Condensation Polymerization of Anthocyanin Biomolecule and its Effect on Polymers

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Abstract: This study deals with pure Iraqi red beet juice which is extracted mechanically into two states, the pure anthocyanin juice and its solid state obtained by evaporation the pure anthocyanin juice until obtained the pure solid anthocyanin. By boiling the juice at 102°C and heating the solid at 110°C both of them show dimerization product which are confirmed from the weight of liberated water and the degree of polymerization is 2 (dimer formation). The pure solid anthocyanin and dimer anthocyanin (by heating and boiling) added to biopolymers (poly vinyl alcohol, poly methyl methacrylate, starch and cellulose) and noticed that this pigment effected on the glass transition temperature, density and hardness of biopolymers.

Keywords: Condensation polymerization, anthocyanin, biopolymers and poly methyl methacrylate

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INTRODUCTION

Anthocyanin is Greek word means anthos-flower and kyanos-blue and it used originally for describing a blue and red pigment, is the significant group in plant pigments which has high solubility in polar solvents and water (Miraj *et al.*, 2015). Anthocyanin is an eminent color pigment which found in several vegetables, fruits and flowers giving a juice its purple hue (Alongi, 2010). It is kind of flavonoids that are phenolic compounds and have the C6-C3-C6 skeleton (Ferreiro-González *et al.*, 2014). It was known as a functional material in human health and was used widely in medicine and food industries (Mei *et al.*, 2014). The antioxidant activity is the most important function of anthocyanin which reduces a singlet oxygen (free radical) in the oxidation reactions of chelate metal and lipid with a wide extent of pharmacological, anti-allergenic, anti-microbial, anti-arteriogenic, anti-thrombosis, anti-inflammatory properties, anticancer activity, anti-oedema properties, cardio-protection and vasodilator (Clemente and Galli, 2011). The free anthocyanins act as monomeric pigments which are unbounded to other sorts. The polymeric anthocyanins can be created in the processes of copigmentation and direct condensation. The pigments which formed have a chemical stability more than a free monomeric and the wine color was stabilizing by variation to the more brick red (Tsanova-Savova *et al.*, 2002). More resistant for decoloration when anthocyanins are polymerized (Predrag *et al.*, 2003). The changes in pH are not effecting on polymeric pigments. The polymerized anthocyanin was creating through storage and thermal processing in a temperature and time-dependent method which

accelerated by the presence of light, oxygen, flavanol derivatives and ascorbic acid (Palencia, 2009). The polymer term was used to signify a particular class from macromolecules which consist from set of orderly repeated chemical units of a same kind or possibly from very limited number of various kinds, often only two, linked end to end or by more complex ways to form the chain molecule by polymerization process. In case of only one type from chemical unit the identical polymer is a homo-polymer, if there was more than one kind it is a copolymer (Bower, 2002). During development science of polymer, two categories were used. The first was based on composition of polymer, which divided polymers into addition and condensation polymers but the second category was based on a polymerization mechanism, which divided into chain and step polymerizations but these terms are usually used synonymously because generality condensation polymers were produced through step polymerizations and generality addition polymers were produced through chain polymerizations but this is not always a case (Odián, 2004). Condensation polymers were obtained from a random reaction of two molecules can be monomer, oligomer or higher molecular weight average each having integral functional end units such as hydroxyl groups or carboxylic acid (Fried, 2014). The repeated units number in a separated polymer chain is named as a degree of polymerization, x_n (Mitchell, 2004). The science of macromolecules was divided into biological and non-biological matters, which considered a large importance. Biological polymers have very institution in a life and intelligence and also provide much of a food on which human exists (Billmeyer, 1984). Polyvinyl alcohol is a hydrophilic polymer is

water-soluble, biodegradable, translucent and colored or white granular powder (Maiti *et al.*, 2012). It is a biggest volume synthetic resin generated in a world which represented by a formula $(C_2H_4O)_n$ (Rowe *et al.*, 2009). The PMMA is one of a most important commercial polymers which was known as Plexiglas and Lucite in America, Plexiglas and Perplex in Europe. The PMMA is a most important homo-polymer in a series of acrylics with adequate high temperature of glass transition (Sankar *et al.*, 2004). Cellulose is a major component in the plant cell wall thus it forms the most abundant polymer on earth (Olatunji, 2015). Cellulose is a crystalline matter which insoluble in all organic solvents and infusible (Vroman and Tighzert, 2009). Starch is environmentally friendly material and is fully biodegradable. In addition a starch has low cost, nevertheless, because starch has comparatively poor mechanical properties compared to other petrochemical polymers and has high sensible towards a water, its use was limited (Vroman and Tighzert, 2009). Pure form of starch is tasteless, white and odorless powder. It is polysaccharide which consists from two types of a molecules, amylose and amylopectin (Olatunji, 2015).

Experimental procedure: Iraqi red beet is used after removing the leaves and cleaning, then cuts as slices and squeezes mechanically so concentrated juice is obtained in two states, the pure anthocyanin juice and its solid state obtained by evaporation the juice at 50°C then drying in vacuum oven at 50°C until obtained the pure solid anthocyanin.

The polymerization process of mechanically extracted anthocyanin is done by two ways as shown in Fig. 1:

- By boiling the juice at 102°C for 15 minute until deposit particles appeared then the deposit particles filtered and then dried in vacuum oven at 50°C
- By heating the pure solid anthocyanin in oven at 110°C until a constant weight (weight loss is liberation of water by condensation polymerization)

Four types of biopolymers are used, synthetic biopolymers poly methyl methacrylate and polyvinyl alcohol and natural biopolymers starch and cellulose. They are different in samples preparation and their testing due to their differences in properties. Sample preparation is done on many steps and there are differences and similarities for each type of used biopolymers which depend on its blending portability with pigment. The sample preparation steps are:

- Addition of pigment (pure solid anthocyanin and dimer anthocyanin by heating and boiling) to the biopolymers in the following percent (1 and 7%)
- For mixing the pigment with biopolymers, ethanol was added, with a continuous stirring on a hot plate at 50°C until getting homogeneous material. Ethanol addition to mixture of PMMA with pigment lead to PMMA soluble and create stick materials
- Evaporating the solvent in vacuum oven at 50°C
- The result is a colored polymeric material in solid form, but the result of PMMA blending was a hard solid material

Fig. 1(a-d): (a) Pure anthocyanin juice, (b) Pure solid anthocyanin, (c) Dimer anthocyanin by boiling the juice at 102°C and (d) Dimer anthocyanin by heating the pure solid anthocyanin at 110°C

RESULTS AND DISCUSSION

The water loss from heating 3g of pure solid anthocyanin at 110°C until no change in weight (constant weight) was 0.5 g. Figure 2 shows the general formula of anthocyanin.

$$\text{Loss} = (0.5/3) \times 100 = 16.67\% \quad (1)$$

Mw = 256 g/mol (molecular weight of anthocyanin biomolecule)

Molecular weight of water molecule = 18 g mol⁻¹

There are several probabilities of links in polymerization of anthocyanin biomolecule:

- One link between two anthocyanin molecules liberates one water molecule:

$$X = (3 \times 18) / 512 \rightarrow X = 10.55\% \quad (2)$$

- Two links between two anthocyanin molecules liberates two water molecules:

$$X = (3 \times 36) / 512 \rightarrow X = 21.09\%$$

- Two links between three anthocyanin molecules liberates two water molecules

$$X = (3 \times 36) / 768 \rightarrow X = 14.06\%$$

- Three links between two anthocyanin molecules liberates three water molecules

$$X = (3 \times 54) / 512 \rightarrow X = 31.64\%$$

- Three links between three anthocyanin molecules liberates three water molecules:

$$X = (3 \times 54) / 768 \rightarrow X = 21.09\%$$

- Three links between four anthocyanin molecules liberates three water molecules

$$X = (3 \times 54) / 1024 \rightarrow X = 15.82\%$$

where, X is water loss percent.

Conclusion from experimental and calculation results that is condensation polymerization occurs between two anthocyanin molecules by hydroxyl groups which agrees with the above probability item 2 shows 21.09% the closest to the actual 16.67% and the least steric effect as well as FTIR shows two new links are ether C-O and ketone C=O where ketone results from breaking the emerging ether linkage during polymerization process. Figure 3 shows the expected polymerization process between anthocyanin molecules:

$$p = \frac{100 \times 0.1667}{0.2109}$$

Fig. 2: General formula of anthocyanin (Oancea and Oprean, 2011)

Fig. 3: Expected dimer formation

$$p = (100 \times 0.1667) / 0.2109 \quad (3)$$

p = 79.04% (percent of anthocyanin in the juice).

From Fig. 4 noticed that the absorption spectrum of dimer anthocyanin (heating) is different from that of dimer anthocyanin (boiling) because the absorption is dependent on the polymerization process and film thickness of samples.

Figure 5 represents the FTIR spectra of pure anthocyanin juice, pure solid anthocyanin, dimer anthocyanin (heating) and dimer anthocyanin (boiling). It is clear from the Table 1 that the absorption pattern of anthocyanin spectra is matching to the standard.

From Fig. 5b noticed that the absorption peaks of pure solid anthocyanin are similar to that of pure anthocyanin juice so it was not polymerized at 50°C. There are new peaks arise after polymerization processes of anthocyanin where in case of dimer anthocyanin (heating) as shown in Fig. 5c there are two new peaks are ether (C-O) at 1325 cm⁻¹ and ketone (C=O) at 1541.12 cm⁻¹ where ketone results from breaking the emerging ether linkage during polymerization process. Appearance of the two new absorption bands at 1541.12 and 1325 cm⁻¹ indicates that there is dimer anthocyanin is formed by two links and this supports the calculation results. In case of dimer anthocyanin (boiling) as in Fig. 5d also there are ether (C-O) at 1319.31 cm⁻¹ and ketone (C=O) at 1558.48 cm⁻¹ but new cyclic ether (C-O) at 1250 cm⁻¹ arises because the molecules of anthocyanin in juice state are more free in motion and rotational so the polymerization process is more easier (three links between two anthocyanin molecules).

Fig. 4: Absorption spectra of dimeranthocyanin (by heating and boiling)

Table 1: Spectra (cm^{-1}) of pure anthocyanin juice

Type of bond (Sankar <i>et al.</i> , 2004)	Experimental absorption peak (cm^{-1})	Stander absorption peak (cm^{-1}) (Stuart, 2004)
Cyclic ether	1051.20	1250-900
C-H bending	1384.89	1380
C=C of benzene ring	1627.92	1680-1600
C-H stretching	2930.73	2930
OH group	3385.07	3920-3280

Figure 6 show the XRD results of pure solid anthocyanin, dimer anthocyanin (heating) and dimer anthocyanin (boiling).

From Fig. 6a noticed that the crystalline structure of pure solid anthocyanin is polycrystalline so the material with high crystalline especially at angles (7.5, 11.5, 13, 17, 19, 19.5, 24.5 and 25). In case of dimer anthocyanin (heating), as in Fig. 6b there is change in the crystalline structure where there is shifting to amorphous state and drooping in all crystallization values and there are new crystallization trends at angles 16, 28, 29.5, 31.5 and 40.5 which refer to changing in structure of dimer anthocyanin (heating). In Fig. 6c of dimer anthocyanin (boiling), there are new crystallization states at angles 14-15, 18-21, 31-32, 37-38.5, 40 and 43-44, which mean that, there is recrystallization happened in dimer anthocyanin (boiling) but also there is tendency to amorphous state. These differences in crystallization trends lead to changing in some physical properties such as color

and this is happened really. A conclusion from XRD results that the crystalline structure of pure solid anthocyanin is quite different from that of dimer anthocyanin (by heating and boiling) and there is tendency to amorphous state in dimer anthocyanin (by heating and boiling). Can indicates that, the polymerization process was occurred but not all anthocyanin polymerized.

Figure 7 show the DSC analysis of pure poly methyl methacrylate and with 7% of pure solid anthocyanin, dimer anthocyanin (heating) and dimer anthocyanin (boiling).

The procedures from Eq. 1 will follow (Housecroft and Sharpe, 2005):

$$\Delta E_a = \Delta H^\ddagger + RT \quad (4)$$

Where:

RT = Thermal energy (work)

pd/v = RT (kJ mol^{-1})

Fig. 5(a-d): FTIR spectrum of (a) Pure anthocyanin juice, (b) Pure solid anthocyanin, (c) Dimer anthocyanin (heating) and (d) Dimer anthocyanin (boiling)

Fig. 6(a-c): XRD of (a) Pure solid anthocyanin, (b) Dimer anthocyanin (heating) and (c) Dimer anthocyanin (boiling)

According to molecular models (an activation energy of chain rupture is about 60 kcal mol⁻¹ (251 kJ mol⁻¹) so at room temperature the value of a decisive factor (D_f) for chain rupture as a thermal fluctuation process is about 101 for 1 mole (Mohammed, 2012):

$$\therefore E_a = H^\ddagger + RT = RTD_f \quad (5)$$

$$E_{a \text{ at R.T}} = 251 \text{ kJ mol}^{-1}$$

$$E_a = \Delta H^\ddagger + RT = 251$$

Because RT value is small at room temperature where equal to 2.47 kJ mol⁻¹ comparing with $\Delta H^\ddagger$:

$$\therefore \Delta H^\ddagger \gg RT \rightarrow E_a \approx \Delta H^\ddagger \quad (6)$$

$$E_a = \Delta H^\ddagger = RTD_f \quad (7)$$

$$E_a = 0.0083 \text{ kJ K}^{-1} \text{ mol}^{-1} \times 298.15 \text{ K} \times D_f = 251 \text{ kJ mol}^{-1}$$

$$D_f = 101.43$$

$$\text{Each unit of } D_f = \frac{251}{101.43} = 2.475 \text{ kJ mol}^{-1}$$

Fig. 7(a-d): Continue

Fig. 7(a-d): DSC analysis of (a) Pure PMMA with (b) 7% pure solid anthocyanin, (c) 7% dimer anthocyanin (heating) and (d) 7% dimer anthocyanin (boiling)

Poly Methyl Methacrylate Powder (PMMA):

T_g of pure PMMA = 264.45°C

$$E_a = RTD_f$$

$$251 = 0.0083 \times 537.6 \times D_f \rightarrow D_f = 56.25$$

$$\Delta_{df} = D_{f \text{ at } R.T} - D_{f \text{ at } 264.45^\circ\text{C}} = 101.43 - 56.25 = 45.18$$

$$\text{Addition } E_a \text{ from different temp.}_{(25 \text{ \& } 264.45^\circ\text{C})} = \Delta D_f \times \text{Each unit of } D_f \text{ (8)}$$

$$= 45.18 \times 2.475 = 111.82 \text{ kJ mol}^{-1}$$

$$E_a \text{ at } (264.45^\circ\text{C}) = 251 - 111.82 = 139.18 \text{ kJ mol}^{-1}$$

$$\Delta T = 264.45 - 25 = 239.45^\circ\text{C}$$

$$1^\circ\text{C} \equiv \frac{\text{Addition } E_a}{\Delta T} \equiv \frac{111.82}{239.45} \equiv 0.47 \text{ kJ mol}^{-1} \quad (9)$$

By addition 7% pure solid Anthocyanin to PMMA:
The temperature of chain rupture increases to 300.4 due to addition 7% pure solid anthocyanin.

$$\therefore T_g = 300.4^\circ\text{C}$$

$$E_a = RTD_f$$

$$251 = 0.0083 \times 573.55 \times D_f \rightarrow D_f = 52.73$$

$$\Delta T = 300.4 - 25 = 275.4^\circ\text{C}$$

$$\text{Addition } E_a \text{ from different temp.}_{(25 \text{ \& } 300.4^\circ\text{C} + 7\% \text{ pure solid An.})} = 275.4^\circ\text{C} \times 0.47 \frac{\text{kJ}}{^\circ\text{C}} = 129.44 \text{ kJ mol}^{-1}$$

Fig. 8(a-d): Effect of pure solid anthocyanin, dimer anthocyanin (heating) and dimer anthocyanin (boiling) as increasing their percent on the density of (a) Polyvinyl alcohol, (b) Poly methyl methacrylate, (c) Starch and (d) Cellulose

$$\text{Given energy by addition pigment} = 111.82-129.44 \quad (10)$$

$$= -17.62 \text{ kJ mol}^{-1} \text{ (this given by 7\% pure solid An.)}$$

$$E_{a \text{ at } (300.4^\circ\text{C})} = 139.18 - (-17) = 156.18 \text{ kJ mol}^{-1}$$

By addition 7% dimer anthocyanin (heating) to PMMA: The temperature of chain rupture increases to 368.85 due to addition 7% dimer anthocyanin (heating).

$$\therefore T_g = 368.85^\circ\text{C}$$

$$E_a = \text{RTD}_f$$

$$251 = 0.0083 \times 642 \times D_f \rightarrow D_f = 47.104$$

$$\Delta T = 368.85 - 25 = 343.85^\circ\text{C}$$

$$\text{Addition } E_a \text{ from different temp.}_{(25 \& 368.85^\circ\text{C} + 7\% \text{ dimer An. (heating)})} = 343.85^\circ\text{C} \times 0.47 \frac{\text{kJ}}{\text{mol}^\circ\text{C}} = 161.61 \text{ kJ mol}^{-1}$$

$$\text{Given energy by addition pigment} = 111.82-161.61$$

$$= -49.79 \text{ kJ mol}^{-1} \text{ (this given by 7\% dimer An. (heating))}$$

$$E_{a \text{ at } (368.85^\circ\text{C})} = 139.18 - (-49.79) = 188.97 \text{ kJ mol}^{-1}$$

By addition 7% dimer Anthocyanin (boiling) to PMMA: The temperature of chain rupture increases to 360 due to addition 7% dimer anthocyanin (boiling).

$$\therefore T_g = 360^\circ\text{C}$$

$$E_a = \text{RTD}_f$$

$$251 = 0.0083 \times 633.15 \times D_f \rightarrow D_f = 47.76$$

$$\Delta T = 360 - 25 = 335^\circ\text{C}$$

Noticed that the glass transition temperature and activation energy of poly methyl methacrylate (PMMA) increase after addition of anthocyanin pigment which due to H-bonding formation between PMMA and pigment (Table 2).

From Fig. 8 noticed that density of biopolymers decreases as increasing percent of pure solid anthocyanin

Fig. 9(a-d): Effect of pure solid anthocyanin and dimer anthocyanin (by heating and boiling) on the hardness of (a) Polyvinyl alcohol, (b) Poly methyl methacrylate, (c) Starch and (d) Cellulose

Table 2: Effect of natural pigment on PMMA at R.T (25°C) and E_a (251 kJ mol⁻¹) with D_f (101.43)

Substance	T_g (°C)	D_f	E_a at T_g (kJ mol ⁻¹)	Addition E_a (kJ mol ⁻¹)	Given energy by addition pigment (kJ mol ⁻¹)
Pure PMMA	264.45	56.250	139.18	111.82	-
7% pure solid anthocyanin	300.40	52.730	156.18	129.44	-17.62
7% dimer An. (heating)	368.85	47.104	188.97	161.61	-49.79
7% dimer An. (boiling)	3600	47.760	184.81	157.45	-45.63

due to the presence of exchangeable proton which reacts with functional groups in biopolymers and destroys the lattice structure of biopolymers, but in case of the blends composed from biopolymers and dimer anthocyanin (by heating and boiling), the diffusion of dimer anthocyanin is take placing through the biopolymers which makes vacancies thus the densities decrease. As the dimer anthocyanin percent increase the vacancies full with the exceed dimer anthocyanin and so the interaction with the biopolymers increased thus the densities increase.

$$\text{Addition } E_a \text{ from different temp. } (25 \& 335^\circ\text{C} + 7\% \text{ dimer An. (boiling)}) = 335^\circ\text{C} \times 0.47 \frac{\text{kJ}}{\text{mol}^\circ\text{C}} = 157.45 \text{ kJ mol}^{-1}$$

$$\text{Given energy by addition pigment} = 111.82 - 157.45$$

$$= -45.63 \text{ kJ mol}^{-1} \text{ (this given by 7\% dimer An. (boiling))}$$

$$E_a \text{ at } (360^\circ\text{C}) = 139.18 - (-45.63) = 184.81 \text{ kJ mol}^{-1}$$

As shown in Fig. 9 the hardness values of biopolymers rises as increasing percent of pure solid anthocyanin and dimer anthocyanin (by heating and boiling) due to the interaction between hydroxyl groups in anthocyanin and functional groups in biopolymers where strong bonding is formed, so the added anthocyanin acts as filler and binder material leads to reduce the ability of a molecule in biopolymers to slide on each other's and then rises the hardness degree.

CONCLUSION

It seems that the highest percent of the red pigment (anthocyanin) in red beet juice is $\approx 79\%$. Boiling of the red beet juice results in dimerization which results in losing the exchangeable proton activity. Polymerization by boiling the juice at 102°C or by heating the solid anthocyanin at 110°C results in dimer anthocyanin. Slightly solubility of dimer anthocyanin pigment in water, acetone and ethanol. Glass transition temperature (T_g) of poly methyl methacrylate (PMMA) increases upon addition of pure solid anthocyanin and dimer anthocyanin (by heating and boiling) due to H-bonding formation. Activation energy of poly methyl methacrylate (PMMA) also increases after addition of anthocyanin pigment. Density of biopolymers decreases upon addition of pure solid anthocyanin and dimer anthocyanin (by heating and boiling). Hardness of biopolymers increases with increasing percent of both pure solid anthocyanin and dimer anthocyanin (by heating and boiling).

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