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Evaluation of the pH and thermal stabilities of rosella anthocyanin extracts under solar light

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ABSTRACT

Anthocyanin is considered as an unstable pigment, undergoes gradual degradation processes throughout the storage or use. The degradation of Rosella anthocyanin extract (RAE) in different pH solutions was studied under solar light and compared with solutions kept in the dark. It appears that RAE solutions under solar light were suffering rapid degradation than those kept in the dark. Moreover, RAE samples at higher pH values showed faster rates of degradation than those with lower pH values. A mechanism for degradation was suggested through the formation of hydroxyl radical which is responsible for demineralization process. It is also appears that as the initial concentration of the dye increases, the requirement needed for degradation increased as well. The thermal degradation behavior of RAE at temperature range 25–60 °C under solar light showed first order kinetics with low activation energies.

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1. Introduction

Anthocyanin pigments are responsible for the attractive red to purple to blue colors of many fruits and vegetables. Water soluble anthocyanins have become increasingly important and have received great interests in research as they not only impart beautiful coloration to the food system, but also have antioxidant properties and health benefits such as enhancement of sight acuteness, antioxidant capacity, controlling type II diabetes, reducing coronary heart disease and prevention of cancer (Bagchi et al., 2004; Fossen et al., 1998; Gulcin et al., 2004). Anthocyanin pigments are relatively unstable and often undergo degradative reactions during processing and storage. The stability of anthocyanin is correlated with structural features of anthocyanin and is also affected by factors such as heat, pH, light, the presence of enzymes, phenolic acids, oxygen, sugar, sulfur dioxide and metal ion (Harborne, 1958; Mazza and Brouillard, 1987). The thermal stability of anthocyanin extracts of *Clitoria ternatea* flower has been reported (Lee et al., 2011). The thermal degradation showed first order kinetics only at 70, 100 and 160 °C. The presence of benzoic acid in the extract lengthened the stabilization period for degradation. It was concluded that high temperature (~35 °C) reduced total anthocyanin content in grape skin to less than half of that in the control berries (Mori et al., 2007). To our knowledge, reports on

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anthocyanin extract degradation under solar light are scarce. The objective of this study was to evaluate the pH and thermal stabilities of rosella anthocyanin extracts under solar light.

2. Experimental

Chemicals of high purity were obtained from BDH. Experiments were conducted in February 2012 for six months at the ground floor of the Chemistry Department building in the University of Basrah-Iraq in an open atmosphere between 9.00 am and 1.00 pm.

2.1. *Reageants and solutions*

Buffer solutions of pH 1, 4, 7 and 9 were prepared according to literature methods (Robinson and Stokes, 1968). These solutions are stable at room temperature for a few months, but the pH should be checked and ajusted prior to use.

2.2. *Preparation of Rosella Anthocyanin Extract (RAE)*

The flower petals were purified from impurities, cut into small pieces, dried at 50 °C for 24 h and kept in a glass container prior to use. Dry petals (4.0 g) of rosella were extracted with buffer solution (100 ml). The suspension was filtered from the solid impurities and the red to purple solution was used for measurements (Cisse et al., 2009).

The absorbance of the extract was measured at λ_{max} in the visible region of the spectrum and altered to absorbance equal to one by addition of the properate buffer solution.

2.3. *UV–Vis spectroscopy*

UV–Vis spectra of RAE were recorded at wavelengths 200–800 nm. All measurements were made after sample preparation directly, since longer standing after about 1 h tends to increase observed reading (Mónica Giusti and Worlstad, 2001).

Absorbance readings were made against water blanks even if the sample was in buffer solution. For each pH value, a calibration curve was conducted between the absorbance and anthocyanin concentration. Calculations of the monomeric anthocyanin pigment concentation were made on the basis of cyanidin-3-glucoside where *MW = 449.2 g/mol* and *molar absorptivity of 26,900* $mol^{-1}L\ cm^{-1}$ as it is considered as the major constituent of anthocyanin in nature (Mónica Giusti and Worlstad, 2001).

2.4. *Irradiation of RAE solutions*

One hundred milliliters of the solution in a beaker (400 ml) was exposed to irradiation under solar light at ambient temperature. At specific time intervals, 2.0 ml of sample were taken out to measure the absorbance at λ_{max}. The rate of decolorization was performed in terms of change in intensity at λ_{max} with time. As the absorbance is directly proportional to the concentration of anthocyanin pigment, the decolorization efficiency or the percent of degradation has been calaulated as:

$$\%\ \text{of degradation} = \frac{A^\circ - A}{A^\circ} \times 100$$

where: A° = the initial absorbance of the sample solution. A = the absorbance at specific time intervals after photoiiradiation.

2.5. *Thermodynamic studies*

Thermal stability of RAE in aqueous solutions at different pH was studied by monitoring the absorbance of anthocyanin pigment at λ_{max} with time at different tempetatures. The sample was warmed in a pyrex tube (15 × 1.6 cm) inside a water bath for 5 min under solar light. Two milliliters of the samples were taken out to measure the absorbance at specific time intervals. All experiments were repeated at least 3 times to insure the reproducibility of the reading.

3. Results and discussion

3.1. *UV–Vis spectra of RAE*

The UV–Vis spectrum of RAE in aqueous solution (pH 1.0) (Fig. 1) exhibits maximum absorption bands (λ_{max}) at 220, 282, 335(sh), 450(sh) and 518 nm. The detailed analysis of this spectrum was described elsewhere (Mónica Giusti and Worlstad, 2001).The UV–Vis spectral data of RAE at different pH solutions are shown in Table 1.

The difference in the maximum absorption bands indicates the presence of different structural forms of anthocyanin at different pH values (Mónica Giusti and Worlstad, 2001). In this study, λ_{max} was chosen to be in the visible region of the spectrum.

3.2. *Degradation studies*

Anthocyanin is considered as an unstable pigment, undergoes gradual degradation processes throughout the storage or use (Cabrita et al., 2000; Dyrby et al., 2001). The degradation pofiles of RAE solutions at different pH for samples kept in the dark at room temperature are shown in Fig. 2. It seems that anthocyanin undergoes slow degradation depending on the pH of the solution and the most rapid degradation was found for pH 9 (20%, after a time period of 180 min). However, the least degradation appeared for pH 1 (6%) for the same period of time. These results might indicate that the presence of hyoxide ions

Table 1 – UV–Vis spectral data for Rosella anthocyanin extracts at different pH values.

RAE at pH	Maximum absorption bands (nm)
1	220, 282, 335(sh), 450(sh), 518
4	233, 278, 288, 323(sh), 519
7	233, 279, 289, 323(sh), 520
9	225, 284, 341, 455, 544

(sh) = shoulder.

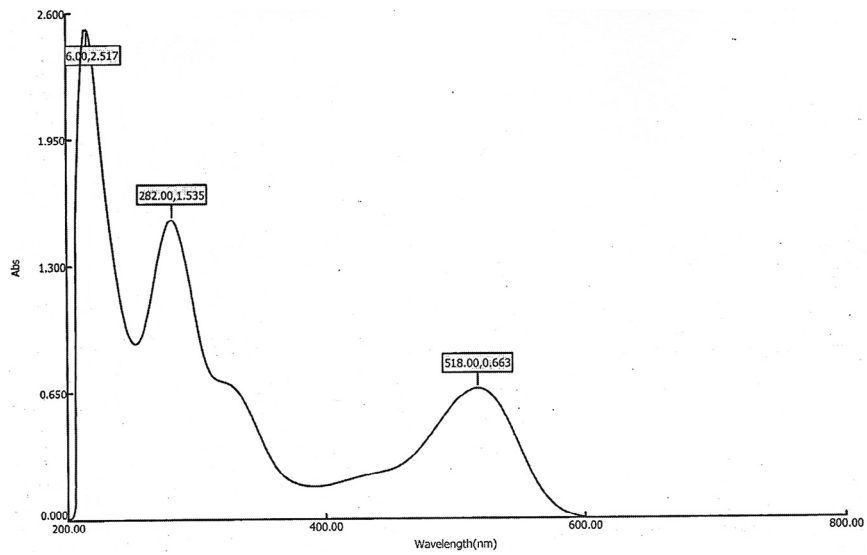

Fig. 1 – UV–Vis spectrum of anthocyanin extract solution at pH 1 at room temperature.

may induce and cooperate the increase in the degradation process.

As light would cause photo degradation of a dye, the effect of light on the stability of RAE was conducted at different pH samples. Figure 3 shows the degradation profiles of RAE solutions at different pH values under solar light at room temperature. A significant observation is that the degradation occurs by a faster rate than those samples kept in the dark. Table 2 shows the degradation rates for RAE samples kept in the dark and others exposed to solar light.

At pH 1, the rate of degradation of RAE is low, indicating that the extract is more stable than those at higher pH values which suffer faster rates. At this pH, the predominant form of anthocyanin is the stable red oxonium form. However, it seems that the most rapid degradation occurs at pH 4 (acidic medium), but this is not true, as the predominant structural form of anthocyanin at this pH is the colorless hemiketal form.

As there is no photocatalyst and no photo-excitation of a semiconductor, followed by the formation of electron–hole pair on the surface of the catalyst, the oxidation of the dye to reactive intermediate will possibly take place *via* the hydroxyl radical that is induced by exposing solution samples to solar light.

On the basis of these observations, it is suggested that anthocyanin aqueous solutions were suffering photo degradation as they are exposed to solar light. A possible mechanism may

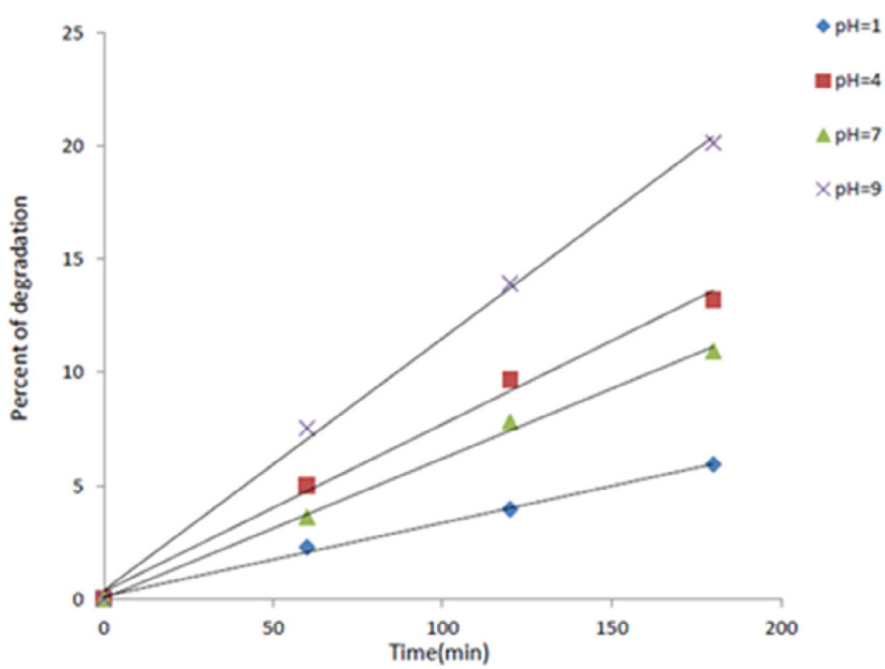

Fig. 2 – The percent degradation of RAE solutions kept in the dark at room temperature.

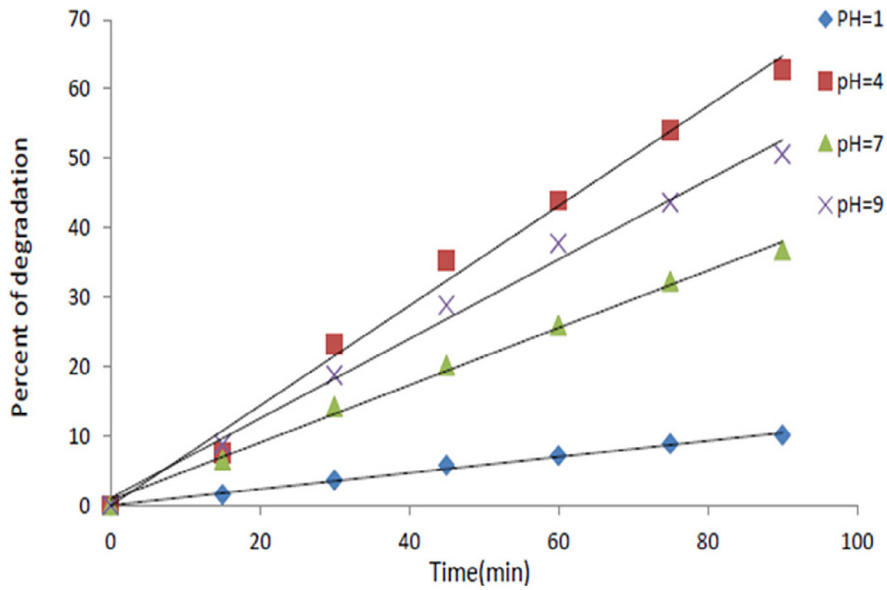

Fig. 3 – The percent degradation of RAE solutions under solar light at room temperature.

take place through the excitation of the dye by visible light to appropriate singlet or triplet states, subsequently followed by electron injection from the excited dye molecule onto hydroxyl ion:

$$\text{Dye} + h\upsilon(\text{solar light}) \rightarrow {}^{1\,\text{or}\,3}\,\text{Dye}^* \quad (1)$$

If oxygen molecules which have been dissolved in water are the only molecules of acceptor, so

$${}^{1\,\text{or}\,3}\text{Dye}^* + O_2 \rightarrow \text{Dye}^{+\bullet} + O_2^{\bullet-} \quad (2)$$

The primary products of photo-oxidation ($O_2^{\bullet-}$) can react further with electrons that are still being photo produced (Sobczynski and Dobosz, 2001):

$${}^{1\,\text{or}\,3}\text{Dye} + O_2^{\bullet-} + H^+ \rightarrow \text{Dye}^{+\bullet} + HO_2^{\bullet-} \quad (3)$$

$$HO_2^{\bullet-} + H^+ \rightarrow H_2O_2 \quad (4)$$

The formed hydrogen peroxide can undergo a further reaction to form hydroxyl radicals:

$$H_2O_2 + e^- \rightarrow HO^\bullet + HO^- \quad (5)$$

Hydroxyl radicals can be also produced from the reactions:

$$\text{Dye}^{+\bullet}(\text{or } RH_2^{+\bullet}) + HO^- \rightarrow \text{Dye}(\text{or } RH_2) + HO^\bullet \rightarrow {}^\bullet RH + H_2O \quad (6)$$

$$\text{Dye}^{+\bullet} + H_2O \rightarrow \text{Dye} + HO^\bullet + H^+ \quad (7)$$

The produced protons can be introduced in reaction (3) or react with hydroxide ion in solution:

$$H^+ + HO^- \rightarrow H_2O \quad (8)$$

Thus, the production of water will lead to a decrease in the hydroxide ion in the solution. A significant observation is that the decolorization of the dye was accompanied with a depression in pH by 2–3 units.

Experimental results revealed that higher degradation of the dye occurred in basic medium than in acidic medium. The rate of photo-decolorization increased with increase in pH and a possible reason for this behavior might be due to the formation of more hydroxyl radicals (Ochoa et al., 2001). A similar behavior was also observed in earlier studies on Acid brown dye (Daneshvar et al., 2003; Sakthivel et al., 2003) and methyl orange (Mehra and Sharma, 2012). In acidic medium, the concentration of hydroxide ions is quite low to form hydroxyl radicals enhancing the degradation process as shown for extract solutions at pH 1.

$^\bullet RH$ (equation 6) can react with oxygen to form:

$$^\bullet RH + O_2 \rightarrow \text{Products} + CO_2 \quad (9)$$

$$\text{and } {}^\bullet RH + {}^\bullet RH \rightarrow HR - RH \quad (10)$$

$$\text{or } {}^\bullet RH + {}^\bullet OH \rightarrow R + H_2O \quad (11)$$

Table 2 – The rate of degradation of anthocyanin extracts for samples kept in the dark and others exposed to solar light.

Process	Time (min)	% Degradation			
		pH 1	pH 4	pH 7	pH 9
Dark	60	2.273	5.011	3.598	7.525
Solar light	60	7.175	43.909	25.925	37.732
Dark	120	3.954	9.687	7.798	13.913
Solar light	120	7.283	63.818	34.649	50.244

Table 3 – The % degradation of RAE solutions at ambient temperature.

Time (min)	% degradation of anthocyanin			
	pH 1		pH 4	
	1.75×10^{-5} M	3.00×10^{-5} M	1.75×10^{-5} M	3.00×10^{-5} M
30	4.00	3.65	23.18	17.02
60	10.91	7.18	43.91	32.05
90	12.27	10.14	60.72	48.31
120	13.40	7.28	63.82	61.46

However, in a study on anthocyanin (Daravingas and Cain, 1968) and in our study, the absorbance of the solution tends to increase after irradiation with solar light after about 60 min. This observation may be due to the formation of polymeric species (equation 10) especially at high concentration of anthocyanin pigment.

However, dilute solutions show more rapid rates than concentrated ones (Table 3), thus at higher dye concentration, degradation decreases at sufficiently long distances form the light source or the reaction zone due to the retardation in the penetration of light. Hence it is concluded that as the initial concentration of the dye increases, the requirement needed for the degradation also increases.

3.3. *Thermal degradation studies*

Thermal stability studies of anthocyanin extracts (Dyrby et al., 2001) or pure anthocyanin (Cabrita et al., 2000) as well as for different nutrition systems (Cemeroglu et al., 1994; Daravingas and Cain, 1968; García-Viguera et al., 1999; Gradinaru et al., 2003) were found to follow first order kinetics.

The thermal degradation of red RAE was studied at a temperature range 25–60 °C under solar light. All measurements have been made between 15 min and 1 h after sample preparation.

Table 4 – Kinetic data of degradation of RAE solutions at 298 K under solar light.

pH	k (min^{-1})	E_a (kJ/mol)	$t_{1/2}$ (min)
1	0.002	4.66	346
4	0.010	0.99	69.3
7	0.005	1.91	144.4
9	0.007	1.	97.6

It has been agreed, with minor doubts, that the rate of photodegradation of organic substances such as dyes for a process take place in solution follows a first order equation:

$$\ln(A/A) = kt$$

where: A_0 = the initial absorbance of the sample solution:
A = the absorbance at specific time intervals after irradiation.
k = the rate constant.
t = irradiation time.

A useful indication for the rate of a first order reaction is the half-life, $t_{1/2}$, of the dye:

$$t_{1l2} = \frac{\ln 2}{k}$$

Also, a main point to note is that, for a first order reaction, the half-life of the dye is independent of its initial concentration.

The thermal degradation profiles of RAE solutions at pH 1 and 9 under solar light are shown in Figs 4 and 5. The kinetic parameters are summarized in Table 4. The activation energy for the degradation process is calculated by using Arrhenius equation:

$$\ln k = \ln A - E_a/RT$$

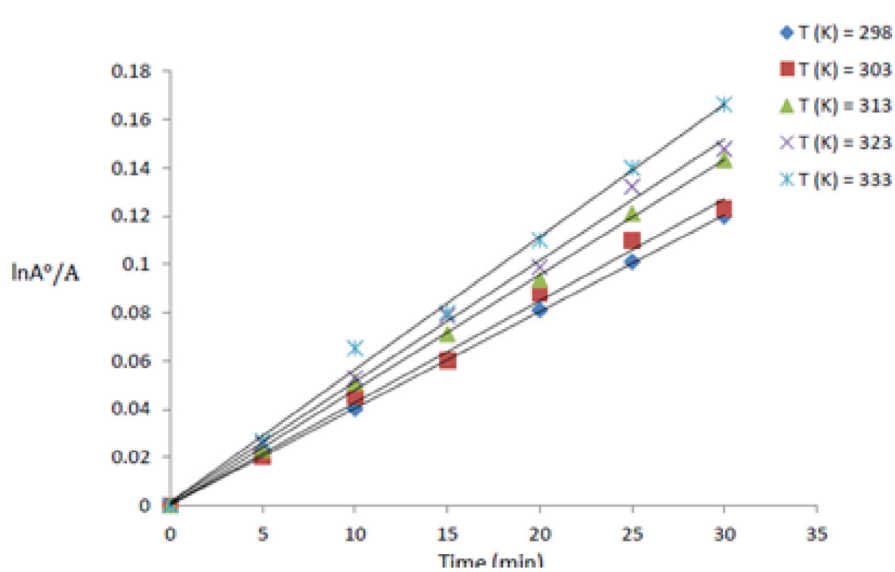

Fig. 4 – The thermal degradation profiles of RAE solutions at pH 1 under solar light.

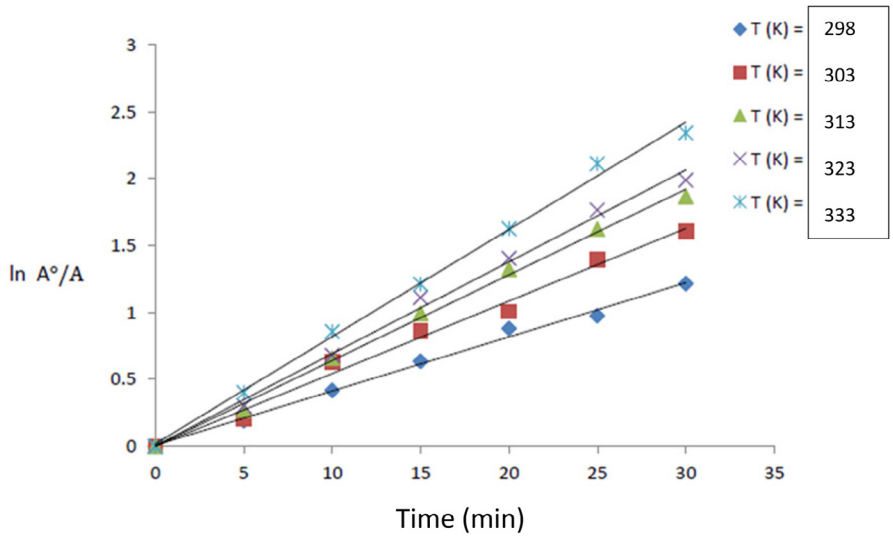

Fig. 5 – The thermal degradation profiles of RAE solutions at pH 9 under solar light.

A plot of ln k against 1/T gives a straight line. The parameter A, which is given by the intercept of the line at 1/T = 0 is called the frequency factor. The activation energy, E_a, is obtained from the slope of the line ($-E_a/R$). Figures 4 and 5 indicate that anthocyanin degradation responds to high temperature. According to Table 4, the RAE solution at pH 1 shows higher activation energy and longer half-life than the other extracts indicating the higher stability of the dye at this pH. It seems also that the degradation profiles of RAE solutions are faster than that observed in other studies (Lee et al., 2011; Sobczynski and Dobosz, 2001), which may be explained by the presence of different constituent species in different extracts.

4. Conclusion

On the basis of this study, it seems properly that anthocyanin degradation is affected by pH, temperature, light, oxygen and the structural forms of anthocyanin extract. Therefore, care must be taken as anthocyanin is relatively unstable and often undergoes degradation during processing and storage. This is in accordance with other works found in literature.

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