

HEAT-INDUCED DEGRADATION KINETICS OF MONOMERIC ANTHOCYANINS AND COLOR OF POMEGRANATE (*PUNICA GRANATUM* L.) CONCENTRATE

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Abstract

The aim of present study is to analyze physicochemical properties and to investigate thermal degradation kinetics of anthocyanins and color of Pomegranate (*Punica granatum* L.) concentrates (40.66 °Brix) heating at 60, 70 and 80°C. Titratable acidity, pH, total phenolic matter and total antioxidant activity of pomegranate concentrates were determined as 1.18 % (as percent citric acid), 3.02, 1190 mg GAE/L and 94 % reduction, respectively. Pomegranate concentrate has initial 1101 mg/L monomeric anthocyanin content and decrease in anthocyanin content was 47.9 %, 66.9 % and 82.5 % for heating at 60, 70, 80 °C, respectively. Anthocyanin degradation showed first-order reaction kinetics and activation energy, E_a , was calculated as 52.27 kJ/mol between 60-80°C ($R^2=0.9973$). Change in TCD* was 0.78, 0.91 and 1.01 heating at 60, 70, 80 °C, respectively and TCD* was fitted to zero-order, first-order and combined kinetics model.

Key words: pomegranate, concentrate, anthocyanin, color, kinetics, thermal degradation

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits widely grown in many tropical and subtropical countries. Over 1000 cultivars of *Punica granatum* exist, originating from the Middle East, extending throughout the Mediterranean, eastward to China and India, and on to the American Southwest, California and Mexico in the New World (Çam, Hışıl & Durmaz 2009). The edible parts of the pomegranate fruits can be consumed fresh or used for the preparation of fresh juice, canned beverages, jelly, jam and paste and also for flavouring and coloring agents for beverages (Mousavinejad et al. 2009). Pomegranate juice is known to be a major source of phenolic compounds comparison with other fruit juices. They contain anthocyanins, ellagic acid, phytoestrogenic flavonoids, tannins, and organic acids (Fazaeli, Yousefi & Emam-Djomeh 2013). Alighourchi, Barzegar and Abbasi (2008) reported that among the polyphenols, anthocyanins have utmost interest. Besides their various health benefits (de Pascual-Teresa & Sanchez-Ballesta 2008), anthocyanins are responsible for the appealing bright red color of juice and other products of pomegranate fruit (Jaiswal, DerMarderosian & Porter 2010). In addition, pomegranate juice contains high levels of antioxidants - higher than most other fruit juices and beverages (Tezcan et al. 2009). Moreover, valuable compounds in different parts of the fruit have functional and medicinal effects (Mousavinejad et al. 2009) and, pomegranate juice has been proposed as chemopreventive, chemotherapeutic, anti-atherosclerotic and anti-inflammatory agent (Çam, Hışıl & Durmaz 2009). Some clinical research studies suggest that pomegranate juice changes the blood parameters such as LDL, HDL, and cholesterol; increase the prostate specific antigen (PSA) and may be helpful against heart disease, Alzheimer's disease, cancer, improvement of sperm quality and erectile dysfunction in male patients (Tezcan et al. 2009).

Color is one of the crucial parameter when making a sensorial evaluation of food quality at the first sight. The bright color of pomegranate is due to its anthocyanin content, so stability of anthocyanins during processing is of major importance (Miguel et al. 2004). In this respect, the purpose of the current study is to analyze physicochemical properties and to investigate thermal degradation kinetics of anthocyanins and color of Pomegranate (*Punica granatum* L.) concentrates (40.66 °Brix) heating at 60, 70 and 80°C.

2. MATERIAL & METHODS

2.1. Materials

Pomegranate (*Punica granatum* L.) fruits were collected in Gaziantep, Southeast Anatolia, Turkey. Pectolytic enzyme, Panzym XXL, was kindly gifted by Sinerji A.Ş., Mersin, TURKEY. The free radical 2,2-dipheynl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu’s reagent, and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the other reagents were of analytical grade.

2.2. Concentrate preperation

All the foreign materials such as pieces of branches and leaves and also unripe and damaged fruits were removed by hand. The cleaned fruits were washed under cold tap water, stalks and seeds were removed. Fruits were ground by using a laboratory blender. Juice was immediately filtered through muslin to remove pulp from the juice. Then the juice was depectinized with 1.0 % (v/w) Panzym XXL at 50 °C for 2 h. The depectinized juice was allowed to rest at 4°C for 24 h. The juice was again filtered through five layer muslin and finally double layer filter paper to obtain a clear juice. Clear juices were concentrated using BÜCHI Rotary Evaporator (Rotavapor R-3 model, BÜCHI Labortechnik AG, Flawil, Switzerland) at 40 °C.

2.3. Physicochemical properties

Total soluble solid content (TSS) was measured with a digital refractometer (PTR 46X, England) at 20 °C. pH measurements were done by using Nel-890 Model pH meter (Ankara, Turkey) calibrated with pH 4 and 7 buffers. Total acidity was determined potentiometrically by titrating the sample with 0.1 NaOH until the pH reached 8.2 and expressed as grams citric acid per liter.

2.4. Determination of total phenolics

The total phenolic content was determined colorimetrically by the Folin-Ciocalteu method (Singleton & Rossi, 1965). 100 µL of sample (diluted 1:5 (v:v) with methanol) was mixed with 6 ml of twice distilled water and 500 µL of Folin-Ciocalteu reagent was added. After waiting 5 minutes at room temperature, 1.5 mL of sodium carbonate (20% w/v) was added to adjust optimum pH for the reaction. The mixture was vortexed and incubated at room temperature (~23°C) for 2 h and then the absorbance was measured at 765 nm using a UV-VIS Lambda 25 spectrophotometer (Perkin Elmer, Shelton, USA). Gallic acid was used as a standard and total phenolic content was expressed in mg gallic acid equivalents (GAE) per liter of concentrate. A mixture of water and reagents was used as a blank. All analyses were done in triplicate ($n = 3$).

2.5. Determination of total anthocyanins

Total anthocyanin content was determined using the pH-differential method described by Giusti and Wrolstad (2001), using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M), and sodium acetate buffer, pH 4.5 (0.4 M). The concentrate samples were diluted in a ratio of 1:10 with twice distilled water. A 0.4 ml of diluted sample was mixed with 3.6 ml of corresponding buffers and allowed to equilibrate for 15 minutes at room temperature. The absorbance of each equilibrated solution was measured at 510 nm ($\lambda_{\max}$) and 700 nm for haze correction, using UV-VIS Lambda 25 spectrophotometer (Perkin Elmer, Shelton, USA). Total monomeric anthocyanins (TMA) were calculated as mg cyanidin-3-glucoside per liter concentrate according to the following equation:

$$\text{Total anthocyanins (mg/L)} = A \times \text{MW} \times \text{DF} \times 1000 / (\epsilon \times l) \quad (1)$$

where $A = (A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor (10) as final volume per initial volume; l = pathlength in cm; ϵ = 26,900 molar extinction coefficient in L/mol/cm for cyanidin-3-glucoside; 1000 = conversion factor from g to mg. All analyses were done in triplicate ($n = 3$). Glass cuvettes of 1 cm path-length were used and all measurements were carried out at room temperature (~23°C). Absorbance readings were made against twice distilled water as blank.

2.6. Determination of antioxidant activity

DPPH assay was done according to the method of Brand-Williams, Cuvelier and Berset (1995) with some modifications. The stock solution was prepared daily by dissolving 1.2 mg DPPH with 50 mL methanol and special care was taken to minimize the loss of free radical activity of the stock solution during the course of sample preparation. 100 μ L of diluted sample in the ratio of 1:10 (v:v) with methanol was mixed with 3900 μ L of 6×10^{-5} mol L⁻¹ DPPH in methanol. The mixture was vortexed and left to stand for 30 min in dark place at room temperature. Then the absorbance was measured spectrophotometrically at 515 nm using UV-VIS Lambda 25 spectrophotometer (Perkin Elmer, Shelton, USA). The percent of reduction of DPPH was calculated by the formula reported by Tural and Koca (2008).

$$\% \text{ DPPH reduction} = [(A_C - A_S)/A_C] \times 100 \quad (2)$$

where A_C = absorbance of a control ($t = 0$ min), A_S = absorbance of a tested sample at the end of the reaction ($t = 30$ min). Glass cuvettes of 1 cm path-length were used and all measurements were carried out at room temperature ($\sim 23^\circ\text{C}$). Absorbance readings were made against methanol as blank and control sample was prepared with the same volume of methanol mixed with DPPH stock solution. All analyses were done in triplicate ($n = 3$).

2.7. Color measurements

The visual color was evaluated using a HunterLab Colourflex (A-60-1010-615 Model Colourimeter, Hunter Associates Lab. Inc. Reston VA, USA). The instrument was standardized each time with a black and a white ($L = 91.10$, $a = 1.12$, $b = 1.26$) tile. The color values were expressed as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness). Total color difference (TCD^*) parameters was calculated and modeled. Color values were the means of triplicate measurements.

2.8. Degradation kinetics

Thermal degradation of pomegranate concentrate was studied in 40.66° Brix concentrate at 60, 70 and 80°C . Aliquots of 10 mL of pomegranate concentrates were put into screw-cap test tubes to prevent evaporation and test tubes were placed into oven preheated to a given temperature. At regular time intervals (0, 2, 4, 6, 8, and 10 h), samples were removed from the oven (NÜVE EN500, Ankara, Turkey) and rapidly cooled by plunging into an ice bath to stop further degradation. The anthocyanin contents and color values of the samples were measured immediately.

2.8.1. Degradation kinetics of anthocyanins

Previous studies showed that thermal degradation of anthocyanins followed a first-order reaction (Garzon & Wrolstad 2002; Kara & Erçelebi 2013; Kırca, Özkan & Cemeroğlu 2006; Wang & Xu 2007; Yalçınöz & Erçelebi 2015). This kinetic type was expressed by the following equation:

$$C = C_o * \exp (\pm k_1 * t) \quad (3)$$

where C_o is the initial anthocyanin contents and C is the anthocyanin contents after time t (min) of heating at the given temperature while k_1 is the first-order rate constant. The parameters of first-order kinetic model (Eq. (3)) were estimated by non-linear regression iterative procedure of the SigmaPlot (SigmaPlot 10.0 Windows version, SPSS Inc.).

Half-lives ($t_{1/2}$) which is the time needed for 50% degradation was calculated by the following equation:

$$t_{1/2} = -\ln 0.5 / k_1 \quad (4)$$

where $t_{1/2}$ is the half-lives and, k_1 is the first-order degradation rate constant (h^{-1}).

The effect of temperature on the degradation rate constants was expressed by the linearized Arrhenius equation by plotting $\ln k$ against $1/T$ in which the temperature dependence of k was quantified by the activation energy E_a according to Eq. (5).

$$\ln k = \ln A_o - \frac{E_a}{RT} \quad (5)$$

where the k is the rate constant (min^{-1}), A_o is the frequency factor (min^{-1}), E_a is the activation energy (kJ/mol), R is the universal gas constant (8.314 J/mol/ K) and T is the absolute temperature (Kelvin, K). The E_a value was calculated from the slope of the straight lines given by Eq. (5), using a linear regression procedure of the SigmaPlot (SigmaPlot 10.0 Windows version, SPSS Inc.).

2.8.2. Degradation kinetics of visual color

The complexity of fruit juices and derivatives implies a wide range of enzymatic and non-enzymatic browning reactions caused by thermal treatments. Consequently it is difficult to establish a reaction mechanism and to obtain a kinetic model describing the global process adequately (Ibarz, Pagan & Garza 1999). There are numerous references on the kinetics of color of food materials in the literature. The majority of these works report zero-order (Eq. (6)) or first-order (Eq. (3)) degradation reaction kinetics.

$$C = C_o \pm k_o * t \quad (6)$$

Sometimes the relatively simple models described do not adequately represent color change mechanism. That is why a combined kinetics has been developed, in which the non-enzymatic color change reactions are considered to consist of two stages. A first stage of colored polymeric compound formation following zero-order kinetics, the second stage supposes decomposition of the colored polymers into non-colored compounds following a first-order kinetics. According to this combined kinetics, the color change mechanism can be expressed by ((Garza et al. 1999; Ibarz, Pagan & Garza 1999):

$$C = \frac{k_o}{k_1} - \left[\frac{k_o}{k_1} - C_o \right] \exp(\pm k_1 * t) \quad (7)$$

The terms C and C_o are the concentrations of color parameters at any time t and initial concentration, respectively; k_o is the zero-order kinetics constant and k_1 is the first-order kinetics constant in Eqs. (3), (6),(7).

Total color difference (TCD^*) was calculated by using L^* , a^* , b^* values (Eq. (8)) (Loughrey 2002).

$$TCD^* = \sqrt{(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2} \quad (8)$$

where, L_o^* (0.63), a_o^* (0.84) and b_o^* (-0.62) refer to initial values, and L^* , a^* and b^* refer to color values at various times during heat treatment.

2.9. Statistical Analysis

All measurements were performed on three freshly prepared and are reported as means and standard deviations. The parameters of kinetic models and Arrhenius equation were estimated by either linear regression procedure or non-linear regression iterative procedure of the SigmaPlot (SigmaPlot 10.0 Windows version, SPSS Inc.).

3. RESULTS AND DISCUSSION

3.1. Physicochemical Properties

Pomegranate juice was concentrated from 15.33 to 40.66 °Brix. Titratable acidity and pH of pomegranate concentrates were determined as 1.18 and 3.02 (Table 1), respectively and, these results were relevant with previous findings (Akbarpour, Hemmati & Sharifani 2009; Miguel et al. 2004). In addition, total monomeric anthocyanin, total phenolic matter and total antioxidant activity of concentrates were determined as 1101 mg/L, 1190 mg GAE/L and, 94 % reduction, respectively (Table 1). When compared to the studies of Tezcan et al. (2009) on commercial pomegranate juices sold in Turkish markets, total phenolic matter was lower while antioxidant activity was higher than

their findings. Ozgen et al. (2008) have been reported that phenolic content of six pomegranate arils from Mediterranean region of Turkey are between 1245 and 2076 mg/L, which was close to our findings. Poyrazoğlu, Gökmen and Artık (2002) have been reported that the pomegranate juices prepared from pomegranates grown in different regions of Turkey had different amount and composition of phenolic matter. In addition, total monomeric anthocyanin content of our pomegranate concentrate (1101 mg/L) was found to be higher than previous studies on different pomegranate genotypes by Çam, Hışıl and Durmaz (2009) (8.1-36.9 mg/100ml) and Ozgen et al. (2008) (6.1-219.0 mg/L). They claimed that the differences in anthocyanin contents were probably caused by the differences due to genotype, harvesting time and ecological conditions. Similar assumptions could also be made for other physicochemical properties. Besides, Tezcan et al. (2009) and Gil et al. (2000) suggested that anthocyanins play only minor role in total antioxidant activity while punicalagin originating from the peels of pomegranate is one of the major contributors of this activity. So, it can be concluded that total monomeric anthocyanin content may not have direct impact on total antioxidant activity of pomegranate concentrate.

Table 1. Physicochemical properties of pomegranate concentrate.

	Pomegranate
pH	3.02
Total soluble solids (°Brix)	40.66
Titrateable acidity (as percent citric acid) (%)	1.18
Color	
L_o^*	0.63
a_o^*	0.84
b_o^*	-0.62
Total Monomeric Anthocyanins(TMA) (mg/L)	1101
Total phenolics (mg GAE /L)	1190
Total antioxidant activity (% reduction)	94

Values represent means calculated from three replicates.

3.2. Degradation kinetics of total monomeric anthocyanins

The use of a first-order model to describe the thermal degradation of anthocyanins has previously been reported (Cemeroglu, Velioglu & Isik 1994; Patras et al. 2010; Reyes & Cisneros-Zevallos 2007; Verbeyst et al. 2011; Yang et al. 2008).

Change in total monomeric anthocyanins (mg/L) content of pomegranate concentrate during heating at 60, 70, 80 °C for 600 minutes were given in Table 2 and degradation of anthocyanins was plotted as a function of time (Fig. 1) and fitted to first-order (Eq. (3)) reaction kinetics. Pomegranate concentrate has initial 1101 mg/L TMA content and decrease in anthocyanin content was 47.9 %, 66.9 % and 82.5 % for heating at 60, 70, 80 °C at the end of 600 minutes, respectively. The extent of anthocyanin degradation increased with increasing temperature and time. Moreover, it can be concluded that as anthocyanin concentration decreased the degradation rate also decreased which agree with those of previous studies reporting first-order degradation kinetics of anthocyanins (Cemeroglu, Velioglu & Isik 1994; Garzon & Wrolstad 2002; Kirca, Özkan & Cemeroglu 2006; Patras et al. 2010; Reyes & Cisneros-Zevallos 2007; Shao-gian, Liang & Pan 2011).

Table 2. Change in total monomeric anthocyanins (mg/L) content of pomegranate concentrate during heating at 60, 70, 80 °C for 600 minutes.

Time (min)	Temperature (°C)		
	60 °C	70 °C	80 °C
	TMA ^a ±SE	TMA ^a ±SE	TMA ^a ±SE
0	1101±78	1101±78	1101±78
120	1004±71	727±51	664±47
240	859±61	595±42	483±34
360	755±53	481±34	325±23
480	635±45	416±29	262±19
600	573±41	364±26	193±14

^a Total Monomeric Anthocyanins (mg/L), SE: Standard error

The first-order reaction rate constants (k_1), half-life of anthocyanins ($t_{1/2}$) and activation energy for temperature range of 60-80 °C were shown in Table 3. k_1 values for anthocyanin degradation were 0.0011, 0.002 and 0.0032 min⁻¹ in pomegranate concentrate (40.66 °Brix) at 60, 70 and 80 °C, respectively (Table 3). $t_{1/2}$ values, calculated by Eq. (4), for anthocyanin degradation were 10.5, 5.8 and 3.6 h in pomegranate concentrate at 60, 70 and 80 °C, respectively (Table 3). Kara and Erçelebi (2013) reported that k_1 and $t_{1/2}$ values for anthocyanin degradation were 0.0014 min⁻¹ and 8.3 h; 0.0026 min⁻¹ and 4.4 h; 0.0036 min⁻¹ and 3.2 h in Urmu mulberry (*Morus nigra* L.) concentrate (45.20 °Brix) at 60, 70 and 80 °C, respectively. Yalçınöz and Erçelebi (2015) reported that k_1 and $t_{1/2}$ values for anthocyanin degradation were 0.002 min⁻¹ and 5.7 h; 0.0027 min⁻¹ and 4.3 h; 0.0054 min⁻¹ and 2.1 h in Cornelian cherry (*Cornus mas* L.) concentrate (43.52 °Brix) at 60, 70 and 80 °C, respectively. Cemeroglu, Velioglu and Isik (1994) reported that k_1 and $t_{1/2}$ values for anthocyanin degradation were 0.011 min⁻¹ and 24.0 h; 0.024 min⁻¹ and 10.9 h; 0.057 min⁻¹ and 4.4 h in sour cherry (*Prunus cerasus* L.) concentrate (45° Brix) at 60, 70 and 80 °C, respectively. In the current study having good accordance with literature, as the temperature increased heat-induced degradation rate increased and $t_{1/2}$ values decreased in consistent with faster reaction rates accompanied by higher k_1 values. Major anthocyanins in pomegranate, Urmu mulberry, Cornelian Cherry and sour cherry are delphinidin, cyanidin, and pelargonidin 3-glucosides and their 3,5-diglucosides (Alighourchi, Barzegar & Abbasi 2008); cyanidin-3-glucoside and cyanidin-3-rutinoside (Fazaeli, Yousefi & Emam-Djomeh 2013); pelargonidin 3-O-glucoside, cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside (Tural & Koca 2008); cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside (Wang & Xu 2007), respectively. These different anthocyanidin content may lead to different heat susceptibilities of anthocyanins (Wang & Xu 2007; Yang et al. 2008).

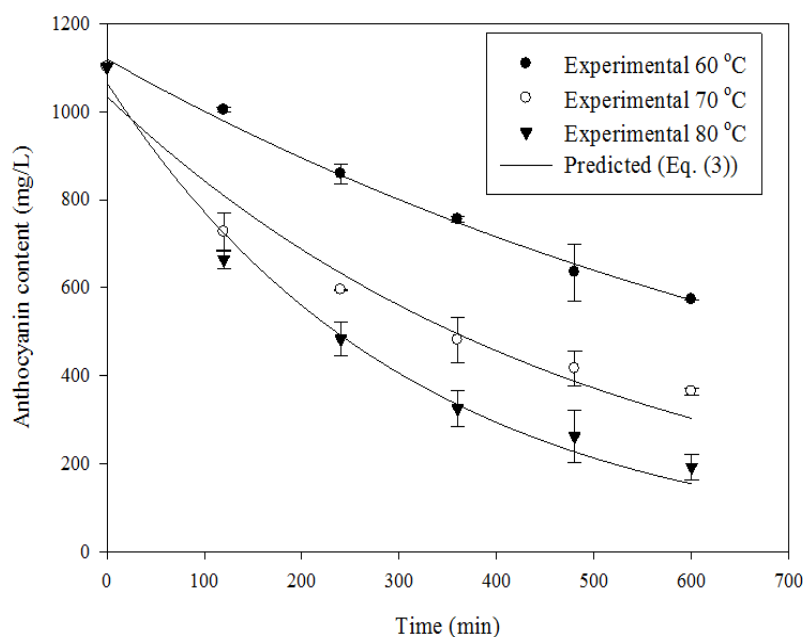


Fig. 1. Anthocyanin degradation in pomegranate concentrate during heating at 60, 70 and 80 °C for 600 minutes.

Table 3. Effect of temperature on k_1 , $t_{1/2}$ and E_a values of anthocyanin degradation in pomegranate concentrate (40.66 °Brix).

Concentrate type	Temperature (°C)	$k_1^a \times 10^3$ (min ⁻¹)	$t_{1/2}$ (h) ^b	E_a (kJ/mol) ^c
Pomegranate	60	1.1 (0.9935)	10.5	52.27 (0.9973)
	70	2.0 (0.9529)	5.8	
	80	3.2 (0.9865)	3.6	

^a Rate constant.

^b Half-life.

^c Activation energy.

^d Numbers in parentheses are the correlation coefficients.

To determine the effect of temperature on degradation rate constants, the constants (k) obtained from Eq. (3) were fitted to Arrhenius equation (Eq. (5)) at 60, 70 and 80 °C (Fig. 2). The calculated activation energy E_a for pomegranate concentrate (40.66 °Brix) was 52.27 kJ/mol with 0.9973 correlation coefficient (Table 3). At 60-80 °C, the activation energy for degradation of anthocyanins for Urmu mulberry (*Morus nigra* L.) concentrate (45.20 °Brix), Cornelian cherry (*Cornus mas* L.) concentrate (43.52 °Brix) and sour cherry (*Prunus cerasus* L.) concentrate (45° Brix) were 46.32 kJ/mol, 48.38 kJ/mol and 75.85 kJ/mol, respectively. These activation energies signify the greater thermal sensitivity of sour cherry anthocyanins followed by pomegranate anthocyanins, cornelian cherry anthocyanins and Urmu mulberry anthocyanins. Change in activation energy values could be due to different soluble solid contents (Cemeroglu, Velioglu & Isik 1994), whose effect was minimized since literature examples were chosen in close to pomegranate concentrate' total soluble solid content of 40.66 °Brix and, different composition of samples (Kırca, Özkan & Cemeroglu 2007).

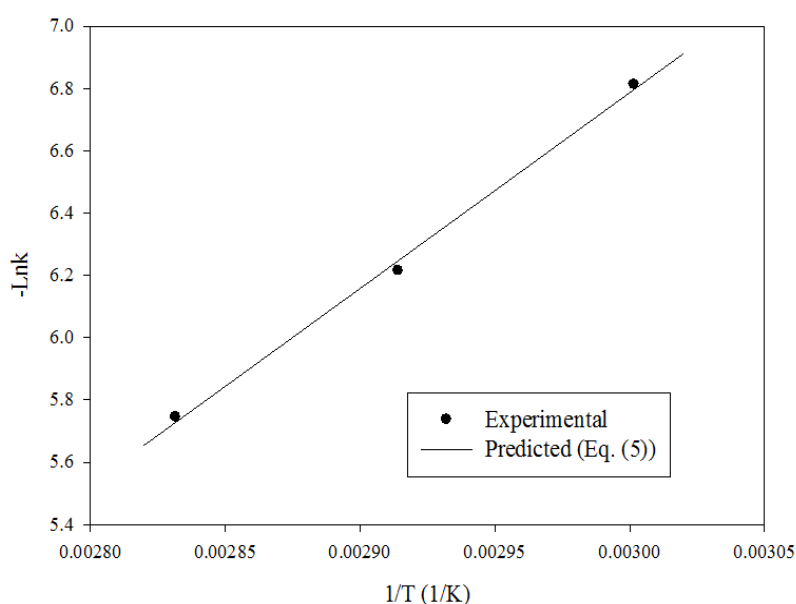


Fig. 2. The Arrhenius plots (Eq. (5)) for degradation of anthocyanins in pomegranate concentrate during heating.

3.3. Degradation kinetics of visual color

Since perception of color is the result of three coordinate axes of L^* , a^* , b^* and, difficult to interpret independently, an overall examination of these three coordinate axes of CIELAB colourimetric space was needed (Loughrey 2002; Wrolstad, Durst & Lee 2005). One of the best parameters for describing the color variation is the total color difference (TCD^*) since it is the combination of parameters L^* , a^* and b^* (Ibarz, Pagan, & Garza, 1999). Therefore, TCD^* parameters were calculated by using L^* , a^* , b^* values and calculated by Eq. (8).

Table 4. Change in total color difference (TCD^*) value of pomegranate concentrate during heating at 60, 70, 80 °C for 480 minutes.

Time (min)	Temperature (°C)		
	60 °C	70 °C	80 °C
	$TDC^* \pm SE$	$TDC^* \pm SE$	$TDC^* \pm SE$
0	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
60	0.2502±0.0177	0.2166±0.0153	0.2860±0.0202
120	0.3662±0.0259	0.3977±0.0281	0.3865±0.0273
180	0.4841±0.0342	0.5004±0.0354	0.5250±0.0371
240	0.5559±0.0393	0.6169±0.0436	0.6924±0.0490
300	0.6539±0.0462	0.7298±0.0516	0.7949±0.0562
360	0.7142±0.0505	0.8206±0.0580	0.8868±0.0627
480	0.7826±0.0553	0.9060±0.0641	1.0143±0.0717

SE: Standard error

There are numerous references on the kinetics of color of food materials in the literature. The majority of these works report zero-order kinetics (Eq. (6)) (Chutintrasri & Noomhorm 2007; Tiwari et al. 2009), first-order kinetics (Eq. (3)) (Ahmed, Shivhare & Raghavan 2000; Shao-gian, Liang & Pan 2011) and combined kinetic model (Eq. (7)) (Garza et al. 1999; Ibarz, Pagan & Garza 1999). Combined model consist of two stages: the first one includes color formation based on Maillard reactions and it follows zero-order kinetics and the second includes the color destruction (pigment destruction) which follows a first-order kinetics (Garza et al. 1999; Ibarz, Pagan & Garza 1999). Variation in TCD^* value of pomegranate concentrate was 0.7826, 0.9060 and 1.0143 at the end of 480 minutes heating at 60, 70, 80 °C, respectively (Table 4). Experimental data for change in TCD^* were fitted to zero-order (Eq. (6)), first-order (Eq. (3)) and combined kinetic model (Eq. (7)). Kinetic parameters of zero, first and combined model were given in Table 5, Table 6 and Table 7, respectively. Model graphs of zero, first and combined kinetic model were given in Fig. 3, Fig. 4 and Fig. 5, respectively.

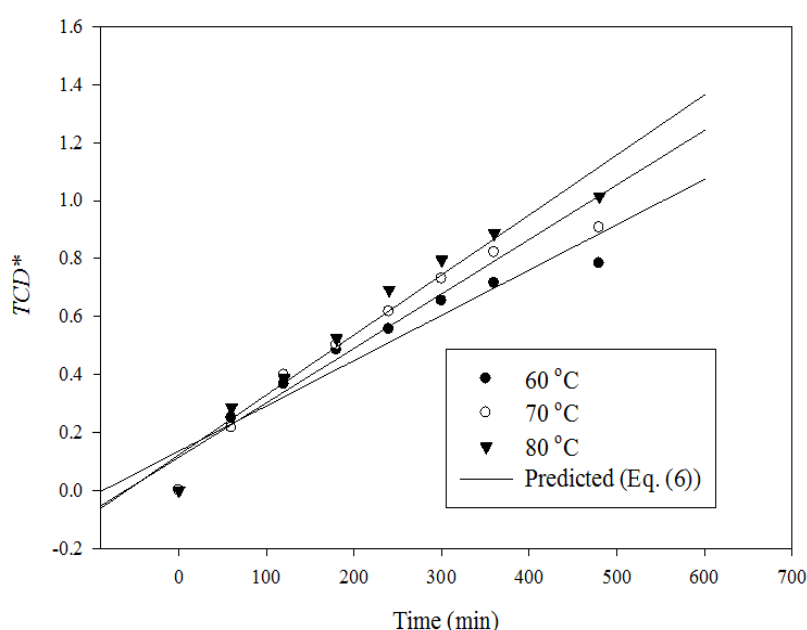


Fig. 3. Change in total color difference (TCD^*) value of pomegranate concentrate during heating at 60, 70, 80 °C for 480 minutes fitted to zero-order (Eq. (6)) kinetics.

Table 5. Kinetics parameters of zero-order model (Eq. (6)) for TCD^* values.

Concentrate type	Temperature (°C)	$C_o \pm SE$	$ko \pm SE$	R^2
Pomegranate	60	0.1362 ± 0.0513	0.0016 ± 0.0002	0.9152
	70	0.1150 ± 0.0491	0.0019 ± 0.0002	0.9445
	80	0.1234 ± 0.0485	0.0021 ± 0.0002	0.9548

SE: Standard error, R^2 : correlation coefficients.

Table 6. Kinetics parameters of first-order model (Eq. (3)) for TCD^* values.

Concentrate type	Temperature (°C)	$C_0 \pm SE$	$k_1 \pm SE$	R^2
Pomegranate	60	1.0275 ± 0.0242	-0.0033 ± 0.0003	0.9902
	70	1.0055 ± 0.0246	-0.0042 ± 0.0003	0.9933
	80	1.0331 ± 0.0545	-0.0045 ± 0.0008	0.9729

SE : Standard error, R^2 : correlation coefficients.

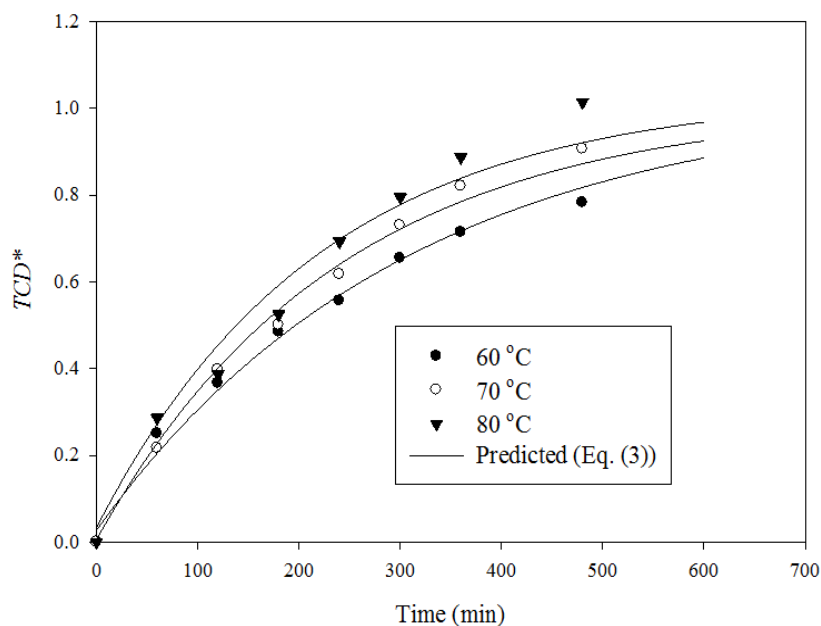


Fig. 4. Change in total color difference (TCD^*) value of pomegranate concentrate during heating at 60, 70, 80 °C for 480 minutes fitted to first-order (Eq. (3)) kinetics.

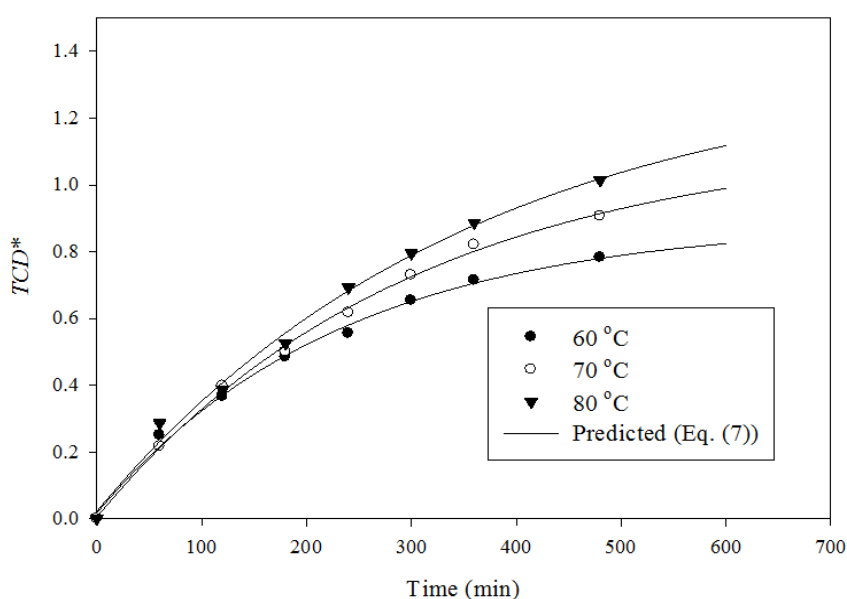


Fig. 5. Change in total color difference (TCD^*) value of pomegranate concentrate during heating at 60, 70, 80 °C for 480 minutes fitted to combined kinetics (Eq. (7)) kinetics.

Table 7. Kinetics parameters of combined model (Eq. (7)) for TCD^* values.

Concentrate Type	Temperature (°C)	$C_o \pm SE$	$k_o \pm SE$	$k_i \pm SE$	R^2	k_o / k_i
Pomegranate	60	0.0167±0.0188	0.0038±0.0003	0.0043±0.0005	0.9955	0.88
	70	0.0046±0.0148	0.0038±0.0002	0.0033±0.0003	0.9979	1.15
	80	0.0215±0.0282	0.0039±0.0003	0.0028±0.0005	0.9936	1.39

SE: Standard error, R^2 : correlation coefficients.

Correlation coefficients (R^2) ranging between 0.9152-0.9548 (Table 5) for zero-order kinetics, 0.9729-0.9933 (Table 6) for first-order kinetics and 0.9936-0.9979 (Table 7) for combined kinetics, respectively. Correlation coefficients (R^2) of TCD^* fitted to first-order (0.9729-0.9933) (Table 6) and combined model (0.9936-0.9979) (Table 7) were not considerably different from each other for pomegranate concentrates. So, both first-order and combined model was found to be appropriate to describe change in color of pomegranate concentrates. As defined in literature (Ibarz, Pagan & Garza 2000), normally temperature increase gives rise to rates of the both two steps of combined model (color formation and pigment destruction). In pomegranate concentrate, as temperature increased, rate of color formation (k_o) did not changed considerably while rate of pigment destruction (k_i) decreased resulting in more pronounced effect on TCD^* by Maillard reaction rather than pigment destruction (Table 7). According to Ibarz et al. (2000), when the ratio of kinetic constants k_o (color formation) and k_i (pigment destruction) is greater than unity, Maillard reaction predominates over pigment destruction. For pomegranate concentrate, Maillard reaction start to predominate over pigment destruction as temperature increased from 60 to 70 and 80 °C (Table 7). These results are correlated with the results of previous studies (Garza et al. 1999; Ibarz, Pagan & Garza 1999, 2000).

4. CONCLUSION

In the present study, physicochemical properties and to thermal degradation kinetics of anthocyanins and color of Pomegranate (*Punica granatum* L.) concentrates (40.66 °Brix) heating at 60, 70 and 80°C were investigated. Titratable acidity, pH, total monomeric anthocyanin, total phenolic matter and total antioxidant activity of concentrates of pomegranate concentrates were determined as 1.18 %, 3.02 , 1101 mg/L, 1190 mg GAE/L and, 94 % reduction, respectively. Anthocyanin degradation followed first-order reaction kinetics. Temperature dependency of anthocyanin degradation was expressed as activation energy, E_a , and E_a calculated as 52.27 kJ/mol ($R^2=0.9973$). Change in visual color was expressed in terms of total color difference (TCD^*) and TCD^* was fitted to zero-order, first-order and combined kinetics model by non-linear regression iterative procedure. Regression analysis revealed that experimental data well fitted to first-order and combined kinetic models due to having good correlation coefficients (R^2) and reasonable C_o values. So, change in pomegranate concentrate color during heating at 60, 70, 80 °C for 480 minutes can be described by either first or combined kinetic model.

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