

# Characteristics and kinetics of catalpol degradation and the effect of its degradation products on free radical scavenging

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## ABSTRACT

**Background:** The dried and steamed roots of *Rehmannia glutinosa* have different pharmacological functions and indications. Catalpol, the main active component of the dried root, was found to be entirely degraded together with amino acids and some oligosaccharides during preparation of the steamed root. Its degradation may contribute to the differences between dried and steamed roots. **Objective:** To reveal the characteristics and kinetics of catalpol degradation, and evaluate its influence on the antioxidant properties of steamed *Rehmannia* roots. **Materials and Methods:** Purified catalpol was heated under different pH and temperature values for different times, alone or with sugars or amino acids. Catalpol concentration was determined by high-performance liquid chromatography. Browning was expressed by the absorbance at 420 nm ( $A_{420}$ ), and antioxidation was displayed by 2,2-diphenyl-1-picrylhydrazyl free radical scavenging ability ( $SA_{DPPH}$ ). Activation energy was calculated using Arrhenius plotting. **Results:** Catalpol was stable in neutral conditions and sensitive to acidic pH under high temperatures. Sugars had no influence on catalpol degradation; however, most amino acids, except for proline, could promote the degradation, and were associated with an increase in  $A_{420}$  and  $SA_{DPPH}$  values. These changes were proved to be mainly related with catalpol aglycone and were dependent on the presence of amino acids. Catalpol degradation was found to obey first-order kinetics. The activation energies were 81.7, 88.8 and 98.7 kJ/mol at pH 4.0, 5.0, and 6.0 respectively, and 70.7 kJ/mol at pH 4.0 value and in the presence of glycine. **Conclusions:** Catalpol degradation, especially, in the presence of amino acids can substantially boost antioxidant properties of the products; therefore, the traditional method for processing *Rehmannia* root seems rather apt.

**Key words:** Antioxidation, catalpol degradation, kinetics, *Rehmannia*

## INTRODUCTION

The root of *Rehmannia glutinosa* Libosch. (*Plantaginaceae*) has been traditionally used as medicine in Asian countries such as China, Korea, and Japan. It is commonly used in the dried and steamed forms. The dried *Rehmannia* root, which has cooling properties, is mainly used to treat heat syndrome, which is characterized by symptoms such as crimson tongue, fidgety, thirst, and bleeding. The steamed form, which is prepared by steaming the dried root with or without rice wine for one to several days, does not have any cooling properties but is used to treat lumbago, tinnitus, anemia, dizziness, night sweat, etc.<sup>[1]</sup> Chemical changes during the process of steaming are responsible

for the alterations of its properties. In a previous work, it was reported that steaming resulted in the decomposition of some components such as catalpol, stachyose, raffinose, sucrose, and amino acids, and the formation of fructose, manninotriose and 5-hydroxymethyl furfural; moreover, the pH changes from 5.5 to 3.8 and the root acquires a black color from the original yellowish-brown color.<sup>[2]</sup>

Catalpol is classified as an iridoid, which represents a group of naturally occurring monoterpenoids characterized by the cyclopentano [c] pyran ring system. This class of secondary metabolites, especially iridoid glycosides, are frequently isolated from medicinal plants used as bitter tonics, sedatives, antipyretics, hypotensives etc.<sup>[3-6]</sup> Extensive investigations have revealed that they have a wide range of biological and pharmacological activities.<sup>[4-7]</sup> Therefore, iridoids are probably present as active components in a number of herbal preparations. It is well-known that iridoid

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glycosides are susceptible to degradation by  $\beta$ -glucosidases and acids.<sup>[7,8]</sup> Their degradation may occur at various stages, including postharvest drying, processing, storage, extraction, and even metabolism in the human body. Their bioactivity may be brought about by themselves directly or by their aglycones and other reaction products. Accordingly, it is important to clarify their degradation characteristics, and the relation between their degradation, and changes in their biological activities.

Catalpol has been proved to have anti-apoptosis, antioxidant, neuroprotective, antihyperglycemic, anti-cancer functions, etc.,<sup>[9-18]</sup> and has been used as a marker in the quality control of dried *Rehmannia* root in Chinese Pharmacopoeia.<sup>[11]</sup> However, there is little information on catalpol degradation during the preparation of steamed *Rehmannia* root. In this study, the effects of acidity, concentration, heating time, temperature, and coexisting compounds on catalpol degradation were investigated; the activation energy ( $E_a$ ) was calculated; and the mechanism underlying catalpol degradation was also discussed. Catalpol degradation may contribute to the antioxidant activity of steamed *Rehmannia* root, which has been well-studied;<sup>[18-21]</sup> therefore, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity ( $SA_{DPPH}$ ) of the reaction solution was also determined.

## MATERIALS AND METHODS

### Chemicals

Catalpol was isolated from fresh *Rehmannia* root according to the method, previously, established in our laboratory. Briefly, water extract of fresh *Rehmannia* root was firstly passed through macroporous resin, and the 20% alcohol fraction was repeatedly chromatographed on silica gel, and the eluent containing catalpol was collected. Its purity was 97.74% as determined by high-performance liquid chromatography (HPLC). Glucose, fructose, galactose, sucrose, raffinose, stachyose and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd. The other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd.

### Degradation of catalpol

A series of 0.1 M phosphate buffer solution (pH value ranged from 4.0 to 9.0) were prepared by mixing 0.1 M  $Na_2HPO_4$  and 0.1 M  $NaH_2PO_4$  in different volumes to achieve the indicated pH value; some of them were adjusted by concentrated NaOH or  $H_3PO_4$  solution, along with monitoring using a pH meter. A plastic freezing tube with a screw cap containing 450  $\mu$ l of 0.1

M phosphate buffer solution was heated to 100°C in a water bath, and intensively shaken after addition of 50  $\mu$ l of catalpol stock solution; this mixture (catalpol concentration ranged from 0.3 to 30 mM) was incubated in the water bath. The reaction was stopped by adding 4.5 ml of cold water after 1, 2, 4 and 8 h, or at intervals of 2-4 h within the reaction time of 28 h. The resulting solution (10-fold dilution) was subject to immediate catalpol determination by HPLC. The remaining solution was frozen for later determination of  $A_{420}$  and  $SA_{DPPH}$ .

### HPLC analysis of catalpol

The content of catalpol in the 10-fold dilution of the reaction solution was analyzed by a Shimadzu 10A HPLC instrument equipped with a UV detector (SPD-10A, Shimadzu Corporation, Japan). The chromatography was carried out on a  $C_{18}$  column (250 mm  $\times$  4.6 mm, 5  $\mu$ m; Phenomenex, USA) at a column temperature of 25°C with methanol-0.1% phosphoric acid solution (8:92, v/v) as the mobile phase. The flow rate was set at 1.0 ml/min. An aliquot of 20- $\mu$ l diluted solution was injected into the HPLC system, with the detection wavelength set at 210 nm. The peak area is linearly related to catalpol concentration in the range of 30.9-1234.8  $\mu$ g/ml ( $R^2 = 0.9990$ ), the limits of detection and quantification of this method were 0.2  $\mu$ g/ml and 0.5  $\mu$ g/ml, respectively.

The degradation rate of catalpol was calculated as follows: Degradation rate (%) =  $(C_0 - C)/C_0 \times 100$ ,

Where,  $C$  is the concentration of catalpol at a given time, and  $C_0$  is the initial concentration of catalpol in a reaction solution. All the reactions were performed in triplicate.

### Determination of $A_{420}$ and $SA_{DPPH}$

The  $A_{420}$  value of the diluted reaction solution was spectrophotometrically determined at 420 nm, which was used to reflect the extent of browning.  $SA_{DPPH}$  was assessed to determine the total antioxidant activity of the diluted reaction solution as described by Chawla *et al.*<sup>[22]</sup> with slight modifications. Briefly, 0.5 ml of DPPH ethanol solution (0.2 mM) was added to 0.5 ml of diluted reaction solution and kept at 25°C for 30 min in the dark. In the control treatment, the diluted reaction solution was replaced by phosphate solution (0.1 M, pH 7.0). Spectrophotometric measurements were obtained at 517 nm.

$SA_{DPPH}$  was calculated as follows:  $SA_{DPPH}$  (%) =  $(A_0 - A)/A_0 \times 100$ ,

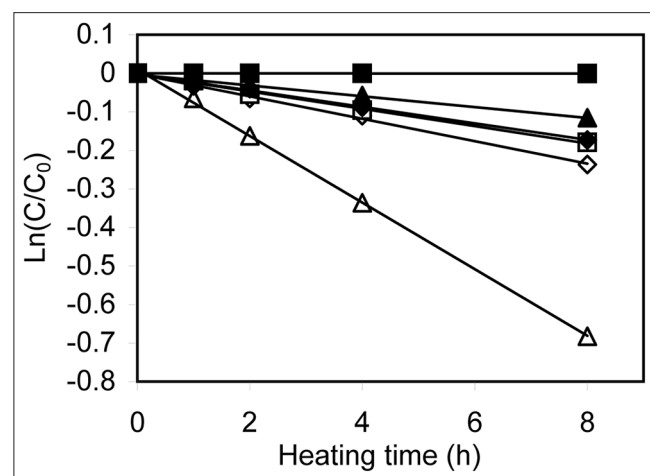
Where  $A$  and  $A_0$  are the absorbance values at 517 nm for the mixture containing diluted reaction solution and the control, respectively.

### Effects of sugars and amino acids on catalpol degradation

To examine the influence of glucose released from catalpol hydrolysis, catalpol was replaced by the corresponding amount of glucose generated in each reaction. To evaluate the effect of sugars, 450  $\mu\text{l}$  phosphate solution was replaced by 450  $\mu\text{l}$  sugar solution (containing glucose, fructose, galactose, sucrose, raffinose or stachyose), which was prepared by dissolving tested sugar in 0.1 M pH 4.0 citro-phosphate buffer. When evaluating the effect of amino acids, the solutions containing amino acids were prepared as follows: The required amino acid [19 were used; the list is shown in Figure 1] was dissolved in 15 ml pH 4.0 citro-phosphate buffer solution by heating, the pH value was again adjusted to 4.0 by addition of 0.1 M disodium hydrogen phosphate or 0.1 M citric acid, and then transferred to a 25-mL volumetric flask and made up the volume by the pH 4.0 buffer solution. The final molar ratio of catalpol and the test compound was set to be 1:1. The reaction was stopped by addition of 4.5 mL cold phosphate buffer (pH 7.0, 0.1 M), and the resulting solution together with further 4- and 8-times diluted solutions (10-, 40- and 80-fold dilutions) were used for determination of catalpol,  $\text{SA}_{\text{DPPH}}$  and  $A_{420}$ , respectively. To confirm the effects of amino acid concentration and acidity on catalpol degradation, catalpol (30 mM) was reacted with different concentrations of glycine (3, 30, and 300 mM) at pH values of 3.0-5.0 and 90°C for 4 h, and the stopped solutions were further diluted 5- and 10-times (50- and 100-fold dilutions) for measurement of  $\text{SA}_{\text{DPPH}}$  and  $A_{420}$ , respectively.

### Effect of temperature on catalpol degradation

Temperature dependency of catalpol degradation was determined at pH 4.0, 5.0, and 6.0, and in the presence of glycine (3 mM) at pH 4.0. The rate constants ( $k$ ) were calculated based on the changes of catalpol concentration at 70°C, 80°C, 90°C, and 100°C. The apparent activation



**Figure 1:** Degradation of catalpol (30 mM) at 100°C, and pH 4.0 ( $\Delta$ ), 5.0 ( $\circ$ ), 6.0 ( $\square$ ), 7.0 ( $\blacksquare$ ), 8.0 ( $\blacktriangle$ ) and 9.0 ( $\blacklozenge$ ) (at column width)

energy and pre-exponential factor were estimated from Arrhenius plotting of the obtained  $k$  values against  $1/T$ , where  $T$  is the temperature in Kelvin.

### Statistical analysis

Results were expressed as mean  $\pm$  S.D of values from three independent experiments. Correlation coefficient (Pearson's R) between different variables was calculated using Microsoft Excel.

## RESULTS

### Effects of acidity, concentration and heating time on catalpol degradation

After heating for 8 h, the catalpol concentration was found to be reduced by 0.1-49.5% at the pH values ranged from 4.0 to 9.0 [Table 1], and some of the reaction solutions became brown. The  $A_{420}$  value of the 10-fold diluted reaction solution was found to be positively correlated with the degradation rate of catalpol [Table 1;  $R = 0.741$ ]. Plotting the natural logarithm of the degradation rate of catalpol at any time to that at the initial time ( $\ln C/C_0$ ) versus heating time ( $t$ ) gives a series of straight lines with  $R^2$  greater than 0.99 [Figure 1]. The results indicate that catalpol degradation obeys first-order reaction kinetics, and the rate constants ( $k$ ) at different pH values, the slopes of the lines, are listed in Table 1. When catalpol was replaced by corresponding amount of glucose released by catalpol degradation, no obvious browning could be detected, especially, at acidic condition,  $A_{420}$  values after heating for 8 h were shown in Table 1.

In another experiment conducted at pH 4.0 and 100°C, the heating time was prolonged to 28 h. The linear relationship between  $\ln C/C_0$  and  $t$  still existed ( $R^2 = 0.9966$ ). By decreasing the concentration of catalpol in the reaction solution from 30 mM to 15.0, 7.0, 3.0, 1.5, 0.7, and 0.3 mM, the catalpol degradation rate was found to increase from 15.8% to 74.2% [Figure 2]. In addition, the catalpol degradation rate, as shown in Table 2, was found to be

**Table 1: Degradation of catalpol (30 mM) at pH 4.0-9.0 and 100°C in 0.1 M phosphate solutions for 8 h**

pH value	Degradation rate (%)	$A_{420}^a$ (catalpol)	$A_{420}^b$ (glucose)	Rate constant ( $\text{h}^{-1}$ )
4.0	49.5 $\pm$ 2.07	0.221 $\pm$ 0.017	0.002 $\pm$ 0.001	0.0864
5.0	21.1 $\pm$ 0.67	0.156 $\pm$ 0.003	0.002 $\pm$ 0.001	0.0291
6.0	16.4 $\pm$ 0.14	0.128 $\pm$ 0.004	0.007 $\pm$ 0.001	0.0225
7.0	0.1 $\pm$ 0.01	0.001 $\pm$ 0.001	0.001 $\pm$ 0.001	0.0001
8.0	10.9 $\pm$ 0.16	0.190 $\pm$ 0.007	0.019 $\pm$ 0.003	0.0140
9.0	15.0 $\pm$ 0.17	0.157 $\pm$ 0.006	0.043 $\pm$ 0.004	0.0206

<sup>a</sup>Absorbance at 420 nm of the 10-fold diluted reaction solution containing 30 mM catalpol; <sup>b</sup>Catalpol was replaced by the corresponding amount of glucose released by catalpol degradation in the reaction system

highly correlated with  $A_{420}$  and  $SA_{DPPH}$  ( $R = 0.9631$  and  $0.9773$ , respectively).

### Effect of sugars and amino acids on catalpol degradation

In systems in which sugars were added, the catalpol degradation rate was found to be almost identical to that in the system no sugar added after heating at  $90^{\circ}\text{C}$  and pH 4.0 for 4 h; however, it increased by 11.7-88.9% in those systems to which amino acids, except proline, were added [Figure 3a]. Moreover, the  $A_{420}$  value of the 80-fold dilution increased by 12.7-46.2 times, and the  $SA_{DPPH}$  value of the 40-fold dilution increased 4.0-8.9 times due to amino acid addition [Figure 3b and c]. When the molar ratio of catalpol to glycine decreased from 1:1 to 1:10 at pH 4.0, the degradation rate increased by 17.1%, and if the ratio increased to 10:1, the degradation reduced by

39.4%. When the pH value changed from pH 4.0 to 5.0 with the molar ratio being 1:1, catalpol degradation rate reduced from 48.6% to 10.1%, if the pH value reduced to pH 3.0, the degradation rate raised up to 96.6%, as shown in Figure 4a. The changing tendency of  $A_{420}$  or  $SA_{DPPH}$  was similar to that of catalpol degradation rate when alteration of both the molar ratio of catalpol to glycine and pH value of the system, as shown in Figure 4b or c.

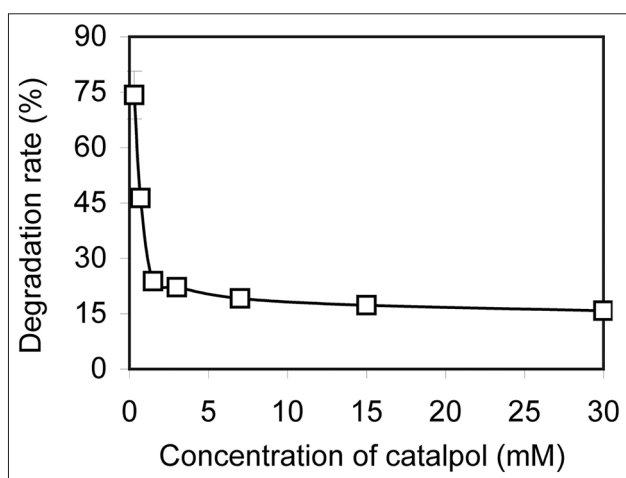
### Effect of temperature on catalpol degradation

Catalpol degradation rates increased with the increase of reaction temperature instead of the acidity and whether glycine was present or not, as shown in Table 3. When the reaction was conducted at a higher pH value, the degradation rate reduced; when 3 mM glycine was added in the system, the degradation rate increased. The obtained  $k$  values for the reactions at temperatures of  $70\text{-}100^{\circ}\text{C}$  varied from  $0.0014\text{ h}^{-1}$  to  $0.1898\text{ h}^{-1}$ . As shown in the Arrhenius plot [Figure 5], the relationship between logarithm of rate constants and inverse temperatures is linear with  $R^2$  values higher than 0.99, and the calculated activation energy and pre-exponential factor increased with pH value and decreased by glycine addition [Table 3].

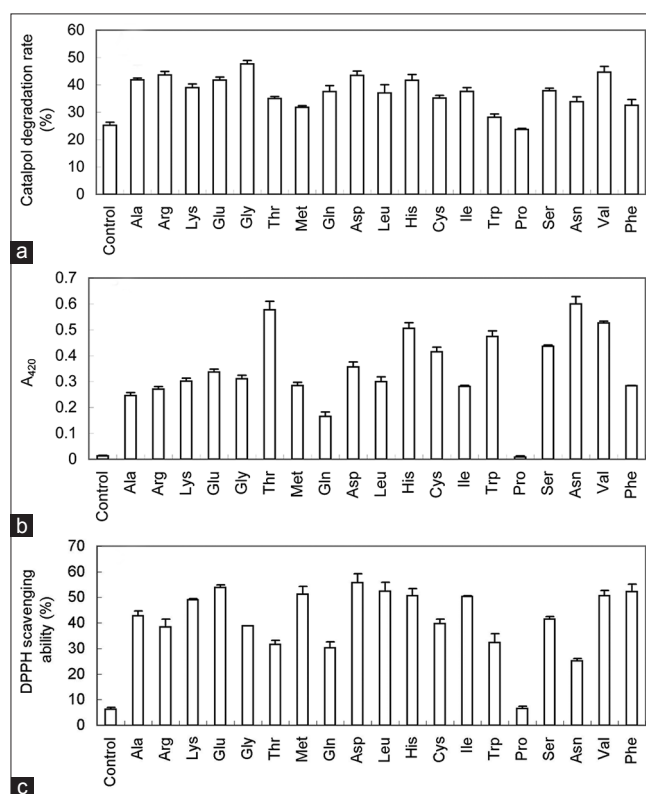
**Table 2: Effect of heating time on catalpol degradation (30 mM), browning and 2-diphenyl-1-picrylhydrazyl free radical scavenging ability at  $100^{\circ}\text{C}$  and pH 4.0**

Heating time (h)	Catalpol degradation rate (%)	$A_{420}$	DPPH scavenging ability (%)
0	/	$0.006\pm 0.002$	$3.2\pm 0.7$
2	$9.9\pm 0.9$	$0.030\pm 0.003$	$5.2\pm 2.0$
4	$24.9\pm 0.5$	$0.070\pm 0.003$	$7.9\pm 1.0$
6	$39.5\pm 0.5$	$0.082\pm 0.007$	$13.0\pm 3.6$
8	$53.1\pm 0.7$	$0.184\pm 0.008$	$21.8\pm 2.0$
10	$58.7\pm 1.6$	$0.222\pm 0.006$	$29.9\pm 1.8$
12	$66.7\pm 0.8$	$0.284\pm 0.011$	$31.5\pm 3.2$
14	$72.6\pm 0.3$	$0.306\pm 0.005$	$35.9\pm 0.9$
17	$77.2\pm 1.1$	$0.365\pm 0.004$	$44.6\pm 1.8$
20	$82.2\pm 0.4$	$0.46\pm 0.012$	$47.8\pm 2.1$
24	$88.0\pm 0.7$	$0.486\pm 0.017$	$49.6\pm 0.8$
28	$91.0\pm 0.2$	$0.497\pm 0.012$	$54.0\pm 1.6$

DPPH: 2-diphenyl-1-picrylhydrazyl



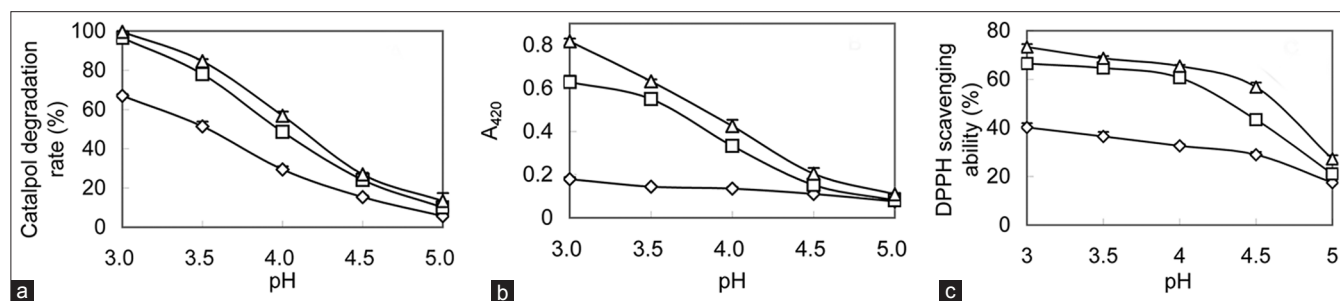
**Figure 2:** Catalpol degradation (0.3-30 mM) at  $90^{\circ}\text{C}$  and pH 4.0 for 4 h (at column width)



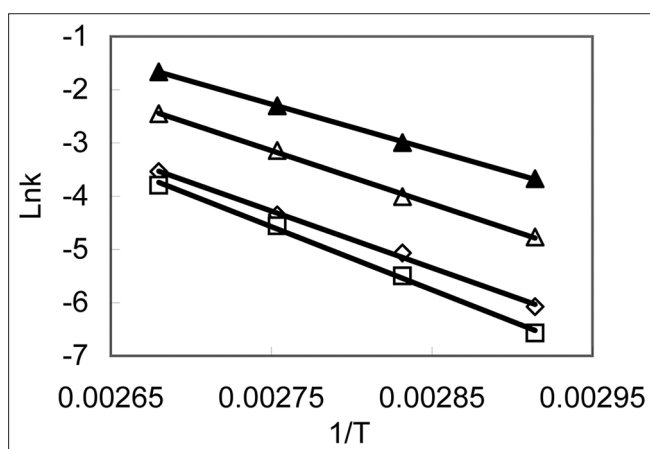
**Figure 3:** Catalpol degradation (30 mM) at  $90^{\circ}\text{C}$  and pH 4.0 for 4 h in the presence of amino acids (30 mM). (a) degradation rate; (b) absorbance at 420 nm of the 80-fold dilution reaction solution; (c) 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging ability of the 40-fold dilution reaction solution (at full page width)

**Table 3: Temperature dependence of catalpol degradation, degradation rate and kinetic parameters**

Reactants	pH	Temperature (K)	Degradation rate (%)	Rate constant (h <sup>-1</sup> )	R <sup>2</sup>	Activation energy (kJ/mol)	Pre-exponential factor (h <sup>-1</sup> )
Catalpol alone	6	373	16.4±0.1	0.0225	0.9982	98.7	1.63×10 <sup>12</sup>
		363	8.3±0.5	0.0105	0.9915		
		353	3.3±0.7	0.0041	0.9939		
		343	1.1±0.3	0.0014	0.9976		
	5	373	21.1±0.5	0.0291	0.9979	88.8	7.79×10 <sup>10</sup>
		363	10.2±0.6	0.0129	0.9992		
		353	5.0±0.1	0.0063	0.9956		
		343	1.9±0.4	0.0023	0.997		
	4	373	49.5±1.7	0.0864	0.9992	83.3	3.95×10 <sup>10</sup>
		363	29.2±0.5	0.0432	0.9991		
		353	13.5±0.2	0.0182	0.9966		
		343	6.6±0.7	0.0085	0.9964		
Catalpol and glycine	4	373	77.7±0.5	0.1898	0.9956	71.6	1.49×10 <sup>9</sup>
		363	56.0±0.7	0.1002	0.9957		
		353	33.3±0.6	0.0501	0.9966		
		343	19.0±1.5	0.0254	0.9985		



**Figure 4:** Degradation of catalpol (30 mM) at 90°C and different pH values for 4 h in the presence of different concentrations of glycine ( $\Delta$ , 3 mM;  $\square$ , 30 mM,  $\Delta$ , 300 mM) (a) catalpol degradation rate; (b) absorbance at 420 nm of the 100-fold dilution reaction solution; (c) 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging ability of the 50-fold dilution of the reaction solution (at column width)



**Figure 5:** Arrhenius plot of the degradation of catalpol (30 mM) at pH 4.0 ( $\Delta$ ), 5.0 ( $\diamond$ ) and 6.0 ( $\square$ ), and at pH 4.0 ( $\blacktriangle$ ) in the presence of glycine (3 mM) (at column width)

## DISCUSSION

Traditionally, fresh *Rehmannia* root is dried at 70-90°C, and the dried root is steamed to produce steamed *Rehmannia* root. The pH value decreases from 6.4 to 5.5 during hot air

drying of the root, and it is further reduced to 3.8 after the steam processing.<sup>[2]</sup> So the conditions for the degradation of catalpol in this investigation were set such that they simulated the traditional conditions for root drying and processing.

Our results indicate that catalpol has excellent stability in a neutral solution, and is sensitive to both acids and alkalis, which is in agreement with the observations in published literatures.<sup>[23,24]</sup> Moreover, our data showed that more catalpol was degraded under lower pH conditions, which means that acid hydrolysis of catalpol occurred. The activation energy for catalpol degradation under different pH values are all below those of acid-catalyzed hydrolysis of general glycosides (about 138-142 kJ/mol),<sup>[25]</sup> which indicated that the underlining mechanism may be different. Bianco<sup>[26]</sup> and Bianco *et al.*<sup>[27]</sup> have proposed a pathway for acid hydrolysis of iridoid glycosides, which involves the formation of an intermediate product, 1,5-cyclopentandialdehyde, through hydration of the C-3/C-4 double bond. The intermediate is highly active, and may further polymerize to form colored compounds and result in

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 rehmaglucin B and D and jioglucin A, B, and C. Unfortunately, all these elucidated components are colorless or off-white,<sup>[28,29]</sup> 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.<sup>[8]</sup> 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.<sup>[2]</sup>

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.<sup>[2]</sup> 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.<sup>[2]</sup> 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  $E_a$  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<sup>[30]</sup> and Hayashi *et al.*,<sup>[31]</sup> 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.<sup>[32-34]</sup> 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,<sup>[32,33]</sup> 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.<sup>[35-37]</sup> According to the linear relationship between  $E_a$  and logarithm of the pre-exponential factor, the same reaction mechanism was assumed.<sup>[35]</sup> 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.<sup>[36-37]</sup>

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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