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Effects of Gibberellic Acid and Calcium Chloride on Colour, Phenolic Compounds, Carotenoids and Quality Attributes of White Cabbage (*Brassica oleracea*) during Storage under Refrigeration

Research Article

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Abstract

The effects of gibberellic acid (150 and 300 mg/L) and calcium chloride (40 g/L) on chlorophylls a and b, carotenoids, total phenolic compounds, colour and physiological weight losses of white cabbage (*Brassica oleracea* L. var. capitata) during storage at 10 ± 1 °C were investigated. Results showed that chlorophyll a and total phenolic compounds decreased in all samples and no significant difference was noted between treated heads and control. Chlorophyll b and carotenoids did not vary much during storage and a steady state was noted in all samples. Treatments maintained well the total colour difference compared to control, and GA treated cabbages maintained better the colour after the fourteen days storage. The chemical treatments also reduced significantly the physiological weight loss which was much greater in controls than in GA treated heads after the storage duration.

Keywords: Gibberellic Acid; Calcium Chloride; Refrigeration; Storage; *Brassica oleracea*.

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Introduction

White Cabbage (*Brassica oleracea* L. var. capitata) is one of the most commonly cultivated vegetables across the entire island of Jamaica. This crop is cultivated mainly in rural parishes and is a staple food for many Jamaican families. In this context it is customary for the immature leaves within the cabbage head to be consumed while the mature outer leaves are usually rejected. Cabbage as a vegetable contains chlorophylls, carotenoids, vitamins and phenolic compounds that are linked with dietary activities, colour, characteristic flavour and therapeutic value.

Chlorophylls, carotenoids and phenolic compounds are important biological compounds found in all vegetables and contribute to the overall visual quality attributes of cabbage. Degradation of these compounds will lead to quality losses of cabbage heads including yellowing of outer leaves, core elongation, internal yellowing in the apex region, and sometimes in rootlet development at the core-end. These quality defects may affect sales as they are obvious to the consumer when the cabbage has reached market [1]. Gibberellic acid (GA_3) has been widely used in agriculture. It was reported that GA_3 plays a role in the development of colours and metabolic activities in the ripening process of fruits and vegetables, and delays shrivelling and senescence of some fruits [2]. It has also been reported that gibberellic acid reduces physiological weight loss of treated mangoes [3] and lemon [4] during storage.

Calcium chloride is also used to extend the shelf-life of crop commodities [5]. The application of calcium chloride maintains the rigidity of cell walls; and delays membrane lipid catabolism and senescence [6]. Thus, tissues remain firm longer due to the inhibition of many enzymes such as polygalacuturonase which is involved in softening, therefore, ripening is delayed and shelf-life extended [2, 7]. Many investigations have been conducted on the postharvest application of calcium chloride to extend the shelf-life of crops. These have shown that calcium chloride reduces physiological weight loss and delays softening of mangoes [8] and rotting of apples [9] during storage.

Although interesting data exist on the application of gibberellic acid and/or calcium chloride during the pre and the postharvest stages of some commodities, no work reported the application of these two chemicals on cabbage and their potential use to extend the shelf-life of this crop. Therefore, the goal of this study is to investigate the impact of gibberellic acid and calcium chloride on the colour, chlorophylls and carotenoids of cabbage heads during storage under refrigerated temperatures.

Materials and Methods

Plant material

Freshly harvested cabbage heads of uniform size, and free from damage, disease or spoilage, were obtained from the local fresh crops market in Kingston. Cabbages were transported immediately to the laboratory and packed in perforated polyethylene bags. From the total cabbages, fifty were selected and 10 heads per bag were packed and kept few hours at 10 ± 1°C prior to chemical treatments.

Chemicals treatments and storage

Prior to packaging and storage, cabbages were sprayed six (6) times using spray bottles with the following solutions: gibberellic acid of 150 mg/L (GA1) and 300 mg/L (GA2), and calcium chloride (40 g/L) (CC). Control samples were sprayed with distilled water. After spraying, samples were left to dry at room temperature, then stored at 10 ± 1°C and 90-95 % RH during fourteen days.

Chlorophyll a, b and carotenoids assessments

Chlorophylls a, b and carotenoids are determined according to the method described by Costache et al [10]. One gram of cabbage leaf is homogenised in 50 mL of 90% methanol using a mortar and pestle. The homogenate was filtered through two layers of cheesecloth, then centrifuged 1,000 × g to obtain a clear extract prior to being analysed. The absorbances of the solutions are read at 653 nm and 666 nm against 90 % methanol blank.

Determination of total phenolic compounds (TPC)

Total phenolic compounds were extracted and assayed as described by Singleton and Rossi [11] with some modifications. Samples (5 g) were homogenized in 70% ethanol containing Na-metabisulfite ($Na_2S_2O_5$, 20 g/L) and extracted using an ultrasound assisted extraction (UAE) with an ultra sound sonicator at room temperature. The extracts were centrifuged at 1,000 × g for 10 min and the supernatant collected for TPC assay. Total phenolic compounds (TPC) of extracts were quantified colorimetrically using Folin-Ciocalteu reagent and chlorogenic acid as standard. Five millilitres of Folin-Ciocalteu (diluted ten-fold in distilled water), 2 mL of sodium bicarbonate (200 g/L) and 2 mL of distilled water were added to 1 mL of extract. After 15 min incubation at room temperature, the absorbance was read at 730 nm using Thermo Scientific™ GENESYS 10S UV-VIS spectrophotometer, and results expressed in chlorogenic acid equivalents (mg CAE/g fresh weight).

Colour analysis

The colour of the arils was measured on the fruit surface using a Konica & Minolta CR-400 chromameter (Konica & Minolta Sensing Inc., Osaka, Japan) and the data were expressed in, L^*, a^* and b^* values (CIE). The colour reading was taken in triplicate at the equatorial region of each fruit and averaged to give a value for each cabbage head. The values a^* and b^* were used to calculate chroma ($C^* = [a^*2 + b^*2]1/2$), which indicates the intensity or colour saturation, and hue angle (H^* = arctangent [b*/a*]), where 0° = red-purple, 90° = yellow, 180° = bluish-green, and 270° = blue [12].

Physiological weight loss (PWL)

Weight loss was calculated as a percentage (%) of the initial mass, with an electronic scale. The physiological weight loss was estimated from the difference between the initial and the final weight after each storage period.

Statistical analysis

Experimental work was duplicated and experiments were run in triplicate. Experimental results were averaged and reported as mean ± standard deviation (SD). A single factor analysis of variance (ANOVA) was performed using GraphPad Prism 4.03 (GraphPad Software, Inc., 2236 La Jolla, CA, USA). Treatment means were compared by least significant difference (*LSD*) at $P \leq 0.05$.

Results and Discussion

Variation of chlorophylls a, b and carotenoids

Chlorophyll a varied significantly during storage as shown in Figure 1A. Chlorophyll a decreased significantly in control, CC and GA1 after 11 days storage, while in GA2, the decrease was significant after 14 days storage. After 4 days, chlorophyll a decreased by 46%, 27% and 50% in control, CC and GA1, respectively, and the decrease reached 81%, 76% and 81% after 14 days storage, respectively. In GA2 treated cabbage, chlorophyll a decreased by 12% after four days, but the decrease was similar to that observed in other treated cabbage and reached 80%.

Surprisingly, chlorophyll b in cabbage did not show significant variation during storage (Figure 1B). Chlorophyll b varied from an initial value of 2.49 mg/g DW and averaged 2.53, 2.56 and 2.63 mg/g DW after four, eleven and fourteen days storage, respectively. Similarly, total carotenoids decreased slightly but did not vary significantly in cabbage during storage (Figure 1C). Total carotenoids yielded 2.71mg/g DW in fresh cabbage heads, and decreased by 14%, 3%, 15% and 4% in control, CC, GA1 and GA2 after fourteen days storage, respectively. On the other hand, carotenoids in cabbage averaged 2.62, 2.55 and 2.46 mg/g DW after four, eleven and fourteen days, respectively.

Comparatively, chlorophylls a/b ratio was estimated and data showed the ratio decreased progressively from 0.31 to 0.17, 0.22, 0.15 and 0.28 after four days, to 0.13, 0.07, 0.15 and 0.16 after eleven days, and to 0.06, 0.07, 0.06 and 0.06 after fourteen days in control, CC, GA1 and GA2, respectively (Figure 2A). On the other hand, the chlorophylls [a+b]/carotenoids ratio varied very slightly and decreased from 1.20 to 1.21, 1.21, 1.07 and 1.17 after four days, to 1.18, 1.07, 1.15 and 1.11 after eleven days, and to 1.19, 1.09, 1.19 and 1.07 after fourteen days in control, CC, GA1 and GA2, respectively (Figure 2B).

The use of gibberellic acid as a preharvest treatment is extensively documented. While the postharvest treatment reported is mainly on the use of GA to extend the shelf-life and longevity of ornamental flowers, few reports are available on fruits and vegetables. However, some discrepancies have been reported on the effect of postharvest application of GA on chlorophyll content. Siddiqui et al [13] reported a decrease of c.a. 50% in chlorophylls of mango fruits stored twelve days at room temperature of 28 ± 2°C, and no difference in effect was noted between the different GA con-

Figure 1. Chlorophylls a(A), chlorophylls b(B) and carotenoids (C) concentration in cabbage heads stored at 10 ± 1°C during 14 days. □Control, ▒Calcium Chloride, ▓GA1 ■ GA2

Figure 1A

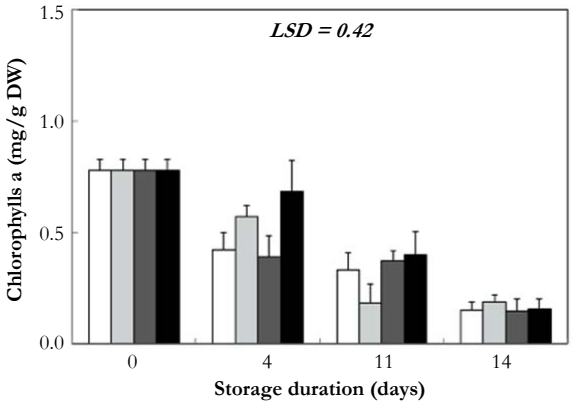


Figure 1B

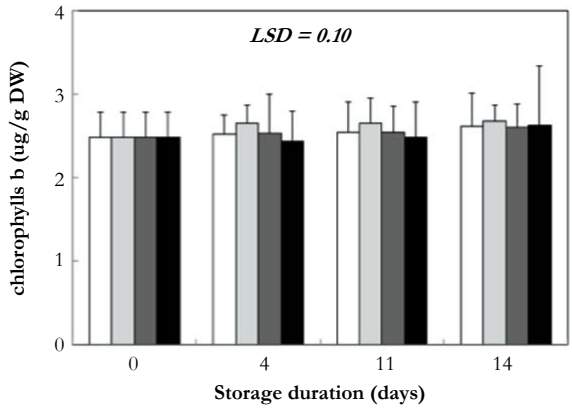


Figure 1C

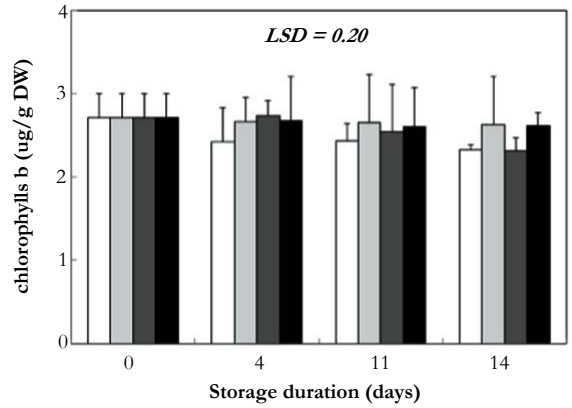
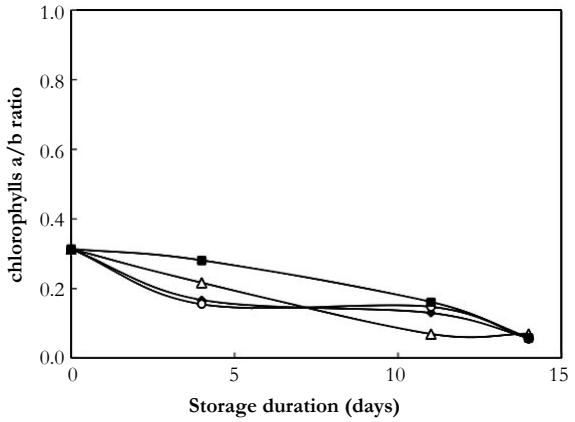
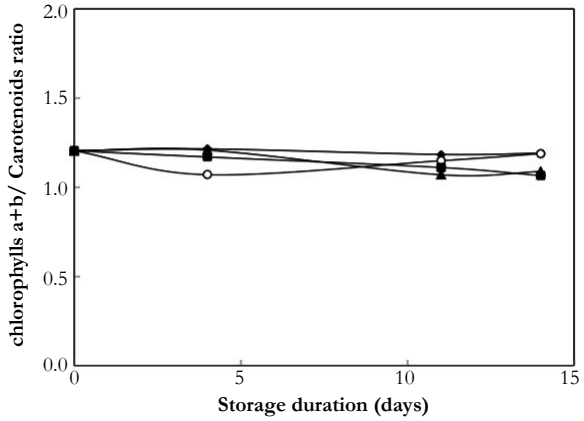


Figure 2. Chlorophylls a/b (A) and Chlorophylls a+b/carotenoids (B) ratios in cabbage heads stored at 10 ± 1°C during 14 days. ◆Control, Δ Calcium Chloride, O GA1. ■ GA2





centrations used.

On the contrary, many studies reported that GA retained green colour of fruits and chlorophylls. Gross et al [14] reported that GA treatments delayed the senescence and retained chlorophyll in persimmon for long periods. Pre and postharvest applications of GA also retained the green fruit colour of citrus fruits. Mahajan et al [7] used different concentrations of GA_3 ranging from 25 to 75 ppm on guava fruit and no significant difference in effect was noted among these concentrations. Singh and Dankhar [15] reported that postharvest application of GA retained chlorophylls in okra during storage and retention was higher even with visible senescence. They also noted that chlorophyll a decreased more rapidly than chlorophyll b which surprisingly exceeded the content of chlorophyll a in some fruits.

Janowska and Jerzy [16] investigated the effects of GA on the longevity of *Zantedeschia elliottiana* leaves and noted that GA reduced the degradation of chlorophylls, but no significance was noted between the concentrations and exposure time. Indeed, the mechanisms of GA on chlorophylls are not clearly understood, but it is likely that GA delays the activity of chlorophyllase during ripening of GA treated strawberry fruit as reported by Martínez et al [17], and/or promotes chlorophyll synthesis [14]. Indeed, the visible colour of plants results from the predominant pigments, even though other pigments are present but are not visible unless the predominant is degraded. Consequently, the degradation of chlorophylls causes colours change in the tissues from green to many other colours such as yellow and orange observed in ripe and senescent tissues [18].

Calcium chloride is widely used in agriculture and food preservation as well. Investigations showed that postharvest use of calcium chloride maintains firmness and visual quality, therefore, extending the shelf life of the fresh produce. It was reported that dipping fruits in calcium chloride was effective in delaying senescence and extended figs [19] and jujube [20] shelf-life during storage. On the other hand, calcium chloride treatments have also been reported as effective in reducing chlorophyll [21]. Calcium chloride has also been used in combination with gibberellic acid and other organic acids. Bhanja and Lenka [22] combined gibberellic acid and calcium chloride treatments of sapota (*Achraszapota*) fruit, and they noted that treatments reduced the physiological weight losses and percentage rotting of fruits during storage, and the shelf-life was extended to 36 days compared to control (8 days).

Indeed, these results are not in contradiction with our results, as we noted that chlorophyll a content is much lower than chlorophyll b. Even though chlorophyll a decreased chlorophyll b did not vary much, therefore, making the change in total chlorophylls (a+b) not significant as shown in Figure 2.

Variation of total phenolics content (TPC)

The total phenolics content decreased progressively during storage of cabbage heads, however, the decrease was significant in the control after four days, while in GA2 TPC decreased significantly after fourteen days (Figure 3). After four days, TPC decreased from 50.91 to 40.20, 39.97, 45.13 and 47.26 mg/g DW in control, CC, GA1 and GA2, respectively. After fourteen days storage, TPC decreased significantly to 26.17, 26.38, 28.91 and 27.10 mg/g DW in control, CC, GA1 and GA2, respectively, and no significant difference was noted between control and treatments.

Many investigations have reported on TPC of white cabbage heads, and different TPC has been determined. For example and among literature cited, Leja et al [23] reported values ranging between 12.58 and 47.6 mg GAE/100 g FW. The large variations of TPC in white cabbages vary with different factors such as the botanical cabbage group [24], variety [25], extraction solvents used [26], and growing region [27].

Many works reported on the total phenolics of cabbage, but few exist on the variation of TPC during storage, and no work reports on the effects of GA and calcium chloride on TPC in stored cabbage. Hounsome et al [28] studied the variation of different phenolic compounds during storage and they noted that long-term storage significantly reduced the content of many phenolic compounds such as caffeic, syringic, gallic and trihyroxybenzoic acids, as well as flavonoids, while other phenolics such as cinnamic, dimethoxybenzoic acid, chalcone and flavone were less affected by storage. Hagen et al [29] stored another Brassica variety called curly kale (*Brassica oleracea* L. var. *acephala*) and noted that total phenolics increased slightly after six weeks storage at 1°C. Nevertheless, in stored lettuce, escarole and rocket salad tissues, TPC varied differently. TPC increased after one day but decreased after three days storage at 4°C. In escarole, TPC increased significantly after three days, while in rocket tissues TPC decreased after three days at the same temperature [30].

Colour variation

During storage, colour of cabbage heads showed different variations (Table 1). Data of a^* are indicating that cabbage heads are less after eleven days storage, and the lightness decreased after the same storage period as indicated by the decrease of L^*. The b^* decreased from positive to negative values and the H^* increased after fourteen days storage indicating that the yellowish colour of cabbages is decreasing and the heads are turning darker and did not retain the yellow colour (as shown in Figure 5). However, no significant difference was noted between control and treated cabbages during the storage period.

Moreover, the total colour difference between all three coordinates was determined by the following equation:

$$\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$$

Data are showing that control, CC, GA1 and GA2 averaged 12.00, 25.56, 24.69 and 22.92 after four, eleven and fourteen days storage indicating that the colour of control and GA2 are lighter than CC and GA1.

Few works have reported on the variation on colour of cabbage during storage, while no reference is readily available on the effects of gibberillic acid and calcium chloride on stored cabbage heads. Manolopoulou and Varzakas [31] reported similar results and noted that lightness L^* and H^* values of fresh cabbage stored at 0°C decreased after 15 days storage, but L^* decreased after 20 days storage, and these data are in agreement with our results indicating that the reduction was limited regardless of the chemical treatments and the initial colour was to some extent preserved. The increase of H^* value observed after fourteen days might be due to browning caused by the senescence [32]. On the other hand, it was suggested that the total colour difference (ΔE) could

Figure 3. Total phenolic compounds concentration in cabbage heads stored at 10 ± 1°C during 14 days. For legends see Figure 1.

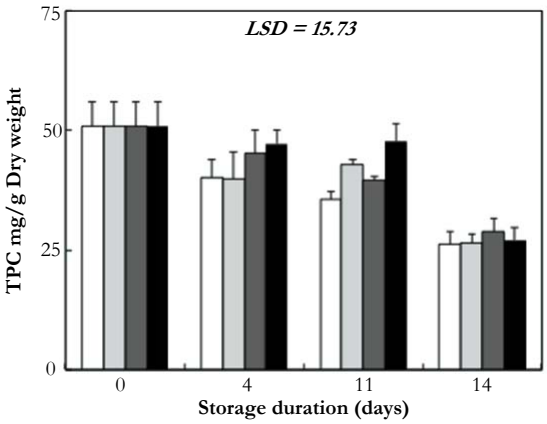


Figure 4. Weight losses in cabbage heads stored at 10 ± 1°C during 14 days. For legends see Figure 1.

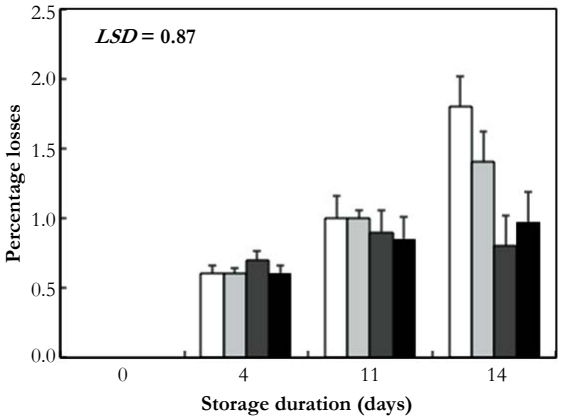


Figure 5. Cabbage heads stored at 10 ± 1°C during 14 days.

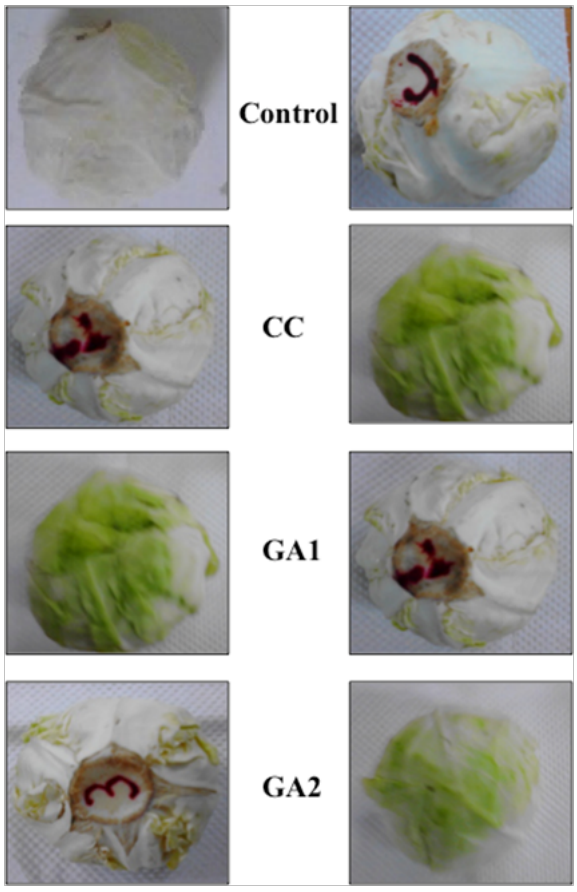


Table 1. Variation of colour parameters of cabbage heads during storage. Values with different superscript letters within the same columns are significantly different.

	*a**	*b**	*L**	*C**	*H**
	Freshly harvested				
	70.60 ± 2.90	10.46 ± 0.30	31.94 ± 1.97	33.62 ± 1.90	108.23 ± 1.02
	After 4 days storage at 10 ± 1°C				
Control	70.60 ± 2.90^{a}	10.46 ± 0.30^{a}	31.94 ± 1.97^{a}	33.62 ± 1.90^{a}	108.23 ± 1.02^{a}
CC	69.66 ± 6.54^{a}	11.14 ± 0.86^{b}	25.98 ± 1.35^{b}	28.30 ± 1.33^{b}	113.26 ± 9.78^{a}
GA1	70.28 ± 3.94^{a}	14.25 ± 0.74^{b}	30.70 ± 2.07^{a}	33.85 ± 2.17^{a}	114.96 ± 0.60^{a}
GA2	72.44 ± 2.68^{a}	9.63 ± 4.85^{a}	28.61 ± 3.87^{ab}	35.25 ± 2.27^{a}	123.59 ± 6.96^{b}
	After 11 days storage at 10 ± 1°C				
Control	58.16 ± 7.75^{b}	-10.63 ± 1.16	18.94 ± 2.01^{c}	24.26 ± 2.12^{b}	113.05 ± 2.22^{a}
CC	61.79 ± 5.96^{b}	-13.61 ± 1.11	30.96 ± 1.67^{a}	33.83 ± 1.98^{b}	113.67 ± 8.55^{a}
GA1	57.47 ± 8.04^{b}	-9.96 ±1.07	22.90 ± 1.94^{b}	24.99 ± 2.10^{b}	113.47± 7.10^{a}
GA2	49.76 ± 7.53^{c}	-13.75 ± 3.44	27.17 ± 6.05^{ab}	30.46 ± 6.94^{ab}	126.42 ± 11.4^{b}
	After 14 days storage at 10 ± 1°C				
Control	70.60 ± 2.90^{a}	10.46 ± 0.30	19.94 ± 1.97^{a}	23.62 ± 1.90^{b}	116.23 ± 1.02^{a}
CC	63.36 ± 3.02^{b}	-12.85 ± 2.28	24.37 ± 1.66^{b}	27.61 ± 2.50^{ab}	117.29 ± 2.86
GA1	71.82 ± 6.08^{a}	-13.20 ± 1.53	24.99 ± 3.57^{b}	28.26 ± 5.72^{ab}	117.84 ± 9.72
GA2	72.72 ± 2.54^{a}	-10.63 ± 1.20	23.21 ± 1.72^{b}	25.55 ± 2.01^{b}	124.50 ± 13.5^{b}

be used as a discoloration index [33], and our data are suggesting that all treatments did not preserve the colour of cabbage.

Physiological weight loss (PWL)

During storage, PWL increased progressively during storage, however, control and CC treated cabbages showed higher PWL than GA treated ones (Figure 4). After four days storage, PWL averaged 0.6% to 0.7% in control and treated cabbages, and after eleven days PWL averaged 1.00%, 1.00%, 0.90% and 0.85% in control, CC, GA1 and GA2, respectively, with no significant difference between control and all treated cabbages. Nevertheless, after fourteen days storage, PWL averaged 1.80%, 1.40%, 0.80% and 0.97% in control, CC, GA1 and GA2, respectively. By fitting linear regression lines to weight losses vs time, results showed average losses of 0.13%, 0.11%, 0.06% and 0.07% per day in control, CC, GA1 and GA2, respectively, indicating that GA treatment was more efficient in reducing PWL than CC treatment.

Extensive literature exists on PWL of fresh crops during storage. Weigh losses of fresh crops is the consequence of respiration and oxidative reactions, mainly sugars. However, degradation of energy-source compounds is correlated to many factors such as the physiological stage, temperatures and storage duration, as well as other factors such as gas composition (eg., modified atmosphere packaging), and even the microbial charge of the stored crops [34]. Manolopoulou and Varzakas [31] reported that weight losses of cabbage averaged 3% after twenty-three days storage at 4°C. In minimally processed cabbage, weight losses were higher and averaged 2.81% and 3.32% in control, and 2.28% and 3.35% in calcium chloride treated heads stored during twenty-two days at 0°C and 5°C, respectively [35].

Conclusions

The application of gibberellic acid and calcium chloride to extend the shelf-life of cabbage heads stored under refrigeration showed that these chemical treatments did not reduce chlorophyll a and total phenolic compounds losses but maintained chlorophyll b and carotenoid levels which contributed to better colour preservation although no statistical difference was noted. However, all the chemical treatments reduced significantly the physiological weight losses which were much higher in the control. We can conclude that gibberellic acid and calcium chloride could be used to maintain the quality attributes of fresh cabbage heads stored under refrigerated temperature, however, the optimal storage period should not exceed two weeks.

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