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Effect of Spermine on Bioactive Components and Antioxidant Properties of Sliced Button Mushroom (*Agaricus bisporus*) during Storage

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ABSTRACT

Role of exogenous application of polyamine spermine as dipping treatment at various levels (0, 0.1, 1 & 2 mM) as a potential tool for maintenance of bioactive components and antioxidant properties of sliced button mushroom (*Agaricus bisporus*) during storage was investigated. Total phenolics and flavonoids, and assay of DPPH activity as well as assay of reducing power were evaluated as markers of bioactive components and antioxidant properties of sliced button mushroom. Spermine treatment effectively maintained higher levels of bioactive components and antioxidant properties (as depicted by higher retention of phenolics & flavonoids & lower EC_{50} values for DPPH & reducing power) as compared to control during whole storage period. Ultrastructural studies further revealed higher incidence of crystalline phenolic compounds in spermine treated mushrooms. Our results suggest that dipping in 1 mM polyamine spermine solution prior to storage can serve the purpose of maintenance of bioactive components and antioxidant properties of sliced button mushroom during storage.

Key Words: Quality; Antioxidant capacity; Transmission electron microscopy; Fresh cut

INTRODUCTION

A cumulative body of evidence suggests inverse association regarding various fruits and vegetables consumption and risk of diseases (Hung *et al.,* 2004). These health-related benefits of different fruits and vegetables have been related to various antioxidants and other functional compounds inherent to these fruits and vegetables (Genkinger *et al.,* 2004). White button mushrooms are not an exception and are believed to be prominent source of various antioxidant compounds e.g., phenolics, carotenoids, flavonoids, tocopherols, ascorbic acid etc. (Mattila *et al.,* 2000; Iqbal *et al.,* 2005). Although button mushroom is reported to possess considerably higher levels of antioxidant properties, these antioxidant properties of button mushroom tend to decrease during post harvest storage with the passage of time.

Polyamines are present in microorganisms, human breast milk (Kalac & Krausova, 2005), legumes, citrus, broccoli and cauliflower etc. (Eliassen *et al.,* 2002) and are involved in numerous physiological processes like cell expansion, cell division, flowering, fruit development, ripening, chilling injury (Zhang *et al.,* 2009) and seedling growth etc. (Farooq *et al.,* 2007). Moreover, recently polyamines have been successfully used to maintain storage quality of different horticultural crops e.g., plum, mango and apricot etc. (Martínez Romero *et al*., 2002; Malik & Singh, 2005; Khan *et al*., 2007).

At present white button mushroom is grown world wide in more than 70 countries and with the passage of time production and consumption is steadily increasing including China (Chang & Bushwell, 2008). Button mushroom is fantabulous source of protein, essential amino acids, minerals, vitamins, specially riboflavin, thiamine, biotin, niacin etc and is medically beneficial for diseases regarding depressed immune function, frequent flu and colds, heart diseases, bronchial inflammation, hyperlipidemia, hypertension, diabetes and modulating urinary inconsistencies.

Based on previous evidence suggesting that polyamines effectively maintained storage quality of various horticultural commodities during storage, herein we report possible role of exogenous application of polyamine spermine as dipping treatment on bioactive components and antioxidant properties of sliced white button mushroom during storage, as sporadic and inconclusive information is reported earlier in this regard.

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MATERIALS AND METHODS

Fresh button mushrooms(*Agaricus bisporus*) were washed initially with sodium hypochlorite solution (0.5% v/v) for one min and then mushrooms were soaked in chilled spermine solution (3 L, 4°C) at desired concentrations (0.1, 1 & 2 mM) for 10 min or dipped in chilled distilled water as control. Afterwards, mushrooms were drained on absorbent paper and were air dried using a fan for 15 min to remove excess water and then hand sliced and aseptically packed in polystyrene plastic food trays (200 ± 5 g/tray) of internal dimensions 18.5×11.3×4 cm and over-wrapped with low density polyethylene films and were refrigerated (4°C) for 17 days. On each designated day mushroom samples were taken out for further analysis.

Sample preparation: Extraction of freeze dried mushroom samples (3 g) was conducted through stirring (150 rpm, 100 mL methanol, 24 h) with subsequent filtration by filter paper. Later on extraction of same samples were conducted twice in the similar way. These methanolic extract samples were mixed together and later on rotary evaporated and eventually mixed in methanol @100 mg/mL. Later on, these samples were used for determination of various biochemical assays. Determination of phenolic compounds was accomplished as suggested by Barros *et al.* (2007). In order to estimate total phenolics, mushroom extract (1 mL) was combined with Folin and Ciocalteu's phenol reagent (1 mL^{l}). Later on saturated sodium carbonate solution (1 mL) was added to the mixture after 3 min and total volume of mixture was adjusted to 10 mL with distilled water. This reaction mixture was then kept in dark for 90 min and then absorbance was read at 725 nm. Standard curve was calculated by using gallic acid.

For estimation of flavonoids contents, Barros *et al.* (2008) method was followed. 250 µL mushroom extract was added to 1.25 mL of distilled water and 75 µL of (5%) $NaNO_2$. Later on, 150 µL of (10%) $AlCl_3.H_20$ solution was added after 5 min. In the end, after 6 min, 500 µL NaOH (1 M) and 270 µL distilled water were also added to the mixture. The solution was mixed well and the absorbance was spectrophotometrically measured at 510 nm. Standard curve was calculated by using (+)-catechin. The DPPH activity assay was assessed following the procedure of Ferreira *et al.* (2007). Different mushroom extract concentrations (0.3 mL each) were combined with methanolic solution containing DPPH radicals (6×10^{-5} mM, 2.7 mL). The mixture was well shaken and was kept in darkness for 60 min in order to obtain stable absorption values. The reduction of the DPPH radicals was determined by measuring absorption at 517 nm. The radical scavenging activity (RSA) was determined as a percentage of DPPH discoloration by using following equation:

$$\% \text{ RSA} = [(A_{DPPH}-A_S)/_{ADPPH}] \times 100$$

Where A_S describes the absorption of the solution when the sample extract has been added at a certain level and A_{DPPH} represents the absorbance of the DPPH solution. The extract concentration, which can provide 50% of radicals scavenging activity (EC_{50}) was estimated from the graph of RSA percentage against extract concentration. Standards used were BHA and α–tocopherol. RSA was reported in terms of EC_{50} (mg/mL) values i.e., effective concentration, which can scavenge 50% of DPPH radicals.

Reducing power was quantified as recommended by Mau *et al.* (2002). Different methanolic mushroom extracts concentrations (2.5 mL each) were combined with sodium phosphate buffer (pH 6.6, 200 mM, 2.5 mL) and potassium ferricyanide (1%, 2.5 mL), respectively. Later on this mixture was incubated at 50°C for 20 min and then 2.5 mL of trichloroacetic acid (10%) was added to the mixture and the mixture was further centrifuged for 8 min at 1000 rpm. The upper layer (5 mL) was combined with 5 and 1 mL of deionised water and 0.1% ferric chloride respectively. In the end, the absorbance was measured spectrophotometrically at 700 nm. The concentration of extract which can provide 0.5 of absorbance (EC_{50}) was determined from the graph of absorbance against concentration of extract. Standards used were α–tocopherol and BHA.

Transmission electron microscopy: Transmission electron microscopy was conducted in order to take an insight regarding localization of phenolic compounds in mushroom tissues. Samples for electron microscopy were obtained on day 17 of storage from control and spermine treated mushrooms. Small sections of mushroom slices (2×2 mm) were cut and dipped in glutaraldehyde containing phosphate buffer and washed thrice for 15 min each wash. Later on mushroom slices were dipped in 1% osmium tetraoxide and were again washed with same buffer. Mushroom samples were dehydrated in succession for 15 min each with ethanol and in the end with absolute acetone. Later on Spurr's resin was used to embed mushroom tissues. (TEM-1230-EX-JEOL) electron microscope was used for ultra-structural observations of mushroom tissues (Stefanowska *et al.,* 2002).

Statistical analysis: DPS (version 6.85) was used for analysis of the data. Sources of variance were treatments and storage time. Least significant difference test (LSD) was employed to determine the statistical difference ($P \leq 0.05$) among the treatments means.

RESULTS

Effect of polyamine spermine on phenolics of *A. bisporus* during storage: *A. bisporus* contains substantial amount of total phenolics (Table I). During first five days of storage, levels of total phenolics remained relatively stable rather it increased and reached to maximum values (5.39 mg/g), (5.76 mg/g), (5.88 mg/g) and (5.94 mg/g) in control, 0.1 mM spermine, 1 mM spermine and 2 mM spermine treated mushrooms, respectively. Later, the levels of total phenolics declined. However, significantly higher amounts were still present in spermine treated mushrooms from day 9 till the end of storage.

Table I: Effect of polyamine spermine on total phenolics (mg/g) of *Agaricus bisporus* during storage at 4°C

Treatments	Day1	Day 5	Day 9	Day13	Day17
Control	5.36±0.25a	5.39±0.04b	4.80±0.04c	3.50±0.02c	3.04±0.05c
Spermine0.1 mM	5.35±0.17a	5.76±0.03a	5.21±0.05b	4.03±0.02b	3.46±0.03b
Spermine1 mM	5.36±0.03a	5.88±0.25a	5.60±0.27a	4.70±0.13a	4.13±0.28a
Spermine2 mM	5.36±0.01a	5.94±0.04a	5.66±0.19a	4.80±0.03a	4.17±0.10a

Within same column, different letters are indication of significant differences

Table II: Effect of polyamine spermine on total flavonoids (µg/g) contents of *Agaricus bisporus* during storage at 4°C

Treatments	Day1	Day 5	Day 9	Day13	Day17
Control	17.88±0.34a	15.52±0.22c	14.39±0.22c	13.12±0.17c	11.02±0.22c
Spermine0.1 mM	17.82±0.11a	16.90±0.11b	16.02±0.15b	14.99±0.65b	13.04±0.20b
Spermine1 mM	17.87±0.22a	17.47±0.28a	16.87±0.22a	16.53±0.22a	14.81±0.32a
Spermine2 mM	17.90±0.02a	17.54±0.34a	16.98±0.30a	16.76±0.22a	14.92±0.23a

Within same column, different letters are indication of significant differences

Table III: Effect of polyamine spermine on assay of DPPH activity (EC_{50} value) of *Agaricus bisporus* during storage at 4°C

Treatments	Day1	Day 5	Day 9	Day13	Day17
Control	2.99±0.10a	3.09±0.13a	3.30±0.05a	3.44±0.04a	3.47±0.06a
Spermine0.1 mM	2.73±0.02b	2.75±0.03b	3.20±0.03b	3.26±0.05b	3.35±0.04b
Spermine1 mM	2.27±0.02d	2.28±0.04d	3.04±0.04c	3.06±0.03c	3.20±0.01c
Spermine2 mM	2.55±0.03c	2.57±0.03c	3.14±0.02b	3.12±0.01c	3.16±0.02c

EC_{50} (mg/mL): effectual concentration which is able to scavenge 50% of DPPH radicals. Within same column, different letters are indication of significant differences

Table IV: Effect of polyamine spermine on essay of reducing power (EC_{50} value) of *Agaricus bisporus* during storage at 4°C

Treatments	Day1	Day 5	Day 9	Day13	Day17
Control	2.04±0.02a	2.01±0.01a	2.12±0.01a	2.22±0.04a	2.32±0.04a
Spermine0.1 mM	1.98±0.05b	1.93±0.00b	2.00±0.04b	2.01±0.03b	2.21±0.03b
Spermine1 mM	1.49±0.02d	1.57±0.01d	1.66±0.03d	1.70±0.03d	1.90±0.04c
Spermine2 mM	1.80±0.01c	1.81±0.00c	1.87±0.06c	1.89±0.04c	1.92±0.02c

EC_{50} (mg/mL): effectual concentration necessary to yield absorbance level of 0.5. Within same column, different letters are indication of significant differences

Effect of polyamine spermine on flavonoids contents of *A. bisporus* during storage: *A. bisporus* flavonoid contents declined during storage at 4°C (Table II). However, mushrooms treated with various concentrations of spermine exhibited significantly higher amounts of flavonoid contents as compared to control from day 5 till day 17 of storage. At day 17 of storage, mushrooms treated with 2 mM spermine contained highest (14.92 µg/g) levels of flavonoids as compared to lowest levels (11.02 µg/g) in control.

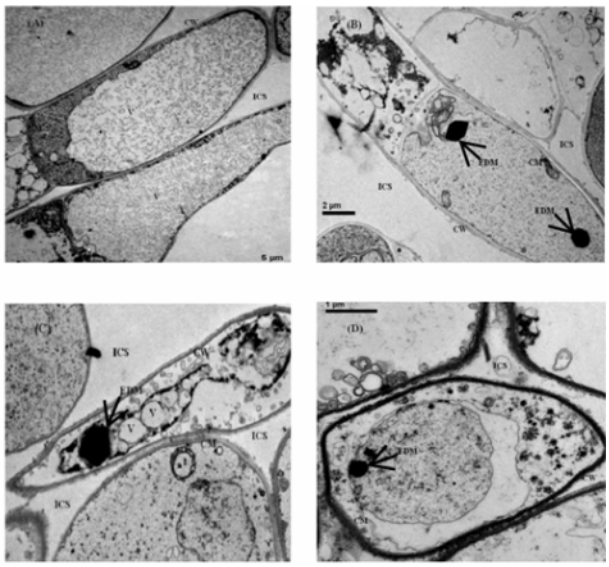
Effect of polyamine spermine on DPPH assay of *A. bisporus* during storage: Activity of DPPH assay of white button mushroom during storage at 4°C is presented in Table III in terms of EC_{50} values. During storage EC_{50} values gradually increased, however EC_{50} values were significantly lower for mushrooms treated with spermine irrespective of the concentrations as compared to control during whole storage period.

Effect of polyamine spermine on reducing power of *A. bisporus* during storage: Reducing power of *A. bisporus* during storage at 4°C is delineated in terms of EC_{50} values (Table IV). During whole storage period, gradual increase in EC_{50} values was observed. However, mushrooms treated with various concentrations of spermine exhibited significantly lower increase in EC_{50} values than control.

DISCUSSION

Phenolic compounds are known to exhibit an array of biological functions such as anti-carcinogenic, antiviral, antibacterial, antithrombotic, hepatoprotective, antiallergic, anti inflammatory and vasodilatory functions etc. (Soobrattee *et al.,* 2005). Gradual decrease in phenolic compounds in water treated mushrooms during post harvest storage with the passage of time could be due to natural senescence process of mushrooms occurring during storage. Significant higher retention of phenolic compounds in spermine treated mushrooms could be attributed to anti-senescence properties of polyamine spermine (Lester, 2000). Flavonoids are nutritionally important and beneficial components normally present in various plants (Marchand, 2002). Flavonoids contents of button mushroom declined during storage, which is in accordance with the findings of

Fig. 1: Transmission electron micrographs of (A) Control (B) 0.1 mM (C) 1 mM and (D) 2 mM spermine washed mushrooms. The incidence of electron dense materials (marked by arrows), which are supposed to be phenolics is higher in spermine treated mushroom tissues while such electron dense materials are not prominent in control. EDM= electron dense material, ICS= inter cellular space, CW= cell wall. CM= cell membrane, V= vacuole



previous researchers (Jiang *et al.,* 2010). However, spermine treated mushroom maintained significantly higher levels of flavonoids compounds during whole storage period. Such decline in flavonoids is regarded as a consequence of natural aging process of button mushroom (Barros *et al.,* 2007). Mirdehghan *et al.* (2007) also described a higher retention of various bioactive compounds in pomegranate arils during storage after exogenous polyamine application.

Transmission electron microscopy (TEM) was conducted in order to find the intracellular localization of phenolic compounds in mushroom tissues. Phenolics reaction with Osmium tetroxide can lead to electron dense materials formation in cellular ultra structure (Nielson & Griffth, 1978). It is apparent that electron dense materials, supposedly phenolics, not prominent in water treated mushroom tissues (Fig. 1a) were highly evident in transmission electron micrographs of spermine treated mushroom tissues and were mostly present inside vacuoles (Fig. 1b-d). Moreover, greater retention of phenolics and flavonoids in spermine treated mushrooms seen in TEM micrographs strengthen our stand point.

Both DPPH as well as assay of reducing power are important indicators of measurement of antioxidant capacity (Tsai *et al.,* 2006). Lower EC_{50} values represent higher antioxidant capacity (Huang & Mau, 2007). Polyamine spermine dipping resulted in higher antioxidant capacity as evidenced by lower EC_{50} values for assay of DPPH activity and also for assay of reducing power while water treated mushrooms exhibited lower antioxidants properties as shown by higher EC_{50} values for assay of DPPH activity and also for assay of reducing power. During post harvest storage, higher retention of antioxidant properties of spermine treated mushroom could be due to eminent retention levels of phenolics as compared to water treated mushrooms. Gradual overall decline in antioxidant properties (as evidenced by higher EC_{50} values for essay of DPPH activity & assay of reducing power) of button mushroom during storage could be explained due to possible involvement of these bioactive components in defense associated mechanisms related to natural senescence of button mushroom during storage.

In conclusion, dipping in polyamine spermine solution prior to storage can maintain bioactive components and antioxidant properties of sliced button mushrooms during storage.

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