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Effects of nitric oxide treatment on flavour compounds and antioxidant enzyme activities of button mushroom (Agaricus bisporus) during storage

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Received 26 May 2020; Revised 14 July 2020; Editorial decision 15 July 2020.

Abstract

Button mushroom (Agaricus bisporus) is sold well for its unique flavour and nutritional benefits. However, the mushroom flavour deteriorates quickly during storage because of its delicate structure and high moisture. In this study, the effects of nitric oxide (NO) application on flavour compounds and antioxidant enzyme activities of stored button mushrooms were investigated. The button mushrooms were immersed in the NO donor sodium nitroprusside (15 μmol/L) for 3 min and then stored under the condition of 4 °C, 90% relative humidity for 12 days. Results showed that the treated mushrooms have reduced weight loss rate, uniform white colour, and higher firmness during storage. Compared to the control, the ketones, alcohols, esters, and aldehydes in the NO-treated button mushroom increased sharply at 3 days of storage and then showed a continuing decline trend, except ester compounds which reached the peak value at 6 days of storage. In addition, NO treatment increased the total phenolics and catalase activity and inhibited the polyphenol oxidase activity in the stored button mushroom. These results indicated that NO treatment is an alternative storage technology to enhance antioxidant capacity and maintain flavour and consumer acceptance of stored button mushroom.

Key words: button mushroom; nitric oxide; flavour; antioxidant enzyme activity; postharvest storage.

Introduction

Button mushroom is a nutrient-rich edible fungus that sells well in the international and domestic markets. In recent years, the production and consumption of button mushroom continued to grow not only for the abundant nutrients (e.g. amino acids, polysaccharides, and vitamins) and health-promoting phytochemicals (e.g. phenolics, terpenes, and sterols), but also for its unique flavour (Mau et al., 2002; Geosel et al., 2011; Elena et al., 2015; Papoutsis et al., 2020). The smell of button mushroom is characterized by flavour compounds, particularly C8 compounds. It has been reported that 1-octen-3-ol, a dominant C8 component, contributed to the typical mushroom smell (Lin et al., 2017). There is an increasing consumption in mushrooms in our daily diet for its nutritional value and hedonic palatability.

Due to high water content and overall structure, button mushroom is prone to browning, open umbrellas, and wrinkles during storage, thus reducing the quality and commercial value of button mushroom (Mohebbi et al., 2012). Many technical means have been conducted on button mushroom to prolong storage life, such as brassinolide treatment (Ding et al., 2016), gallic acid grafted...
chitosan film packaging (Liu et al., 2019), cinnamondehyde fumigation (Gao et al., 2014), high CO₂ treatment (Lin et al., 2017), and UV-C treatment (Wu et al., 2016).

Nitric oxide (NO) treatment is an effective method for postharvest storage of fresh products. It has been reported that NO mainly delays the senescence of fruit and vegetable by regulating the activity of enzymes to protect the enzyme system during the aging of plant tissues and regulating the content of antioxidants (Leshem and Wills, 1998). Previous reports also showed that NO application can enhance the content of phenolic and flavonoid in mushroom (Russula griseocarnosa) during storage (Dong et al., 2012). In addition, NO plus modified atmosphere treatment can prolong the button mushroom storage time up to 12 days at low temperature (Jiang et al., 2011). For now, the effects of NO treatment on flavour compounds and antioxidant enzyme activities in button mushroom are lack of research.

In the present research, button mushrooms were treated with 15 μmol/L NO donor sodium nitroprusside for 3 min. The physical indexes, volatile components, and antioxidant enzyme activities of button mushroom in NO treatment and the control were studied. The purpose of this work was to evaluate the effects of NO treatment on flavour compounds and antioxidant enzyme activities of the stored button mushroom.

Materials and methods
Plant material and NO treatment
Fresh button mushrooms used in this research were obtained from a planting base located in Beijing, China. A total number of 240 (40 fruits in each basket, 3 baskets in each group) intact, closed, and uniform mushrooms were collected. Before analyses, mushrooms were pre-cooled at 20 °C for 30 min. The button mushrooms were divided into two groups randomly. One group (three baskets) was soaked in a 15 μmol/L sodium nitroprusside (Solarbio, Beijing, China) aqueous solution for 3 min. Another group (three baskets) was soaked in water and considered as control. After drying the surface water with an electric fan, all the mushrooms were transferred to cold storage under the condition of 4 °C, 90% relative humidity. Fifteen mushrooms from each group were placed separately to determine weight loss and respiration rate. The physical and physiological data were taken at 3 days intervals up to 12 days. Each sampling point consisted of 15 mushrooms and were considered as three replications (5 mushrooms in each replication). Mushroom caps were taken separately in each sampling point and stored at ~80 °C immediately after freezing in liquid nitrogen.

Respiration evaluation
Respiration rate detection was performed with a handheld three-gas analyzer (F950, FELIX, USA). Five sets of button mushrooms were randomly selected and placed in a closed container. Two needle-sized holes were opened at the top of the container and sealed with sealing tape to prevent air leakage. After 4 h, the volume percentage of CO₂ in the closed container was measured to characterize the rate of respiration. Three replicates were conducted in each group.

Weight loss measurement
The weight loss rate was measured through the following equation: Weight loss (%) = (m₀ − m)/m₀ × 100. ‘m₀’ represents sample weight at 0 days of storage while ‘m’ represents the weight of each sample at each sampling point. Fifteen mushrooms for each replication were used for calculation, and three replicates were conducted.

Firmness evaluation
The firmness was detected using a texture analyzer (TA-HDplus; Stable Micro Systems, UK). The top side of the mushroom was penetrated by the probe at a uniform speed, and the penetrated length was 10 mm. Fifteen mushrooms in each group were used for measurement at 3 days intervals.

Colour analysis
The external colour of caps was measured using a WSCS Colorimeter (Shanghai Precision Instrument Co., Ltd, China). Three points of epidermal cap were measured and the values were compared with the ideal colour of mushroom (L* = 97, a* = −2, b* = 0) according to the following equation: ∆E = [(L* – L₀)² + (a* – a₀)² + (b* – b₀)²]¹/² (Gao et al., 2014). The lower ∆E value indicates a better colour quality of mushroom.

Malondialdehyde content analysis
The contents of malondialdehyde (MDA) were analyzed using the method from Heath and Packer (1968). The MDA contents were calculated as follows: MDA (mol/L) = [6.45 × (A₅₃₂ − A₆₀₀) − 0.56 × A₄₅₀] × 10⁻⁶. ‘A₄₅₀’, ‘A₅₃₂’, and ‘A₆₀₀’ represent absorbencies of the aqueous phase at 450, 532, and 600 nm, respectively.

Volatile compounds analysis
Volatile were detected by headspace solid phase microextraction-gas chromatography-mass spectrometry method according to the report from Lin et al. (2017). An AOC-5000 autosampler and GC-MS QP2010 Plus system (Shimadzu, Kyoto, Japan) were used. Samples were ground and 1 g powder was weighed into a 10 ml vial. Five millilitres of solution contained 200 mM ethylenediaminetetraacetic acid and CaCl₂ (20%) was added. Then the internal standard phenylethyl acetate was added and the mixture was homogenized. Solid-phase micro-extraction test was performed on a 65 μm polydimethylsiloxane/divinylbenzene fibre, and headspace extraction was conducted at 50 °C for 30 min under agitation. A DB-WAX column (30 m × 0.25 mm × 0.25 μm) was used in this experiment. Temperature program was as follows: 40 °C for 2 min, then increased to 120 °C at 3 °C/min, finally ramp 5 °C/min to 200 °C and held for 5 min. The carrier gas was helium (He), and the flow rate was 1.0 ml/min.

The volatiles were identified using the database of NIST/EPA/NIH Mass Spectral Library (NIST-11). Relative contents were calculated by an internal standard method and the final results were expressed as μg/g.

Total phenolic content analysis
The total phenolics content was measured as described by Gao et al. (2014). Generally, 80% ethanol was mixed with 5 g mushroom powder, then the mixture was centrifuged at 10 000 g for 15 min. One millilitre of collected supernatant liquid was mixed with 1 ml of Folin–Ciocalteu reagent and 10 ml of 7% sodium carbonate, which was finally topped up to 25 ml. The absorbance of this mixed liquid was detected at 750 nm. Gallic acid was used as a standard for qualification.

Enzyme activity analysis
The Polyphenol oxidase (PPO), Peroxidase (POD), and Catalase (CAT) activity was detected using kits (Solarbio, China, Beijing). The operating steps were conducted following protocols described in the manufacturer’s instructions.
Statistical analysis

Origin 8.6 software and MultiExperiment Viewer software (MeV v4.8.1) were used to draw the figures in this research. One-way analysis of variance was conducted by IBM SPSS Statistics 21 and Student’s t-test was used to compare the mean averages. Significant differences were considered at 0.05 level.

Results

Effects of NO treatment on respiration rate and weight loss in stored button mushroom

The respiration rate of button mushrooms increased slightly upon storage (Figure 1A). The NO-treated group had a relatively stable respiration rate, whereas the control group showed an increased trend during the third day and then was stable. The respiration rate in NO-treated mushroom was significantly lower than that of the control group during the whole storage period \((P < 0.05)\).

The weight loss rate increased gradually with storage time in both the control and treated groups (Figure 1B). NO-treated mushroom had a significantly lower weight loss rate as compared to the control group from 6 days of storage \((P < 0.05)\).

Effects of NO treatment on firmness and colour in stored button mushroom

Firmness plays a vital role in determining the shelf life of button mushrooms. The firmness gradually decreased with time in both the NO-treated and the control button mushrooms (Figure 2A). Compared with the control, the NO-treated button mushroom showed higher firmness value during the whole storage. The total colour variation was showed as \(\Delta E\) value, and a lower value indicates a better colour quality of mushroom. As shown in Figure 2B, \(\Delta E\) value of the control group increased continuously, while the NO-treated mushroom kept stable and was significantly lower than that of control \((P < 0.05)\). At the end of storage, the \(\Delta E\) values of the control and NO-treated mushroom were 36.91 and 21.97, respectively. Therefore, NO treatment could maintain the external colour of the button mushroom.

Effects of NO treatment on MDA content in stored button mushroom

MDA is mainly produced from lipid peroxidation of the cell membrane (Gurbuz and Heinonen, 2015). The MDA content in both the control and NO-treated samples increased during...
the whole storage period as showed in Figure 3. Button mushroom had a significantly lower MDA content after NO treatment during storage time, which indicated that NO treatment may be beneficial to minimize oxidative injury of button mushroom during storage.

Effects of NO treatment on volatile compounds in button mushroom during storage

Flavour, which is mainly decided by the volatile compounds of food, is a key factor that impacts consumers’ preferences. In this research, the variations of the volatile compounds of button mushroom after the treatment of NO were investigated (Figure 4). A total of 28 volatile metabolites were found in the stored button mushroom, and these compounds were classified as ketone (1 compound), alcohols (10 compounds), esters (8 compounds), aldehydes (2 compounds), and others (7 compounds) according to the chemical structure. Among them, C8 compounds including 3-octanone, 3-octanol, 1-octen-3-ol, and (Z)-2-octen-1-ol were considered as the dominant volatile compounds of button mushrooms. The clustering result suggested that the volatile profile of NO-treated mushroom at 3 days of storage was different from others. At 3 days of storage, almost half of the compounds (e.g., 1-octen-3-ol, 3-octanol, and 3-octanone) increased significantly, while that in the control group maintained at the same level in mushroom at 0 days of storage. The results may indicate that NO treatment was beneficial for promoting the flavour of a button mushroom.

Figure 5 showed the variations of different types of volatile compounds in button mushrooms during storage. After NO treatment, ketones, alcohols, esters, and aldehydes increased sharply before 3 days of storage and then showed a declining trend, except ester compounds which reached the peak value at 6 days of storage. As for the control group, different kinds of volatile compounds showed a similar variation trend. The control group kept steady during the
first 3 days. Then an increasing trend was observed between 3 and 6 days of storage, but declined after the sixth day. Although the NO-treated group increased sharply at an early stage, at 9 days of storage, the control group had a higher content of ketone, alcohol, ester, and aldehyde compounds, respectively.

Effects of NO treatment on total phenolic content and antioxidant enzyme activities in stored button mushroom

The total phenolic content of button mushroom showed an increasing trend during storage after NO treatment, while the control group maintained much stable during storage (Figure 6A). From 6 days of storage, NO-treated button mushroom had a significantly increased total phenolic content as compared to the control mushroom (P < 0.05). At the end of storage, the total phenolic content in NO-treated mushroom and the control was 1.37 and 0.71 mg/g, respectively.

PPO enzyme is the main factor causing the browning of button mushroom during the postharvest period. The PPO activity first increased and then decreased during storage and peaked at 6 days of storage in both the NO-treated and the control mushrooms during the storage period (Figure 6B). Results showed that the button mushroom treated with NO significantly reduced the activity of PPO from 6 days of storage compared with the control mushroom, but did not change the storage time of the peak of PPO activity (P < 0.05). POD is an enzyme mainly account for the enzymatic browning as well as the antioxidant defense. The POD activity of button mushroom in the control group increased before 3 days of storage and then showed a decreasing trend until 12 days of storage (Figure 6C).

The peak of PPO activity appeared at 6 days of storage in NO-treated mushrooms. The POD activity was significantly decreased by NO treatment at 3 and 12 days of storage (P < 0.05) as compared to the control samples. CAT plays an antioxidant role in mushroom during storage. The activity of CAT first increased and then decreased during storage and peaked at 3 days of storage in both the control and NO-treated groups during storage (Figure 6D). Results also suggested that NO treatment could significantly increase the CAT activity in button mushroom during the storage period (P < 0.05).

Discussion

NO plays a crucial role in different physiological processes of plants as considered to be a multifunctional signaling molecule. Exogenous NO treatment could improve postharvest quality by alleviating postharvest diseases, controlling chilling injury, and delaying senescence in various products (Zhang et al., 2020). Respiratory metabolism, dehydration, softening, and browning are the main processes that resulted in quality loss in mushrooms during the storage period. NO has been proved to inhibit the biosynthesis of ethylene and delay the physiological process, which leads to decreased respiration rate (Jiang et al., 2011; Lai et al., 2011). Water transpiration is commonly observed in mushroom because of its thin epidermal structure. A delayed physiological process could also reduce the water loss in mushrooms. Besides, the lower cap opening may contribute to a decreased water loss after NO treatment (Jiang et al., 2011). Firmness is also regarded as an important factor to determine the quality of the mushroom, which would decrease gradually with the senescence of fleshy products. Studies have shown that application of NO is beneficial to

Figure 5. Effects of nitric oxide (NO) treatment on contents of ketones (A), alcohols (B), esters (C), and aldehydes (D) in stored button mushroom. Vertical bars are standard errors of the means of three replicates. The single asterisk (*) indicates significant difference at P<0.05.
maintaining the firmness in blueberry (Grozeff et al., 2017), peach (Han et al., 2018), and winter jujube (Zhao et al., 2019) mainly by reducing the activity of enzymes which are related to cell wall degradation. During the postharvest storage, white mushrooms would lose their uniform white colour and become brown gradually catalyzed by PPO. NO treatment showed positive effects on maintaining the colour of mushroom according to the previous study (Jiang et al., 2011; Lin et al., 2017). According to this work, NO could delay the senescence of button mushroom by decreasing the respiration rate and water loss. In addition, NO treatment maintained higher firmness and better colour than that in the control during storage. These results suggested that postharvest application of NO is beneficial to maintaining a higher commercial value of button mushroom.

Button mushroom favours market with its special flavour and high nutritional value. The characteristic flavour of mushrooms is mainly decided by volatiles. Previous reports have shown that C8 volatiles contributed significantly to the special mushroom flavour (Mau et al., 1992; Wen et al., 2020). Our results also found that C8 ketones and alcohols, such as 3-octanone, 3-octanol, 1-octen-3-ol, and (Z)-2-octen-1-ol, were the dominant contributors of button mushroom, which was consistent with the previous studies (Chen and Wu, 1984; Cho et al., 2008).

The flavour compound profiles of mushrooms could be affected by a series of factors, such as environmental conditions, maturity, storage, and processing. There have been many studies focussed on the effects of processing and postharvest storage on the volatile compounds of mushroom (Cho et al., 2006, 2007; Schmidtberger and Schieberle, 2020). For example, it has been reported that the early stage of hot air drying decreased ketone as well as alcohol concentrations and generated the cyclic organosulfur chemicals, which accounted for the characteristic odour of shiitake mushroom (Qin et al., 2020). Freeze drying decreased C8 chemical contents and generated alkanes and heterocyclic compounds in the latter stage (Pei et al., 2020). During low-temperature storage, the high CO2 in-package treatment was proved to maintain a higher content of 1-octen-3-ol, which was considered as a typical mushroom-like flavour compound (Lin et al., 2017). Pulsed light treatment increased the content of C8 compounds, such as 1-octene-3-ol, 1-octen-3-one, and 3-octan, in stored shiitake mushrooms (Wen et al., 2020). According to our results, although ketones, alcohols, esters, and aldehydes in button mushroom increased sharply after NO treatment during the early stage, the control group had a higher content of these compounds at the end of storage. For NO-treated mushrooms, the sharply increasing of volatiles, especially C8 compounds might be due to the cleavage of linoleic acid after NO treatment.

NO is generally regarded as an important signaling molecule in secondary metabolites biosynthesis and accumulation in plants (Xu, 2007). NO has been proved to mediate brassinosteroid-induced flavonoid biosynthesis in Camellia sinensis L. and UV-B-induced flavonoids metabolism in leaves of Betula pendula (Zhang et al., 2011; Li et al., 2017). As typical secondary metabolites, phenolic compounds exhibit strong antioxidant activity and could respond to biotic and abiotic stress. The study has reported that pulsed light treatment could enhance selected individual phenolic compound contents in shiitake mushroom (Wen et al., 2020). It has been proved that NO could activate the biosynthetic pathway to induce the accumulation of phenolics in mushroom (Dong et al., 2012). And the same result was found in this study, NO treatment significantly enhanced the phenolics contents in button mushroom during storage.

Previous reports showed that activities of antioxidant enzymes were affected by various postharvest treatments in button
mushrooms and other plants. For example, fresh-cut burdock showed a longer shelf life after short-term CO₂ treatment, which decreased the PPO activity and enhanced the activities of superoxide dismutase (SOD), POD, and CAT (Dong et al., 2015). Application of sodium nitroprusside improved arsenic stress tolerance of *Isatis capradora* Desv. Shoots through enhancing antioxidant enzymes activity (Souri et al., 2020). It has been found that NO gas fumigation would increase the activities of SOD, POD, and CAT in Hami melon (Zhang et al., 2017). Our results also suggested that NO treatment was beneficial to maintaining the CAT activity, while the activity of PPO was inhibited, which was in accordance with the previous study (Jiang et al., 2011; Lin et al., 2017). These results suggested that NO treatment enhanced antioxidant capacity by inducing the CAT activity and promoted flavour and consumer acceptance in stored button mushrooms.

**Conclusions**

The present study showed that NO treatment is an effective measure to maintain the quality of button mushroom during postharvest storage. NO-treated mushrooms have reduced weight loss rate, uniform white colour, and higher firmness during storage. After NO treatment, ketones, alcohols, esters, and aldehydes increased sharply before 3 days of storage and then showed a continuing decline trend, except ester compounds which reached the peak value at day 6. As for the control group, different kinds of volatile compounds showed a similar variation trend. In addition, NO treatment increased the total phenolics and CAT activity, inhibited the PPO activity in stored button mushrooms. These results indicated that NO treatment was a promising storage technology to enhance the antioxidant ability and maintain contents of flavour-related 1-octen-3-ol for postharvest button mushroom.

**Funding**

This work was supported by the Science and Technology Innovation Project of the Chinese Academy of Agricultural Sciences (CAASASTIP-201X-IAPPST), China.

**Conflict of Interest**

The authors declare no conflict of interest.

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