

Anti-Browning of Mushroom (*Agaricus bisporus*) Slices by Glutathione during Hot Air Drying

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Abstract: Browning of mushroom tends to occur during hot air drying due to Poly Phenol Oxidase (PPO), while glutathione is known for its ability to inhibit the activity of PPO and browning. In this study, the efficacy of glutathione in inhibiting browning on mushroom slices was estimated. Browning of mushroom slices treated with glutathione was monitored during hot air drying. PPO activity in mushroom was inhibited by 98.2 with 0.08% glutathione. Compared with the control, mushroom slices treated with glutathione showed no browning during hot air drying. These results indicate that application of glutathione is a promising method of Anti-browning of mushroom by glutathione during hot air drying.

Keywords: Browning, drying, glutathione, mushroom

INTRODUCTION

Mushrooms, a special group of macroscopic fungi, have been used as a food source since ancient times. Mushrooms are liked for their delicious flavor, low calorific value, high protein contents (20-40%), vitamins of B-group and minerals (Walde *et al.*, 2006).

Mushrooms are highly perishable commodities and they start deteriorating immediately within a day after harvest. In view of their highly perishable nature, the fresh mushrooms have to be processed to extend their shelf life for off-season use. Among the various methods employed for preservation, drying is one of the common methods used for mushrooms (Argyropoulos *et al.*, 2011; Kar *et al.*, 2004; Kotwaliwale *et al.*, 2007; Kumar *et al.*, 2013; Walde *et al.*, 2006).

Colour of the product is of prime importance to the consumer as product quality criteria (Matser *et al.*, 2000). Thus, browning in mushrooms during drying, which is ascribed to the activity of phenolic compounds, oxygen and Poly Phenol Oxidase (PPO), decreased the sensory quality and commercial value of the dried mushrooms (Kotwaliwale *et al.*, 2007; Kumar *et al.*, 2013). Though application of advanced drying methods, e.g., microwave drying and freeze-drying, can alleviate this problem, it needs expensive equipments and high production cost. Therefore, using color protecting agents is a practical way to inhibit browning in mushrooms during drying.

Glutathione is known for its ability to inhibit the activity of PPO and browning (Jiang and Fu, 1998). Thus, the aim of this study is to investigate the inhibitory effects of glutathione on the activity of PPO and browning in mushroom during hot air drying.

MATERIALS AND METHODS

Materials: Fresh mushrooms (*Agaricus bisporus*) were purchased from a local supermarket and stored at 4°C until used. Glutathione was purchased from Fuchen Chemical reagents Co. (Tianjin, China). L-β- (3, 4-dihydroxyphenyl) alanine (L-DOPA), Brij-35, sodium phosphate and ammonium sulphate were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of PPO extract from mushroom: Mushroom (100 g) was powdered by grinding with liquid nitrogen in a Waring blender (JJ-2, Wuxi Woshin Instruments Co., Ltd. and Wuxi, China). The powder was kept in a polyethelene bag and stored at -20°C until use (not more than 24 h). The isolation of PPO was carried out according to the method described by Nirmal and Benjakul (2009a) with slight modifications. The powder (50 g) was mixed with 150 mL of the extracting buffer (0.05 M sodium phosphate buffer, pH 7.2, containing 1.0 M NaCl and 0.2% Brij-35). The mixture was stirred continuously at 4°C for 30 min, followed by centrifugation at 8000×g and 4°C for 30 min using a refrigerated centrifuge (GTR10-1, Beijing Era Beili Centrifuge Co., Ltd., Beijing, China). Solid ammonium sulphate was added into the supernatant to obtain 40% saturation and allowed to stand at 4°C for 30 min. The precipitate was collected by centrifugation at 12,500×g at 4°C for 30 min using a refrigerated centrifuge. The pellet obtained was dissolved in a minimum volume of 0.05 M sodium phosphate buffer, pH 7.2 and dialyzed against 15 volume of the same buffer at 4°C with three changes of dialysis buffer. The insoluble materials were removed by centrifugation at 3000×g at 4°C for 30 min and the supernatant was used as “crude PPO extract”.

Determination of PPO activity: The activity of PPO was determined using L-DOPA as a substrate according to the method described by Nirmal and Benjakul (2009b) with a slight modification. The assay system consisted of 100µL of crude PPO extract, 600 µL of 15 mM L-DOPA in deionised water, 400 µL of 0.05 M phosphate buffer (pH 6.0) and 100 µL of deionised water. The PPO activity was determined for 3 min at 45°C by monitoring the formation of dopachrome at 475 nm using a UV spectrophotometer (752, Nanjing Qilin Instruments Co., Ltd., Nanjing, China). One unit of PPO activity was defined as an increase in the absorbance by 0.001 at 475 nm/min/mL. Enzyme and substrate blanks were prepared by excluding the substrate and enzyme, respectively, from the reaction mixture and deionised water was used instead.

Effect of glutathione on the activity of PPO: Glutathione was dissolved in distilled water to various concentrations (0.04, 0.08, 0.12, 0.16, 0.20 and 0.24%, respectively, w/v). Glutathione solution (100 µL) was mixed with crude PPO extract (100 µL) and the mixture was incubated at room temperature for 30 min and then, the assay buffer (400 µL, 0.05 M phosphate buffer, pH 6.0) was added. To initiate the reaction, 600 µL of preincubated 15 mM L-DOPA (45°C) were added. The reaction was conducted at 45°C and the absorbance at 475 nm was monitored for 3 min. The control was run in the same manner, except the deionized water was used instead of glutathione solution. The sample blank was prepared by using distilled water instead of L-DOPA. One unit of PPO activity was defined as that causing an increase in the absorbance by 0.001 at 475 nm/min/mL. Residual activity was determined and expressed as the activity relative to the control (without glutathione) as follows:

$$\text{Relative activity (\%)} = (B/A) \times 100$$

where,

A : PPO activity of control

B : PPO activity in the presence of glutathione (Nirmal and Benjakul, 2009a)

Treatments of mushroom: Fresh mushrooms with uniform size and color were washed with tap water, destemmed and cut into slices with equal thickness using a sharp stainless steel knife. All slice cut from each mushroom was pooled together and treated in one of the dipping solutions (water for control and 0.08% glutathione solution) for 5 min, drained on a clean paper towel, taken for drying and spread over perforated aluminum trays and trays were kept in the drying chamber at different temperatures (50, 55, 60, 65, 70 and 75°C, respectively) for varying time (20, 40, 60, 80, 100, 120, 140, 160 and 180 min, respectively). During the drying, color and moisture content of the sample was recorded.

Color and moisture determination: The color of mushroom slices was measured using a Minolta color imeter (CS-100A, Minolta Co. Ltd., Japan) according to Lu *et al.* (2007) with slight modifications (2007). The degree of browning was expressed by the changes in the Lightness (L) value. Moisture content of mushroom was determined by vacuum oven method.

Statistical analysis: All of the tests were performed in triplicate and data were reported as means±S.D. Analysis of variance and significant differences among means were tested by one-way ANOVA using SPSS software (version 17.0 for Windows, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of glutathione on PPO inhibition: Glutathione is the most abundant non-protein thiol compound present in living organisms and used as a pharmaceutical compound and can be used in food additives and the cosmetic industries (Li *et al.*, 2004). It was used for the inhibition of PPO in apple (Billaud *et al.*, 2004; Brun-Mérimée *et al.*, 2004; Gacche *et al.*, 2004), Loquat Fruit (Ding *et al.*, 1998), litchi fruit (Jiang and Fu, 1998), must (Cheyner *et al.*, 1990) and

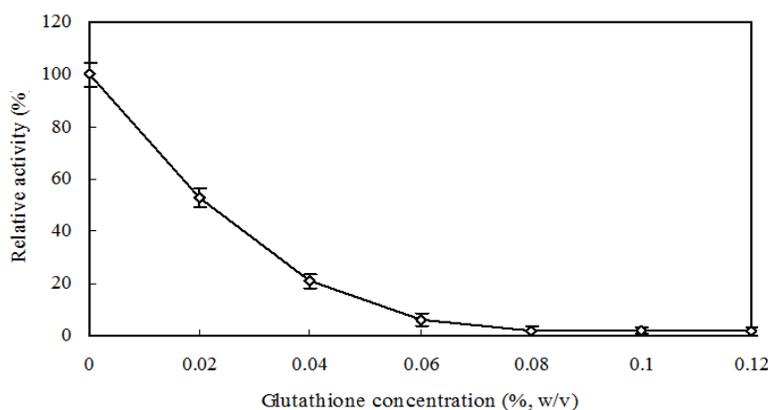


Fig. 1: Effect of glutathione on inhibition of PPO activity of mushroom

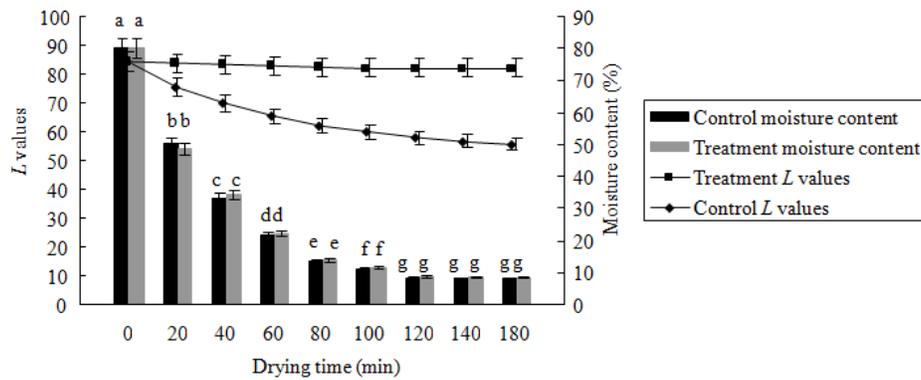


Fig. 2: Effect of glutathione on inhibition of browning formation and moisture content in mushroom during hot air drying at 70°C for varying time

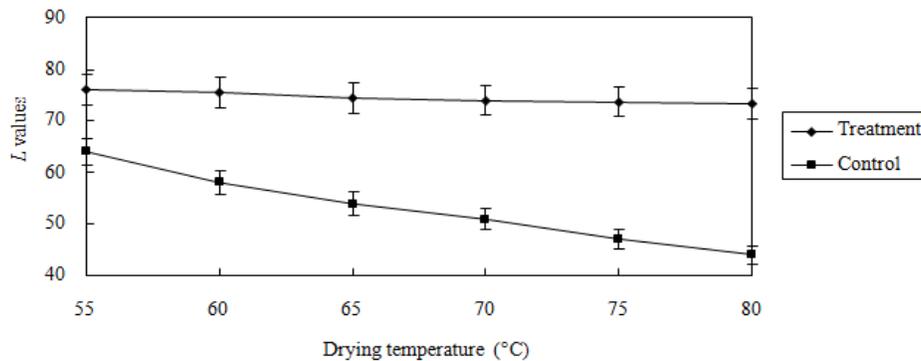


Fig. 3: Effect of glutathione on inhibition of browning formation in mushroom during hot air drying at different temperatures for 120 min

Fruit juices and protein-containing foods (Molnar-Perl and Friedman, 1990).

The effect of glutathione on the inhibition of PPO in mushroom is shown in Fig. 1. The relative activity of PPO decreased sharply with the increase in the level of glutathione up to 0.06% in the reaction mixture and decreased slowly with the amount of glutathione between 0.06 and 0.08%. No decrease in relative activity of PPO was observed when the amount of glutathione was further increased, indicating that at 0.08% of glutathione, all PPO was saturated by the glutathione. It is therefore that the optimum amount of glutathione under this condition was 0.08% (Fig. 1). Therefore, 0.08% (w/v) glutathione, which shows the highest PPO inhibitory effect, was used for further study.

Effect of glutathione on inhibition of browning formation in mushroom during hot air drying:

Browning formation decreases the sensory quality of mushroom products. Recently, many antioxidants have been applied to the anti-browning of mushroom, such as ascorbic acid (Zhang *et al.*, 2004), ascorbic acid derivatives (Hsu *et al.*, 2006), cystein (Dorantes-

Alvarez *et al.*, 1998; Huang *et al.*, 2008), tetra sodium pyrophosphate (Dorantes-Alvarez *et al.*, 1998), Benzoic Acid, Glutathione, EDTA, 4-Hexylresorcinol and Sodium Chloride (Weemaes *et al.*, 1999). However, using glutathione to inhibit browning formation of mushroom during hot air drying is not frequently reported. Effect of glutathione on the anti-browning of mushroom during hot air drying at 70°C for varying time is shown in Fig. 2. Both control and 0.08% glutathione samples showed identical drying characteristics: the moisture content in both samples decreased to the lowest levels in 120 min; there was no significant difference in the final moisture contents between the two samples (~9%, $p > 0.05$). The L values of control and 0.08% glutathione samples were 74 and 51 on 120 min at 70°C, respectively (Fig. 2). The L values of control samples decreased with the decreasing of the moisture contents within 120 min and decreased further after 120 min even the moisture maintained unchanged. However, the L values of 0.08% glutathione samples did not decrease significantly during the whole drying time course ($p > 0.05$).

Effect of glutathione on the anti-browning of mushroom during hot air drying at different temperatures for 120 min is shown in Fig. 3. The L

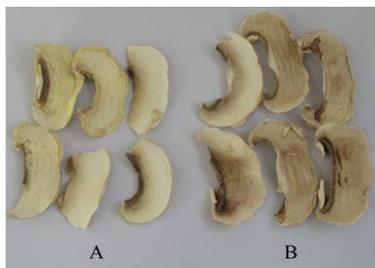


Fig. 4: Color quality of 0.08% glutathione (a) and control (b) samples

values of control samples decreased with the increasing of temperature. In the case of 0.08% glutathione samples, the *L* values did not affected significantly by drying temperature ($p>0.05$). The results indicate that higher drying temperature could be used to dry mushroom pretreated with 0.08% glutathione to get higher drying efficiency. The color quality of control and 0.08% glutathione samples is shown in Fig. 4. For 0.08% glutathione samples, color showed faint yellow features (Fig. 4a), while that of the control got serious gray and brown (Fig. 4b). Results showed that glutathione as anti-browning agent prevented mushroom slices from browning during hot air drying, indicating that glutathione is an effective anti-browning agent. Previous studies indicated that glutathione inhibits browning in apple (Jiang and Fu, 1998). The inhibition of glutathione of mushroom browning coincided with the ability of the treatment to inhibit PPO activity (Fig. 1).

CONCLUSION

Glutathione treatment could be used to inhibit PPO activity in mushroom and the efficacy was associated with dose. PPO activity in mushroom was inhibited by 98.2 with 0.08% glutathione. Pretreatment with 0.08% glutathione could be used to prevent browning from forming in mushroom during hot air drying, while that of the control got serious gray and brown. Therefore, pretreatment with 0.08% glutathione means high quality of dehydrated mushrooms.

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