

Relationship in between Chemical Oxidation and Browning of Flavanols

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Abstract. Catechin, epicatechin and chlorogenic acid are widely distributed in the plant kingdom. At present, influencing factors of phenol chemical oxidation is little research. In order to study non-enzymatic browning factors, this research utilized catechin, epicatechin and chlorogenic acid to establish simulation systems. The browning degree and products of flavanols were investigated by transmittance and high-performance liquid chromatography (HPLC). The main results and conclusions were follows. The brown generation is increased after phenols of chemical oxidation at 50°C and at pH 3.7 phosphate buffered saline, the sequence of influencing factor of browning is pH > kind of phenol > temperature. Oxidation of compounds of catechin and epicatechin results in formation of their Methylene quinone or *o*-Quinones. In addition, oxidation products of catechin, epicatechin and chlorogenic acid were mixture of different molecular sizes. The research has showed that brown generation correlated well with chemical oxidation of phenols and chemical oxidation reaction generated larger molecular weight polymers.

Keywords: catechin, epicatechin, chlorogenic acid, browning, chemical oxidation



1. Introduction

Polyphenols are broadly distributed in the plant kingdom, especially fruits and vegetables. Phenolic oxidation mainly includes chemical oxidation and enzymatic oxidation. In fact, sterilization and heat treatment of fruit and vegetable processing have made phenoloxidase passivation, so for processed fruits and vegetables, phenolic reaction mainly is chemical oxidation. The research only is applicable to oxidation of phenols that have a greater influence on the browning of fruits and vegetables.

Catechin, epicatechin and chlorogenic acid are widely distributed in the plant kingdom. Recently, lots of researches have been showed that phenolic compounds largely contribute to oxidative browning of fruits and vegetables. The phenol oxidation polymerization is the cause of producing browning of lotus root [1], and the results showed that pH had the biggest biggest effect on browning of polyphenols, followed by kind of polyphenols, the temperature had less effect on browning [2]. Phenolic compounds are directly related to the browning phenomenon of white wines [3]. The study found that catechin and epicatechin play crucial role at the early stages of cider making [4]. The degree of browning chlorogenic acid is associated with the browning of fruit juice [5]. Catechin, epicatechin and chlorogenic acid were more potent than others in fruit wine in enhancing browning [6-8]. However, at present, influencing factors of phenol chemical oxidation is little research. This research utilized catechin, epicatechin and chlorogenic acid to develop model systems in order to study the influence factors of phenols oxidation on browning degree. In this way, it can help to provide theoretical support for better control of browning of fruit and vegetable products in the future.

2. Materials and methods

2.1 Chemical oxidation

Catechin (Sigma), epicatechin (Sigma) and chlorogenic acid (Sigma) were dissolved in phosphate buffer solution at different pH 3.7, 7, 10 (0.02M) by heating on a steam bath. After the mixture was filtered with 0.45 μ m membrane filters, 20 mL of phenols solution at different pH's Phosphate buffer solution was added to test tube with plug. The solution was then spared with sufficient oxygen with oxygen cylinder. The test tube were kept at the a water bath with different temperature.

In the experiment, oxidation of catechin, epicatechin, chlorogenic acid and mixture of epicatechin and chlorogenic acid were carried out at pH 3.7, 7, 10 and at temperature 37, 50 and 90°C. Phenols oxidation reaction were investigated according to $L_9(3)^4$ orthogonal design (Table 1).

Table 1. $L_9(3)^4$ orthogonal design of chemical oxidation about phenolics.

	pH	temperature(°C)	Phenols
1	3.7	37	epicatechin
2	7	50	chlorogenic acid
3	10	90	catechin

2.2 Sampling

The reaction was terminated by adding 0.5 μ L of concentrated sulfuric acid to 0.75 mL sample. To prevent precipitation and any loss of the oxidation products, 0.75 mL of 100% acetonitrile was added and the vial sealed.

2.3 HPLC

Shimadzu SCL-10AVP HPLC (SHIMADZU, Japan) was used for analytical separation. Column temperature was set at 40°C. The volume injected was 100 μ L. The wavelengths 280 nm was monitored simultaneously, and scanning was done from 230-650 nm by spectrophotometer (721B, China). The mobile phase was filtered with 0.45 μ m membrane filters and degassed. Mobile phase A was 40% acetonitrile in double distilled water. A linear gradient was run from 5%-100% mobile phase A. Mobile phase B was 5% acetic acid. A linear gradient was run from 95%-0% mobile phase B in 30 min.

2.4 Browning

Transmittance was measured at 420 nm with double beam UV-visible light spectrophotometer. The experiment was repeated 3 times.

3. Results and discussion

3.1 The influence factors of phenol chemical oxidation

Table 2. Results and analysis of $L_9(3)^4$ orthogonal design of chemical oxidation about phenolics.

	pH	temperature(°C)	phenols	$T_{420nm}(\%)$
1	3.7	37	epicatechin	96.30
2	3.7	50	chlorogenic acid	98.20
3	3.7	90	catechin	96.00
4	7	37	chlorogenic acid	97.90
5	7	50	catechin	96.00
6	7	90	epicatechin	66.70
7	10	37	catechin	16.40
8	10	50	epicatechin	65.10
9	10	90	chlorogenic acid	80.80
Range(browning degree)	42.1	16.23	22.83	

Xidizing reaction continued for 5 h. Phenolate anion concentrations was 200 mg/L

Oxidation of catechin, epicatechin, chlorogenic acid were carried out separately at pH 3.7, 7, 10 and at temperature 37, 50 and 90°C (Table 2). Table 2 shows that the sequence of influencing factor of browning is pH>kind of phenols>temperature. This phenomenon is in conformity with previous works [2]. The intensity of brown produced was highest at pH 10. The intensity of brown produced was lowest at 50°C with the largest T_{420nm} . The effect of oxidation of different phenols on browning was different. Intensity of brown produced was higher after catechin and epicatechin oxidation than

chlorogenic acid. The means were compared by analysis of variance at a significance level of 0.05. The analysis of the effects of pH, temperature and kind of phenol on browning. The results showed that only pH has a significant influence ($F=3.135>0.117$) on the browning. In this experiment the browning degree was investigated by measuring T_{420nm} , the greater the transmittance, the lighter the browning.

3.2 Effect of pH on browning degree of chemical oxidative products

In order to study effect of pH on browning degree of chemical oxidative products, oxidation of catechin, epicatechin, chlorogenic acid were carried out separately in various values of pH at 50°C (Figure 1, Figure 2, Figure 3). It can be observed that the amount of pigmentation produced was higher at pH 10, 7 with small T_{420nm} after oxidizing catechin and epicatechin. For chlorogenic acid, browning degree was bigger at pH 10 than pH 7, 3.7 significantly. This suggests that phenolic compounds of chemical oxidation is more likely to occur in alkaline solution. This phenomenon is in conformity with Cilliers and Singleton's works [9]. So we can think that the effect of the phenol chemical oxidation on the browning of fruit juice (pH 3-4) is small.

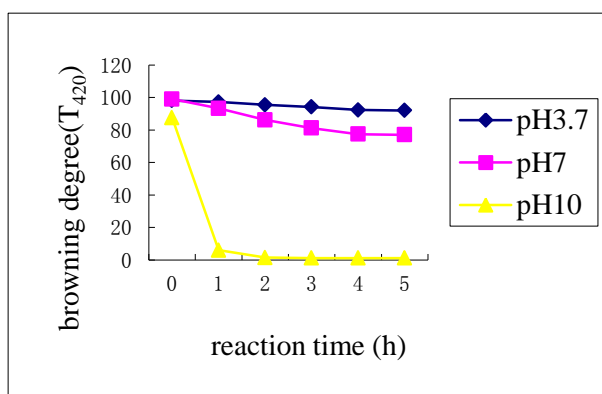


Figure 1. Browning degree of chemical oxidative products by catechin (500 mg/L) in various values of pH (50°C) .

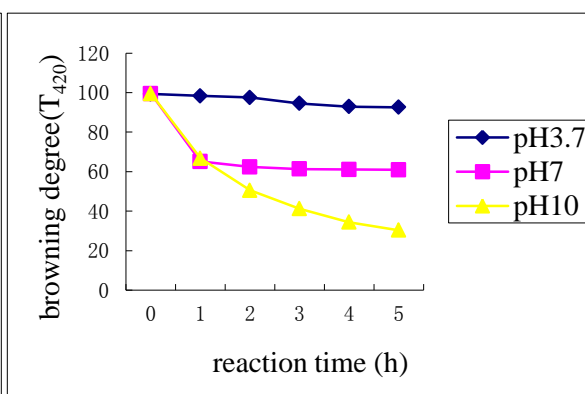


Figure 2. Browning degree of chemical oxidative products by epicatechin (500 mg/L) in various values of pH (50°C) .

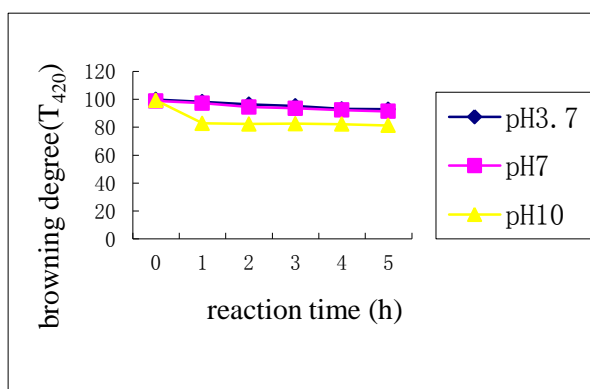


Figure 3. Browning degree of chemical oxidative products by chlorogenic acid (500 mg/L) in different pH (50°C) .

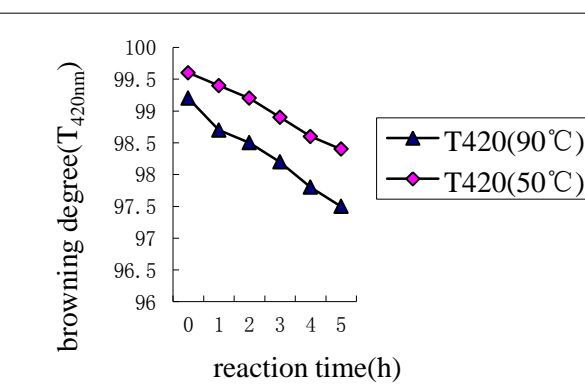


Figure 4. Browning degree of chemical oxidative products by 500mg/L catechin in various temperature (pH3.7).

3.3 Effect of temperature on browning degree of chemical oxidative products

In order to study effect of temperature on browning degree of chemical oxidative products, oxidation of catechin, epicatechin, chlorogenic acid were carried out separately in various temperature at pH 3.7. (Figure 4, Figure 5, Figure 6). It can be seen that with the extension of reaction time, browning degree of three kinds of phenols oxidation products is more serious at 90°C than 50°C. This suggests that oxidation condensation reaction of phenols are more likely to occur at higher temperatures. Liu Jing studied the influence of temperature on the jujube juice concentrate browning in the softening process. Liu Jing's research have found that total phenol content gradually decreases with the increase of temperature, the higher the temperature, the faster the decline[10].

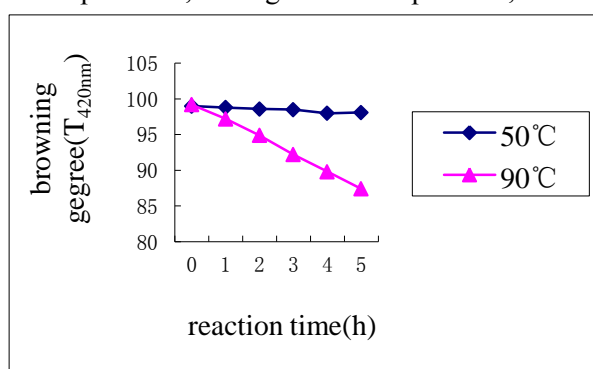


Figure 5. Browning degree of chemical oxidative products by 500mg/L epicatechin in various temperature (pH3.7) .

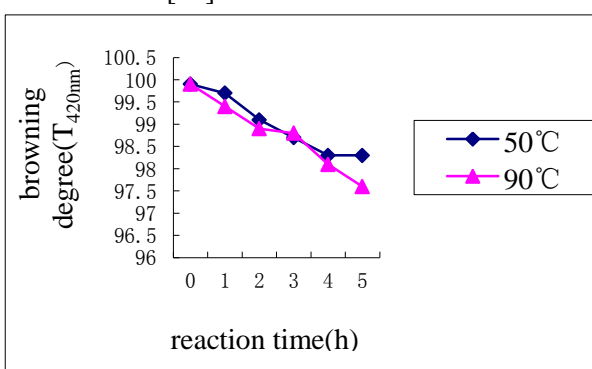


Figure 6. Browning degree of chemical oxidative products by 500 mg/L chlorogenic acid in various temperature (pH3.7) .

3.4 Chemical oxidation of phenol reaction rate

In order to indicate the rate of oxidation reaction of catechin, epicatechin and chlorogenic acid, phenolate anion concentrations was monitored at various reaction time by HPLC.

Table3 shows rate constants ($\times 10^{-5} \text{ h}^{-1}$) for the first-order reaction of catechin, epicatechin and chlorogenic acid in various values of pH (200 mg/L). It can be seen that the reaction rate of epicatechin was the fastest at 50°C and pH 3.7, followed by catechin and then chlorogenic acid. The chemical oxidation rate of chlorogenic acid is relatively small. Oxidation of catechin, epicatechin and chlorogenic acid was faster at pH 10 than pH 3.7. This suggests that phenolic oxidation can occur rapidly under alkaline conditions. Reaction rate was higher at 90°C than at 50°C, which is consistent with the change trend of browning.

Table 3. Rate Constants ($\times 10^{-5} \text{ h}^{-1}$) for the First-Order Reaction of phenols in various values of pH .

	catechin		epicatechin		chlorogenic acid	
	50°C (r)	90°C (r)	50°C (r)	90°C (r)	50°C (r)	90°C (r)
pH3.7	33.05(0.99)	35.5(0.98)	35.62(0.94)	51.5(0.99)	28.96(0.92)	44.5(0.81)
pH10	74.15(0.97)		61.2(0.96)		75(0.85)	

number of data points used in calculating the first-order rate constant is 8. r =correlation coefficient of the straight line fitting.

3.5 Effect of oxidation of mixture on browning degree of products

3.5.1 Browning degree of mixed phenols. Browning rate of mixtures of chlorogenic acid and epicatechin is larger than pure 500 mg/L chlorogenic acid or 500 mg/L epicatechin by chemical oxidation at pH 3.7 and at 50°C (Figure 7). The possible explanation is that oxidative condensation reaction is more likely to happen due to the interaction between different phenolics to produce brown pigment than pure phenol.

3.5.2 Reaction rate of mixed phenols. In chlorogenic acid-epicatechin mixtures, the reaction rate of epicatechin was much faster than pure epicatechin, whereas chlorogenic acid rate was slower than that of single oxidation (Table 4). The possible explanation is that the rate of epicatechin is enhanced by chlorogenic acid quinone that chlorogenic acid oxidation generate, with reduction of the chlorogenic acid quinone back to chlorogenic acid. At the same time, it can also explain the phenomenon of the increase of browning in chlorogenic acid-epicatechin mixtures.

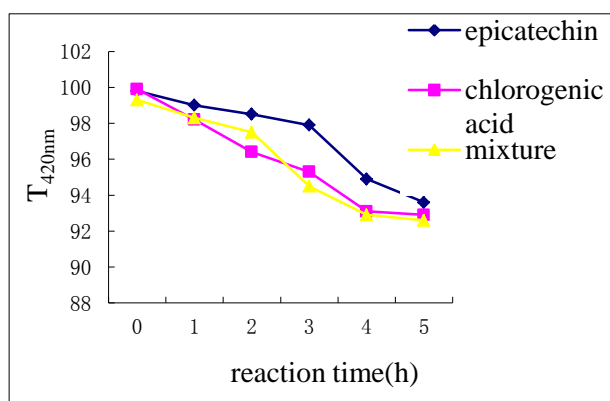


Figure 7. Browning rate of 500 mg/L chlorogenic acid, 500 mg/L epicatechin and mixture by chemical oxidation (pH 3.7, 50°C).

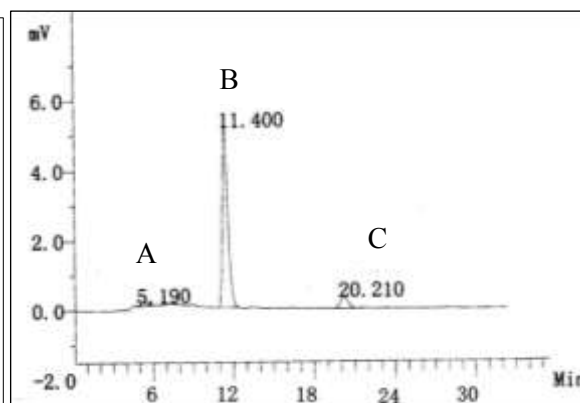


Figure 8. Chemical oxidative products of catechin (500 mg/L) at pH 3.7 at 50°C after 12h in 100% oxygen.

Table 4. Rate Constants ($\times 10^{-5} \text{ h}^{-1}$) for the First-Order Reaction of 500 mg/L chlorogenic acid, 500 mg/L epicatechin and the mixture.

pH	epicatechin (r)	chlorogenic acid (r)	mixture	
			epicatechin (r)	chlorogenic acid (r)
3.7	35.62(0.942)	28.96(0.923)	49.64(0.925)	19.75(0.938)

number of data points used in calculating the first-order rate constant is 8. r =correlation coefficient of the straight line fitting.

3.6 Research on phenols oxidation products

3.6.1 Research on chemical oxidative products of catechin. Chemical oxidative products of catechin at pH 3.7 at 50°C after 12h in 100% oxygen was monitored by HPLC (Figure 8). Peak A has chromatographic elution time shorter than that of catechin (peak B). This would indicate that the oxidation products are more polar than catechin. Meanwhile, Peak C has chromatographic elution time longer than that of catechin. This would indicate that the oxidation products are less polar than catechin. These phenomenon demonstrate that oxidation products are mixture. Chemical oxidative products of catechin were scanned from 200-600 nm (Figure 9). We can think that oxidation products with absorbance at 305 nm have the structure of Methylene quinone. This indicate that catechin with absorbance at 280 nm can react with Methylene quinone to yield a condensation product. Oxidation products with a shoulder peak at 400-500 nm have the structure of *o*-Quinones. These data show that oxidation products have the structure of Methylene quinone and *o*-Quinones after catechin was oxidized, then these products have potential to react further with catechin and other phenols. Customarily, polymer molecular with molecular weight 500-3000 is known as condensed tannins, and polymer of larger molecular weight called phenolic acids [11].

3.6.2 Research on chemical oxidative products of epicatechin. Chemical oxidative products of epicatechin (500 mg/L) at pH 3.7 at 50°C after 12h in 100% oxygen was monitored by HPLC (Figure 10). Peak A has chromatographic elution time shorter than that of epicatechin (peak B). In all other things being equal, the longer the time of peak flowing out, the bigger the molecular weight. This would indicate that the oxidation products are more polar and smaller molecular weight than epicatechin. Meanwhile, Peak C has chromatographic elution time longer than that of epicatechin. This would indicate that the oxidation products are less polar and bigger molecular weight than epicatechin. Oxidation products of epicatechin were scanned from 200-600 nm (Figure 11). From Figure 11, it can be concluded that oxidation products have the structure of Methylene quinone and *o*-Quinones after epicatechin was oxidized.

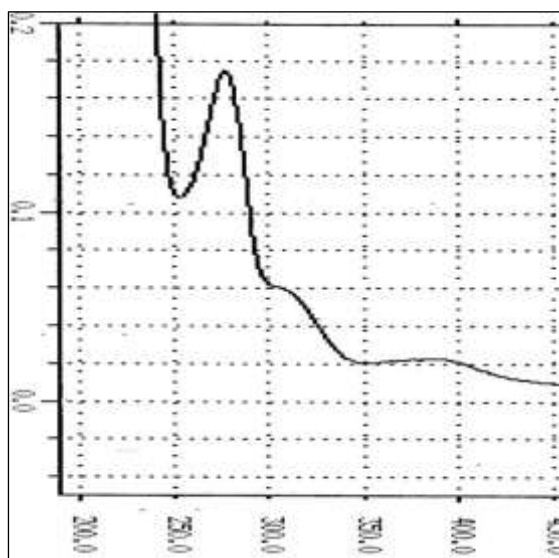


Figure 9. Absorbtion curve of chemical oxidative products of catechin (500 mg/L) at pH 3.7 at 50°C after 12 h in 100% oxygen.

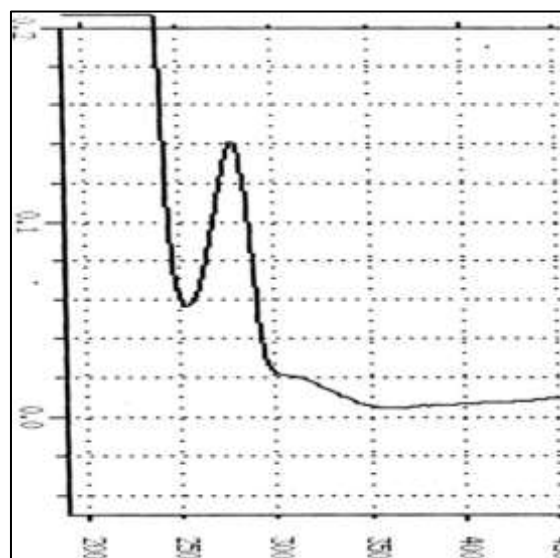


Figure 11. Absorbtion curve of chemical oxidative products of epicatechin (500 mg/L) at pH 3.7 at 50°C after 12 h in 100% oxygen.

3.6.3 Research on chemical oxidative products of chlorogenic acid. More products were produced after chlorogenic acid was oxidized (Figure 12). Peak A, B has chromatographic elution time shorter than that of chlorogenic acid (peak C). This would indicate that the oxidation products are more polar and smaller molecular weight than chlorogenic acid. Meanwhile, Peak D, E has chromatographic elution time longer. This would indicate that the oxidation products are less polar and bigger molecular weight than chlorogenic acid. Typical absorption peak was not found by scanning the each peak.

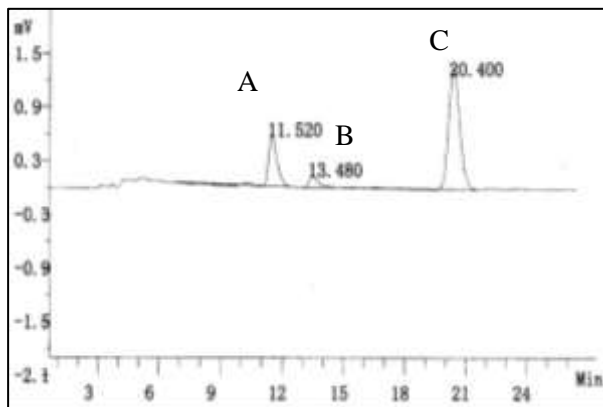


Figure 10. Chemical oxidative products of epicatechin (500 mg/L) at pH 3.7 at 50°C after 12 h in 100% oxygen.

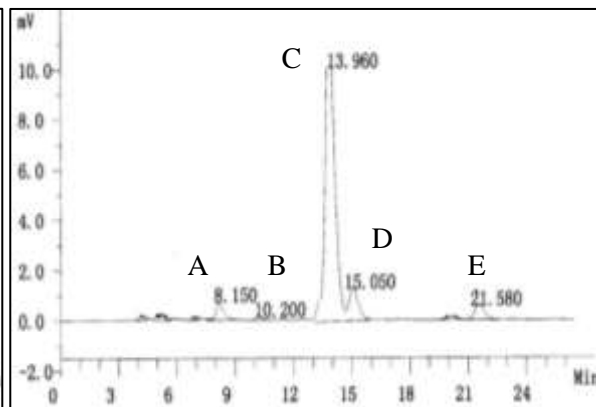


Figure 12. Chemical oxidation of chlorogenic acid (500 mg/L) at pH 3.7 at 50°C after 12 h in 100% oxygen.

4. Conclusion

The brown generation is increased after phenols of chemical oxidation, the sequence of influencing factor of browning is pH > kind of phenol > temperature.

Phenols are more likely to occur chemical oxidation under alkaline conditions. Epicatechin had the fastest oxidation reaction rate than catechin and chlorogenic acid at pH 3.7 and at 50°C.

Catechin and epicatechin generated oxidative products with structure of methylene quinone and *o*-quinones after catechin and epicatechin were oxidized in phosphate buffer pH 3.7 and at 50 °C.

The oxidative products of catechin, epicatechin and chlorogenic acid were the mixture with different molecular weight after occurring chemical oxidation in phosphate buffer pH 3.7 and at 50 °C.

Acknowledgments

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