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The Influence of Hot Water and Calcium Chloride on the Changes in Color, Phenolics and Polyphenol Oxidase Activity of Mushroom During Postharvest Storage

Mantarda Sıcak Su ve Kalsiyum Klorürün Hasat Sonu Depolama Sırasında Renk, Fenolikler ve Polifenol Oksidaz Aktivitesindeki Değişimler Üzerine Etkisi

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ABSTRACT

Objective: This study was performed to determine the effects of CaCl₂ and hot water on the changes in color, phenolics and polyphenoloxidase activities of mushrooms during storage.

Material and Methods: Mushrooms undergo significant changes in quality after harvest due mainly to changes in color. Mushrooms were treated with various concentrations of CaCl₂ and hot water at various degrees for different time periods and stored for 12 days at 10°C.

Results: Significant changes were observed in color components during storage. Color change was associated with the increases in total soluble phenolics, especially increases in chlorogenic acid and polyphenol oxidase activity, suggesting that both phenolics and polyphenol oxidase contribute significantly to the browning in mushrooms. Hot water and CaCl₂ treatments significantly reduced color change possibly through reductions in total soluble phenolics and the activity of polyphenol oxidase.

Conclusion: The results suggest that CaCl₂ and hot water treatments could be used to reduce color change after harvest and extend the shelf life of mushrooms.

ÖZ

Amaç: Bu çalışma, mantarda depolama sırasında sıcak su ve kalsiyum klorürün renk, fenolikler ve polifenol oksidaz aktivitesi üzerindeki etkilerini belirlemek amacıyla yapılmıştır.

Materyal ve Metot: Mantarlar hasattan sonra çoğunlukla renk değişiminden kaynaklanan önemli kalite değişimleri gösterirler. Bu amaçla mantarlar değişik konsantrasyonlarda kalsiyum klorür ve farklı sıcaklık dereceleri ve sürelerinde sıcak suya daldırılmış ve 10°C'lik depoda 12 gün depolanmıştır.

Bulgular: Depolama sırasında renk bileşenlerinde önemli değişiklikler gözlenmiştir. Renk değişiminin toplam çözünebilir fenoliklerdeki, özellikle de klorojenik asit içeriğindeki ve polifenol oksidaz enzim aktivitesindeki artışlarla birlikte ortaya çıkması, hem fenoliklerin hem de polifenol oksidazın mantarlarda ortaya çıkan kahverengileşmeye önemli katkılar sağladığını göstermektedir. Sıcak su ve CaCl₂ uygulamaları, muhtemelen toplam çözünebilir fenoliklerde ve polifenol oksidaz enzim aktivitesinde azalmalar meydana getirmek suretiyle renk değişimini önemli ölçüde azaltmışlardır.

Sonuç: Elde edilen sonuçlar, CaCl₂ ve sıcak su uygulamalarının hasattan sonraki renk değişimini azaltmak için kullanılabileceğini ve böylece mantarın hasat sonu ömrünün uzatılabileceğini göstermektedir.

INTRODUCTION

Mushroom is known with its short postharvest life which is generally attributed to its high respiration rate and rapid quality loss. The major factors responsible for quality loss in mushrooms are color change, changes in texture, weight loss, stipe elongation, cap opening and off flavor development [Donker and Braaksma, 1997; Çetin and Eren, 2017]. Color change mainly occurs through the generation of brown pigments. The development of browning pigments has been attributed to several parameters including changes in enzymatic activities and in the levels of total phenolic compounds [Stussi and Rats, 1981], the alteration of membrane permeability [Beaulieu and Lacroix, 1992], the action of bacteria and mold on the mushroom tissues [Soler-Rivas et al., 1999] and disruption of the cellular integrity through abrasions, washing, senescence and mechanical damage [Hughes, 1958]. Among enzymes responsible for browning, polyphenol oxidase (PPO) present in the pileus (cap) and stipe (stalk) of mushrooms plays an important role in the browning [Soler-Rivas et al., 1999]. PPO catalyses the hydroxylation of monophenols to o-dihydroxy phenols, and oxidation of o-diphenols to o-quinones. The quinones then polymerize to form brown melanin pigments [Vámos-Vigyázó, 1981]. Mushroom browning might also occur by the action of bacteria and mold on the mushroom tissues. For instance, *Pseudomonas tolaasii* and *Verticillium maltousei* infections could generate brown spots on mushrooms [Royse and Wuest, 1980; Salunkle and Desai, 1984].

In order to protect color loss in mushrooms, various postharvest applications have been evaluated [Eissa et al., 2009]. Irrigation with calcium chloride containing water enhanced the white color at harvest and decreased postharvest browning [Philippoussis et al., 2001] modified atmosphere packaging reduced browning rate [Liu et al., 2010].

Browning of fresh sliced mushrooms was also inhibited by immersion in citric acid (4%) or hydrogen peroxide (5%) [Brennan et al., 2000], irradiation (2.0 kGy) and sulphitation (0.1 %) [Wani et al., 2009]. Moreover, inhibition of enzyme activity through the use of chemicals [Martinez and Whitaker, 1995; Kubo and Kinst-Hori, 1998], heat treatment, decreasing O₂ (10-20%) and increasing CO₂ concentrations (2.5%) [Simon and Gonzalez-Fandos, 2010] have also been practiced. Among other postharvest applications, dipping in CaCl₂ resulted in an increased shelf-life especially in fresh cut products by preventing firmness loss and maintaining physical quality [Philippoussis et al., 2001; Wszelaki and Mitcham, 2003]. In addition, it has been reported that hot water dips has extended the shelf life in different fruit and vegetable species by preventing chilling

injury, firmness loss, pathogen development and postharvest diseases [Wszelaki and Mitcham, 2003; Karabulut et al., 2004].

The objective of this study was to determine the influence of CaCl₂ and hot water dips on the changes in color, phenolics and polyphenol oxidase activity in mushroom during postharvest storage.

MATERIAL and METHOD

Mushrooms (*Agaricus bisporus*) were purchased from a local grower in Isparta, Turkey. They were harvested from the second flush when their pilei dimateres were 3 or 4 cm. Mushrooms were graded on a seven point scale based on their developmental stage on the basis of cap opening [Guthrie et al., 1984]. The second flush mushrooms from the first and second developmental stages were used in the study. After harvest, mushrooms were transported to the laboratory in a freezer at 4°C and some of them were used for the control. The rest of the mushrooms were separated into different groups and treated with CaCl₂ and hot water. For CaCl₂ treatment, 1% and 2% solutions were prepared and mushrooms were dipped into the solutions for 2 minutes [Aguayo et al., 2006]. Hot water treatments were conducted at different temperatures and various incubation periods. For this purpose, 45, 50, 55 and 60°C temperatures and 45, 60 and 75 seconds incubation times were employed [Karabulut et al., 2004]. After treatments mushrooms were stored at 10°C until decayed. Five replications with 10 mushrooms in each replication were used for each treatment. Samples were taken from storage with 2 day intervals (0, 2, 4, 6, vs.) from each treatment and the control, frozen in liquid nitrogen and stored at -80°C.

The color of the mushroom cap was measured using a Minolta Chroma meter (Model CR-300, Minolta Camera Co., Osaka, Japan) in the L*, a*, b* mode. The color was measured at 3 equi-distant points on each mushroom cap. Measured values of L*, a*, b* were compared to ideal mushroom color values of L* = 97, a* = -2 and b* = 0 [Ajilouni et al., 1993] based on ΔE color parameter described by the following equation:

$$\Delta E = [(L-97)^2 + \{\alpha - (-2)\}^2 + b^2]^{1/2}$$

ΔE shows the degree of overall color change as compared to color values of an ideal mushroom.

Total soluble phenolics and chlorogenic acid contents and polyphenol oxidase activity were determined as described [Karakurt et al., 2000]. Chlorogenic acid was determined by the procedure described by Coseteng and Lee using authentic chlorogenic acid as a standard. Total soluble phenolics were determined as described by Coseteng and Lee using tannic acid as a Standard. One unit of polyphenol

oxidase activity was defined as a change in absorbance of 0.001 min⁻¹ ml⁻¹ enzyme extract.

RESULTS and DISCUSSION

Mushroom browning occurs via two distinct mechanisms of phenol oxidation: (a) activation of polyphenoloxidase (PPO) (b) spontaneous oxidation [Jolivet et al., 1998]. In order to determine the influence of treatments in the prevention of browning, we have evaluated the changes in the levels of phenolic compounds and the activity of polyphenol oxidase, the two major contributors of browning in mushroom. There were significant differences in whiteness between the control and hot water and CaCl₂-treated mushrooms during storage (Table 1, Table 2). The L* value of control decreased sharply during storage and it was 76.1 on the day 8 of storage which may not be

considered as commercially acceptable since an L* value of 80 is regarded as the lowest value for a mushroom to be commercially acceptable [Lopez-Briones et al., 1992]. CaCl₂ and hot water treatments significantly delayed the change in color (Table 1, Table 2). In 2 % CaCl₂ and 55°C and 60 °C hot water treatments, L* value was still acceptable after 12 days of storage (Table 1). The highest L* values were observed from 60°C 60 s hot water (87.3) and 2% CaCl₂ treatments after 12 days of storage. At the end of the storage, the mushrooms of CaCl₂ and hot water treatments browned slightly, but they also had commercial value and edibility. Compared with control, all treatments significantly inhibited the browning of mushrooms (Table 1, Table 2). The least color change was observed from 60°C 60 s (29.1) and 55°C 75 s (30.1) treatments during 12 days of storage period (Table 2).

Table 1. Changes in color lightness (L*) value during storage in mushroom

Çizelge 1. Mantarın depolanması sırasında renk değerindeki (L) değişimler*

Treatment	Days in storage						
	0	2	4	6	8	10	12
Control	96.2a	95.3a	89.2b	81.4c	76.1d	70.4e	65.1f
CaCl ₂ %1	96.2a	95.1a	92.8a	87.3b	83.8cb	81.2c	79.4c
CaCl ₂ %2	96.2a	94.8a	94.2ab	92.5ab	89.6bc	85.4cd	83.3d
45 °C 45 s	96.2a	93.4a	88.3b	84.2b	78.4c	72.1d	68.2d
45 °C 60 s	96.2a	94.0a	87.3b	86.2b	77.4c	73.9c	67.1d
45 °C 75 s	96.2a	94.8a	89.5b	83.3c	76.5d	75.1d	69.4e
50 °C 45 s	96.2a	94.9ab	90.3bc	87.7c	81.3d	77.4d	72.3e
50 °C 60 s	96.2a	95.2a	92.2ab	87.9bc	84.1c	78.3d	73.7d
50 °C 75 s	96.2a	94.6a	91.3ab	88.3bc	84.1cd	80.0de	75.2e
55 °C 45 s	96.2a	95.3a	93.2ab	89.6b	84.5c	82.3cd	79.2d
55 °C 60 s	96.2a	94.4a	91.6ab	89.4b	87.6bc	85.2c	84.2c
55 °C 75 s	96.2a	95.2ab	93.8ab	91.0bc	88.6cd	86.9d	85.4d
60 °C 45 s	96.2a	93.6ab	92.5abc	90.1bc	88.3cd	86.7d	84.1d
60 °C 60 s	96.2a	94.9ab	93.2ab	90.4bc	89.3c	88.7c	87.3c
60 °C 75 s	96.2a	95.3a	92.1ab	89.6bc	84.8cd	83.3d	82.0d

Means within each column followed by different letters are significantly different at 5 % level of significance.

Table 2. Changes in ΔE color value during storage in mushroom

Çizelge 2. Mantarın depolanması sırasında ΔE renk değerindeki değişimler

Treatment	Days in storage						
	0	2	4	6	8	10	12
Control	11.8e	12.6e	18.8d	26.9c	35.3b	40.5ab	45.9a
CaCl ₂ %1	11.8e	12.1e	15.3de	19.2cd	23.2bc	28.4ab	32.4a
CaCl ₂ %2	11.8e	12.0de	14.6de	17.9cd	21.8bc	26.4ab	30.3a
45 °C 45 s	11.8e	12.7ed	18.1d	26.3c	33.1b	39.4a	43.8a
45 °C 60 s	11.8c	12.9c	17.7c	24.8b	29.9b	36.3a	41.5a
45 °C 75 s	11.8e	13.1ed	18.6d	25.4c	30.2bc	35.4ab	40.9a
50 °C 45 s	11.8d	13.3d	17.9d	24.3c	29.2bc	33.3ab	38.4a
50 °C 60 s	11.8d	12.9d	16.6dc	21.3c	28.1b	34.2a	39.6a
50 °C 75 s	11.8e	12.8e	16.1ed	22.0cd	27.4bc	33.2ab	37.3a
55 °C 45 s	11.8e	12.3e	14.4de	20.2cd	24.8bc	30.2ab	35.1a
55 °C 60 s	11.8e	12.4e	15.5de	19.2cd	23.7bc	27.1ab	30.6a
55 °C 75 s	11.8e	12.2e	14.8de	18.3cd	22.3bc	26.0ab	30.1a
60 °C 45 s	11.8e	12.0e	14.9de	19.2cd	23.8bc	27.4ab	31.2a
60 °C 60 s	11.8e	12.5de	13.7de	17.9cd	21.6bc	25.3ab	29.1a
60 °C 75 s	11.8d	12.7d	14.1d	16.9cd	22.7bc	26.1ab	30.6a

Means within each column followed by different letters are significantly different at 5 % level of significance.

Total soluble phenolic compounds showed significant increases during storage in all treatments and the control (Table 3). There was a 22.5% increase in soluble phenolics content of the control mushrooms during 12 days storage period as compared to the Day 0.

The increase in total phenolics was significantly reduced by treatments. 1% CaCl₂ and 55°C 75 s hot water-treated mushrooms demonstrated 17.6 and 17.8%

increases in total soluble phenolics during storage, respectively.

Chlorogenic acid, the major phenolic compound, showed a similar increasing trend during storage (Table 4). The chlorogenic acid content in the control mushrooms increased 44% during storage, but this increase was 34.6% in 1% CaCl₂ treatment, and 34.9% in 55°C 75 s hot water treatment.

Table 3. Changes in total soluble phenolics (µg/g fresh weight) during storage in mushroom

Çizelge 3. Mantarın depolanması sırasında toplam çözünebilir fenoliklerin (µg/g taze ağırlık) değişimleri

Treatment	Days in storage						
	0	2	4	6	8	10	12
Control	204.0d	219.0c	236.0b	252.0a	246.6ab	250.3a	250.0a
CaCl ₂ %1	204.0d	219.6c	233.3b	245.6a	243.0ab	242.0ab	240.0b
CaCl ₂ %2	204.0d	221.3c	232.6b	245.3a	244.0a	241.6ab	241.0ab
45 °C 45 s	204.0d	217.3c	232.3b	249.0a	244.3a	248.3a	242.0ab
45 °C 60 s	204.0d	219.3c	234.6b	250.0a	247.0a	249.6a	248.6a
45 °C 75 s	204.0d	215.6c	232.3b	251.6a	245.3a	250.3a	250.3a
50 °C 45 s	204.0c	210.0c	222.3b	241.3a	241.3a	244.3a	243.3a
50 °C 60 s	204.0c	210.3c	222.3b	240.6a	241.3a	243.6a	241.6a
50 °C 75 s	204.0c	211.0c	223.6b	240.0a	240.3a	243.6a	243.0a
55 °C 45 s	204.0d	216.6c	228.3b	236.3ab	245.3a	246.3a	245.6a
55 °C 60 s	204.0c	217.0b	225.0b	237.3a	246.3a	247.3a	247.0a
55 °C 75 s	204.0d	211.6cd	218.3bc	227.0b	242.0a	241.3a	240.3a
60 °C 45 s	204.0e	209.6de	215.6cd	226.6b	239.0a	241.6a	241.0a
60 °C 60 s	204.0d	217.0c	233.3b	248.3a	246.0a	249.0a	249.6a
60 °C 75 s	204.0d	217.3c	232.0b	249.0a	246.3a	249.3a	250.0a

Means within each column followed by different letters are significantly different at 5 % level of significance.

Table 4. Changes in chlorogenic acid content (µg/g fresh weight) of mushroom during storage

Çizelge 4. Mantarın depolanması sırasında klorojenik asit içeriğindeki (µg/g taze ağırlık) değişimler

Treatment	Days in storage						
	0	2	4	6	8	10	12
Control	104.0d	119.0c	136.0b	152.0a	146.6ab	150.3a	150.0a
CaCl ₂ %1	104.0d	119.6c	133.3b	145.6a	143.0ab	142.0ab	140.0ab
CaCl ₂ %2	104.0d	221.3c	132.6bc	145.3a	144.0ab	141.6ab	141.0ab
45 °C 45 s	104.0d	117.3c	132.3b	149.0a	144.3ab	148.3a	148.6a
45 °C 60 s	104.0d	119.3c	134.6b	150.0a	147.0a	149.6a	148.6a
45 °C 75 s	104.0c	115.6c	132.3b	151.6a	145.3a	150.3a	150.3a
50 °C 45 s	104.0c	110.0c	122.3b	141.3a	141.3a	144.3a	143.3a
50 °C 60 s	104.0c	110.3c	122.3b	140.6a	141.3a	143.6a	141.6a
50 °C 75 s	104.0c	111.0c	123.6b	140.0a	140.3a	143.6a	143.0a
55 °C 45 s	104.0d	116.6c	128.3b	136.3ab	145.3a	146.3a	145.6a
55 °C 60 s	104.0c	117.0b	125.0b	137.3a	146.3a	147.3a	147.0a
55 °C 75 s	104.0d	111.6cd	118.3bc	127.0b	142.0a	141.3a	140.3a
60 °C 45 s	104.0d	109.6cd	115.6c	126.6b	139.0a	141.6a	141.0a
60 °C 60 s	104.0d	117.0c	133.3b	148.3a	146.0a	149.0a	149.6a
60 °C 75 s	104.0d	117.3c	132.0b	149.0a	146.3a	149.3a	150.0a

Means within each column followed by different letters are significantly different at 5 % level of significance.

Polyphenol oxidase activity in the control, CaCl₂-treated and 45°C hot water-treated mushrooms increased during first 2 days of storage and then decreased (Table 5). However, 55°C and 60°C hot water-treated mushrooms demonstrated decreases in the activity from the beginning of storage. The less browning observed in these treatments were possibly due to their lower PPO activities [Nan-Yi et al., 2011]. PPO catalyses the hydroxylation of monophenols to o-dihydroxy

phenols, which in turn oxidizes to o-quinones. The o-quinones condense to form the brown melanin pigments [Nan-Yi et al., 2011]. The role of PPO in the browning of mushrooms were also shown by irradiation which inhibits PPO activity and thus reduces browning [Fry and Strothkamp, 1983]. Likewise, the decrease in the L* value during storage confirmed the loss of whiteness of the mushrooms due, most probably, to the formation of melanin [Beaulieu and Lacroix, 1992].

Table 5. Changes in polyphenol oxidase activity (units/mg protein) during storage in mushroom
Çizelge 5. Mantarın depolanması sırasında polifenol oksidaz aktivitesindeki değişimler

Treatment	Days in storage						
	0	2	4	6	8	10	12
Control	748.6b	853.3a	289.0c	108.6d	51.3e	31.6e	47.0e
CaCl ₂ %1	748.6a	785.3a	248.6b	108.6c	50.0d	36.6d	52.3d
CaCl ₂ %2	748.6a	780.6a	258.0b	114.6c	49.3d	35.3d	63.3d
45 °C 45 s	748.6b	847.6a	295.0c	110.3d	55.3e	34.6e	48.6e
45 °C 60 s	748.6b	858.6a	296.3c	113.0d	54.3e	38.6e	49.3e
45 °C 75 s	748.6a	789.6a	256.6b	106.6c	44.6d	38.0d	47.0d
50 °C 45 s	748.6a	786.6a	249.0b	103.6c	45.6d	38.6d	48.0d
50 °C 60 s	748.6a	786.0a	253.6b	111.0c	48.6d	35.3d	49.6d
50 °C 75 s	748.6a	511.6b	204.3c	90.6d	38.0e	26.6e	28.0e
55 °C 45 s	748.6a	525.3b	219.6c	107.0d	52.6e	45.6e	43.3e
55 °C 60 s	748.6a	611.6b	255.3c	180.0d	93.0e	43.6ef	38.0f
55 °C 75 s	748.6a	630.6b	379.6c	186.3d	93.3e	47.6f	39.6f
60 °C 45 s	748.6a	429.0b	187.0c	79.6d	30.3e	24.0e	25.3e
60 °C 60 s	748.6a	419.0b	186.3c	87.0d	33.0e	25.0e	23.6e
60 °C 75 s	748.6a	440.3b	204.3c	85.0d	43.0de	22.3e	17.0e

Means within each column followed by different letters are significantly different at 5 % level of significance

CONCLUSION

In conclusion, the data suggested that color change, an important quality criteria in mushroom, was mainly associated with the increases in total soluble phenolic contents especially increases in chlorogenic acid content, and high polyphenol oxidase activity suggesting that both soluble phenolics and polypehnol oxidase contributed significantly to the color loss in mushrooms. The effect of hot water treatments on the

color change was significant. They showed this effect by reducing the levels of total soluble phenolics and decreasing the activity of polyphenol oxidase. CaCl₂ treatment also reduced browning possibly through inhibition of polyphenol oxidase activity and reduction of total soluble phenolics. The results suggest that CaCl₂ and hot water treatments could be used to delay the color change after harvest, and extend the shelf life of mushroom.

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