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Prevention of Browning in Mushroom by Onion Extract

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Abstract

The inhibitory effect of onion extract on mushroom tyrosinase activity was investigated. The enzyme activity was inhibited by 96.3% with the addition of onion extract. The inhibitory action of onion extract toward mushroom tyrosinase activity slightly increased after heat treatment at 100°C for 10 min (97.5%) and decreased after incubation of the extract at pH 2.3 for 3 hrs (79.9%). However, the inhibitory action of the extract after dialysis decreased to 18.8%. The onion extract showed drastic inhibition of the browning in mushroom.

Key words: mushroom tyrosinase, inhibition, onion extract

INTRODUCTION

Tyrosinase (monophenol, o-diphenol : oxygen oxidoreductase, EC 1.14.18.1) is a copper-containing mono-oxygenase widely distributed in micro-organisms, plants and animals (1,2). The enzyme catalyzes two distinct reactions involving molecular oxygen : the hydroxylation of monophenols to o-diphenols and the oxidation of the o-diphenols to o-quinones (1,3). Tyrosinase also termed polyphenol oxidase is usually associated with the biosynthesis of brown melanin pigments in animals (melanogenesis) and enzymatic browning in plants (melanosis) (4). Browning reactions in fruits and vegetables are recognized as a serious problem in the food industry (5). The reaction occurs due to the oxidation and dehydrogenation of colorless polyphenols present in plants (6). Scientists in the food industry are currently investigating innovative ways to control or inhibit enzymatic browning in foods (7-11).

In mushrooms, the major enzyme responsible for the reaction is tyrosinase although many mushroom species also contain laccase activity (12). Mushroom tyrosinase has been extensively studied in crude extracts, purified forms and in commercial preparations with regard to its physical and enzymatic properties (13). Many papers have reported the inhibition activity of tyrosinase, but little research has been conducted on the inhibitory action of the tyrosinase produced by a natural food source. The application of natural tyrosinase inhibitors to food and cosmetics is somewhat limited due to off-flavors, off-odors, toxicity, and lack of economic feasibility (14,15). Therefore, the development of alternative safe and efficient tyrosinase inhibitors is needed. The objective of this work is to study the inhibitory action of onion extract on mushroom tyrosinase, and browning of the mushroom.

MATERIALS AND METHODS

Materials

Mushroom and onion were purchased from a local market in Pusan, Korea. Mushroom tyrosinase, L-DOPA, L-tyrosine and cellulose membrane dialysis tubing were obtained from Sigma Chemical Co.

Preparation of onion extract

Onion (100 g) was homogenized with 100 mL of 50 mM phosphate buffer (pH 6.6) for 5 min. The homogenate was centrifuged at 15,000×g for 20 min, and the supernatant was collected. All steps were carried out at 4°C.

Enzyme assay

Tyrosinase activity was assayed with L-DOPA as a substrate using spectrophotometric procedure (16). The assay mixture contained 0.1 mL of mushroom tyrosinase (780 unit), 1.9 mL of 50 mM phosphate buffer (pH 6.6) and 1 mL of 0.03% L-DOPA. The onion extract (1.0 mL) was added into the assay mixture and the inhibitory effect was measured. The total assay volume was 3 mL. The increase in absorbance at 475 nm with or without onion extract was measured. One unit of enzyme activity was defined as the amount of enzyme which caused a change of 0.001 in absorbance per minute.

Dialysis of onion extract

The crude onion extract (10 mL) was dialyzed against 4 L of H_2O for 4 hrs, and onion extract inside the dialysis tube after dialysis was used as a dialyzed onion extract. The molecuar weight cutoff of dialysis membrane used was 12,000.

Browning of mushroom

Mushroom was cut into 5 mm slices and each slice was placed in individual petri dish. Subsrate solution (1 mL of 0.01 M L-tyrosine/0.1 M Tris-HCl pH 9.2) and the substrate solution with onion extract were spread over the whole surface of

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the slices. Lids were placed on the petri dishes to minimize evaporation. All slices were incubated at 30°C for 30 min.

RESULTS AND DISCUSSION

Inhibitory effect of onion extract on mushroom tyrosinase activity

Fig. 1 shows the effect of onion extract in inhibiting the activity of mushroom tyrosinase with L-DOPA used as a substrate. The enzyme activity was drastically inhibited (96.3%) by addition of onion extract. Fig. 2 reveals the inhibition of the mushroom tyrosinase activity by onion extract treated with heat at 100°C for 10 min. As shown in Fig. 2, heated onion extract also had similar inhibitory action toward mushroom tyrosinase; 97.5% of the enzyme activity was inhibited by the addition of heat treated onion extract. The onion extract was incubated at pH 2.3 for 3 hours and the stability of inhibitory action of onion extract was also examined. As shown in Fig. 3, 79.9% of the enzyme activity was inhibited by the presence of onion extract previously incubated at the acidic pH. Therefore, the compounds responsible for this inhibition in onion extract seem to be heat and acid stable.

The onion extract was dialyzed, and the inhibitory activity of the extract after dialysis was investigated (Fig. 4). The enzyme activity was partially inhibited (18.8%) by the onion extract after dialysis. This finding suggests that the ingredients responsible for tyrosinase inhibition in onion extract have small molecular weight. Recently, a number of tyrosinase inhibitors have been isolated and identified from natural and microbial origins (17-19). The thiol compounds, including L-cysteine, glutathione, dithiothreitol, mercaptoethanol and thiourea, are effective inhibitors of many polyphenol oxidases from plant sources (20-24). In a previous study, the inhibitory effect of honey on polyphenol oxidase and on the browning of apple

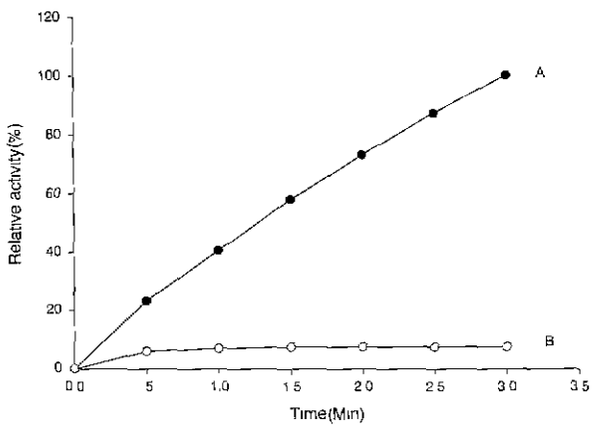

Fig. 1. Inhibitory effect of onion extract on mushroom tyrosinase activity. The enzyme activity was measured at 25°C for 3 min, using the spectrophotometric method.
A (●-●) : control
B (○-○) : addition of onion extract

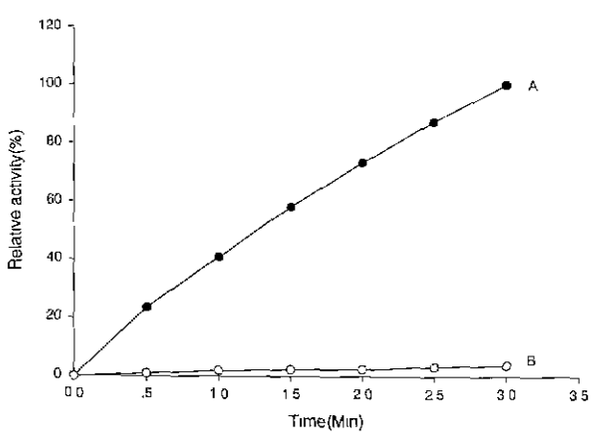

Fig. 2. Inhibitory effect of onion extract heated at 100°C for 10 min on mushroom tyrosinase activity.
A (●-●) : control
B (○-○) : addition of heated onion extract

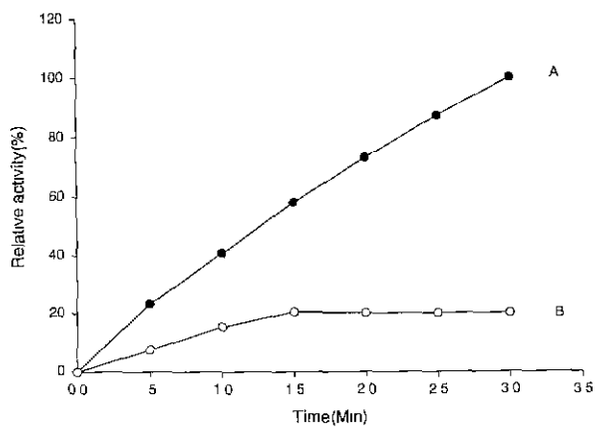

Fig. 3. Inhibitory effect of onion extract incubated at pH 2.3 for 3 hours on mushroom tyrosinase activity.
A (●-●) : control
B (○-○) : addition of acid-treated onion extract

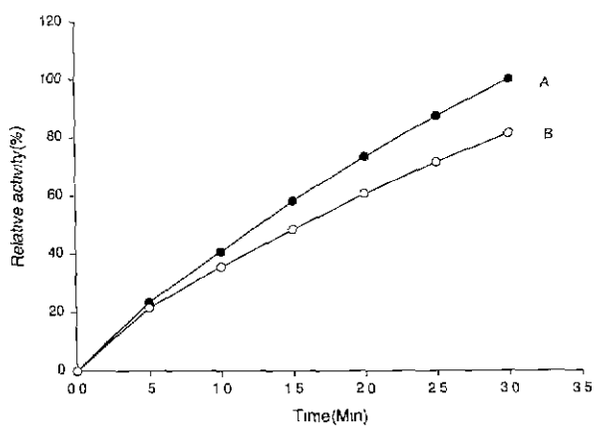

Fig. 4. Inhibitory effect of dialyzed onion extract on mushroom tyrosinase activity.
A (●-●) : control
B (○-○) : addition of dialyzed onion extract

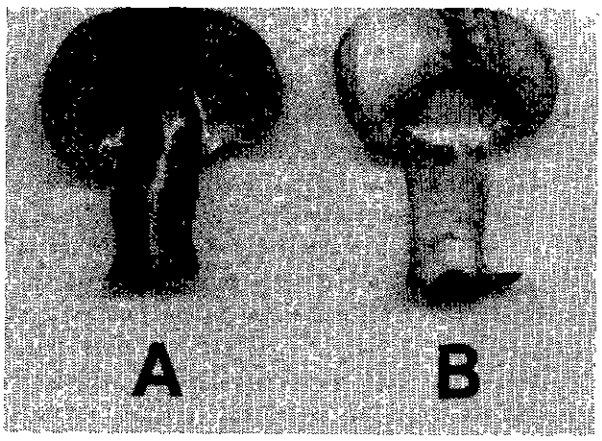

Fig. 5. Effect of onion extract on browning of mushroom. A : addition of substrate solution (0.01 M L-tyrosine in 0.1 M Tris-HCl pH 9.2), B : addition of substrate solution with onion extract.

slices, grape juice and in model systems was attributed to a small peptide with a molecular weight of 600 (25). Therefore the reducing compounds or small peptides present in onion might be responsible for the inhibition of browning in mushroom. Further research is needed to identify the compounds present in onion responsible for the inhibitory effect on browning.

Effect of onion extract on browning of mushroom

Fig. 5 shows the effect of onion extract on browning of mushroom. The control slice (Fig. 5-A) underwent a brown color change, whereas the addition of onion extract (Fig. 5-B) reduced the intensity of brown color formation in the mushroom. The above data confirms that onion extract has a strong inhibitory effect on mushroom tyrosinase action. Onion extract could be utilized as a natural inhibitor of browning in mushroom and other food sources such as prepeeled potato, and juice manufacturing from various plants and vegetables.

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