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Effects of aqueous chlorine dioxide treatment on polyphenol oxidases from Golden Delicious apple

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Abstract

Effects of chlorine dioxide (ClO_2) treatment on the activity and characteristics of polyphenol oxidase (PPO) in Golden Delicious apples were studied. The treatment with 50 mg/l ClO_2 for 1 h did not affect some characteristics of the PPO, including its optimum pH value (5.0) and temperature (40 °C) as well as the maximum absorption wavelength (412 nm) of the final products. With increasing ClO_2 concentrations from 0 to 100 mg/l, the V'_{max} value reduced and K'_m value changed irregularly. When the concentration of ClO_2 increased from 0 to 60 mg/l, residual PPO activities significantly decreased, showing a negative linear-correlation with ClO_2 concentration. For 10 and 50 mg/l ClO_2 treatments, partial inhibition of PPO was achieved within 0.5 h and the PPO activities did not significantly decrease after 0.5 h. The inhibition and inactivation of PPO by ClO_2 treatment were observed at processing temperatures (30 and 70 °C) and storing temperatures (20, 0–4, and −18 °C).

Keywords: Inhibition; Anti-browning; Enzyme; Chemical treatment; Enzymatic kinetics

1. Introduction

Browning reactions in fruits and vegetables are recognized as a serious problem during handling, processing and storage. Polyphenol oxidases (PPO, EC 1.14.18.1) activity that has been extensively reported is the main factor involved in browning (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994; Walker & Ferrar, 1998). Since enzymatic browning causes deterioration of sensory and nutritional quality and affects appearance and organoleptic properties, inactivation of PPO is desirable for preservation of foods (Hendrickx, Ludikhuyze, Vandenbroeck, & Weemaes, 1998). Several methods, such as the use of anti-browning agents and the exclusion of oxygen as well as thermal processing, have been used to inhibit the enzymatic browning (Hsu, Shieh, Bills, & White, 1988). Inactivation of PPO by thermal processing has shown its limitation due to loss of sensory and nutritional quality of food products (Soliva-Fortuny, Elez-Martinez, Sebastian-Caldero, & Martin-Belloso, 2002; Sun, Lee, & Song, 2002). Although exclusion of oxygen can inhibit PPO activity, browning may restart when oxygen is reintroduced (Langdon, 1987). A common approach for enzymatic browning prevention is the use of anti-browning agents, which act on the enzyme or react with substrates and/or products of enzymatic catalysis so that browning pigment formation is inhibited (Arslan & Dogan, 2005). Chemical additives, such as sulfites in any of their forms (sulfur dioxide, sodium or potassium metabisulfite, sodium or potassium bisulfite), acidifiers (citric, malic and phosphoric acids), chelators (EDTA), reducing agents (ascorbic acid, alone or in combination), and cysteine have been used for controlling browning in fruits and vegetables (Fayad, Marchal, Billaud, & Nicolas, 1997; Richard-Forget, Goupy, & Nicolas, 1992; Sapers, 1993). Calcium ascorbate is widely used by the fresh-cut industry to control browning of cut apples (Abbott, Saftner, Gross, Vinyard, & Janick, 2004; Bhagwat, Saftner, & Abbott, 2004; Karaibrahimoglu, Fan, Sapers, & Sokorai, 2004). The use of these anti-browning agents in the food industry is, however, constrained due to their high cost and low effectiveness

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(McEvily, Iyengar, & Otwell, 1992). Therefore, research and development studies for finding effective substitutes are still ongoing (Girelli, Mattei, Messina, & Tarola, 2004).

In recent years, some new techniques (low temperature blanching, gamma irradiation, and high-pressure treatment) and anti-browning agents (dipeptides and 2,3-diaminopropionic) have also been reported for the control of PPO activities (Arslan & Dogan, 2005; Gautam, Sharma, & Thomas, 1998; Girelli et al., 2004; Sun et al., 2002; Yemenicioglu, 2002).

Chlorine dioxide (ClO_2) is a strong oxidizing and sanitizing agent that has broad and high biocidal activity, including algae, animal planktons, food spoilage organisms and foodborne pathogens (Bundgaard-Nielsen & Nielsen, 1996; Du, Han, & Linton, 2002; Du, Han, & Linton, 2003; Han, Linton, Nielsen, & Nelson, 2000; Han, Linton, Nielsen, & Nelson, 2001; Han, Sherman, Linton, Nielsen, & Nelson, 2000; Junli, Li, Nanqi, Fang, & Juli, 1997; Junli, Li, Nanqi, Li, et al., 1997; Narkis & Kott, 1992; Roberts & Reymond, 1994). ClO_2 is widely used in postharvest handling of fruits and vegetables. It was effective in killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *yeasts*, *molds* and *Alicyclobacillus acidoterrestris* spores on fresh and fresh-cut produce (Lee, Gray, Dougherty, & Kang, 2004; Sy, McWatters, & Beuchat, 2005; Sy, Murray, Harrison, & Beuchat, 2005), while maintaining quality and extending shelf life (Han, Selby, Schultze, Nelson, & Linton, 2004). Aqueous ClO_2 as an antimicrobial has been approved by FDA to wash fruits and vegetables at a residual concentration of 3 mg/l (FDA, 1998). However, information is limited regarding to the effects of ClO_2 on enzyme activities in produce.

Besides its strong antimicrobial activities, ClO_2 may have synergistic bleaching effects on color since it has been widely used to bleach pulp in the paper industry. It has drawn interests to investigate if ClO_2 affects those enzymes involving in browning activity in produce. Youm et al. used aqueous ClO_2 to treat minimally processed lettuce and found the activity of PPO in lettuce was decreased by 30% (Youm, Lee, Jang, Kim, & Song, 2004). Our previous studies found that 4, 6, and 8 mg/l aqueous ClO_2 treatment exhibited some anti-browning effects on the Fuji apple juices, while the 10 and 12 mg/l dosage had little effect on the juice browning. To further explore this phenomenon, we investigated the inhibitory effects of aqueous ClO_2 treatments on PPO in Golden Delicious apples.

2. Materials and methods

2.1. Materials

Golden Delicious apples were purchased from local market at Taian, P.R. China. The fruits were stored at 0–4 °C immediately after purchase. Some indexes of the apples after analysis in our laboratory were: pH value at 4.04 ± 0.01, soluble solid content at 11.4 ± 0.15°Brix, and titratable acidity at 0.24 ± 0.01 g malic acid/100 ml juices.

2.2. Extraction of PPO

PPO was extracted from the apples using a modified procedure developed by Rocha (Rocha & Morais, 2001). Apple samples and extraction buffer (0.2 mol/l pH7.4 sodium phosphate-citric acid buffer) were stored at 4 °C. The samples were blended using a homogenizer (Saikang Inc, Shanghai, China) with extraction buffer for 3 min at 1 min intervals using 1.6 ml buffer/g fruit ratio, including 20 g/kg polyvinylpolypyrrolidone (PVPP, insoluble) and 2.5 ml/kg TritonX100. The homogenates were centrifuged for 10 min at $1795 \times g$ with an 80-2 bench top centrifuge (Zhongda, Jiangsu, China). After extracting for 30 min at 4 °C, the enzyme solution was centrifuged again in a TGL-16G-A superspeed centrifuge (Anting Inc, Shanghai, China) for 30 min at 4 °C and $12{,}000 \times g$. The supernatants were collected as the PPO samples.

2.3. Assay for PPO activity

The enzymatic activity was determined by measuring the increase in absorbance at 412 nm for catechol at 40 °C using a UV-2100 spectrophotometer (Unico Inc, Shanghai, China). This method has been reported to determine PPO activity in apples (Podsedek, Wilska-Jeszka, Anders, & Markowski, 2000; Rocha & Morais, 2001) and other products (Arslan, Erzengin, Sinan, & Ozensoy, 2004; Pilar Cano, Begona de Ancos, & Gloria Lobo Mariana Santos, 1997; Yemenicioglu, 2002). The reaction mixture contained 2.8 ml substrate solution and 0.2 ml enzyme. The substrate solution was 0.05 mol/l catechol in 0.2 mol/l sodium phosphate-citric acid buffer (pH 5.0). The reference cuvette contained only the substrate solution. The straight-line section of the activity curve as a function of time was used to determine the enzyme activity [units/(min ml enzyme)] (Rocha & Morais, 2001). A unit of enzyme activity was defined as the change of 0.001 in the absorbance value under the conditions of the assay (Galeazzi & Sgarbieri, 1981). When a lag phase occurred, the reaction rate was measured after the lag phase (Rocha & Morais, 2001). All the measurements were performed in triplicate.

2.4. Aqueous ClO_2 treatment

A stabilized ClO_2 solution product (Shanda Huate Inc, Shandong, China) was used in the research, which contained 1.90 g stabilized ClO_2 in 100 g solutions. After activation by hydrochloric acid, the active ClO_2 solution was further diluted with distilled water to 250, 500 or 1000 mg/l ClO_2 solution (active ingredient). The concentration of ClO_2 was measured over time in capped samples at 360 nm wavelength using a UV-2100 spectrophotometer (Unico Inc, Shanghai, China) (Fu, Wang, & Du, 2005). The PPO samples were treated by aqueous ClO_2 at different concentration (0–100 mg/l) for different times (0–24 h). The PPO samples treated by 0 mg/l ClO_2 were used for control. The samples after treatment were used for