK_h 2.6 for malvidin 3-glucoside, Brouillard & Delaporte, 1977) in contrast to the current study with the food relevant interval pH 3.0–3.8 where the colourless carbinol pseudobase, cf. Fig. 1, will dominate. However, better light stability of elderberry extract caused by structural differences between cyanidin and malvidin, and/or co-pigmentation of the anthocyanins in the elderberry extract, known to stabilize the quinoidal base and the flavylium

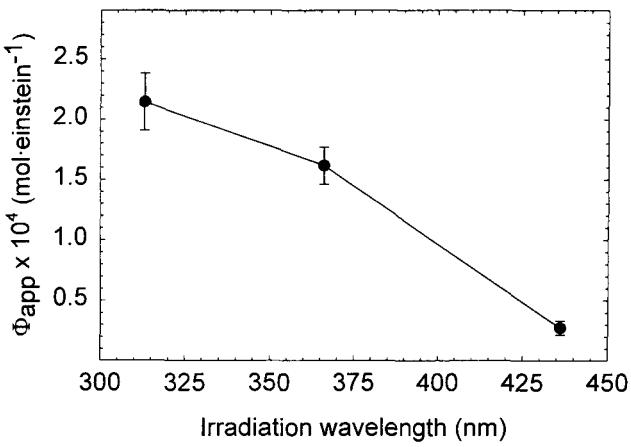

Fig. 3. Photodegradation quantum yields, Φ_{app}, at 25°C for Elderberry Red WS at pH 3.0–3.8. Results shown are means of at least six determinations ± standard error.

cation in anthocyanin solutions (cf. Brouillard & Dangles, 1993), cannot be excluded.

The high degree of photodegradation found in the present study appears to be in contrast to findings of Giusti and Wrolstad (1996) who found only a slightly, yet significant, higher rate of bleaching in anthocyanin syrup exposed to fluorescent light than for syrup stored in the dark. This difference is likely a consequence of the Stark-Einstein law outlining that only absorbed photons can lead to photochemical reactions, and of the relationship between the rate constant for photodegradation of anthocyanin (-d[A]/dt), the absorbed intensity (I_{abs}) and the photodegradation quantum yield (Φ_{app}): $-d[A]/dt = \Phi_{app} \cdot I_{abs}$ (Wayne, 1988). Consequently, results concerning wavelength dependence of photodegradation and rate constants for photodegradation are only directly comparable when photon fluxes at specific wavelengths (i.e. lamp spectrum and intensity) and absorbances are taken in account. For pH 3.0–3.8, the light stability of elderberry extract at 436 nm, as measured by photodegradation quantum yield, is equal to another red water-soluble natural colorant carminic acid which, however, is approximately fifty per cent more stable at 366 nm (Jørgensen & Skibsted, 1991). Using packaging material with a uv-absorber, the industrial manufacturer is thus given two red colorants with almost equal light stability and the specific choice can consequently be made focusing on costs, product and product characteristics. However, intermolecular association is well known for both carminic acid (Stapelfeldt *et al.*, 1993) and anthocyanins (cf. Osawa, 1982) which is expected to affect the the colour stability positively (Mazza & Miniati, 1993). This may also partly explain the findings of Giusti and Wrolstad (1996) on storage of anthocyanin syrup containing more than 100 times higher concentration of anthocyanin than used in the present study. For such highly coloured products, which do not obey Lambert-Beer's law, intermolecular associations have to be taken into account, since the quantum yield for photodegradation is expected to be lower for these products than estimated in the present study, in effect underestimating their long-term light stability.

CONCLUSIONS

The pH of solution has been found not to affect the photochemical breakdown of elderberry extract for pH 3.0–3.8 and standardization of product pH will therefore only affect the colour intensity. The wavelength of irradiation has a profound effect on the colour stability of the product, as measured by photodegradation quantum yield, and anthocyanin-coloured foodstuffs should preferably be exluded from uv-light by incorporation of an efficient barrier in the packaging material in order to ensure long-life colour acceptability in display.

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