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Effect of Sugar on Anthocyanin Degradation and Water Mobility in a Roselle Anthocyanin Model System Using ^{17}O NMR

PI-JEN TSAI,* YU-YUAN HSIEH, AND TZOU-CHI HUANG

Department of Food Science, National Pingtung University of Science and Technology, 1 Hsueh Fu Road, Nei Pu Hsiang, 91207 Pingtung, Taiwan, Republic of China

The purpose of this study was to try to elucidate the relationship between anthocyanin degradation and water mobility using ^{17}O NMR. A model system containing anthocyanin from roselles with different sugar concentrations (20, 40, 60%) was used to compare the effect of sugar (sucrose and honey) on the kinetics of anthocyanin degradation and water mobility after heating. Data on the anthocyanin degradation index (DI), half-life of anthocyanin, and activation energy of anthocyanin degradation showed that sucrose was a good anthocyanin protector, especially at high concentration. However, honey led to a severe anthocyanin degradation after its concentration reached 40% or it was heated to temperatures >50 °C. Spin−spin relaxation rates (R_2) of water using ^{17}O NMR were further used to monitor the water mobility in two sugar systems to explain the differences in browning under the same concentration and water activity. R_2 in sucrose was significantly higher than that in honey after the concentration reached 40%. Apparently, increasing the ability to bind with water molecules favored the stability of anthocyanin in sucrose solution.

KEYWORDS: Anthocyanin degradation; water mobility; roselle; sucrose; honey

INTRODUCTION

Anthocyanins are responsible for the red color of roselles (*Hibiscus sabdariffa* L.) (*1*) and are quite unstable. The stability of anthocyanin is affected by storage temperature, sugar, pH value, ascorbic acid, water activity, etc. Among these, water availability is essential for anthocyanin degradation (*2*). The degradation index (DI) and water activity have commonly been used to assess anthocyanin stability.

Sugar has been reported to stabilize the red color of strawberry anthocyanin with 40% sucrose concentration or at temperatures below 55 °C due to reduced water activity, hyperchromic effect, enzymes inhibition, or steric interference (*3*, *4*). Similarly, honey has been reported to be able to inhibit the browning of apples (*5*) and raisins (*6*) through inactivation of polyphenol oxidase (PPO). However, in our previous work, roselles preserved with honey as an antibrowning agent browned badly after storage, whereas sucrose acted as a good color protector at the same level of water activity (A_w). More research is needed to understand the difference in antibrowning between sucrose and honey.

Because the effect of sugars on chemical changes in the food systems is related to their A_w suppression properties (*7*), water mobility and the percentage of bound water or free water could be measured through NMR to elucidate the mechanism of sugar−water interaction (*8*, *9*). According to these studies, increasing sucrose concentration was found to increase the gelatinization temperature of starch and binding between water and starch, and ^{17}O was suggested as the best monitor of water mobility in the sugar−water system. Sucrose was also reported to be more effective than glucose in restricting the water mobility in a starch−water−sugar system (*10*). The aim of this study was (1) to determine how sucrose or honey inhibits or accelerates the degradation of roselle anthocyanin and their related sugar−water interaction and (2) to give a plausible explanation for the stability of anthocyanins through water mobility detection by ^{17}O NMR.

MATERIALS AND METHODS

Preparation of Samples. Four grams of freeze-dried roselle was immersed in 200 mL of acidified ethanol (1.5 mol/L HCl), and the extract was collected and freeze-dried. The freeze-dried powder was then mixed with pH 3.2 citric acid−Na_2HPO_4 buffer (0.1 M citric acid and 0.2 M Na_2HPO_4) containing different concentrations of sugar until the concentration reached 2 mg/mL. After filtering through a 0.45 μm filter, the extract was ready to use as a model system and was heated at different levels of temperature (30−60 °C) for 96 h.

Anthocyanin Degradation Index. Samples for each treatment were applied to UV−vis spectroscopy, and the absorbances at 420 and 520 nm were collected. DI was calculated from A_{420nm}/A_{520nm} (*1*).

Activation Energy. Samples were heated at 30, 40, 50, or 60 °C, and their anthocyanin contents were calculated at intervals. By determining the change in anthocyanin content versus time, the rate of degradation was calculated. The activation energy was then calculated by $\mathrm{Ln}\ K = -E_a/RT$, where K is a rate constant, $R = 1.986$, and $T = (°C + 273)$.

Half-Life of Anthocyanin. Anthocyanin content was quantified by an HPLC system consisting of a Hitachi L-6000 pump, an RP C18 column (250 × 4.6 mm), and a photodiode array detector (Hitachi Ltd.) according to the method described by Tsai et al. (*1*). The time needed for anthocyanin degradation to 50% residual was recorded as the half-life of anthocyanin.

* Author to whom correspondence should be addressed (telephone +886-8-7740408; fax +886-8-7740378; e-mail pijen@mail.npust.edu.tw).

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Table 1. DI Values of Anthocyanin–Sugar–Water Model Systems at Various Concentrations after 96 h of Heat Treatment at Different Temperatures[a]

	DI at			
sugar[b] (%)	30 °C	40 °C	50 °C	60 °C
S20	0.87c	1.31c	2.63a	2.69d
S40	0.60e	0.78d	2.57b	2.83c
S60	0.55f	0.63e	2.48b	2.57e
H20	0.66d	0.77d	2.55c	2.83c
H40	1.13b	1.39b	2.58b	2.90b
H60	1.46a	1.53a	2.57b	2.96a

[a] Values in a column with different letters indicate significant difference ($p < 0.05$). [b] S20, 20% sucrose; S40, 40% sucrose; S60, 60% sucrose solution; H20, 20% honey; H40, 40% honey; H60, 60% honey solution.

Water Activity. The water activity of each sample was detected by a water activity measuring machine (Aqualab, model CX-2, Decagon Devices Inc., Pullman, WA) at 25 °C after balancing.

Water Mobility (*11*). Samples prepared as described under Preparation of Samples were applied to a NMR machine to collect the data of water mobility. A NMR analyzer (Bruker AMX400 FT-NMR) was used to acquire the free induction decay (FID) curves that indicate the rate of signal decay (T_2). Observations were at a frequency of 16 kHz. The $\pi/2$ pulse width for the ^{17}O nucleus was 10 μs. Spectra were accumulated 16 times. Water mobility expressed as R_2 was calculated from the reverse of T_2 ($R_2 = 1/T_2$). The higher R_2 is associated with a higher water mobility.

Statistical Analysis. Statistical analyses of the data were carried out using SAS (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Changes of anthocyanin DI, activation energy of anthocyanin degradation, A_w, and R_2 in the anthocyanin−sugar−water system were measured and compared for different sugar types, concentrations, and heating temperatures.

Effect of Sugar Type, Concentration, and Temperature on Anthocyanin Degradation. Comparative DI values are shown in **Table 1**. Results showed that anthocyanin degradation decreased when sucrose concentration increased or heating temperature was below 40 °C. Conversely, in the honey system, DI increased as honey concentration or heating temperature increased. Honey seemed to show its antibrowning function only when the concentration was under 20% or when the heating temperature was below 40 °C. Moreover, browning was much more rapid in honey than in sucrose samples when the temperature was >50 °C or when the sugar concentration was >40%. This can be further studied by the retention percentage and the half-life of anthocyanin.

There was <5% anthocyanin left after heating at >50 °C for 96 h. For the 40 and 60% sucrose samples, the anthocyanin residual was 60% after heating at 30 and 40 °C, whereas the residual was <25% in the 40 and 60% honey samples. Furthermore, increasing the concentration of sucrose increased the time needed to degrade the 50% anthocyanin. As shown in **Table 2**, the half-lives of anthocyanin in the 20, 40, and 60% sucrose systems were 110.21, 139.39, and 144.39, at 30 °C and 13.63, 19.87, and 23.4 h at 60 °C, respectively. In the honey systems, the half-life of anthocyanin decreased when honey concentration increased (131.03, 93.89, and 88.3 h for 20, 40, and 60% honey, respectively). This indicates that the mechanism of degradation of anthocyanin versus sugar concentration differed greatly between sucrose and honey solutions.

Effect of Sugar on the Activation Energy of Anthocyanin Degradation. Activation energy was further used to interpret the browning capacity of the anthocyanin−sugar−water system. As shown in **Figure 1**, the activation energy was 14.78, 17.32, and 18.21 kcal/mol for the 20, 40, and 60% sucrose systems, respectively, but only 9.27, 8.58, and 7.83 kcal/mol for the honey. It seems that honey favors browning due to a lower activation energy of anthocyanin degradation, and the higher the honey concentration, the lower the activation energy became. It was found that sucrose, especially in high concentration, was better than honey in inhibiting the degradation of roselle anthocyanins under this model system.

Table 2. Half-Life of Anthocyanin in Honey and Sucrose Model Systems at Different Temperature

	half-life (h) at			
sugar[a] (%)	30 °C	40 °C	50 °C	60 °C
S20	110.21	92.59	62.49	13.63
S40	139.39	118.66	68.56	19.87
S60	144.39	131.52	91.01	23.42
H20	131.03	75.07	26.05	11.61
H40	93.89	48.91	25.76	11.47
H60	88.32	24.83	13.13	10.34

[a] S20, 20% sucrose; S40, 40% sucrose; S60, 60% sucrose solution; H20, 20% honey; H40, 40% honey; H60, 60% honey solution.

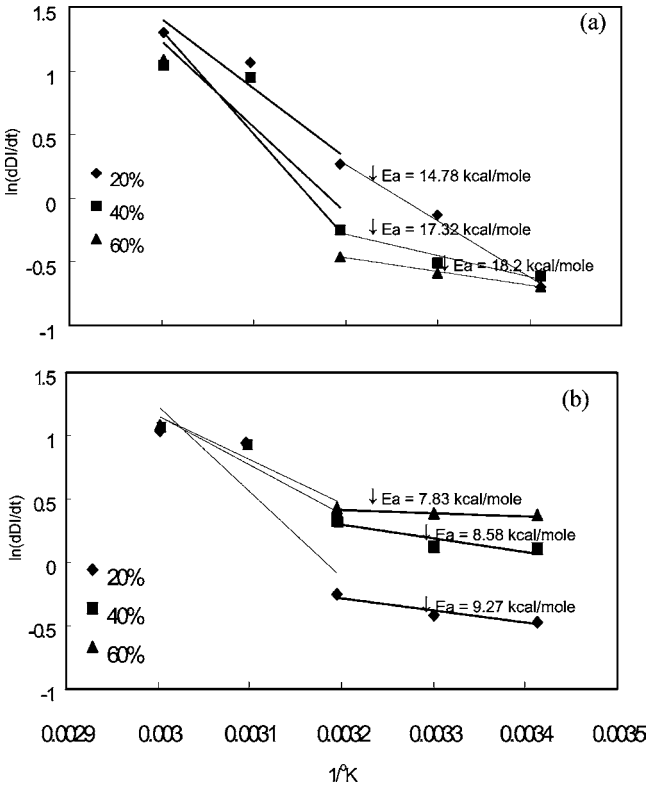

Figure 1. Correlation among reaction rate, activation energy of anthocyanin degradation, and concentration of sugar solution in (**a**) sucrose and (**b**) honey model systems.

Effect of Sugar on Water Mobility. Water availability is essential for anthocyanin degradation. In the report of Chinachoti and Stengle (*12*), water activity, water mobility, and percentage of bound or free water were used to monitor the effect of sucrose on the interaction of the starch water system by using ^{17}O NMR to detect the relaxation rate (R_2) (*12*). In this study, R_2 was determined at room temperature. As shown in **Table 3**, R_2 increased in both sucrose and honey solutions when the sugar concentration increased. BecauseSince sucrose has strong power to release the water that was originally tightly bound to starch (*12*), this might be the result of the increasing competition for water molecules among the sugar molecules as the concentration of sugar is increased. Furthermore, some of the sugar molecules

Table 3. Water Activity (A_w) and Spin–Spin Relaxation Rate (R_2) in Different Sugar–Anthocyanin–Water Model Systems at Various Concentrations

	A_w		R_2	
sugar (%)	sucrose	honey	sucrose	honey
20	0.93a	0.95a	625.39a	640.17a
30	0.92a	0.94a	643.22a	648.35a
40	0.91a	0.93a	666.67a	658.16a
50	0.90a	0.91a	947.45a	856.23b
60	0.84a	0.88a	1213.59a	1076.43b

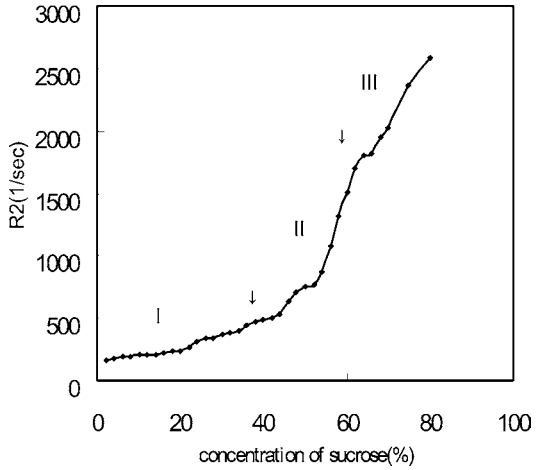

Figure 2. Relationship between sucrose concentrations and spin–spin relaxation rate (R_2) in sucrose solution using ^{17}O NMR: (I) sucrose concentration = 5–40%; (II) sucrose concentration = 40–60%; (III) sucrose concentration = 60–80%.

Table 4. Correlation Analysis among DI Values, Sugar Concentrations, Water Activity, Temperature, and Water Mobility in Honey Model System[a]

	DI	honey (%)	A_w	temp (°C)	R_2
DI	1.00	0.99**	0.95*	0.93*	0.84*
honey (%)		1.00	–0.95	0.08	0.83**
A_w			1.00	0.36	–0.90
temp (°C)				1.00	0.53
R_2					1.00

[a] *,**, significant at 5 and 1% levels, respectively.

might begin to bond with each other and form sucrose clusters (*10*). Thus, water available to participate in a chemical reaction such as browning decreased when sugar concentration increased.

This can be further explained by changes in bound water percentage. **Figure 2** shows the curve of R_2 versus sucrose concentration at three levels from 5 to 80%. The percentage of bound water, calculated from R_2, increased from 24 to 54% when the sucrose concentration increased from 20 to 60%. Obviously, increasing the sucrose concentration may decrease the availability of water to participate in the anthocyanin degradation and favors the stability of anthocyanin in the system.

Correlation among Color, Sugar Type, Concentration, Temperature, A_w, and Water Mobility. Further analysis was conducted to elucidate the relationship among all of the parameters mentioned above. Results are shown in **Tables 4** and **5**. In the sucrose−water system, anthocyanin degradation is significantly positively related to A_w and temperature but negatively related to the sucrose concentration and R_2 value. This confirmed that sucrose affects the mobility of water in the anthocyanin−sugar−water system and affects the potential for anthocyanin degradation. However, in the honey−water system, anthocyanin degradation was significantly positively related to honey concentration and relaxation rate, which suggested that increasing the concentration of honey could lead to heavier browning despite the higher R_2 in the samples. That is, the relaxation behavior could not entirely explain what happened in the honey system. Perhaps, the tendency of the reducing sugar from the honey (*13*) to favor browning strongly dominate the effect of sugar in restricting water mobility.

Table 5. Correlation Analysis among DI Values, Sugar Concentrations, Water Activity, Temperature, and Water Mobility for Sucrose Model System[a]

	DI	sucrose (%)	A_w	temp (°C)	R_2
DI	1.00	–0.97**	0.93*	0.96*	–0.92*
sucrose (%)		1.00	–0.92**	0.13	0.99**
A_w			1.00	–0.17	–0.96**
temp (°C)				1.00	0.57
R_2					1.00

[a] *,**, significant at 5 and 1% levels, respectively.

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