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Colour of a xanthylium pigment in aqueous solutions at different pH values

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Abstract

A xanthylium salt pigment was synthesized from the reaction involving glyoxylic acid and (+)-catechin. After isolation, its spectral characteristics and colour intensity, influenced by pH, were studied. When varying pH from acidic to alkaline values, a steady bathochromic displacement of the visible wavelength was observed while no noticeable change was observed in the UV absorbance wavelengths. A concomitant increase in the intensity of the visible absorption wavelength was also observed. The pink reddish taint obtained in the alkaline pH values may allow the studied xanthylium compound to be regarded as a possible colorant for some slightly pink coloured alkaline products.

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1. Introduction

Natural colorants have attracted widespread interest because of their general health and safety image and numerous other important properties. Besides, their eventual potential health effects are attracting considerable interest in the international scientific community. The development of natural food colorants from natural sources is thus receiving increasing attention as natural pigments tend to replace synthetic ones.

In addition to anthocyanins, which are the most abundant polyphenolic pigments in plants, the presence of yellow pigments bearing the xanthylium skeleton has already been reported (Dangles & Brouillard, 1994). Their importance in the colour change during storage of fruit-derived beverages has also been reported (Es-Safi, Le Guernevé, Cheynier, & Moutounet, 2003). Moreover, some natural pigments contain chromophores that consist of xanthylium structures (Robertson, Whalley, & Yates, 1950).

The colour of anthocyanin and xanthylium pigments is generally easily affected by a number of reactions occurring in food products, and the major problems associated with the storage of food natural colorants is their instability caused by temperature, oxygen, pH, light and some enzymes (Francis, 1989). While effect of pH on anthocyanin colour is well documented (Cabrita, Fossen, & Andersen, 2000; Fossen, Cabrita, & Andersen, 1998), little is known about the colour and structural modification influenced by pH, of xanthylium salts. Dangles and Brouillard (1994) showed that in contrast to flavylium ions, which are essentially monomeric and readily hydrate to give colourless species according to a reversible process, the structural transformations of 1,3,6,8-tetrahydroxylxanthylium ions were dominated by dimerization and xanthenol formation was insignificant.

In the present paper the colour change of a xanthylium salt pigment in aqueous solutions at different pH values was investigated. The studied compound was formed through a reaction involving (+)-catechin and glyoxylic acid.

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2. Materials and methods

2.1. Reactions

Reaction of (+)-catechin (11.9 mg) with glyoxylic acid (19.0 mg) was conducted in hydroalcoholic solution constituted of H_2O-EtOH (9/1, v/v). After 2.5 h of incubation at 40 °C, the major colourless dimer, in which two (+)-catechin units are linked through their 8 positions by a carboxymethine bridge, was purified by semipreparative HPLC. Its evolution was monitored in a pH 3.5 aqueous solution at 39 °C, yielding the studied xanthylium salt as previously described (Es-Safi et al., 1999).

2.2. Absorption spectra

Spectrophotometric measurements were carried out using a GBC 111 UV–Visible spectrophotometer fitted with a 10 mm pathlength quartz cell and equipped with GBC Scan Master manager software.

2.3. Buffer solutions

The different pH used values were prepared using usual buffer solutions. The accurate pH for each buffer solution was measured with a Bioblock pH meter.

2.4. Measurements of colour

The isolated pigment was dissolved in acidified methanol to make stock of solutions with concentrations of 1 mg/ml and aliquots of 2 and 4 ml of these solutions were transferred to sample tubes, evaporated to dryness, and dissolved in 20 ml of each buffer solution to give a final concentration of 0.1 mg/ml. The sample tubes were sealed with parafilm and the pigment stored in air atmosphere. UV–Vis spectra were recorded between 250 and 700 nm on a GBC 111 spectrophotometer equipped with GBC Scan Master manager software and a computer unit. Pure buffers were used as reference cell solutions. The colour intensities were measured as absorbance values at $\lambda_{\max}$ at each pH value and expressed as molar absorptivity (ϵ, in $M^{-1}\,cm^{-1}$) as recently described for anthocyanins (Cabrita et al., 2000). For measures reproducibility, each experiment was conducted in triplicate.

3. Results and discussion

3.1. Pigment preparation

In the presence of aldehydes, flavanols bind to each other through bridges established between the 6 and/or 8 A ring nucleophilic positions (Es-Safi, Cheynier, & Moutounet, 2002; Timberlake & Bridle, 1976). Thus, the condensation between (+)-catechin and glyoxylic acid yields four dimeric colourless compounds (Es-Safi, Le Guernevé, Fulcrand, Cheynier, & Moutounet, 2000). These compounds have been demonstrated to correspond to diastereoisomers with structures consisting of two flavanols linked by carboxy-methine bridge (CHCOOH) through their A ring 6 and 8 positions. Isolation of the 8–8 colourless dimer, and its further evolution in a pH 3.5 hydroalcoholic medium, yielded the studied yellow pigment according to Scheme 1 reactions pathways. Purification of the major one was achieved by semi-preparative HPLC and its structure was elucidated through 1D and 2D NMR and mass spectroscopy showing its xanthylium-based nature (Es-Safi et al., 1999).

3.2. Colour variation of xanthylium salt at different pH values

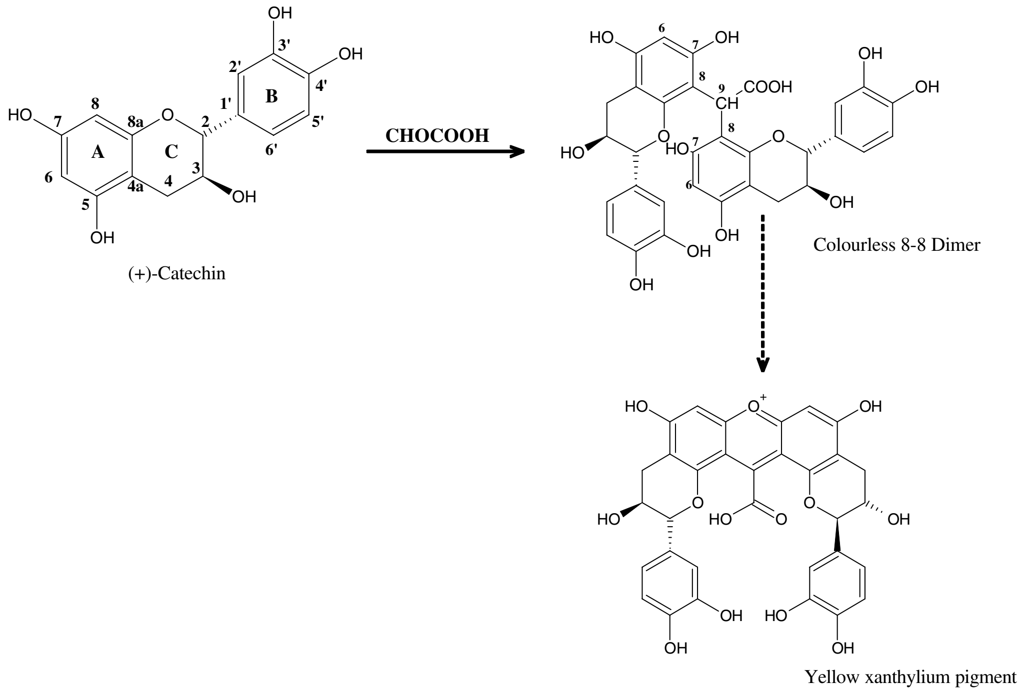
At pH 2.2, the UV–Visible spectrum of the isolated xanthylium salt was characterized by the presence of two major absorption maxima (Fig. 1), the first in the visible region located around 440 nm and the second in the UV region located around 270 nm. In addition, another less intense maximum located at 307 nm was also observed in accordance with previously reported data concerning spectral characteristics of xanthylium salts (Dangles & Brouillard, 1994; Hrazdina & Borzell, 1971).

When increasing pH from acid to alkaline values, the visible absorption maxima for the isolated xanthylium salt suffered a bathocromic shift from 440 to 489 nm (Fig. 2(a)). At pH values higher than 9 the shape of the spectrum was unchanged but a steady increase of the absorption intensity was registered, as can be seen in Fig. 2(b).

The observed blue-shift with respect to pH increase is in accordance with the work of Dangles and Brouillard (1994) on tetrahydroxyxanthylium ion and that of Hrazdina and Borzell (1971) on an unidentified natural xanthylium salt detected in a grape extract.

From pH 2.2 to 4.0, few variations of the visible absorption maximum were observed (Fig. 3(a)). At pH 5.0, in addition to the maximum at 450 nm, a shoulder at 486 nm was also observed. At pH 5.6 two visible absorption maxima with equivalent absorption intensities were observed. From pH 6.0 to 8.9 a gradual increase of the maximum absorption at 486 nm was observed, concomitant with the disappearance of the maximum located around 450 nm which became only a shoulder as shown Fig. 2(a).

In the case of the maximum located around 307 nm, an equivalent evolution was observed (Fig. 3(b)). Thus a small variation in the pH, ranging from 2.0 to 5.0, was recorded. From pH 5.6 to 7.0, two maxima were ob-



Scheme 1. Synthesis of the xanthylium pigment from (+)-catechin via the colourless dimer.

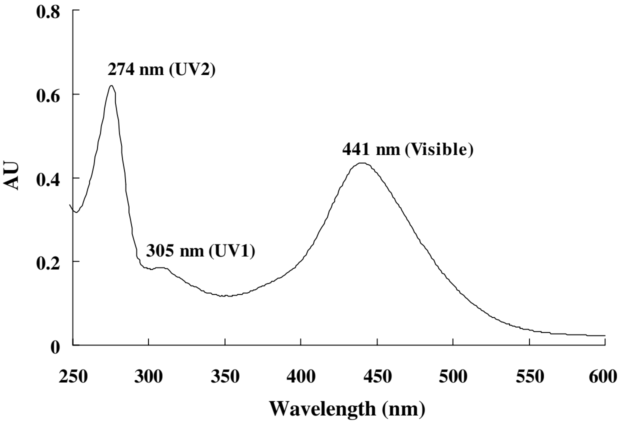


Fig. 1. UV–Visible spectrum of the xanthylium pigment in a buffer solution at pH 2.2.

served, respectively, at 307 and 322 nm. Only the latter was observed from pH 7.6 to 8.9. In contrast, no noticeable variation was observed in the case of the absorption maximum around 271 nm which remained almost constant in the studied pH range (Fig. 3(c)).

3.3. Colour intensity of xanthylium salt at different pH values

The pH variation affects the colour intensities of the xanthylium salts. Thus Fig. 4, showed the increase of the visible maximum absorption intensity, while few varia-

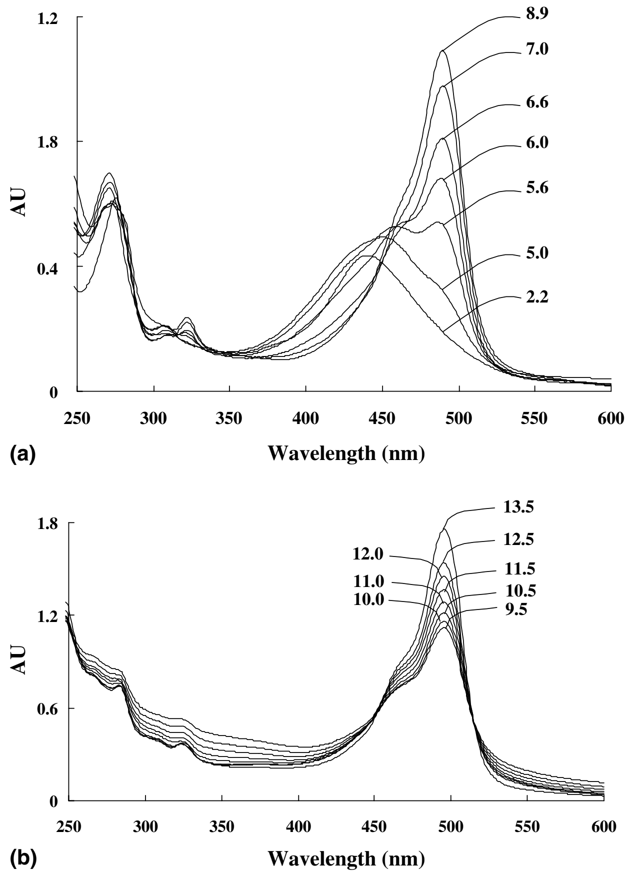


Fig. 2. UV–Visible spectra of the xanthylium salt recorded at different pH values: (a) from 2.2 to 8.9; (b) from pH 9.5 to 13.5.

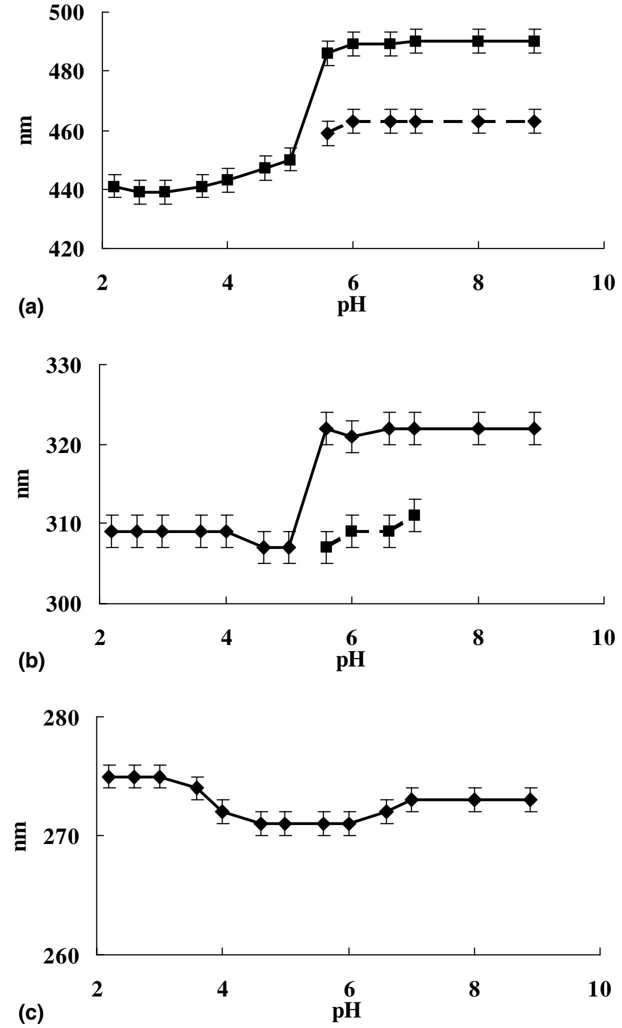


Fig. 3. UV–Visible absorption maxima at different pH values for the xanthylium pigment: (a) visible; (b) UV1; (c) UV2.

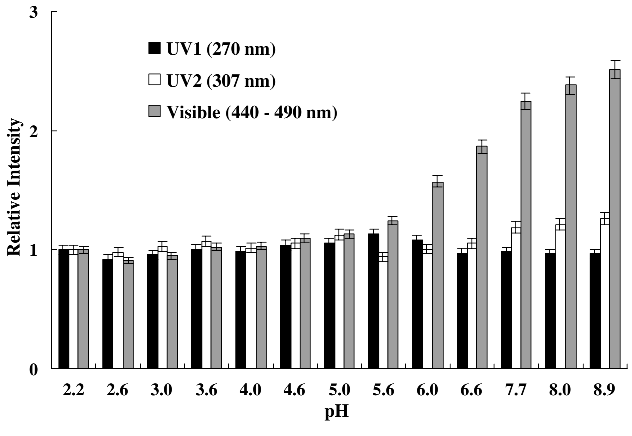


Fig. 4. Absorption intensities relating to pH 2.2 values for the xanthylium pigment.

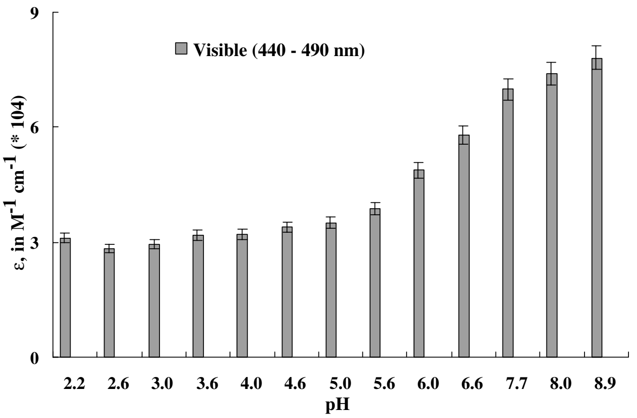


Fig. 5. Absorbance measurements conducted at the visible wavelength at different pH values.

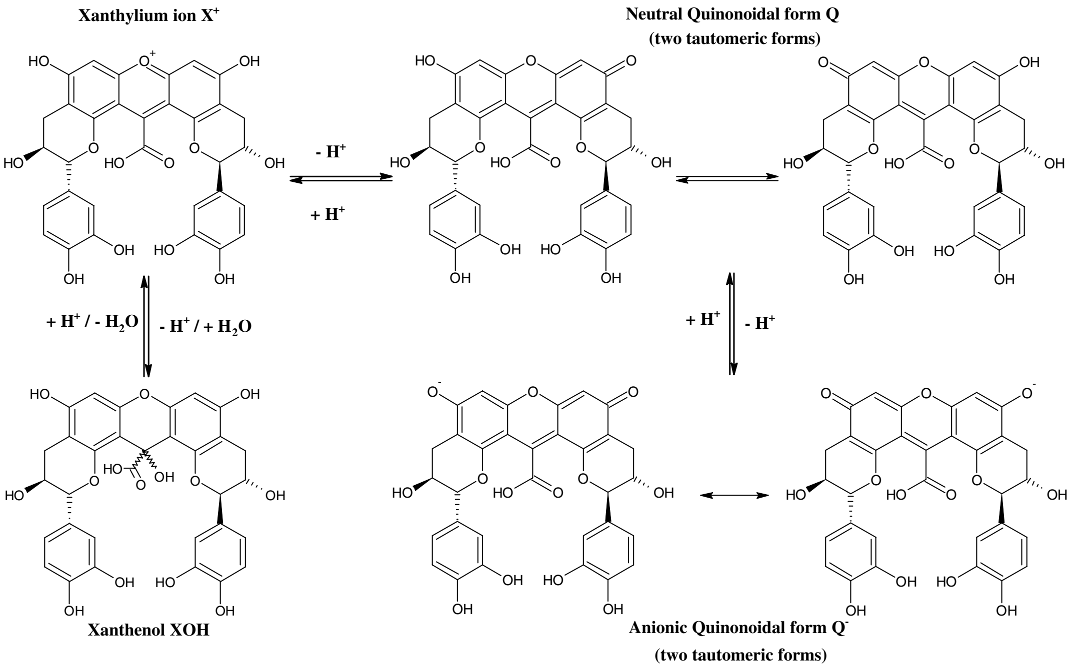
tions were observed, in the case of the UV absorption maxima, by increasing pH values.

The maximum colour intensity for the studied xanthylium salt was reached in the alkaline region, giving a reddish-pink colour ($\lambda = 490$ nm). Few variations were observed in the intensity of the absorption maxima located at 270 and 307 nm, which remained constant in all of the studied range of pH values. The curve representing the intensity of the visible absorption maximum began to increase at pH 5.6 and continued with the increase of pH (Fig. 4).

The variation of the visible absorption maxima molar extinction coefficient is shown in Fig. 5. The molar extinction was constant for acid pH values, ranging from 2.2 to 5.6. From pH 5.6 to 8.9, a steady increase was observed which continued with increase of pH.

3.4. Structural transformations

Like flavylium ions, polyhydroxylated xanthylium salts were shown to suffer (in aqueous solution media) various structural transformations (Dangles & Brouillard, 1994). Thus the studied pigment may exist in xanthylium (X^+), neutral (Q) and anionic (Q^-) quinonoidal forms, depending on the pH (Scheme 2). In addition to these transformations, self-association which is a dominant feature of xanthylium-type pigments could also occur. This was shown by varying the pigment concentration, with concomitant recording of the UV–Visible spectra. A deviation from Beer's law was observed. This can be interpreted as a dimerization of the pigment, as previously reported for 1,3,6,8-tetrahydroxyxanthylium ion (Dangles & Brouillard, 1994) which was reported to exist in acidic medium as both monomer X^+ and dimer $(X^+)_2$ ions. In addition to the xanthylium structure, the dimerization process could also involve the neutral and the anionic forms of the pigment.



Scheme 2. Hypothetical structural transformations of the xanthylium pigment.

The transformations indicated are obviously influenced by pH and thus explained the variation of the absorbance of the pigment (at a given wavelength) with pH as observed above. When going from pH 2.2 to 8.9 the wavelength of the visible absorption maximum is steadily displaced from 441 to 490 nm. This variation could be interpreted by successive changes in absorbing species within the series $(X^+)_2$– XQ^+–Q_2–QQ^-–$(Q^-)_2$. From pH 2.2 to 5.6, the observed spectral modifications were rather small and could be ascribed to the first two deprotonation steps leading to the Q_2 form. From pH 5.6 to 8.9, the observed spectral changes were much more significant and attributable to the Q^- form in the dimers QQ^- and $(Q^-)_2$.

4. Conclusion

This work showed that the colour and intensity of the studied xanthylium compound were significantly influenced by pH. In relatively acidic aqueous medium, the pigment occurred as an intense yellow colour, typical of the xanthylium form. From pH 5.6 the pigment colour intensity increased, while there was a gradual bathochromic shift to more pink reddish colour. The possible use of the studied pigment as a food colorant in slightly alkaline products should therefore be considered.

This work finally opens perspectives for further investigations of similar compounds. It should be borne in mind that other factors, not directly addressed in this study, may influence the pH-dependency of the xanthylium salt colour in aqueous media and should therefore be investigated. A number of other properties, such as temperature stability and copigmentation, remain of a high interest and should be investigated.

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