Effect of malic acid on bioactive components and antioxidant properties of sliced button mushroom (*Agaricus bisporus*) during storage

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Abstract:

BACKGROUND: White button mushroom (*Agaricus bisporus*) is one of the most sensitive agricultural crops after harvesting and its antioxidant properties tend to decrease during post-harvest storage with the passage of time. **OBJECTIVES:** The possible role of exogenous application of malic acid as dipping treatment on bioactive components and antioxidant properties of sliced white button mushroom during storage was investigated. **METHODS:** The mushrooms were soaked in chilled malic acid solution at desired concentrations (1%, 3% and 5%) for 10 minutes or were dipped in chilled distilled water as control and were refrigerated (4°C) for 15 days. On each designated day (every 5 days) mushroom samples were taken out for further analysis. Total phenolic, chelating ability, and DPPH assay, as well as assay of reducing power, were evaluated as markers of bioactive components and antioxidant properties of sliced button mushroom. **RESULTS:** Malic acid treatment effectively maintained higher levels of bioactive components and antioxidant properties (as depicted by higher retention of phenolic and lower EC50 values for DPPH and reducing power) as compared to control during the whole storage period. On the other hand, chelating ability of mushrooms was not influenced by the malic acid treatment and a weak correlation was found between malic acid treatment and chelating ability retention. **CONCLUSIONS:** Our results suggest that dipping in 3% malic acid solution prior to storage can serve the purpose of maintenance of bioactive components and antioxidant properties of sliced button mushroom during storage.

Introduction

Increased consumption of various fruits and vegetables has been recommended as a key component of a healthy diet for reducing risks and preventing diseases due to various antioxidants and other functional compounds inherent in fruits and vegetables (Genkinger et al., 2004). White button mushroom (*Agaricus bisporus*) is not an exception and is used not only as food, but also as functional food and medicine due to its high amount of proteins and minerals and low starch and cholesterol contents. It is also believed to be prominent source of various antioxidant compounds, e.g., phenolic, carotenoids, flavonoids, tocopherols, ascorbic acid, etc. (Wani et al., 2010). Although button mushroom is reported to pos-

sess considerably higher levels of antioxidant properties, from the point of view of post-harvest physiology, mushroom is one of the most sensitive agricultural crops after harvesting and these antioxidant properties of button mushroom tend to decrease during post-harvest storage with the passage of time (Jahangir et al., 2011).

The water content of *Agaricus bisporus* mushroom is about 90%, which accelerates the microbial spoilage and water-dependent reactions (Hershko and Nussinovitch, 1998). Storage of mushrooms at low temperatures (1–4 ^{o}C) is the main factor to extend the shelf-life by virtue of decrease in microbial growth and physiological reactions, such as enzymatic browning, and especially the respiration rate, which is extremely high in mushrooms compared to other fruits and vegetables (Kim et al., 2006). Moreover, in some studies, the effects of different anti-microbial and anti-browning solutions and hydrocolloid-based substances on the sensory attributes and microbial quality of the whole or sliced mushrooms have been analyzed. These solutions include ascorbic acid and its derivatives as anti-browning agents (Hsu et al., 1988); H2O2 as an anti-bacterial agent along with sodium erythorbate as an anti-browning agent (Sapers et al., 2001); H2O2 and ClO2 as anti-bacterial agents and sodium D-isoascorbate monohydrate as an enzymatic browning inhibitor (Cliffe-Byrnes and O'berrne, 2008); and citric acid along with sodium D, L-isoascorbate as anti-browning agents (Simon and Gonzalez Fandos, 2009). In this regard, Nussinovitch and Kampf (1993) utilized alginate-based coating to prolong the shelf-life of mushroom. Hershko and Nussinovitch (1998) coated mushrooms with alginate and alginate-ergosterol to investigate the structural changes of mushrooms during storage. Kim et al. (2006) studied the effect of MAP on the shelf-life of the chitosan-coated mushrooms. Sedaghat and Zahedi (2012) evaluated acidic washing and coating with gum arabic (GA), carboxymethyl cellulose, and emulsified gum arabic (EGA) on the weight loss, firmness, and color of mushroom.

Based on the previous studies focused on the retarding of mushroom decadence through coating and treatment with anti-browning solutions, in this study the possible role of exogenous application of citric acid on bioactive components and antioxidant properties of sliced white button mushroom during storage was investigated. To the best of our knowledge, there is no prior report in this regard.

Materials and Methods

Fresh button mushrooms (*Agaricus bisporus*) were prepared from a local grower, transported to the laboratory within 2 hours and stored in refrigerator before experiments. Washed initially with distilled water for one minute, the mushrooms were soaked in chilled malic acid (Merck, Germany) solution at desired concentrations (1%, 3% and 5%) for 10 minutes or dipped in chilled distilled water as control. Afterwards, mushrooms were drained on absorbent paper and were air dried using a fan for 15 minutes to remove excess water. Then they were hand sliced, aseptically packed in polystyrene plastic food trays (250±5 g/tray), over-wrapped with low density polyethylene films, and refrigerated (4°C) for 15 days. On each designated day (every 5 days) mushroom samples were taken out for further analysis.

Sample preparation: Extraction of freeze dried mushroom samples was prepared according to the method suggested by Shyamala et al. (2005) with some modifications. In brief, 15 grams of dried and chopped samples was extracted with 100 mL methanol (Dr Mojallali Chemical Laboratories, Iran) for 24 hours with occasional shaking and then were further filtered and evaporated to dryness in a vacuum dryer (Rotary Evaporator, RE-52AA, China). Later on, these methanolic extract samples

were used for determination of various biochemical assays.

Determination of total phenolic contents: Determination of phenolic compounds was accomplished as suggested by Barros et al. (2007). In order to estimate total phenolic, 1 mL mushroom extract (5 mg/mL) was combined with 1 mL Folin and Ciocalteu's phenol reagent (Merck, Germany). Then, 1 mL saturated sodium carbonate solution (Merck, Germany) was added to the mixture after 3 minutes and total volume of mixture was adjusted to 10 mL with distilled water. This reaction mixture was then kept in dark for 90 minutes and then absorbance was read at 725 nm. Standard curve was calculated by using gallic acid.

Measurement of reducing power: The reductive potential of methanol extracts of mushroom samples was determined according to the method described by Yen & Duh (1994). The different concentrations of the extracts were made (0.1-4 mg/mL) in 0.2 M phosphate buffer pH 6.6 containing 1% potassium ferrocyanid (Merck, Germany). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of trichloroacetic acid (10% w/v) was added to the mixture which was then centrifuged at 3000 g for 10 minutes. The upper layer was separated and mixed with 2.5 mL of distilled water containing 0.5 mL of ferric chloride 1% (Merck, Germany). The absorbance of this mixture was measured at 700 nm. The intensity in absorbance showed the antioxidant activities of the extracts. The concentration of extract, which can provide 0.5 of absorbance (EC50), was determined from the graph of absorbance against concentration of extract.

DPPH assay: The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay was done using the stable radical DPPH as a reagent, according to the method suggested by Burits and Bucar (2000). In brief, 50 µL of the extracts (various concentrations) were added to 5 mL of the DPPH (Sigma-Aldrich GmbH, Steinheim, Germany) solution (0.004% methanol solution). After 30 minutes of incubation at room temperature, the absorbance was read against pure methanol at 517 nm. The radical-scavenging activities of the samples were calculated as percentage of inhibition according to the following equation:

I (%) = (Ablank - Asample/Ablank) x 100, where Ablank is the absorbance of the control (containing all reagents except the test compound) and Asample is the absorbance of the test compound.

The extract concentration, which can provide 50% of radicals scavenging activity (EC50), was estimated from the plot of inhibition percentages against essential oil concentration using PHARM/PCS version 4. All tests were carried out in triplicate, and the average results and standard deviations were calculated.

Metal chelating assay: The chelating effects of ferrous ions from *A. bisporus* extracts was estimated by the method suggested by Chua et al. (2008) with slight modifications. In brief, 200 µL of different concentrations of the extracts and 740 µL methanol were added into 20 µL of 2mM FeCl2 (Merck, Germany). The reaction was initiated by adding 40 µL of 5 mM ferrozine (Sigma, Germany) to the mixture, which was then shaken vigorously and left standing at ambient temperature for 10 minutes. The ratio of inhibition of ferrozine Fe2+ complex formation was calculated as follow:

% inhibition=[(absorbance of control-absorbance of test sample)/absorbance of control]x100

A lower absorbance indicates higher chelating ability. The extract concentration providing 50% chelating ability (EC50) was calculated from the graph of antioxidant activity percentage against extract concentration.

Statistical analysis: All determinations

were done in triplicate. The results were reported as means ± standard deviation (SD). The significance of differences among treatment means was determined by one-way analysis of variance (ANOVA) and Tukey test using SigmaStat software (version 2.03). A p value less than 0.05 was statistically considered significant.

Results

Effect of malic acid treatment on phenolic extracts of *A. bisporus* during storage: Variations in the values of total phenolic extracts of *A. bisporus* during storage are depicted in Table 1.

During the first five days of storage, levels of total phenolic remained relatively stable in treatment groups and there was no significant difference between them ($p>0.05$). Later, the levels of total phenolic in all samples showed a tendency to decrease. For the control sample, the gradual decrease in total phenolic from day 5 to the end of the storage period was significantly ($p<0.05$) higher than treated samples. There was no difference between 3% and 5% treated samples throughout the entire storage period ($p>0.05$).

Effect of malic acid treatment on reducing power of *A. bisporus* during storage: Reducing power of *A. bisporus* during storage at 4°C is delineated in terms of EC50 values (Table 2).

During the whole storage period, gradual increase in EC50 values was observed. However, mushrooms treated with 3% and 5% concentrations of malic acid exhibited significantly lower increase in EC50 values than the control ($p<0.05$). In this respect, the reducing power of control and 1% treated samples during beginning until day 10 of the storage period were similar ($p>0.05$) and also there was no difference between 3% and 5% treated samples throughout the entire storage period ($p>0.05$).

Effect of malic acid treatment on DPPH assay of *A. bisporus* during storage: White button mushroom during storage at 4°C is presented in Table 3 in terms of EC50 values. During storage, EC50 values gradually increased. However, EC50 values were significantly lower for mushrooms treated with malic acid irrespective of the concentrations as compared to control during whole storage period ($p<0.05$). Similar to reducing power assay results, 3% and 5% treated samples were not statistically different ($p>0.05$) during the beginning until the end of the storage period.

Effect of malic acid treatment on Metal

Table 1. Effect of malic acid treatments on total phenolics (mg/g) of *Agaricus bisporus* during storage at 4°C. Results are the average of three replications ± standard deviation. Different letters within columns represent significant differences ($p<0.05$).

Treatments	Day 0	Day 5	Day 10	Day 15
Control	5.82±0.22 [a]	5.10±0.03 [b]	4.30±0.14 [c]	3.12±0.11 [c]
Malic acid 1%	5.81±0.11 [a]	5.73±0.15 [a]	5.01±0.09 [b]	4.10±0.04 [b]
Malic acid 3%	5.83±0.22 [a]	5.69±0.16 [a]	5.33±0.06 [a]	4.85±0.08 [a]
Malic acid 5%	5.84±0.17 [a]	5.78±0.07 [a]	5.41±0.04 [a]	4.97±0.12 [a]

Table 2. Effect of malic acid treatments on essay of reducing power (EC50 value in mg/mL) of *Agaricus bisporus* during storage at 4°C. Results are the average of three replications ± standard deviation. Different letters within columns represent significant differences ($p<0.05$).

Treatments	Day 0	Day 5	Day 10	Day 15
Control	1.21±0.03 [a]	1.55±0.07 [a]	1.86±0.01 [a]	2.23±0.09 [a]
malic acid 1%	1.22±0.06 [a]	1.51±0.06 [a]	1.83±0.06 [a]	1.93±0.08 [b]
malic acid 3%	1.20±0.05 [a]	1.30±0.04 [b]	1.41±0.04 [b]	1.63±0.04 [c]
malic acid 5%	1.24±0.07 [a]	1.27±0.03 [b]	1.33±0.08 [b]	1.54±0.11 [c]

Table 3. Effect of malic acid treatments on assay of DPPH activity (EC50 value in µg/mL) of *Agaricus bisporus* during storage at 4°C. Results are the average of three replications ± standard deviation. Different letters within columns represent significant differences ($p<0.05$).

Treatments	Day 0	Day 5	Day 10	Day 15
Control	244.67±2.31 [a]	284.61±4.13 [a]	359.62±7.90 [a]	420.42±8.23 [a]
malic acid 1%	246.62±5.12 [a]	271.77±2.98 [b]	353.49±6.73 [a]	382.65±6.88 [b]
malic acid 3%	242.75±4.92 [a]	257.11±3.52 [c]	284.41±4.96 [b]	301.23±7.12 [c]
malic acid 5%	245.15±4.10 [a]	248.48±2.89 [c]	279.53±5.22 [b]	299.65±6.44 [c]

Table 4. Effect of malic acid treatments on Metal chelating assay (EC50 value in µg/mL) of *Agaricus bisporus* during storage at 4°C. Results are the average of three replications ± standard deviation. Different letters within columns represent significant differences ($p<0.05$).

Treatments	Day 0	Day 5	Day 10	Day 15
Control	2022.52±33.23 [a]	2031.41±44.88 [a]	2086.58±34.71 [b]	2121.88±54.65 [b]
malic acid 1%	2079.40±51.11 [a]	2086.75±23.55 [a]	2156.22±34.43 [ab]	2403.64±54.16 [a]
malic acid 3%	2080.20±53.87 [a]	2089.42±33.18 [a]	2201.35±58.45 [a]	2445.76±38.46 [a]
malic acid 5%	2026.11±41.12 [a]	2043.66±41.24 [a]	2197.25±39.56 [ab]	2423.46±28.75 [a]

chelating assay of *A. bisporus* during storage: The effects of malic acid treatments on the chelating ability of the mushrooms are presented in Table 4. During storage, EC50 values gradually increased. However, the use of malic acid did not lead to preserving the chelating ability in treated mushrooms compared to the control during the whole storage period.

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 (Soobrattee et al., 2005). Accordingly, Luximon-Ramma et al. (2002) showed linear correlation between antioxidant activity and phenolic contents of plant, fruits, and beverages extracts. It has been reported that among antioxidant sources, total phenols are the major antioxidant components found in the mushrooms. Other antioxidants, such as ascorbic acid, β-carotene, and lycopene, are only found in small or vestigial amounts (Barros et al., 2007 b).

Total phenolic content of *Agaricus bisporus* in this study (5.82±0.22 mg/g of dried mushroom) was higher than those reported by Jahangir et al. (2011) (5.36±0.25 mg/g) on *Agaricus bisporus* and Hung and Nhi (2012) (4.1 mg/g) on Straw mushroom (Volvariella volvacea).

Gradual decrease in phenolic compounds in water treated mushrooms during post-harvest storage with the passage of time (Table 1) could be due to natural senescence process of mushrooms occurring during storage, (Lester, 2000) and especially the effect of polyphenoloxidase (PPO) enzyme on their phenolic compounds (Hsu et al., 1988). According to Sedaghat and Zahedi's study, (2012) malic acid can retard the activity of the polyphenoloxidase (PPO) enzyme by reducing the pH of fruit tissue and through reduction of Cu2+ to Cu+ in PPO. Significant higher retention of phenolic compounds in malic acid treated mushrooms could be attributed to anti-browning and anti-senescence properties of malic acid by this mechanism.

Because of accommodating many samples in a short period and enough sensitivity for detecting ingredients at low concentration, DPPH assay has been extensively used to evaluate the antioxidant activities of proton-donating substances according to hydrogen donating ability. DPPH radicals accept electrons or hydrogen

radicals to form stable diamagnetic molecules. The antioxidant activity of substances can be expressed as the reduction capability of DPPH radical at 517 nm (Chua et al., 2008; Elmastasa et al., 2007).

According to the results reported by Jahangir et al. (2011) and Yeh et al. (2011), methanol extract of *A. bisporus* and cold water extract of Grifola Frondosa (an edible mushroom) were found to possess significant DPPH radical scavenging activity with EC50 values of 2990 and around 20000 µg/mL, respectively. The results indicated that methanol extract of *A. bisporus* in our study showed much better performance (EC50= 244.67±2.31 µg/mL) against DPPH radical.

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductants in the solution causes the reduction of the Fe3+/ferricyanide complex to ferrous form (Chua et al., 2008).

Jahangir et al. (2011) and Ferrari et al. (2012) reported that *A. bisporus* extracts had EC50 values of 2.04±0.02 and 1.65±0.07 mg/mL, respectively, in reducing power assay. Comparing the results shows that the reducing power of *A. bisporus* extract in this study (EC50 = 1.21±0.03 mg/mL) is better than that of the mentioned studies.

According to tables 2 and 3, malic acid dipping resulted in higher antioxidant capacity as evidenced by lower EC50 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 EC50 values for assay of DPPH activity and also for assay of reducing power. Many studies have revealed that the antioxidant activities of phenolic compounds are probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Chang et al., 2001). Therefore, during post-harvest storage, higher retention of antioxidant properties of malic acid treated mushroom could be due to eminent retention levels of phenolic as compared to water treated mushrooms. Gradual overall decline in antioxidant properties (as evidenced by higher EC50 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.

The chelating effects of various extracts on Fe^{2+} were determined by the formation of ferrozine-Fe^{2+} complexes. Chelating agents are able to capture ferrous ion before ferrozine, thus hindering the formation of ferrozine-Fe^{2+}. Therefore, measurement of the rate of color reduction helps to estimate the chelating activity of the samples. The metal chelating capacity is important since it reduces the concentration of transition metals that may act as catalysts to generate the first few radicals to initiate the radical-mediated oxidative chain reactions in biological and/or food systems. Ion chelating agents may also inhibit the Fenton reaction and hydroperoxide decomposition (Liu et al., 2010).

As shown in Table 4, malic acid dipping resulted in poor metal chelating activity as evidenced by higher EC50 values for metal chelating assay, while water treated mushrooms exhibited better chelating properties as shown by lower EC50 values for assay of chelating activity from day 10^{th} to end of the storage period.

It has been reported that compounds with structures containing two or more of the -OH, -SH, -COOH, -PO3H2, -C=O, -NR2, -S-, and –O- functional groups in a favorable structure-function configuration can show metal chelating activity (Lindsay, 1996). In this respect, Ferrari et al. (2012) found that there is a weak correlation between the phenolic content

and chelating activity against Fe^{2+} that is in a same agreement with our results.

In conclusion, dipping in malic acid solution prior to storage can maintain bioactive components and antioxidant properties of sliced button mushrooms during storage. In this study there was no significant difference between 3% and 5% malic acid solutions. Thus, because of economical considerations, the lower concentration of acid (3%) is suggested.

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چکیده

زمینه مطالعه: قارچ دکمه‌ای سفید (*Agaricus bisporus*) یکی از حساس‌ترین محصولات کشاورزی در زمان پس از برداشت می‌باشد و با گذشت زمان در طول مدت نگهداری ویژگی‌های آنتی اکسیدانی آن کاهش می‌یابد. **هدف:** در این مطالعه نقش احتمالی تیمار خارجی اسید مالیک به صورت غوطه وری بر ترکیبات فعال زیستی و ویژگیهای آنتی اکسیدانی قارچ سفید دکمه‌ای در زمان نگهداری مورد ارزیابی قرار گرفته است. **روش کار:** قارچها در محلول اسید مالیک سرد با غلظت‌های ۱٪، ۳٪ و ۵٪ به مدت ۱۰ دقیقه و همچنین در آب مقطر سرد به عنوان کنترل غوطه ور شده و در دمای $4^{\circ}C$ به مدت ۱۵ روز نگهداری شدند. در هر ۵ روز نمونه‌های قارچ مورد آزمایش قرار گرفتند. جهت ارزیابی ترکیبات فعال زیستی و ویژگی‌های آنتی اکسیدانی قارچ دکمه‌ای، تست‌های تعیین ظرفیت فنلی تام، قدرت شلاته کنندگی، دی پی پی اچ و همچنین قدرت احیا کنندگی روی قارچها انجام شد. **نتایج:** با کسب ظرفیت فنلی بالاتر، و EC_{50} پایین‌تر در آزمون‌های دی پی پی اچ و قدرت احیا کنندگی، تیمار اسید مالیک توانست ویژگی‌های آنتی اکسیدانی و ترکیبات زیستی قارچ دکمه‌ای را در طول مدت نگهداری در مقایسه با کنترل به طور مؤثرتری حفظ کند. از طرف دیگر قدرت شلاته کنندگی قارچها تحت تاثیر تیمار اسید مالیک قرار نگرفت و ارتباط ضعیفی بین تیمار اسید مالیک و بقای قدرت شلاته کنندگی قارچ دکمه‌ای مشاهده گردید. **نتیجه‌گیری نهایی:** نتایج این تحقیق نشان داد که غوطه وری قارچ دکمه‌ای در محلول ۳٪ اسید مالیک قبل از نگهداری به خوبی می‌تواند ترکیبات فعال زیستی و ویژگی‌های آنتی اکسیدانی قارچ دکمه‌ای را در زمان نگهداری حفظ کند.

واژه‌های کلیدی: قارچ دکمه‌ای، ویژگی‌های آنتی اکسیدانی، زمان نگهداری، تیمار اسید مالیک

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