browning. In addition, the double bond may also be attacked by intra-molecular hydroxymethyl group at C-8 of the catalpol molecule, or MeOH during isolation manipulation and the epoxy structure of catalpol can therefore, open up in acidic conditions. All these modifications will lead to the formation of various catalpol derivatives, which may include rehmaglutin B and D and jioglutin A, B, and C. Unfortunately, all these elucidated components are colorless or off-white,[28,29] and cannot explain the discoloration of the reaction solutions observed in this experiment.

When the assumed released amount of glucose was heated [Table 1], there was no browning, which means that the browning is probably a result of changes in catalpol aglycone. This result is in line with the finding that iridoid glycosides are characterized by the instability of their aglucones in the presence of acid, which leads to the production of compounds of various colors, and finally to the formation of black polymeric substances.[8] The browning indicated that new colored compounds were generated, which should, at least in part, be responsible for the darkening and antioxidation of steamed *Rehmannia* root.[2]

In addition, we found that lower concentration of catalpol led to a higher degradation rate [Figure 2], which suggested that the early elementary reaction of catalpol degradation was reversible, and high dilution of catalpol does not favor the reverse reaction according to the collision theory.

During the processing of *Rehmannia* root, catalpol was found to be entirely decomposed after 12 h of steaming.[2] By contrast, catalpol was degraded at a much slower rate in our simplified system because catalpol only reduced by 66.7% when heated for 12 h at pH 4.0. This result indicates that coexistent substances can facilitate the degradation of catalpol. Oligosaccharides including, stachyose, raffinose, and sucrose, the main components of *Rehmannia* root, decomposed during the processing of the root.[2] Our data indicate that they have no influence on catalpol degradation; however, most tested amino acids could promote the degradation of catalpol, except for proline, which indicated that free amino groups are involved in the reaction. This indicates that the reactions between amino acids and catalpol contribute to the browning and antioxidation much more than degradation of catalpol alone. Furthermore, the extent of the effect of different amino acids is quite different; for example, threonine, tryptophan and asparagine resulted in relatively higher A_{420} and lower SA_{DPPH} values, while phenylalanine, methionine and leucine resulted in lower values. In addition, the effects of amino acids can also be significantly affected by their concentration and the acidity of the reaction system as shown in Figure 4.

At the same acidity and temperature, adding glycine can greatly lower the *Ea* value, which indicates that the reaction mechanism is different from that for catalpol alone. Under acidic conditions (pH 4.0), the amino group of glycine is protonized, and nucleophilic attack may not explain the reaction mechanism. As proved by Namiki and Hayashi[30] and Hayashi *et al*.,[31] free radicals are involved in the reactions of sugars with amino acids or amines; therefore, we believe that free radicals might have been involved in the reaction between catalpol and amino acids, leading to the substantial decrease in its activation energy.

Amino acids may react with the aldehyde group of 1.5-cyclopentandialdehyde or glucose and the epoxy group of catalpol. The reaction between an amino acid and a reducing sugar is the well-known non-enzymatic browning reaction, Maillard reaction, which is of vital importance in the food industry for the production of specific colors, aromas, and flavors.[32-34] In this experiment, strong browning was found in systems to which amino acids were added. To determine whether the browning resulted from the reaction of amino acids with catalpol aglycone or glucose, a corresponding amount of glucose generated in each reaction system was allowed to react with the amino acid. The A_{420} value of the resulting solutions varied from 0.006 to 0.010. This result confirms that Maillard reaction of sugar with amino acids is very slow under water-rich conditions,[32,33] and thus, the browning is mainly due to the reaction between catalpol aglycone and amino acids.

The phenomenon of enthalpy-entropy compensation has been widely documented for temperature-dependent data to determine kinetic parameters.[35-37] According to the linear relationship between *Ea* and logarithm of the pre-exponential factor, the same reaction mechanism was assumed.[35] Our data on catalpol degradation also fit the relationship, with and without the presence of glycine. However, the reaction mechanism could not be the same as that discussed above. For this reason, we think that enthalpy–entropy compensation is merely a statistical artifact.[36-37]

Traditionally, the end point of *Rehmannia* root processing is "black as pitch" and "sweet as maltose." According to our data in this investigation, the strong browning reaction between catalpol and amino acids should be mainly responsible for the blackening of the root. In view of the potent antioxidation of the resulting reaction solution, the reaction of catalpol or other iridoids with amino compounds may be a new source of antioxidants, and catalpol degradation should therefore, be encouraged during the traditional drying

and processing of *Rehmannia* root. Due to the different effects of different amino acids, specific amino acids can be added to boost the amount of antioxidants present in *Rehmannia* root. In ancient times, rice wine was brewed in the open, with lactic acid, amino acids, and/or proteins as its intrinsic components. Steaming the root with rice wine may therefore, accelerate the degradation of catalpol and enhance the rate of antioxidation of the produce. In this sense, the traditional technique for processing *Rehmannia* root seems apt. However, additional studies are needed to isolate and elucidate these newly generated antioxidants, clarify the reaction mechanism of catalpol degradation, and determine other biological and pharmacological activities of catalpol degradation products.

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