

Developing a Thermochromic Material and Study of Kinetics of Degradation of Anthocyanin from Local Grapes

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Abstract— Thermochromic materials undergo a colour change with temperature which finds numerous practical applications in food and medical industry. Since most thermochromic materials contain at least one toxic compound, their application in medical and food industry is limited. The present study intended to develop a thermochromic material based on natural pigment, anthocyanin, extracted from local grapes. Different biodegradable polymers were incorporated into anthocyanins and thermochromic effect was monitored. A kinetic study was conducted to identify conditions which enhance anthocyanin stability. Thermochromic ability of anthocyanin from local grapes was investigated using agar, agarose and chitosan biopolymers as matrices, while kinetic study was carried out for different solvents, temperatures and pH values. The concentration of anthocyanin was measured using pH differential method. Although for agar and agarose biopolymers, a considerable thermochromic effect was not observed, for chitosan – acetic acid – anthocyanin mixture, positive results were observed. At isothermal conditions, chitosan film which was originally wine red changed gradually to violet, then brownish yellow and finally to brown. Depending on temperature, pH and the solvent, colour of anthocyanin changed. At low pH values, low temperatures and with polar solvents, high colour retention was observed than at high pH values, high temperatures and with less polar solvents. Colour retention of samples had a direct relationship with degradation rate of anthocyanin. The kinetic results proved that acidified ethanol extracts of anthocyanin from local grapes are stable at low temperatures. Results indicated that chitosan – acetic acid – anthocyanin mixture has potency for use as thermochromic material. Further, by enhancing the conditions identified in the kinetic study, anthocyanin from local grapes can be used as a food colorant in other food items.

Keywords— Anthocyanin, Thermochromic, Chitosan

I. INTRODUCTION

Currently, thermochromic materials have many applications. Almost all of the thermochromic materials in use are synthetic and highly sensitive to temperature and they change colour at precise temperatures. These materials are easy to apply and the colour change is reversible. Synthetic thermochromic materials have been incorporated into baby bottles, kettles, sensors, thermometers, optical switches, temperature sensitive light filters and thermal printing (Chen & Yang, 2009). Major drawbacks of these synthetic materials are that most of them are carcinogenic or at least one of the components in the material exhibits toxic effects. Heavy metal salts, leuco dye systems, which even can contain bisphenol A (BPA) are some compounds that are used in synthetic thermochromic materials (Seeboth, Löttsch, & Ruhmann, 2013). Therefore, the importance of developing a non-toxic thermochromic material has become essential.

If a natural organic molecule can change its spectral properties reversibly with temperature and if the thermo-optical change is detectable to the human eye, that particular material can replace the synthetic thermochromic material. Recently most researchers have developed non-toxic thermochromic materials using anthocyanidin and their derivatives (Seeboth et al., 2013), (Löttsch, Ruhmann, & Seeboth, 2013), by using the changes in their molecular structure which depend on the chemistry of the surroundings. Anthocyanins are one of the major classes of pigments which are the most important natural pigments after chlorophyll that are visible to the human eye (Brouillard, Chassaing, Isorez, Kueny-stotz, & Figueiredo, 1993).

In aqueous environment, anthocyanins are sensitive to the pH of the solution and temperature. At high temperatures anthocyanins degrade into phenolic compounds. In the presence of a proper matrix, molecular arrangement of anthocyanin in space changes at different temperatures (Matsushima, Ogiue, & Fujimoto, 2000). This process leads to a reversible colour change of the mixture resulting in a thermochromic effect. This stacking ability makes anthocyanin, a thermochromic material. Almost all of the

thermochromic materials which are based on anthocyanin are developed using synthetic anthocyanidins, a high cost organic compound. In this research project, we have attempted to develop a thermochromic material which is based on natural anthocyanin extracted from local grapes.

The stability of anthocyanin is an important factor if it is to be used as a thermochromic material. In the current project, the stability of anthocyanin was studied using a spectroscopic method, since anthocyanin contains a chromophore which absorbs ultraviolet and visible radiation. The pH differential method was used to determine the monomeric anthocyanin content (Moldovan, David, Chisbora, & Cimpoi, 2012) which is based on the structural changes of the anthocyanin chromophore between pH 1.0 and 4.5.

II. METHODOLOGY AND EXPERIMENTAL DESIGN

A. Sample preparation

Local grapes were purchased from the public market. They were first washed with tap water and then with distilled water after the stems were removed. They were wiped with clean paper towels. Then the skin was peeled off by hand and dried in air for four hours. Dried grape skin (10 g) was chopped with mortar and pestle while introducing the appropriate acidified solvent. This mixture was sonicated for 60 minutes at room temperature. A nylon mesh was used to filter out the grape skin and the residue was washed with 50 ml of solvent. Filtrate was vacuum filtered three times. Resultant solution was centrifuged at 3200 rpm for 15 minutes and the solvent was evaporated under low pressure at 35 °C until 70% of the initial volume was reached. Solvent extraction was carried out for each 50 ml portion of the extract with 10 ml of hexane until the hexane layer became colourless. Prepared samples were stored at 2 °C until the kinetic study was performed.

B. Kinetic study

For each solvent, four temperatures (2, 30, 60, 90 °C ± 1 °C) and four pH values (1, 2, 5 and 8) were studied in triplicates. Therefore, for one solvent system 4×4×3 samples were prepared by adding 2.5 ml of the extract and diluting the sample in a 10 ml volumetric flask (DF = 4) with the corresponding buffer. Aliquots were kept for 30-45 minutes at room temperature to equilibrate and then initial readings (A_0) were taken with a single beam UV-Visible spectrophotometer. For this measurement two portions (0.5 ml × 2) were removed from each extract. Prepared samples were kept at required storage temperatures. For the kinetic study, similar portions were removed at regular time intervals and anthocyanin concentrations were determined by pH differential method as follows.

$$\left. \begin{array}{l} \text{Anthocyanin pigment} \\ \text{Concentration} \end{array} \right\} = A \times MW \times DF \times 10^3 / (\epsilon \times l)$$

Pigment concentration in terms of (malvidin-3-glucoside equivalents, mg/L)

$$A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}$$

A = absorbance

MW (molecular weight) = 449.2 g/mol for malvidin-3-glucoside

DF = dilution factor

l = pathlength of the cuvette in cm

ϵ = molar extinction coefficient in $\text{L mol}^{-1} \text{cm}^{-1}$,
(for malvidin-3-glucoside $\epsilon = 26\,900 \text{ L mol}^{-1} \text{cm}^{-1}$)

10^3 = factor for conversion from g to mg

1) Heat treatment

Thermal degradation of grape anthocyanin was studied at two storage temperatures (2 °C, 30 °C) and two high temperatures (60 °C, 90 °C). Portions of 30 ml (10 ml × 3) of anthocyanin were stored under corresponding storage temperatures after recording the A_0 value. For high temperatures (60 °C, 90 °C) 30 ml portions were heated to the corresponding temperatures in round bottom flasks fitted with a condenser. This set up was employed to minimize losses due to evaporation. The initial reading was taken after 10 minutes when thermal equilibrium was reached. Small portions (1 ml × 3) from each solution mixture were removed at regular time intervals and rapidly cooled in an ice bath.

2) Determination of monomeric anthocyanin content:

Anthocyanin concentration of grape extract was determined by pH differential method. To each of two 5 ml volumetric flasks, a 0.5 ml portion of the solution was transferred. One portion was diluted with pH 1 potassium chloride buffer and the other portion (0.5 ml) with pH 4.5 sodium acetate buffer. Absorbance of both of these samples was measured at 520 nm and 700 nm using a UV-Visible spectrophotometer. The corresponding buffer solutions (pH 1 or pH 4.5) were used as the blank solutions.

All experiments were done in triplicate and the statistics (mean and standard deviation) were analysed using Microsoft® office Excel 2007. Linear regressions were obtained using SigmaPlot 2001 version 7.0 for Windows.

C. Thermochromic study

1) Preparation of chitosan films

For the thermochromic study, 0.100 g of chitosan was measured into a 100 ml beaker and 10.00 ml of 1 M acetic acid was added to the beaker. Mixture was continuously swirled at room temperature until chitosan completely dissolved. Resultant mixture was transferred

into a Petri dish (diameter 6 cm) and then it was kept at 110 °C for 30 minutes. Mixture was taken out and 2 ml of anthocyanin (acidified aqueous extraction, concentration = 33.88 ppm) was added to the mixture. Resultant mixture was cooled in air. After cooled to room temperature, mixtures were kept at 2 °C, 30 °C, 60 °C and 90 °C and the colour change was observed.

2) Preparation of agar films

Agar 0.100 g was measured into a 100 ml beaker and 10.00 ml of distilled water was added to the beaker. Mixture was covered by a watch glass and continuously swirled and heated in a water bath until it boiled. Then 1 ml of anthocyanin (acidified aqueous extraction, concentration = 33.94 ppm) was added to the mixture and swirled at room temperature for 2 minutes. The resultant mixture was transferred into a Petri dish (diameter 6 cm) and air dried for 15 minutes. Mixtures were kept at 2 °C, 30 °C, 60 °C and 90 °C temperatures and the colour change was observed.

3) Preparation of agarose films

Agarose films were prepared in the same manner as agar films and thermochromic effect was studied under similar conditions.

III. RESULTS

A. Kinetic study

Previous work on anthocyanin degradation has shown that the anthocyanin degradation follows first order kinetics. For a first order reaction, variation of the concentration with time can be written as in equation 1.

$$c_t = c_0 e^{-kt} \quad (1)$$

where, c_t and c_0 are the anthocyanin concentrations at time t and $t=0$ respectively; k is the first order rate constant and t is the storage time (hours or days). Variation of anthocyanin concentration as a function of time can be plotted according to equation 2 (Figure 1).

$$\ln \frac{A_t}{A_0} = -kt \quad (2)$$

A_t – absorbance at time t , A_0 – absorbance at $t=0$

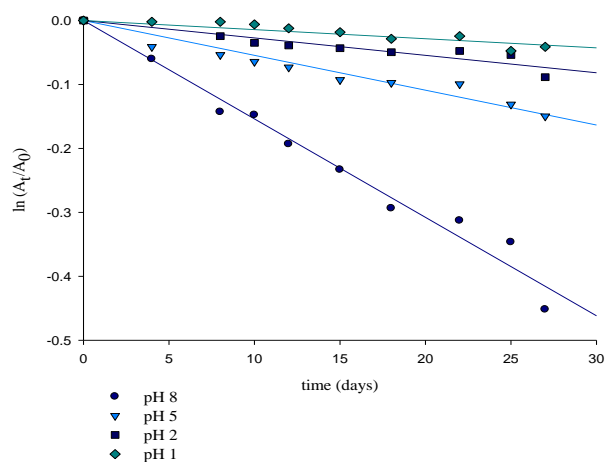


Figure 1. Variation of anthocyanin concentration with time for water at 2 °C

Anthocyanin degradation was studied using Arrhenius equation.

$$k = A e^{\frac{-E_a}{RT}} \quad (3)$$

k - first order rate constant, A - pre-exponential factor, R - universal gas constant, E_a – Arrhenius activation energy, T – temperature in Kelvin. Equation 3 can be rearranged to,

$$\ln(k) = \left(\frac{E_a}{R}\right) \times \frac{1}{T} + \ln(A) \quad (4)$$

By plotting a graph of $\ln(k)$ vs. $1/T$ activation energy for each process was calculated.

Colour degradation was observed with increasing pH. Initially bright and attractive colours were observed, but only the extracts at low pH values retained their colour with increasing temperature and time. These observations were compatible with calculated rate constants. To the best of our knowledge, kinetic study of anthocyanin from local grapes has not been reported in literature prior to this study. The calculated half life values were compared to the half life values from literature, for the degradation process of anthocyanin obtained from other sources.

When considering aqueous extraction, at 2 °C, half life values varied from 55.1 days to 486 days, when pH changes from 1 to 8. Moldovan *et al* have reported (Moldovan, David, Chisbora, & Cimpoi, 2012) that half life values at 2 °C for anthocyanin from European Cranberry bush were 48.1 and 10.3 days, in water, at pH 3 and 7 respectively. When comparing with their values,

anthocyanins from local grapes are more stable (254, 55.1 days).

At higher pH values anthocyanins turn to quinonoid base form or its anionic form which is blue-violet in colour. Data above implies that at high pH values, quinonoid base is unstable. Therefore, at low pH, degradation of anthocyanin is less than at high pH values (pH 5 and pH 8).

Temperature is another parameter which affects the colour stability of anthocyanin. When the temperature increases, stability of anthocyanin decreases, hence a colour change can be observed. Wang *et al.* have found that anthocyanins in blackberry juice (65.0 Brix) has a half life of 330.1 days at 5 °C and at pH 2.86 and more diluted juice (8.9 Brix*) has a half life of 16.7 and 2.9 hrs at 60 °C and 90 °C respectively. pH of the juice was 2.89. In the aqueous extraction from local grapes half lives were found to be 254 days, 26.2 hrs and 2.48 hrs at 2 °C, 60 °C and 90 °C respectively at the same pH. This implies that anthocyanins are stable at low temperatures and low pH values.

To evaluate the most appropriate solvent, and to predict the solvent sensitivity, variation of the rate constant was plotted against the pH, for each temperature (figures 2-5).

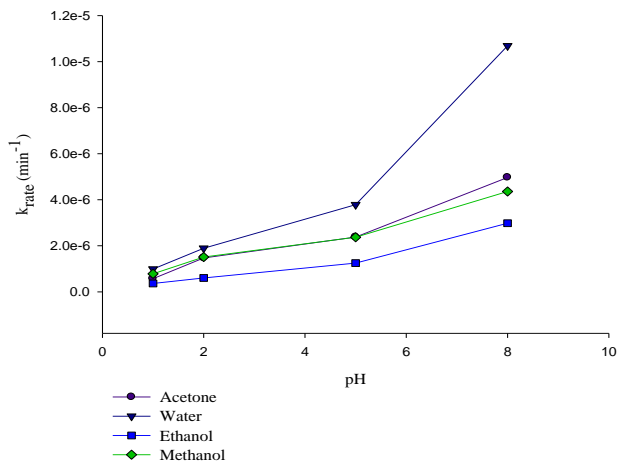


Figure 2. Degradation rate of anthocyanin in different solvents as a function of pH at 2 °C.

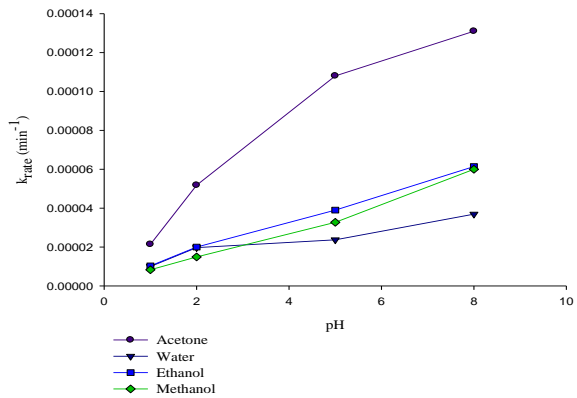


Figure 3. Degradation rate of anthocyanin in different solvents as a function of pH at 30 °C.

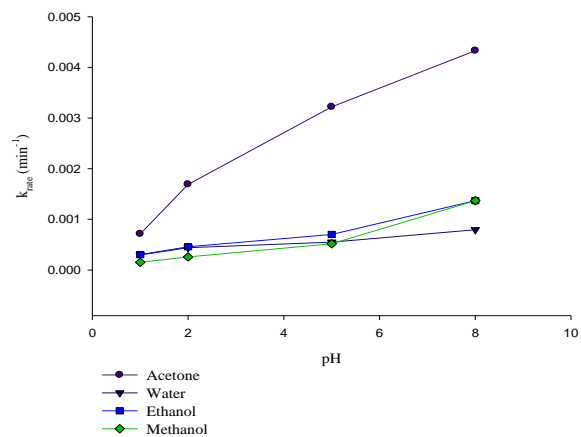


Figure 4. Degradation rate of anthocyanin in different solvents as a function of pH at 60 °C.

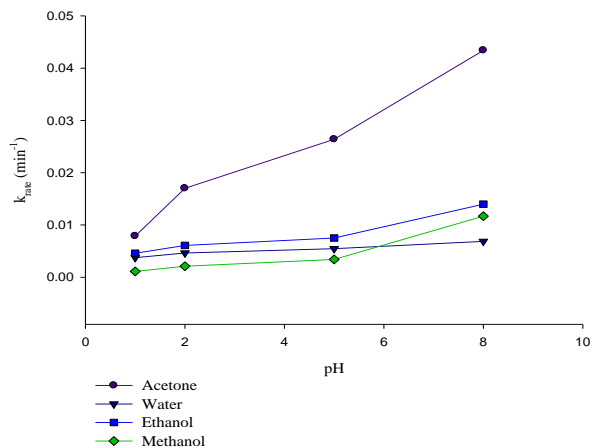


Figure 5. Degradation rate of anthocyanin in different solvents as a function of pH at 90 °C.

The kinetic study revealed that, anthocyanin had the highest degradation rate in water at 2 °C (Figure 2) while in ethanol it showed the lowest rate of degradation at both high and low pH values. When the temperature was increased anthocyanin degraded more significantly in acetone at all pH values (figures 3-5). But at high pH values (pH 5 and pH 8) anthocyanin had the highest resistance for degradation in water while in methanol it had the lowest degradation rate at low pH values (pH 1, pH 2). Meanwhile, the acetone extract showed the highest sensitivity for the all pH values and all temperatures except 2 °C. Water extract was sensitive to pH at 2 °C and it was less sensitive at 30, 60 and 90 °C. At 2 °C ethanol had the lowest sensitivity. Therefore, all these results conclude that acetone extract was the most sensitive extract for pH while ethanol and water extracts were comparatively less sensitive towards pH.

Activation energy for the degradation of anthocyanin can be obtained by plotting $\ln k$ vs $1/T$ as follows.

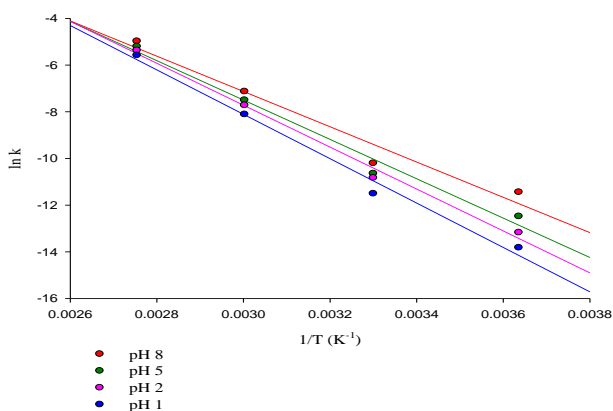


Figure 6. Variation of $\ln k$ with $1/T$ for acetone extract

As mentioned previously, there are no reported literature data on local grapes. Therefore calculated activation energy values were compared with activation energies for other food sources and different grapes species. The activation energies reported for blood orange juice for 11.2, 45 and 69 Brix were, 73.6, 84.5, 89.5 kJ/mol respectively. Patras *et al* have reported the activation energy for degradation of anthocyanin extract from grapes as 76.61 kJ/mol. In the present study, calculated values for aqueous extraction of local grapes varied from 62.8 to 79.0 kJ/mol in the pH range 1-8. Calculated activation energy values for anthocyanin from local grapes (Table 1) are therefore in the same order with blood orange and grapes from other sources.

High activation energy values imply that anthocyanin at low pH is more susceptible to degradation than in high

pH media. Increasing the pH results in a decrease in the activation energy hence, an increment in the rate of degradation.

Table 1. Activation energies and pre-exponential factors.

solvent	pH	E_a (kJ/mol)	A (min^{-1})
Acetone	8	86.8	1.48×10^{11}
	5	88.9	2.09×10^{11}
	2	89.3	1.38×10^{11}
	1	90.8	1.05×10^{11}
Water	8	62.8	5.65×10^6
	5	70.1	5.33×10^7
	2	74.6	2.20×10^8
	1	79.0	3.29×10^{10}
Ethanol	8	80.4	5.14×10^9
	5	82.0	4.95×10^9
	2	87.1	2.06×10^{10}
	1	89.5	3.29×10^{10}
Methanol	8	75.6	8.78×10^8
	5	69.5	3.56×10^7
	2	69.3	1.84×10^7
	1	69.8	1.20×10^7

B. Thermochromic study

Anthocyanin was incorporated into agar, agarose and chitosan films and the colour change of the film was monitored at different temperatures as a function of time.

For agar and agarose, similar results were obtained and at low temperatures (2 °C, 30 °C) a colour change was not observed. Chitosan – anthocyanin – acetic acid system showed a potential colour change with temperature. However, none of these systems exhibited reversibility.

Agarose is the pure form of agar. Agar has a slight yellow colour whereas, agarose is a white powder. Initial colours of both films were much similar (light pink) and a colour change was not prominent even at high temperatures.

A previous study which has been done with synthetic anthocyanin derivative (7-diethylamino-4'-dimethylaminoflavylum perchlorate) – agar system, has shown a reversible colour change with time (Matsushima, Ogiue, & Fujimoto, 2000). Anthocyanin (from local grapes) – agar system did not exhibit any thermochromic effect. Anthocyanin – agarose system was the same as anthocyanin – agar system. At high temperatures, with

isothermal heating agar mixture was discoloured due to the formation of thermally degraded phenolic compounds and this process was irreversible.

In contrast to anthocyanin-agar and anthocyanin-agarose films, prominent colour changes were observed as a function of time, at higher temperatures for chitosan-acetic acid-anthocyanin films. At high temperatures (90 °C and 60 °C) colour change was rapid and at low temperatures, (2 °C and 30 °C) prominent colour changes were observed after 18 hrs. (figures 7-10)

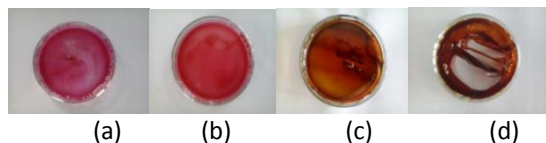


Figure 7. Colour change for chitosan mixture at 90 °C. (a) t=0, (b) 1 hr, (c) 3 hr, (d) 4 hr

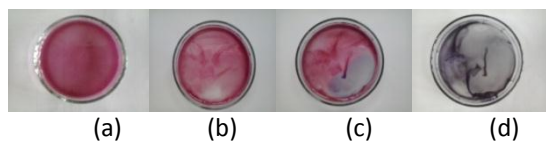


Figure 8. Colour change for chitosan mixture at 60 °C. (a) t=0, (b) 1 hr, (c) 3 hr, (d) 4 hr

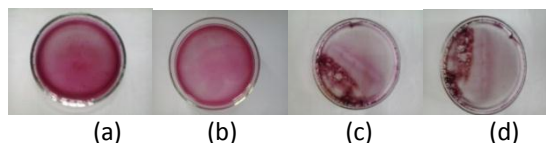


Figure 9. Colour change for chitosan mixture at 30 °C. (a) t=0, (b) 6 hr, (c) 18 hr, (d) 24 hr

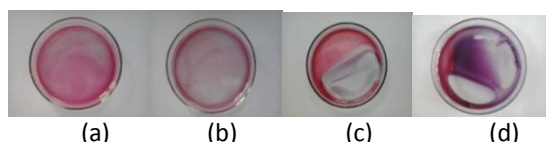


Figure 10. Colour change for chitosan mixture at 2 °C. (a) t=0, (b) 6 hr, (c) 18 hr, (d) 24 hr

The film thickness was observed to be not uniform and the film tend to shrink as the temperature was increased. This may be avoided by having a thicker chitosan film. These studies are currently in progress.

When considering the chitosan – acetic acid – anthocyanin mixture, initially a wine red colour was observed. With time and temperature, it turned purple and then brownish yellow. These results were much similar to the results reported in literature on a PLA (poly lactic acid) – anthocyanidin system (Seeboth et al., 2013). However, irreversibility of the natural anthocyanin – chitosan system is a problem. With natural anthocyanin, reversibility is impossible because of sugar moieties attached to the anthocyanidin ring system. These large sugar moieties prevent the proper rearrangement of the molecule which is responsible for a reversible colour change. Thus, molecular stacking and formation of hydrogen bonding become impossible to a certain extent. Colour changes observed for chitosan – anthocyanin – acetic acid films were the same as that for anthocyanin in buffer solutions. Comparing the time taken for colour changes in chitosan – anthocyanin – acetic acid films (few hours) with those in kinetic study (few days), it implies that the colour changes are more prominent and faster in chitosan – anthocyanin – acetic acid films than some of the systems in solution.

IV. DISCUSSION AND CONCLUSION

According to the results of the kinetic study of anthocyanin from local grapes, the degradation of anthocyanin follows first order reaction kinetics while the variation of degradation rate constant with temperature obeyed the Arrhenius relationship. Further, these results show that stability of anthocyanin strongly depends on temperature, pH and the solvent.

Anthocyanins are less stable at high pH values for all solvents. When comparing the rate constants of high and low pH values, in most cases rate constant for pH 8 is approximately ten times greater than the rate constant for pH 1. These results imply that the pigment stability can be enhanced by lowering the storage pH. Furthermore, at high temperatures the pigment degrades faster. Hence the degradation rate is a maximum at 90 °C and a minimum at 2 °C. Therefore, low storage temperatures will be more appropriate to preserve the anthocyanin stability.

We can conclude that at low pH values, low temperatures and in the presence of ethanol (solvent), anthocyanin stability of local grapes is maximized while with high temperatures, high pH values and in acetone environment, anthocyanin stability was low. Therefore, 1% acidified ethanol is the most efficient solvent system because anthocyanin extraction efficiency and the anthocyanin stability is highest under these conditions.

Ethanol is a non-toxic material in small doses. Therefore, above results exhibit the potential of developing a food colourant based on anthocyanins from local grapes or developing a thermochromic material based on natural anthocyanin-chitosan system which could be used in food packaging. Further, using acidified ethanol in the extraction process will be cost-effective in the industrial scale. Further studies on this system are required before it is commercialized as a thermochromic material.

ACKNOWLEDGMENT

We thank the Department of Chemistry, University of Colombo for providing the laboratory facilities to conduct this research.

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