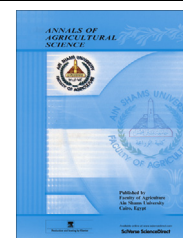




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## ORIGINAL ARTICLE

# Effects of anti-coloring agents on blackening inhibition and maintaining physical and chemical quality of fresh-cut okra during storage

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## KEYWORDS

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**Abstract** Fresh-cut okra pods were stored in sealed polypropylene bags at 5 °C and 95% RH for 8 days. Pods were dipped in 0.5% solution of cysteine, ascorbic acid, CaCl<sub>2</sub>, or citric acid for 5 min before storage. The main observed undesirable physiological and morphological alterations were weight loss, increasing microbial load, softening texture, and decreasing the phenolic content with blackening in color. CaCl<sub>2</sub> was effective in increasing cell membrane integrity leading to improving texture, minimizing weight loss, decreasing microbial load, and preventing polyphenoloxidase (PPO) from contacting its phenolic substrates and thus reducing blackness. Ascorbic acid and cysteine were best anti-coloring agents since their strong ability to inhibit PPO and reacting with the resulted colored quinones to give colorless products. Reducing blackness was found parallel to decreasing phenolic content, indicating the role of the phenolic oxidation in the blackening process in okra pods during storage. Citric acid was less effective in enhancing the examined physical and chemical properties.

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## Introduction

Okra (*Abelmoschus esculentus* L), a tropical and African origin vegetable, is produced in many warm-weather countries e.g. India, Pakistan, Turkey, Iran, Nigeria, Ghana, Greece, and southern USA and is considered one of the most important vegetables in Egypt (Arapitsas, 2008; Falade and Omojola, 2010) because of its nutritional content and medicinal potentials against inflammation, gastric irritation (Arapitsas, 2008), and colon cancer (Babarinde and Fabunmi, 2009). Okra is a

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good source of viscous mucilage, proteins (Karakoltsidis and Constantinides, 1975), and dietary fibers (Adom et al., 1996) in addition to contents of vitamin C, polyphenols (Arapitsas, 2008), fats, carbohydrates (Baxter and Waters, 1990), and many minerals e.g. Na, K, Mg, Ca, Fe, Zn, and Mn (Al-Wandawi, 1983); Recently, there has been an increasing market demand for minimally processed fresh-cut fruits and vegetables. However, okra has a short shelf-life and extremely perishable because of the high water contents and respiration rate (Finger et al., 2008; Falade and Omojola, 2010); in addition, the damage in tissues caused by fresh-cut preparation is a major contributor to induce undesirable biochemical pathways, leading to browning or blackening of surface cutting, lowering nutritive value (Brecht, 1995; Queiroz et al., 2008), providing sites for microbial infection, accelerating respiration rate, water loss, and ethylene production (Finger et al., 2008; Falade and Omojola, 2010). Phenolic compounds are well known to play an important role in the browning process since polyphenoloxidase (PPO) can catalyze their oxidation by molecular oxygen in two steps: first, hydroxylation in the *ortho* position to give a catecholic structure and second oxidation of catechols to *o*-quinones that can be self-condensed or polymerized with other biochemicals to produce brown pigments responsible for the undesirable color change. Therefore, damaged tissues in fresh-cut products expose phenolic compounds in vacuoles to the membrane-bound polyphenoloxidase resulting in color darkening (Piližota and Subarić, 1998; Yoruk and Marshall, 2003; Gacche et al., 2006).

Various approaches to control the extent of browning or blackening have been investigated; in general, enzymatic browning can be avoided or minimized by thermal inactivation of PPO or by using chemical additives. Treatments with ascorbic acid, cysteine, citric acid, and oxalic acid can inhibit polyphenoloxidase during storage; ascorbic acid can also reduce the resulted quinones back to the starting catechols before browning process takes place, in addition to its strong antioxidant activity (Abo-Shady et al., 2007; Ali et al., 2013), while cysteine, under certain conditions, may react with quinones to give colorless products (Altunkaya and Gökmen, 2008; Queiroz et al., 2008; Chang, 2009). Other treatments such as calcium chloride (CaCl<sub>2</sub>) maintain visual quality by keeping the integrity of the cell wall and retarding vegetable flesh softening (Luna-Guzmán and Barrett, 2000).

The objective of this study was to determine the effect of some anti-coloring agents on reducing browning or blackening, preserving phenolic and water contents, maintaining physical quality, and inhibiting pathogen infection of fresh-cut okra during storage.

## Materials and methods

Freshly harvested okra (Zara cultivar) was obtained from a commercial farm in Esmailia Governorate, Egypt. Pods were harvested at immature stage in the first week of August in 2010 and 2011 seasons and transported immediately, under cooling condition, to the postharvest laboratory, Horticulture Research Institute, Giza. Samples were selected free of visual damage or defects and uniform in color and size (40–50 mm long and 12–15 mm diameter).

All cutting utensils (knife and cutting board) used in removing okra pod calyx were washed with soap and water then rinsed with 100 ppm sodium hypochlorite solution prior to

use. Fresh-cut okra was randomly divided into 5 groups for the following treatments:

- (1) Dipping in 0.5% solution of cysteine for 5 min.
- (2) Dipping in 0.5% solution of ascorbic acid (AA) for 5 min.
- (3) Dipping in 0.5% solution of calcium chloride (CaCl<sub>2</sub>) for 5 min.
- (4) Dipping in 0.5% solution of citric acid (CA) for 5 min.
- (5) Dipping in distilled water (control).

All fresh-cut okra samples were packed in sealed polypropylene bags (15 × 15 cm and 20 μ thickness). Each bag contained 20 pods and stored at 5 °C and 95% RH; a complete randomized design was used. At each interval, three bags were used as replicates for the following measurements:

- (1) Weight loss percentage (WL) was calculated by the following formula:  $WL = [(W_i - W_f)/W_i] \times 100$ , where  $W_i$  is initial fruit weight (gm) and  $W_f$  is final fruit weight (gm) at a given time.
- (2) General appearance (GA) was determined visually using a scale from 1 to 9; where 9 = excellent, 7 = good, 5 = fair, 3 = poor and 1 = unusable. Samples rating below 5 were considered unmarketable.
- (3) Texture was measured by using TA-1000 texture analyzer instrument with a penetrating cylinder of 1 mm diameter. Penetration to a constant distance (3 and 5 mm) inside the pulps with a constant speed 2 mm/s. was performed and the peak of resistance was recorded (gm/cm<sup>3</sup>).
- (4) Color change was determined by using a Minolta Chroma meter model CR-400 for measuring the  $L^*$  and Hue angle ( $h^\circ = 180 + \tan^{-1}(b^*/a^*)$  where  $L^*$ ,  $a^*$  and  $b^*$  are Hunter parameters).
- (5) Total microbial count was obtained by the pour plate method using nutrient agar as the growth medium. In sterile Petri dishes, 1 ml of the homogenized diluent sample was poured then 10 ml of nutrient agar was gently dispensed and swirled. The plates were inverted after solidification and incubated at 37 °C for 24 h. The colonies were counted and the number of colonies per plate was multiplied by the dilution factor to obtain the total viable counts per ml of the original sample. Microbial colonies were counted using a Protos Colony Counter (Model 50000, Synoptics Ltd., Cambridge, UK) and reported as log CFU/g of tissue.
- (6) Total soluble phenols were determined spectrophotometrically by the method of Folin–Ciocalteu (Folin and Ciocalteu, 1927; Hyodo et al., 1978); results were reported as mg (gallic acid)/g fresh weight.
- (7) Statistical analysis was performed where data were analyzed by two-way analysis of variance (ANOVA) method using SPSS version 16. Fischer's least significant difference (LSD) at 5% probability level was used to examine significant effects.

## Results and discussion

To examine the effects of different treatments on preserving the physical and chemical conditions of fresh-cut okra during

storage, pods were stored, as recommended previously, in polypropylene pages (Babarinde and Fabunmi, 2009) at 95% RH and 5 °C (Finger et al., 2008).

#### Weight loss

Data in Table 1 showed a progressive increase in the weight loss percentage of fresh-cut okra during storage. The increasing weight loss is in consistence with the report of Gupta and Mukherjee (1982) and could be attributed to the loss of moisture through transpiration and dry matter contents through respiration processes (Adetuyi et al., 2008). The rate of weight loss was least after 8 days when fresh-cut okra was treated with calcium chloride in the two seasons (0.92% and 0.87% for seasons 2010 and 2011, respectively) which could be due to strengthen the cell wall (Bolin and Huxsoll, 1989) and reducing the respiration rate in the stored vegetables (Fallik et al., 1999).

#### Texture

Data in Table 2 revealed that significant reduction in pod texture had occurred by prolongation of the storage period. These results agree with those obtained by Sargent et al. (1996). The decrease in pod texture was attributed to gradual breakdown of protopectin to water soluble lower molecular weight fractions leading to the increase in the rate of pod softening (Wills et al., 2007).

Among the examined compounds, CaCl<sub>2</sub> was distinctive among other treatments and significantly reduced the texture loss of fresh-cut okra during storage relative to control group. The favorable effect of CaCl<sub>2</sub> could be due to stabilization of membrane systems through the formation of Ca-pectates which increase the rigidity of the middle lamella and cell wall (Poovaiah, 1986). Generally, any treatment capable of delaying softening is potentially helpful in extending the postharvest shelf-life and maintaining the product quality. The interaction between treatments and storage periods indicates that CaCl<sub>2</sub> treatment was superior in maintaining fresh-cut texture during the storage period.

#### General appearance (GA)

Data in Table 3 indicate that the general appearance of the control fresh-cut okra samples was deteriorated severely with

a poor grade (3 and 2.33 in both seasons, respectively) after 8 days. The decrease in GA during storage period resulted from shriveling, wilting, and color change in pods (Sargent et al., 1996). However, treatments by cysteine, ascorbic acid and CaCl<sub>2</sub> significantly enhanced the general appearance relative to control after the storage period in both seasons. However, okra samples treated with cysteine showed the best general appearance where pods kept their good GA (7 and 7.67) till the end of the storage period in both seasons, respectively. Moreover, ascorbic acid or CaCl<sub>2</sub> treated samples showed good appearance till 6 days of storage then dropped to a fair level at the end of the storage period in both seasons.

#### Total microbial count

Data in Table 4 showed that microbial growth was increased with increasing the storage period particularly in control samples; however, samples of all treatments had significant lower level of microbial load after 8 days relative to control samples. Ascorbic acid and CaCl<sub>2</sub> were the most effective treatments for reducing the total microbial count in both seasons, while cysteine and citric acid were less effective. These results are in agreement with those reported by Luna-Guzmán and Barrett (2000); CaCl<sub>2</sub> may have provided an inhibitory effect on microbial growth, while water alone (control) allowed for spore spreading and thus increasing microbial load; besides, calcium chloride and ascorbic acid can lower intracellular pH or reduce water activity (Shelef, 1994; Whitaker, 1994), which provides a protective antimicrobial barrier against food borne pathogens in products (Weaver and Shelef, 2007). In addition, microflora is usually restricted to fungal and lactic acid bacteria at low pH (Luna-Guzmán and Barrett, 2000). Also, calcium could enhance tissue texture leading to providing protection from fungal or bacterial attack by stabilizing or strengthening cell walls (Bolin and Huxsoll, 1989).

#### Color and total phenolic content changes

The color was measured by recording the lightness/darkness parameter ( $L^*$  value) and hue angle ( $h^\circ$ ). Darkness of control pods was observed clearly during storage period (Table 5) where there was not only significant but also remarkable decrease in the  $L^*$  value from 58.29 and 55.64 at the zero time to 45.71 and 40.28 after 8 days of control samples in the two

**Table 1** Effect of anti-coloring agents on weight loss percentage of fresh-cut okra during storage in 2010 and 2011 seasons.

Treatments	2010 Season				Mean	2011 Season				Mean
	Storage period (days)					Storage period (days)				
	2	4	6	8		2	4	6	8	
Control	0.70	0.98	1.30	1.46	1.11	0.58	0.89	1.16	1.39	1.01
Cysteine	0.64	0.94	1.14	1.38	1.03	0.52	0.84	1.09	1.20	0.91
Ascorbic acid	0.67	0.90	1.20	1.42	1.05	0.55	0.90	1.13	1.22	0.95
Calcium chloride	0.42	0.63	0.79	0.92	0.69	0.32	0.54	0.68	0.87	0.60
Citric acid	0.66	0.92	1.26	1.41	1.06	0.57	0.86	1.11	1.30	0.96
Mean	0.62	0.87	1.14	1.32		0.51	0.83	1.03	1.19	
L.S.D	Season 2010					Season 2011				
Treatment	0.04					0.05				
Storage period	0.09					0.07				
Treatment × storage period	0.11					0.09				

**Table 2** Effect of anti-coloring agent on texture of fresh-cut okra during storage in 2010 and 2011 seasons.

Treatments	2011 Season					Mean	2010 Season					Mean
	Storage period (days)						Storage period (days)					
	0	2	4	6	8		0	2	4	6	8	
Control	13.21	12.58	12.64	11.82	11.03	12.31	15.34	14.97	14.40	13.74	12.98	14.29
Cysteine	13.21	12.96	12.52	11.94	11.12	12.35	15.34	15.06	14.41	13.91	13.15	14.37
Ascorbic acid	13.21	12.82	12.41	11.75	11.20	12.28	15.34	15.00	14.52	13.82	13.04	14.34
Calcium chloride	13.21	13.13	12.97	12.54	12.17	12.80	15.34	15.21	15.06	14.87	14.65	15.03
Citric acid	13.21	12.93	12.57	11.54	11.15	12.28	15.34	14.92	14.45	13.85	13.11	14.33
L.S.D	Season 2010						Season 2011					
Treatments	0.06						0.07					
Storage period	0.12						0.14					
Treatments × storage period	0.17						0.19					

**Table 3** Effect of anti-coloring agent on general appearance (score) of fresh-cut okra during storage in 2010 and 2011 seasons.

Treatments	2011 Season					Mean	2010 Season					Mean
	Storage period (days)						Storage period (days)					
	0	2	4	6	8		0	2	4	6	8	
Control	9.00	8.33	7.00	5.00	3.00	6.46	9.00	8.33	6.33	4.33	2.33	6.19
Cysteine	9.00	9.00	9.00	8.33	7.00	8.46	9.00	9.00	9.00	9.00	7.67	8.73
Ascorbic acid	9.00	9.00	8.33	7.00	5.00	7.66	9.00	9.00	9.00	7.67	5.67	8.07
Calcium chloride	9.00	9.00	8.33	7.67	6.33	8.06	9.00	9.00	9.00	7.67	5.00	7.93
Citric acid	9.00	8.33	7.00	5.67	4.33	6.86	9.00	9.00	7.67	5.00	3.67	7.01
Mean	9.00	8.73	7.93	6.73	5.13		9.00	8.86	8.20	6.73	5.13	
L.S.D	Season 2010						Season 2011					
Treatments	0.21						0.26					
Storage period	0.25						0.31					
Treatments × storage periods	0.31						0.36					

**Table 4** Effect of anti-coloring agent on total microbial count (log<sub>10</sub> CFU) of fresh-cut okra during storage in 2010 and 2011 seasons.

Treatments	2011 Season					Mean	2010 Season					Mean
	Storage period (days)						Storage period (days)					
	0	2	4	6	8		0	2	4	6	8	
Control	0.45	1.24	1.72	3.14	4.86	1.96	0.53	2.27	3.08	4.12	5.23	3.05
Cysteine	0.45	0.96	1.79	2.82	3.78	1.96	0.53	1.98	2.32	3.15	3.87	2.37
Ascorbic acid	0.45	0.83	1.02	1.91	2.52	1.35	0.53	1.36	1.96	2.24	2.79	1.82
Calcium chloride	0.45	0.72	1.10	1.82	2.64	1.35	0.53	1.54	1.84	2.31	2.68	1.78
Citric acid	0.45	1.10	1.66	2.91	3.56	1.94	0.53	1.84	2.45	3.12	3.92	2.37
Mean	0.45	0.97	1.45	2.52	3.47		0.53	1.79	2.33	3.03	3.69	
L.S.D	Season 2010						Season 2011					
Treatment	0.18						0.12					
Storage period	0.22						0.18					
Treatment × storage period	0.26						0.20					

seasons, respectively, while other treatments showed also decrease in the  $L^*$  value but at lesser extent. The best treatments were cysteine, ascorbic acid, and  $\text{CaCl}_2$  where the  $L^*$  value remained closer to initial value (~56.5 and 54.5 in the two seasons, respectively) at the end of the experimental period, while citric acid was less effective.

Hue angle ( $h^\circ$ ) is a useful parameter to show the color difference (Voss, 1992). In consistent with the  $L^*$  value results, hue angle of control samples showed also much color

deterioration after 8 days (from 120.38 and 123.24 to 110.69 and 112.84 in the two seasons, respectively). Cysteine, ascorbic acid, and  $\text{CaCl}_2$  treatments gave again the best results where the hue angle dropped only to ~118 and 122 in both seasons, respectively, during the storage period while citric acid was also less effective in preventing color change (Table 6).

Total soluble phenolic content was decreased throughout the storage period (Table 7) in a parallel trend to that observed

**Table 5** Effect of anti-coloring agent on color ( $L^*$  value) of fresh-cut okra during storage in 2010 and 2011 seasons.

Treatments	2010 Season						Mean	2011 Season					
	Storage period (days)					Mean		Storage period (days)					Mean
	0	2	4	6	8			0	2	4	6	8	
Control	58.29	53.17	50.61	48.24	45.71	51.2	55.64	53.39	50.17	45.22	40.28	48.94	
Cysteine	58.29	57.62	57.21	57.06	56.94	57.42	55.64	55.23	55.04	54.90	54.72	55.11	
Ascorbic acid	58.29	57.46	57.00	56.82	56.61	57.24	55.64	55.11	54.82	54.62	54.32	54.90	
Calcium chloride	58.29	57.45	56.94	56.72	56.51	57.18	55.64	55.16	54.71	54.69	54.12	54.86	
Citric acid	58.29	56.03	55.12	53.11	50.14	54.54	55.64	54.11	50.29	46.17	40.73	49.39	
Mean	58.29	56.34	55.37	54.39	53.18		55.64	54.6	53.01	51.12	48.83		
L.S.D	Season 2010							Season 2011					
Treatment	0.28							0.26					
Storage period	0.31							0.30					
Treatments $\times$ Storage period	0.34							0.34					

**Table 6** Effect of anti-coloring agent on hue angle ( $h^\circ$ ) of fresh-cut okra during storage in 2010 and 2011 seasons.

Treatments	2010 Season						Mean	2011 Season					
	Storage period (days)					Mean		Storage period (days)					Mean
	0	2	4	6	8			0	2	4	6	8	
Control	120.38	117.92	115.23	114.71	110.69	115.79	123.24	120.27	117.14	114.39	112.84	117.58	
Cysteine	120.38	120.10	119.94	119.61	119.23	119.85	123.24	122.92	122.75	122.41	122.16	122.70	
Ascorbic acid	120.38	119.75	119.23	119.06	118.75	119.43	123.24	122.27	122.03	121.78	121.54	122.17	
Calcium chloride	120.38	119.43	119.00	118.82	118.54	119.23	123.24	122.04	121.83	121.64	121.13	121.97	
Citric acid	120.38	118.20	117.13	115.28	112.42	116.68	123.24	120.78	117.71	115.27	113.68	118.14	
Mean	120.38	119.08	118.11	117.50	115.93		123.24	122.86	120.29	119.01	118.27		
L.S.D	Season 2010							Season 2011					
Treatment	0.32							0.42					
Storage period	0.37							0.46					
Treatment $\times$ storage period	0.41							0.49					

**Table 7** Effect of anti-coloring agent on total phenolic content (mg (gallic acid)/g fresh weight) of fresh-cut okra during storage in 2010 and 2011 seasons.

Treatments	2010 Season						Mean	2011 Season					
	Storage period (days)					Mean		Storage period (days)					Mean
	0	2	4	6	8			0	2	4	6	8	
Control	10.62	9.75	9.10	8.23	7.91	9.12	11.29	10.31	9.73	9.2	8.42	9.79	
Cysteine	10.62	10.25	10.04	9.52	9.14	9.91	11.29	11.02	10.82	10.41	9.94	10.69	
Ascorbic acid	10.62	10.12	9.91	9.35	9.07	9.81	11.29	10.95	10.71	10.35	9.62	10.58	
Calcium chloride	10.62	10.17	9.87	9.39	9.0	9.81	11.29	10.87	10.52	10.17	9.57	10.48	
Citric acid	10.62	10.02	9.17	8.45	8.03	9.26	11.29	10.54	10.12	9.64	8.73	10.06	
Mean	10.62	10.06	9.62	8.99	8.63		11.29	10.74	10.38	9.95	9.26		
L.S.D	Season 2010							Season 2011					
Treatment	0.18							0.28					
Storage period	0.22							0.30					
Treatment $\times$ storage period	0.26							0.32					

of color change where the phenolic content of control samples was decreased remarkably during the experimental period from 10.62 and 11.29 to 7.91 and 8.42 in the two seasons,

respectively. In addition, cysteine, ascorbic acid, and  $\text{CaCl}_2$  treatments were still better in preserving the phenolic content till the end of storage period ( $>9$  mg/g fresh weight in both

seasons) than that of citric acid (8.03 and 8.73 mg/g fresh weight) in the two seasons, respectively.

The decrease in phenolic content upon fresh-cut treatment is due to the oxidation by polyphenoloxidase to give the colored quinones (Chang, 2009). During storage, the enzymatic oxidation is continued, and the resulted quinones are polymerized non-enzymatically to give darker pigments, which explains the parallel consumption of phenols with the development of blackness throughout the storage period. The superiority of ascorbic acid and cysteine treatments is attributed to their dual roles in inhibiting color formation; while ascorbic acid reduces quinones back to the colorless catechols, cysteine reacts with quinones to give colorless products; besides, both of them are PPO inhibitors (Piližota and Šubarić, 1998; He and Luo, 2007; Chang, 2009). The main function of CaCl<sub>2</sub> is strengthen the cell wall and stabilizing the cell membrane (Picchioni et al., 1995; Luna-Guzmán and Barrett, 2000), 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 (Jiang et al., 2004; Queiroz et al., 2008; Huang et al., 2012); in addition, chloride ion is known PPO inhibitor (Piližota and Šubarić, 1998). Citric acid acts mainly as enzyme inhibitor by chelating the copper from the enzyme active site; besides, any used acidulates may lower the suitable pH for maximum PPO activity (He and Luo, 2007).

In conclusion, the main undesirable changes occurred in stored okra pods were texture softening, blackening in color and increasing microbial load. Softening was relieved by treatment with CaCl<sub>2</sub> while microbial load was best treated with ascorbic acid and CaCl<sub>2</sub>. In the meantime, cysteine, ascorbic acid, and CaCl<sub>2</sub> could preserve color changes till the end of the experimental period. Therefore, deterioration in the general appearance, weight, texture, color and phenolic content could be mostly preserved in fresh-cut okra pods in polypropylene bags up to 8 days at 9 °C and 95% RH upon dipping in 0.5% solution of cysteine, ascorbic acid, or CaCl<sub>2</sub> for 5 min.

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