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Maintaining Postharvest Quality of Fresh Button Mushrooms by Application of Hydrogen Peroxide and Antibrowning Agent Treatments

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Abstract

This experiment was conducted to study the effect of postharvest dipping of fresh button mushrooms (*Agaricus bisporus*) in hydrogen peroxide (H_2O_2) 5% alone or in combination with antibrowning agents (calcium chloride at 3 or 5%, ascorbic acid 3 or 5% and citric acid 2 or 4%) beside dipping in distilled water (control) to inhibition browning, had the lower level of microbial load and maintaining quality during storage for 12 days at 4 ± 1°C and 90 ± 2% relative humidity during two successive seasons of 2016 and 1017. Results showed that all studied treatments reduced weight loss percentage, browning index and Polyphenol oxidase activity, total bacteria counts, yeast and mold counts and maintaining of phenolic and flavonoids contents of fresh button mushrooms during cold storage comparing with untreated control. However, dipping button mushrooms in solution H_2O_2 5% + calcium chloride 5% was the most effective in this concern, which reducing browning, maintaining quality and inhibiting microbial counts for 12 days at 4 ± 1°C and 90 ± 2% relative humidity.

Keywords: Button mushrooms (*Agaricus bisporus*), Hydrogen peroxide, Calcium chloride, Ascorbic acid, Citric acid, Browning, Quality, Cold storage.

Introduction

Button mushrooms (*Agaricus bisporus*) have been used throughout the world for many centuries not only for their delicacy, flavor and nutritional value but also for functional aim In fact, mushrooms contain necessary nutrients and bioactive components such as several essential amino acids, vitamins (riboflavin, niacin and folate), minerals (potassium, phosphorus, zinc, copper, magnesium and selenium) (Tao *et al*., 2006 and Singla *et al*., 2012), unsaturated fatty acids and dietary fibers. Moreover, they contain different polyphenolics and flavonoids, which are recognized as excellent antioxidants (Singla *et al*., 2012).Button mushrooms has a very high metabolic activity that can result in deteriorative changes, such as increased respiration rate and ethylene production and also high water content (Mohebbi *et al*., 2012), hence having a short shelf life of 3 – 4 days at ambient temperatures (Tao *et al*., 2006). Postharvest browning of mushrooms is a severe problem that reduces the shelf life because of respiration and biochemical activities (Singla *et al*., 2012). The high tyrosinase and phenolic content of mushrooms makes them prone to enzymatic browning which is the major cause of quality loss and accounts for reduction in their market value (Brennan *et al*., 2000). Discoloration reactions of mushrooms are complex and depend on raw material condition, washing, cutting, handling and packaging practices and bacterial growth during storage. Color, fresh and clean appearance and uniform closed buttons have high importance for mushroom quality and consumer preferences (Vizhanyo and Jozsef, 2000).

Most strategies of controlling the browning process are either inhibiting polyphenol oxidase (PPO) activity or converting quinones to colorless materials (Stohs and Miller, 2014) in order to controlling the enzymatic browning and the quality losses of button mushrooms, it is necessary to use some antibrowning agents (ascorbic acid, citric acid and calcium chloride) and hydrogen peroxide as a postharvest treatment in conjunction with low temperature to extend shelf life of button mushrooms.

Citric acid is widely used as an additive and as an antimicrobial by virtue of their low pH in food industry dipping in citric acid solution prior to storage can maintain bioactive components and antioxidant properties of sliced button mushrooms during storage. Citric acid is able to retard 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 (Javan *et al*., 2015).

Applying calcium chloride treatment may contribute to cell wall integrity by increasing the amount of endogenous calcium available to bind with deesterified pectic residues. In addition to preserving firmness, postharvest calcium dips reduce respiration, decrease ethylene production and delay senescence in fresh produce such as carrots (Izumi and Watada, 1994), strawberries (Rosen and Kader, 1989) and tomatoes (Artes *et al*., 1999). The potential of calcium in imparting stability to vacuole membranes and further slowing the enzymatic browning was studied by (Beelman *et al*., 1987). They also indicated that $CaCl_2$ inhibited browning of mushrooms. Calcium dips raise the possibility of producing fruit less susceptible to flesh browning symptoms. Rosen and Kader (1989) reported that 1% $CaCl_2$ dip reduced softening and browning rates of 'Bartlett' pear slices. This study also indicates that $CaCl_2$ treatments had

lower browning index (BI) compared to control. This could be due to the fact that calcium helps to maintain membrane stability as mentioned by Poovaiah, (1988) and Picchioni *et al*. (1995).

Ascorbic acid is a good antioxidant (Ali *et al*., 2013) ascorbic acid was considered as suitable enzymatic browning inhibitors in fresh cut apple, pineapple and potato in many literatures (Kwak and Lim, 2005; Limbo and Piergiovanni, 2006; Jeong *et al*., 2008). McEvily *et al*. (1992) suggested that ascorbic acid inhibits PPO activity by reducing o-quinones to diphenols. Some reports attributed its action to the reduction of the formed quinones to the original colorless diphenols (Limbo and Piergiovanni, 2006) and considered ascorbic acid as a PPO inhibitor (Altunkaya and Gökmen, 2008). Ascorbic acid could be effective in reducing the respiration rate and ethylene production in fresh cut product (Gil *et al*., 1998).

Hydrogen peroxide (H_2O_2) is strong oxidizer proposed as an alternative means of decontaminating fruits and vegetables. It has been effective in extending the shelf life of cantaloupe, cucumber, zucchini, bell peppers, grapes, and mushrooms (Sapers and Simmons, 1998). It is generally used as a bleaching and antimicrobial agent and could control the bacterial blotch and browning of mushrooms (Raffellini *et al*., 2008). Moreover, washing in H_2O_2 solution can markedly reduce human pathogens *E. coli* and *Salmonella* spp. (Ukuku *et al*., 2005).

The objectives of this study are to investigate the maintaining postharvest quality of fresh button mushrooms by application of hydrogen peroxide and antibrowning agent treatments (calcium chloride, ascorbic acid and citric acid) during 12 days of storage at 4°C.

Material and methods

Freshly harvested, button mushrooms (*Agaricus bisporus*) were purchased from Food Technology Research Institute at Giza, Egypt, during two successive seasons of 2016 and 2017 in 4th and 6th of December in the first and second seasons respectively, then delivered to laboratory of Vegetable Crops Postharvest and Handling Department, Horticulture Research Institute at Giza, Egypt. Button mushrooms uniform size and intact veil and free from blemishes were selected for postharvest experiment. Button mushrooms were washed with tap water and then treated with the following, treatments.

Treatments:

1. Dipping in solution of H_2O_2 5% for 5 min then $CaCl_2$ 3% for 10 min.
2. Dipping in solution of H_2O_2 5% for 5 min then $CaCl_2$ 5% for 10 min.
3. Dipping in solution of H_2O_2 5% for 5 min then Ascorbic acid 3% for 10 min.
4. Dipping in solution of H_2O_2 5% for 5 min then Ascorbic acid 5% for 10 min.
5. Dipping in solution of H_2O_2 5% for 5 min then Citric acid 2% for 10 min.
6. Dipping in solution of H_2O_2 5% for 5 min then Citric acid4% for 10 min.
7. Dipping in solution of H_2O_2 5% for 5 min.
8. Control (untreated).

All treatments were air dried and packed of button mushrooms in polypropylene bags (25 × 30 cm) and 20mm thickness. Each bag contain 100 g mushrooms represented as experimental unit (EU). Twelve EU were prepared for each treatment and stored at 4 ± 1°C and 90 ± 2% RH for 12 days. Samples were taken randomly in three replicates EU and were arranged in complete randomized design. Measurement were examined immediately after treatment and after 3, 6, 9 and 12 days for the following characteristics.

Weight loss (%):

It was calculated according to the following equation:

Weight loss % = [(Initial weight of mushrooms– weight of mushrooms at sampling date)/ Initial weight of mushrooms] x 100

Polyphenol oxidase (PPO) activity:

Polyphenol oxidases (PPO, E.C. 1.14.18.1) activity in mushrooms extract, during the storage period was determined according to Pizzocaro *et al.* (1993).

Total phenolic and flavonoids contents:

Total phenolic contents were measured according to Singleton and Rossi, (1965).

Flavonoids were extracted and determined according to the methods of Barros *et al*. (2008).

Browning Index (BI):

The color of the mushroom cap was measured using a Minolta Chroma Meter (Model CR-155, Minolta Camera Co., Osaka, Japan), using the Hunter Lab Color Scale. Four mushrooms were randomly selected from each sample and the color was measured at four equidistant points on each mushroom cap using an aperture diameter of 4mm. Mushroom color has been commonly measured using the L* value of the Hunter scale (Cliffe-Byrnes and O'Beirne, 2007). Also to changes in other parameters of the hunter scale (a* and b*) related to browning (Aguirre *et al*., 2008). In order to capture this variation in a single index that would be related to a turn towards brown color, browning index (BI) was calculated according to Bozkurt and Bayram, (2006) and Ruangchakpet and Sajjaanantakul, (2007) using the following equation:

Browning Index (BI) = [100 (x - 0.31)] / 0.17, where x = (a* + 1.75L*) / (5.645L* + a* – 0.3012b*), L* value indicates lightness of the color, which range from 0 (dark) to 100 (white). The positive value of a* indicates red color, while negative value indicates green color. The positive value of b* indicates yellow color, while negative value indicates blue color.

Total bacteria counts (TBC) and total yeast and mold counts (TYMC):

The population of total bacteria counts (TBC) and total yeast and mold counts (TYMC) were determined by the method of Gonulalan *et al*., (2003).

All data were subjected to the statistical analysis according to the method described by Sendecor and Cochran, (1980).

Results and discussion

Weight loss (%)

Water loss is an important physiological process that affects the main quality attributes of fresh mushrooms. In mushrooms, the phenomenon causes shrinkage when it becomes excessive. The weight reduction of button mushroom during storage is shown in Table (1). Generally speaking, there was reduction in weight in all samples as the time (days) increased. Weight loss in mushrooms is a common phenomenon which occurs mainly due to moisture loss and loss of carbon reserves due to respiration (Jauathunge and Illeperuma, 2001). Concerning the effect of postharvest treatments, data revealed that were significant differences among treatments in weight loss% during storage, all treatments reduced weight loss % as compared with untreated control. Moreover, button mushrooms dipped in solution of H_2O_2 5% +$CaCl_2$ 5% was the most effective treatment for reducing the weight loss during storage, followed by $CaCl_2$ 3% or citric acid (4 and 2%) or ascorbic acid (5 and 3%) in combination with H_2O_2 5%. Hydrogen peroxide treatment alone was less effective in this concern; while untreated control gave the highest value of weight loss%. These results were true in the two seasons and in agreement with Koushki *et al.* (2011) for $CaCl_2$, Ali *et al.* (2015) for ascorbic acid and Gupta and Bhat, (2016) for H_2O_2 and citric acid. This may be associated to the contribution of calcium to maintain the cellular organization and to regulate enzymatic activities, thereby retarding the moisture loss caused by the senescence (Koushki *et al.*, 2011). Hydrogen peroxide and citric acid treatment of mushrooms had a significant effect in controlling weight loss and it might be due to these materials helped in reducing the rate of respiration and transpiration ,which in turn reducing weight loss during storage (Gupta and Bhat, 2016). Brennan *et al*. (2000) found the application of citric acid at 1% exhibited a beneficial effect on the quality of fresh mushrooms which reducing weight loss and microbial growth and keeping the fresh color and increasing the shelf life period.

Table 1. Effect of hydrogen peroxide and anti-browning agent treatments on weight loss (%) of fresh mushrooms during cold storage at 4 °C in 2016 and 2017 seasons.

Treatments	2016					2017				
	Storage period in days									
	3	6	9	12	M	3	6	9	12	M
H_2O_2 5%+ $CaCl_2$ 3%	0.71	1.20	1.55	2.41	1.47	0.73	1.24	1.71	2.50	1.54
H_2O_2 5%+ $CaCl_2$ 5 %	0.34	0.83	1.34	2.25	1.19	0.35	0.85	1.38	2.32	1.23
H_2O_2 5%+ Ascorbic acid 3%	0.91	1.54	2.17	3.21	1.96	0.94	1.59	2.24	3.30	2.02
H_2O_2 5%+ Ascorbic acid 5%	0.95	1.55	2.14	3.14	1.95	0.98	1.59	2.21	3.23	2.00
H_2O_2 5%+ Citric acid 2%	0.93	1.40	1.87	2.74	1.74	0.96	1.44	1.92	2.82	1.79
H_2O_2 5%+ Citric acid 4%	0.85	1.26	1.65	2.56	1.58	0.87	1.24	1.70	2.63	1.61
H_2O_2 5%	0.95	1.65	2.30	3.40	2.08	0.98	1.70	2.37	3.50	2.14
Control	0.98	1.91	2.58	3.97	2.36	1.10	1.97	2.66	4.66	2.60
M	0.83	1.42	1.95	2.96		0.86	1.45	2.02	3.12	
LSD at .05 level	2016					2017				
Treatments(T)	0.15					0.10				
Storage periods(S)	0.43					0.33				
S *T	0.21					0.19				

Concerning the interaction between postharvest treatments and storage periods, data indicated that button mushrooms dipped in H_2O_2 5% + $CaCl_2$ 5% was the most effective treatment in reducing the weight loss% after 12 days of storage, followed by H_2O_2 5% + $CaCl_2$ 3%, citric acid 4 and 2 % in combination with H_2O_2 5% with no significant differences between them at the same period.

Browning index (BI)

Browning index as well as white color measurement is good indicators for discoloration. Browning index formula considered the changes in a* (red and green color), b* (yellow and blue color) and L* (white color) which was more suitable for color changes measurements. The browning index of mushrooms is related to change in color of mushrooms. As the L* value decreased and a* and b* value increased, the browning index increased (Gupta and Bhat, 2016).

Storage time is important factors in BI deterioration of mushrooms, though with the increase in storage period BI increased in both seasons (Table 2). These results were in agreement with Koushki *et al*. (2011).

Data also showed that all postharvest treatments reduced the BI compared to untreated control. Samples treated with H_2O_2 5% + $CaCl_2$ 5% had significantly the lowest browning index throughout the whole storage time. This reduction was significant at each data point in both seasons followed by H_2O_2 5% in combination with $CaCl_2$ 3% or critic acid 4%. After the 12th day of storage, fresh mushrooms dipped in H_2O_2 5%+ $CaCl_2$ 5% gave the lowest value of BI while, the highest ones was obtained from untreated control. At the end of storage, a different browning index (BI) was observed in mushrooms treated with different treatments with values ranged from 5.19 to 15.95 in the first season and 5.29 to 15.87 in the second season that was attributed to a generalized enzymatic browning in mushroom during storage. The browning index (BI) of untreated mushrooms (control) was significantly higher than other treatments.

Previous research has shown that increased levels of calcium within membranes can slow senescence and maintain the selective permeability of membranes (Ferguson, 1984). Chikthimmah *et al*. (2005) indicate that the increasing levels of calcium in mushrooms treated with $CaCl_2$ may have decreased browning by increasing vacuolar membrane integrity, thereby reducing the opportunity for tyrosinase to react with its phenolic substrates. Rosen and Kader, (1989) reported that 1% $CaCl_2$ dip reduced softening and browning rates of 'Bartlett' pear slices and had lower BI compared to control. This could be due to the fact that calcium helps to maintain membrane stability as mentioned by Poovaiah, (1988) and Picchioni *et al*. (1995). However, calcium salts, particularly calcium chloride and calcium ascorbate have been reported to maintain firmness, inhibit browning, and extend the shelf life of fresh produce and fresh-cut produce such as melons (Luna-Guzman and Barrett, 2000). Citric acid reduced browning index, pre storage dips in citric acid used to prevent oxidative browning due to *o*-diphenol oxidase (Brennan *et al*., 2000). Hydrogen peroxide could be interacted with these explanations. It was reported that hydrogen peroxide reduced non-enzymatic browning (Nerya *et al.,* 2005), enzymatic browning and microbial activity (Raffellini *et al.,* 2008)**.** Sharaf-Eldin and Geösel, (2016) indicated that immersing mushrooms after harvest in H_2O_2 reduced significantly discoloration during storage, especially with 2% concentration.

Table 2. Effect of hydrogen peroxide and antibrowning agent treatments on browning index of fresh mushrooms during cold storage at 4 °C in 2016 and 2017 seasons.

Treatments	2016						2017					
	Storage period in days											
	0	3	6	9	12	M	0	3	6	9	12	M
$H_2O_2$5%+ $CaCl_2$ 3%	0.62	1.11	2.51	4.10	6.80	3.03	0.59	1.13	2.56	4.17	6.94	3.08
H_2O_2 5%+ $CaCl_2$ 5 %	0.62	1.50	2.74	3.68	5.19	2.75	0.59	1.50	2.79	3.72	5.29	2.78
H_2O_2 5%+ Ascorbic acid 3%	0.62	1.94	4.38	6.90	11.20	5.01	0.59	1.96	4.47	7.00	11.42	5.09
H_2O_2 5%+ Ascorbic acid 5%	0.62	1.79	3.16	5.47	10.70	4.35	0.59	1.81	3.22	5.61	10.91	4.43
H_2O_2 5%+ Citric acid 2%	0.62	1.11	2.97	4.76	8.76	3.64	0.59	1.12	3.03	4.86	8.94	3.71
H_2O_2 5%+ Citric acid 4%	0.62	1.36	3.13	4.89	7.90	3.58	0.59	1.37	3.19	4.99	8.06	3.64
H_2O_2 5%	0.62	2.03	3.86	7.37	14.19	5.61	0.59	2.05	3.94	7.52	12.48	5.31
Control	0.62	2.78	5.99	10.88	15.95	7.24	0.59	2.82	6.11	11.10	15.87	7.30
M	0.62	1.70	3.59	6.01	10.09		0.59	1.72	3.66	6.12	9.99	
LSD at .05 level	2016						2017					
Treatments(T)	0.03						0.05					
Storage periods(S)	0.97						1.08					
S *T	1.80						2.73					

Polyphenol oxidase (PPO) activity:

Data in Table (3) showed that PPO activity of fresh mushrooms significantly increased with the prolongation of storage period in the two seasons.

These results are in agreement with those obtained by Espin *et al.* (1998) suggested that PPO enzyme activity increased in button mushrooms with the increase in storage period and that the activity of phenols was closely associated with the development of browning by catalysing hydroxylation of monophenols to *o*-di phenols and dehydrogenation of *o*-diphenols to *o*-quinones in the presence of oxygen (Espin *et al*., 1998).

Data also, revealed that all postharvest treatments reduced the activity of PPO during storage as compared with untreated control. Moreover, fresh mushrooms dipped in H_2O_2 5% + $CaCl_2$ 5% was the most effective in reducing PPO activity, following by H_2O_2 5% in combination with $CaCl_2$ 3% or citric acid 4% with no significant differences between them, while the other treatments were less effective in this

concern. Untreated control had the higher increase in the activity of PPO enzyme. These results were true in the two seasons and in agreement with Gupta and Bhat, (2016) for calcium and Brennan *et al*. (2000) for citric acid and hydrogen peroxide.

Enzymatic reactions present a serious processing problem during postharvest period of mushroom and this usually occur on the surface in the presence of oxygen. The role of calcium might be to preserve membrane structure, and thereby keep PPO in its latent form, or to prevent cellular decompartmentalization and the mixing of soluble phenolic substrates, which are vacuolar, and membrane-bound PPO. In addition, PPO could be directly inhibited by $CaCl_2$.Calcium chloride treatment decreased polyphenoloxidases (PPO) activity (Tomas-Barberan and Espin, 2001) and by such way prevents polyphenols oxidation. Antioxidant potential are well correlated in most cases (Poovaiah, 1988). Citric acid exerts its inhibitory on PPO activity by lowering the pH as well as by chelating the copper at the active site of the enzyme (Sedaghat and Zahedi, 2012). Calcium reduced PPO activity through complexion the copper at the active site of the enzyme (Bolin and Huxsoll, 1989).

Regarding the interaction between postharvest treatments and storage periods, data showed that after 12 days of storage fresh mushrooms dipped in $CaCl_2$ 5% or $CaCl_2$ 3% or citric acid 4% in combination of H_2O_2 5% were reduced PPO activity with significant differences between them in the first season.

Table 3. Effect of hydrogen peroxide and antibrowning agent treatments on Polyphenol oxidase activity (PPO) (Unit/g fresh weight) of fresh mushrooms during cold storage at 4 °C in 2016 and 2017 seasons.

Treatments	2016						2017					
	Storage period in days											
	0	3	6	9	12	M	0	3	6	9	12	M
$H_2O_2$5%+ $CaCl_2$ 3%	1.65	2.02	2.19	2.33	2.42	2.12	1.59	1.95	2.13	2.26	2.34	2.05
H_2O_2 5%+ $CaCl_2$ 5 %	1.65	1.80	1.87	2.05	2.19	1.91	1.59	1.73	1.80	1.99	2.11	1.84
H_2O_2 5%+ Ascorbic acid 3%	1.65	2.29	2.88	3.14	3.46	2.68	1.59	2.23	2.81	3.07	3.39	2.62
H_2O_2 5%+ Ascorbic acid 5%	1.65	2.28	2.74	3.03	3.33	2.60	1.59	2.21	2.67	2.96	3.26	2.54
H_2O_2 5%+ Citric acid 2%	1.65	2.17	2.70	2.93	3.09	2.51	1.59	2.12	2.64	2.86	3.02	2.45
H_2O_2 5%+ Citric acid 4%	1.65	2.15	2.29	2.39	2.57	2.21	1.59	2.08	2.22	2.33	2.51	2.15
H_2O_2 5%	1.65	2.35	2.89	3.28	3.64	2.76	1.59	2.29	2.83	3.22	3.57	2.70
Control	1.65	2.40	2.97	3.67	4.18	2.97	1.59	2.33	2.91	3.60	4.11	2.91
M	1.65	2.18	2.57	2.85	3.11		1.59	2.12	2.50	2.79	3.04	
LSD at .05 level				2016						2017		
Treatments(T)				0.06						0.07		
Storage periods(S)				0.23						0.24		
S *T				0.35						N.S.		

Total phenolic and flavonoids contents

Data in Tables (4 and 5) showed that total phenolic and flavonoids contents were decreased with the prolongation of storage period. The decrease in phenolic content on fresh mushrooms may be due to the oxidation of PPO enzyme to give the colored quinones and quercetin was oxidized directly by PPO (Queiroz *et al*., 2008). Moreover, Robards *et al*. (1999) found that phenolic compounds had a significant role in oxidation process as antioxidants and as substrates in browning reactions.

Concerning the effect of postharvest treatments, data revealed that all treatments were effective in maintaining total phenolic and flavonoids contents during storage compared with untreated control. Moreover, button mushrooms dipped in H_2O_2 5% + $CaCl_2$ 5% or H_2O_2 5% + $CaCl_2$ 3% or H_2O_2 5% + Citric acid 4% were the most effective treatments in reducing the loss of total phenolic and flavonoids contents with significant differences between them in the two seasons, however, the other treatments were less effective in this concern. The lowest value was obtained from untreated control. These results were true in the two seasons and in agreement with Jones and Jacobsen, (1983) for calcium chloride and Ali *et al*. (2015) for citric acid. The main function of $CaCl_2$ is strengthened the cell wall and stabilizing the cell membrane (Jones and Jacobsen, 1983), thus keeps PPO, which is membrane bound enzyme, away from its phenolic substrates present mainly in vacuoles leading to preserving phenolic content and inhibiting browning process (Koushki *et al*., 2011). Citric acid acts mainly as enzyme inhibitor by chelating the copper from enzyme active site, beside any used acidulates may lower the suitable pH for maximum PPO activity(Javan *et al.,* 2015).

Table 4. Effect of hydrogen peroxide and antibrowning agent treatments on total phenolic (mg g^{-1}) of fresh mushrooms during cold storage at 4 °C in 2016 and 2017 seasons.

Treatments	2016						2017					
	Storage period in days											
	0	3	6	9	12	M	0	3	6	9	12	M
$H_2O_2$5%+ $CaCl_2$ 3%	1.44	1.28	1.14	1.01	0.92	1.16	1.23	1.09	0.95	0.82	0.72	0.96
H_2O_2 5%+ $CaCl_2$ 5 %	1.44	1.34	1.26	1.16	1.08	1.26	1.23	1.15	1.07	0.97	0.89	1.06
H_2O_2 5% + Ascorbic acid 3%	1.44	1.10	0.75	0.69	0.60	0.92	1.23	0.91	0.56	0.50	0.41	0.72
H_2O_2 5% + Ascorbic acid 5%	1.44	0.93	0.87	0.76	0.65	0.93	1.23	0.74	0.68	0.57	0.46	0.74
H_2O_2 5%+ Citric acid 2%	1.44	0.97	0.88	0.78	0.67	0.95	1.23	0.78	0.69	0.59	0.48	0.75
H_2O_2 5%+ Citric acid 4%	1.44	1.21	1.12	0.95	0.85	1.11	1.23	1.02	0.93	0.76	0.66	0.92
H_2O_2 5%	1.44	0.89	0.81	0.71	0.56	0.88	1.23	0.70	0.62	0.52	0.39	0.69
Control	1.44	0.80	0.72	0.65	0.51	0.82	1.23	0.61	0.53	0.44	0.28	0.62
M	1.44	1.07	0.94	0.84	0.73		1.23	0.88	0.75	0.65	0.54	
LSD at .05 level	2016						2017					
Treatments(T)	0.03						0.02					
Storage periods(S)	0.07						0.07					
S*T	0.14						0.16					

Table 5. Effect of hydrogen peroxide and antibrowning agent treatments on total flavonoids (mg g^{-1}) of fresh mushrooms during cold storage at 4 °C in 2016 and 2017 seasons.

Treatments	2016						2017					
	Storage period in days											
	0	3	6	9	12	M	0	3	6	9	12	M
$H_2O_2$5%+ $CaCl_2$ 3%	0.94	0.89	0.85	0.80	0.59	0.81	0.92	0.87	0.83	0.78	0.59	0.80
H_2O_2 5%+ $CaCl_2$ 5 %	0.94	0.91	0.88	0.85	0.69	0.85	0.92	0.89	0.86	0.83	0.71	0.84
H_2O_2 5%+ Ascorbic acid 3%	0.94	0.87	0.66	0.55	0.40	0.68	0.92	0.75	0.64	0.53	0.38	0.64
H_2O_2 5%+ Ascorbic acid 5%	0.94	0.78	0.68	0.59	0.43	0.68	0.92	0.76	0.66	0.57	0.41	0.66
H_2O_2 5%+ Citric acid 2%	0.94	0.77	0.71	0.61	0.46	0.70	0.92	0.75	0.69	0.58	0.44	0.67
H_2O_2 5%+ Citric acid 4%	0.94	0.88	0.80	0.72	0.53	0.77	0.92	0.86	0.78	0.70	0.51	0.75
H_2O_2 5%	0.94	0.73	0.62	0.55	0.31	0.63	0.92	0.71	0.60	0.50	0.29	0.60
Control	0.94	0.68	0.55	0.49	0.26	0.58	0.92	0.66	0.53	0.41	0.21	0.55
M	0.94	0.81	0.72	0.65	0.46		0.92	0.78	0.70	0.61	0.44	
LSD at .05 level	2016						2017					
Treatments(T)	0.02						0.01					
Storage periods(S)	0.05						0.07					
S*T	0.09						0.10					

For the interaction between postharvest treatments and storage period, After 12 days of storage, results indicated that button mushrooms dipped in H_2O_2 5% + $CaCl_2$ 5% had significantly higher values of total phenolic and flavonoids contents compared with other treatments, followed by H_2O_2 5% + $CaCl_2$ 3%, H_2O_2 5% + Citric acid 4% or H_2O_2 5% + Citric acid 2 % with no significant differences between them. These results were true in the two seasons.

Total bacteria counts (TBC) and yeast and mold counts (TYMC)

Table (6) presents growth of total bacteria counts (TBC) (expressed as log $cfug^{-1}$) of fresh mushroom during 12 days of storage. Gradual growth of microorganisms was seen during storage in all samples. TBC of mushrooms were usually due to the growth of *Pseudomonas* bacteria (Brennan *et al.*, 2000). As these bacteria grow, they break down the mushroom fibers which soften and lead to enzymatic browning (Brennan and Gormley, 1998). However, some treatments retarded the microbial growth more than others. The highest amount of TBC was observed in control samples. Samples combined H_2O_2 5% + $CaCl_2$ 5% were found to be significantly effective in

delaying TBC in mushroom during the 12 days of cold storage, followed by H_2O_2 5% in combination with $CaCl_2$ 3% and citric acid 4%. The other treatments were less effective in this concern. These results were in agreement with Brennan *et al.* (2000) and Simon and Gonza´lez-Fandos (2009).

Concerning the interaction between postharvest treatments and storage periods, data revealed that, after 12 days of cold storage, the amount of TBC in the H_2O_2 5%+ $CaCl_2$ 5% and H_2O_2 5%+ $CaCl_2$ 3% treatments were 0.40 and 1.00 log cfu g^{-1} respectively (average of the two seasons), while in untreated control was 3.25 log cfu/g^{-1} (average of the two seasons).(Table 6)

Table 6. Effect of hydrogen peroxide and antibrowning agent treatments on total bacteria counts (cfu/ g^{-1}) of fresh mushrooms during cold storage at 4 °C in 2016 and 2017 seasons.

Treatments	2016						2017					
	Storage period in days											
	0	3	6	9	12	M	0	3	6	9	12	M
$H_2O_2$5%+ $CaCl_2$ 3%	0.00	0.00	0.20	0.30	1.00	0.30	0.00	0.00	0.30	0.40	1.00	0.34
H_2O_2 5%+ $CaCl_2$ 5 %	0.00	0.00	0.00	0.20	0.40	0.12	0.00	0.00	0.00	0.20	0.40	0.12
H_2O_2 5%+ Ascorbic acid 3%	0.00	0.90	1.20	1.50	1.90	1.10	0.00	1.10	1.20	1.50	1.90	1.14
H_2O_2 5%+ Ascorbic acid 5%	0.00	0.70	1.00	1.40	1.80	0.98	0.00	0.50	1.00	1.70	1.90	1.02
H_2O_2 5%+ Citric acid 2%	0.00	0.40	0.80	1.30	1.50	0.80	0.00	0.40	0.90	1.30	1.50	0.82
H_2O_2 5%+ Citric acid 4%	0.00	0.40	0.50	0.80	1.30	0.60	0.00	0.30	0.50	0.80	1.30	0.58
H_2O_2 5%	0.00	1.20	1.50	1.50	1.80	1.20	0.00	1.20	1.50	1.70	1.90	1.26
Control	0.00	1.50	1.80	2.40	3.10	1.76	0.00	1.60	1.70	2.60	3.40	1.86
M	0.00	0.64	0.88	1.18	1.60		0.00	0.64	0.89	1.28	1.66	

LSD at .05 level	2016	2017
Treatments(T)	0.09	0.18
Storage periods(S)	0.13	0.20
S *T	1.17	0.39

Data in Table (7) showed that the initial total yeast and mold count (TYMC) mushrooms increased with the prolongation of storage period particularly in untreated control in the two seasons. Similar results were obtained by (Beelman *et al*., 1987).

Concerning the effect of postharvest treatments, data revealed that there were significant differences in total yeast and mold count between all treatments and untreated control. Button mushrooms dipped in H_2O_2 5% in combination with $CaCl_2$ at 5% or 3% treatments had significantly the lowest count in TMYC with significant differences between them ,followed by H_2O_2 5% in combination with citric acid at 4% or 2% with significant differences between them, while the other treatments were less effective in reducing this character. Untreated control had higher levels of TYMC during storage. These results were true in two seasons and in agreement with Chikthimmah *et al.* (2005) for H_2O_2 and $CaCl_2$ and Gad El-Rab, (2013) for citric acid.

Citric acid can lower intracellular pH (Aboul-Anean *et al*., 2013) and calcium reduced water activity (Beelman *et al.*,1987) which provides a protective antimicrobial barrier against food borne pathogens in products (Crowe *et al*.,2005) in addition, microflora is usually restricted to fungal and lactic acid bacteria at low pH (Chikthimmah *et al*., 2005). Also, calcium enhanced tissue develops resistance to fungal attack by stabilizing or strengthening cell walls, thereby making them more resistant to harmful enzymes produced by bacteria and fungi, and that it also delays aging of fruits (Picchioni *et al*.,1995). Moreover, hydrogen peroxide performs multiple functions in early defense response against pathogens (Hu *et al.*, 2003) and suppressed microorganisms activity (Crowe *et al.*, 2005; Raffellini *et al.*, 2008). The antimicrobial action stems from its ability to form reactive oxygen species such as the hydroxyl radical and singlet oxygen, which can damage bacterial protein, DNA and cellular membrane components (Juven and Pierson, 1996), and remove protein coats of bacterial spores.

The interaction between postharvest treatments and storage periods data in Table 7 revealed that after 12 days of storage button mushrooms dipped in H_2O_2 %5 in combination with $CaCl_2$ at 5% or 3% and citric acid at 4% were the most effective treatments in reducing TYMC with no significant differences between them in the two seasons.

Table 7. Effect of hydrogen peroxide and antibrowning agent treatments on yeast and mold counts (cfu/$g^{-1)}$ of fresh mushrooms during cold storage at 4 °C in 2016 and 2017 seasons.

Treatments	2016						2017					
	Storage period in days											
	0	3	6	9	12	M	0	3	6	9	12	M
$H_2O_2$5%+ $CaCl_2$ 3%	0.00	0.00	0.00	0.10	0.60	0.14	0.00	0.00	0.00	0.30	0.70	0.20
H_2O_2 5%+ $CaCl_2$ 5 %	0.00	0.00	0.00	0.10	0.20	0.06	0.00	0.00	0.00	0.20	0.30	0.10
H_2O_2 5%+ Ascorbic acid 3%	0.00	0.40	0.60	0.80	1.40	0.64	0.00	0.40	0.50	0.70	1.50	0.62
H_2O_2 5%+ Ascorbic acid 5%	0.00	0.70	1.00	1.60	1.90	1.04	0.00	0.60	1.10	1.80	2.00	1.10
H_2O_2 5%+ Citric acid 2%	0.00	0.20	0.40	0.60	1.00	0.44	0.00	0.20	0.40	0.50	1.20	0.46
H_2O_2 5%+ Citric acid 4%	0.00	0.20	0.30	0.50	0.90	0.38	0.00	0.10	0.30	0.40	1.00	0.36
H_2O_2 5%	0.00	1.40	1.50	1.80	1.80	1.30	0.00	1.30	1.60	1.80	1.90	1.32
Control	0.00	0.60	1.50	2.70	3.60	1.68	0.00	0.50	1.70	2.50	3.20	1.58
M	0.00	0.44	0.66	1.03	1.43		0.00	0.39	0.70	1.03	1.48	
LSD at .05 level				2016					2017			
Treatments(T)				0.03					0.08			
Storage periods(S)				0.18					0.15			
S *T				1.10					0.87			

Conclusion

From the previous results it could be concluded that button mushrooms dipped in solution of H_2O_2 5%+ $CaCl_2$ 5% significantly reduced weight loss%, browning index, total bacteria counts, total yeast and mold counts and PPO activity and maintained total phenolic and flavonoids contents during storage for 12 days at 4 ± 1ºC and 90 ± 2% relative humidity.

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المحافظة على جودة فطر عيش الغراب الكروى بعد الحصاد عن طريق إضافة فوق أكسيد الهيدروجين ومثبطات اللون البنى

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أجريت هذة التجربة لدراسة تأثير غمر فطر عيش الغراب الكروى بعد الحصاد فى فوق أكسيد الهيدروجين 5٪ بمفردة أو مع مركبات مضادة للتلون باللون البنى (كلوريد الكالسيوم 3 أو 5٪ أو أسكوربيك أسيد 3 أو 5٪ أو ستريك أسيد 2 أو 4٪) بجانب الغمر فى الماء المقطر (كنترول) وذلك لتثبيط التلون البنى وتقليل الحمل الميكروبى والمحافظة على الجودة أثناء التخزين المبرد على درجة 4 ± 1 م ورطوبة نسبية 90 ± 2٪ لمدة 12 يوم خلال موسمى 2016 و 2017. أظهرت النتائج أن جميع المعاملات التي تمت دراستها قللت من نسبة الفقد فى الوزن ونشاط أنزيم بولي فينول أوكسيديز والحمل الميكروبى (بكتيريا وفطر) كما حافظ على المحتوى من المركبات الفينوليه والفلافونويدز أثناء التخزين المبرد مقارنتاً بالمعاملة كنترول. أوضحت النتائج أن المعاملة بفوق أكسيد الهيدروجين 5٪ + كلوريد الكالسيوم 5٪ أكثر فاعلية فى تقليل التلون البنى والمحافظه على الجودة وتثبيط الحمل الميكروبى وذلك بعد 12 يوم من التخزين المبرد على درجة 4 ± 1 م ورطوبة نسبية 90 ± 2٪ .