JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 2 (2015) 20-25

Effect of Citric Acid Dipping Treatment on Bioactive Components and Antioxidant Properties of Sliced Button Mushroom (*Agaricus bisporus*)

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Article type
Original article

Keywords
Citric Acid
Agaricales
Antioxidants

Received: 18 Oct 2014
Revised: 13 Nov 2014
Accepted: 23 Dec 2014

Abstract

Background: White button mushroom (*Agaricus bisporus*) is a sensitive agricultural crop whose antioxidant properties are decreased during post-harvest storage. In this study, the effect of citric acid dipping treatment on bioactive components and antioxidant properties of sliced button mushroom (*Agaricus bisporus*) was investigated.

Methods: Fresh mushrooms soaked in chilled citric acid solution at different concentrations (1%, 3% and 5% w/v) for 10 min were categorized as treatment groups and those dipped in chilled distilled water were considered as control. Bioactive components and antioxidant properties of sliced button mushroom were assessed on each designated day (every 5 days up to 15 days) of storage using chemical evaluations including total phenolics, chelating power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as well as assessment of reducing power. The significance of differences among treatments was determined by one-way analysis of variance (ANOVA) and Tukey tests using Sigma Stat software (version 2.03).

Results: Incorporation of 3% w/v citric acid treatment effectively maintained bioactive components and antioxidant properties [with 4.89 mg/g, 304.31 µg/ml (EC_{50}) and 1.69 mg/ml (EC_{50}) in total phenolic, DPPH and reducing power assays, respectively] at higher levels compared to control [with 3.12 mg/g, 420.42 µg/ml (EC_{50}) and 2.23 mg/ml (EC_{50}) in the mentioned evaluations] at the last day of the experiment ($p<0.05$). No significant difference was observed between 3% and 5% w/v citric acid solution treated samples ($p>0.05$).

Conclusion: These results showed that dipping in citric acid solution prior to storage can maintain bioactive components and antioxidant properties of sliced button mushrooms 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 to these 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 a functional food due to its high amount of protein, minerals,

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low starch and low cholesterol. Previous studies have demonstrated that the regular consumption of white button mushroom or consumption of its isolated bioactive constituents and antioxidant compounds (phenolics, carotenoids, flavonoids, tocopherols, ascorbic acid, etc.) is beneficial to health (Wani et al., 2010). From a post-harvest physiology point of view, mushroom is one of the most sensitive agricultural crops and its antioxidant properties tend to decrease during post harvest storage over time (Jahangir et al., 2011).

The water content of *Agaricus bisporus* mushroom is about 90% which accelerates the microbial spoilage and water-dependant reactions (Hershko and Nussinovitch, 1998). The storage of these mushrooms at low temperatures (1-4 ^{o}C) is the main factor of extension of their shelf life due to the decrease in the microbial growth rates and physiological reactions such as enzymatic browning and especially respiration (Kim et al., 2006). It should be noted that in some studies, the effects of different anti-microbial and anti-browning solutions along with hydrocolloid-based substances on the sensory attributes and microbial quality of a whole or sliced mushroom have been examined. These solutions include ascorbic acid, citric acid, hydrogen peroxide, sodium erythorbate, chlorine dioxide, sodium D-isoascorbate monohydrate, sodium D, L-isoascorbate and their derivatives as anti-bacterial and anti-browning agents (Cliffe-Byrnes and O'berrne, 2008; Hsu et al., 1988; Sapers et al., 2001; Simon and Gonza´ lez-Fandos, 2009).

Based on previous studies on the slowing of mushroom spoilage through coating and treatment with anti-browning solutions, this study investigated the effect of citric acid dipping treatment on the bioactive components and antioxidant properties of sliced button mushroom (*Agaricus bisporus*). To the best of our knowledge there is no prior report.

Materials and methods

Sampling

Fresh button mushrooms (*Agaricus bisporus*) were prepared from a local grower, transported to the laboratory within 2 h and stored in a refrigerator before experiments. After initially washing with distilled water for 1 min, the mushrooms were soaked in chilled citric acid (Merck, Germany) solution at concentrations of 1%, 3% and 5% w/v 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 were hand sliced and aseptically packed in polystyrene plastic food trays (250±5 g/tray) and over-wrapped with low density polyethylene films and were refrigerated (4 °C) for 15 days. At 5-day intervals, mushroom samples were taken out for further analysis.

Extraction

Extract of freeze dried mushroom samples was prepared following the method of Shyamala et al. (2005) with some modifications. Briefly, 15 g of dried and chopped mushroom was treated with 100 ml methanol (Chemical laboratories, Iran) for 24 h with occasional shaking and then was further filtered and evaporated to dryness in a vacuum dryer (Rotary evaporator, RE-52AA, China). Later, 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. (2007a). In order to estimate total phenolic compounds, 1 ml mushroom extract (5 mg/ml) was combined with 1 ml Folin and Ciocalteu's phenol reagent (Merck, Germany). One ml saturated sodium carbonate solution (Merck, Germany) was added to the mixture after 3 min and the total volume was adjusted to 10 ml with distilled water. This reaction mixture was then kept in the dark for 90 min before absorbance was read at 725 nm. A standard curve was calculated using gallic acid.

Measurement of reducing power

The reductive potential of methanolic mushroom extracts was determined following the method of Yen and Duh (1994). 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 min. A portion (2.5 ml) of trichloroacetic acid (10% w/v) was added to the mixture that was then centrifuged at 3000 xg for 10 min. The upper layer was separated and mixed with 2.5 ml 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 absorbance of 0.5 (EC_{50}) was determined from the graph of absorbance against concentration of extract.

DPPH assay

The hydrogen atom or electron donation abilities of the extracts were measured from the bleaching of the purple-colored methanol solution of 2, 2-di phenyl-1-picryl hydrazyl (DPPH, Sigma- Aldrich GmbH, Steinheim, Germany). This spectrophotometric assay was done using the stable radical DPPH as a reagent, according to the method of Burits and Bucar (2000). Briefly, 50 µl of the extracts (various concentrations) were added to 5 ml of the DPPH

solution (0.004% methanol solution). After 30 min 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 (I) according to the following equation:

$$I\ (\%)=(A_{blank}-A_{sample}/A_{blank})\times 100$$

Where A_{blank} is the absorbance of the control (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

The extract concentration, which can provide 50% of radicals scavenging activity (EC_{50}) 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 were estimated by the method of Chua et al. (2008) with slight modifications. Briefly, 200 µl of the extracts (different concentrations) and 740 µl methanol were added into 20 µl of 2 mM $FeCl_2$ (Merck, Germany). The reaction was initiated by the addition of 40 µl of 5 mM ferrozine (Sigma, Germany) into the mixture, which was then shaken vigorously and left standing at ambient temperature for 10 min. The ratio of inhibition of ferrozine (Fe^{2+}) complex formation was calculated as follows:

I (%)=[(absorbance of control-absorbance of test sample)/absorbance of control]×100

A lower absorbance indicates higher chelating ability. The extract concentration providing 50% chelating ability (EC_{50}) 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 difference among treatment means was determined by one-way analysis of variance (ANOVA) and Tukey test using Sigma Stat software (version 2.03). A *p* value less than 0.05 was statistically considered significant.

Results

Variations in values of total phenolics of *A. bisporus* extracts during storage are depicted in Table 1. During first five days of storage, levels of total phenolic compounds remained relatively stable in treated groups and there was no significant difference between them ($p>0.05$). Later, the levels of total phenolic compounds in all samples showed a trend to decrease. For the control sample the gradual decrease in total phenolic compounds from day 5 to end of the storage period was significantly ($p<0.05$) higher than treated samples. There was no difference between samples treated by different concentration of citric acid throughout the entire storage period ($p>0.05$).

Reducing power of *A. bisporus* during storage at 4 °C is delineated in terms of EC_{50} values (Table 2). During the whole storage period, a gradual increase in EC_{50} values was observed. However, mushrooms treated with solutions containing 3% and 5% (w/v) citric acid exhibited significantly lower increase in EC_{50} values compared to control ($p<0.05$). In this respect, the reducing power of control and treated samples with 1% citric acid from the 1th day until the 10th day of storage period were similar ($p>0.05$) and also there was no difference between 3% and 5% treated samples throughout the entire storage period ($p>0.05$).

Results of the DPPH assay of white button mushroom during storage at 4 °C are presented in Table 3 in terms of EC_{50} values. During storage EC_{50} values gradually increased, however EC_{50} values were significantly lower for mushrooms treated with citric acid irrespective of the concentrations as compared to control during whole storage period ($p<0.05$). Similar to the results of reducing power assay, 3% and 5% w/v citric acid treated samples during the storage period were not statistically different ($p>0.05$).

The effects of citric acid treatments on the chelating power of the mushrooms are presented in Table 4. Results indicated that EC_{50} amounts gradually increased during storage. It should be noted that citric acid treatment did not maintain the chelating power of mushrooms compared to the control groups during all days of storage.

Table 1: Effect of citric acid treatments on total phenolic compounds (mg/g) of *A. bisporus* during storage at 4 °C

Citric acid concentration (%)	Day 0	Day 5	Day 10	Day 15
0	5.82±0.22 [a*]	5.10±0.03 [b]	4.30±0.14 [c]	3.12±0.11 [c]
1	5.82±0.11 [a]	5.63±0.15 [a]	4.90±0.09 [b]	4.10±0.04 [b]
3	5.85±0.22 [a]	5.59±0.16 [a]	5.28±0.06 [a]	4.89±0.08 [a]
5	5.84±0.17 [a]	5.68±0.07 [a]	5.31±0.04 [a]	4.95±0.12 [a]

*Results are the average of triplicate±SD. Different letters within columns represent significant differences ($p<0.05$)

Table 2: Effect of citric acid treatments on power (EC_{50} value in mg/ml) of *A. bisporus* during storage at 4 °C

Citric acid concentration (%)	Day 0	Day 5	Day 10	Day 15
0	1.21±0.03 [a*]	1.57±0.08 [a]	1.81±0.01 [a]	2.23±0.09 [a]
1	1.24±0.04 [a]	1.48±0.08 [a]	1.78±0.09 [a]	1.97±0.10 [b]
3	1.23±0.05 [a]	1.31±0.04 [b]	1.45±0.04 [b]	1.69±0.06 [c]
5	1.24±0.07 [a]	1.29±0.06 [b]	1.36±0.10 [b]	1.58±0.11 [c]

*Results are the average of triplicate±SD. Different letters within columns represent significant differences (p<0.05)

Table 3: Effect of citric acid treatments on DPPH activity (EC_{50} value in µg/ml) of *A. bisporus* during storage at 4 °C

Citric acid concentration (%)	Day 0	Day 5	Day 10	Day 15
0	244.67±2.3 [a*]	284.61±4.1 [a]	359.62±7.9 [a]	420.42±8.2[a]
1	241.62±5.1 [a]	274.77±3.7 [b]	361.44±6.8 [a]	397.21±6.3[b]
3	243.75±4.9 [a]	261.11±3.12[c]	289.21±5.5 [b]	304.31±8.1[c]
5	245.15±4.1 [a]	252.48±2.84[c]	285.71±4.9 [b]	307.26±4.4[c]

*Results are the average of triplicate±SD. Different letters within columns represent significant differences (p<0.05)

Table 4: Effect of citric acid treatments on metal chelating (EC_{50} value in µg/ml) of *A. bisporus* during storage at 4 °C

Citric acid concentration (%)	Day 0	Day 5	Day 10	Day 15
0	2022.52±323 [a*]	2031.41±47.88 [a]	2086.58±34.41 [b]	2121.88±54.12[b]
1	2088.40±51.11 [a]	2091.86±37.21 [a]	2145.22±22.46[ab]	2399.31±62.10[a]
3	2080.20±53.87 [a]	2087.36±24.23 [a]	2193.39±48.20 [a]	2421.73±24.90[a]
5	2013.11±41.12 [a]	2054.79±38.44 [a]	2188.52±41.20 [a]	2404.62±27.22[a]

*Results are the average of triplicate±SD. Different letters within columns represent significant differences (p<0.05)

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 the antioxidant activity and phenolic contents of plants, fruits and beverages. It has been reported that among antioxidant sources, total phenols are the major antioxidant components found in the mushrooms and other antioxidants such as ascorbic acid, β-carotene and lycopene are only found in small or vestigial amounts (Barros et al., 2007b).

The total phenolic content of *A. 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 for *A. bisporus* and Hung and Nhi (2012), 4.1 mg/g for straw mushrooms (*Volvariella volvacea*).

A gradual decrease in phenolic compounds in water treated mushrooms during post-harvest storage (Table 1) could be due to the natural senescence process of mushrooms during storage (Lester, 2000) and especially the effect of the enzyme poly phenol oxidase (PPO) on their phenolic compounds (Hsu et al., 1988). According to Sedaghat and Zahedi (2012), citric acid is able to retard the PPO enzyme activity by reducing the pH of fruit tissue and reduction of coenzyme. Significantly, higher retention of phenolic compounds in citric acid treated mushrooms could be attributed to the anti-browning and anti senescence properties of citric acid.

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 (Chua et al., 2008). 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 (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) had significant DPPH radical scavenging activity with EC_{50} values of 2990 and around 20 mg/ml, respectively. Our results indicate that methanol

extract of *A. bisporus* has much better performance (EC_{50}=244.67±2.31 µg/ml) against DPPH radical.

The reducing power of a compound may serve as an 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 the specimen. The presence of reductant in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to ferrous form (Chua et al., 2008).

Jahangir et al. (2011) reported that *A. bisporus* extracts had EC_{50} values of 2.04±0.02 mg/ml. The reducing power of *A. bisporus* extract in our study (EC_{50}=1.21±0.03 mg/ml) is better than that in those previous works.

According to Tables 2 and 3, citric acid dipping treatment resulted in higher antioxidant capacity as evidenced by lower EC_{50} values for DPPH activity and reducing power assay while water treated mushrooms exhibited lower activity of antioxidants as shown by higher EC_{50} values for DPPH activity and reducing power assay. 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, the antioxidant properties of citric acid treated mushrooms could be due to better retention of phenolics than in water treated mushrooms. A gradual decline in antioxidant properties (as evidenced by higher EC_{50} values for DPPH activity and reducing power assay) of button mushrooms during storage could be explained by possible involvement of these bioactive components in defense-associated mechanisms related to natural senescence during storage. The chelating effects of various extracts on Fe^{2+} were determined by the formation of ferrozine-Fe^{2+} complexes. Since chelating agents capture ferrous ion before ferrozine, the formation of ferrozine-Fe^{2+} will be postponed. Therefore, measuring the rate of color reduction helps to estimate the chelating activity of the samples. The metal chelating capacity is important since it reduces the transition metals concentration 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 also may inhibit the Fenton reaction and hydroperoxide decomposition (Liu et al., 2010). As shown in Table 4, citric acid dipping resulted in poor metal chelating activity as evidenced by higher EC_{50} values for metal chelating assay while water treated mushrooms exhibited better chelating properties as shown by lower EC_{50} values for chelating activity from day 10^{th} to the end of the storage period.

It has been reported that compounds with structures containing two or more of the following functional groups: -OH, -SH, -COOH, $-PO_3H_2$, -C=O, $-NR_2$, -S- and -O- in a favorable structure-function configuration can show metal-chelating activity (Lindsay, 1996) and it was shown that there is a weak correlation between the phenolic content and chelating activity against Fe^{2+} that is in agreement with our results.

Conclusion

These results indicated that dipping in citric acid solution prior to storage can maintain bioactive components and antioxidant properties of sliced button mushrooms during storage. In this study, no significant difference was observed between 3% and 5% w/v citric acid solution treated samples. Therefore, the lower concentration of acid (3% w/v) is suggested on economic points of view.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors would like to acknowledge the Damghan branch of Islamic Azad University and Semnan University Research Offices for the financial supports.

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