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The control of Maillard reaction in white grape molasses by the method of reducing reactant concentration

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Abstract

The aim of this study was to investigate the impact of removing sugar and/or amino groups by applying one ion exchange (Dowex 50Wx8) and two adsorbent (Activated Carbon and Amberlite XAD-16) resins to grape juice in the control of Maillard reaction. Grape juices, a control sample and separately applied with each resin, were concentrated up to 50, 60 and 70 °Bx and stored at three temperatures (50, 65 and 75 °C) for 10 days. HMF accumulation was lower in the grape juices which were treated with Amberlite or activated carbon throughout the boiling. Rate constant, activation energy and Q_{10} values indicated that activated carbon application has a decelerating effect on HMF formation in molasses, particularly molasses with 50 °Bx. Dowex was more effective than Amberlite in controlling of brown pigment formation. Analysing the results of HMF and brown pigment, activated carbon was the best resin to control the Maillard reaction.

Keywords: grape molasses; 5-hydroxymethylfurfural (HMF); kinetic parameters; non-enzymatic browning.

Practical Application: This work provides useful information to control the Maillard reaction by reagent reduction using specific adsorbents to prevent the formation of harmful intermediate products for human health such as 5-hydroxymethylfurfural, which occur during long-term heat treatment applied during grape juice concentration.

1 Introduction

Maillard reaction is defined, with a general expression, as the reaction between the carbonyl and amino groups (Oh et al., 2018). As previously reported by Van Boekel (2001), Maillard reaction starts with the condensation of carbonyl and amino groups and then N-glycosylamine or N-fructosylamine is formed. After Amadori or Heyns rearrangements, the reaction progresses with different paths according to factors such as pH. Diversity of intermediate and/or end products in a food or model system in which Maillard reaction occurred shows differences according to pathway the reaction (Ames, 1990). Though it was first described by Louis Camille Maillard in 1912, Maillard reaction keeps up to date because new technologies find out different effects of the reaction on physical, chemical, sensorial and nutritional properties of foods. Despite its simplistic definition, the reaction is intensely complex and it is affected by several factors such as kind of sugar and amino group which is undergoing the reaction, pH, temperature and metal ions found in reaction media (Ames, 1990; Van Boekel, 2001). Previous studies which have been generally carried out on model systems focused on the mechanism of the reaction and provide insight to current researches.

In some cases Maillard reaction is undesirable because of the losses in the nutritional and physical quality of the foods. On the contrary, Maillard reaction is encouraged to form colour, flavour and/or aroma in some foods such as roasted coffee (Albouchi & Murkovic, 2018). Grape molasses differs from concentrated grape juice, primarily, from the point of sensorial properties owing to flavor substances which are formed by non-enzymatic browning reactions depending on the difference in heat treatment. Caramelisation and Maillard reaction are desirable in molasses in terms of the formation of typical flavor of the molasses. On the other hand, particularly in recent years, researchers have been working out to keep Maillard reaction under control due to the forming of toxic compounds such as HMF, acrylamide, Nε-carboxymethyl-lysine and pyrraline (Oh et al., 2018). Most common methods for inhibition and/or controlling of the Maillard reaction are the reducing of processing and/or storage temperature, regulating the water activity and pH (Ames, 1990; Jaeger et al., 2010). Beside the above mentioned methods, removal of reactants is also evaluated as one of the controlling methods, even if not prevalent (Jaeger et al., 2010). Oxidising of the reducing group in the sugar with hexose oxidase (Soe & Petersen, 2005) and removing sugar and amino acids by soaking in water and ethanol (Martin & Ames, 2001) are the experimental techniques previously practiced by researchers in removing a reactant from potato to reduce Maillard reaction.

Adsorption and ion exchange technologies are widely used in pharmacology, cosmetics, water and food industry for the purposes of purification, enrichment and solvent recovery. The adsorbents used in technological applications are porous solids with high adsorption capacity and expanse specific surface area such as activated silica, activated clay and activated carbon (Bansal & Goyal, 2005). Ion exchange resins are water insoluble and cross-linked carrier polymers which contains ionizable functional groups. Ion exchange reaction could be defined as

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reversible exchanging of ions between resin and liquid phase (Harland, 2007). Adsorbent and ion exchange resins are used in edible oils, fruit juices and sugar syrup to decolour, in fruit juice industry to remove bitter compounds and in drinking water industry to remove the odour-causing compounds (Vera et al., 2003; Arslanoğlu et al., 2005; Marsh & Reinoso, 2006; Çoklar & Akbulut, 2010; Kammerer et al., 2011). To the best of our knowledge, in spite of a large number of studies on non-enzymatic browning reaction, there is no research on controlling the Maillard reaction by decreasing the content of reactant using resins.

The aims of this research were to determine whether the resin application affected the reducing sugar and free amino groups of white grape juice and, if the application is effective on removing reactants, to find out whether the removing ratio was sufficient to control the reaction both in concentration and storage. In addition to these, to find out the best resin to control the reaction was aimed.

2 Materials and methods

2.1 Resin application to grape juice and concentration

White grape juices used in this research was produced in the factory of TARGID Food and Agricultural Products Industry and Trade A.S (Mersin, TURKEY). Granular activated carbon, Amberlite® XAD-16 and Dowex® 50Wx8 were supplied from Sigma-Aldrich.

Granular activated carbon, Amberlite® XAD-16 and Dowex® 50Wx8 resins were separately added to white grape juice at a dose of 5 g/L. Grape juices were placed in a reciprocating shaking waterbath (200 stroke min-1 and 30 mm amplitude) at 30 °C. After 120 min resins were removed by filtration. Resin applied and non-applied grape juices were concentrated up to 50, 60 and 70 °Bx by heating at atmospheric pressure. Grape molasses were put into glass jars (200 mL) and then placed into incubators at temperatures 50, 65 and 75 °C (Nuve, Turkey) for storage. Figure 1 summarizes the production steps from grape juice to storage. Samples were removed and analyzed at different time intervals during the 10 days storage.

2.2 Determination of pH

pH values of molasses diluted to 20 °Bx with ultra-pure water and grape juices were measured with a pH meter (WTW, Weilheim, Germany). pH measurement were carried out at 25 °C (Cemeroglu, 1992).

2.3 Determination of total protein

Total protein content of grape juices and molasses were determined by Bradford method (Kruger, 2009). Five mL of Comassie Blue G250 reactive were added to 100 μL of diluted sample and absorbance value at 595 nm was recorded after 5 min by using a spectrophotometer (Hitachi, UV 1800, Japan). Amount of protein in the samples was calculated according to the curve prepared with bovine serum albumine (10-1000 μg/mL) and the results were given as mg/kg dry weight (DW).

2.4 Determination of free amino groups

Method of Matmaroh et al. (2006) was used to determine the amount of free amino groups in grape juices and molasses. Briefly, 0.5 mL of adequately diluted sample was added to 2 mL of phosphate buffer (0.2125 M, pH at 8.2) and 1 mL of 2,4,6,-trinitrobenzenesulfonic acid solution (0.01%). After the incubation at 50 °C for 30 min, 2 mL of sodium sulphide solution (0.1 M) was added to mixture and the absorbance value at 420 nm was recorded. Results were calculated according to the L-leucine standard curve (20-2000 μg/mL) and given as g L-leucine equivalent/kg DW.

2.5 Determination of glucose and fructose content by HPLC

Glucose and fructose concentrations in grape juices and molasses were determined according to the method of Nordic Committee on Food Analysis (1993). Analysis was performed by using an Agilent 1100 HPLC (Agilent 1100, Waldbronn, Germany) system with refractive index detector. Water and acetonitrile mixture (1:3;v/v) was used as mobile phase at a flow rate of 1.5 mL/min. Ten μl of the diluted sample was injected to the system. Separation was achieved with a carbohydrate column (Agilent, 4.6 × 250 mm, 5 μ). Column and detector temperatures were set at 30 °C. Data were analysed by ChemStation software.

2.6 Determination of 5-hydroxymethylfurfural (HMF)

Hydroxymethylfurfural content of samples was measured by Winkler method (Winkler, 1955). According to the method, the intensity of red colour generated by the reaction among barbituric acid, p-toluidine and HMF was spectrophotometrically measured. Two mL of diluted samples and 5 mL of p-toluidine solution were measured into two separate tubes. One mL of

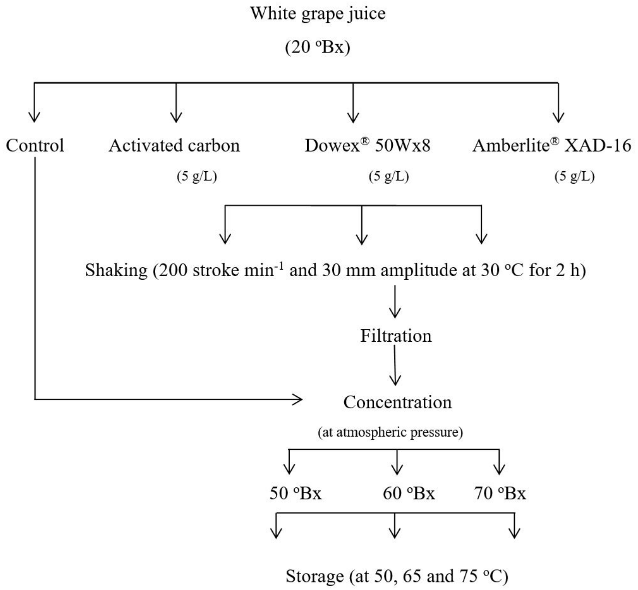

Figure 1. Flow chart of grape molasses production from grape juice to storage.

distilled water was added to one of them and 1 mL barbituric acid (0.5%) to the other. Absorbances were read at 550 nm and the results were given as mg/100 g DW.

2.7 Browning index measurment

Twenty five mL of acetic acid:water (2% v/v)-formaldehyde solution was added to 0.5 g of the sample and centrifuged for 10 min at 4500 *x* g. Five mL of supernatant was mixed with 5 mL of ethanol and centrifuged. Absorbances of the supernatant at 420 and 600 nm were measured. The difference between two absorbance values was used as browning index (Özhan et al., 2010).

2.8 Calculation of kinetic parameters

Rate law for zero order reactions

Zero order reaction law is defined by Equation 1 and taking integration of the Equation 1 gives the Equation 2 for integrated zero order reaction rate law.

$$-\frac{d[A]}{dt} = k[A]^n \tag{1}$$

$$[A] = -kt + [A]_0 \tag{2}$$

where: k = Rate constant; $[A]_0$ = Initial concentration; [A] = Concentration after t; t = Time.

A linear line was obtained from the plotted values of concentration versus time in a cartesian coordinate. An equation for HMF or brown pigment formation was obtained from the curve which fitted to Equation 2. According to the graph, it was determined that both HMF and brown pigment formation in grape molasses followed a zero order reaction law. Reaction rate constants of HMF and brown pigment formation were obtained from the slope of the line.

Activation energy (Ea)

To determine the effect of temperature on HMF and brown pigment formation throughout the storage, activation energy (Ea) values were calculated via Arrhenius equation in logarithmic form (Equation 3).

$$ln\left(\frac{k_2}{k_1}\right) = \frac{E_a}{R} \cdot \frac{(T_2 - T_1)}{T_1 \cdot T_2} \tag{3}$$

where: k_1 = Rate constant at T_1; k_2 = Rate constant at T_2; R = Ideal gas constant, 8.314 J/mol °K; E_a = Activation energy; J/mol °K.

Temperature coefficient (Q_{10})

Q_{10} value represents the temperature dependence of a reaction and indicates how a reaction rate changes by temperature increase of 10 °C. Temperature Coefficient (Q_{10}) values for HMF and brown pigment formation were calculated according to the Equation 4.

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{(T2-T1)}} \tag{4}$$

2.9 Statistical analyses

The results were expressed as mean ± SD and subjected to ANOVA to determine whether there was an effect of resin application and concentration degree on sugars, HMF content, pH, total protein and free amino content of grape juice. Duncan's multiple range test was used to compare and interpret the difference between means. $p<0.05$ was considered statistically significant. The statistical analyses were performed using MINITAB (Release 14, Minitab Inc. USA) and MSTAT-C (MSTAT Development Team, 1989).

3 Results and discussions

3.1 Effect of resin application on glucose, fructose, protein and free amino contents and pH values

Amounts of fructose, glucose, total protein and free amino groups in grape juices before and after resin application are shown in Table 1. Resin non-applied grape juice was used as control to see the effect of resins on aforementioned compounds. Amounts of fructose and glucose in the control were found to be 43.31 ± 4.38 and 37.37 ± 3.53 g/100 g DW, respectively. According to the results, resin application and concentration processes affected the fructose content of the grape juice.

Table 1. Some chemical properties of grape juice after the resin application and concentration.

		Glucose (g/100 g DW)	Fructose (g/100 g DW)	Protein (mg/kg DW)	Free Amino group content (g L-leucine equivalent /kg DW)	HMF (mg/100 g DW)	pH
Brix	20	44.54 ± 1.01a**	38.96 ± 0.88a	526.37 ± 144.75a	1.99 ± 0.18a	nd	4.93 ± 0.38a
	50	45.40 ± 1.56a	37.25 ± 1.39b	385.01 ± 63.44b	1.44 ± 0.26b	11.65 ± 3.40c	4.77 ± 0.29b
	60	42.34 ± 1.91b	36.66 ± 1.85b	347.52 ± 59.24b	1.37 ± 0.29b	17.16 ± 7.20b	4.72 ± 0.28c
	70	39.25 ± 3.27c	34.28 ± 2.92c	303.34 ± 43.00b	1.26 ± 0.27c	24.49 ± 10.16a	4.70 ± 0.26d
Resins	Control	43.31 ± 4.38a	37.37 ± 3.53a	437.80 ± 178.14	1.87 ± 0.17a	17.58 ± 4.21b	4.91 ± 0.12c
	Activated carbon	42.29 ± 1.34b	36.43 ± 1.15b	364.80 ± 79.40	1.46 ± 0.39b	11.61 ± 3.35d	4.98 ± 0.12a
	Dowex	43.67 ± 1.39a	37.30 ± 1.55a	405.90 ± 81.16	1.26 ± 0.29c	28.11 ±11.03a	4.30 ± 0.02d
	Amberlite	42.26 ± 4.35b	36.06 ± 3.14b	353.80 ± 111.87	1.47 ± 0.37b	13.77 ± 4.72c	4.94 ± 0.13b

**Results were given as mean ± Standard Deviation (n=2). Different letters in the same column indicate statistically significant differences between resins and concentration degrees ($p<0.05$).

Amounts of fructose in the control and Dowex® 50Wx8-100 applied grape juice were found to be nearly the same. On the other hand, statistically significant decreases in the value of fructose present in the grape juice after Amberlite® XAD-16 and activated carbon application occurred ($p<0.05$), with no differences between these two grape juices. Similar to fructose results, lower glucose values were found in Amberlite® XAD-16 and activated carbon grape juices than that in control, while no statistically significant change occurred in the value of Dowex® 50Wx8-100 applied grape juice.

According to the results of Ajlouni & Iyer (2003), adsorbent (XAD 16) and weak-base (IRA 95) resins application did not affect the glucose, fructose, sucrose and sorbitol content of pear juice. Unfortunately, due to limited data on resin-sugar and/or resin-amino content interaction, we couldn't compare our results to previous studies to determine the efficiency of our resins in removing sugars or amino group-contained compounds.

Amounts of protein and free amino groups in grape juice were found as 437.8 ± 178.14 mg/kg DW and 1.87 ± 0.17 g L-leucine equivalent/kg DW, respectively. Resin application made a reduction in the free amino content of the grape juice. While the lowest value was found in Dowex applied grape juice, the difference among the activated carbon and Amberlite applied juices was statistically insignificant. Contrary to free amino content, total protein concentration did not affect from the resin application.

pH value of resin non-applied grape juice was found to be 4.57 ± 0.13. While the value decreased to 4.10 ± 0.08 after Dowex® 50Wx8-100 application, higher pH values were obtained in activated carbon (4.65 ± 0.11) and Amberlite® XAD-16 (4.59 ± 0.12) applied grape juices.

Resins are generally applied to fruit juices for the purposes of reducing the bitterness and acidity and improving the color. While desired improvement occurring with resin application, some physical and chemical changes could also be occurred in fruit juices after the application. In a previous study on reduction of acidity from passion fruit juice, increases in pH values of the juices were obtained after applying 10 weak anionic resins (Vera et al., 2003). Similarly, lower acidity value was reported in activated carbon applied apple juice by Çoklar & Akbulut (2010).

Dowex® 50Wx8-100 is a strongly acidic cation-exchange resin and it contains H^+ as active group. In this study, a decrease in pH of Dowex applied grape juice could be resulted from the transfer of H^+ ions from the resin to grape juice.

3.2 Effect of concentration on glucose, fructose, protein and free amino contents and pH values

Results for fructose, glucose, total protein, free amino contents and pH values obtained in molasses and non-concentrated grape juices are given in Table 1. Fructose and glucose contents of grape juice were determined as 44.54 ± 1.01 and 38.96 ± 0.88 g/100 g DW, respectively. Concentration process had a reducing effect on glucose content of the grape juice. As concentration degree increases, decreasing glucose content of grape juice was observed. Concentrated juices had lower glucose values when compared to control, except the grape juice with concentrated value of 50 °Bx. As distinct from glucose changes, it was found that decreases in fructose began at lower concentration degrees and gradually increases with increase in concentration. Glucose and fructose contents of the juice which was concentrated to 70 °Bx respectively decreased to nearly 11.88 and 12.01%, with respect to the control sample. Total protein and free amino contents of the molasses can be seen in Table 1. According to the results, a decreasing amount of protein in grape juices by the order 20>50>60>70 °Bx was observed. On the other hand, the differences in protein content of the grape molasses which were concentrated to 50, 60 and 70 °Bx were found to be statistically insignificant.

Amounts of protein and free amino groups in the control were found as 526.37 ± 144.75 mg/kg DW and 1.99 ± 0.18 g L-leucine equivalent/kg DW, respectively. Total free amino content of grape juice decreased from 1.99 g L-leucine equivalent/kg DW to 1.26 g L-leucine equivalent/kg DW after the concentration up to 70 °Bx.

It is well known that sugars, especially reducing ones, are the reactants of the caramelization and Maillard reactions and their concentrations decrease after these reactions (Van Boekel, 2001; Laroque et al., 2008). On the other hand, there are differences in the reactivity of the sugars in non-enzymatic browning (Reyes et al., 1982). According to the results of Göğüş et al. (1998) who studied on HMF accumulation and browning formation in acidic model systems, fructose was more reactive than glucose in terms of HMF accumulation and brown pigment formation. Previous studies reported that fructose is more reactive than glucose at low pH values (Kato et al., 1969). Presumably, high temperature and prolonged heat application to increase the dry matter content of molasses may have increased the degree of Maillard reaction in this study.

pH value of the control was measured to be 4.93 ± 0.38. Regarding to the concentration degree, there were statistically significant differences between the pH values of molasses at different Brix values (50, 60 and 70 °Bx). As concentration degree increased, lower pH values were obtained in molasses. Decreasing pH level during concentration could be arisen from Maillard reaction due to the long-term exposure of high temperature to remove the water from the grape juice. Previous studies indicate that acidity of the reaction media could be changed in accordance to the degree of Maillard reaction. Acetic acid and formic acid are two organic acids which have been identified at glucose and fructose contained model systems (Van Boekel & Brands, 1998). Van Boekel (2001) attributed decreasing of the pH from 6.7 to 5.6 in glucose-glycine solution heated at 100 °C to Maillard reaction.

As reported by previous studies, Maillard reaction is also affected by the pH of the media (Ames, 1990). Under strong acidic condition (at pH 2), D-fructose decomposes to 3-deoxy-D-glucosone and then to HMF by caramelization reaction. On the other hand D-glucose is more stable to degradation at pH 2. Furthermore, while at the range of pH 3-7 reducing sugars are stable to decomposition by caramelization, the interaction of carbonyl groups with amino groups could be taken place (Kato et al., 1969).

3.3 Effect of concentration and resin application on HMF formation

As an intermediate product of Maillard reaction, HMF can be formed via both sugar degradation and carbonyl-amino reaction.

In our study, as expected, since these samples were not subjected to any heat treatment, HMF was not determined in both resin non-applied and non-concentrated grape juices (Table 1). Concentration process induced the HMF formation because of higher temperature. On the other hand, higher values were observed as concentration degree was increased from 50 to 70 °Bx.

In relation to effect of resin application, lower HMF values were observed in the molasses which were produced from resin applied juices, except for the Dowex applied grape juice, than that of control sample. The lowest value was found to be 11.61 ± 3.35 mg/100 g DW in the molasses produced from activated carbon applied grape juice.

Similar results were reported by Makhlouf-Gafsi et al. (2018), who studied the effect of ultrafiltration and temperature on HMF levels in date palm sap syrups. They reported higher HMF values in the date syrup prepared from sap juice than prepared from its permeate. They have attributed their results to reduction in protein and sugar, the reactants of The Maillard reaction, by ultrafiltration.

When we take into the consider the amounts of glucose, fructose, free amino group and pH values of grape juices, Dowex application could be induced the HMF formation as a result of decreasing of the pH. Though the lowest free amino content value was observed in Dowex applied grape juices, higher sugar content and lower pH values may have accelerated the rate of HMF formation and also Maillard reaction. Considering that lower HMF contents were occurred in molasses produced from activated carbon or Amberlite applied grape juices, resin application could be an effective method to control the reaction.

3.4 5-hydroxymethylfurfural (HMF) accumulation throughout the storage

HMF contents were analysed and kinetic parameters were estimated in molasses produced from resin applied and non-applied grape juices during the storage at three different temperatures (50, 65 and 75 °C). Amounts of HMF in control samples which had 50, 60 and 70 °Bx values were 13.34, 15.73 and 22.26 mg/100 g DW, respectively, at the beginning of the storage. HMF values of all grape molasses increased throughout storage at all temperatures (Figure 2) and its accumulation data obtained from the control and other molasses were fitted to zero order kinetic models.

HMF accumulation as a function of heating time or storage has been modelled via zero and first order models (Garza et al., 1999; Ibarz et al., 1999; Buglione & Lozano, 2002; Tosun, 2004; Arslanoğlu et al., 2005; Rattanathanalerk et al., 2005).

Similarly to results of this study, Özhan et al. (2010) reported that HMF accumulation in carob molasses during storage followed a zero-order rate law. Göğüş et al. (1998) investigated the Maillard reaction progress in grape molasses and model system (L-arginine, L-proline, L-glutamine, D-glucose, D-fructose) during storage. They found that the order of HMF formation was pseudo first for model system and zero for molasses.

Rate constant for HMF formation in control sample stored at 50 °C was observed as 4.903, 6.6379, and 8.6741 mg/100 g DW/day for 50, 60 and 70 °Bx, respectively. It was discovered that rate constant for HMF accumulation increased according to the increase in concentration and temperature (Table 2).

In molasses produced by using Activated carbon and Amberlite® XAD-16, HMF accumulation was lower than that in the control. Among all the molasses with 70 °Bx, Amberlite® XAD-16 applied grape molasses had the lowest rate constant value. On the contrary, rate constant for HMF accumulation increased in grape molasses produced from Dowex® 50Wx8-100 applied juices. Activation energy values for HMF accumulation ranged between 83.589-134.396 kJ/mol in molasses stored at three different temperatures. It was found that activation energy for molasses decreased with increasing concentration.

Other two important kinetic parameters for predicting the rate of reaction are activation energy and temperature coefficient (Q_{10}) values. These parameters enable us to have an idea about dependence of a reaction to temperature and temperature changes. In other words, activation energy is defined as the minimum energy that molecules should have to cause a reaction. Activation energies in food systems range from 4.2 to 630 kJ/mol. Some researchers noted that activation energies for molasses are between 102.67-158.63 kJ/mol. Hawkes & Villota (2006) noticed that the activation energy for non-enzymatic browning reaction changed in the range of 37.8-168 kJ/mol. Tosun (2004) also determined the activation energy for HMF formation in hard molasses as 158.63 kJ/mol. Activation energies for HMF formation of pineapple juice and peach puree were recorded as 29.401 and 117 kJ/mol, respectively. Each system has a specific E_a (activation energy) and it can be change according to the water activity (Bell, 1997).

Q_{10} values indicate the temperature sensitivity of a reaction and make us to interpret how reaction sensitivity change when there is a 10 °C increase in temperature. The lowest Q_{10} value among the molasses which had 50 °Bx was Amberlite applied grape juices. Highest Q_{10} values were determined in molasses concentrated after Dowex application. Unfortunately, higher Q_{10} values observed in control samples which had 60 and 70 °Bx values, except 50 °Bx, as compared to all other molasses used in this study.

Considering the kinetic parameters for HMF accumulation during storage, we could conclude that Dowex application induced the reaction and didn't comply with controlling the Maillard reaction.

3.5 Brown pigment formation throughout the storage

The brown pigment formation in molasses throughout the storage was evaluated by absorbance measurements at 420 nm. As expected, browning in all molasses increased with increasing storage temperature and time (Figure 3). Brown colored pigment formation in foods occurs in both caramelization and Maillard reactions. Caramelization requires higher temperatures, particularly above 80 °C, to develop the brown color (Davies & Labuza, 1997). Whereas, Maillard reaction occurs at lower temperature, even at room temperature. Therefore, browning in stored foods, most probably, arises from Maillard reaction. Just as for HMF, browning index is accepted as an indicator of Maillard reaction (Özhan et al., 2010).

There was no brown pigment formation in molasses, which were stored at 50 °C. Thereby, kinetic parameters for browning index were determined for the molasses stored at 65 and 75 °C. It was observed that brown pigment formation in white grape molasses followed a zero-order rate law. Table 3 indicates that the rate constants for browning index increased with increasing temperature in all molasses during storage. In grape molasses, reactants for Maillard reaction increased as a

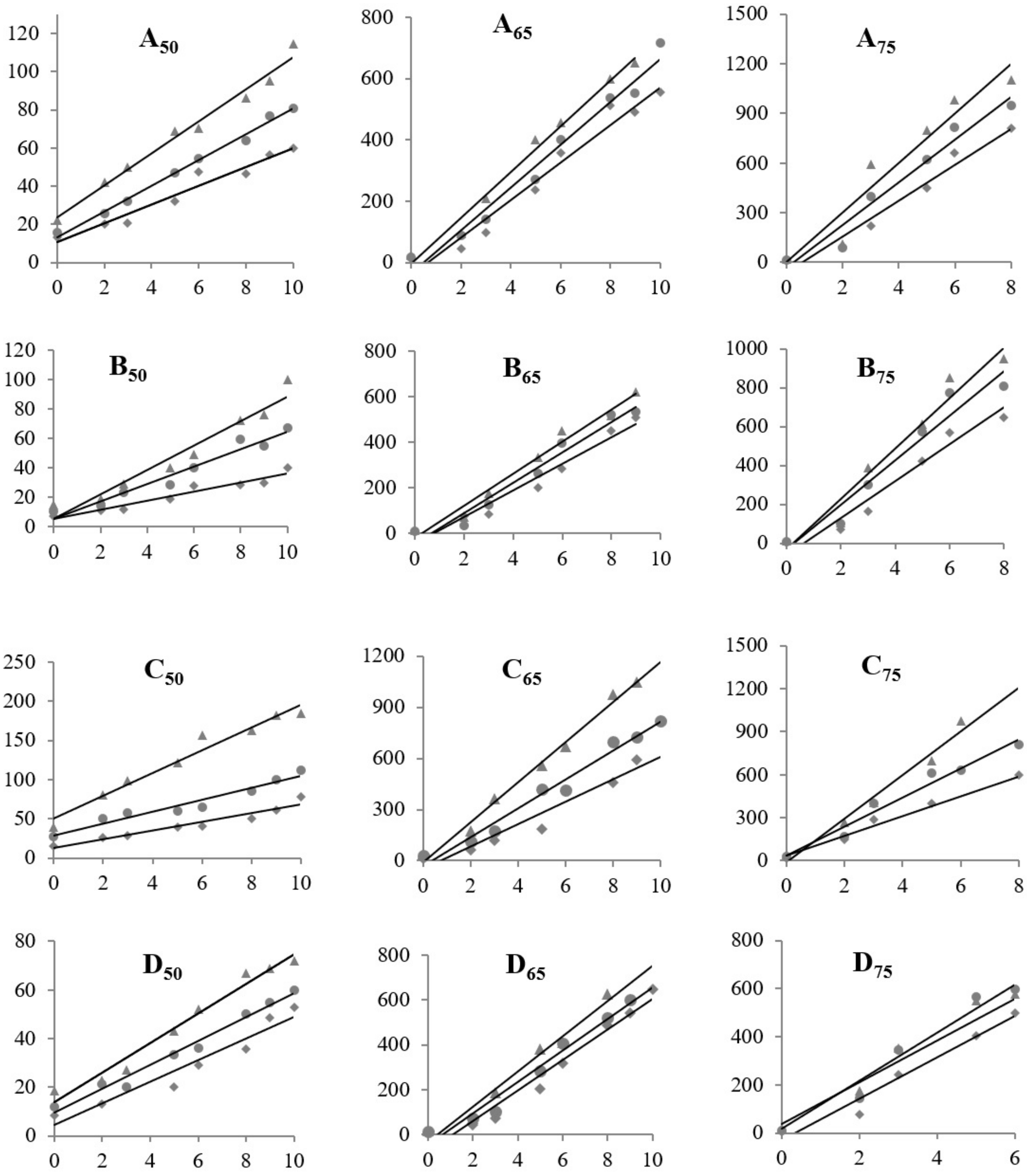

Figure 2. HMF accumulation in grape molasses at storage temperature. x (horizontal) and y (vertical) axes of the graphic show storage time (day) and HMF content (mg/100 g DW), respectively. Geometric figures point out the Brix values of molasses (o: 70 °Bx; Δ: 60 °Bx; and ◊:50 °Bx). While capital letters indicate molasses produced from resin non-applied and resin applied grape juices ((A) Resin non-applied or control; (B) Activated carbon applied; (C) Amberlite applied; and (D) Dowex applied), subscripts of the capital letter shows the storage temperature (such as A_{50}: Control sample stored at 50 °C).

Table 2. Rate constant (*k*), activation energy (E_a) and Q_{10} value for HMF content in white grape molasses stored at different temperatures.

Sample	Concentration (oBx)	T (°C)	*k* (mg/100 g DW/ day	R^2	Q_{10} (50-75 °C)	E_a (kJ mol^{-1})
Control	**50**	**50**	4.9003	0.9363	4.03	119.289 (R^2 0.954)
		65	6.6379	0.9902		
		75	8.6741	0.9832		
	60	**50**	61.179	0.9622	3.75	116.329 (R^2 0.972)
		65	69.151	0.9716		
		75	77.684	0.9700		
	70	**50**	108.43	0.9651	3.30	112.996 (R^2 0.982)
		65	138.80	0.9453		
		75	168.63	0.7074		
Activated Carbon	**50**	**50**	2.9873	0.9210	3.88	134.396 (R^2 0.926)
		65	5.7847	0.9327		
		75	8.2981	0.9236		
	60	**50**	61.833	0.9486	4.19	115.066 (R^2 0.953)
		65	66.684	0.9631		
		75	69.664	0.9671		
	70	**50**	94.794	0.9515	3.89	102.113 (R^2 0.960)
		65	114.54	0.9446		
		75	118.24	0.9170		
Dowex® 50Wx8-100	**50**	**50**	4.7664	0.9853	4.31	105.937 (R^2 0.869)
		65	7.4260	0.9353		
		75	14.466	0.9659		
	60	**50**	65.832	0.9275	3.80	104.864 (R^2 0.911)
		65	84.241	0.9785		
		75	113.86	0.9912		
	70	**50**	69.862	0.9453	4.02	83.5890 (R^2 0.872)
		65	109.05	0.9582		
		75	120.52	0.9977		
Amberlite® XAD-16	**50**	**50**	4.4190	0.9378	3.64	114.833 (R^2 0.895)
		65	5.6959	0.8973		
		75	6.3911	0.9565		
	60	**50**	68.261	0.9608	3.87	106.511 (R^2 0.901)
		65	70.300	0.9705		
		75	78.382	0.9714		
	70	**50**	82.671	0.9389	3.59	107.400 (R^2 0.907)
		65	86.557	0.9209		
		75	99.806	0.9775		

T and R^2 indicate the storage temperatures and R-squared of the models, respectively.

result of concentration process owing to removing water. Thus, as in the case of temperature, browning occurred faster in the molasses which had higher dry matter contents.

In many studies, kinetics of brown pigment formation as a result of caramelisation or Maillard reaction was described by zero or first order kinetics (Toribio & Lozano, 1986; Cohen et al., 1994; Vaikousi et al., 2008).

As regards to resin application on browning index, lowest rate constant was calculated for the molasses produced from Dowex applied grape juices which had 50 °Bx values and stored at 65 °C. Similarly, molasses produced from either Amberlite or activated carbon applied grape juices had lower rate constant values for brown pigment formation than those of the control.

E_a values of control samples at 50, 60 and 70 °Bx values were calculated as 86.931, 65.716 and 49.320 kJ/mol, respectively. It was observed that activation energy values for brown pigment formation in molasses decreased as concentration degree increased. Dowex or activated carbon applied molasses exhibited much higher activation energies than those of control and Amberlite applied molasses.

Similar to our results Özhan et al. (2010) found that the activation energy for brown pigment formation in carob molasses stored at different temperature was 75.86 kJ mol^{-1}. Turkmen et al. (2006) calculated activation energy for brown pigment formation in honey, which were stored at 50, 60 and 70 °C for 12 days, to be 122 kJ mol^{-1}. Activation energies for colour formation in Maillard reaction occurred in different sugar and amino acid

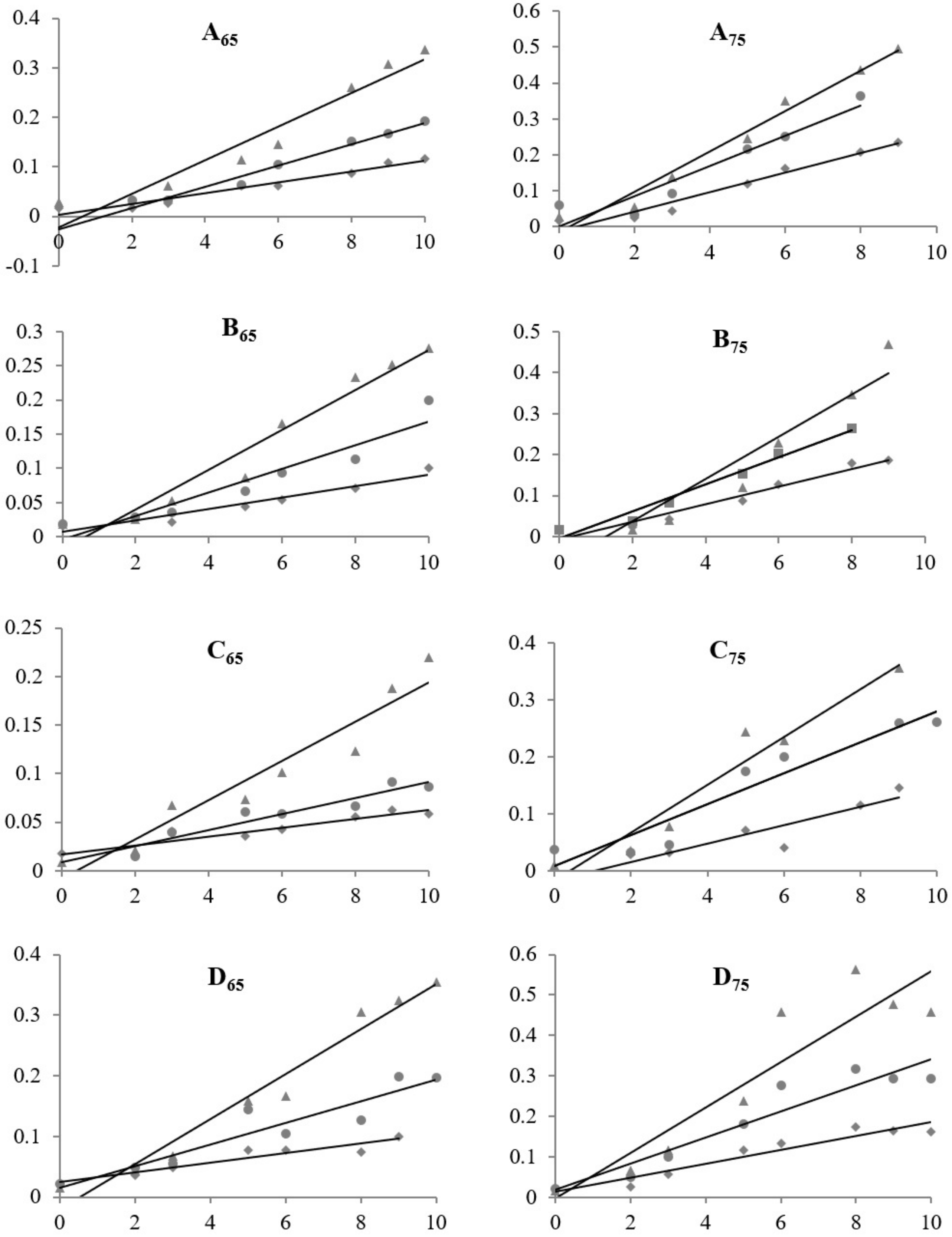

Figure 3. Brown pigment formation in grape molasses during storage. x and y axes of figures show storage time (day) and absorbance value at 420 nm, respectively. Geometric figures point out the Brix values of molasses (o: 70 °Bx; Δ: 60 °Bx; and ◊:50 °Bx). While capital letters indicate molasses produced from resin non-applied and resin applied grape juices ((A) Resin non-applied or control; (B) Activated carbon applied; (C) Amberlite applied; and (D) Dowex applied), subscripts of the capital letter shows the storage temperature.

Table 3. Rate constant (k), activation energy (E_a) and Q_{10} value for browning index (BI) in white grape molasses stored at different temperatures.

Sample	Concentration (°Bx)	T (°C)	k (A_{420}/day)	R^2	E_a (kJ mol^{-1})	Q_{10} (65-75 °C)
Control	**50**	**65**	0.0111	0.9589	86.931	2.43
		75	0.0270	0.9591		
	60	**65**	0.0215	0.9752	65.716	1.96
		75	0.0421	0.9007		
	70	**65**	0.0340	0.9403	49.320	1.66
		75	0.0563	0.9745		
Activated carbon	**50**	**65**	0.0083	0.9396	93.533	2.60
		75	0.0216	0.9577		
	60	**65**	0.0173	0.9061	63.155	1.91
		75	0.0330	0.9721		
	70	**65**	0.0291	0.9480	56.204	1.78
		75	0.0517	0.8920		
Dowex-50W-100	**50**	**65**	0.0046	0.8683	122.507	3.50
		75	0.0161	0.8198		
	60	**65**	0.0082	0.8987	96.068	2.67
		75	0.0219	0.8012		
	70	**65**	0.0203	0.9242	71.100	2.07
		75	0.0420	0.9448		
Amberlite XAD-16	**50**	**65**	0.0080	0.9095	74.287	2.14
		75	0.0171	0.9176		
	60	**65**	0.0179	0.8854	57.724	1.81
		75	0.0323	0.9059		
	70	**65**	0.0372	0.9657	40.350	1.51
		75	0.0562	0.8593		

T and R^2 indicate the storage temperatures and R-squared of the models, respectively.

containing model system were varied between 23-147 kJ mol^{-1} (Van Boekel, 2001). The results indicate that molasses produced from Dowex applied grape juices required higher energy to initiate the browning. Browning is more likely not to occur if the product fails to gain the energy required for activation. Passing over the lower energy barrier is easy and the reactions exhibiting lower activation energy, initiates more easily.

Q_{10} values for control which had different Brix values were found to be in the range of 2.43-1.66. It was found that Q_{10} values for browning formation decreases as the concentration degree increases. Higher Q_{10} values in Dowex applied grape molasses compared to other molasses showed that Dowex applied molasses were more sensitive to temperature changes.

Liu et al. (2008) reported Q_{10} values for browning in galactose/glycine model system were in the range of 1.98-2.00. On the other hand, higher Q_{10} values ranging between 1.59 and 15.88 were reported for brown colour formation in citrus juice concentrates (Koca et al., 2004).

It could be said that activated carbon application slowed up brown pigment formation in molasses during the storage when the rate constant, Q_{10} value and activation energy are taking into consideration.

4 Conclusions

Non-enzymatic browning is desirable, providing that it is not extreme, in molasses production due to the formation of specific taste and flavor. While both caramelisation and Maillard reaction occurs during open vessel concentration of grape juice, increments in HMF content and brown color of the molasses during storage are mostly originated from Maillard reaction. To inhibit the extensive accumulation of Maillard reaction products, the reaction should be kept under control.

This research aimed to control the Maillard reaction in grape molasses by removing of reactants, namely glucose, fructose, protein and/or free amino content, using adsorbent (activated carbon and Amberlite) and ion exchange (Dowex) resins. Activated carbon and Amberlite were effective in reducing the amounts of fructose, glucose and free amino groups.

At the same time lowest HMF content was determined in molasses which were produced from activated carbon applied grape juice. Amberlite application was also as effective as activated carbon in terms of reducing HMF formation. While Dowex application was most efficient to reduce brown pigment formation during storage, HMF production rate was lower in activated carbon applied molasses than Amberlite applied samples. Molasses produced from grape juice had higher HMF contents after Dowex application in both throughout concentration and storage than the control.

Activate carbon could be applied to grape juices before concentration to keep under control the HMF and brown colour formation during the concentration and the storage period. Furthermore, it could be more effective to keep the intended limits of HMF content and brown colour formation by combining the resins at favourable ratios.

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