# Stability of Anthocyanins in Frozen and Freeze-dried Raspberries during Long-Term Storage

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Stability of Anthocyanins in Frozen and Freeze-dried Raspberries during Long Term Storage in Relation to Glass Transition

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## Abstract

Anthocyanins, natural plant pigments in the flavonoid group, are responsible for the red color and some of the nutraceutical benefits of raspberries. This study explores anthocyanin degradation in frozen and freeze-dried raspberries during storage in relation to glass transition temperatures. Frozen raspberries were stored at -80, -35 and -20°C, while freeze-dried raspberries were stored at selected water activity values ($a_w$) ranging from 0.05 to 0.75 at room temperature (23°C) for more than a year. The characteristic glass transition temperatures ($T_g$') of raspberries with high water content and glass transition temperature ($T_g$) of raspberries with small water content were determined using a differential scanning calorimeter. The pH differential method was used to determine the quantity of anthocyanins in frozen and freeze-dried raspberries at selected time intervals. The total anthocyanins in raspberries fluctuated during 378 days of storage at -20 and -35°C, and -80°C. Anthocyanin degradation in freeze-dried raspberries ranged from 27-32% and 78-89% at water activity values of 0.05-0.07 and 0.11-0.43, respectively, after one year. Anthocyanins were not detectable in freeze-dried raspberries stored at water activity values of 0.53-0.75 after 270 days. First order and Weibull equations were used to fit the anthocyanin degradation in freeze-dried raspberries. The first order rate constant ($k$) of anthocyanin degradation ranged from 0.003-0.023 days$^{-1}$ at the selected water activities. Significant anthocyanin degradation occurred in both the glassy and rubbery states of freeze-dried raspberries during long-term storage. However, the rate of anthocyanin degradation in freeze-dried raspberries stored in the glassy state was significantly smaller than the rate of anthocyanin degradation in the rubbery state.

*Key words: Glass transition, maximally freeze-concentrated matrix, water activity,*

*Weibull equation*

## Introduction

Color is one of the primary attributes determining the quality of fruits. Anthocyanins are polyphenolic compounds present in raspberry fruits, responsible for the attractive red color. Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium salt. The concentrations of anthocyanins in raspberries range between 0.2-2.2 g/g fresh weight (de Ancos and others 1999; Sablani and others 2010a). The main anthocyanins present in red raspberries include cyanidin-3-glucoside and cyanidin-3-sophoroside (de Ancos and others 1999). These bioactive compounds are significant because of their nutraceutical benefits, antioxidant and anticarcinogenic properties (Zhang and others 2005; Wang and Lin 2000). Anthocyanins can also be used as natural food colorants in the food industry (Gradinaru and others 2003). However, anthocyanins are labile in nature and susceptible to deterioration during processing and storage (Francis 1989). Raspberry fruits are commonly preserved by freezing and drying. Frozen and dried raspberries are used as ingredients in many food formulations such as jam, jelly, sauce, puree, topping, syrup, juice concentrates, bakery and dairy products. In freezing, the retention of anthocyanins depends on the freezing rate, composition, pH, cultivar, temperature, and the presence/absence of oxygen (Wrolstad and others 1970; Mazza and Miniati 1993). Raspberries are often quick-frozen at very low temperatures (-80°C) for long term preservation with minimal deterioration of quality. The major parameters determining the stability of anthocyanins during dried storage are water content, water activity, temperature, presence/absence of oxygen, light and relative humidity of the environment (Francis 1989).

Mechanisms of anthocyanin degradation during processing and storage were proposed by Markakis and others (1957) and by Erlandson and Wrolstad (1972). Water may enhance hydrolysis of the glycosidic linkage in anthocyanin molecules, yielding unstable anthocyanidins with subsequent opening of the pyrilium ring to form chalcones and brown end products (Markakis and others 1957; Erlandson and Wrolstad 1972). Water in the presence of oxygen advances the oxidation rate of anthocyanins (Jackman and Smith 1992). Anthocyanin degradation was attributed to oxidation by Jackman and Smith (1992). During storage, oxygen may diffuse into the dry raspberry matrix inducing reactions between anthocyanins and quinones resulting in subsequent formation of brown pigments (Gradinaru and others 2003). The effect of oxygen on anthocyanin degradation was small in freeze-dried strawberries at low water activities (Erlandson and Wrolstad 1972). Enzymes such as polyphenol oxidase, peroxidase and anthocyanase may be also responsible for anthocyanin degradation. Water may facilitate enzyme reactivity and increase enzyme substrate complexes and product formation rates. Erlandson and Wrolstad (1972) reported the anthocyanin degradation rate in freeze-dried strawberries before enzyme inactivation was comparable to the anthocyanin degradation rate after enzyme inactivation.

As temperature is reduced during freezing, maximum-freeze-concentrated matrices characterized by large viscosities are formed (Goff and Sahagian 1996). At maximum-freeze-concentration, maximum ice crystallization also occurs within the residual unfrozen water (Goff and Sahagian 1996; Sablani and others 2010b). The maximum-freeze-concentration conditions are described by two temperatures, i.e. the glass transition temperatures of the maximum-freeze-concentrated matrices ($T_g'$) and the onset of ice melting temperature ($T_m'$) (Goff and Sahagian 1996). The transition from the reversible liquid/rubber state to the glassy state starts in the food matrix below the temperature corresponding to the onset of ice melting temperature ($T_m'$). More details on $T_g'$ and $T_m'$ are presented elsewhere (Goff and Sahagian 1996; Kasapis 2009; Roos 2010; Sablani and others 2010b).

According to the glass transition concept, foods are most stable in the glassy state, i.e. at temperatures below their glass transition temperature. Below $T_g'$, viscosity becomes great enough to inhibit the rates of chemical reactions. Physical and chemical degradation reactions of frozen food systems may be related to molecular mobility and thus $T_g'$ (Torreggiani and others 1999; Goff and Sahagian 1996; Rahman 2009). Akkose and Aktas (2008) observed a significant difference in total volatile basic nitrogen (TVB-N) and the thiobarbituric acid-reactive substance (TBARS) values in ground beef during 6 months storage at temperatures greater than and less than its $T_g'$. Brake and Fennema (1999) reported the rate of formation of malonaldehyde using TBARS in minced mackerel was significantly different at temperatures greater than and less than its $T_g'$. The $T_g'$ and $T_m'$ value of raspberry fruit were identified as -47 and -38°C, respectively (Syamaladevi and others 2009). The raspberry matrices consist of ice and glass at temperatures less than their $T_g'$ (< -47°C), recommended for long term storage (Champion and others 2000; Roos and others 1991; Syamaladevi and others 2009). Maximum ice formation in the matrices take place when the food is stored between its $T_m'$ and $T_g'$ ($-47°C < T < -38°C$). But in this temperature range, the molecular mobility and reaction rates are greater than the reaction rates at temperatures less than $T_g'$. At temperatures greater than the $T_m'$ (> -38°C), raspberry matrices are plasticized by melting ice. Melting of ice may result in a partially-freeze-concentrated raspberry matrix, characterized by significantly smaller viscosity and larger molecular mobility than the raspberry matrix below $T_g'$. The partial freeze concentrated condition is not suitable for long term food storage systems (Syamaladevi and others 2009; Champion and others 2000; Roos and others 1991).

As water is gradually removed during drying of food systems, a rubbery to glassy state transformation occurs. The glass transition temperature ($T_g$) is a characteristic temperature range wherein the glass to rubber transition occurs in an amorphous food system at a specific water content. The $T_g$ is an essential consideration during processing and storage of food. Food quality may be attributed to dramatic changes in physical, mechanical, electrical and thermal properties during glass transitions (Roos and Karel 1991; Slade and Levine 1991). Molecular mobility and diffusion are considerably reduced in the glassy state of food systems. Rates of degradation reactions may be enhanced when foods are stored at temperatures greater than their $T_g$. For instance, rates of thiamin and vitamin C degradation, lipid oxidation and the Maillard reaction are smaller in the glassy state than the rubbery state of selected food systems (Bell and others 1998; Bell and White 2000; Drusch and others 2006; Sablani and others 2007). Food storage in the glassy state is recommended to minimize the reaction rates of unwanted physical and chemical degradations. However, molecular motion and selected degradation reactions may occur at slower rates in the glassy state (Bell 1996; Bell and Hageman 1994; Gradinaru and others 2003). Bell (1996) reported that polyvinylpyrrolidone exhibits sufficient molecular mobility and browning reactions in its glassy state, even though these reaction rates are much smaller than in the rubbery state. The influence of $T_g$ on the rates of selected chemical reactions is not clearly understood. Additional research is needed to understand the influence of molecular mobility at temperatures surrounding $T_g$ on chemical reaction rates.

Since frozen and dried storage are two important methods of long term storage of raspberries, it is important to determine the retention of anthocyanins in the glassy and rubbery state of frozen and dried raspberries during storage. This will help in identifying adequate frozen and dried storage conditions for raspberries and maximal retention of the functional qualities of raspberry fruits. The objective of the current study was to evaluate the stability of anthocyanins in glassy and rubbery states of frozen and dried raspberries during long term storage.

## Materials and methods

Fresh raspberries (*Rubus idaeus*) grown in Washington State were generously supplied by Milne fruit products (Milne Fruit Products Inc. Prosser, WA). Fresh raspberries were frozen by keeping in a freezer room maintained at -35°C. A portion of the frozen raspberries were layered on metal pans and placed inside a freeze dryer (Virtis freeze mobile 24 with Unitop 600L, VirTis SP Industries Co., New York, NY). The shelf temperature was set at -10°C with a pressure of 20 Pa. The temperature of the condenser was adjusted at -60°C. After freeze drying (2 days), the raspberries were ground immediately to a fine powder using a mortar and pestle. A vacuum oven method was used for water content determination of fresh and freeze-dried raspberry powder equilibrated with selected relative humidities. Raspberry powder was weighed into aluminum cups and placed in a vacuum oven at 80°C for 10 h and pressure of 10 kPa

(Syamaladevi and others 2009). The experiments were conducted in triplicate.

### Selection of frozen and dried storage conditions

The selection of frozen storage temperatures was determined considering the $T_g'$ (-47°C) and $T_m'$ (-38°C) of raspberries (Syamaladevi et al., 2009). One temperature (-80°C) was selected far below the $T_g'$ (-47°C) of raspberries. A second temperature of -35°C was selected as close to the $T_m'$ but greater than the $T_g'$ of raspberries. A third storage temperature of -20°C was selected to represent commercial frozen storage at a temperature greater than the $T_g'$ and $T_m'$ of raspberries.

The water content, water activity and glass transition temperature of the freeze-dried raspberry powder was determined as 0.052 kg water/kg raspberry, 0.2 and 4.55°C respectively. No endotherms associated with sugar crystallization or melting were observed suggesting the amorphous nature of the freeze dried raspberries. This behavior of freeze dried raspberries was also observed in our previous study (Syamaladevi et al., 2010). After freeze drying, the raspberry powders were placed in open weighing bottles and equilibrated with saturated salt solutions of constant water activity in airtight containers at room temperature (23°C). To store freeze-dried raspberry powder in the glassy state at room temperature, $P_2O_5$ and CsFl salts were used for moisture equilibration. Saturated solutions of LiCl, $CH_3COOK$, $MgCl_2$, $K_2CO_3$, $MgNO_3$, $NaNO_2$, and NaCl ($a_w$ ranging from 0.113 to 0.750) were used to achieve the rubbery state in freeze-dried raspberry powder at room temperature. Water activity values for these solutions were obtained from Greenspan (1977). A small amount (1-2 g) of thymol was kept inside the air tight glass containers with raspberry powders to avoid microbial growth in the raspberry powders. Weights of raspberries were taken periodically (3-4 days) until constant weights (the change in weights < 0.1%) were obtained during equilibration. Constant raspberry weights were obtained in 37 days of equilibration. After equilibration, the quantity of anthocyanins was determined and reported as the initial value of anthocyanins in raspberry powder. At selected time intervals, the quantity of anthocyanins in the stored raspberry powders was determined.

**Extraction and quantification of total anthocyanins**

The fresh/frozen raspberries were pulverized and homogenized with a stainless steel fruit blender (Guisti and Wrolstad, 1996a; Plessi and others, 2007). One g of dried or 5 g of fresh/frozen fruit was mixed with 50 ml of 1% HCl-Methanol (v/v) at room temperature (23°C). The homogenized mixture was held overnight at 4°C. The mixture was centrifuged at 10000 g for 10 min at 4°C and the supernatant was collected. The pellet was removed and mixed with acidified methanol and held for 1 h. The pellet solution was centrifuged again at equivalent conditions and the supernatant collected and mixed with the original solutions. The dilution factor for extraction was determined. Extraction of anthocyanins was conducted in triplicate.

The total anthocyanin content was quantified using the pH differential method (Guisti and Wrolstad 1996a). Specific quantities of extracts were diluted in pH 1.0 and pH 4.5 buffers, and absorbance determinations were conducted at 530 and 700 nm with a Shimadzu 300 UV spectrophotometer, using 1 cm path length cells. The dilution factor used was 10. The anthocyanin content was calculated and expressed as cyanidin-3-glucoside (Cyd-3-glu)/100 g dry solids using an extinction coefficient of 34300 L $cm^{-1}$ $mol^{-1}$ and a molecular weight of 449.2 g $mol^{-1}$ (Giusti and Wrolstad 2001).

**Kinetics of quality degradation**

The kinetics of anthocyanin degradation data was modeled using zero, first, second and Weibull equations. The general equation describing quality degradation is

$$-\frac{dC}{dt} = kC^n \qquad (1)$$

where $C$ is the concentration of the quality parameter, $k$ is the reaction rate constant and $n$ is the order of the reaction. The half life ($t_{1/2}$) of a reaction is obtained assuming first order kinetics as

$$t_{1/2} = \frac{\ln 0.5}{k} \qquad (2)$$

The Weibull equation is more flexible in fitting degradation reaction kinetics since it includes a scale factor (Cunha and others 1998). The Weibull equation is equal to the first order equation when the shape factor $\gamma$ is equal to 1. The Weibull equation is used to fit the microbial, enzymatic and other degradation reactions in foods (Odriozola-Serrano and others 2009; Cunha and others 1998). The Weibull equation is

$$C = C_o \exp\left[-\left(\frac{t}{\alpha}\right)^{\gamma}\right] \qquad (4)$$

where $C$ is the retention of the quality parameter after time $t$, $C_o$ is the initial concentration of the quality parameter, $\alpha$ is the scale factor and $\gamma$ is the shape parameter determining the shape of the curve. A $\gamma$ value greater than 1 indicates the curve is convex (forming shoulder), while a $\gamma$ value less than 1 indicates the curve is concave (forming tail). The values of $\alpha$ and $\gamma$ are determined by non-linear optimization for curve fitting.

Anthocyanin degradation data were analyzed for statistical significance using SAS 9.1 (SAS Institute, Inc., Cary, NC, USA). A value of $P<0.05$ was selected as statistically significant using two-way ANOVA by Tukey's LSD method.

## RESULTS AND DISCUSSION

### Stability of raspberry anthocyanins during frozen storage

The water content of fresh raspberries was 0.86 g water/g raspberry. The quantity of total anthocyanins in fresh raspberries was 0.75 mg anthocyanins/g of dry raspberry solids, comparable to values found in the literature (de Ancos and others 1999, Sablani and others 2010a). A significant two-way interaction ($P < 0.05$) between time and temperature of frozen storage on total anthocyanins in raspberries was observed in the statistical analysis by two-way ANOVA. Statistical differences in total anthocyanins during frozen storage are not shown in the figures as the effect of storage temperature on total anthocyanins depended on time of storage and *vice versa*. Hence, the changes in total anthocyanins with time are described for each frozen storage temperature. No significant difference ($P \geq 0.05$) in the concentration of anthocyanins was observed for frozen raspberries immediately after freezing when compared to fresh raspberries. The total anthocyanins in raspberries immediately after freezing at -20, -35 and -80°C were not significantly different ($P \geq 0.05$). In general, an increasing trend with some fluctuation in the total anthocyanins of frozen raspberries was observed during storage of 378 days at -20 and -35°C. An increase of 15, 38, 12, and 21% in total anthocyanin concentration was observed in frozen raspberries stored at -20°C after 0, 158, 278, and 378 days respectively in comparison with the total anthocyanins in fresh raspberries (Figure 1). Similarly, at -35°C, an increase of 8, 32, 25 and 29% in total anthocyanins was observed in frozen raspberries after 0, 158, 278, and 378 days respectively in comparison with the total anthocyanins in fresh raspberries (Figure 1). At -80°C, an increase of 33 and 24% in total anthocyanin concentration was observed in frozen raspberries after 158, 278 days respectively while a decrease of 16% in total anthocyanins was observed after 378 days compared to the anthocyanins in fresh raspberries (Figure 1). This decrease in total anthocyanins at -80°C may be due to experimental variability as fluctuating trend was also observed at higher storage temperatures of -20 and -35°C. The greater quantity of anthocyanins in frozen raspberries may be attributed to better extraction efficiency of anthocyanins from frozen raspberries than extraction from fresh raspberries because of cellular disruption during freezing and thawing (de Ancos and others 2000). Previous studies report no significant differences between total anthocyanin quantity in fresh and frozen (-20°C) raspberries (de Ancos and others 2000). Also, an increase (7-23%) in the quantity of total anthocyanins in frozen raspberries at selected temperatures (-20 and -35°C) after 378 days of storage was observed compared to the total anthocyanins in raspberries immediately after freezing (Figure 1). Storage temperature exhibits no significant influence on the degradation of anthocyanins although the selected temperatures were greater than and less than the $T_g'$ of raspberry matrices. Rizzolo and others (2003) report no significant difference in total anthocyanin content of blueberry juices frozen at -10, -20 and -30°C after 6 months of storage. No influence of glass transition and storage temperatures on degradation of anthocyanins was observed during the frozen storage of blueberry juice with/without the addition of selected sugars (Rizzolo and others 2003). Torreggiani and others (1999) reported a significant loss of strawberry anthocyanins at -10°C during four months of storage, and no existence of a direct relationship between anthocyanin loss and $T_g'$ was observed. There is no evidence than the degradation of anthocyanins in frozen raspberries is diffusion-limited or dependent on molecular mobility.

The viscosity of the unfrozen raspberry matrices may be large enough to restrict mobility of anthocyanins during storage at the selected temperatures. Specifically, the selected low storage temperatures may result in small molecular relaxations and mobility, reducing anthocyanin degradation rates. Enzymatic degradation of anthocyanins in frozen raspberries may be inhibited by large viscosity and resultant slow diffusion rate of substrates in the frozen raspberry matrices. The highest selected temperature (-20$^{o}$C) may be used without significant degradation of anthocyanins during storage of frozen raspberries.

### Stability of raspberry anthocyanins during storage of dried raspberry powder

Raspberry powder equilibrated at selected water activities for 37 days were considered as the initial point of storage study. The initial glass transition temperatures ($T_{gi}$) and water content of freeze-dried raspberry powders stored at selected water activities are presented in Table 1 (Syamaladevi and others 2010). Anthocyanin degradation kinetics in the glassy state storage of dry raspberry powder did not follow a kinetic order due to increases and decreases in the quantity of anthocyanins observed during storage (Figure 2). The two-way ANOVA analysis indicated a significant interaction ($P < 0.05$) between water activity and time of storage on total anthocyanins. Statistical differences in total anthocyanins during storage in the glassy state are not shown in the Figure 2 as the effect of water activity on total anthocyanins depended on time of storage and *vice versa*. Hence, the changes in total anthocyanins are described for each water activity with storage time. There was a decrease in total anthocyanins in dried raspberry powder after 370 days storage (Figure 2). A decrease of 52, 51, 27 and 27% in total anthocyanin concentration was observed in dried raspberry powder stored at water activity value of 0.05 after 114, 177, 267, and 370 days respectively in comparison with the total anthocyanins in dried raspberry powder before storage (Figure 2). Similarly, a decrease of 37, 35, 32, and 30% in total anthocyanins was observed in powder stored at water activity value of 0.07 after 114, 177, 267, and 370 days, respectively (Figure 2). During equilibration (37 days), the quantity of anthocyanins in freeze-dried raspberries in the rubbery state increased for selected water activity values (0.11-0.75) and started to decrease after equilibration. The anthocyanin content in dry raspberry powder after water activity equilibration was considered as the initial value of anthocyanin concentration. Total anthocyanin degradation ranged between 78-89% at water activity values of 0.11-0.43 after 370 days of storage at 23°C (Table 2, 3 and Figure 3). Complete degradation of anthocyanins was observed in dry raspberry powder stored at higher water activity values (0.53-0.86) after 270 days of storage at 23°C (Table 2 and 3).

Gradinaru and others (2003) observed degradation of encapsulated and free anthocyanins in Hibiscus attributed to the occurrence of reactant mobility in the glassy state. Bell (1996) observed slower rates of brown pigment formation in glassy polyvinylpyrrolidone. Sablani and others (2007) reported significant difference in the rates of vitamin *C* degradation in the glassy and rubbery states of fortified formula. Glassy amorphous systems exhibit adequate molecular mobility to allow diffusion limited reactions (Bell 1996; Roozen and others 1991). Erlandson and Wrolstad (1972) reported anthocyanin degradation in freeze-dried strawberry powder in the rubbery state during storage at selected relative humidities at 37ºC. Gradinaru and others (2003) observed an increase in the rate of degradation of Hibiscus anthocyanins during storage with an increase in selected water activities. The limited diffusion of molecules in the highly viscous glassy raspberry matrices during dried storage may result in the slower rates of anthocyanin degradation. The physical state of the food system is more influential than available water in the glassy state since no significant differences ($P \geq 0.05$) were observed between the quantities of anthocyanins found in freeze-dried raspberry powder stored at the two selected water activity values of 0.05 and 0.07 (Figure. 2) after 370 days. The slower rates of anthocyanin degradation in the glassy raspberry powder indicate the importance of glassy state in the storage of freeze-dried raspberry powder. Water acts as a solvent or a reactant in many chemical reactions in foods. Water may act as a reactant rather than a solvent at lower water activity values (0.05-0.43), enhancing anthocyanin degradation. At higher water activity values, diffusion limited reactions are enhanced which is attributed to the combined effect of enzymatic activity, oxidation, and molecular mobility as a result of more available water.

The first order and Weibull equations were used to fit anthocyanin degradation kinetics in freeze-dried raspberry powder equilibrated at $a_w$ ranging between 0.11-0.75 (Figure 4 and 5). A number of studies report first order kinetics for anthocyanin degradation in selected fruits and vegetables (Baublis and others 1994; Guisti and Wrolstad 1996b; Garson and Wrolstad 2001, 2002). The reaction rates of anthocyanin degradation at the selected water activity values (0.11-0.75) were calculated assuming first order kinetics (Figure 4). Greater retention of anthocyanins was observed at water activity values of 0.11 and 0.23 for the selected storage periods. The first order equation parameters for freeze-dried raspberry powder in the current study are listed in Table 4. The reaction rate constant (*k*) values of anthocyanin degradation increased as water activity values of freeze-dried raspberry powder increased. The *k* values of anthocyanin degradation in freeze-dried raspberry powder at selected water activity values are comparable to the *k* values of freeze-dried strawberries of similar water activity values (Garzon and Wrolstad 2001). However, limited number of anthocyanin measurements may influence the accuracy of *k* values of anthocyanin degradation and prediction capability of first-order kinetic equation. The half life ($t_{1/2}$) of anthocyanin degradation in dried raspberry powder was determined using a first order kinetics equation and calculated *k* values at selected water activities during storage (Table 4). The small differences in $t_{1/2}$ of anthocyanin degradation in freeze-dried raspberry and strawberry powders may be attributed to the difference in the stability of specific anthocyanins present and the nature of the anthocyanin determination methods used (Garzon and Wrolstad 2001). They found that the kinetics of anthocyanin degradation were not linear in freeze-dried strawberry powders.

The Weibull equation better fit the anthocyanin degradation data in freeze-dried raspberries at selected water activities than did a first order equation with larger $R^2$ (0.93 compared to 0.91) values (Figure 5). Odriozola-Serrano and others (2009) reported the Weibull equation was effective in predicting the degradation of anthocyanins and antioxidants during the storage of fresh cut strawberries. Oms-Oliu and others (2009) reported that degradation kinetics of vitamin *C* and antioxidant capacity of fresh cut water melon fruits fit well with the Weibull equation. Weibull equation parameters for freeze-dried raspberry powders determined by nonlinear optimization using Statistica software are presented in Table 4. The scale factor, $\alpha$, determined for raspberry anthocyanin degradation ranged between 72 to 311 days. The rate constants of raspberry anthocyanin degradation (the inverse of the scale factor) obtained using the Weibull equation were smaller than the rate constants obtained from the first order equation (Table 4). A value of $\gamma$ less than 1 represents concavity of the kinetic data curve and loss of anthocyanins during the initial stages of storage (Odriozola-Serrano and others 2009). Convexity ($\gamma$ greater than 1) of the anthocyanin degradation curve represents greater degradation rates during storage (Tiwari and others 2009). Larger $\gamma$ values (19.6 and 21.8) were observed for freeze-dried raspberry powders stored at water activity values of 0.66 and 0.75 (Table 4). However, limited number of anthocyanin concentration measurements during 378 days of storage may influence $\alpha$ and $\gamma$ values and prediction performance of anthocyanin degradation.

An alternative to the Arrhenius approach for expressing quality degradation kinetics is by relating the reaction rate to ($T$-$T_g$), where $T$ is the storage temperature (Sablani and others 2007). Although a 27-32% reduction in anthocyanin content was observed in the glassy state of freeze-dried raspberry powders, no clear trend of anthocyanin degradation was observed. Consequently, the anthocyanin degradation rate constants of freeze-dried raspberry powders were estimated only in the rubbery state ($a_w$ ranging between 0.11 and 0.75) (Figure 6). The minimum rate constant observed was around ($T$-$T_g$) = 5°C and 15°C, close to the glass transition temperature. The anthocyanin degradation rate constants in the rubbery state of freeze-dried raspberries are similar even though a 10°C difference (when ($T$-$T_g$) = 5°C and 15°C) in experimental storage temperature ($T$) (Figure 6). However, the anthocyanin degradation rate constants in the rubbery state increased with a further increase of ($T$-$T_g$). The greatest rate of anthocyanin degradation was observed when ($T$-$T_g$) ranged between 35-53°C (Figure 6). A smaller rate constant was observed when ($T$-$T_g$) = 80°C. This may be attributed to the presence of water acting as a solvent hindering the degradation reactions and reducing the rates of degradation reactions (Bell 1996). ($T$-$T_g$) is a useful approach relating storage temperature and stability of anthocyanins in freeze-dried raspberry powders. Glassy state storage of freeze-dried raspberry powders is recommended to avoid increased rates of anthocyanin degradation. Sablani and others (2007) reported significant reductions in vitamin *C* degradation rates with decreasing ($T$-$T_g$) and water activity. Bell (1996) observed the rate of brown pigment formation in polyvinylpyrrolidone decreased seven times when the system changed from rubbery to the glassy state (i.e. ($T$-$T_g$) > 0°C). Paterson and others (2005) reported that stickiness of amorphous lactose increased considerably at large ($T$-$T_g$). The cake strength of skim milk powder increased considerably for higher positive values of ($T$-$T_g$), such as 20°C (Fitzpatrick and others 2007).

Similarly, increasing water activity ($a_w$) in freeze-dried raspberry powders increased the anthocyanin degradation rate except for $a_w$ = 0.11 and 0.23. A maximum anthocyanin degradation rate was observed between $a_w$ = 0.53 and 0.66 (Figure 6). The slower rates of anthocyanin degradation at low water activity values may be attributed to the presence of monolayer moisture and limited mobility of reactants, while the slower reaction rates at high water activity ($a_w$ = 0.75) may be attributed to the dilution of reactants in freeze-dried raspberry powders (Bell 1996).

## Conclusions

Storage temperatures of -20°C and -35°C may be used for long-term storage of frozen raspberries, providing better retention of anthocyanins over extended periods. A significant reduction (27-32%) in the quantity of anthocyanins was observed in the glassy state of freeze-dried raspberry powders during 270 days storage, indicating that degradation reactions continue to occur in the glassy state of amorphous raspberry powder. Approximately 79-100% degradation in the quantity in anthocyanins was observed in the rubbery state of freeze-dried raspberry powders stored at 23°C for more than one year. The Weibull equation yielded a better fit to anthocyanin degradation kinetics than the first order equation for freeze-dried raspberry powders in the rubbery state. An increase in both the molecular mobility and availability of water as a reactant enhanced anthocyanin degradation in the rubbery state of freeze-dried raspberry powder. A slower anthocyanin degradation rate for freeze-dried raspberry powder in the glassy state indicates that the glass transition concept is important for identifying suitable storage conditions of freeze-dried raspberry powder.

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# LEGENDS TO FIGURES

Figure 1. Retention of total anthocyanins in fresh and frozen (-20, -35 and -80°C) raspberries during long term storage.

Figure 2. Quantity of total anthocyanins in freeze-dried raspberry powders in the glassy state during long term storage.

Figure 3. Percentage retention of total anthocyanins in freeze-dried raspberry powders at selected water activity values (0.11-0.75) in rubbery state after 224 days of storage at 23°C

Figure 4. Anthocyanin degradation kinetics in the rubbery state of freeze-dried raspberry powders fit with first order equation

Figure 5. Anthocyanin degradation kinetics in the rubbery state of freeze-dried raspberry powders fit with Weibull equation

Figure 6. Variation of first order reaction constant ($k$) of anthocyanin degradation in freeze-dried raspberry powders with ($T$-$T_g$) and $a_w$

Table 1. Initial glass transition temperatures and water contents of freeze-dried raspberry stored at selected water activities (Modified from Syamaladevi and others 2010)

| Water activity, $a_w$ (fraction) | Water content (kg water/kg raspberry) | Initial glass transition temperature, $T_{gi}$ ($^{o}$C) | State of raspberries when stored at room temperature (23$^{o}$C) |
|---|---|---|---|
| 0.05 | 0.022±0.000 | 39.6±1 | Glassy state |
| 0.07 | 0.019±0.000 | 38.9±0 | Glassy state |
| 0.11 | 0.034±0.000 | 17.1±1 | Rubbery state |
| 0.23 | 0.046±0.001 | 7.3±1 | Rubbery state |
| 0.33 | 0.069±0.001 | -5.03±1 | Rubbery state |
| 0.43 | 0.086±0.001 | -12.0±5 | Rubbery state |
| 0.53 | 0.112±0.001 | -19.4±6 | Rubbery state |
| 0.66 | 0.134±0.003 | -29.7±6 | Rubbery state |
| 0.75 | 0.175±0.001 | -57.0±0 | Rubbery state |

Table 2. Anthocyanin concentrations in the rubbery state of freeze-dried raspberry powders at selected water activities during storage

| Storage time (days) | Anthocyanins concentration (mg/g raspberry solids) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.11 | 0.23 | 0.33 | 0.43 | 0.53 | 0.66 | 0.75 |
| 0 | 1.28±0.010 | 1.36±0.017 | 1.12±0.014 | 0.702±0.018 | 1.26±0.059 | 0.829±0.031 | 0.891±0.01 |
| 37 | 1.06±0.021 | 0.735±0.003 | 0.893±0.006 | 0.513±0.012 | 0.771±0.032 | 0.473±0.022 | 0.181±0.009 |
| 187 | 0.996±0.009 | 0.818±0.006 | 0.448±0.008 | 0.422±0.006 | 0.185±0.005 | 0.013±0.003 | 0.031±0.006 |
| 233 | 0.510±0.002 | 0.504±0.002 | 0.333±0.001 | 0.201±0.000 | 0 | 0 | 0 |
| 333 | 0.288±0.002 | 0.195±0.004 | 0.164±0.004 | 0.079±0.004 | 0 | 0 | 0 |

Table 3. Percentage retention of anthocyanins in the rubbery state of freeze-dried raspberry powders at selected water activities during storage

| Storage time (days) | Percentage retention of anthocyanins | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.11 | 0.23 | 0.33 | 0.43 | 0.53 | 0.66 | 0.75 |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 37 | 82.5±1.8 | 54.1±0.8 | 80.0±1.2 | 73.1±2.3 | 61.4±4.1 | 57.1±3.3 | 20.3±10.7 |
| 187 | 77.9±1.0 | 60.2±1.0 | 40.1±0.9 | 60.1±1.6 | 14.8±0.9 | 1.6±0.4 | 3.4±4.8 |
| 233 | 39.9±0.4 | 37.1±0.6 | 29.8±0.4 | 28.6±0.6 | 0 | 0 | 0 |
| 333 | 22.5±0.3 | 14.4±0.4 | 14.7±0.4 | 11.3±0.8 | 0 | 0 | 0 |

Table 4. First order reaction rate constants ($k$) and Weibull parameters ($\alpha$, $\gamma$) for anthocyanin degradation during the storage of dried raspberry powders at selected water activities

| | Water activity | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.11 | 0.23 | 0.33 | 0.43 | 0.53 | 0.66 | 0.75 |
| $k$ (days$^{-1}$) | 0.003 | 0.003 | 0.004 | 0.005 | 0.010 | 0.023 | 0.016 |
| $t_{1/2}$ (days) | 231 | 231 | 173 | 139 | 69 | 30 | 43 |
| $\alpha$ (days) | 311 | 267 | 237 | 258 | 139 | 76 | 72 |
| $\gamma$ | 2.64 | 0.969 | 1.48 | 1.52 | 1.59 | 19.6 | 21.8 |

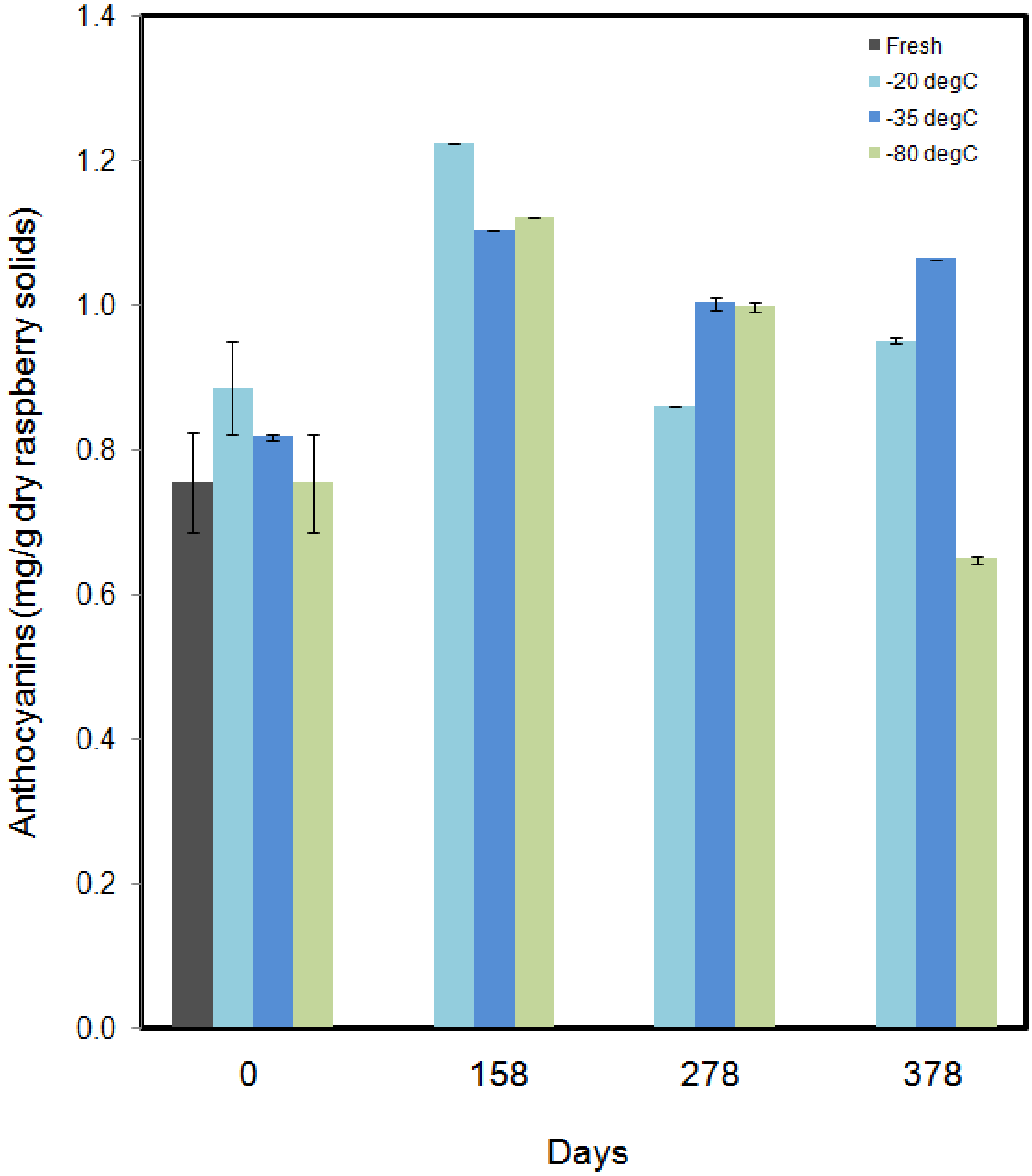

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Anthocyanins (mg/g dry raspberry solids)
Fresh
-20 degC
-35 degC
-80 degC
0
158
278
378
Days

Figure 1

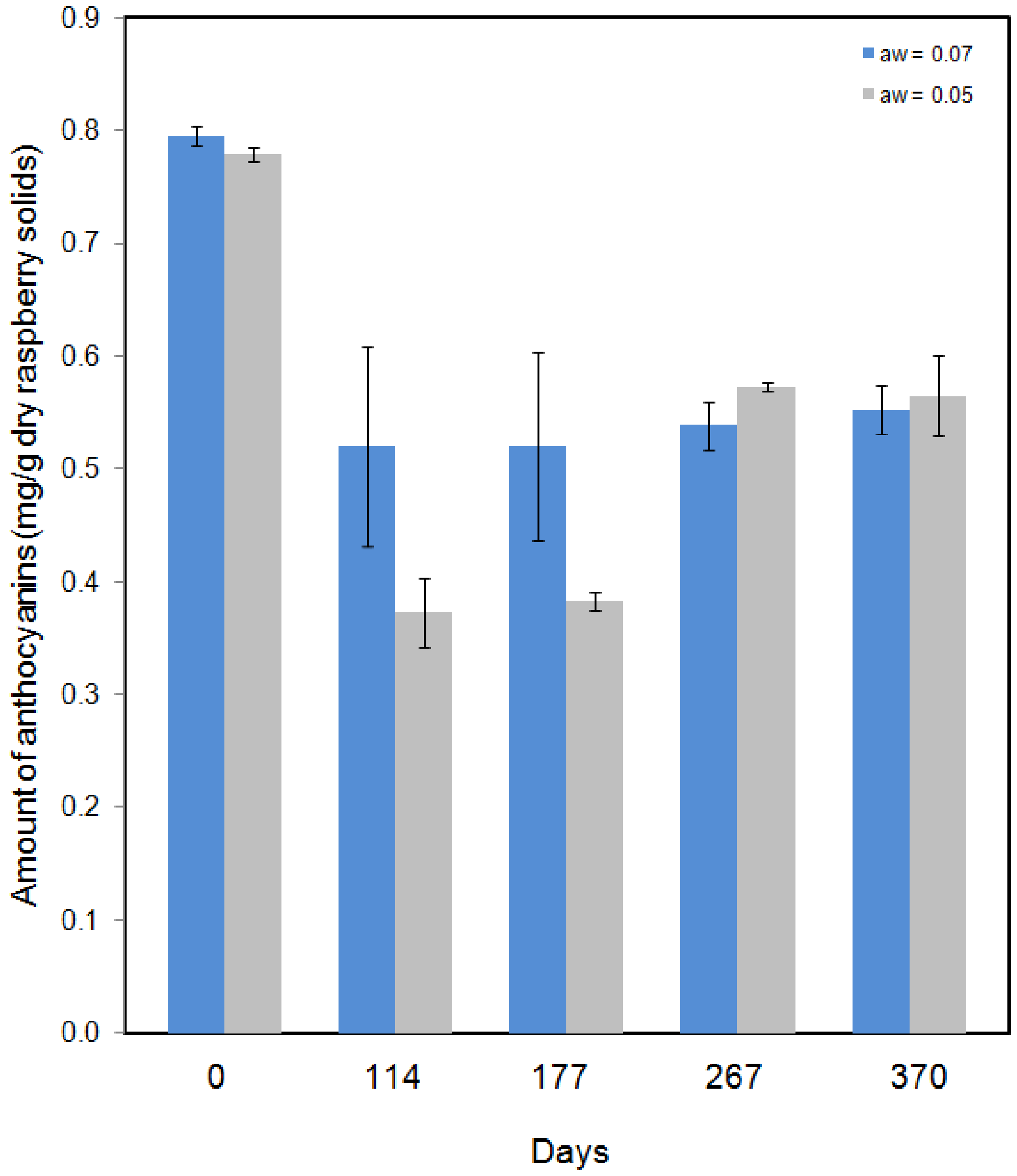

aw = 0.07
aw = 0.05
Amount of anthocyanins (mg/g dry raspberry solids)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
114
177
267
370
Days

Figure 2

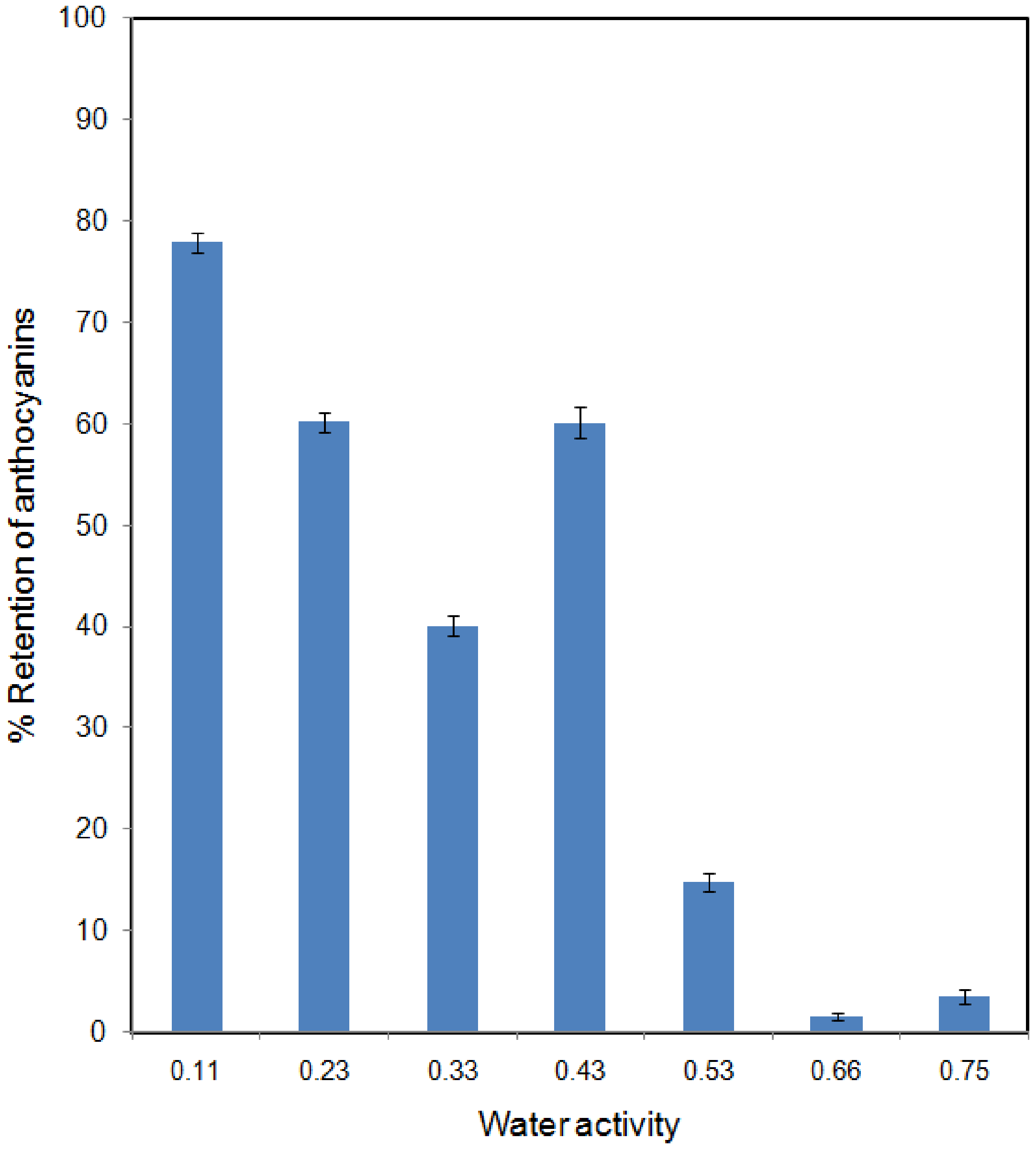
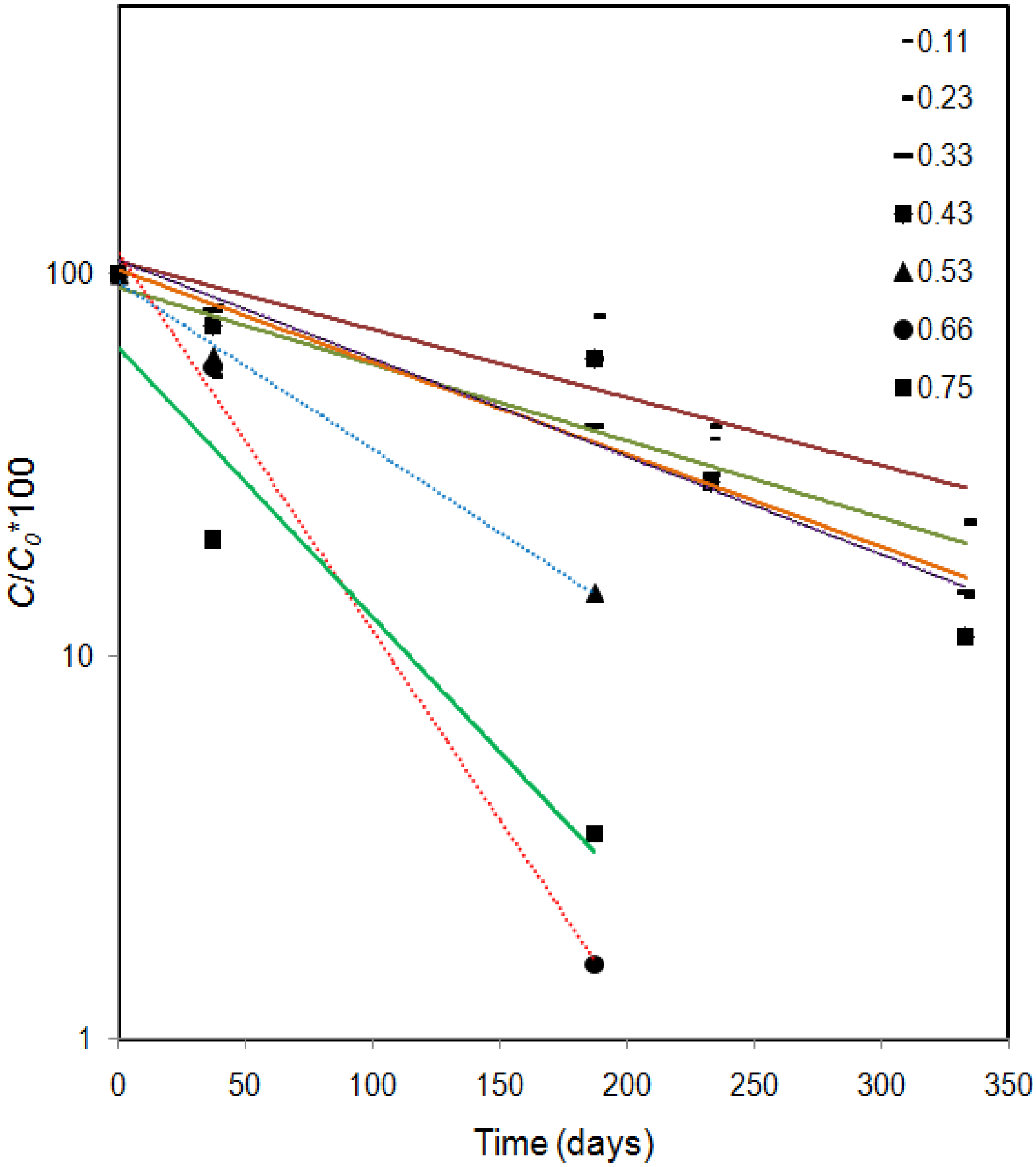

100
90
80
70
60
50
40
30
20
10
0
% Retention of anthocyanins
0.11
0.23
0.33
0.43
0.53
0.66
0.75
Water activity

Figure 3

613

614



615

616 Figure 4

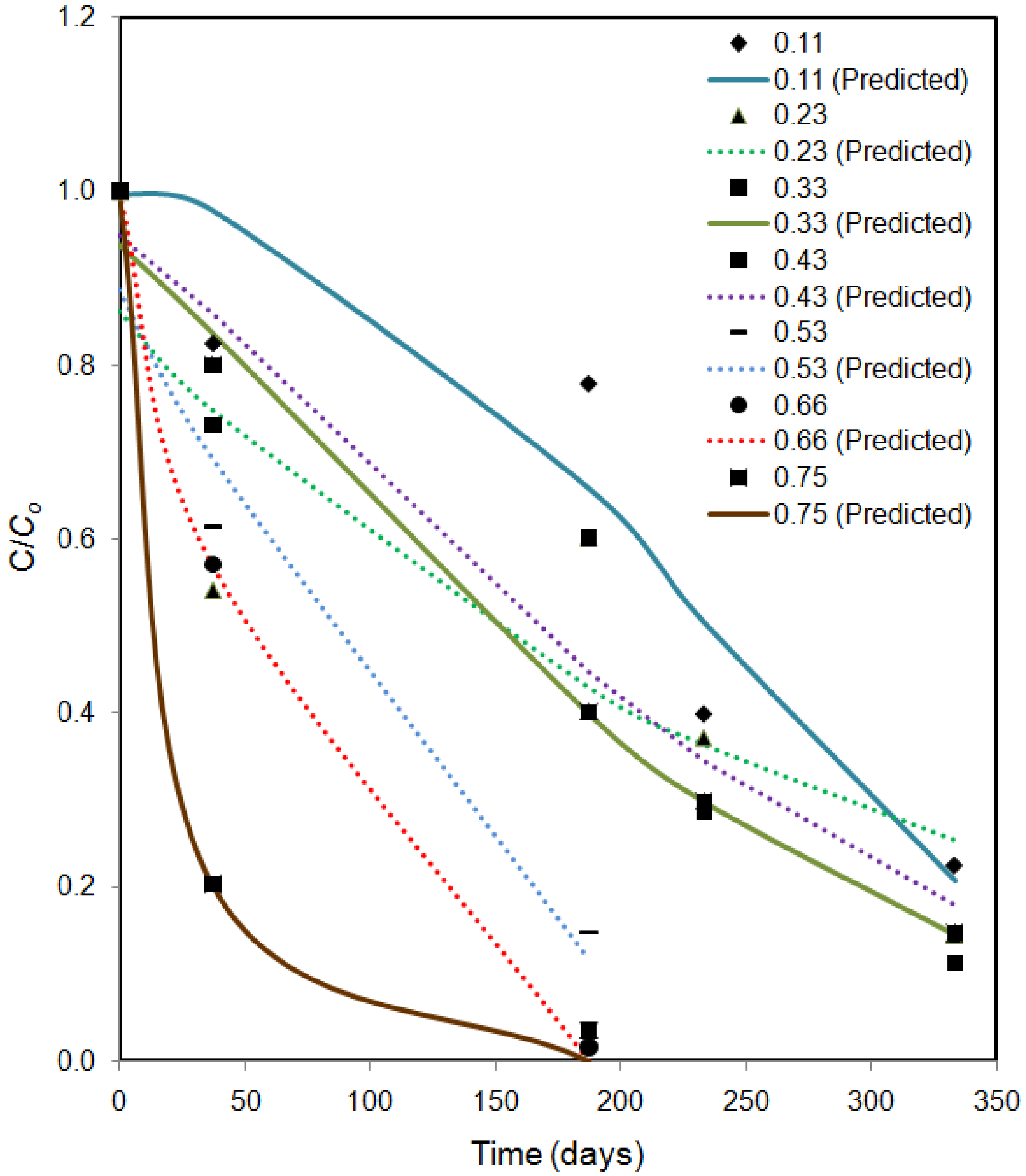
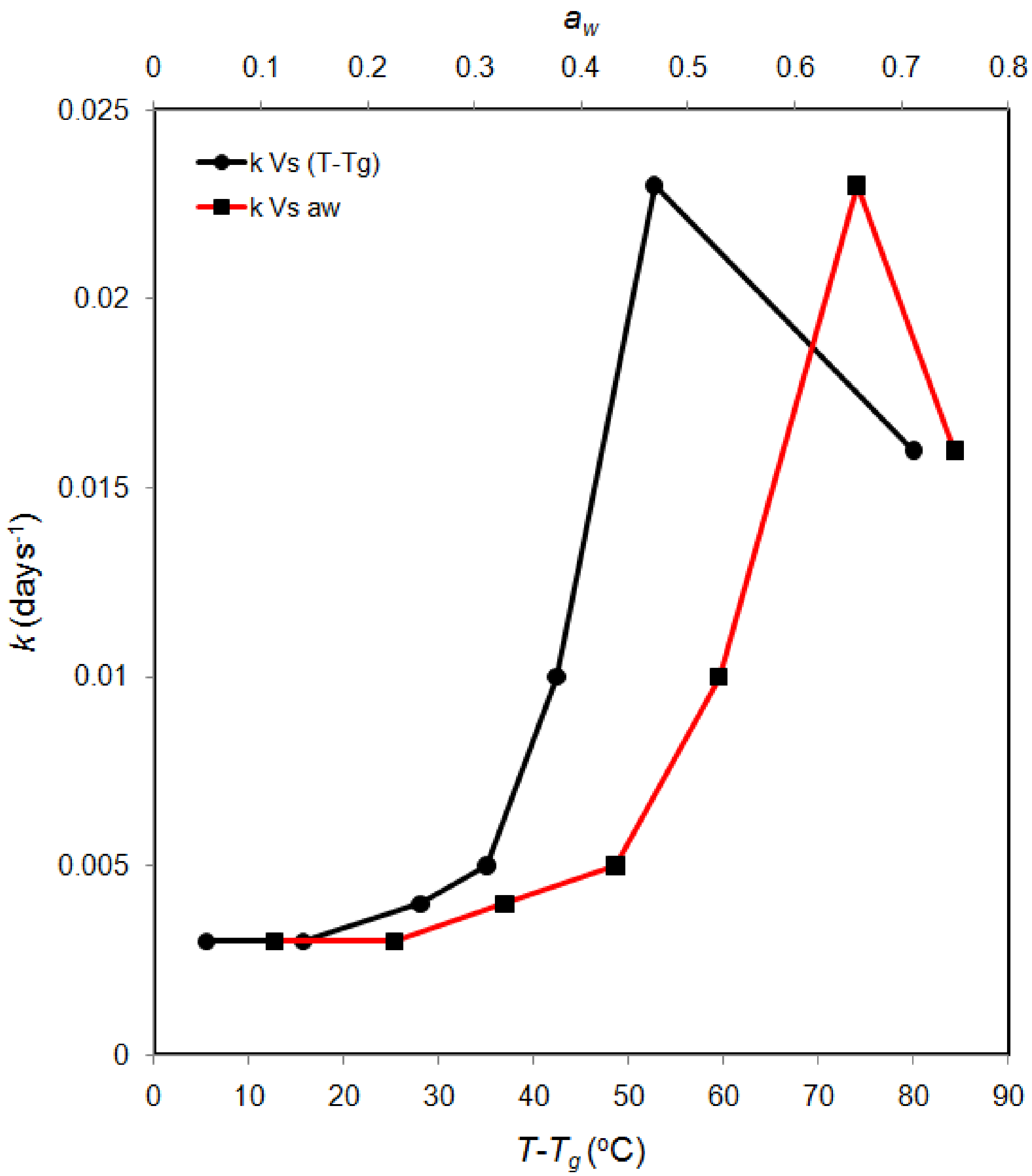

1.2
1.0
0.8
0.6
0.4
0.2
0.0
$C/C_o$
0
50
100
150
200
250
300
350
Time (days)
0.11
0.11 (Predicted)
0.23
0.23 (Predicted)
0.33
0.33 (Predicted)
0.43
0.43 (Predicted)
0.53
0.53 (Predicted)
0.66
0.66 (Predicted)
0.75
0.75 (Predicted)

Figure 5



$a_w$
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.025
0.02
0.015
0.01
0.005
0
k Vs (T-Tg)
k Vs aw
$k$ (days$^{-1}$)
0
10
20
30
40
50
60
70
80
90
$T$-$T_g$ (°C)

Figure 6