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Delay Yellowing and Maintain Quality of Fresh Broccoli Florets During Refrigerated Storage by Hot Water and Calcium Chloride Treatments

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Abstract: Broccoli (*Brassica oleracea var. italica Imperial hybrids*) was obtained from experimental Farm for Central Laboratory for Agricultural Climate (CLAC) Dokki site, ARC, Giza Governorate, Egypt, during the two successive seasons of 4th and 6th of December in 2018 and 2019 winter seasons respectively. Broccoli heads were harvested at the proper stage of marketing and then transported to the laboratory of Handling of Vegetable Crops Department, Horticulture Research Institute, Giza governorate. Fresh broccoli heads were separated into florets and stems and only blemish and defect free heads were used, then disinfected with sodium hypochlorite 150 ppm for 10 min, followed by repeated washing with distilled water and then dipped in the following treatments [Hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 4 min plus $CaCl_2$ solution at 2%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 2%, hot water at 45°C for 4 min, hot water at 45°C for 8 min, $CaCl_2$ solution 1% for 10 min, $CaCl_2$ solution 2% for 10 min and distilled water represented as control form 5 min]. All treatments were dried and packed in trays sealed with polypropylene film 15µm thicknesses and each package was approximately 100-120g and stored at 5°C and 95% relative humidity for 16 days. The obtained results revealed that broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ 2% was the most effective treatment in reducing weight loss percentage, not induced off odor, gave the lowest deterioration and more retained of total chlorophyll content, reducing the total sugar loss and green color loss (high hue angle), reducing the increase of lightness and gave excellent to good appearance after 16 days of storage at 5°C and 95% RH.

Key words: Broccoli · Hot water · Calcium Chloride · Color · Yellowing · Senescence · Storage · Quality

INTRODUCTION

Broccoli (*Brassica oleracea var. italica*) is a Brassica genus vegetable worldwide used vegetable with high nutritional value and health benefits, due to its richness in vitamins, antioxidants, anti-carcinogenic substance and health-promoting phytochemicals such as glucosinolates [1, 2]. Epidemiological studies have shown an inverse association between the consumption of Brassica vegetables and the risk of cancer [3]. Broccoli is a highly perishable vegetable that senesces quickly after harvest and thus its postharvest life is quite short due to yellowing, softening, water loss, decay and off-odor incidences a storage life of 3 to 4 weeks in 0°C and 95% RH and 2 to 3 days at 20°C [4, 5, 6]. Yellowing caused by either chlorophyll loss or blooming of the buds is a common problem intimately related to ethylene concentration and temperature of storage [7]. During the postharvest period, inflorescences lose their green color, turn yellow and decrease their nutritional and quality, diminishing the concentration of proteins, sugars, ascorbic acid and glucosinolates [8, 9]. This high sensitivity and perishability caused a high rate of metabolism and consequently, a high respiration rate is extremely sensitive to ethylene. Its deterioration rate appears to be affected by the storage temperature [10]. The degreening of broccoli heads is one of the significant features of their senescence and quality deterioration and directly determines consumer acceptance. De greening and yellowing of sepals accompanied by chlorophyll breakdown is the main visible symptoms of broccoli senescence [11]. The loss of green color in broccoli florets occurs due to chlorophyll breakdown and this is stimulated by exogenously applied and endogenously

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produced ethylene [12, 13]. Also, broccoli senescence is associated with a rapid loss of proteins and sugars [14, 15].

Recently a consumer tended to reduce the use of chemical preservatives to extend postharvest life of fruit and vegetables, since these compounds may be environmental contaminants or harmful to human health. Thus, the utilization of new and alternative physical technologies to extend postharvest life of fruit and vegetables has become important. Heat treatments (HT) can be used as a postharvest technique to maintain the postharvest quality and exhibited remarkably beneficial effects on the maintenance quality of many horticultural crops, reduction of the yellowing of broccoli florets and suppression of the activities of Chl-degrading enzymes, as chlorophyllase, Chl-degrading peroxidase and decay without negative effects on off-odors or weight loss [16]. Also, it shows a lower loss of sucrose and proteins [17-19]. Also, in this concern, immersion in hot water slowed the loss of soluble proteins and L-ascorbic acid and reduced the rate of respiration and ethylene production [20]. Additionally, HW treatment of 47°C for 7.5 min was the optimal treatment for maintaining the color of green florets and preventing heat damage on fresh-cut broccoli [21]. Funamoto *et al.* [22] revealed that treatment of broccoli with hot water could reduce chlorophyll degradation due to the suppression of chlorophyll degrading enzyme activities with inhibition of floret yellowing.

Calcium treatments are known as a potential postharvest approach used to maintain quality and to extend shelf life of fresh commodities because of its effects in controlling physiological disorders and delaying senescence in fresh commodities [23]. Calcium chloride ($CaCl_2$) has been effectively used to inhibit postharvest diseases, reduce weight loss and delay ripening in many fresh fruits [24]. Calcium chloride enters cell walls where it is involved in the cross-linking of pectin, making cell walls more resistant to cell wall degrading enzymes [25]. Hence, Calcium chloride dips can maintain visual quality by keeping the integrity of the cell wall and retarding flesh softening of fruits resulting in the longer shelf life of fresh-cut [26, 27].

Therefore, the main objectives of the present study were to investigate the effect of dipping in hot water, calcium chloride solution treatments and its combinations as postharvest treatments to maintaining the quality, delay yellowing and extending the shelf life of broccoli florets during refrigerated storage at 5°C and 95% RH.

MATERIALS AND METHODS

Broccoli (*Brassica oleracea var. italic Imperial F1 hybrids*), was harvested at the proper stage of marketing on 4th and 6th of December in 2018 and 2019 winter season respectively, from Experimental Farm for Central Lab. for Agric. Climate in Giza Governorate, to investigate the effect of hot water immersion, calcium chloride and it's combination on maintains quality and extend the shelf life of fresh-cut broccoli florets during cold storage. Broccoli heads were transported immediately to the laboratory of the Department of Vegetable Handling and Postharvest Research Section, Horticultural Research Institute, Agriculture Research Center, Giza Governorate, Egypt. Fresh broccoli heads were separated into florets and stems and only blemish and defect free heads were used. The florets were disinfected with sodium hypochlorite at the rate of 150 ppm for 10 min, followed by repeated washing with distilled water. Afterward, the broccoli florets were divided into 9 groups and dipped in hot water (HW), a solution containing calcium chloride 1% and 2% and its combinations between hot water and calcium chloride solution treatments as follows: 1- [Hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%], 2- [hot water at 45°C for 4 min plus $CaCl_2$ solution at 2%], 3-[hot water at 45°C for 8 min plus $CaCl_2$ solution at 1%], 4- [hot water at 45°C for 8 min plus $CaCl_2$ solution at 2%] , 5- [hot water at 45°C for 4 min], 6- [hot water at 45°C for 8 min], 7- [$CaCl_2$ solution 1% for 10 min], 8- $CaCl_2$ solution 2% for 10 min], 9- [distilled water represented as control form 5 min].

The broccoli florets were immediately immersed into cool water at 10°C for 5min after hot water treatments. As for combined treatments between hot water treatments (HWT) and calcium chloride solution, the broccoli florets were dipped in hot water then immediately immersed into cool water at 10°C for 5min then immersed in a solution containing calcium chloride 1%, 2% for 10 min. All treatments of fresh-cut broccoli florets were dried in a well-ventilated room under the sterilized condition for 20 min and packed in trays sealed with polypropylene film 15 µm thicknesses and each packed was approximately 100-120g represented as an experimental unit (EU). Twelve EU were prepared for each treatment. Samples were arranged in a complete randomized design. All treatments were placed inside carton boxes (40 × 30 ×12.5 cm) and stored at 5°C and 95% RH, for 16 days. Samples of 3 replicates (EU) were randomly taken and examined immediately after treatment and at four days intervals (0, 4, 6, 12 and 16 days) for the following properties:

Physical Analysis

Weight Loss (%): Weight of each sample of the three replication of each experimental unit was recorded after harvest and after every 4 days of storage period. Cumulative weight losses were expressed as percentage loss of original weight [11].

General Appearance: It was determined according to the scale of scoring system 9: Excellent, 7: good, 5: fair, 3: poor and 1: unsalable, depending on morphological defects such as flower bud yellowing, texture (crisp to very soft) and pathological defects [28].

Off Odor Score: It was evaluated on a scale of scoring system of 1 to 5 where 1= none, 2 = slight, 3 =moderate, 4= sever and 5= external sever [29].

External Surface Color: It was Color was evaluated by a color meter (Minolta model CR400, Konica Minolta, Tokyo, Japan), to measure the L* (lightness) and hue angle [30].

Chemical Composition:

Total Chlorophyll Content: The content of chlorophylls was measured according to Moran [31].

Total Sugars Content: The content of sugars was measured according to Lemoine *et al.* [32].

Statistical Analysis: The experiment was factorial with 2 factors in complete randomized design (CRD) with 3 replicates. Comparison between means was evaluated by Duncan's Multiple Range Test at 5% level of significance. The statistical analysis was performed according to Snedecor and Cochran [33].

RESULTS AND DISCUSSION

Weight Loss Percentage: Weight loss is one of the most important postharvest issue which has a direct impact on marketability and quality of broccoli [34]. Data in Table (1) show the effect of dipping in hot water and calcium chloride solution treatments on weight loss percentage of broccoli florets during storage at 5°C and 95% RH. Data revealed that there were significant increases in weight loss percentage with the extend of the storage period during the two seasons. The loss in weight may be attributed to respiration and other senescence related metabolic processes during storage [35]. Similar results were obtained by Jia et *al.* [36]; Pedro *et al.* [37] and Lemoine *et al*. [11] on broccoli florets.

Concerning the effect of dipping of hot water, calcium chloride solution and its combined applications, results indicated that there were significant differences among treatments in weight loss percentage during storage. All treatments reduced weight loss % during storage as compared with untreated control. Furthermore, broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% was the most effective treatment in reducing weight loss % during storage at 5°C in both seasons, followed by dipped in $CaCl_2$ solution % at 2, hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%, $CaCl_2$ solution % at 1, hot water at 45°C for 4 min and hot water at 45°C for 8 min plus $CaCl_2$ solution at 1% and hot water at 45°C for 8 min which were less effective in this concern. Meanwhile untreated control gave the highest values of weight loss%. in the two seasons. These results were in agreement with those reported by Perini *et al.* [38] for hot treatment and Fadhel *et al.* [39] for calcium chloride. Hot water treatments reduced the rate of respiration and ethylene production, consequent reducing weight loss [20]. As well hot water treatments caused a delay in senescence and hence reduction in weight loss during storage [11, 38]. Yan *et al.* [40] indicated that kojic acid and $CaCl_2$ treatments inhibited ethylene production and reduced the respiration rate in stored broccoli. The positive effects of Calcium treatment in retarding weight loss of broccoli is presumably due to its importance in maintaining cell wall structure [41]. Moreover, Calcium cross-linking effects in the cellular matrix, which affects the water permeability of the cell structures and affects the active water transport through the cell membrane [42].

In other studies, Calcium applications are effective in terms of membrane functionality and integrity maintenance which may also be the reason for the lower weight loss found in calcium treated fruits [43]. Also, delaying of natural physiological processes like respiration, the onset of the climacteric, ripening process and senescence due to $CaCl_2$ as reported by Hussain *et al*. [44].

In general, the interaction among hot water and calcium chloride solution and its combination treatments and storage periods were a significant effect on weight loss percentage after 16 days of storage at 5°C and 95% RH, the lowest value of weight loss was recorded from the

Table 1: Effect of dipping in hot water and calcium chloride solutions treatments on weight loss % of broccoli florets during cold storage at 5°C and 95% RH in 2018 and 2019 seasons

	Storage periods (days)				
	2018 season				
Treatments (dipping in)	4	8	12	16	Mean
HWT at 45°C for 4 min plus $CaCl_2$ 1%	1.17 x	1.41 r-t	1.94 l	2.75 e	1.82 F
HWT at 45°C for 4 min plus $CaCl_2$ 2%	1.03 y	1.26 w	1.82 m	2.59 g	1.68 H
HWT at 45°C for 8 min plus $CaCl_2$1%	1.33 uv	1.66 o	2.24 i	3.03 b	2.06 C
HWT at 45°C for 8 min plus $CaCl_2$ 2%	1.25 w	1.54 p	2.10 j	2.85 c	1.94 D
HWT at 45°C for 4 min	1.29 vw	1.49 pq	2.00 k	2.77 de	1.89 E
HWT at 45°C for 8 min	1.40 st	1.73 n	2.29 i	3.07 b	2.12 B
Dipping in $CaCl_2$ solution at 1%	1.12 x	1.47 qr	2.04 jk	2.83 cd	1.87 E
Dipping in $CaCl_2$ solution at 2%	1.00 y	1.38 tu	1.91 l	2.68 e	1.74 G
Control (distilled water)	1.46 q-s	1.80 m	2.35 h	3.15 a	2.19 A
Mean	1.23 D	1.53 C	2.08 B	2.86 A	
	2019 season				
HWT at 45°C for 4 min plus $CaCl_2$ 1%	1.23 v	1.35 tu	2.02 lm	2.62 e	1.81 G
HWT at 45°C for 4 min plus $CaCl_2$ 2%	1.08 y	1.19 vw	1.87 n	2.42 gh	1.64 I
HWT at 45°C for 8 min plus $CaCl_2$1%	1.41 st	1.58 pq	2.31i	2.88 b	2.05 C
HWT at 45°C for 8 min plus $CaCl_2$ 2%	1.32 u	1.51r	2.16 j	2.78 c	1.94 D
HWT at 45°C for 4 min	1.35 tu	1.43 s	2.07 kl	2.70 d	1.89 E
HWT at 45°C for 8 min	1.47 rs	1.64 p	2.36	2.90 b	2.09 B
Dipping in $CaCl_2$ solution at 1%	1.16wx	1.41st	2.09 k	2.72 cd	1.84 F
Dipping in $CaCl_2$ solution at 2%	1.10 xy	1.31 u	1.97 m	2.51 f	1.72 H
Control (distilled water)	1.53 qr	1.75 o	2.44 g	3.06 a	2.19 A
Mean	1.29 D	1.46 C	2.14 B	2.73 A	

Means in the same column having the same letter are not significantly different at 0.05 levels by Duncan's multiple rang test.

broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution % at 2 in the two seasons. While the highest ones were obtained from untreated control in the two seasons.

General Appearance (Score): Data presented in Table (2) show that the general appearance (score) of broccoli florets was decreased with the prolongation of the storage period. These results were in agreement with those obtained by Dong *et al.* [45]; Forney [16] and Yan *et al.* [40]. The decreases in general appearance during storage might be due to the degradation of chlorophyll [29, 9]. Branchless and florets lose turgor and become flaccid if water loss is excessive [46]. Decay commonly occurs on florets, primarily during advanced stages of senescence [47].

Concerning the effect of hot water, calcium chloride solution and its combination treatments on general appearance, data revealed that the broccoli florets with all treatments had significantly the highest score of appearance as compared with untreated control. The broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% was the most effective treatments for maintaining general appearance during storage at 5°C and 95% RH, followed by dipped in $CaCl_2$ solution % at 2, hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 4 min, $CaCl_2$ solution % at 1 and hot water at 45°C for 8 min which were less effective in this concern in the both seasons. Whereas untreated control recorded the lowest value of general appearance. These results agreed with Forney [16]. Who indicated that hot water treatments are effective in extending the shelf life of fresh broccoli immersion in 50°C water for 2 min seems to be most effective in reducing yellowing and decay while not inducing off-odors or weight loss. Calcium chloride plays an important role in the protection of cell integrity and has a positive effect on inhibiting the degradation of chlorophyll [25]. Application of kojic acid plus $CaCl_2$ of broccoli resulted in the maintenance of a higher level of quality and prolonged shelf life due to the inhibiting chlorophyll degradation and color changes during storage [40].

The effect of interaction among hot water and calcium chloride solution and its combination treatments and storage periods at 5°C and 95% RH on general appearance (score), results indicated that the broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2%, gave excellent to good appearance at the

Table 2: Effect of dipping in hot water and calcium chloride solutions treatments on general appearance (score) of broccoli florets during cold storage at 5°C and 95% RH in 2018 and 2019 seasons

	Storage periods (days)					
	2018					
Treatments (dipping in)	0	4	8	12	16	Mean
HWT at 45°C for 4 min + $CaCl_2$ 1%	9.00 a	9.00 a	7.00 a-d	7.00 a-d	6.33 b-d	7.67 B
HWT at 45°C for 4 min+ $CaCl_2$ 2%	9.00 a	9.00 a	9.00 a	9.00 a	8.33 ab	8.87 A
HWT at 45°C for 8 min + $CaCl_2$ 1%	9.00 a	9.00 a	7.67 a-c	5.67 c-e	5.00 d-f	7.27 BC
HWT at 45°C for 8 min+ $CaCl_2$ 2%	9.00 a	9.00 a	7.67 a-c	5.67 c-e	5.00 d-f	7.27 BC
HWT at 45°C for 4 min	9.00 a	9.00 a	7.67 a-c	5.67 c-e	5.67 c-e	7.40 BC
HWT at 45°C for 8 min	9.00 a	9.00 a	7.00 a-d	5.00 d-f	5.00 d-f	6.87 C
Dipping in $CaCl_2$ 1%	9.00 a	9.00 a	8.33 ab	5.67 c-e	5.00 d-f	7.40 BC
Dipping in $CaCl_2$ 2%	9.00 a	9.00 a	8.33 ab	7.00 a-d	6.33 b-d	7.93 B
Control (distilled water)	9.00	8.33 ab	6.33 b-d	3.67 ef	3.00 f	6.07 D
Mean	9.00 A	8.85 A	7.67 B	6.04 C	5.52 D	
	2019					
HWT at 45°C for 4 min + $CaCl_2$ 1%	9.00 a	9.00 a	7.00 a-d	6.33 b-d	5.67 c-e	7.40 BC
HWT at 45°C for 4 min+ $CaCl_2$ 2%	9.00 a	9.00 a	9.00 a	9.00 a	8.33 ab	8.87 A
HWT at 45°C for 8 min + $CaCl_2$ 1%	9.00 a	9.00 a	7.67 a-c	5.67 c-e	5.00 de	7.27 BC
HWT at 45°C for 8 min+ $CaCl_2$ 2%	9.00 a	9.00 a	7.67 a-c	5.67 c-e	5.00 de	7.27 BC
HWT at 45°C for 4 min	9.00 a	9.00 a	7.67 a-c	6.33 b-d	5.67 c-e	7.53 BC
HWT at 45°C for 8 min	9.00 a	9.00 a	7.00 a-d	5.67 c-e	5.00 de	7.00 C
Dipping in $CaCl_2$ 1%	9.00 a	9.00 a	8.33 ab	6.33 b-d	5.00 de	7.53 BC
Dipping in $CaCl_2$ 2%	9.00 a	9.00 a	8.33 ab	7.00 a-d	6.33 b-d	7.93 B
Control (distilled water)	9.00 a	8.33 ab	6.33 b-d	3.67 ef	2.33f	5.93 D
Mean	9.00 A	8.85 A	7.67 B	6.19 C	5.37 D	

Means in the same column having the same letter are not significantly different at 0.05 levels by Duncan's multiple rang test.

end of storage period 16 days at 5°C and 95% RH. Followed by dipped in $CaCl_2$ solution at 2% was less effective in this concern. On the other side, untreated control had the unsalable appearance at the end of the storage period in both seasons.

Off Odor Score: Results in Table (3) including the effect of dipping in hot water, $CaCl_2$ solution and its combination treatments on off-odor, showed that there were significant differences in the off-odor of broccoli florets during storage at 5°C and 95% RH. It was noticed that all used treatments did not show any changes in their off-odor inside package till 4 days at 5°C, then increased with the prolongation of the storage period in the two seasons. These results were in agreement with those obtained by Yan *et al.* [40]; Shehata *et al.* [48] and Forney [16].

Concerning the effect of dipping in hot water, calcium chloride solution and its combination applications on off-odor score results indicated that there were significant differences among treatments on the off-odor score during storage periods in in both seasons.

The broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% and $CaCl_2$ solution at 2% was closer to the initial off-odor followed by dipped in $CaCl_2$ solution at 1%, hot water at 45°C for 4 min plus $CaCl_2$ solution at 1% and hot water at 45°C for 4 min with no significant differences each other. While, hot water at 45°C for 8 min, hot water at 45°C for 8 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 2% and untreated control which were less effective in this concern with no significant differences among them in the two seasons. These results were agreed with those found by Shehata *et al.* [48] and Forney [16].

Off-odor induced by high-temperature long duration, hot-water treatments appeared to be the physiological injury caused by the heat treatments. The nature of odor produced by heat-treated broccoli was different by anaerobic stress. Also, hot water treatments are effective in extending the shelf life of fresh broccoli in case immersion of broccoli in 50°C water for 2 min seems to be most effective in reducing yellowing and decay while not inducing off odors or weight loss [16]. Furthermore, heat treatments may have contributed to improving the sensory attributes of fresh-cut cantaloupe through heat shock proteins [50].

Table 3: Effect of dipping in hot water and calcium chloride solutions treatments on off-odor (score) of broccoli florets during cold storage at 5°C and 95% RH in 2018 and 2019 seasons

	Storage periods (days)					
	2018 Season					
Treatments (dipping in)	0	4	8	12	16	Mean
HWT at 45°C for 4 min plus $CaCl_2$ 1%	1.00 g	1.00 g	1.00 g	1.33 fg	2.00 d-f	1.27 B
HWT at 45°C for 4 min plus $CaCl_2$ 2%	1.00 g	1.00 g	1.00 g	1.00 g	1.33 fg	1.07 B
HWT at 45°C for 8 min plus $CaCl_2$ 1%	1.00 g	1.00 g	1.33 fg	3.00 a-c	3.00 a-c	1.87 A
HWT at 45°C for 8 min plus $CaCl_2$ 2%	1.00 g	1.00 g	1.33 fg	2.67 b-d	3.00 a-c	1.80 A
HWT at 45°C for 4 min	1.00 g	1.00 g	1.00 g	1.67 e-g	2.00 d-f	1.33 B
HWT at 45°C for 8 min	1.00 g	1.00 g	2.00 d-f	3.00 a-c	3.67 a	2.13 A
Dipping in $CaCl_2$ solution at 1%	1.00 g	1.00 g	1.00 g	1.67 e-g	2.00 d-f	1.33 B
Dipping in $CaCl_2$ solution at 2%	1.00 g	1.00 g	1.00 g	1.00 g	1.67 e-g	1.13 B
Control (distilled water)	1.00 g	1.00 g	1.33 fg	2.33 c-e	3.33 ab	1.80 A
Mean	1.00 C	1.00 C	1.22 C	1.96 B	2.44 A	
	2019 Season					
HWT at 45°C for 4 min plus $CaCl_2$ 1%	1.00 e	1.00 e	1.00 e	1.33 de	1.67 c-e	1.20 B
HWT at 45°C for 4 min plus $CaCl_2$ 2%	1.00 e	1.00 e	1.00 e	1.00 e	1.33 de	1.07 B
HWT at 45°C for 8 min plus $CaCl_2$1%	1.00 e	1.00 e	1.33 de	2.67 a-c	2.67 a-c	1.73 A
HWT at 45°C for 8 min plus $CaCl_2$ 2%	1.00 e	1.00 e	1.33 de	2.67 a-c	2.67 a-c	1.73 A
HWT at 45°C for 4 min	1.00 e	1.00 e	1.00 e	1.67 c-e	2.00 b- e	1.33 B
HWT at 45°C for 8 min	1.00 e	1.00 e	2.00 b-e	3.00 ab	3.33 a	2.07 A
Dipping in $CaCl_2$ solution at 1%	1.00 e	1.00 e	1.00 e	1.67 c-e	2.00 b-e	1.33 B
Dipping in $CaCl_2$ solution at 2%	1.00 e	1.00 e	1.00 e	1.33 de	1.67 c-e	1.20 B
Control (distilled water)	1.00 e	1.00 e	1.33 de	2.33 a-d	3.00 ab	1.73A
Mean	1.00 C	1.00 C	1.22 C	1.96 B	2.26 A	

Means in the same column having the same letter are not significantly different at 0.05 levels by Duncan's multiple rang test

Di Pentima *et al.* [51] indicated that changes in the odor of broccoli florets could be partially attributed to changes in the volatile emission of oxynitride, sulfur compounds and aromatic and organic sulfur compounds. Also, the objectionable odor produced by broccoli during senescence has been attributed to the enzymatic production of volatile sulfur compounds. Yan *et al.* [40] indicated that through the combined treatment of broccoli florets with kojic acid and calcium chloride resulted to maintain postharvest quality was associated with a significant delay in the production of the offensive odor from broccoli during storage and inhibits off-odor production, relative to the untreated broccoli. Moreover, reduce the respiration rate inhibited ethylene production and reduced the respiration rate in stored broccoli. Hence, this may lead to delay in the production of the offensive odor from broccoli during storage.

Regarding the effect of interaction among hot water and calcium chloride solution and its combination treatments and storage periods on off-odor (score), results indicated that the broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2%, was closer to the initial off odor and delay in the production of the offensive odor from broccoli. followed by dipped in $CaCl_2$ solution at 2%, hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 4 min and dipped in $CaCl_2$ solution at 1% with no significant differences each other during storage till 16 days at 5°C and 95% RH in the both seasons

Color (Hue Angle H°): Data presented in Table (4) reflected a decrease in hue angle value of broccoli florets with the prolongation of the storage period at 5°C and 95% RH during the two seasons. The loss of green color in broccoli florets occurs due to chlorophyll breakdown and this is stimulated by exogenously applied and endogenously produced ethylene [12]. These results were in agreement with those obtained by Tain *el al.* [21] and Perini *et al*. [38].

Yellowing is reflection to decline in the hue angle of the flower buds. As well the increase in yellowing rates and the decrease in chlorophyll concentrations during storage very important factor to storage life of broccoli [45, 16]. Hue angle decreased significantly during storage at 4 °C reflecting the transition from green color to yellow [39]. Yellowness is generally related to senescence which is characterized by chlorophyll degradation during storage [52].

Table 4: Effect of dipping in hot water and calcium chloride solutions treatments on color (hue angle h°) of broccoli florets during cold storage at 5°C and 95% RH in 2018 and 2019 seasons

	Storage periods (days)					
	2018 Season					
Treatments (dipping in)	0	4	8	12	16	Mean
HW at 45°C for 4 min plus $CaCl_2$ 1%	132.33 a	128.33 b-d	124.00 e-h	120.67 h-j	117.33 j-l	124.53 C
HWT at 45°C for 4 min plus $CaCl_2$ 2%	132.33 a	130.67 AB	129.12 a-c	126.33 c-f	123.50 f-h	128.39 A
HWT at 45°C for 8 min plus $Cacl_2$ 1%	132.33 a	126.00 c-f	121.33 hi	117.33 j-l	113.53 mn	122.11 DE
HWT at 45°C for 8 min plus $Cacl_2$ 2%	132.33 a	127.00 c-e	123.33 f-h	118.67 i-k	114.33 l-n	123.13 D
HWT at 45°C for 4 min	132.33 a	128.67b-d	126.33 c-f	121.00 hi	118.77 i-k	125.42 C
HWT at 45°C for 8 min	132.33 a	125.33 d-g	121.50 hi	116.33 k-m	113.50 mn	121.80 E
Dipping in $Cacl_2$ solution at1%	132.33 a	125.67 d-f	121.67 hi	116.67 k-m	113.67 mn	122.00 DE
Dipping in $Cacl_2$ solution at 2%	132.33 a	128.67 b-d	127.67 b-d	123.67 e-h	121.00 hi	126.67 B
Control (distilled water)	132.33 a	122.00g-i	116.33k-m	112.33 no	109.50 o	118.50 F
Mean	132.33 A	126.93 B	123.48 C	119.22 D	116.13 E	
	2019 Season					
HW at 45°C for 4 min plus $CaCl_2$ 1%	134.20 a	130.00 cd	128.33 d-g	124.50 k-m	120.30 op	127.47 C
HWT at 45°C for 4 min plus $CaCl_2$ 2%	134.20 a	132.33 ab	130.53 bc	127.57 e-h	123.80 k-m	129.69 A
HWT at 45°C for 8 min plus $Cacl_2$ 1%	134.20 a	128.33 d-g	126.53 g-j	122.83 mn	118.80 p	126.14 DE
HWT at 45°C for 8 min plus $Cacl_2$ 2%	134.20 a	129.04 c-f	127.43f-i	123.33 mn	119.60 p	126.72 D
HWT at 45°C for 4 min	134.20 a	130.83 bc	128.60 d-f	123.83 k-m	121.63 no	127.82 C
HWT at 45°C for 8 min	134.20 a	127.33 f-i	125.43 j-l	123.37 mn	119.53 p	125.97 E
Dipping in $Cacl_2$ solution at1%	134.20 a	127.70 e-h	125.63 i-k	123.60 lm	119.73 p	126.17 DE
Dipping in $Cacl_2$ solution at 2%	134.20 a	130.83 bc	129.33 c-e	126.40 h -j	122.63 mn	128.68 B
Control (distilled water)	134.20 a	123.37 mn	119.67 p	115.33 q	112.23 r	120.96 F
Mean	134.20 A	128.86 B	126.83 C	123.42 D	119.81 E	

Means in the same column having the same letter are not significantly different at 0.05 levels by Duncan's multiple rang test

Concerning the effect of dipping in hot water, calcium chloride solution and its combination treatments on hue angle value, data indicated that there were significant differences among all treatments during storage periods at 5°C and 95% RH in both seasons. The broccoli florets which dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% was the most effective in retention of green color (high hue angle) that is an indicator of greenness during storage. Followed by dipped in $CaCl_2$ solution at 2, hot water at 45°C for 4 min, hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 1%, dipped in $CaCl_2$ solution at 1% and hot water at 45°C for 8 min were less effective in this concern with significant differences each other. Whereas untreated control was gave lowest value in the both seasons. These results were agreement with Tian *et al.* [18], who reported that dipping the broccoli in hot water at 42-55°C could reduce yellowing during storage. Similarity, immersion of broccoli in hot water 50°C water for 2 min was most effective in reducing yellowing [16]. The loss of chlorophylls during postharvest broccoli senescence correlated with an increment in the activities of enzymes involved in their catabolism such as chlorophyllase and Mg-dechelatase [53]. Treating broccoli with hot water treatments could reduce chlorophyll degradation due to the suppression of chlorophyll degrading enzyme activities with inhibition of floret yellowing [11, 22]. On the other side, kojic acid with calcium chloride treatment reduce oxidative damage in broccoli and maintenance of color quality in stored broccoli might be partly attributed to enhanced redox homeostasis, leading to a positive effect on maintaining the quality of harvested broccoli [40].

As for The interaction between hot water, calcium chloride solution and its combination treatments and storage periods at 5°C and 95% RH on hue angle value, results indicated that the broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% followed by dipped in $CaCl_2$ solution at 2% were the most effective in retention of green color (high hue angle) after 16 days of storage with no significant differences between them in the two seasons.

Color (Lightness): Results in Table (5) showed that there were significant increases in lightness of broccoli florets with the prolongation of the storage period at 5°C and 95% RH during the two seasons. These results were in agreement with those obtained by Perdo *et al.* [37] and Fadhel *el al.* [39].

Table 5: Effect of dipping in hot water and calcium chloride solutions treatments on color (Lightness L. Value) of broccoli florets during cold storage at 5°C and 95% RH in 2018 and 2019 seasons

	Storage periods (days)					
	2018 Season					
Treatments	0	4	8	12	16	Mean
HWT at 45°C for4 min plus $CaCl_2$ 1%	35.88 u	38.70 pq	39.80 lm	40.67 i	41.36 gh	39.28 F
HWT at 45°C for4 min plus $CaCl_2$ 2%	35.88 u	36.37 t	38.03 s	38.60 q	39.03 op	37.58 I
HWT at 45°C for8 min plus $CaCl_2$ 1%	35.88 u	39.75 m	40.71 i	41.87 f	42.35 e	40.11 C
HWT at 45°C for 8 min plus $CaCl_2$ 2%	35.88 u	39.03 op	40.10kl	41.30 gh	41.86 f	39.64 E
HWT at 45°C for 4 min	35.88 u	38.40 qr	39.40 n	40.60 i	41.20 h	39.10 G
HWT at 45°C for 8 min	35.88 u	40.77 i	41.77 f	42.80 d	43.30 c	40.90 B
Dipping in $CaCl_2$ solution at1%	35.88 u	39.40 n	40.20 jk	41.30 gh	42.88 d	39.93 D
Dipping in $CaCl_2$ solution at 2%	35.88 u	38.20 rs	39.07 no	40.03 k-m	40.53 ij	38.74 H
Control (distilled water)	35.88 u	41.63 fg	42.40 e	43.70 b	44.80 a	41.68 A
Mean	35.88 E	39.14D	40.16 C	41.21 B	41.92 A	
	2019 Season					
HWT at 45°C for 4 min plus $CaCl_2$ 1%	38.53 s	39.50 q	40.73 kl	41.76 i	42.43 gh	40.59 F
HWT at 45°C for 4 min plus $CaCl_2$ 2%	38.53 s	37.00 t	38.90 r	39.73 pq	39.97 op	38.83 I
HWT at 45°C for 8 min plus $CaCl_2$ 1%	38.53 s	40.60 lm	41.63 i	42.87 f	43.43 e	41.41 C
HWT at 45°C for 8 min plus$CaCl_2$ 2%	38.53 s	40.08 no	41.03 jk	42.43 gh	42.87 f	40.99 E
HWT at 45°C for 4 min	38.53 s	39.07 r	40.34 mn	41.73 i	42.35 h	40.40 F
HWT at 45°C for 8 min	38.53 s	41.54 i	42.63 f-h	43.87 d	44.40 c	42.19 B
Dipping in $CaCl_2$ solution at1%	38.53 s	40.10 no	41.13 j	42.43 gh	43.87 d	41.21 D
Dipping in $CaCl_2$ solution at 2%	38.53 s	39.03 r	39.99 op	41.13 j	41.63 i	40.06 H
Control (distilled water)	38.53 s	42.73 fg	43.53 de	44.77 b	46.03 a	43.12 A
Mean	38.53 E	39.96 D	41.10 C	42.30 B	43.00 A	

Means in the same column having the same letter are not significantly different at 0.05 levels by Duncan's multiple rang test

The deterioration of color during the postharvest storage of broccoli, is a result of losses in total chlorophyll [54, 55].

Concerning the effect of dipping in hot water, calcium chloride solution and its combination treatments on lightness value, data demonstrated that there were significant differences among all treatments during storage periods at 5°C and 95% RH in tested seasons. The broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% was the most effective in reducing the increases of lightness during storage, followed by dipped in $CaCl_2$ solution at 2, hot water at 45°C for 4 min, hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 1%, dipped in $CaCl_2$ solution at 1% and hot water at 45°C for 8 which were less effective, in this concern. Meanwhile the untreated control recorded the highest mean value. These results were achieved in the two seasons and were agreement with those reported by Costa *et al*. [53] and Funamoto *el al*. [22]. Heat treatments delayed chlorophyll catabolism in broccoli during postharvest senescence by reducing the activities of the enzymes involved in chlorophyll degradation. Similarity maintenance of color quality in stored broccoli as a result of $CaCl_2$ combined with kojic acid treatment might be partly attributed to enhanced redox homeostasis, which would have a positive effect on maintaining the quality of harvested broccoli as reported by Yan *et al*. [40]. Also Fadhel *el al*. [39] noted that the applied treatments of $CaCl_2$ with alginate coating allowed a slight color change and broccoli florets could be considered as visually acceptable for consumption.

In relation to interaction between dipping in hot water, calcium chloride solution and its combination treatments and storage periods at 5°C and 95% RH on lightness value, results showed that the broccoli florets dipped in hot water at 45° for 4 min plus $CaCl_2$ solution at 2% was the most effective treatment in reducing the increases of lightness after 16 days of storage followed by dipped in $CaCl_2$ solution at 2 % in both seasons.

Table 6: Effect of dipping in hot water and calcium chloride solutions treatments on total chlorophyll content (mg/100g F.W) of broccoli florets during cold storage at 5°C and 95% RH in 2018 and 2019 seasons

	Storage periods (days)					
	2018 Season					
Treatments (dipping in)	0	4	8	12	16	Mean
HWT at 45°C for 4 min plus $CaCl_2$ 1%	99.70 a	97.03 ef	95.73 h	94.47 k-m	93.17pq	96.02 D
HWT at 45°C for 4 min plus $CaCl_2$ 2%	99.70 a	98.90 b	97.77 cd	96.60 e-g	95.53 hi	97.70 A
HWT at 45°C for 8 min plus $CaCl_2$ 1%	99.70 a	98.00 c	96.63 e-g	95.47 h-j	94.33 k-n	96.83 C
HWT at 45°C for 8 min plus $CaCl_2$ 2%	99.70 a	96.50 fg	95.43 h-j	94.83 jk	93.80 n-p	96.05 D
HWT at 45°C for 4 min	99.70 a	96.50 fg	95.40 h-j	94.10 i-o	93.60 o-q	95.86 D
HWT at 45°C for 8 min	99.70 a	96.73 ef	95.63 hi	94.47 k-m	93.50 o-q	96.01 D
Dipping in $CaCl_2$ solution at 1%	99.70 a	96.03 gh	94.70 kl	93.60 o-q	92.40 r	95.29 E
Dipping in $CaCl_2$ solution at 2%	99.70 a	98.30 bc	97.17 de	96.03 gh	94.98 i-k	97.24 B
Control (distilled water)	99.70 a	95.80 h	94.03 m-o	93.03 qr	91.17 s	94.75 F
Mean	99.70 A	97.09 B	95.83 C	94.73 D	93.61 E	
	2019 Season					
HWT at 45°C for 4 min plus $CaCl_2$ 1%	102.40 a	99.06 ef	97.74	96.07 n-p	95.20 p-s	98.09 D
HWT at 45°C for 4 min plus $CaCl_2$ 2%	102.40 a	100.90 b	99.80 c-e	98.10 g-j	97.28 j-l	99.70 A
HWT at 45°C for 8 min plus $CaCl_2$ 1%	102.40 a	100.03 b-d	98.64 fg	96.97 k-m	95.93 n-q	98.79 C
HWT at 45°C for 8 min plus $CaCl_2$ 2%	102.40 a	98.57 f-h	97.47 i-l	95.77 o-r	95.10 q-s	97.86 D
HWT at 45°C for 4 min	102.40 a	98.17 g-i	97.03 k-m	95.73 o-r	95.70 o-r	97.81 D
HWT at 45°C for 8 min	102.40 a	98.73 fg	97.66 i-k	95.97 n-q	94.98 rs	97.95D
Dipping in $CaCl_2$ solution at 1%	102.40 a	98.04 g-j	96.70 l-n	95.03 rs	94.03 t	97.24E
Dipping in $CaCl_2$ solution at 2%	102.40 a	100.33 bc	99.20 d-f	97.50 i-l	96.40 m-o	99.17 B
Control (distilled water)	102.40 a	97.63 i-k	96.05 n-p	94.37 st	93.03 u	96.70 F
Mean	102.40 A	99.05 B	97.81 C	96.17 D	95.30 E	

Means in the same column having the same letter are not significantly different at 0.05 levels by Duncan's multiple rang test

Total Chlorophyll Content: Data presented in Table (6) revealed that the total chlorophyll content of broccoli florets was decreased and gradually deteriorated during storage at 5°C and 95% RH with the extend of the storage period during the two seasons. These results were in agreement with those obtained by Perini *et al*. [38]; Buchert *et al.* [56] and Nath *et al*. [34]. Chlorophyll degradation is the most obvious visual change and this is accompanied by losses in membrane lipid and proteins eventually resulting in cell death [9].The decreased and gradual deterioration could be attributed to the gradual increase of destruction by chlorophyll degrading peroxidase POD activity which is the transformation of chloroplasts to chromoplasts [45].

Concerning the effect of dipping in hot water, calcium chloride solution and its combination treatments on total chlorophyll content, results indicated that there were significant differences among treatments during storage periods at 5°C in both seasons. The broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% exhibited the lowest deterioration and more retained of total chlorophyll content during storage periods. Followed by dipped in $CaCl_2$ solution at 2%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 2%, hot water at 45°C for 4 min, hot water at 45°C for 8 min and $CaCl_2$ solution at 1% were less effective in this concern. Meanwhile the untreated control recorded the lowest mean value in the both season. These results were in agreement with Yan *et al.* [40] and Tian *et al.* [21]. For hot water treatment Funamoto *et al.* [22] observed that treating broccoli with heat treatment could reduce chlorophyll degradation due to the suppression of chlorophyll degrading enzyme activities with inhibition of floret yellowing. Calcium chloride treatments lead to the protection of cell integrity and have a positive effect on inhibiting the degradation of chlorophyll [25]. In other study, a reduction in the content of chlorophyll and inhibiting chlorophyll degradation in broccoli florets during storage was reduced as a result of kojic acid and $CaCl_2$ treatments than untreated control [40]. Also, Guo *et al.* [57] reported that application of $CaCl_2$ reduced the decline of total chlorophyll content during storage.

As for the effect of interaction between hot water, calcium chloride solution and its combination treatments and storage periods at 5°C on total chlorophyll content, results revealed that the broccoli florets dipped in hot

Table 7: Effect of dipping in hot water and calcium chloride solutions treatments on total sugar (mg/100g F.W) of broccoli florets during cold storage at 5°C and 95% RH in 2018 and 2019 seasons

	Storage periods (days)					
	2018 Season					
Treatments	0	4	8	12	16	Mean
HWT at 45°C for 4 min plus $CaCl_2$ 1%	1.99 a	1.79 c	1.69 de	1.57 hi	1.49 j-l	1.71 C
HWT at 45°C for 4 min plus $CaCl_2$2%	1.99 a	1.92 b	1.81 c	1.72 d	1.66 ef	1.82 A
HWT at 45°C for 8 min plus $CaCl_2$1%	1.99 a	1.60 gh	1.50 j-l	1.39 m-o	1.31 qr	1.56 F
HWT at 45°C for 8 min plus $CaCl_2$2%	1.99 a	1.63 fg	1.53 ij	1.41 m	1.33 pq	1.58 E
HWT at 45°C for 4 min	1.99 a	1.70 d	1.59 gh	1.48 kl	1.39 mn	1.63 D
HWT at 45°C for 8 min	1.99 a	1.58 h	1.46 l	1.35 o-q	1.29 r	1.53 G
Dipping in $CaCl_2$ solution at 1%	1.99 a	1.70 d	1.59 h	1.46 l	1.36 n-p	1.62 D
Dipping in $CaCl_2$ solution at 2%	1.99 a	1.81 c	1.72 d	1.59 gh	1.51 jk	1.72 B
Control (distilled water)	1.99 a	1.52 j	1.38 m-o	1.28 r	1.21 s	1.48 H
Mean	1.99 A	1.70 B	1.59 C	1.47 D	1.40 E	
	2019 Season					
HWT at 45°C for 4 min plus $CaCl_2$ 1%	1.95 a	1.73 b-d	1.62 e-h	1.50 i-l	1.40 m-p	1.64 C
HWT at 45°C for 4 min plus $CaCl_2$ 2%	1.95 a	1.88 a	1.76 bc	1.65 d-g	1.53 h-k	1.75 A
HWT at 45°C for 8 min plus $CaCl_2$ 1%	1.95 a	1.57 f-j	1.46 k-m	1.35 o-r	1.24 st	1.51 F
HWT at 45°C for 8 min plus $CaCl_2$ 2%	1.95 a	1.59 e-i	1.52 i-l	1.39 m-q	1.29 r-t	1.55 E
HWT at 45°C for 4 min	1.95 a	1.67c-e	1.56 g-j	1.45 k-n	1.32 p-s	1.59 D
HWT at 45°C for 8 min	1.95 a	1.51 i-l	1.40 m-p	1.30 q-t	1.21 tu	1.47 G
Dipping in $CaCl_2$ solution at 1%	1.95 a	1.66 c-e	1.54 h-k	1.43 l-o	1.33 p-s	1.58 D
Dipping in $CaCl_2$ solution at 2%	1.95 a	1.77 b	1.65 d-g	1.53 h-k	1.43 l-o	1.67 B
Control (distilled water)	1.95 a	1.48 j-m	1.36 n-r	1.21 tu	1.12 u	1.42 H
Mean	1.95 A	1.65 B	1.54 C	1.42 D	1.32 E	

Means in the same column having the same letter are not significantly different at 0.05 levels by Duncan's multiple rang test

water at 45°C for 4 min plus $CaCl_2$ solution at 2% was more retained and reduced the decline of total chlorophyll content after 16 days of storage at 5°C followed by dipped in $CaCl_2$ solution at 2% in two seasons.

Total Sugars Content: Results in Table (7) indicated that total sugar content was decreased in broccoli florets with the prolongation of the storage period during the two seasons. These results were in agreement with those obtained by Page *et al.* [9]; Perini *et al.* [38] and Costa *et al.* [19]. The decrease in total sugars content during storage might be due to the consumption of sugars through respiration [11].

Concerning the effect of dipping in hot water, calcium chloride solution and its combination treatments on total sugars content, data revealed that there were significant differences among all treatments during storage periods at 5°C in both seasons. The broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% was the most effective treatments in reducing the total sugar loss during storage periods at 5°C. Followed by in $CaCl_2$ solution at 2%, hot water at 45°C for 4 min plus $CaCl_2$ solution at 1%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 2%, hot water at 45°C for 8 min plus $CaCl_2$ solution at 1% and $CaCl_2$ solution at 1% were less effective in this concern Meanwhile the untreated control recorded the lowest mean value in the both season. These results were true in the two seasons and were in agreement with Costa *et al.* [19] found that the heat treatments broccoli induced a slight delay in the consumption of sugars. Additionally heat treatment causes a temporary stress that stops the normal metabolism of tissue, which recovers after a certain time. Perini *et al.* [38] revealed that the content of both total and reducing soluble sugars maintained higher in treated samples when compared with the controls after five days. Lemoine *et al.* [11] indicated that the higher level of total sugars could be due to lower tissue consumption caused by the stress produced by the heat shock. An increase in the level of sucrose in florets dipped in hot water was noticed as supply of single sugars is necessary for respiration, which maintains the quality of the product and delays senescence [18]. On the other hand dipping in $CaCl_2$ induces a delay in consumption of sugar during storage [58]. Furthermore, mode of action of $CaCl_2$ treatments may be due to delaying of natural physiological processes like respiration [44].

Concerning the interaction between dipping in hot water, calcium chloride solution and its combination treatments and storage periods at 5°C on total sugar content, results indicated that broccoli florets dipped in hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% was more retained and reduced the total sugar loss after 16 days of storage at 5°C in two seasons.

CONCLUSIONS

From the previous results, it could be conclude that broccoli florets dipped in Hot water at 45°C for 4 min plus $CaCl_2$ solution at 2% was the most effective treatment in reducing weight loss percentage, not induced off odor and gave lowest deterioration of total chlorophyll content, reducing the total sugar loss and green color loss (high hue angle), reducing increase of lightness and gave excellent and good appearance after 16 days of storage at 5°C and 95% RH.

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