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Nitric oxide fumigation stimulates flavonoid and phenolic accumulation and enhances antioxidant activity of mushroom

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ABSTRACT

The effects of nitric oxide (NO) on antioxidant activity and contents of phenolics and flavonoids in mushroom *Russula griseocarnosa* were investigated. Freshly harvested mushrooms were fumigated with 0, 10, 20 and 30 μL L^{-1} NO at 20 °C for 2 h and then taken to examine the antioxidant activities using assays of reducing power, chelating effect on ferrous ions, scavenging effect on hydroxyl free radicals, and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity. The results showed that the antioxidant activities of the mushrooms fumigated with NO were significantly increased when compared to the controls. Moreover, NO fumigation significantly enhanced phenolic and flavonoid contents and stimulated the activities of phenylalanine ammonia-lyase and chalcone synthase. The results indicated that NO fumigation might have potential application for enhancing the bioactive compounds and improving antioxidant activities in the mushrooms. Furthermore, the data suggested that the NO-induced phenolic and flavonoid accumulation was due to the activation of the biosynthetic pathways in the mushrooms.

1. Introduction

The consumption of plant foods that contained antioxidants can help to prevent various diseases such as cancer, cardio- and cerebrovascular diseases and neurodegenerative disorders (Aquilano, Baldelli, Rotilio, & Ciriolo, 2008; Halliwell & Gutteride, 1984). This protection is mainly related to the capacity of antioxidants in plant foods to scavenge free radicals which are considered to be responsible for the oxidative damage of lipids, proteins, and nucleic acids (Bimla & Punita, 2006). Phytochemicals, such as phenolics and flavonoids in fruit and vegetables, are suggested to be one of the major bioactive compounds for health benefits (Mallavadhani et al., 2006).

Mushrooms have long been consumed in China and other countries, and are becoming more and more important in our diet for their nutritional value, including high protein and low fat/energy contents (Mattila, Suonpää, & Piironen, 2000). Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes, steroids, etc. (Kim et al., 2008). Recently, mushrooms have become attractive as functional foods and as source of physiologically beneficial medicines, while being devoid of undesirable side effects (Kim et al., 2008). Many of the biological functions, such as anticancer, antiviral, immunopotentiating, and hypolipidemic activities, are considered to be attributed to their free radical scavenging and antioxidant activity. Therefore, the secondary compounds with antioxidant activities such as phenolics and flavonoids in mushrooms are of great interest as possible protective agents to help human health.

It has been well characterized that biosynthesis and accumulation of secondary metabolites in plants are mediated by the endogenous signaling systems in which nitric oxide (NO) has been reported to play critical roles (Hahlbrock et al., 2003; Xu, 2007). NO has been reported to be involved in mediating UV-B-induced flavonoids accumulation in *Betula pendula* leaves (Zhang, Dong, Jin, Sun, & Xu, 2011), fungal elicitor-induced hypericin biosynthesis in *Hypericum perforatum* suspension cells (Xu, Dong, & Zhu, 2005a), methyl jasmonate-induced secondary metabolite production of *Taxus* cell suspension cultures (Wang & Wu, 2005), and ozone induces flavonol production of *Ginkgo biloba* cells (Xu et al., 2011). In previous studies, we reported that exogenous application of NO stimulated catharanthine biosynthesis in *Catharanthus roseus* suspension cells (Xu, Dong, & Zhu, 2005b). These results suggest that NO might be an efficient elicitor to induce secondary metabolite accumulation in plant cells. However, little information is available about the effect of NO on the accumulation of antioxidant compounds in mushrooms.

The objective of this work was to examine the effect of NO on the contents of phenolics and flavonoids and the antioxidant activity in mushroom *Russula griseocarnosa*. The results show that

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postharvest NO figuration significantly enhances the contents of phenolics and flavonoids and stimulates the antioxidant activity in the mushroom. Furthermore, the data suggest that NO-induced secondary compound accumulation might be due to the activation of the biosynthetic pathways in the mushrooms.

2. Materials and methods

2.1. Plant material and NO fumigation

Mushrooms (*R. griseocarnosa*) were harvested in September, 2010 from a farm in Hangzhou, Zhejiang province, China, and transported to the laboratory immediately after harvest. Then, 750 homogeneous mushrooms with uniform size and maturity, and free of visual blemishes were selected, from which three replicates of 10 mushrooms were used to determine the characteristics at harvest, and the remained 720 mushrooms were randomly divided into four lots for the following treatment: control (no treatment) and NO figuration at 10, 20 and 30 μl L^{-1} concentrations. Treatments were performed by fumigating the mushrooms in a sealed plastic container (50 l) with different concentrations of NO (10, 20 and 30 μl L^{-1}) for 2 h at ambient temperature (20 °C). Different concentrations of NO were obtained from a cylinder containing 4500 μl L^{-1} NO in nitrogen (Hangzhou New Century Mixed Gas Co., Ltd., China) and injected into the container using a 50 ml syringe through an injection port on the container lid. Following treatments, both control and treated mushrooms were sorted into samples, each one composed of 10 mushrooms and stored at 20 °C and approx. 95% Relative humidity at temperature-control chamber for up to 6 days. During storage, 3 samples of 10 mushrooms were taken at random every day for measurements of antioxidant activities and phenolic and flavonoid contents respectively.

2.2. Preparation of methanolic extract of mushrooms

Fruit bodies of mushrooms with different treatments were collected and cleaned to remove any residual compost/soil and subsequently air-dried in the centre of oven at 55 °C to constant weight (5 h). All of the dried mushrooms were ground to fine powder and passed through a 40 mesh sieve. One gram of each of dried mushroom samples was mixed with 30 ml of methanol. Samples were stirred for 1 h for effective extraction and centrifuged at 2000xg for 15 min. Supernatants (methanolic extracts) were stored at 4 °C until the completion of the analysis of antioxidant activity and contents of total flavonoids and total phenolics.

2.3. Assays of antioxidant activity

2.3.1. Determination of reducing power

The reducing power was determined according to the method of Oyaizu (1986) with minor modification. Briefly, 2.5 ml of mushroom methanolic extracts were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After 2.5 ml of 10% trichloroacetic acid (TCA) (w/v) had been added, the mixture was centrifuged at 2000g for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. BHT and ascorbic acid were used as standards. A higher absorbance indicates a higher reducing power.

2.3.2. DPPH radical scavenging assay

The capacity of the mushrooms with different treatments to scavenge free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method reported (Hatano, Kagawa, Yasuhara, & Okuda, 1988) with minor modification. Briefly, 0.3 ml of methanolic extract of mushrooms was mixed with 2.7 ml of a methanolic solution containing DPPH radicals (6 μmol /l). The mixture was shaken vigorously and kept for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was measured by monitoring the decrease of absorption at 517 nm continuously. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: RSA (%) = $100(1 - A_C/A_D)$, where A_C is the absorbance of the solution with methanolic extract of the mushrooms and A_D is the absorbance of the DPPH solution.

2.3.3. Scavenging effect on hydroxyl free radicals

·OH was generated by the Fenton reaction and detected by its ability to salicylic acid as described by Smirnoff and Cumbes (1989). The reaction mixture contained, in a total volume of 4 ml, 9 mM $FeSO_4$ (1 ml), 9 mmol/l salicylic acid (1 ml), 8.8 mmol/l H_2O_2 (1 ml), and 1 ml mushroom methanolic extract. One milliliter of methanol instead of sample served as control, and the absorbance was measured spectrophotometrically at 510 nm.

2.3.4. Chelating effect on ferrous ions

The chelating effect of ferrous ions of the mushrooms was examined by the method of Decker and Welch (1990), with some modifications. Briefly, 0.5 ml of mushroom methanolic extracts was mixed with 0.05 ml of 2 mmol/l $FeCl_2$ and 0.2 ml of 5 mmol/l ferrozine. Total volume was diluted with 2 ml of methanol. Then, the mixture was shaken vigorously and kept at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the formula:

$$\text{chelating effect}(\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the ferrozine–Fe^{2+} complex and A_{sample} is the absorbance of the mushroom extract. BHT and TBHQ were used as standards.

2.4. Measurement of total phenolics

The contents of total phenolic contents of the mushrooms were determined using Folin–phenol methods (Folin & Ciocalteu, 1927) with some modification described by Singleton, Orthofer, and Lamuela-Raventos (1999). For the preparation of the calibration curve, l ml aliquot of 0.024, 0.048, 0.072, 0.096, 0.12, and 0.144 mg/ml methanolic gallic solution was mixed with 5 ml Folin–Ciocalteu reagent and 4 ml sodium carbonate (75 g/l). The absorbance at 765 nm was measured after 1 h at 20 °C and the standard curve was drawn. To the same reagent (Folin–Ciocalteu reagent and sodium carbonate), 1 ml methanolic mushroom extract was mixed as described above and the absorbance at 765 nm was measured after 1 h. The total phenolic content in the mushroom was expressed as gallic acid equivalent in milligrammes per gramme of sample.

2.5. Determination of total flavonoids

Flavonoids were expressed as rutin equivalents. Rutin was used to make the calibration curve (0.012, 0.024, 0.048, 0.072 and 0.096 mg/ml in 70% ethanol (v/v)). Total flavonoids were determined using a method described by Lin and Tang (2007) with minor modification. Briefly, the standard solutions or extracts (1 ml) were mixed with 0.3 ml of 5% $NaNO_2$ (w/v). After 6 min, 0.1 ml of 10% Al $(NO_3)_3$ (w/v) were mixed and allowed to stand for another

6 min. Afterwards, 4 ml of 4% NaOH were mixed and then diluted with 70% ethanol (v/v) to 10 ml. After standing for 12 min, the reaction mixture absorbance was measured at 510 nm. Total flavonoid content was expressed as milligrammes of rutin equivalents per g of extract.

2.6. Measurement of PAL and CHS activities

The activity of phenylalanine ammonia-lyase (PAL) was determined as the method reported (Tanak, Kojima, & Uritani, 1974). Five grammes of fresh samples were homogenized in 20 ml of borate buffer (0.1 mol/l, pH 8.0) containing 5 mmol/l β-mercaptoethanol and 2 mmol/l EDTA. Homogenate was centrifuged with 2000x*g* for 20 min and the supernatant was collected for enzyme assay. For determining PAL activity, 0.5 ml of supernatant was incubated for 1 h at 30 °C in 2 ml of 0.1 mol/l borate buffer (pH 8.0) containing 1 ml of L-phenylalanine (0.1 mmol/l, pre-dissolved in 0.01 mol/l borate buffer at pH 8.8). The increase in OD_{290nm} due to the formation of trans-cinnamate was measured spectrophotometrically. PAL activity was expressed as change in OD_{290nm} h^{-1} mg^{-1} protein. Protein was assayed with bovine serum albumin as the standard by the dye-binding method of Bradford (1975).

Chalcone synthase (CHS) activity was measured as the method reported by Kreuzaler and Hahlbrock (1975) with minor modification. Briefly, 0.08 ml of the enzyme extract was pre-incubated for 1 min at 30 °C and then treated with 0.02 ml of CoA-mixture and incubated for 5 min at 30 °C. The CoA-mixture was prepared by mixing 50 μmol/l *p*-coumaroyl-CoA with 42.5 μmol/l [2-^{14}C]-malonyl-CoA (14.7 μCi/μ mol) and its pH was adjusted to 8.0 with KOH, prior to adding the enzyme extract. The reaction was terminated by adding 0.2 ml ethylacetate containing 5 μg naringenin, mixed and then centrifuged (12,000x*g*, 10 min, 25 °C). The organic layer (150 μl) was collected and evaporated. The residue was dissolved in 10 μl ethylacetate and spotted onto an Avicelcellulose thin-layer chromatography plate. Thin-layer chromatography was conducted using 5% 2,5-diphenyloxaazole and 0.025% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene. Radioactivity was measured with a liquid scintillation counter, Model XH27-FJ-2017P (ZHONGTAI Instruments, Beijing). CHS activity was expressed as pkat mg^{-1} protein. Protein was assayed with bovine serum albumin as the standard by the dye-binding method of Bradford, 1976.

2.7. Statistics

Experiments were performed using a completely randomized design. Data from experiments were analyzed by *t*-test for simple comparisons between each treatment and its control taken at the same time point and Tukey test for the multiple comparisons between means. The assumptions of analysis of variance were considered to be statistically significant at $P < 0.05$.

3. Results and discussion

3.1. NO fumigation enhances phenols and flavonoids contents in mushroom

Flavonoids and phenolics are considered to be beneficial antioxidants as they exhibit scavenging activity of harmful active oxygen species (Wootton-Beard & Ryan, 2011). The effects of NO on phenolic and flavonoid contents in the mushrooms have been examined in this work. As shown in Fig. 1, the contents of phenolics and flavonoids in the mushroom fumigated with NO were significantly increased 3 days after treatment ($P < 0.05$), while phenolic and flavonoid contents in the mushroom without NO treatment

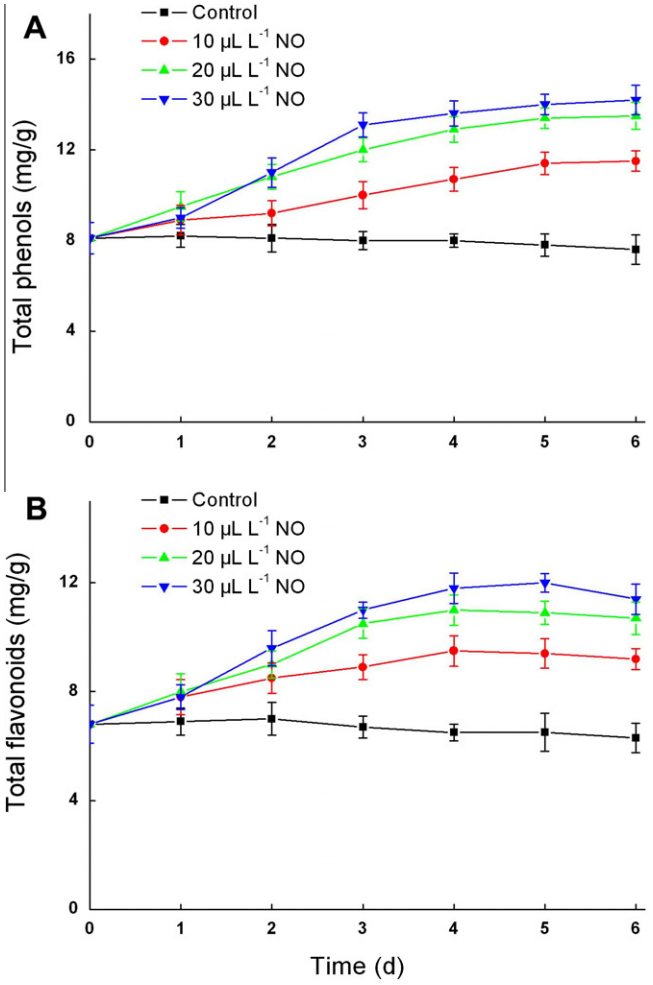

Fig. 1. Effect of NO on total phenolics (A) and total flavonoids (B) in the mushrooms. Mushrooms that were fumigated with different concentrations of NO were taken as the time indicated in the figure to determine total phenolic and total flavonoid contents. Mushrooms without NO treatment served as control. Data are means ± SE of three replicates.

(control) remained relatively constant during the same period (Fig. 1A and B), showing that the increases of phenolic and flavonoid contents in NO-treated mushrooms were not due to the development-dependent changes in these compounds. Therefore, our results indicated that NO fumigation stimulated phenolic and flavonoid accumulation in the mushroom. Moreover, the results showed that the contents of total flavonoids and total phenolics of the mushrooms treated with 20 and 30 μl L^{-1} NO were higher than those treated with 10 μl L^{-1} NO (Fig. 1A and B), which suggested that the effects of NO on flavonoid and phenolic contents of the mushrooms were dose-dependent. The data indicated that 20 and 30 μl L^{-1} NO were optimal for enhancing flavonoid and phenolic accumulation of the mushrooms.

Accumulation of secondary metabolites such as flavonoids and phenolics is one of the common responses of plants to many biotic and abiotic stresses. For example, UV-B irradiation and ozone fumigation have been reported to stimulate flavonoid accumulation in plant cells (Xu et al., 2011; Zhang et al., 2011). It has been well characterized that the stress-induced secondary metabolite accumulation in plant cells is mediated by the endogenous signaling, such as jasmonic acid (JA) and peroxide hydrogen (H_2O_2) (Hahlbrock et al., 2003; Xu et al., 2005a). Therefore, plant-derived signal molecules have been used for eliciting secondary metabolite production in plant cells. For example, JA has been used for

improving alkaloid production of *Catharanthus roseus* hairy root (Ruiz-May et al., 2011). NO has emerged as a key signaling molecule in plants recently. The role of NO in plant defense signaling has been extensively investigated. NO has been reported to be involved in mediating UV-B-induced flavonoid accumulation in *Betula pendula* Roth (silver birch) leaves (Zhang et al., 2011). Recently, Jiang et al. (2011) reported that treatment of button mushroom (*Agaricus bisporus*) with 2,2-(hydroxynitrosohydrzino)-bisethanamine (DETANO), a NO donor, promoted the accumulation of phenolics. Together with the results of the present work, it is suggested that NO might be an efficient elicitor for improving flavonoid and phenolic contents in mushrooms.

3.2. Effect of NO fumigation on antioxidant activity of mushrooms

Mushrooms are well characterized to be of antioxidant activity. This is mainly correlated with their phenolic and flavonoid compounds (Chen, Xia, Zhou, & Qiu, 2010). The mechanisms of action of flavonoids and phenolics are through scavenging and chelating process or act as free radical terminators (Cook & Samman, 1996). Our above results indicated that NO fumigation significantly enhanced the contents of flavonoids and phenolics in the mushroom. It is, therefore, presumed that NO treatment may stimulate the antioxidant activities of the mushroom. In order to check this presumption, the effects of NO on the antioxidant activities of the mushrooms were examined, using several chemical and biochemical assays: reducing power (measuring the conversion of a Fe^{3+}–ferricyanide complex to the ferrous form), scavenging activity on DPPH radicals (measuring the decrease in DPPH radical absorption after exposure to radical scavengers), metal chelating (by measuring colour reduction in the Fe^{2+}–ferrozine complex), and scavenging effect on hydroxyl free radicals (measured by the color intensity of the Fenton reaction system).

Fig. 2A shows the reducing power of the methanolic extracts from the mushrooms treated with NO. The reducing capacity of a compound Fe^{3+}/ferricyanide complex to the ferrous forms may serve as a significant indicator of antioxidant capacity (Meir, Kanner, Akiri, & Philosoph-Hadas, 1995). In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of samples. The presence of reducers (antioxidants) causes the reduction of the Fe^{3+}–ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration (Chen et al., 2010). As shown in Fig. 2A, the reducing powers of methanolic extracts from the mushroom pretreated with NO increased gradually after treatments, while the reducing powers of methanolic extracts from the mushroom without NO treatment decreased slightly during the same period. The results indicated that treatment of NO enhanced the reducing powers of the mushroom. Moreover, the data showed that the reducing powers of the mushroom treated with 20 and 30 μl L^{-1} NO were higher than those treated with 10 μl L^{-1} NO (Fig. 2A), which suggested that the effects of NO on reducing power of the mushrooms were dose-dependent. However, the reducing powers of the mushrooms did not increase significantly when NO concentration was elevated from 20 to 30 μl L^{-1} (Fig. 2A), which suggest that treatment with high dose of NO might have saturation effect on reducing powers. Our results showed that 20 and 30 μl L^{-1} NO were optimal for

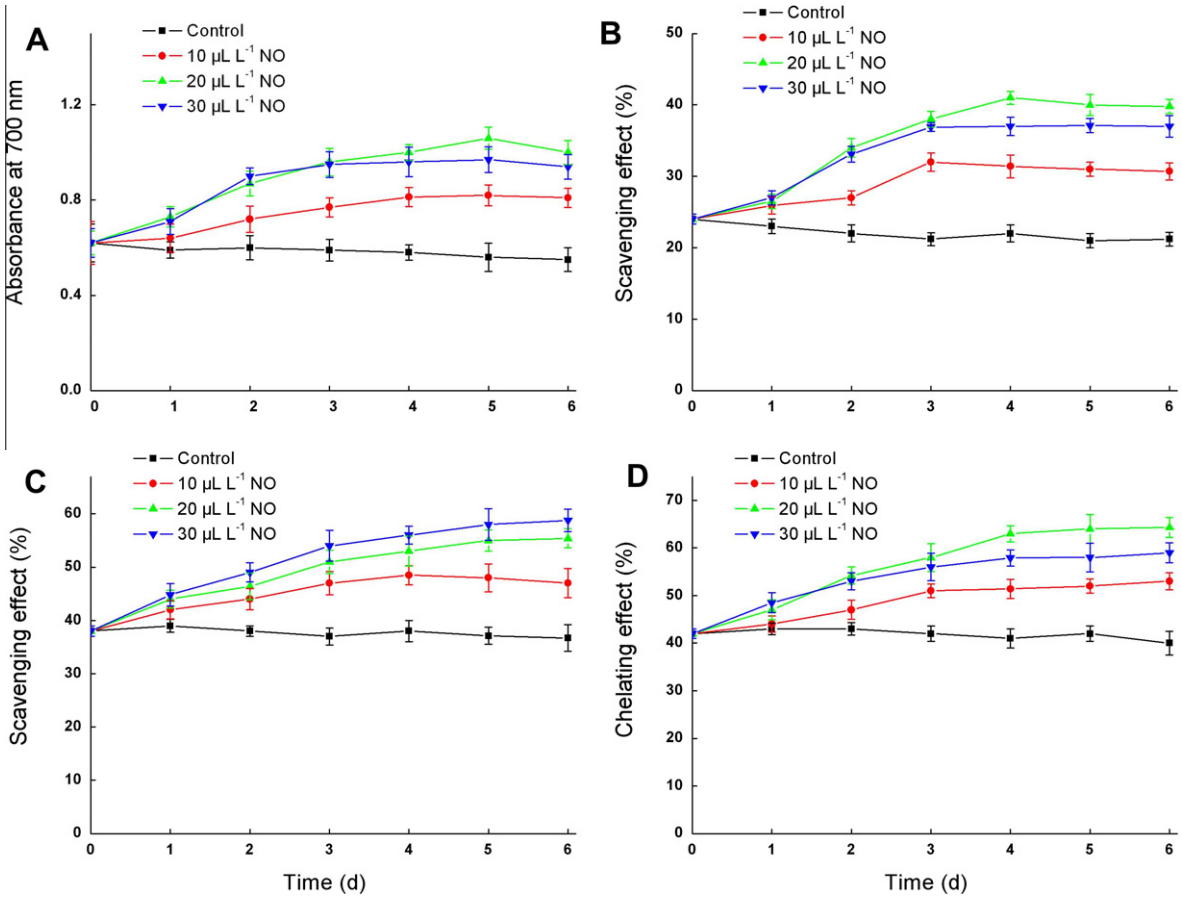

Fig. 2. Effect of NO on reducing power (A), radical scavenging effect (B), ·OH scavenging activity (C), and chelating capacity (D) of the mushrooms. Mushrooms that were fumigated with different concentrations of NO were taken as the time indicated in the figure to determine the antioxidant activities. Mushrooms without NO treatment served as control. Data are means ± SE of three replicates.

enhancing the reducing powers of the mushrooms. In order to examine the relationship between the enhanced reducing powers and the NO-stimulated phenol and flavonoid accumulation in the mushrooms, we analyzed the correlation between the reducing powers and the contents of phenols and flavonoids in the mushroom fumigated with 20 μl L^{-1} NO. The highly positive correlation between the reducing powers and the contents of phenols (R^2 = 0.9780) and flavonoids (R^2 = 0.9702) indicated that phenolic and flavonoid compounds contributed significantly to the enhanced reducing powers in the NO-fumigated mushrooms.

DPPH· is a stable free radical and was widely used to study the radical scavenging effects of extracts. Unlike other free radicals such as hydroxyl radical and superoxide anion, DPPH· has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004). A freshly prepared DPPH solution exhibits a deep purple colour with absorption maximum at 517 nm. The purple colour generally fades or disappears when an antioxidant is present in the medium. Thus, the decrease in absorbance is taken as a measure of the extent of radical scavenging. The scavenging effects of methanolic extracts from the mushrooms on the DPPH radical were examined in this work. The results indicated that the scavenging effects of the mushrooms treated with NO were significantly increased when compared with those of the control (P < 0.05, Fig. 2B), showing that NO treatment stimulated the scavenging effects of the mushroom on DPPH radical. Moreover, the data showed that the extracts obtained from the mushroom treated with 20 and 30 μl L^{-1} NO scavenged DPPH radicals by higher percentages than extracts from the mushroom treated 10 μl L^{-1} NO (Fig. 2B), which suggested that treatment of 20 and 30 μl L^{-1} NO might be optimal for enhancing the scavenging effects of the mushroom on DPPH radical. Furthermore, the correlation between the DPPH· scavenging effects and the contents of phenols and flavonoids in the mushroom fumigated with 20 μl L^{-1} NO was analyzed. The highly positive correlation between the DPPH· scavenging activities and the contents of phenols (R^2 = 0.9625) and flavonoids (R^2 = 0.9776) showed that phenolic and flavonoid compounds contributed significantly to the enhanced DPPH· scavenging capacities in the NO-fumigated mushrooms.

The ·OH scavenging activity of mushroom extracts was assessed by the ability to compete with salicylic acid for ·OH radicals in the ·OH generating/detecting system. As shown in Fig. 2C, the ·OH scavenging activities of the mushrooms treated with NO significantly increased when compared with those of the control, 2 days after NO treatment (P < 0.05). The results indicated that NO treatment promoted the ·OH scavenging activities of the mushrooms. The correlation between the ·OH scavenging activities and the contents of phenols and flavonoids in the mushroom fumigated with 20 μl L^{-1} NO was analyzed. The highly positive correlation between the ·OH scavenging activities and the contents of phenols (R^2 = 0.9914) and flavonoids (R^2 = 0.9572) suggested that phenolic and flavonoid compounds contributed significantly to the enhanced ·OH scavenging activities in the NO-fumigated mushrooms.

The binding of ferrous ions by methanolic extracts from mushroom was estimated. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator (Elmastas et al., 2006). As shown in Fig. 2D, the formation of the Fe^{2+}–ferrozine complex was prevented by methanolic extracts of the control mushroom, indicating that the extracts of mushroom had a marked capacity for iron binding, which is in agreement with the results reported previously (Chen et al., 2010). The absorbance of the Fe^{2+}–ferrozine complex in the presence of methanolic extracts from mushroom treated with NO was significantly lower than that of the control and the chelating effect was significantly increased thereby (Fig. 2D), showing that NO treatment enhanced chelating capacity of the extract of mushroom. The correlation between the chelating capacities and the contents of phenols and flavonoids in the mushroom fumigated with 20 μl L^{-1} NO was analyzed. The highly positive correlation between the chelating capacities and the contents of phenols (R^2 = 0.9914) and flavonoids (R^2 = 0.9572) in the NO-fumigated mushrooms indicated that phenolic and flavonoid compounds contributed significantly to the enhanced chelating capacities in the NO-fumigated mushrooms.

Together, our data suggested that NO fumigation significantly improved the antioxidant capacities of the mushrooms. The antioxidant properties were evaluated using the extract of the mushrooms, which is a complex mixture of phytochemicals with additive and synergistic effects. It has been reported that the reducing power, DPPH·-scavenging activity, hydroxyl free radicals, and Fe^{2+}-chelating extent are mainly related to the contents of total phenols and flavonoids in mushroom. Though other antioxidants were probably present in the mushroom extract, the amounts of ascorbic acid, β-carotene, and lycopene found in the mushroom extract were very low (Chen et al., 2010). Therefore, phenolic compounds and flavonoids are considered to make a significant contribution to the mushrooms' antioxidant activity (Chen et al., 2010). Our results showed that NO treatment significantly enhanced phenolic and flavonoid contents in the mushrooms and the antioxidant activities in the NO-fumigated mushrooms were highly correlated with the contents of phenols and flavonoids.

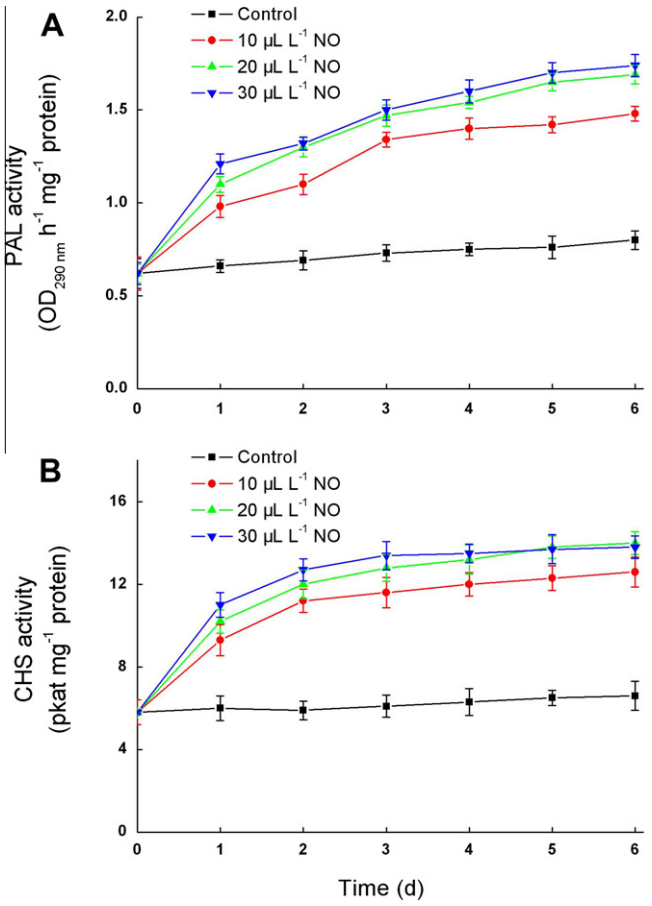

Fig. 3. Effect of NO on the activities of PAL (A) and CHS (B) in the mushrooms. Mushrooms that were fumigated with different concentrations of NO were taken as the time indicated in the figure to determine the enzyme activities. Mushrooms without NO treatment served as control. Data are means ± SE of three replicates.

Thus, it is suggested that NO fumigation may stimulate the antioxidant activities of the mushrooms by enhancing phenolic and flavonoid contents.

3.3. Effect of NO fumigation on PAL and CHS activities

Phenolic and flavonoid compounds are synthesized via the shimimate–phenylpropanoid–flavonoids pathways in plants (Tsai, Harding, Tschaplinshi, Lindroth, & Yuan, 2006). Phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) are key enzymes in the biosynthetic pathways (Tsai et al., 2006). The effect of NO on PAL and CHS activities in the mushrooms has been examined in this work. As shown in Fig. 3A and B, the activities of the mushrooms fumigated with NO rapidly increased when compared with the control, showing that NO fumigation triggered the key enzymes of the biosynthetic pathways in the mushroom.

Plant secondary metabolites are generally associated with plant defense responses against herbivores and pathogens and involved in a broad array of ecological functions (Tsai et al., 2006). Therefore, the biosynthesis of secondary metabolites in plants is easily affected by environmental factors. For example, the expression of *PAL* and *CHS* genes and the accumulation of flavonols in plants can be elicited by UV-B irradiation (Zhang et al., 2011). The results of the present work indicated that NO fumigation significantly enhanced PAL and CHS activities in the mushroom, showing that NO might be an effective elicitor for activating phenylpropanoid–flavonoids pathways in the mushroom. Together with the results of Fig. 1, our data suggested that NO fumigation may stimulate the accumulation of phenolic and flavonoid compounds in the mushroom by activating the biosynthetic pathways.

4. Conclusion

In conclusion, the results indicate that postharvest NO figuration stimulates phenolic and flavonoid accumulation and enhances the antioxidant activities in the mushroom, which suggests that NO fumigation might have potential application for enhancing the bioactive compounds and improving the antioxidant activities in the mushroom. Moreover, our data demonstrate that the NO-induced flavonoid and phenolic accumulation might be due to the activation of phenylpropanoid–flavonoids pathways in the mushroom.

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