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Inhibitory effects of α-$Na_8SiW_{11}CoO_{40}$ on tyrosinase and its application in controlling browning of fresh-cut apples

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ABSTRACT

α-$Na_8SiW_{11}CoO_{40}$ was synthesized and characterized. The inhibitory effects of α-$Na_8SiW_{11}CoO_{40}$ on the activity of mushroom tyrosinase and the effects of α-$Na_8SiW_{11}CoO_{40}$ on the browning of fresh-cut apples were studied. The Native-PAGE result showed that α-$Na_8SiW_{11}CoO_{40}$ had a significant inhibitory effect on tyrosinase. Kinetic analyses showed that α-$Na_8SiW_{11}CoO_{40}$ was an irreversible and competitive inhibitor. The inhibitor concentration leading to a 50% reduction in activity (IC_{50}) was estimated to be 0.239 mM. Additionally, the results also showed that α-$Na_8SiW_{11}CoO_{40}$ treatment could significantly decrease the browning process of apple slices and inhibit the polyphenol oxidase (PPO) activity. Moreover, application of α-$Na_8SiW_{11}CoO_{40}$ resulted in higher peroxidase activity and promoted high amounts of phenolic compounds and ascorbic acid. This study may provide a promising method for the use of polyoxometalates to inhibit tyrosinase activity and control the browning of fresh-cut apples.

1. Introduction

Consumer demand for fresh-cut fruits and vegetables has increased because of consumers' fast-paced lifestyles. Fresh-cut apples are popular due to advantages such as freshness, convenience, nutrition and being pollution free (Allende, Tomás-Barberán, & Gil, 2006). However, browning can easily occur in the processing of fresh-cut apples, which seriously affects the sensory quality and nutritional value of the slices (You et al., 2007). Meanwhile, it is generally accepted that the phenolic compounds and metabolic enzymes of phenolics are the major factors causing the browning of fresh-cut apples (Chung & Moon, 2009). Gil, Gorny, and Kader (1998) reported that browning of fresh-cut 'Fuji' apple occurs via the reaction of polyphenol oxidase (PPO) with phenolic compounds. Rocha and Morais (2002) indicated that browning of 'Jonagored' apple slices during storage is moderately correlated with PPO activity. Chung and Moon (2009) reported that browning in 'Tsugaru' apples is correlated with the amounts of phenolic compounds. Chauhan, Raju, Singh, and Bawa (2011) reported that the browning rate and browning degree of apples were significantly related to PPO and peroxidase (POD) activity. Therefore, inhibiting the enzyme activity involved in browning should be an important method for maintaining quality and extending the shelf life of fresh-cut apples.

Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a copper-containing multifunctional oxidase (Lin et al., 2013). Tyrosinase plays a central role in the transformation of L-tyrosine into dopaquinone, which results in the formation of melanin (Wang, Zhang, Yan, & Gong, 2014). The melanin produced by tyrosinase can protect insects from skin injuries (Lin et al., 2011). In human beings, the production and accumulation of melanin

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can cause a series of dermatological disorders or, more seriously, malignant melanoma (Khan, 2007). In plants, on the one hand, phenolic compounds are oxidized by PPO to form brown pigments, which can cause the browning of fruits and vegetables (Mayer, 2006). On the other hand, the oxidation products of phenolic compounds can combine with other components, including amines, peptides and proteins. These complexes are responsible for the loss of nutrition in fruits and vegetables and simultaneously inhibit the activity of proteinase and glycolytic enzymes (Lin et al., 2011).

Polyoxometalates (POMs) are a rich class of inorganic metal–oxygen cluster compounds that have been widely applied to various fields because of their incomparable structural versatility (Zang, Chen, Long, Cronin, & Miras, 2013). Rhule, Hill, Judd, and Schinazi (1998) confirmed that POMs were high-efficacy and low-toxicity anti-HIV drugs through *in vivo* and *in vitro* studies. Raza et al. (2012) indicated that seven polyoxotungstates had inhibitory potency in the nanomolar range against two major isozymes of alkaline phosphatase. At the same time, they demonstrated that these compounds were very promising anticancer drugs with no cytotoxic effects in normal human cell lines. Turner et al. (2012) reported the inhibitory effects of POMs on several enzymes and concluded that POMs might become effective enzyme inhibitors. POMs have antiviral (Rhule et al., 1998), antitumor (Zhai, Li, Zhang, Wang, & Li, 2008) and anticancer (Raza et al., 2012) aspects that have been widely investigated by scholars. However, there have been few reports about the effects of POMs on tyrosinase and the browning of fresh-cut fruits and vegetables.

In this paper, α-$Na_8SiW_{11}CoO_{40}$ (abbreviated as $SiW_{11}Co$) was synthesized and characterized (Wang et al., 2005). The inhibitory effects of $SiW_{11}Co$ on the activity of mushroom tyrosinase were studied with electrophoresis and kinetics. The effects of $SiW_{11}Co$ on the nutrient composition of fresh-cut apples were explored by measuring the changes in the indexes of browning degree, total phenolics (TP), ascorbic acid (AsA), peroxidase (POD) and polyphenol oxidase (PPO). The goal of the present study is to provide a theoretical foundation for designing novel tyrosinase inhibitors and anti-browning agents.

2. Materials and methods

2.1. Reagents and instruments

$SiW_{11}Co$ was synthesized and characterized according to the published literature (Wang et al., 2005). Mushroom tyrosinase (EC 1.14.18.1) was purchased from Worthington Chemical Co. (USA) (NO. LS003789). The specific activity of the enzyme was 965 U mg^{-1} without further purification. L-3, 4-Dihydroxyphenylalanine (L-DOPA) and dimethyl sulfoxide (DMSO) were the products of Aldrich Chemical Co. (USA). Kits to measure the Coomassie brilliant blue protein, POD and AsA contents were all bought from the Nanjing Jiancheng Bioengineering Institute in Nanjing, Jiangsu province, China. All other reagents were local and were analytical grade, and redistilled and ion-free water was used throughout the experiments. IR spectra were obtained from a sample powder pelletized with KBr on an FT-IR480 spectrometer over a range of 4000–400 cm^{-1} (Fig. S1). The UV–vis spectra were recorded at room temperature with a Cary-50 spectrophotometer in the wavelength range from 190 to 800 nm (Fig. S2).

2.2. Plant materials

Fuji apples (*Malus pumila* Mill.), which were planted in Luochuan of Shanxi province, China, were purchased from the supermarket of Quanzhou Normal College in Fujian province, China. The apples were selected from the same batch with similar shapes and maturity and without injuries, diseases or pests.

2.3. Electrophoresis analysis and PPO activity staining

For the determining the molecular weight of tyrosinase, SDS–PAGE and PPO activity staining were performed according to the method of Zhang et al. (2011) with a slight modification. The samples were 6 μL Marker or 20 μL unboiled tyrosinase, which were loaded onto a polyacrylamide gel made of 12% running gel and 5% stacking gel with a thickness of 1.5 mm. The 14.4–116 kDa Protein Molecular Weight marker was used for SDS–PAGE. The activity of tyrosinase was 1000 U mL^{-1} dissolved in 50 mM phosphate buffer, pH 6.8. The samples for SDS–PAGE were incubated with an equal volume of loading buffer (final SDS concentration was 2% w/v) for 1 h at room temperature. Electrophoresis was run at constant voltage (180 V) until the bromophenol blue front began to run off the gel. The gel was removed and cut into two parts: one part with the Marker and another part with the tyrosinase. The parts were stained with 0.1% Coomassie Brilliant Blue R-250 and 10 mM L-DOPA dissolved in 50 mM phosphate buffer (pH 6.8), respectively. The gel was stained for PPO activity with L-DOPA at 37 °C for 1 h.

Native-PAGE was carried out to study the inhibitory effect of $SiW_{11}Co$ on tyrosinase. The activity of tyrosinase was 1000 U mL^{-1} dissolved in 50 mM phosphate buffer, pH 6.8, and the sample volume was 20 μL per well. The electrophoresis conditions of Native-PAGE and SDS–PAGE were similar. However, the gel, electrophoresis buffer and loading buffer of Native-PAGE did not contain SDS or mercaptoethanol. After electrophoresis, each lane was cut into a piece of gel and soaked in an inhibitor solution with concentrations ranging from low to high. After shaking for 45 min, the solution was discarded, and then the gels were incubated in 10 mM L-DOPA for 1 h at 37 °C. All other processes were carried out at 4 °C.

2.4. Enzyme kinetic analysis

The tyrosinase activity assay was performed as published in the literature (Lin et al., 2013) with slight modifications. The 3 mL reaction media contained 2.8 mL of 0.5 mM L-DOPA solution in 50 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 6.8), which was pre-incubated at 30 °C, and 0.1 mL of different concentrations of the effector dissolved in DMSO solution. Then 0.1 mL of the aqueous solution of the tyrosinase was mixed immediately and sufficiently. The final concentration of mushroom tyrosinase was 17.3 μg mL^{-1} for the diphenolase activity. At a constant temperature of 30 °C, the measurement was performed at 475 nm with a UV spectrophotometer. The enzyme activity was calculated according to Lambert–Beer's law ($\varepsilon = 3700$ L mol^{-1} cm^{-1}). The extent of inhibition by $SiW_{11}Co$ was expressed as the concentration required to reduce tyrosinase activity to 50% (IC_{50}). DMSO without an inhibitor was used as a control. The inhibition type was assayed with a Lineweaver–Burk plot, and the inhibition constant was determined by second plots of the apparent K_m/V_m or $1/V_m$ versus the concentration of the inhibitor (Wang et al., 2014).

2.5. Effect on the browning process of fresh-cut apples

A preliminary study showed that treatment with $SiW_{11}Co$ at 3 mM was the most effective concentration for inhibiting the browning of apples in a range of 0.125–6 mM. Therefore, in this study, 3 mM $SiW_{11}Co$ was used. The apples were cut into pieces (4 mm * 5 mm * 2 mm) and were equally divided into two groups: the control group was dipped in sterile ultrapure water for 1 min at