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Contributed Paper

Effect of L-cysteine, Potassium Metabisulfite, Ascorbic Acid and Citric Acid on Inhibition of Enzymatic Browning in Longan

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ABSTRACT

The effect of different anti-browning agents on partially purified longan polyphenol oxidase (PPO) activity was investigated by spectrophotometry, using catechol as a phenolic substrate. For this purpose, L-cysteine, potassium metabisulfite, ascorbic acid, and citric acid were used to inhibit the activity of longan PPO at different concentrations (0-10 mM). L-cysteine was found to be the most potent anti-browning agent (L-cysteine > potassium metabisulfite > ascorbic acid > citric acid). Pre-incubation of PPO with some anti-browning agents increased the extent of inhibition.

Keywords: enzymatic browning, longan, polyphenol oxidase.

1. INTRODUCTION

The longan *(Dimocarpus longan* Lour.*)* is an attractive subtropical fruit. It has a short shelf-life under normal ambient conditions due to skin color loss (browning) and deterioration during storage and transportation [1]. Browning has been attributed to the oxidation of phenolics by PPO, producing brown-colored byproducts. In the presence of oxygen, enzyme catalyses two different reactions: the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenol to o-quinone. Then quinone may condense and react non-enzymatically with other phenolic compounds, amino acids, and protein to produce colored polymers [2].

The most widespread agents used for control of browning are sulfiting agents. Due to adverse health effects, several studies have been devoted to the non-sulfite anti-browning agents such as reducing agents (ascorbic acid, glutathione, L-cysteine), enzyme inhibitors (aromatic carboxylic acids, substituted resorcinols, anions), chelating agents (phosphate, EDTA, organic acids), acidulants (citric acid, phosphoric acid), complexing agents (cyclodextrins), and enzymes [3-7].

Jiang [8] reported that PPO from longan fruit peel was inhibited by reduced glutathione, L-cysteine, and thiourea, whereas $MnSO_4$ and $CaCl_2$ enhanced PPO activity. To our knowledge no investigation has been carried out on studying the effect of potassium metabisulfite, ascorbic acid, and citric acid on purified longan PPO activity.

In this paper, we describe the effect of L-cysteine, potassium metabisulfite, ascorbic acid, and citric acid on partially purified longan PPO.

2. MATERIALS AND METHODS

2.1 Materials

Mature longan fruit, edor cultivar, was supplied by a major cultivator in Amphur Doisakat, Chiang Mai Province, Thailand.

2.2 Extraction of PPO and Partial Purified Enzyme

All steps were carried out at 4°C. Longan pulp was homogenized in 0.1 M sodium phosphate buffer pH 6.8, and centrifuged at 12,000xg for 20 min. The enzyme solution was fractionated with solid ammonium sulfate (20-80% saturation) and the precipitate was collected by centrifugation at 15,000xg for 20 min. The precipitate was then redissolved in 0.01 M sodium phosphate buffer pH 6.8 and dialyzed against the same buffer.

2.3 Enzyme Assay and Protein Determination

PPO activity was assayed with catechol as a substrate by a spectrophotometric procedure [9]. The assay was performed using 2.0 ml of 0.1 M catechol, 2.0 ml of 0.1 M sodium phosphate buffer pH 6.8, and 1.0 ml of enzyme. The increases in absorbance at 480 nm were recorded every 10 min for 60 min. One unit of enzyme activity was defined as the amount of enzyme which caused a change of 0.001 in absorbance per minute. Protein content was determined according to the dye-binding method of Bradford [10] with bovine serum protein as the standard.

2.4 Effect of Anti-browning Agents on PPO

Anti-browning agents examined included L-cysteine, potassium metabisulfite, ascorbic acid, and citric acid. The tested concentrations of anti-browning agents were 0, 1.0, 2.5, 5.0, and 10.0 mM. Two different conditions: with and without pre-incubation of enzyme in inhibitor solutions for 10 min at room temperature were studied. The experiments were determined in 3 replicates at each concentration of each anti-browning agent.

3. Results and Discussion

The effects of L-cysteine, potassium metabisulfite, ascorbic acid, and citric acid on partially purified PPO activity from longan were studied. The results are shown in Figure 1. The percentage inhibition was compared with that of the control (0% inhibition). L-cysteine was the most effective inhibitor. At the concentrations of 1, 2.5, and 5 mM of L-cysteine, there were 84, 98, and 100% inhibition of PPO activity, respectively. While the results obtained at 1, 2.5, and 5 mM of potassium metabisulfite were 52, 80, and 100% inhibition, respectively. There was significant difference between the degree of enzyme inhibition obtained with and without pre-incubation at low concentration of L-cysteine (1 mM) and potassium metabisulfite (1 and 2.5 mM).

Sulfhydryl compounds such as L-cysteine and potassium metabisulfite have been investigated as inhibitors of enzymatic browning. The formation of quinone-sulphite complexes prevents the quinone polymerization [11]. A further action of sulfhydryl compounds on PPO may directly inhibit the enzyme by combining irreversibly with copper at the active site of the enzyme [12]. The latter effect may explain the difference between the PPO inhibition obtained with and without pre-incubation.

At low ascorbic acid concentrations 1, 2.5, and 5 mM, the inhibitory effect on PPO was 27, 44, and 54% inhibition, respectively, while the result obtained at a higher ascorbic acid concentration (10 mM) was nearly 100% inhibition. At a given ascorbic acid concentration, there was no significant difference between the degree of enzyme inhibition obtained with or without pre-incubation. This is as expected provided that ascorbic acid does not act directly on the enzyme structure. Mardtinez and Whitaker [4] reported that the mechanism of ascorbic acid inhibition involves the reduction of quinone generated by PPO.

At low citric acid concentration, 1, 2.5, and 5 mM, the inhibitory effect on PPO was 16, 28, and 41% inhibition, respectively, while the result obtained at a higher citric acid concentration (10 mM) was 72% inhibition. The inhibitory effect increased as the concentration of citric acid increased. At every level of citric acid concentrations, the inhibitions

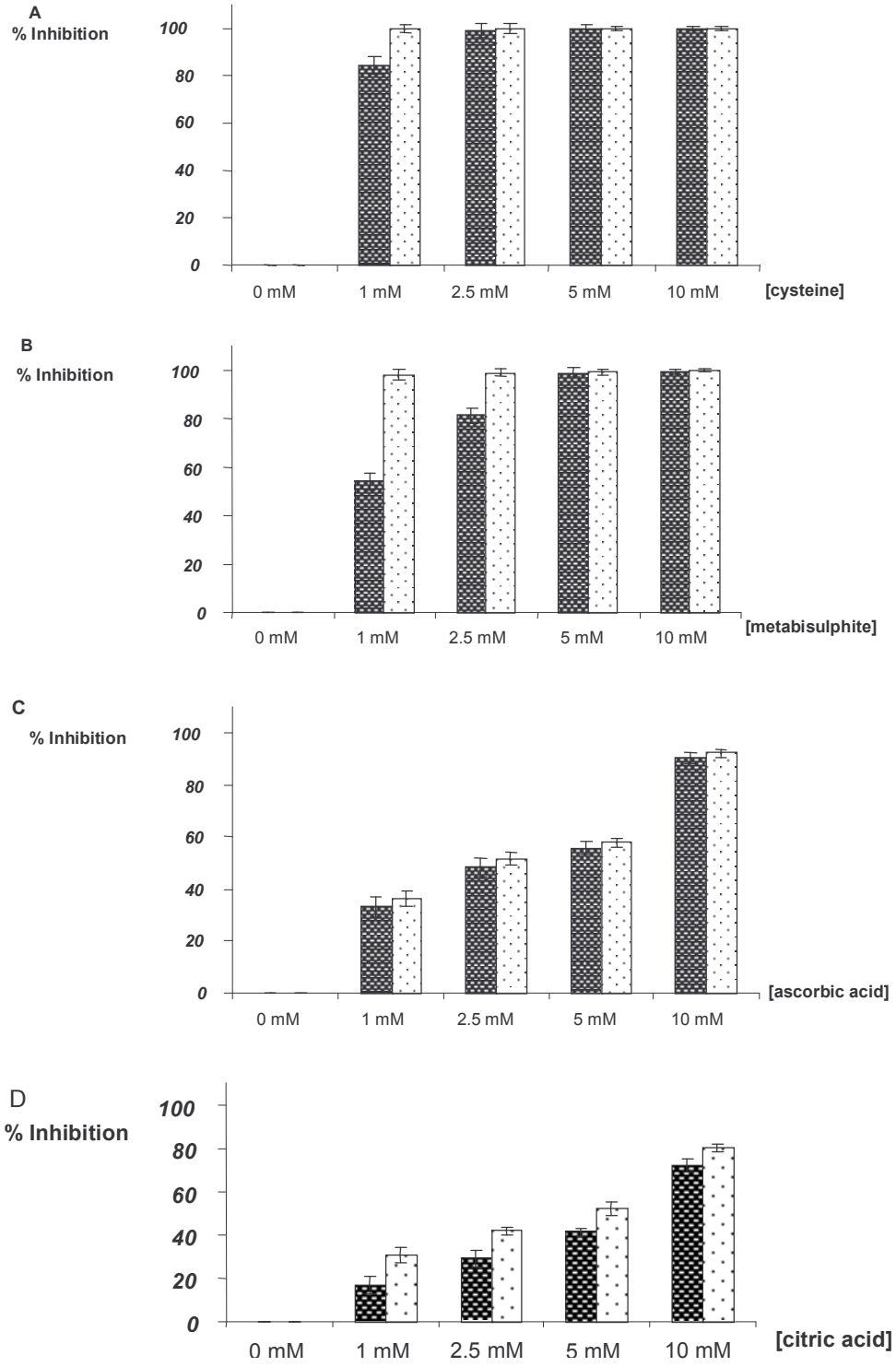

Figure 1. Effect of inhibitors on PPO in 0.1 M phosphate buffer pH 6.8. Inhibitors were; (A) L-cysteine, (B) potassium metabisulfite, (C) ascorbic acid, and (D) citric acid. Results are means of three replicates of each concentration of each inhibitor.
▩ : No pre-incubate; ⊡ : Pre-incubate

of enzyme activities obtained from pre-incubation conditions were higher than those obtained from no pre-incubation conditions by 5-10%.

The results presented demonstrated that L-cysteine appears to be a potent inhibitor of longan PPO, followed by potassium metabisulfite, ascorbic acid, and citric acid, respectively. These results are in agreement with the study of Ding *et al*. [13]. They reported the effectiveness of a series of sulfhydryl compounds in inhibiting PPO activity in a model system (chlorogenic acid) and in loquat (*Eriobotrya japonica* Lindl.) juice. The results showed that L-cysteine is the most effective inhibitor of browning in pure chlorogenic acid solution as well as in loquat juice. The concentration of L-cysteine for 90% browning inhibition depended on loquat cultivars, and ranged from 0.6 mM to 2.0 mM. Similarly, Yaar and Sairolu [14] reported that L-cysteine and ascorbic acid (at the concentration of 2 mM) showed good inhibition of quince (*Cydonia oblonga*) PPO (99 and 98% inhibition, respectively). They also studied the influence of citric acid on the quince enzyme. At 2 and 20 mM of citric acid resulted in little inhibition of quince PPO (9 and 23% inhibition, respectively). Formerly, the effect of citric acid on controlling browning of litchi fruit was studied by Jiang and Fu [15]. They reported that 100 mM of citric acid solution showed 80% inhibition of litchi PPO activity, and the browning grade decreased to 2.1, whereas that of control was higher than 4.0 at 25±2 ºC, 6 days after storage.

Among compounds that inhibit PPO activity, sulfur dioxide (SO_2) is one of the most effective and is used in food industry for many years. However, restrictions of sulfite usage in foods associated with consumer concern about its safety generate the need for substitutes [16]. Therefore, alternative chemicals without toxic effects are needed, such as sulfhydryl (SH or thiol), ascorbic acid, and citric acid. These compounds have potential to be used commercially, to substitute sulfite as anti-browning agent, to prevent enzymatic browning in processed fruit products.

4. CONCLUSION

The effectiveness of some anti-browning agents including L-cysteine, potassium metabisulfite, ascorbic acid, and citric acid on partially purified longan PPO was evaluated. L-cysteine was the most effective browning inhibitor, followed by potassium metabisulfite, ascorbic acid, and citric acid, respectively. Pre-incubation of the enzyme with some of these compounds increased the extent of the inhibition, implying a direct effect on the active site of the enzyme.

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