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Research Paper

Extraction of polyphenol oxidase in shiitake mushrooms (Lentinus edodes) and its enzymatic characteristics

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ABSTRACT: *In this research, polyphenol oxidase in shiitake mushrooms were extracted by various process parameters (PVP addition amount, extraction time, extraction pH, solid-liquid ratio). At the same time, the effects of pH and temperature on the enzymatic characteristics of polyphenol oxidase in shiitake mushrooms were also studied. The research results showed that polyphenol oxidase had the highest activity when 16% PVP was added, the extraction time was 15 minutes, the extraction pH was 7, and the solid-liquid ratio was 1:2. Polyphenol oxidase activity could kept high under condition of pH 5 and 30 ℃*
KEYWORDS: *Polyphenol Oxidase, Extraction, Enzymatic Characteristics*

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I. INTRODUCTION

Shiitake mushrooms contain high nutritional value and are of great help to human body health. But after a period of postharvest, fresh mushrooms will get brown, and this change is mainly caused by the oxidation of polyphenol oxidase for their exposition to the air [1-3]. Phenolic compounds were oxidized by oxygen to quinones substancesin the air, resulted in browning [4,5]. Browning will induce the decrease of mushroom and edible value and commodity value, shorten the shelf life of the mushroom, at the same time also affect the distribution and sale of mushroom [6].

Therefore, in order to clarify the characteristics of polyphenol oxidase in shiitake mushrooms, it is necessary to get the optimal extraction process and process parameters to achieve the purpose of reducing browning phenomenon and improve its commodity value [7,8].

II. MATERIALS AND METHODS

2.1. PVP adding amount optimization

PVP amount was optimized within the range of 8%, 10%, 12%, 14%, 16%, 18%. The optimum adding amount of PVP was analyzed by the enzyme activity accordingly.

2.2. Extraction time optimization

Extraction time range was selected as 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes. Then the optimal extraction time of polyphenol oxidase was analyzed.

2.3. Extraction pH optimization

Extraction pH range was selected as 6, 8, 7, 7.5, 6.5, 8.5. Then the optimal extraction pH of polyphenol oxidase was analyzed.

2.4. Material-liquid ratio

Shiitake mushroom weight:phosphate buffer (material-liquid ratio) was selected as a range of 1:1, 1:2 and 1:3, 1:4, 1:5, 1:6. Then the optimal material-liquid ratio was analyzed.

2.5. Extraction method of polyphenol oxidase in shiitake mushrooms

2g fresh mushroom was mixed with 6ml of phosphate mixture buffer (PBS: pH7.8, containing 5% (w/v) PVP) was put into a precooled mortar for grinding in ice bath, then transferred into a centrifuge tube for low-temperature centrifugation (8000r/min,4℃) for 15min, and the supernatant was taken as crude enzyme extract [9-11].

2.6. Determination of polyphenol oxidase activity of shiitake mushrooms

2.0mL acetic acid buffer with pH4.75 was added with 1.0mL 0.1mol/L pyrocatechol solution as the substrate and 0.5mL crude enzyme extract. Then absorbance at 410nm was recorded every 30 seconds for 3min

continuously [12]. An activity unit (U) was defined as the amount of enzyme required to cause a change in absorbance value of 0.01 per minute under the determination conditions, and the results were expressed as $U \cdot min^{-1} \cdot g^{-1}$ fresh mushroom [13].

III. RESULTS AND DISCUSSION

3.1 The optimal PVP adding amount of polyphenol oxidase in shiitake mushrooms

As can be seen from Fig.1, when the adding amount of PVP is less than 14%, the activity of polyphenol oxidase gradually increased with the increase of the adding amount of PVP, and the effect is relatively obvious. When the adding amount of PVP was between 14% and 16%, with the increase of the addition amount of PVP, the activity of polyphenol oxidase increased little and the growth rate was slow. When the adding amount of PVP exceeded 16%, the activity of polyphenol oxidase decreased with the increase of the adding amount of PVP. The reason for this phenomenon could be explained for PVP is a kind of surfactant, appropriate amount of PVP can promote the extraction effect [14]. Therefore, in a certain range, the PVP can increase the activity of polyphenol oxidase. However, excessive addition of PVP will inhibit the activity of polyphenol oxidase, so when the amount of PVP exceeded 16%, the activity of polyphenol oxidase will decrease. Therefore, the optimal adding amount of PVP is 16%.

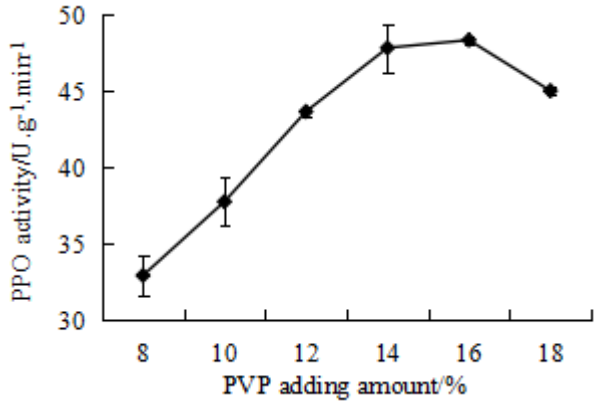

Fig.1 Effect of PVP adding amount on PPO extraction

3.2 influence of extraction time on the extraction effect of lentinus edodes polyphenol oxidase

It can be seen from Fig.2 that different extraction time had different effects on the extraction of polyphenol oxidase in shiitake mushrooms. When extraction time is between 5 minutes and 15 minutes, with the increase of extraction time, the activity of polyphenol oxidase gradually increased. However, the activity of polyphenol oxidase decreased when the extraction time was over 15 minutes. Between 15 and 20 minutes, the activity of polyphenol oxidase decreased slowly with the increase of time. The activity of polyphenol oxidase decreased rapidly after 20 minutes extraction. The reason may be that in a certain period of time, with the increase of extraction time, the polyphenol oxidase in shiitake mushroom is gradually extracted, so the activity of polyphenol oxidase can be measured to increase gradually, until the maximum extraction of polyphenol oxidase, the highest enzyme activity can be measured.After this time, some other proteins or impurities in the mushrooms may be extracted, thus reducing its enzyme activity. Therefore, the optimal extraction time is 15 minutes.

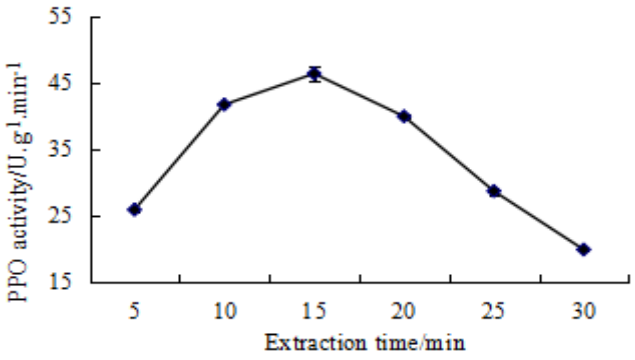

Fig.2 Effect of extraction time on PPO extraction

3.3 Effect of extraction pH on the extraction effect of polyphenol oxidase in shiitake mushrooms

As can be seen from Fig.3, the extraction pH had a great influence on the extraction effect of polyphenol oxidase in shiitake mushrooms. When the pH value was between 6 and 7, with the increase of pH value, the activity of polyphenol oxidase gradually increased, and the enzyme activity increased rapidly between pH 6.5 and 7. The activity of polyphenol oxidase reached the maximum when pH was 7. After pH exceeded 7, the activity of polyphenol oxidase gradually decreased with the increase of pH. Acidity has a greater effect on the activity of polyphenol oxidase than alkali [15]. The reason may be that the polyphenol oxidase is cuprase containing copper, which is easy to be released under acidic conditions. Strong alkaline conditions can partially denatured the polyphenol oxidase, leading to the decrease of enzyme activity [16-18]. Therefore, the optimal extraction pH of polyphenol oxidase in shiitake mushrooms was 7.

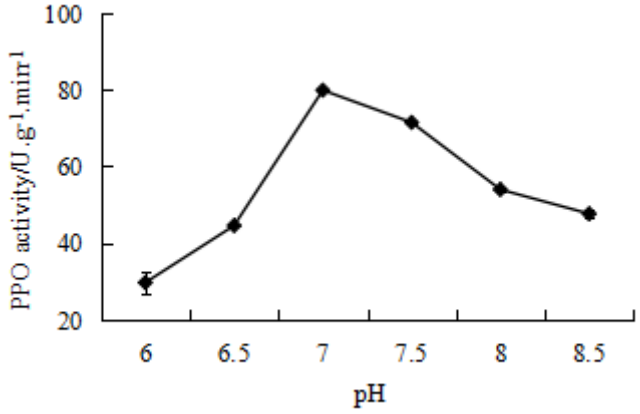

Fig.3 Effect of extraction pH on PPO extraction

3.4 Effect of the ratio of material to liquid on the extraction effect of polyphenol oxidase in shiitake mushrooms

As can be seen from Fig. 4, when the ratio of material to liquid is between 1:1 and 1:2, the activity of polyphenol oxidase gradually increased with the increase of material-liquid ratio. When the material-liquid ratio was 1:2, the activity of polyphenol oxidase reached the maximum value. When the material-liquid ratio was between 1:2-1:6, the activity of polyphenol oxidase decreased. The reason may be that with the increase of material-liquid ratio, the concentration of enzyme was diluted and more hetero-proteins were extracted from mushrooms, leading to the decrease of polyphenol oxidase activity [19,20]. Therefore, material-liquid ratio of 1:2 was selected as the optimal material-liquid ratio for the extraction of polyphenol oxidase in shiitake mushrooms.

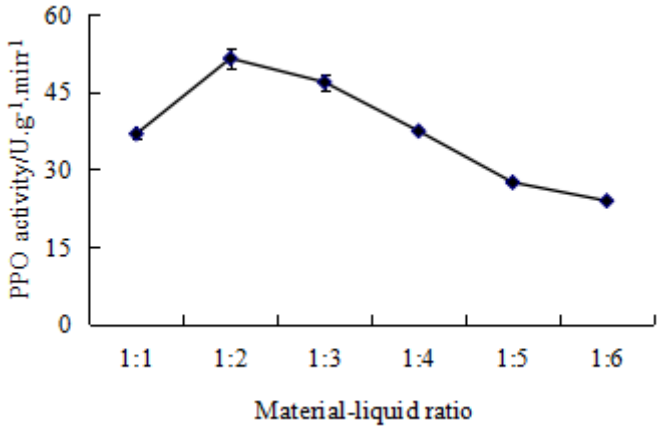

Fig.4 Effect of material-liquid ratio on PPO extraction

3.5 Effect of pH on polyphenol oxidase activity of shiitake mushrooms

As can be seen from Fig.5, pH had a great influence on polyphenol oxidase in shiitake mushrooms. When pH was 3.0, the activity of polyphenol oxidase is very low. When pH was from 3.0 to 3.5, the activity of polyphenol oxidase gradually increased with the increase of pH value. When pH was 3.5-5.0, the activity of polyphenol oxidase increased rapidly. When pH was 5.0-8.0, the activity of polyphenol oxidase decreased with

the increase of pH. Polyphenol oxidase decreased rapidly when pH was 5.5. The activity of polyphenol oxidase reached the maximum when pH was 5.0. Therefore, the optimal pH value of polyphenol oxidase is 5.0.

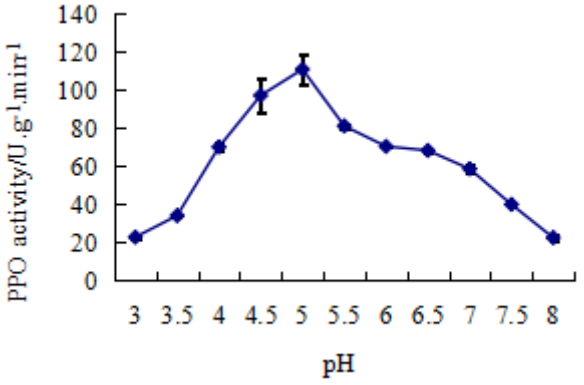

Fig. 5 Effect of pH on PPO activity of shiitake mushrooms

3.6 Effect of temperature on polyphenol oxidase activity of lentinus edodes

As shown in Fig. 6, temperature had a significant effect on the activity of polyphenol oxidase.When the temperature was 30℃, the activity of polyphenol oxidase reached the maximum. When the temperature was between 30℃ and 70℃, the activity of polyphenol oxidase decreased gradually with the increase of low temperature. And when the temperature was between 30℃ and 40℃, the activity of polyphenol oxidase decreased slowly with the increase of temperature. When the temperature was 40℃-60℃, the activity of polyphenol oxidase decreased rapidly with the increase of temperature. When the temperature was 70℃, the activity of polyphenol oxidase was very low. Therefore, the optimum temperature of polyphenol oxidase was 30℃.

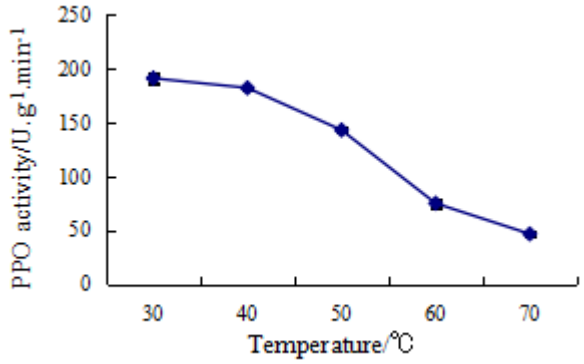

Fig. 6 Effect of temperature on PPO activity of shiitake mushrooms

IV. CONCLUSION

In this research, the optimal extraction conditions of polyphenol oxidase were analyzed and obtained. The enzymatic characteristics of polyphenol oxidase were studied, and the following results were obtained:

16% PVP was added to the extraction buffer, the pH of the extraction buffer was 7.0, the extraction time was 15 minutes, and the ratio of material to liquid was 1:2. The extraction was carried out according to the extraction method of polyphenol oxidase, and the polyphenol oxidase activity was the highest.The study on the enzymatic characteristics of polyphenol oxidase showed that the activity of polyphenol oxidase is affected by temperature and pH, and the optimal pH and temperature of polyphenol oxidase were 5.0 and 30℃respectively.

ACKNOWLEDGEMENT

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