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ORIGINAL PAPER

Aloe vera gel treatment delays postharvest browning of white button mushroom (*Agaricus bisporus*)

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Abstract
Postharvest browning is the limiting factor for the storage of white button mushroom (*Agaricus bisporus*). Aloe vera has been shown to have a wide range of physiological effects in horticultural produce. To investigate the role of Aloe vera coatings on mushroom quality and browning during storage, button mushrooms were treated with Aloe vera gel (0, 25, 50 or 75%) and stored at 4 °C for 15 days. The results showed that treating the mushrooms with Aloe vera gel before storage, lowered weight loss and the levels of surface browning and also increased superoxide dismutase activity, total phenolic content and 1,1-diphenyl-2-picrylhydrazyl scavenging activity. Moreover mushrooms treated with Aloe vera gel treatments also maintained lower thiobarbituric acid-reactive substances, hydrogen peroxide (H_2O_2) and hydroxyl radical (–OH) and decreased polyphenol oxidase activity compared with non-treated mushrooms. These results showed that pre-storage treatment with Aloe vera gel decreased white button mushrooms browning during cold storage and that the optimum treatment was 50% Aloe vera gel for maintaining the quality of button mushroom during storage.

Keywords Polyphenol oxidase · Reactive oxygen species · Storage · Superoxide dismutase · Total phenols.

Introduction

Button mushroom (*Agaricus bisporus*) is one of the most widely used mushrooms in the world which contains important nutrients and phytochemicals which have significant antioxidant abilities and human health benefits [1]. Surface browning is an important factor which limits consumer acceptability of button mushrooms. Enzymatic browning occurs in many horticultural produce and is generally related to polyphenol oxidase (PPO) activity, phenolic substrate availability, membrane integrity and increasing lipid peroxidation [2]. In many horticultural crops, surface browning is the result of senescence and the accumulation of reactive oxygen species (ROS) such as hydrogen peroxide, which leads to an increase PPO and the subsequent surface browning which reduces consumer appeal and quality [3]. Antioxidant enzymes like superoxide dismutase (SOD) have the ability to protect cells from ROS and reduce browning [4].

Postharvest browning is the limiting factor for the storage of white button mushroom (*Agaricus bisporus*) which mostly happens through the oxidation of phenolic substances into quinones by enzymatic action, like PPO, which eventually polymerize to produce browning [5, 6]. A number of postharvest treatments have been examined to reduce the levels of postharvest browning in mushrooms and include treatment with glycine betaine [7], brassinolide [6] and 4-methoxy cinnamic acid [8]. Gel from the *Aloe vera* plant has been used as an ingredient in many foods for many years and has been extensively studied in many food applications such as edible coatings [9]. The leaves of the Aloe vera plant contain the characteristic mucilaginous clear gel, which is applied as Aloe vera gel. The gel works has been shown to be a barrier to O_2 and CO_2 and also acts as moisture barrier and therefore reducing weight loss, browning, softening, and growth of yeast and molds [10]. Aloe vera gel itself comprises a wide range of nutrients and phytochemicals such as vitamins, amino acids, sugars, minerals, and enzymes which contribute to its benefits to human health. Chrysargyris et al.

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[11] and Martínez-Romero et al. [12] showed that the application of Aloe vera gel coating could increase quality of tomato and plum fruit during storage. Limited studies have been conducted on the effects of Aloe vera gel coating on postharvest browning. Supapvanich et al. [13] showed that an Aloe vera gel coating retarded browning on fresh cut wax apple fruit. However detailed examinations on the postharvest effects of Aloe vera gel on browning of mushroom have not been reported. This study examined the effect of Aloe vera gel on postharvest browning of white bottom mushroom during cold storage.

Materials and methods

Plant material

Commercial grade white button mushrooms (*Agaricus bisporus*) were obtained from Meygol company, Ardekan, Fars (Iran) and transported back to the laboratory within 2 h of harvest. Homogeneous color and size (cap diameter 3.5–4 cm) mushrooms without injuries were selected for this study. Aloe vera gel was prepared based on method described by Sogvar et al. [14]. Mature Aloe vera (*Aloe barbadensis* Miller) leaves were taken from a commercial greenhouse in Yasuj province and washed with a mild chlorine solution of 0.03% (v/v). Then matrix was detached from the outer cortex of leaves and blended. The mixture was then filtered to remove the fibers to obtain the fresh Aloe vera gel.

Mushrooms were dipped in control (0%) and Aloe vera gel solutions diluted at 1:3 (25%), 1:1 (50%) and 3:1 (75%) with distilled water at 20 °C for 5 min. After treatment, mushrooms were air-dried for 30 min before cold storage at 4 ± 1 °C and 85–90% RH for up to 15 days. All mushrooms were placed into plastic trays (165 × 125 × 65 mm) over-wrapped with plastic films (0.02 mm thick polyvinyl chloride). Mushrooms were destructively assessed at 0, 5, 10 and 15 days of cold storage.

Weight loss analysis

For determining weight loss, mushrooms were weighed using an electronic balance (A&D Company, Japan). Percentage weight loss was determined by measuring weight at the beginning and the end of storage.

Browning Index (BI)

Minolta colorimeter (CR-300, Osaka, Japan) was used to measure the surface color of mushroom caps where three readings at three points on each mushroom cap were measured. Eight mushrooms were analyzed for each replication. The following equation was used for the BI measurement based on Ding and Ling [15]. BI = [100 (x − 0.31)]/0.17, where x = (a* + 1.75L*)/(5.645L* + a* − 0.3012 b*)). L* represents (light/dark), a*represents (red/green) and b* represents (yellow/blue).

Determination of total phenolic content (TPC) and DPPH scavenging activity

TPC was measured using the method described by Lu et al. [1]. Mushroom caps (5 g) were homogenized with 25 mL 80% ethanol, and the mixture was centrifuged at 1725×*g* for 10 min at 4 °C. 2 mL of sodium carbonate solution (20% w/v) was then mixed with 0.8 mL of diluent, and reacted with 1 mL of Folin Ciocalteu solution. The absorbance of mixture at 760 nm was measured. Gallic acid was used for a standard curve and results expressed as μg g^{-1}FW. 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was measured according to the procedure described by Alothman et al. [16]. Briefly, cap tissues (2 g) were homogenized in 10 mL of methanol and centrifuged at 6000×*g* for 15 min at 4°C. Then 50 μL of supernatant were mixed with 1 mL of DPPH and 1.5 mL of Tris buffer. The absorbance was measured with the spectrophotometer at 517 nm. The following equation was used for DPPH calculation, where Abs control is the absorbance of DPPH solution without extract.

$$\%\ \text{inhibition of DPPH} = (\text{Abs control} - \text{Abs sample})/\text{Abs control} \times 100.$$

Determination of reactive oxygen species

The procedure from Khan et al. [17] was used for the measurement of hydrogen peroxide (H_2O_2) content. Mushroom cap tissues (300 mg) were mixed with 4 mL of 0.1% (w/v) trichloroacetic acid. The mixture was the centrifuged at 12,000×*g* for 15 min at 4°C. After centrifugation, 0.5 mL of the supernatant was diluted with 0.5 mL of 10 mmol L^{-1} potassium phosphate buffers (pH 7.0) and 1 mL of 10 M potassium iodide. The absorbance was measured with the spectrophotometer at 390 nm and expressed as μmol g^{-1} FW.

Hydroxyl radical (–OH) was measured according to Khan et al. [17]. Mushroom cap tissues (50 mg) were homogenized with 1 mL of 10 mmol L^{-1} Na-phosphate buffer (pH 7.4) containing 15 mmol L^{-1} 2-deoxy-D-ribose at 37 °C. Following incubation, 0.7 mL of the mixture was added to the reaction solution containing 3 mL of 0.5% (w/v) thiobarbituric acid (TBA) and 1 mL of glacial acetic acid. The absorbance was recorded with a spectrophotometer at 532 nm, and expressed as μmol g^{-1} FW.

Thiobarbituric acid-reactive substances (TBARS)

TBARS content was measured for estimating the levels of lipid peroxidation according to the method described by Dokhanieh and Aghdam [18]. Cap tissues (2 g) from 10 mushrooms were ground with 15 mL of 50 mmol L^{-1} Tris–HCl buffer (pH 7.8). The mixture was then centrifuged at 10,000×*g* for 20 min at 4 °C and supernatant was used for the determination of TBARS content. A 4 mL sample of the supernatant was mixed with 0.2 mL of 200 g L^{-1} TCA and 3.8 mL of 5 g L^{-1} TBA and incubated in boiling water for 25 min. The mixture was then centrifuged at 10,000×*g* for 10 min and the absorbance measured at 532, 600 and 450 nm. TBARS content was calculated by the following formula, TBARS content = $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$. Results were expressed as nmol g^{-1} FW.

Determination of browning related enzyme activities (PPO and SOD)

The extraction method for the measurement of PPO and SOD was modified from Ding et al. [6]. In brief, frozen mushroom tissue (5 g) were homogenized and extracted with 10 mL 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 5% (w/v) polyvinyl poly pyrrolidone. The mixture was then centrifuged at 12,000×*g* for 30 min at 4 °C, and supernatant was used for the enzymatic assays.

PPO activity was determined according to the method described by Yingsanga et al. [19]. For PPO analysis, 1 mL of supernatant was mixed with 1 mL of sodium phosphate buffer (100 mM, pH 7.0) and 1 mL pyrocatechol (50 mM). The change in absorbance at 410 nm was measured spectrophotometrically and absorbance was recorded at 410 nm and was expressed as units (U) mg^{-1} protein. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of nitroblue tetrazolium and was expressed as U mg^{-1} protein [20]. Protein content was determined according to Bradford [21] using bovine serum albumin as a standard.

Statistical analysis

All the experiments were conducted using completely randomized design (CRD) with four replicates per treatment. A total of 192 mushrooms per treatment were used. Data were subjected to Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS) version 8.2 (SAS Institute Inc., Cary, NC, USA). Sources of variation were storage days and treatments. The means were compared with the Duncan's Multiple Range Test (DMRT) at significance level of $P < 0.05$. The experiment was repeated three times and the data pooled before analysis.

Results and discussion

Weight loss and BI

Weight loss and consequent shrivel is a significant commercial and consumer attribute, as mushrooms are prone to high water loss due their high transpiration rate and lack of a protective structures (e.g. surface layers of cells) to prevent excessive moisture loss and shrivel [13]. As expected in this experiment, weight loss increased for all treatments during storage (Fig. 1a), however all Aloe vera gel treatments retained more weight (water) than the control treatments. At all assessment times, both of the highest Aloe vera gel treatments (50 and 75% Aloe vera gel) resulted in the lowest weight loss, whilst the 25% Aloe vera gel treatment was intermediate between the highest concentrations and

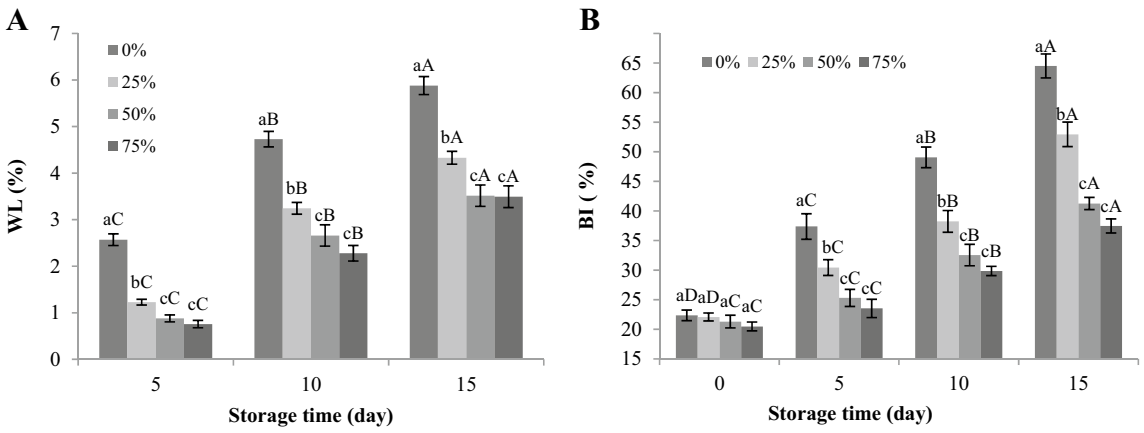

Fig. 1 WL (**a**) and BI (**b**) values in mushrooms treated with Aloe vera gel (0, 25, 50 and 75%). Mean comparison with different small and capital letters indicate significant differences by DMRT at $P < 0.05$ between treatments within each storage time and storage time within each treatment, respectively. Vertical bars represent standard error of means of three experiments with four replicates per experiment

the non-Aloe vera gel control. These results are comparable with Guillén et al. [9] who mentioned that Aloe vera gel could decrease weight loss of peach and plum. These results confirm that Aloe vera coatings act as a semi-permeable barrier against moisture which thereby reduces water loss [9].

Surface browning is the major limiting factor affecting consumer appeal of mushrooms [22]. In this experiment, all concentrations of Aloe vera gel delayed the development of postharvest browning at all storage times (Fig. 1b). Mushrooms untreated (control) always resulted in the highest level of postharvest browning at all storage times. There were no significant differences in the levels of browning after treatment with Aloe vera gel between the higher concentrations (50 and 75%), however the browning of the higher levels of Aloe vera gel was lower compared to that of the 25% Aloe vera gel treatment. These results support the observation that lower browning was associated with lower moisture loss [23].

TPC and DPPH scavenging activity

The postharvest application of Aloe vera gel to mushrooms resulted in higher levels of TPC and DPPH scavenging activity than in control mushrooms during storage at all storage and assessment times (Fig. 2a, b). The rapid accumulation of antioxidant capacity, as measured with DPPH in Aloe vera gel treated mushrooms during storage (Fig. 2b) could be attributed to the strong retention of phenolic compounds of Aloe vera gel treated mushrooms (Fig. 2a). Phenolic compounds have been shown to be major antioxidants in mushrooms [1, 24] while the major phenolic compound in Aloe vera gel itself is aloe emodin (1, 3, 8-trihydroxyanthraquinone), which is thought to contribute to its antioxidant activity. The accumulation of phenolic compounds in mushrooms treated with Aloe vera coating in this experiment may be due to an increase in phenylalanine ammonia lyase activity which has been shown to retain high phenolic and antioxidant capacity than controls [25].These results are comparable with Hassanpor [25] who also showed that Aloe vera coating could increase TPC in raspberry during storage. These results suggest that Aloe vera gel treatments can be used to maintain TPC accumulation in mushroom and lead to increasing DPPH scavenging capacity which may alleviate mushroom cap browning.

H_2O_2 and –OH

The levels of reactive oxygen species, H_2O_2 and –OH, in mushrooms treated with Aloe vera gel and stored for up to 15 days are presented in Fig. 3a, b. The results show that there is an increase in the levels of H_2O_2 in the control mushrooms during storage. H_2O_2 is a strong oxidant which oxidizes membrane lipids and has been shown to be responsible for destruction of cell integrity and mushroom senescence [17, 26]. In this experiment, treatment with Aloe vera gel resulted in lower H_2O_2 levels at all storage assessments, with the highest Aloe vera gel treatment (75%) have similar H_2O_2 levels after 15 days storage, as compared to at the beginning of the storage; whereas the control H_2O_2 levels were nearly doubled (Fig. 3a).

The levels of –OH are thought to play an important role in oxidation processes [17]. In this experiment, Aloe vera gel treated mushrooms always had lower levels of –OH at all storage times (Fig. 3b). Many studies in other crops have shown a close relationship between browning and the accumulation of reactive oxygen species such as H_2O_2 and –OH. For example Chomkitichai et al. [27] demonstrated an increase in the level of browning of longan fruit with increasing of levels of H_2O_2 and –OH. It

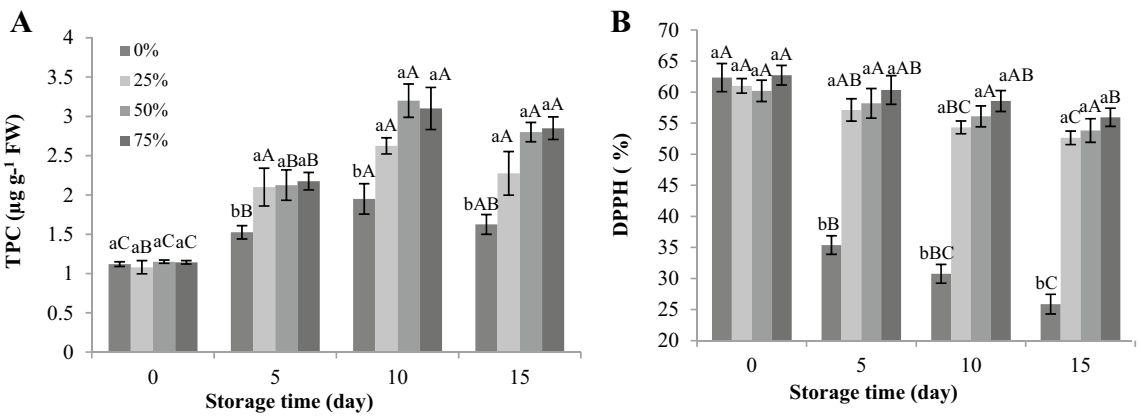

Fig. 2 TPC (**a**) and DPPH scavenging activity (**b**) values in mushrooms treated with Aloe vera gel (0, 25, 50 and 75%). Mean comparison with different small and capital letters indicate significant differences by DMRT at $P<0.05$ between treatments within each storage time and storage time within each treatment, respectively. Vertical bars represent standard error of means of three experiments with four replicates per experiment

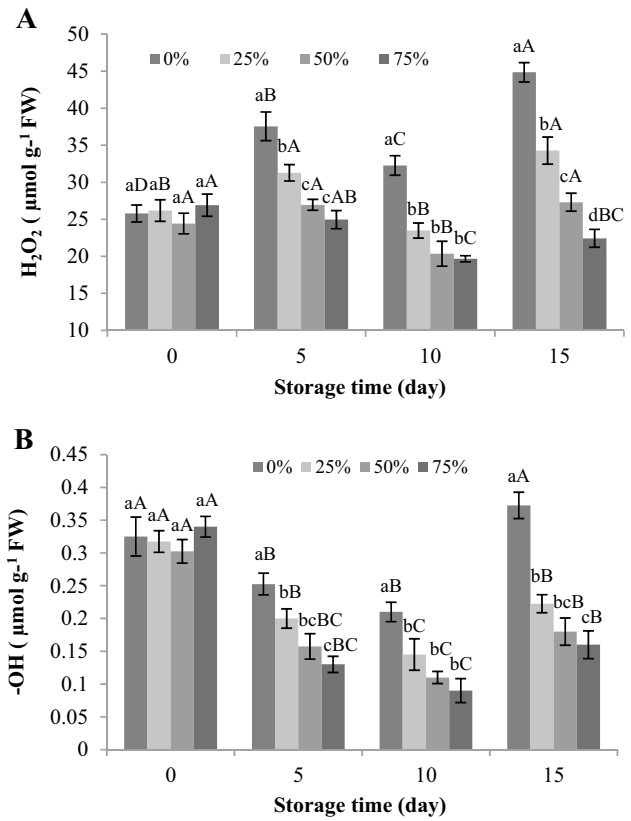

Fig. 3 H_2O_2 (**a**) and (–OH) (**b**) values in mushrooms treated with Aloe vera gel (0, 25, 50 and 75%). Mean comparison with different small and capital letters indicate significant differences by DMRT at $P<0.05$ between treatments within each storage time and storage time within each treatment, respectively. Vertical bars represent standard error of means of three experiments with four replicates per experiment

is thought that this production of ROS (H_2O_2 and –OH) might disrupt the structure of cell membranes to cause the loss of cellular compartmentalization leading to increase in browning enzymes which may reduce the storage quality and marketability of harvested horticultural crops [3, 27, 28].

Membrane integrity indicator

TBARS is a by-product of lipid peroxidation and is used as an indicator of membrane integrity [18, 29]. In this experiment, the level of TBARS increased in the non-Aloe vera gel control mushrooms during storage (Fig. 4). However, significantly lower TBARS levels were measured in both the 50 and 75% Aloe vera gel treatments. The results showed that both the 50 and 75% Aloe vera gel treatments inhibited TBARS accumulation, suggesting the maintenance of membrane integrity. These observations are comparable with Dokhineh and Aghdam [18] who showed

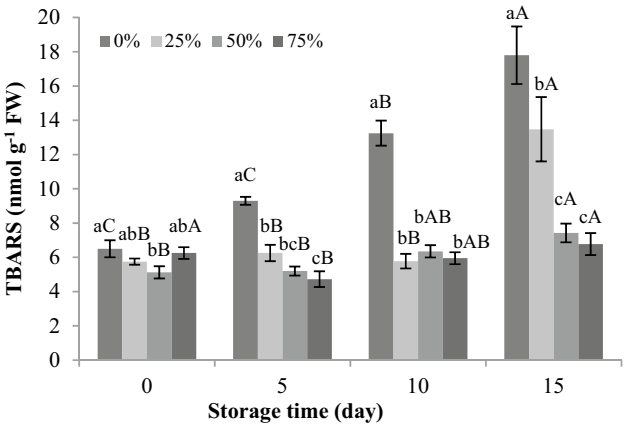

Fig. 4 TBARS values in mushrooms treated with Aloe vera gel (0, 25, 50 and 75%). Mean comparison with different small and capital letters indicate significant differences by DMRT at $P<0.05$ between treatments within each storage time and storage time within each treatment, respectively. Vertical bars represent standard error of means of three experiments with four replicates per experiment

that salicylic acid application could decrease browning of mushroom with lower TBARS content.

PPO and SOD activities

The activities of the browning related enzymes, PPO increased in all treatments during storage (Fig. 5a, b). However the level of PPO activities in Aloe vera gel treatments were always lower than non-Aloe vera gel treated mushrooms during storage (Fig. 5a, b). Enzymatic browning reactions generally negatively affect food quality and therefore inhibiting these reactions are beneficial to maintain quality. PPO activity is often related with tissue browning [30]. In this experiment, the reduction of PPO activity could be related to the increase of total phenols observed in Aloe vera treated mushrooms where the inhibition of mushroom browning is a result oxidation of phenolic compounds [6]. The reduction of browning with decreased PPO has been observed in other browning systems such fresh cut banana [31]. These results are comparable with Supapvanich et al. [13] who mentioned Aloe vera coating could retard PPO activity of wax apple during storage.

The activity of SOD in treated mushrooms are presented in Fig. 5b and the results show that Aloe vera gel treated mushrooms had higher SOD activity during storage compared with control (Fig. 5b). SODs have been shown to possess antioxidant defensive roles against free radicals in many systems [32]. Saba and Moradi [4] showed lower activity of SOD resulted in higher browning of pear cultivars.

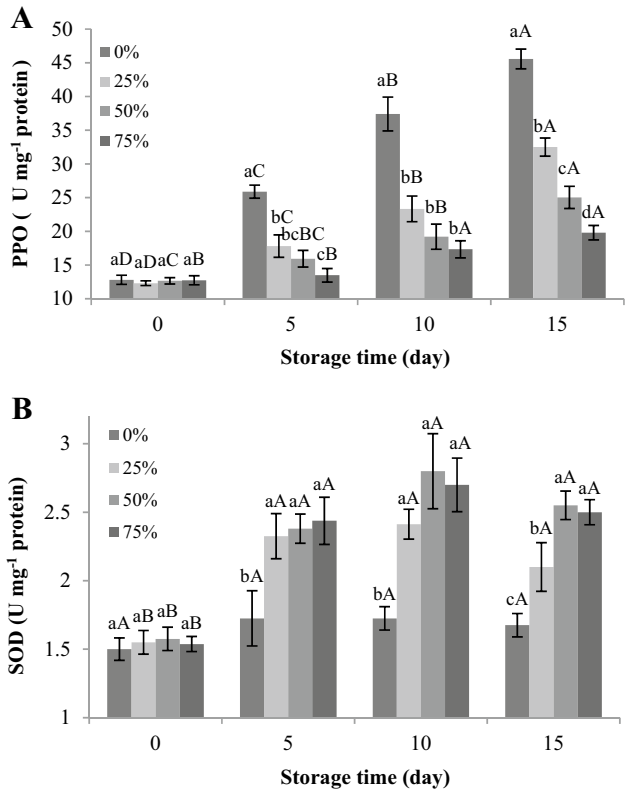

Fig. 5 PPO (**a**) and SOD (**b**) activity values in mushrooms treated with Aloe vera gel (0, 25, 50 and 75%). Mean comparison with different small and capital letters indicate significant differences by DMRT at $P<0.05$ between treatments within each storage time and storage time within each treatment, respectively. Vertical bars represent standard error of means of three experiments with four replicates per experiment

Conclusion

Aloe vera gel has been assessed as a useful coating for maintaining postharvest quality of fresh products. The results of this experiment show that the application of Aloe vera gel dip before storage was effective in reducing postharvest browning, weight loss and PPO activities during cold storage for up to 15 days. Moreover, mushrooms treated with Aloe vera gel showed lower biochemical indicators of oxidation like TBARS, H_2O_2 and –OH. These results demonstrate that a pre-storage Aloe vera gel treatment maybe an environmental friendly treatment for decreasing browning and increasing quality of white bottom mushrooms but more work is required to assess different application methods, times and concentrations. Based on the results of this experiment optimum treatment was 50% Aloe vera gel for maintaining the quality of button mushroom during storage.

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