

## Comment on Degradation of Ascorbic Acid in Ethanolic Solutions

A recent publication by Hsu et al.<sup>1</sup> in *Journal of Agricultural and Food Chemistry* examined the degradation of ascorbic acid in ethanolic solutions stored at various temperatures to understand its stability in alcoholic beverages such as wine. The authors found that the degradation of ascorbic acid in an ethanolic solution follows temperature-dependent, first-order kinetics. A trend of steadily increasing activation energy is observed with increasing ethanol concentration. Departure from this trend occurs in solutions with 40% (v/v) ethanol concentration, which show a 2-fold decrease in activation energy from 30% (v/v) ethanol concentrations as well as significant increase in relative rate of ascorbic acid degradation at all temperatures from all other ethanol concentrations. This departure was postulated to have occurred because of a lower water activity environment observed in solutions with higher ethanol concentrations. The decreased water activity in certain ethanol concentrations has been attributed to an increase in the strength of hydrogen bonding between ethanol and water as well as ethanol–ethanol hydrogen bonding.<sup>2</sup> Degradation rates of ascorbic acid for all ethanol concentrations increased with increasing temperature with complete degradation occurring much more rapidly at the highest temperature, providing a clear indication of temperature-dependent kinetics.

This thermodynamic and kinetic approach to the study of molecular stability in various ethanol concentrations has been previously used for other molecules in ethanol solutions.<sup>3,4</sup> In this paper we show our analysis of the kinetics of degradation of ascorbic acid in ethanolic solutions reported by Hsu et al.<sup>1</sup> to provide a thermodynamic explanation for the decrease in activation energy observed in 40% (v/v) ethanolic solutions based on transition-state theory.

The standard Gibbs free energy of activation ( $\Delta G_a^\circ$ ) at absolute temperature  $T$  (K) was calculated using the Eyring equation (eq 1). A conversion of the rate per day from Table 1 of the paper to the standard units of rate constant per second ( $k$ ) was necessary prior to use of the Eyring equation

$$\Delta G_a^\circ = -RT \ln \frac{kh}{k_B T} \quad (1)$$

where  $R$ ,  $h$ , and  $k_B$  are constant values and correspond to the ideal gas constant (8.3145 J/(K mol)), Planck's constant ( $6.6261 \times 10^{-34}$  J s), and the Boltzmann constant ( $1.3807 \times 10^{-23}$  J/K), respectively.<sup>5</sup> The resulting values of  $\Delta G_a^\circ$  for degradation of ascorbic acid for all ethanol concentrations are shown as a function of temperature in Figure 1A. A similar pattern in  $\Delta G_a^\circ$  is evident for increasing temperature in all ethanol concentrations. But when comparing 30 and 40% (v/v) concentrations of ethanol, a small change in  $\Delta G_a^\circ$  (for example, from 106.5 kJ/mol at 30% (v/v) to 104.3 kJ/mol at 40% (v/v) at 35 °C) is observed. A significant decrease in activation energy (from 94 kJ/mol at 30% (v/v) to 43.3 kJ/mol at 40% (v/v)) was shown by Hsu et al.<sup>1</sup> However, a thermodynamic reasoning for the small change in  $\Delta G_a^\circ$  was not discussed.

To explain this phenomenon using thermodynamics, a linear regression of  $\Delta G_a^\circ$  versus temperature was constructed to

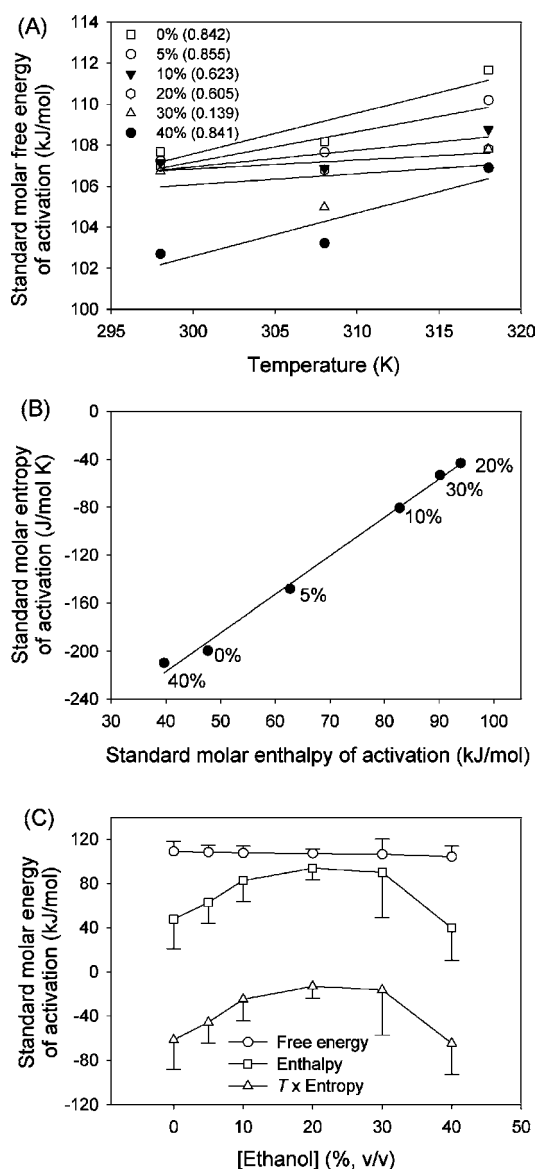
determine molar entropy ( $\Delta S_a^\circ$ ) and enthalpy ( $\Delta H_a^\circ$ ) of activation of ascorbic acid degradation (Figure 1A) using eq 2. In the regression, the slope corresponds to  $-\Delta S_a^\circ$ , whereas the  $y$ -intercept corresponds to  $\Delta H_a^\circ$ :

$$\Delta G_a^\circ = \Delta H_a^\circ - T\Delta S_a^\circ \quad (2)$$

The resulting values of  $\Delta H_a^\circ$  and  $\Delta S_a^\circ$  are shown (Figure 1B). For all ethanolic solutions,  $\Delta S_a^\circ$  is negative, whereas  $\Delta H_a^\circ$  values are all positive. For all ethanolic solutions except 30 and 40% (v/v) ethanol,  $\Delta H_a^\circ$  and  $\Delta S_a^\circ$  become larger with increasing ethanol concentration (Figure 1C). This means that increasing concentrations of ethanol up to 20% (v/v) lower the relative energy of ascorbic acid to the transition state in the solution, and at a higher concentration of ethanol the trend is reversed. The relative contributions of enthalpy and entropy to the degradation kinetics in different ethanol concentrations are shown in Figure 1C. Interestingly, we recognized that the value of  $\Delta H_a^\circ$  strongly correlates with that of  $\Delta S_a^\circ$  throughout the ethanolic solutions as the  $R^2$  value of the linear fitting in Figure 1B has a large value (0.9945). The correlation between enthalpy and entropy changes is possibly due to a phenomenon called enthalpy–entropy compensation, whereby an increase in enthalpy or entropy accompanies a corresponding increase in the other variable, consequently limiting the variation of Gibbs free energy change.<sup>3,6–12</sup> Stronger hydrogen bondings between water and ethanol in 40% (v/v) ethanolic solution<sup>2</sup> may result in the decrease in observed enthalpy of the system. Because  $\Delta G_a^\circ$  remains relatively constant with a relatively large change in  $\Delta H_a^\circ$  at 40% (v/v) ethanol concentration (Figure 1C), we can conclude that a corresponding change in  $\Delta S_a^\circ$  is occurring as a result of enthalpy–entropy compensation. Results of our analysis are summarized in Figure 1C, where the three molar energies of activation ( $\Delta G_a^\circ$ ,  $\Delta H_a^\circ$ , and  $T \times \Delta S_a^\circ$ ) at 35 °C are plotted as a function of ethanol concentration (v/v), and the very highly correlated entropy–enthalpy compensation can be seen in Figure 1B. Hsu et al.<sup>1</sup> were the first to examine the kinetics of degradation of ascorbic acid in ethanolic solutions, and further investigations of this type would prove useful for understanding the behavior of ascorbic acid degradation in ethanolic solutions as well as the examination of deaerated ethanol solutions in the absence of oxygen. Performing a similar experiment at more temperatures between 0 and 45 °C with more frequent data collection would likely improve some of the poor correlations ( $R^2$ ) of the linear regressions in Figure 1A, possibly by including the contribution of heat capacity in the degradation of ascorbic acid. Although our analysis provides thermodynamic characteristics of ascorbic acid degradation in ethanolic solutions, a mechanistic understanding of the behavior requires more detailed studies with quantum and molecular mechanical calculations (for example, see refs 13 and 14).

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**Figure 1.** (A) Temperature-dependent standard molar free energy of activation for degradation of ascorbic acid in ethanolic solutions. Standard molar enthalpy and entropy of activation were obtained by linear fitting.  $R^2$  of the fitting is shown in the figure legend. (B) Relationship between enthalpy and entropy of activation at different ethanol concentrations (v/v) ( $R^2 = 0.9945$ ). (C) Thermodynamic energy values of ascorbic acid degradation at 35 °C. For standard molar enthalpy and  $T \times$  entropy of activation, the error bars represent standard error. For standard molar free energy of activation, the error bars represent the 95% confidence level. Error bars are shown in only one direction for clarity of the graph. SigmaPlot (version 11, Systat Software Inc., San Jose, CA, USA) was used for graph preparation and statistical analysis.

In conclusion, our thermodynamic analysis indicates the importance of both  $\Delta H_a^\circ$  and  $\Delta S_a^\circ$  in the degradation of ascorbic acid in ethanolic solutions. Even though there is significant variation of  $\Delta H_a^\circ$  and  $\Delta S_a^\circ$  between ethanolic concentrations,  $\Delta G_a^\circ$  remains relatively constant due to enthalpy–entropy compensation. Further investigation of this system could provide useful information about the environment observed in ethanolic solutions derived from the fermentation of fruit juices, such as wine, and provide techniques for better preservation. Recently, several other bioactive compounds were

investigated for their stability in ethanolic solutions.<sup>15,16</sup> It will be of great interest to examine those compounds to determine if enthalpy–entropy compensation is applicable as observed in this paper.

During this Journal's reviewing process, several concerns with the Hsu et al. data were brought to our attention by a reviewer. In Figure 2 of the Hsu et al. paper, the correlation for the fit of the first-order kinetics plot of ascorbic acid degradation is relatively poor with some concentrations, such as 40% (v/v) ethanol at 25 °C. The increasing rate of ascorbic acid degradation with increasing ethanol concentration and temperature creates an additional problem. Because the data points were collected every two days, this means that in some cases only one data point for ascorbic acid concentration was collected following the initial concentration measurement. For example, in the case of ascorbic acid degradation in 40% (v/v) ethanol at 35 °C, the additional data collected is at near-zero concentration or possibly when ascorbic acid is entirely degraded. The method of ascorbic acid concentration quantification is not mentioned, which provides uncertainty with regard to the validity of the ascorbic acid concentrations measured at near-zero concentrations. The uncertainty of the actual ascorbic concentrations, due to no mention of the quantification method, and the poorly correlated first-order kinetic plots in turn make some of the very low error values of the rate constants shown in Table 1 of the Hsu et al. paper questionable (i.e.,  $1.583 \pm 0.001 \text{ day}^{-1}$  for 40% (v/v) ethanol at 45 °C). In summary, this could mean that the 30 and 40% (v/v) ethanol concentration anomalies may be due only to poor data collection and not the phenomena we described.

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### Notes

The authors declare no competing financial interest.

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