CHAPTER 4 RESULTS

4.1 Experiment I: The Effects of Sanitary Treatments on Microbial Growth and Visual Quality in Fresh-Cut Broccoli Florets during Cool Storage

4.1.1 Hot Water (HW) Treatments

Hot water treatment is a commonly physical treatment to reduce food-borne pathogens and also to delay senescence (Funamoto et al., 2002). However, the literature on hot water treatment to control microbial and maintain the quality of fresh-cut broccoli florets is limited. Therefore, the aim of this present study was to determine the optimal temperature of hot water required to reduce total bacteria and food-borne pathogens (coliforms and *Salmonella-Shigella* spp.) as well as to maintain the quality of fresh-cut broccoli florets during storage at low temperature.

4.1.1.1 Effect of HW on Microbial Populations

The effect of hot water (HW) treatments at different degrees Celsius (50, 55 and 60 °C for 3 min) on total bacteria, coliforms and *Salmonella-Shigella* spp. counts in fresh-cut broccoli florets during storage at 4 °C is shown in Figure 4.1 (Table A1-A3). The initial population of total bacteria, coliforms and *Salmonella-Shigella* spp. in tap water (25 °C, control) treated broccoli florets averaged 4.73, 3.23 and 3.23 log CFU·g^{-1} FW, respectively. In the initial day storage, the HW treatments at 55 and 60 °C for 3 min completely inhibited both food-borne pathogens, while the HW treatment at 50 °C for 3 min reduced total bacteria, coliforms and *Salmonella-Shigella* spp. by 0.84, 1.0 and 1.0 log CFU·g^{-1} FW, respectively, when compared to the control (Figure 4.1; Table A1-A3). Among the treatments, HW treatment at 50 °C maintained the lowest numbers of microbial counts throughout 10 days of storage in comparison with the control. The highest number of microbial counts was observed in tap water washed on the initial date to day 10 of storage of investigation as well.

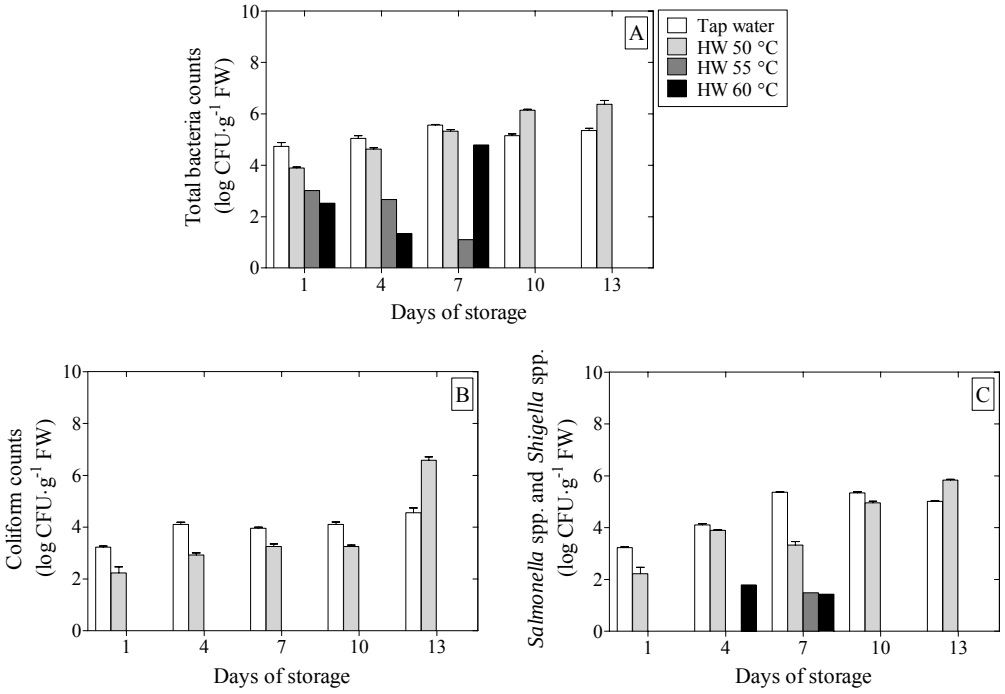

Figure 4.1 Changes in microbial populations of total bacteria (A), coliforms (B) and *Salmonella-Shigella* spp. (C) counts of fresh-cut broccoli florets treated with tap water (control) and hot water (HW) at 50, 55 and 60 °C for 3 min. Florets were stored at 4 °C for 13 days. Vertical bars represent the mean ± standard error.

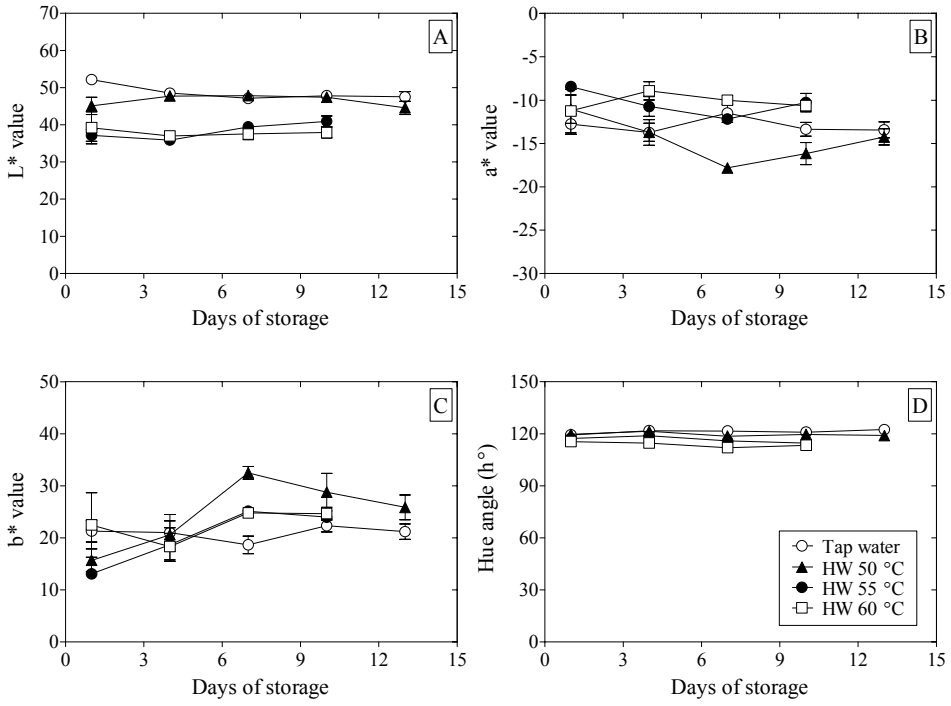

Figure 4.2 Changes in lightness (L*) (A), a* value (B), b* value (C) and hue angle (h°) (D) color of fresh-cut broccoli florets treated with tap water (control) and hot water (HW) at 50, 55 and 60 °C for 3 min. Florets were stored at 4 °C for 13 days. Vertical bars represent the mean ± standard error.

4.1.1.2 Effect of HW on Florets Color

Fresh-cut broccoli florets treated with HW at 55 and 60 °C showed a lower L*, a*, b* value and hue angle than broccoli florets treated with HW at 50 °C and tap water (control) during storage at 4 °C (Figure 4.2). The L* value and hue angle of broccoli florets treated at 50 °C was close to that of tap water (control). The control and treated samples with HW at 50 °C showed nearly constant the L* value throughout storage (within the range of 44.57 to 47.86) (Figure 4.2A; Table A4). The green color of broccoli is indicated by the a* (redness/greenness) value. The fresh-cut broccoli treated with HW at 50 °C showed a lower a* value than the other treatments (within range of -17.83 to -11.04) (Figure 4.2B; Table A5). This indicated that the HW at 50 °C could maintain the green florets color during period storage. The florets color by b* (yellowness/blueness) value showed slight increase in all florets samples, and the florets treated HW were not significantly different when compared with the control during storage at 4 °C for 13 days (within the range of 15.69 to 32.50) (Figure 4.2C; Table A6). The hue angle was approximately 118.70 to 121.38° (green) constantly during storage in the control and florets samples treated with HW at 50 °C. With regards to the color, broccoli florets treated with tap water and the HW at 50 °C maintained the hue angle (h°) during storage periods (Figure 4.2D; Table A7).

4.1.1.3 Effect of HW on O_2 and CO_2 Levels in the Package and Respiration Rate

In the packages of fresh-cut broccoli, a decrease in O_2 and an increase in CO_2 levels were observed in all treatments (Figure 4.3A and B; Table A8-A9). The lowest levels of O_2 (13.2 to 16.2% O_2) and the highest levels of CO_2 (4.2 to 5.4% CO_2) were detected in the package of fresh-cut broccoli treated with HW at 50 °C for 3 min. The gas composition inside the package of fresh-cut broccoli treated HW at 50 °C for 3 min was found to be about 13 to 16% O_2 and 4.0 to 5.8% CO_2, whereas the O_2 and CO_2 levels in the package of the other treatments ranged from about 16 to 21% O_2 and 0.0 to 4.4% CO_2, respectively. High water temperatures increased the respiration rate of fresh-cut broccoli florets (Figure 4.3C; Table A10). Broccoli florets treated with HW at 60 °C for 3 min showed sharply increased respiration rates on the initial day of storage in comparison to other treatments, followed by broccoli florets treated with HW at 55 °C. The respiration rate of the florets treated with HW at 50 °C and the control treatment gradually increased and peaked on day 7 of storage, and the florets treated with HW at 50 °C had a slightly higher respiration rate than the control (Figure 4.3C; Table A10).

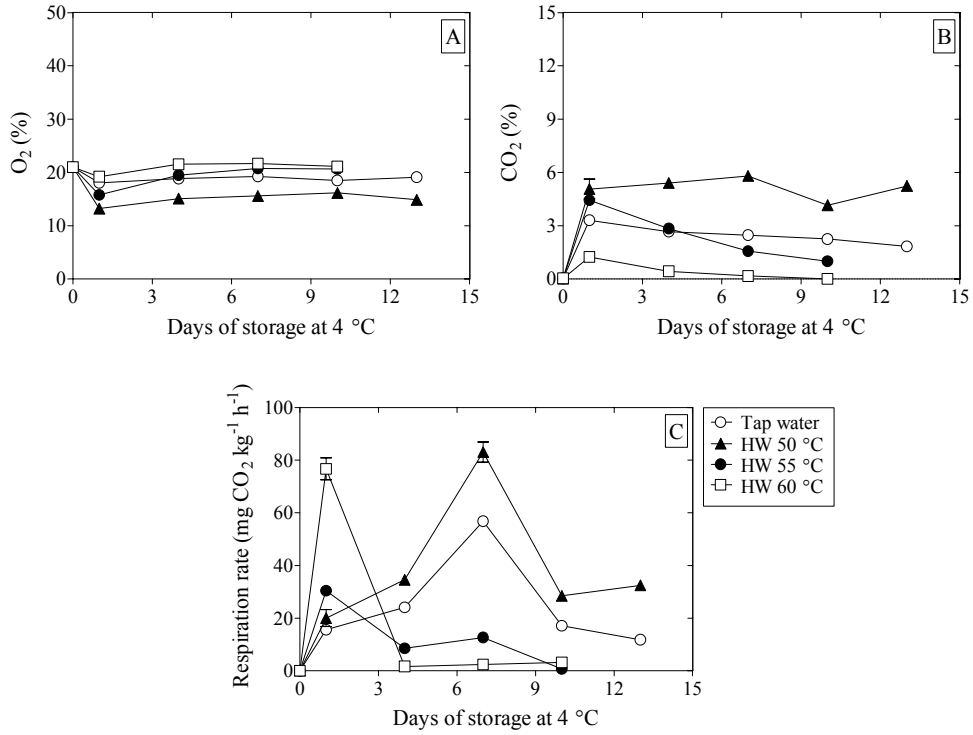

Figure 4.3 Changes in the percentage of oxygen (O_2) (A), carbon dioxide (CO_2) (B) concentration in package, and respiration rate (mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) (C) of fresh-cut broccoli florets treated with tap water (control) and hot water (HW) at 50, 55 and 60 °C for 3 min. Florets were stored at 4 °C for 13 days. Vertical bars represent the mean ± standard error.

4.1.1.4 Effect of HW on Sensory Attributes

In this study, the shelf-life of fresh-cut broccoli florets was determined based on the unacceptable quality limit for consumers (average score above 5) (Allende et al., 2004; Martínez-Sánchez et al., 2006). As shown in Figure 4.4, the sensory attributes of HW treated broccoli florets was compared with that of the control (tap water wash). The higher temperature of HW treatments showed higher scores of visual quality and visual color, but lower scores of odor attributes compared to the control (Figure 4.4; Table A11-A13). However, HW at 55 to 60 °C for 3 min caused softening, scald-like symptoms and off-odor in fresh-cut broccoli florets, which is caused by over cooking. In our study, high temperature (55 and 60 °C) of HW treatment is not recommended in fresh-cut broccoli florets because an abnormal odor was detected after 1 day of storage. However, HW at 50 °C for 3 min showed severe initial abnormal or off-odor after 7 days of storage, probably due to the final gas concentrations managed within packages (Figure 4.4C; Table A13).

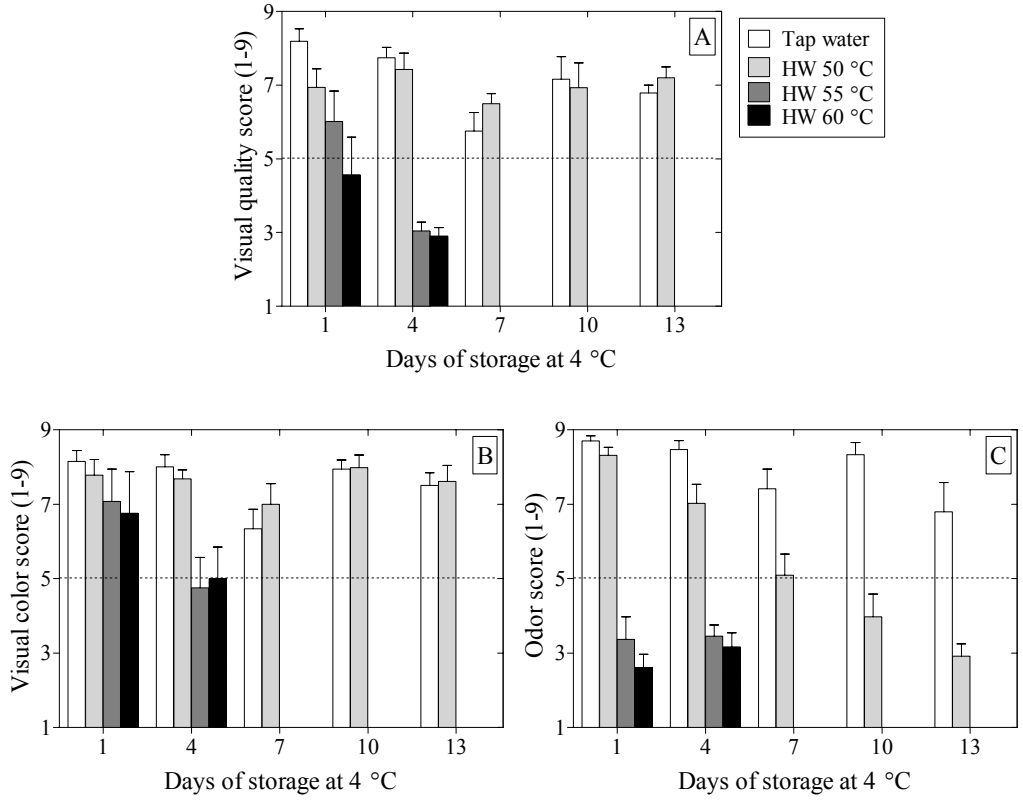

Figure 4.4 Changes in the sensory attributes: overall visual quality (A), visual color (B), and odor (C) score of fresh-cut broccoli florets treated with tap water (control) and hot water (HW) at 50, 55 and 60 °C for 3 min. Florets were stored at 4 °C for 13 days. Vertical bars represent the mean ± standard error. Overall visual quality score: 9 = fresh appearance, 1 = unusable. Color score: 9 = dark green, 1 = 100% yellow. Odor score: 9 = no off-odor, 1 = extreme off-odor. The horizontal line marks indicate the unacceptable quality limit for customers.

4.1.2 Vapor Heat (VH) Treatments

Most research involved in fresh-cut broccoli florets trends using heat treatment techniques such as blanching or hot air treatment have focused on the quality, extending shelf-life and maintaining antioxidants rather than in reducing pathogenic populations. At the present, there is less information about the reduction of microbial populations by VH (90 °C) treatment especially in fresh-cut broccoli florets. Thus, the aim of the present study was to investigate the exposure times of VH required to reduce total bacteria, coliforms, *Salmonella-Shigella* spp. and yeast and mold counts and also to maintain the quality and sensory characteristics of fresh-cut broccoli florets during storage for 6 days at low temperature.

4.1.2.1 Effect of VH on Microbial Population

The effect of exposure times of VH on microbial populations in fresh-cut broccoli florets is shown in Figure 4.5 (Table A14-17). After treatment, VH for 15 sec was able to reduce the initial population of total bacteria, coliforms, *Salmonella-Shigella* spp. and yeast and mold counts by 0.35, 0.51, 0.01 and 0.05 log CFU·g^{-1} FW, respectively, when compared to the control. There was no significant difference (P>0.05) in reducing microbial population between VH (30 and 45 sec). The results of VH had little effect in reducing or eliminating microbial populations (total bacteria, coliforms, *Salmonella-Shigella* spp. and yeasts and molds) in fresh-cut broccoli florets.

4.1.2.2 Effect of VH on Florets Color

Green color of broccoli florets can be observed in L* (lightness) value and hue angle (Lemoine et al., 2010). The florets color (L*, a*, b* value and hue angle) of fresh-cut broccoli florets treated with VH was not significantly different when compared with the control during storage at 4 °C for 6 days (Figure 4.6). The L* value showed slight increase in all florets samples. On day 3 of storage, florets treated with VH for 30 and 45 sec had significantly decreased on L* value when compared to the control (within the range of 35.37 to 43.78) (Figure 4.6A; Table A18). The decrease in the L* value of florets treated with VH for 30 and 45 sec indicated darkening of florets of broccoli, which could be attributed to the florets turning to olive green due to over cooking, while florets treated with VH for 15 sec had no significant difference (P>0.05) during 6 days of storage. In addition, a slight decrease of a* (redness/greenness) (within the range of -13.23 to -8.62) and b* (yellowness/blueness) (within the range of 19.80 to 25.64) value

were observed on florets treated with VH during 6 days of storage (Figure 4.6B and C; Table A19-A20). However, the florets color by a* and b* values had no significant difference between VH treatment and the control during storage. Hue angle ranged from green to yellow (180 to 90°) (McGuire, 1992), initial storage hue values were approximately 115 to 117° (green) in all florets samples and were constant during storage (Figure 4.6D; Table A21).

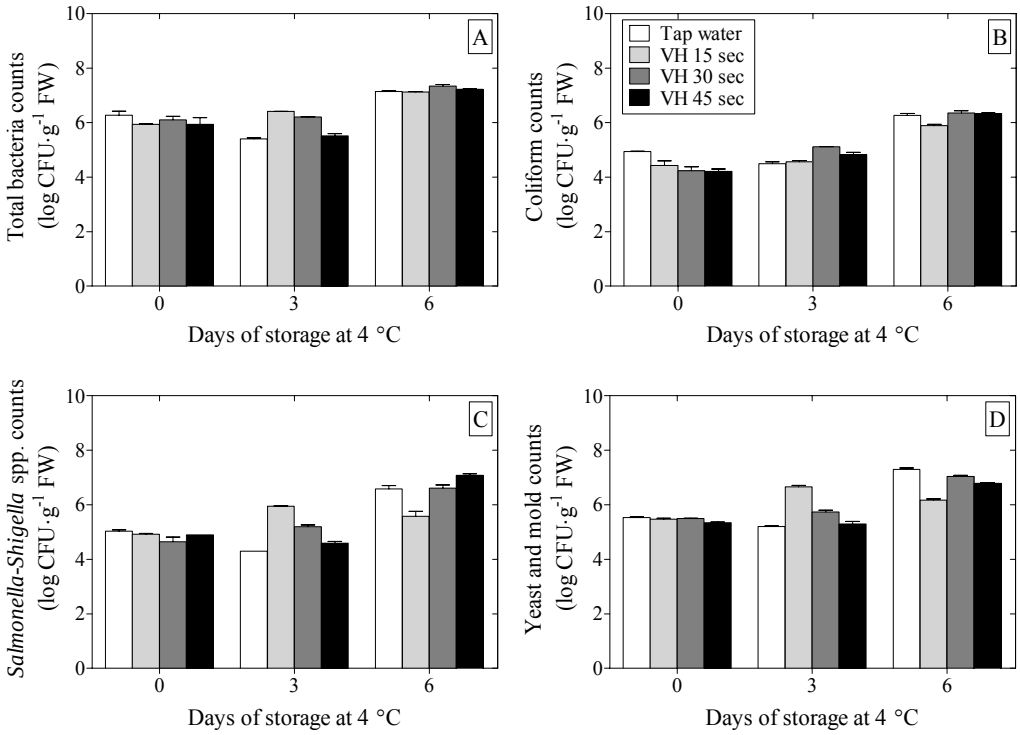

Figure 4.5 Changes in microbial populations of total bacteria (A), coliforms (B) *Salmonella-Shigella* spp. (C), and yeast and mold (D) counts of fresh-cut broccoli florets treated with tap water (control) and vapor heat (VH) treatment at 90 °C for 15, 30 and 45 sec. Florets were stored at 4 °C for 6 days. Vertical bars represent the mean ± standard error.

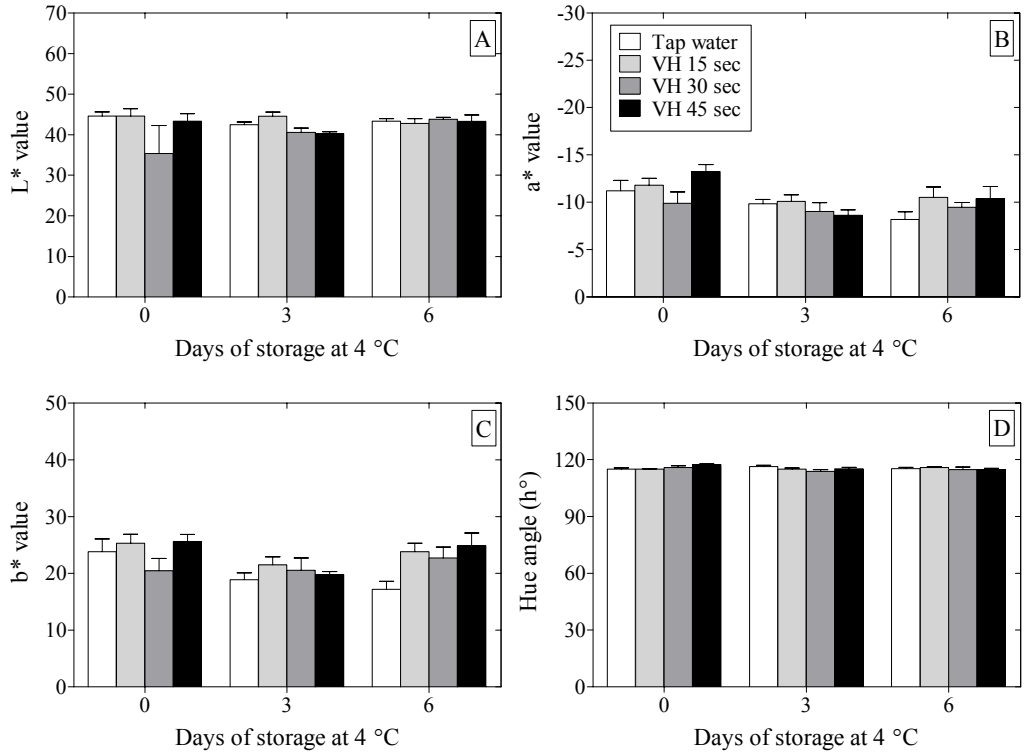

Figure 4.6 Changes in lightness (L*) (A), a* value (B), b* value (C) and hue angle (h°) (D) color of fresh-cut broccoli florets treated with tap water (control) and vapor heat (VH) treatment at 90 °C for 15, 30 and 45 sec. Florets were stored at 4 °C for 6 days. Vertical bars represent the mean ± standard error.

4.1.2.3 Effect of VH on O_2 and CO_2 Levels in the Package and Respiration Rate

In the packages of fresh-cut broccoli, a decrease in O_2 and an increase in CO_2 levels were observed in all treatments (Figure 4.7A and B; Table A22-A23). The lowest levels of O_2 (11.8 to 13.1% O_2) and the highest level of CO_2 (1.7 to 10.8% CO_2) were detected in the package of fresh-cut broccoli treated with VH for 15 sec. The gas composition inside the package of fresh-cut broccoli treated with tap water was observed to be about 18 to 19% O_2 and 1.7 to 7.5% CO_2, whereas the O_2 and CO_2 levels in the package of the VH treatments ranged from about 11.8 to 20.1% and 1.2 to 10.8%, respectively. After treatment, the respiration rate of all samples were increased and decreased after 3 days of storage (Figure 4.7C; Table A24). The respiration rate was significantly different depending on the exposure time of VH. The lowest level of respiration rate was detected in the samples treated with VH for 45 sec. The respiration rate of the control was 40.73 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$, whereas the VH treatment for 15, 30 and 45 sec showed lower respiration rates with 29.58, 27.32, 22.70 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$, respectively.

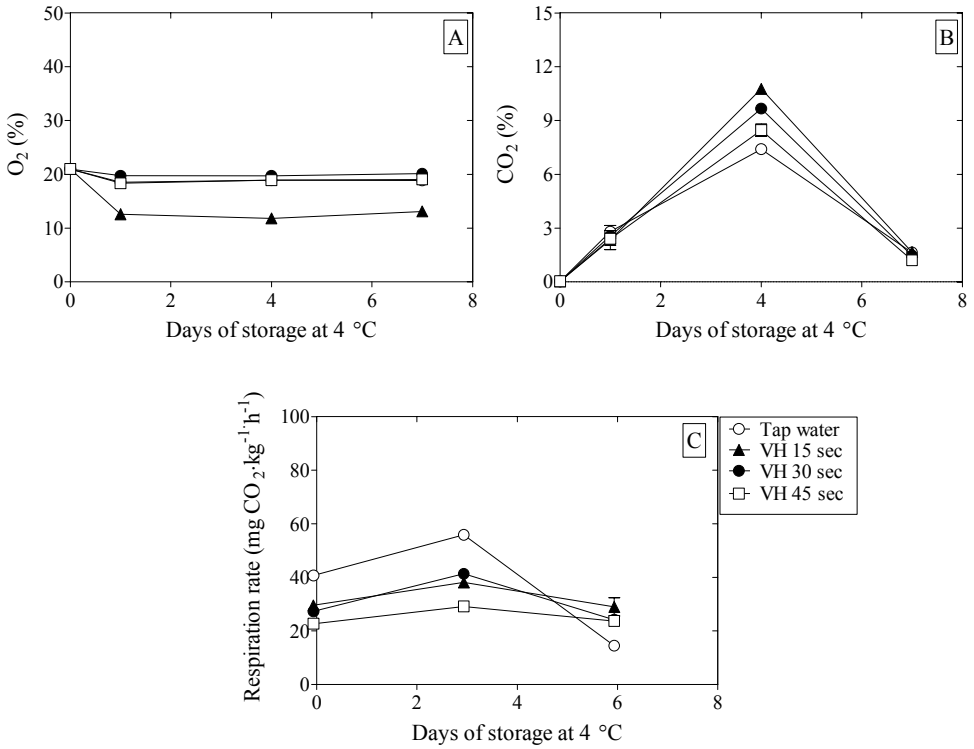

Figure 4.7 Changes in percentage of oxygen (O_2) (A), carbon dioxide (B) concentration in package, and respiration rate (mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) (C) of fresh-cut broccoli florets treated with tap water (control) and vapor heat treatment (VH) at 90 °C for 15, 30 and 45 sec. Florets were stored at 4 °C for 6 days. Vertical bars represent the mean ± standard error.

4.1.2.4 Effect of VH on Sensory Attributes

The sensory attributes including overall visual quality, visual color and odor/off-odors were evaluated during 6 days of storage at 4 °C (Figure 4.8; Table A25-27). The sensory evaluation of VH treated broccoli florets was compared with untreated as the control. The short exposure time of VH (90 °C) for 15 sec showed non-significant (P>0.05) differences in the scores of visual quality and visual color and odor when compared with the control during storage. The florets treated with VH for 30 and 45 sec showed low scores in all sensory attributes. In addition, VH treatment for 30 and 45 sec seemed to decrease florets quality, and caused injury tissue on the fresh-cut broccoli florets. Therefore, VH treatment for 30 and 45 sec are not recommended in fresh-cut broccoli florets due to color change and off-odor during storage at 4 °C.

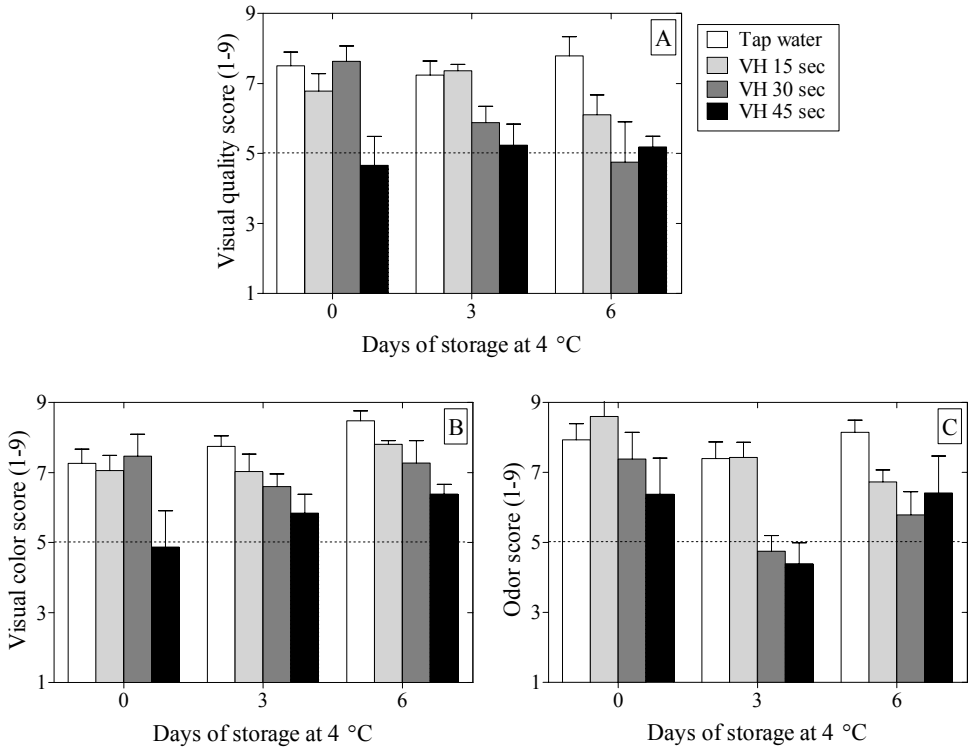

Figure 4.8 Changes in the sensory attributes: overall visual quality (A), visual color (B), and odor (C) score of fresh-cut broccoli florets treated with tap water (control) and vapor heat (VH) treatment at 90 °C for 15, 30 and 45 sec. Florets were stored at 4 °C for 6 days. Vertical bars represent the mean ± standard error. Overall visual quality score: 9 = fresh appearance, 1 = unusable. Color score: 9 = dark green, 1 = 100% yellow. Odor score: 9 = no off-odor, 1 = extreme off-odor. The horizontal line marks indicate the unacceptable quality limit for customers.

4.1.3 Ozonated Water

The use of ozonated water has been applied for reducing microbial populations and increasing safety during fresh-cut processing operations (Wang, et al., 2004; Beltrán, et al., 2005; Ölmez and Akbas, 2009). However, the optimum concentration and contact time of ozonated water for reducing of microbial loads is limited. Thus, the aim of our study was to investigate the optimum concentration and contact time of ozonated water for minimizing the microbial loads and its effects on the quality of fresh-cut broccoli florets during storage at 4 °C.

4.1.3.1 Effect of Ozonated Water on Microbial Population

The effect of ozonated water treatment on microbial loads of fresh-cut broccoli florets is shown in Figure 4.9 (Table A28-31). For initial day storage, ozonated water treatment for 15 min (1.50 ppm) gave the greatest reduction (P<0.05) of total bacteria, coliform, *Salmonella-Shigella* spp. and yeast and mold counts by 2.05, 1.21, 0.38 and 1.80 log CFU·g^{-1} FW, respectively, when compared to the control. The microbial counts of the control and all treated samples steadily increased during storage at 4 °C. After 6 days of storage, microbial population (total bacteria, coliform, *Salmonella-Shigella* spp. and yeasts and molds) were reduced by ozonated water less than 1.0 log CFU·g^{-1} FW when compared to the control. However, from this study I recommend that treating fresh-cut broccoli with ozonated water with increasing contact time and higher ozone concentration can be used to reduce microbial population on the produce before consumption.

4.1.3.2 Effect of Ozonated Water on Florets Color

The florets color (L*, a*, b* value and hue angle) of fresh-cut broccoli florets treated with ozonated water is shown in Figure 4.10. The L* (lightness) and a* (redness/greenness) value showed slight increase in all ozonated water samples, but they were significantly lower than the control sample during storage (Figure 4.10A-4.10B; Table A32-A33). Florets treated with ozonated water for 15 min (1.50 ppm) had significantly (P<0.05) lower L* and a* value (within the range of 38.64 to 39.68 and -12.01 to -12.87, respectively) when compared to the control during storage at 4 °C for 6 days. The lower L* and a* values of treated ozonated water for 15 min indicated maintaining green color of florets. The b* (yellowness/blueness) value on florets treated with ozonated water slightly decreased during storage. Moreover, the florets treated

with ozonated water for 15 min (1.50 ppm) had significantly (P<0.05) lower b* value, within the range of 21.75 to 22.60 (Figure 4.10C; Table A34). This indicated that the application of ozonated water treatment retarded the yellowing of the florets. The change in hue angle (green color) showed slight change (approximately 118 to 120°; still green florets) in all ozonated water samples when compared with the control (Figure 4.10D; Table A35).

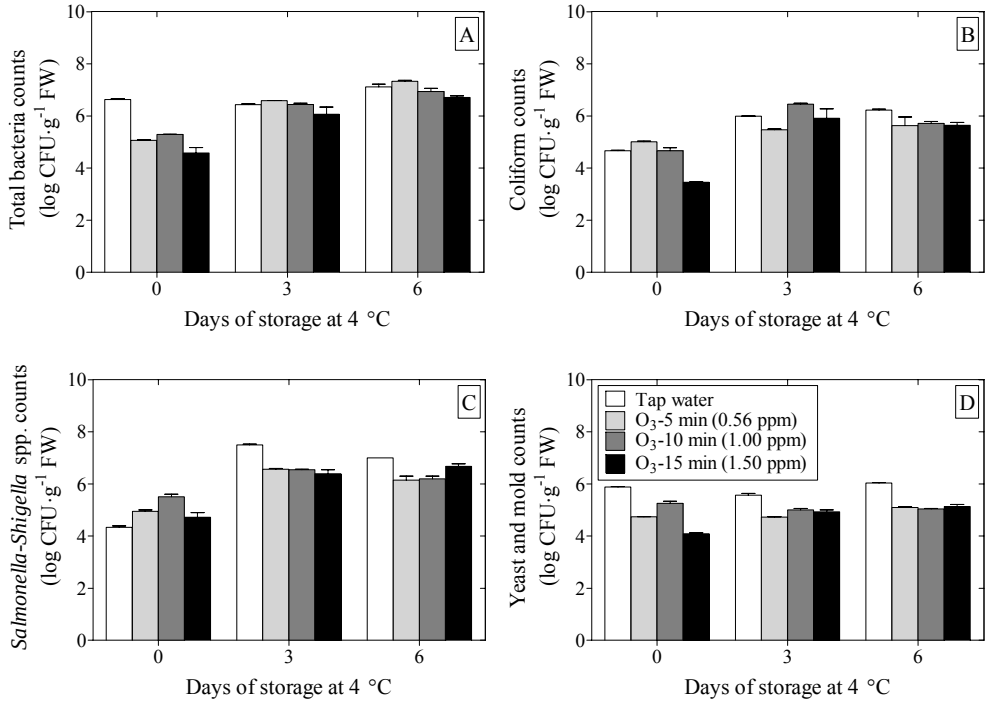

Figure 4.9 Changes in microbial populations of total bacteria (A), coliforms (B) *Salmonella-Shigella* spp. (C), and yeast and mold (D) counts of fresh-cut broccoli florets treated with tap water (control) and ozonated water (O_3) for 5 min (0.56 ppm), 10 min (1.00 ppm) and 15 min (1.50 ppm). Florets were stored at 4 °C for 6 days. Vertical bars represent the mean ± standard error.

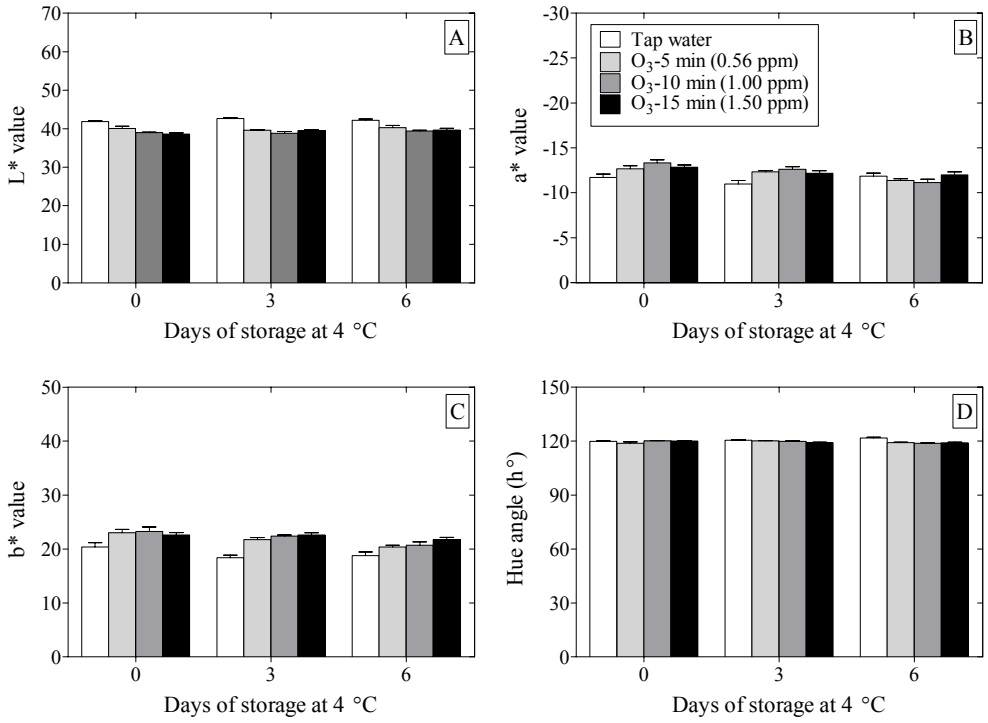

Figure 4.10 Changes in lightness (L*) (A), a* value (B), b* value (C) and hue angle (h°) (D) color of fresh-cut broccoli florets treated with tap water (control) and ozonated water (O_3) for 5 min (0.56 ppm), 10 min (1.00 ppm) and 15 min (1.50 ppm). Florets were stored at 4 °C for 6 days. Vertical bars represent the mean ± standard error.

4.1.3.3 Effect of Ozonated Water on Chlorophyll Changes

After the first day of storage, there was a slight decrease in chlorophyll contents (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid) in all samples treated with ozonated water and the control of fresh-cut broccoli (Figure 4.11; Table A36-A39). The sample treated with ozonated water slightly decreased when compared with the control sample, appearing as a loss in green color and related with L* value of the florets of broccoli (Figure 4.10A; Table A32). At the beginning of storage, total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents in untreated fresh-cut broccoli as the control were 62.73, 47.94, 14.79 and 0.026 mg $100 \cdot g^{-1}$ FW, respectively. At the end of storage, total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents slightly declined in the control florets to 56.43, 43.92, 12.50 and 0.021 mg $100 \cdot g^{-1}$ FW, respectively. The ozonated water treatment for 10 min (1.00 ppm of ozone dissolve) had the higher total chlorophyll (within the range of 57.39 to 49.32 mg $100 \cdot g^{-1}$ FW), chlorophyll *a* (within the range of 43.26 to 37.98 mg $100 \cdot g^{-1}$ FW), chlorophyll *b* (within the range of 14.13 to 11.34 mg $100 \cdot g^{-1}$ FW) and carotenoid (within the range of 0.018 to

0.011 mg 100·g^{-1} FW) contents. However, the ozonated water treated fresh-cut broccoli had significantly (P<0.05) lower chlorophyll (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid) contents than the control. Results showed that ozonated water (0.56, 1.00, and 1.50 ppm of ozone dissolve) may reduce total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents of fresh-cut broccoli florets during storage at 4 °C.

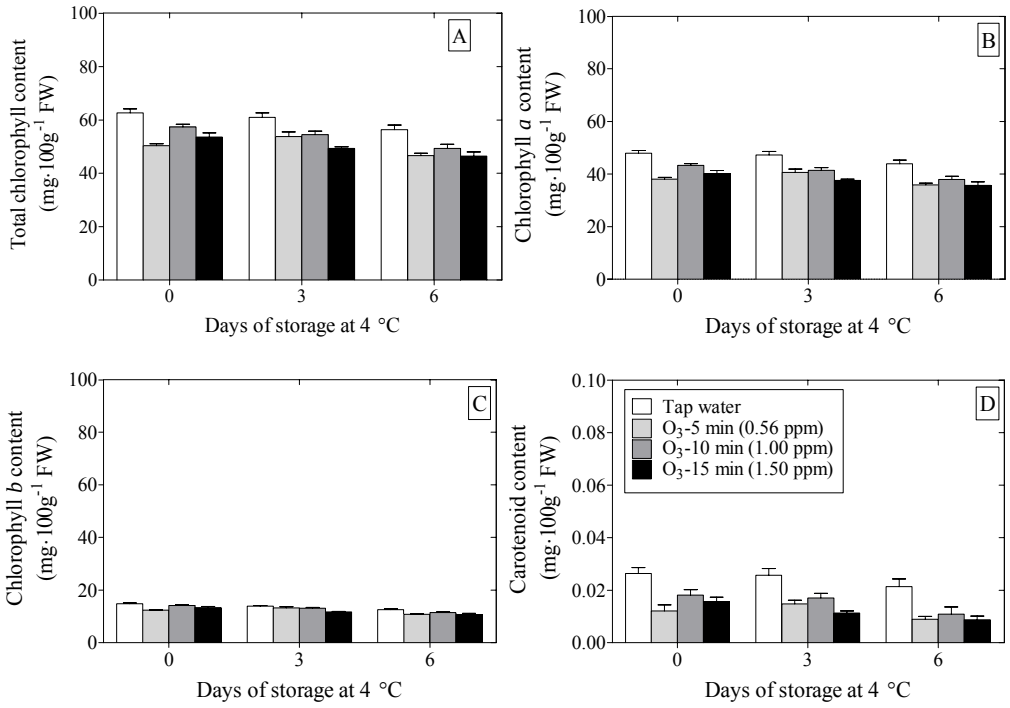

Figure 4.11 Changes in total chlorophyll (A), chlorophyll *a* (B), chlorophyll *b* (C) and carotenoid (D) content of fresh-cut broccoli florets treated with tap water (control) and ozonated water (O_3) for 5 min (0.56 ppm), 10 min (1.00 ppm) and 15 min (1.50 ppm). Florets were stored at 4 °C for 6 days. Vertical bars represent the mean ± standard error.

4.1.3.4 Effect of Ozonated Water on Sensory Quality Attributes

Mean scores for sensory attributes including overall visual quality, visual color and odor of fresh-cut broccoli florets after 6 days at 4 °C are shown in Figure 4.12 (Table A40-A42). The same trend was found for visual quality and color without significant differences among treatments during storage (Figure 4.12A-4.12B; Table A40-A41). The treated ozonated water samples showed similar scores; and were acceptable for consumption up to day 6 of storage. Similar to previous studies in fresh-cut lettuce and rocket leaves (Beltrán et al., 2005; Martínez-Sánchez et al., 2006), no significant (P>0.05) effect of the lower concentration of ozonated water was observed in terms of organoleptic changes. However, in this study an abnormal odor or/and off-odor was

detected in the fresh-cut broccoli florets treated with ozonated water at all concentrations (Figure 4.12C; Table A42). Therefore, the ozonated water treatments (0.56, 1.00, and 1.50 ppm of ozone dissolve) are not recommended in fresh-cut broccoli florets.

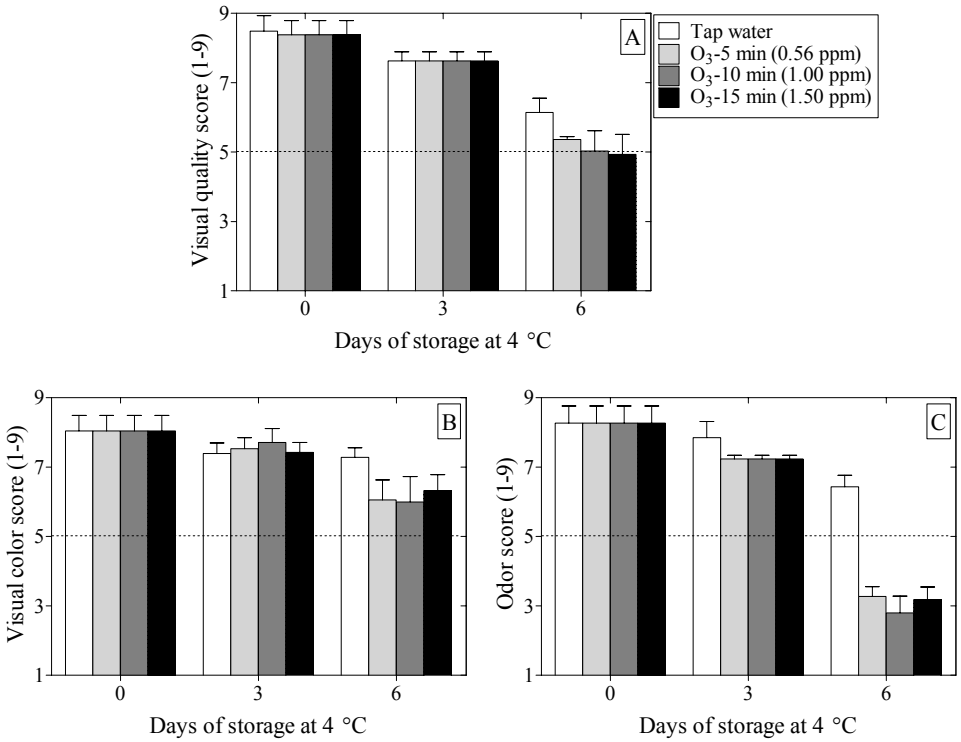

Figure 4.12 Changes in the sensory attributes: overall visual quality (A), visual color (B), and odor (C) score of fresh-cut broccoli florets treated with tap water (control) and ozonated water (O_3) for 5 min (0.56 ppm), 10 min (1.00 ppm) and 15 min (1.50 ppm). Florets were stored at 4 °C for 9 days. Vertical bars represent the mean ± standard error. Overall visual quality score: 9 = fresh appearance, 1 = unusable. Color score: 9 = dark green, 1 = 100% yellow. Odor score: 9 = no off-odor, 1 = extreme off-odor. The horizontal line marks indicate the unacceptable quality limit for customers.

4.1.4 Sodium Chlorite (SC) Solutions

Chlorinated water is usually applied to reduce microbial populations by approximately 1-2 log CFU·g^{-1} FW. However, it can be harmful due to the reaction of organic materials forming toxic chlorine by-products such as trihalomethanes and chloramines (Richardson et al., 2000). Sodium chlorite (SC) solution was described as a sanitizer and effective antimicrobial agent against pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* on fresh-cut carrots, spinach, fresh-cut lettuce and fresh-cut cilantro (Ruiz-Cruz et al., 2007; Lee and Baek, 2008; Allende et al., 2009). However, there are no reports on the effect of SC on fresh-cut broccoli during storage. Therefore, the objective of this study was to evaluate the effects of SC solutions (at 500, 750 and 1,000 ppm) on reducing microbial populations and maintaining quality of fresh-cut broccoli florets during storage for 12 days at low temperature.

4.1.4.1 Effect of SC on Microbial Population

The microbial populations (total bacteria, *Salmonella-Shigella* spp. and yeasts and molds) on the control sample increased with storage life and were generally higher than that of SC-treated samples (Figure 4.13; Table A43-A45). In this experiment, coliform was not detected less than 1.0 log CFU·g^{-1} FW (data not shown). The microbial counts of the control and all treated samples slightly increased during storage at 4 °C. In initial day storage, SC-treated samples at concentrations 500 ppm gave the greatest reduction (P<0.05) of total bacteria, *Salmonella-Shigella* spp. and yeast and mold counts by 0.76, 3.00 and 0.49 log CFU·g^{-1} FW, respectively when compared to the control. Antimicrobial action of SC treatment was very dramatic against *Salmonella-Shigella* spp. growth during the first three days of storage (Figure 4.13B; Table A44). At the end of storage, the microbial (total bacteria, *Salmonella-Shigella* spp. and yeasts and molds) populations were reduced by high concentration of SC-treated samples (750 and 1,000) by approximately 0.50, 1.40 and 0.90 log CFU·g^{-1} FW, respectively when compared to the control. This study recommended that treating fresh-cut broccoli with SC treatment can be used to reduce microbial population on the fresh-cut broccoli florets during 12 days of storage.

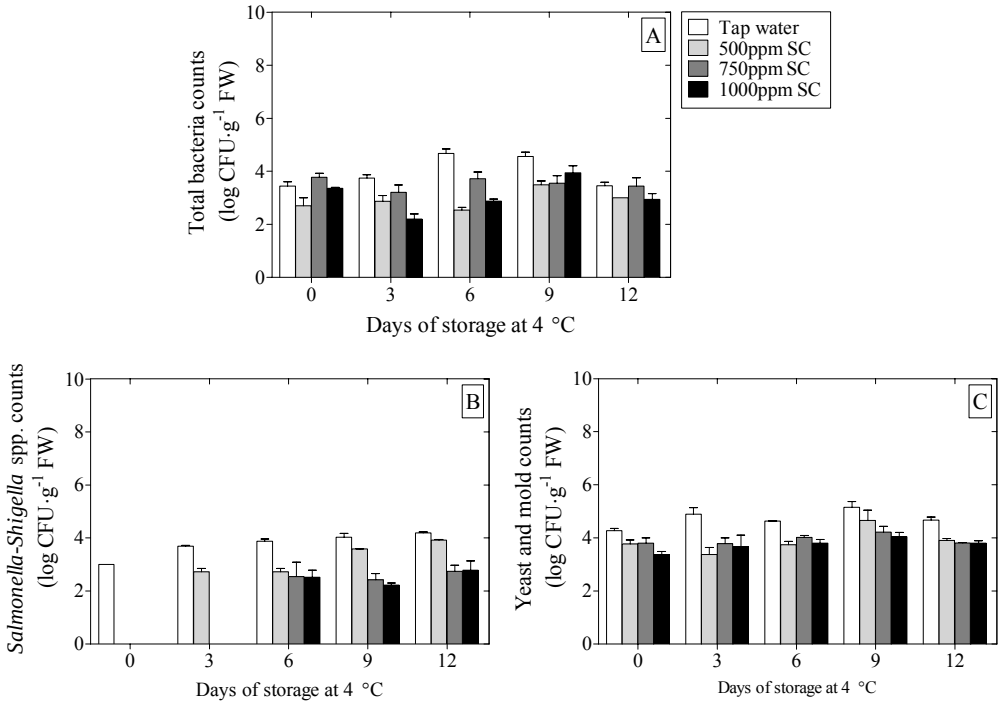

Figure 4.13 Changes in microbial populations of total bacteria (A), *Salmonella-Shigella* spp. (B), and yeast and mold (C) counts of fresh-cut broccoli florets with tap water (control) and 500, 750 and 1,000 ppm of sodium chlorite (SC) solutions. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.1.4.2 Effect of SC on Weight Loss, Respiration Rate and Florets Color

Weight loss of SC-treated and untreated (control) fresh-cut broccoli were not significantly different throughout storage (reaching a maximum 0.28%) (Figure 4.14A; Table A46). On the initially day of storage, the respiration rate of the control was 28.53 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$, whereas 500, 750 and 1,000 ppm of SC treatment showed respiration rates of 26.29, 38.50 and 34.10 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$, respectively (Figure 4.14B; Table A47). Respiration rate of all floret samples gradually increased with storage life, except for florets treated with 1,000 ppm SC, which exhibited a sharp increase after 9 days of storage (84.71 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) and then decreased on day 12 (42.75 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$). SC-treated florets had generally higher respiration rates than the control. The floret color (L*, a*, b* value and hue angle) of fresh-cut broccoli florets treated with SC solutions are shown in Figure 4.15. The L* (lightness), a* (redness/greenness), b* (yellowness/blueness) and hue angle value showed slightly changed in all concentrations of SC-treated samples; these were not significant differences (P>0.05) in the florets color from the control during storage (Figure 4.15; Table A48-A51). The change in hue angle (green color) slightly changed (approximately 120.7 to 121.3°;

green) in all SC-treated samples; these were not significant differences (P>0.05) in the florets color from the control (121.7°; green) at the end of storage (Figure 4.15D; Table A51). This indicated that the application of SC treatment did not have negative effects on florets color change (L*, a*, b* and hue angle).

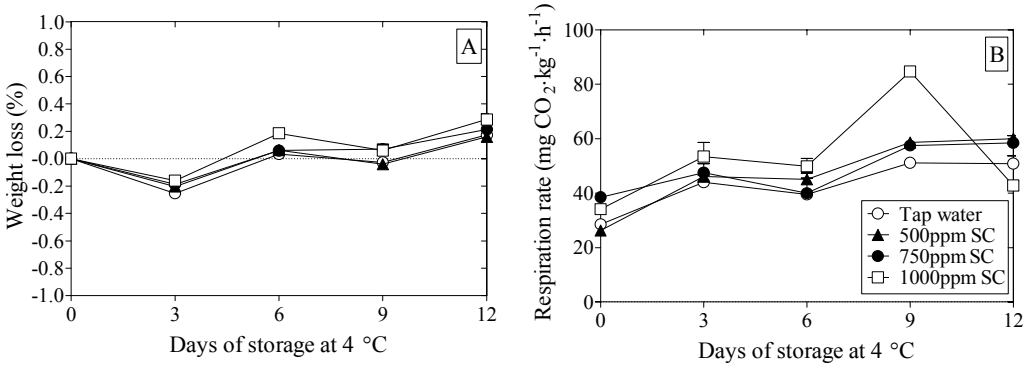

Figure 4.14 Changes in weight loss (A) and respiration rate (B) of fresh-cut broccoli florets treated with tap water (control) and 500, 750 and 1,000 ppm of sodium chlorite (SC) solutions. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

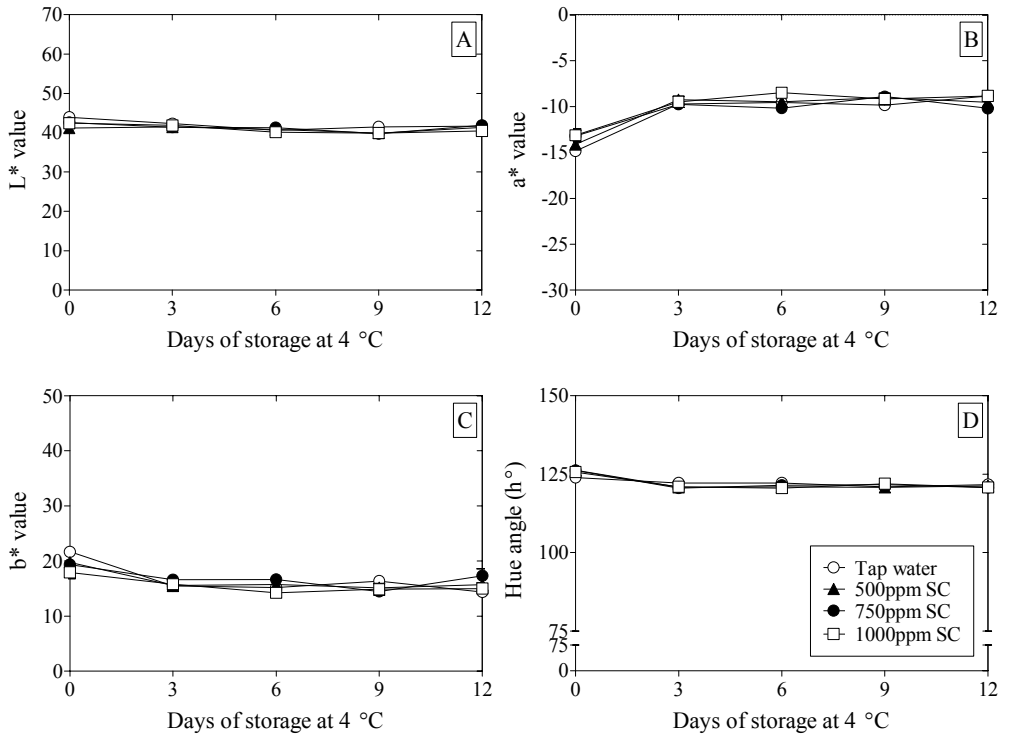

Figure 4.15 Changes in lightness (L*) (A), a* value (B), b* value (C) and hue angle (h°) (D) color of fresh-cut broccoli florets treated with tap water (control) and 500, 750 and 1,000 ppm of sodium chlorite (SC) solutions. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.1.4.3 Effect of SC on Changes of Chlorophyll and Carotenoid Content

Chlorophyll analysis supported the loss of green color. On the initially day of storage, there was slight decrease in chlorophyll contents (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid) of all SC-treated and the control of fresh-cut broccoli (Figure 4.16; Table A52-A55). The starting values of storage total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents in untreated fresh-cut broccoli as the control were 80.29, 62.23, 18.06 and 0.052 mg $100 \cdot g^{-1}$ FW, respectively. At the end of storage, total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents slightly declined in the control florets to 65.87, 50.57, 15.30 and 0.032 mg $100 \cdot g^{-1}$ FW, respectively. At the end of storage (day 12), the SC-treated with 500 and 750 ppm had higher total chlorophyll content (within the range of 65.87 and 67.78 mg $100 \cdot g^{-1}$ FW), chlorophyll *a* (within the range of 51.83 to 53.69 mg $100 \cdot g^{-1}$ FW), chlorophyll *b* (within the range of 15.96 to 16.94 mg $100 \cdot g^{-1}$ FW) and carotenoid (within the range of 0.037 to 0.038 mg $100 \cdot g^{-1}$ FW) contents. The SC-treated fresh-cut broccoli had significantly ($P<0.05$) higher chlorophyll (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid) contents than the control during storage. This indicated that the application of SC (500, 750 and 1,000 ppm) may maintain total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents of fresh-cut broccoli florets during storage at 4 °C.

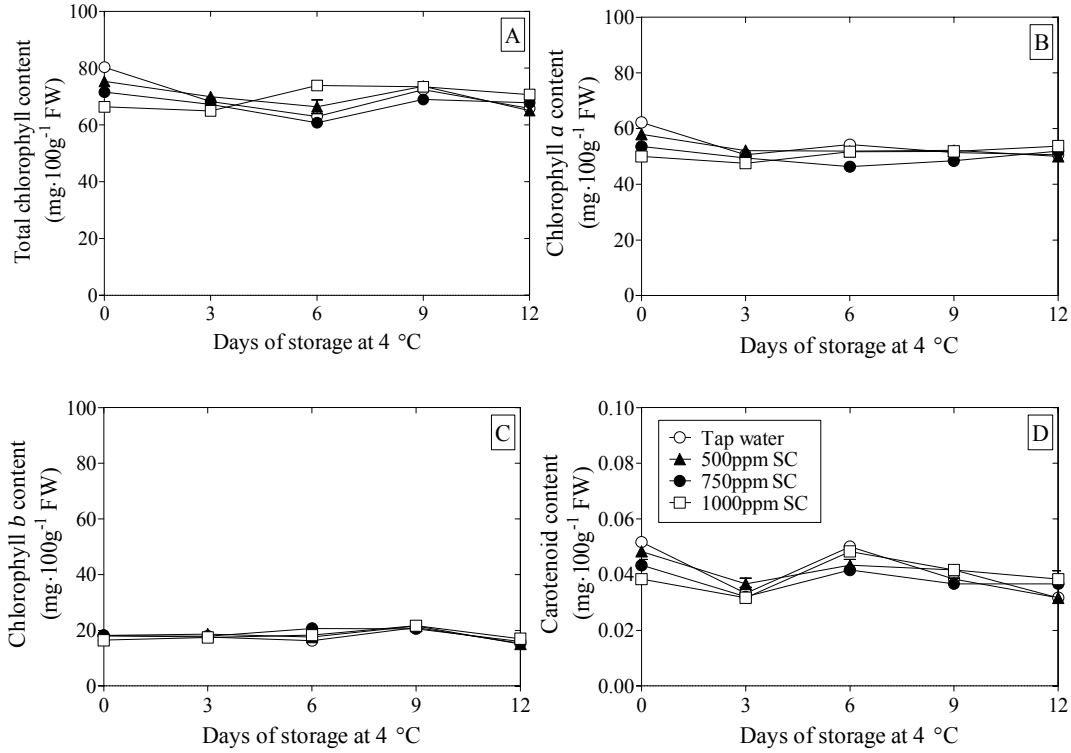

Figure 4.16 Changes in total chlorophyll (A), chlorophyll *a* (B), chlorophyll *b* (C) and carotenoid (D) content of fresh-cut broccoli florets treated with tap water (control) and 500, 750 and 1,000 ppm of sodium chlorite (SC) solutions. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.1.4.4 Effect of SC on Sensory Quality Attributes

Figure 4.17 (Table A56-A58) shows the effects of SC on the visual quality, sensory color and odor of broccoli florets. Overall visual quality changes during 12 days of storage were not significantly different among treatments (Figure 4.17A; Table A56). In general, visual quality scores slightly declined with storage life. Visual color (Figure 4.17B; Table A57) and odor (Figure 4.17C; Table A58) were similarly comparable among treatments, except on day 12 when florets treated with 1,000 ppm SC had the lowest scores for odor (6.29; slightly off-odor). Such reduced scores for odor were due to off-odor development as one appearance of tissue damage.

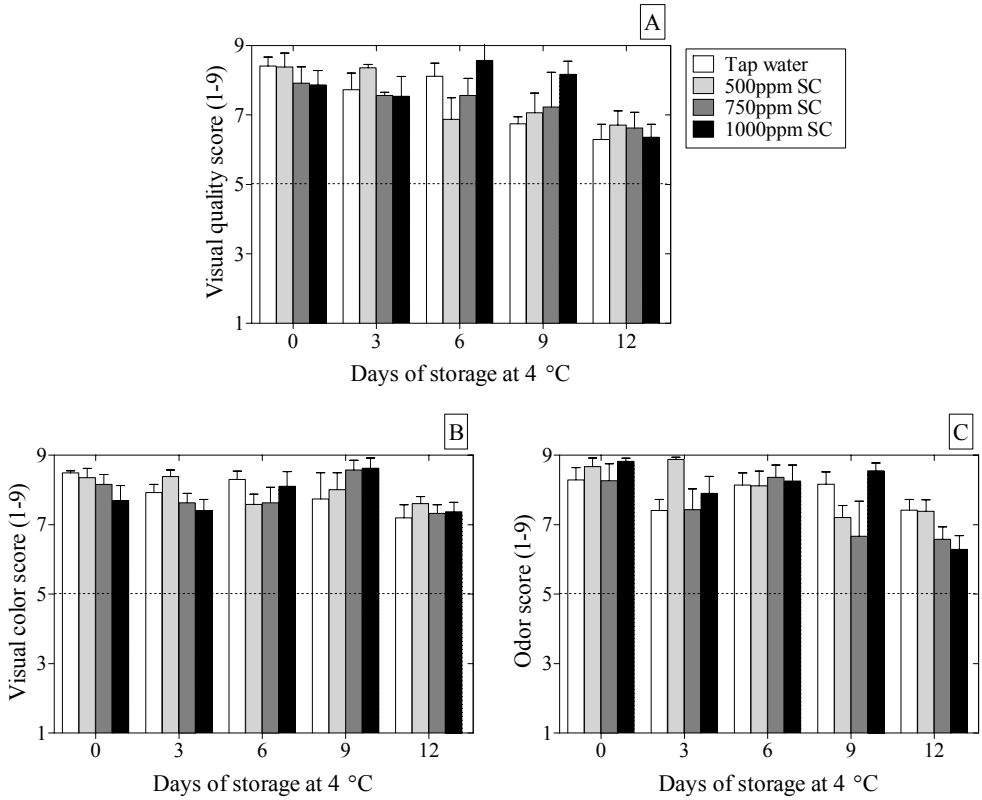

Figure 4.17 Changes in the sensory attributes: overall visual quality (A), visual color (B), and odor (C) score of fresh-cut broccoli florets treated with tap water (control) and 500, 750 and 1,000 ppm of sodium chlorite (SC) solutions. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error. Overall visual quality score: 9 = fresh appearance, 1 = unusable. Color score: 9 = dark green, 1 = 100% yellow. Odor score: 9 = no off-odor, 1 = extreme off-odor. The horizontal line marks indicate the unacceptable quality limit for customers.

4.2 Experiment II: The Effects of Hot Water Combined with Sodium Chlorite on Microbial Growth and Visual Quality in Fresh-Cut Broccoli Florets during Storage

Heat treatments are commonly applied to delay chlorophyll senescence and to maintain green color and antioxidant quality in vegetables. Treating broccoli heads in hot water (HW) at 50 °C for 2 min was the most effective treatment for reducing yellowing and decay without negative effect on off-odors or weight loss (Forney, 1995). HW treatment of 47 °C for 7.5 min was the optimal treatment for maintaining green floret color and preventing heat damage on fresh-cut broccoli (Tian, 1996). Funamoto et al. (2002) observed that treated broccoli heads with hot air at 50 °C for 2 h could reduce chlorophyll degradation due to the suppression of chlorophyll degrading enzyme activities with an inhibition of floret yellowing. Moreover, Munyaka et al. (2010) reported that blanching treatment in 90 °C for 4 min could have higher vitamin C retention in fresh-cut broccoli florets. Also, sodium chlorite ($NaOCl_2$; SC) is an oxidizing agent, which is effective controlling microbial growth and inhibiting the browning reaction on fresh-cut produce such as fresh-cut apple and shredded carrot (Lu et al., 2006, 2007; Cruz et al., 2006). According to FDA, the main application of SC is able to the generate chlorine dioxide gas for use as a powerful antimicrobial agent for disinfecting water and washing fruit, vegetables and poultry (FDA, 2010). The FDA allowed SC use on fresh and fresh-cut produce in the range of 500-1200 ppm for spraying or dipping (FDA, 2000). Moreover, SC does not form carcinogenic products when compared with chlorine (Ruiz-Cruz et al., 2006).

Referring to the results in experiment I of four sanitary treatments (hot water, vapor heat, ozonated water, and sodium chlorite solution), a maximum hot water treatment for fresh-cut broccoli florets at 50 °C for 3 min has the potential to maintain the physical quality of broccoli florets, but it was insufficient to control the growth of food-borne pathogens. Moreover, in experiment I, a maximum of SC treatment for fresh-cut broccoli florets at 500 ppm has the potential to reduce microbial populations and to maintain the quality of fresh-cut broccoli florets during storage at 4 °C.

However, in the experiment I did not study lower temperatures and shorter duration times of hot water treatment and did not study lower concentrations and shorter duration times of SC solutions. Therefore, the maximum HW treatment at 45 °C was applied for 1 min, the sample was cooled with tap water, then dipped in 100 or 300 ppm of SC solution for 1 min at ambient temperature (25±2 °C). This was studied in more detail in experiment II. The effects of these combined treatments were determined on microbial reduction, florets quality and antioxidant properties of fresh-cut broccoli florets during storage at 4 °C.

4.2.1 Effect of HW Combined with SC on Microbial Population

The efficacy of microbial disinfection by the HW dip at 45 °C for 1 min followed by immersing in SC solution at 100 or 300 ppm for 1 min was investigated on fresh-cut broccoli florets and compared with the florets pre-washed with cold tap water (control). The initial counts of total bacteria, coliforms, *Salmonella-Shigella* spp. and yeasts and molds in the tap water wash were 5.38, 4.84, 4.75 and 5.54 CFU·g^{-1} FW, respectively (Figure 4.18; Table A59-62). Both of the combined treatments (HW combined 100 or 300 ppm of SC) and tap water treatments were able to reduce the counts of the investigated microbial immediately after treatments throughout storage. The combined treatments had more effectiveness in reducing the microbial counts than using tap water. The counts of total bacteria, coliforms, *Salmonella-Shigella* spp. and yeasts and molds significantly decreased during storage with ranges of 0.13-0.69, 0.11-0.84 0-0.82, and 0.01-0.47 log CFU·g^{-1} FW, respectively when the fresh-cut broccoli were treated with HW combined with 300 ppm of SC compared with the control (tap water wash).

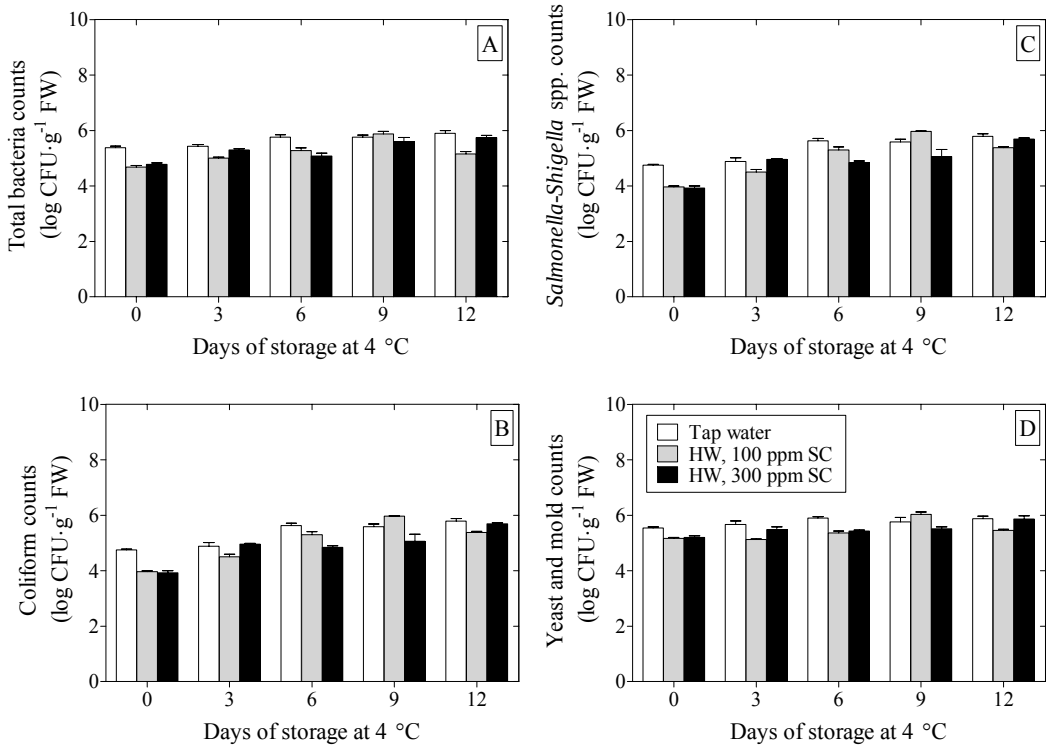

Figure 4.18 Changes in microbial populations of total bacteria (A), coliforms (B), *Salmonella-Shigella* spp. (C), and yeast and mold (D) counts of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 100 or 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.2.2 Effect of HW Combined with SC on Color of Florets

At the beginning of storage, L* values of the control and the HW combined with 100, and 300 ppm of SC samples were 41.34, 40.95 and 39.54, which slightly increased to 40.48, 40.89 and 40.52 at day 12 of storage, respectively (Figure 4.19A; Table A63).

Hue angle gave the best indicator of green floret color in broccoli (Forney, 1995). During 12 days of storage at 4 °C, both the control and HW+SC treated samples showed hue angle values of 120-122, which were not significantly different between all samples (Figure 4.19D; Table A66). Moreover, there were no significant differences between L*, a*, b* values and hue angle of the combined treatment and the control. These results indicate that fresh-cut broccoli treated with HW combined with SC sample showed no effect on green florets color change. Moreover, no visible symptoms of overcooking were found in the combination of the HW and SC treated sample.

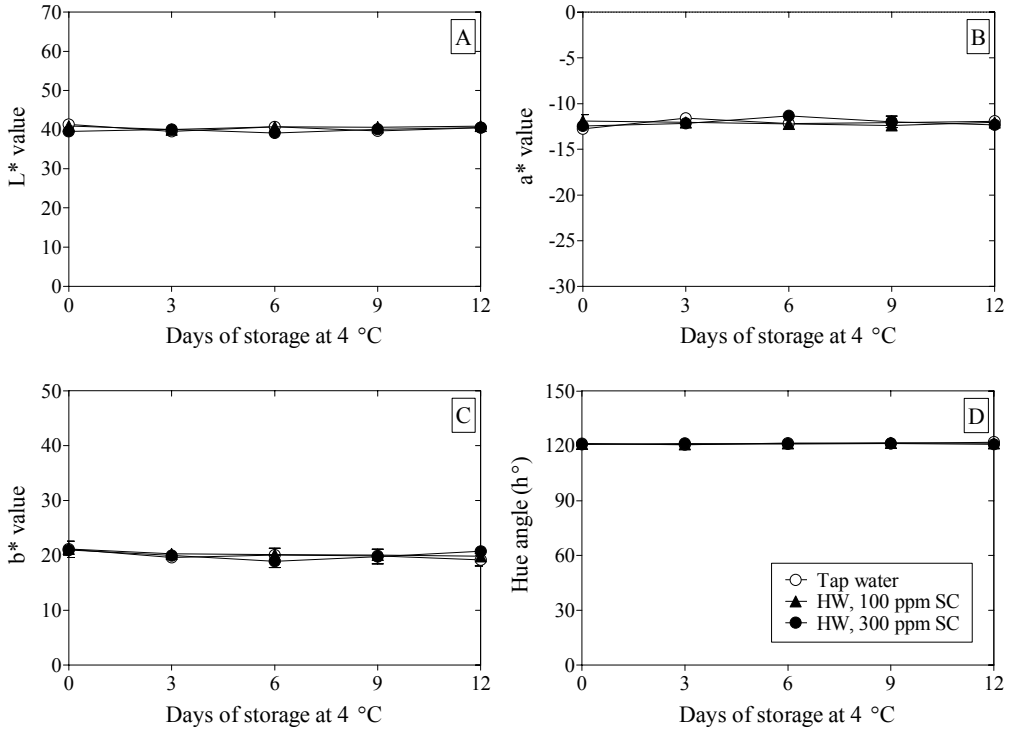

Figure 4.19 Changes in lightness (L*) (A), a* value (B), b* value (C) and hue angle (h°) (D) color of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 100 or 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.2.3 Effect of HW Combined with SC on Weight loss, Respiration Rate and O_2 and CO_2 Levels in Package

After treatment, all samples showed an increase of weight loss, reaching a maximum 0.2% after 12 days (Figure 4.20A; Table A67). HW combined with 100 ppm SC had slightly higher weight loss than the other treatments. In the packages of fresh-cut broccoli, the O_2 concentration slightly increased or maintained a steady level in all samples (Figure 4.20B; Table A68). The CO_2 concentration in all treatments increased up to 2 to 3 %CO_2 during the first 6 days and then decreased slightly; however, the differences were not significant (Figure 4.20C; Table A69). Damage or lesions suffered by plant tissues can induce an enhanced respiratory activity (Lemoine et al., 2009). Among the treatments, a slight decrease in respiration rate was detected during the first 3 days of storage followed by maintaining a steady level in all samples (Figure 4.20D; Table A70). Moreover, HW combined with 100 ppm of SC treated florets had generally slightly higher respiration rates than the control.

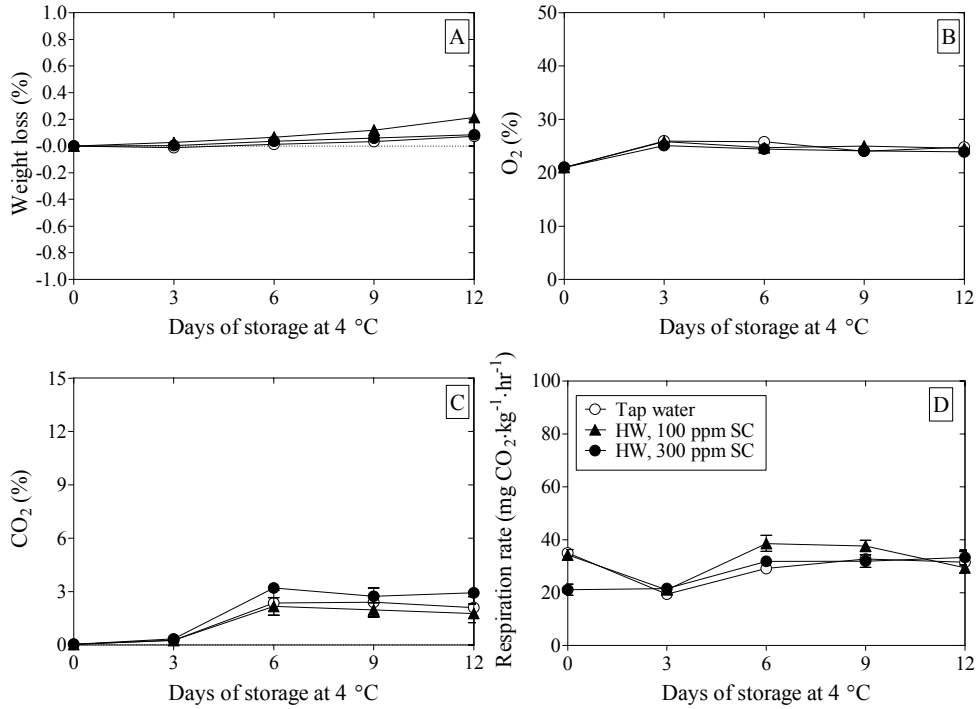

Figure 4.20 Changes in percentage of weight loss (A), oxygen (O_2) (B), carbon dioxide (CO_2) (C) and respiration rate (mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) (D) of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 100 or 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.2.4 Effect of HW Combined with SC on Chlorophyll Content

This study showed that chlorophyll contents (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid) of all fresh-cut broccoli florets samples slightly declined during storage at 4 °C (Figure 4.21; Table A71-A74). At the beginning of storage, total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents in untreated fresh-cut broccoli as the control were 71.65, 56.18, 15.47 and 0.038 mg $100 \cdot g^{-1}$ FW, respectively. At the end of storage, total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents slightly declined in the control florets. Also, at the initial day total chlorophyll content of HW combined with 100 and 300 ppm of SC treated samples was 76.21 and 71.78 mg $100 \cdot g^{-1}$ FW and slightly declined to be 65.65 and 66.01 mg $100 \cdot g^{-1}$ FW, respectively, at the end of storage period (decreased approximately 14 and 8%, respectively) (Figure 4.21A; Table A71). At the initial day, chlorophyll *a* content of HW combined with 100 and 300 ppm of SC treated samples was 59.43 and 55.83 mg $100 \cdot g^{-1}$ FW and slightly declined to 50.54 and 52.10 mg $100 \cdot g^{-1}$ FW, respectively, at the end of storage period (decreased approximately 15 and 7%, respectively) (Figure 4.21B;

Table A72). At the initial day, chlorophyll *b* content of HW combined with 100 and 300 ppm of SC treated samples was 16.78 and 15.96 mg $100 \cdot g^{-1}$ FW and slightly declined to 15.11 and 13.92 mg $100 \cdot g^{-1}$ FW, respectively at the end of storage period (decreased approximately 10 and 13%, respectively) (Figure 4.21C; Table A73). No significant differences in total carotenoid content were observed between the control and fresh-cut broccoli treated samples. In initial total carotenoid content of average 0.04 mg $100 \cdot g^{-1}$ FW, was slightly decreased or maintained a steady level in all samples (Figure 4.21D; Table A74). This indicated that the application of the combined treatment (HW combined with SC solution at 100 and 300 ppm) could maintain the chlorophyll (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid) contents of fresh-cut broccoli florets during storage at 4 °C.

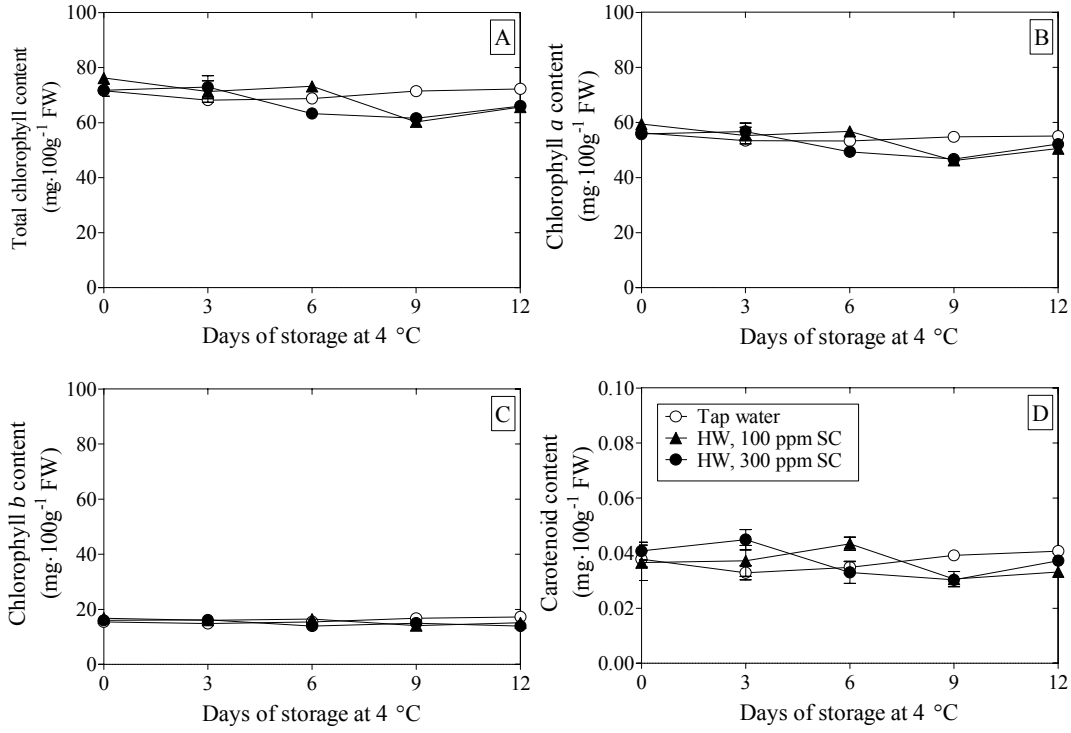

Figure 4.21 Changes in total chlorophyll (A), chlorophyll *a* (B), chlorophyll *b* (C) and carotenoid (D) content of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 100 or 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.2.4 Effect of HW Combined with SC on Sensory Quality Attributes

The sensory attributes including overall visual quality, visual color and odor/off-odors were evaluated during 12 days storage at 4 °C (Figure 4.22; Table A75-A77). In general, all sensory attributes scores slightly declined with storage period at 4 °C. While the HW combined with 300 ppm of SC treated broccoli florets showed good scores of all attributes, there was no significant difference (P>0.05) when compared with tap water sample. Therefore, the HW combined with 300 ppm of SC is recommended for fresh-cut broccoli floret during storage at 4 °C.

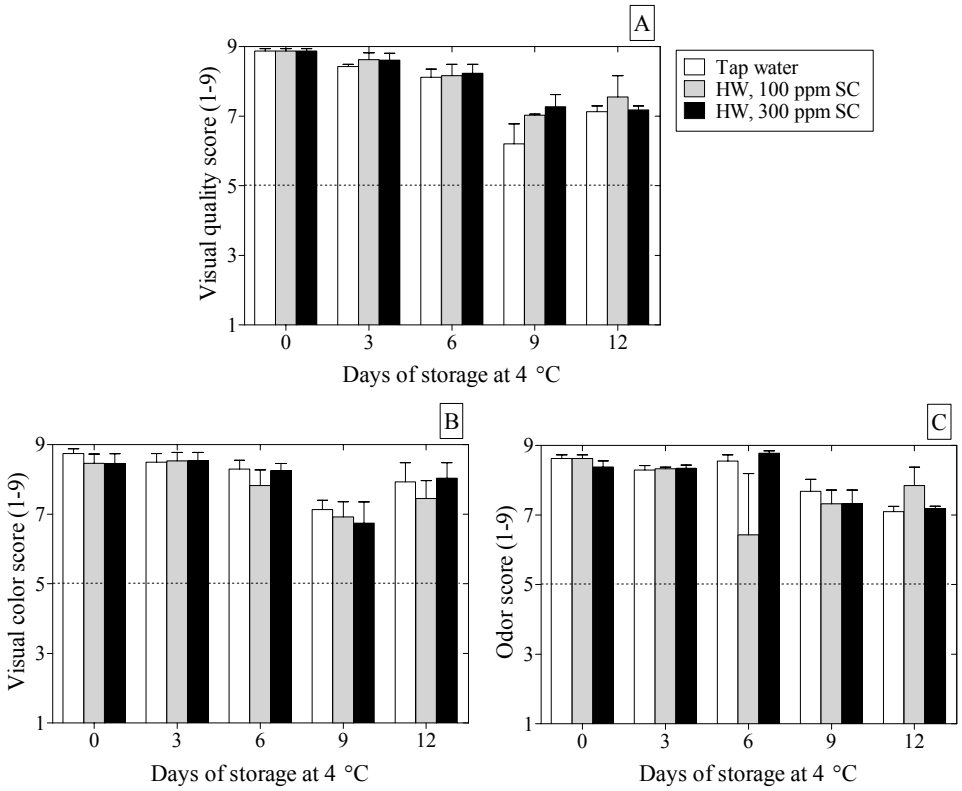

Figure 4.22 Changes in the sensory attributes: overall visual quality (A), color (B), and odor (C) score of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 100 or 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error. Overall visual quality score: 9 = fresh appearance, 1 = unusable. Color score: 9 = dark green, 1 = 100% yellow. Odor score: 9 = no off-odor, 1 = extreme off-odor. The horizontal line marks indicate the unacceptable quality limit for customers.

4.3 Experiment III: The Effects of Hot Water Combined with Sodium Chlorite on the Change of Bioactive Compounds in Fresh-Cut Broccoli Florets during Storage

There is limited information on the influence of HW treatment in maintaining antioxidant quality and on its combined effect with SC solution treatment in the inactivation microorganism of fresh-cut. Therefore, the main objective of this study was to investigate the influence of HW treatment combined with SC solution on the bioactive compounds of fresh-cut broccoli florets throughout storage at low temperature.

4.3.1 Effect of HW Combined with SC on Total Ascorbic Acid, Ascorbic Acid and Dehydroascorbic Acid Content

Broccoli florets are significant sources of dietary ascorbic acid. The main biologically active form is L-ascorbic acid (AsA), although its oxidation product L-dehydroascorbic acid (DHA) is also active. Total ascorbic acid (AsA+DHA), ascorbic acid (AsA) and dehydroascorbic acid (DHA) contents of all fresh-cut broccoli florets samples slightly declined during storage at 4 °C. The initial total ascorbic acid (AsA+DHA) content of fresh-cut broccoli florets slightly declined from 177.66 to 124.44 mg·100^{-1} FW in the control sample and 172.54 to 120.52 mg·$100g^{-1}$ FW in treated samples (Figure 4.23A; Table A78). The loss of total AsA+DHA content from the beginning to the last day of storage was approximately 29.96 and 30.15%, respectively. The initial AsA content of fresh-cut broccoli florets were slightly declined from 139.47 to 112.96 mg·100^{-1} FW in the control sample and 142.66 to 111.44 mg·$100g^{-1}$ FW in treated samples (Figure 4.23B; Table A79). The loss of AsA content from the beginning to the last day of storage was approximately 19.00% and 21.88%, respectively.

In broccoli heads, ascorbic acid content decreased during storage, but the drop was delayed by hot air treatment at 50 °C for 2 h (Shigenaga et al., 2005). Moreover, higher levels of ascorbic acid were found in strawberries treated in an air oven (45 °C for 3 h) (Vicente et al., 2006). In previous studies, it was shown that heat treatments can delay the loss of ascorbic acid content during postharvest of different products.

Dehydroascorbic acid (DHA) content remained almost constant during storage at 4 °C and showed the same pattern of total AsA and AsA. The initial DHA content of fresh-cut broccoli florets slightly declined from 37.06 to 14.57 mg·100^{-1} FW in the control sample and 26.66 to 8.38 mg·$100g^{-1}$ FW in treated samples (Figure 4.23B; Table A80). The loss of DHA content from the beginning to the last day of storage was approximately 60.70 and 68.57%, respectively. Treated samples had lower levels of DHA than the control during experiment but the differences were not significant (P>0.05). During storage, the DHA concentration can increase as a product of oxidation (Wills et al., 1984). The results showed that DHA content increased in the first day of postharvest senescence and then declined during storage. The combined treatment could maintain levels of total ascorbic acid, AsA and DHA of fresh-cut broccoli florets during storage at 4 °C for 12 days.

The loss of total AsA occurs primarily by chemical degradation, which involves oxidation of AsA to DHA, followed by hydrolysis to 2,3-diketogulonic acid and further polymerization to form other nutritionally inactive products (Gregory, 1996). Because heat is known to speed the oxidation process of AsA, thermal processing results in loss of total AsA content in fruits and vegetables (Gregory, 1996). This result suggested the HW at 45 °C combined with 300 ppm of SC treatment might retain or have less effect on the loss of total ascorbic acid, ASA and DHA.

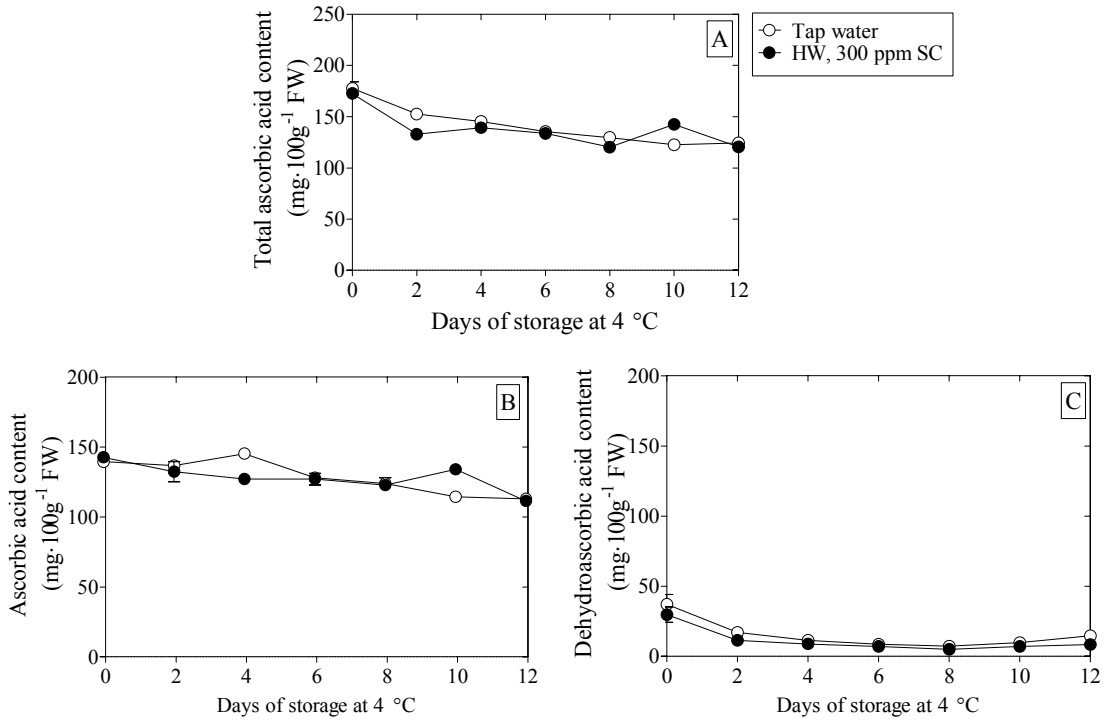

Figure 4.23 Changes in total ascorbic acid (A) and ascorbic acid (B) content of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.3.2 Effect of HW Combined with SC on Chlorophyll Content

During storage period at 4 °C, no significant difference was found in total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents of broccoli florets treated with HW combined with 300 ppm of SC samples when compared with tap water wash (control) (Figure 4.24; Table A81-A84). The chlorophyll (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid) contents slightly declined in both the control and treated sample, and there was no significant differences (P>0.05). At the beginning of storage, total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid contents in untreated fresh-cut broccoli as the control were 71.73, 55.32, 14.24 and 0.030 mg 100·g^{-1} FW, respectively. Total chlorophyll content of all samples within the range of 71.19 to 88.33 mg 100·g^{-1} FW slightly decreased or maintained a steady level in all samples during 12 days of storage (Figure 4.24A; Table A81). The same trend was found in chlorophyll *a*, chlorophyll *b* and carotenoid content without significant difference (P>0.05) among treatments during storage (Figure 4.24B-4.24D; Table A82-A84). Chlorophyll *a* content of all samples within the range of 53.65 to 66.00 mg 100·g^{-1} FW slightly decreased or

maintained a steady level in all samples during 12 days of storage. Chlorophyll *b* content of all samples within the range of 10.98 to 14.24 mg $100 \cdot g^{-1}$ FW slightly decreased or maintained a steady level in all samples during 12 days of storage. Total carotenoid content of an average 0.030 mg $100 \cdot g^{-1}$ FW slightly decreased or maintained a steady level in all samples.

This indicated that the application of combined treatment (HW combined with SC solution at 300 ppm) had no negative effect on the chlorophyll (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoid) contents of fresh-cut broccoli florets during 12 days of storage at 4 °C.

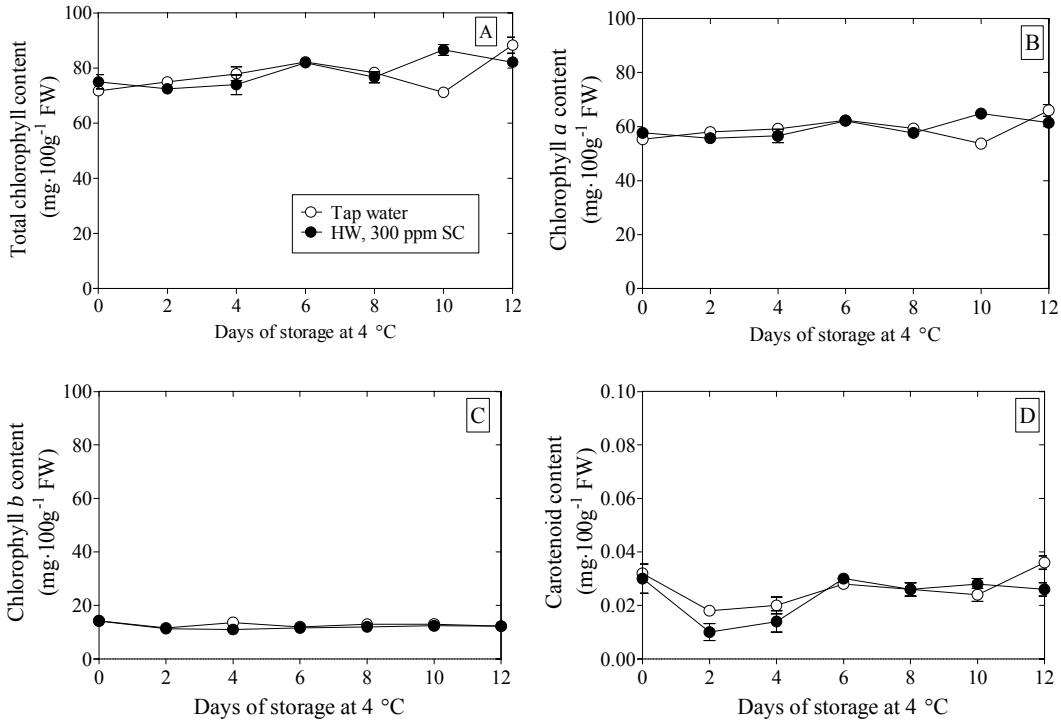

Figure 4.24 Changes in chlorophyll (A) and carotenoid (B) content of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.3.3 Effect of HW Combined with SC on Total Phenolic and Flavonoid Content

Total phenolic and flavonoid contents remained almost constant during storage at 4 °C. In this study, the total phenolic content of fresh-cut broccoli florets slightly declined from 149.14 to 143.34 mg GAE $100 \cdot g^{-1}$ FW in the control sample and 157.56 to 138.47 mg GAE $100 \cdot g^{-1}$ FW in treated samples (Figure 4.25A; Table A85). At the initial day after treatment, total phenolic content in treated samples increased by approximately 6% when compared with the control and maintained a higher level than the control until day 4. After that, levels were similar at day 6 and returned to a high level in treated samples at day 10. The initial flavonoid content of fresh-cut broccoli florets slightly declined from 15.53 to 13.66 mg QE $100 \cdot g^{-1}$ FW in the control sample and 16.12 to 11.23 mg QE $100 \cdot g^{-1}$ FW in treated samples (Figure 4.25B; Table A86). Although the HW combined with SC treated samples had slightly higher contents of total phenolic and flavonoid, there were no significant differences among all treatments. Therefore, there was no loss or gain in the content of both total phenolic and flavonoid in fresh-cut broccoli florets with HW at 45 °C combined with 300 ppm of SC treatment.

The results were no different in total phenolics and total flavonoids content in fresh-cut broccoli florets with HW at 45 °C combined with 300 ppm of SC treatment. Phenolic acids occur in plants as metabolic intermediates, and they also accumulate in the vacuoles (Chism and Haard, 1996). Thermal processing may release more bound phenolic acids from the breakdown of cellular constituents. Although the disruption of cell walls also releases the oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables (Chism and Haard, 1996), HW at 45 °C combined with 300 ppm of SC treatment deactivates these enzymes to avoid the loss of total phenolic and flavonoid contents.

4.3.4 Effect of HW Combined with SC on Total Glucosinolate Content

The total glucosinolate content was determined in broccoli florets over 12 days of storage. The change of the total glucosinolate content was not significant in both the control and combined treatment over the first day of storage, then a slight decrease of total glucosinolate content was observed in florets of the control and combined treatment (Figure 4.26; Table A87). In addition, the deceased rate in treated florets was significantly lower than in the control florets. The total glucosinolate content was reduced by 53.13% after 4 days of storage at 4 °C in the control florets and reduced by 51.61% in the florets treated with HW combined with SC solution. After 4 days of storage, the total glucosinolate in both the control and treated florets increased again thereafter in the range of 0.81 and 0.90 mg sinigrin $100 \cdot g^{-1}$ FW, respectively, at the end of storage at 4 °C. The total glucosinolate content was increased by 62.96 and 66.67% in the control and treated florets, respectively. Glucosinolates are among the most important health-promoting natural products in broccoli florets. They are known to contribute to the anticarcinogenic activity of broccoli. However, higher losses of glucosinolates have been reported after harvesting (Vallejo et al., 2002; Jia et al., 2009). Fresh-cut broccoli florets suffer a series of stresses after processing (washing/cutting) and during storage and are caused by a complex metabolism of glucosinolates. Cutting broccoli heads to fresh-cut broccoli florets brings myrosinase in contact with glucosinolates, which might lead to a high level of glucosinolate hydrolysis. On the other hand, it might also induce the biosynthesis of glucosinolates during HW combined with SC treatment and storage.

The present study showed that HW at 45 °C combined with 300 ppm of SC treatment leads to higher level of glucosinolate, in comparison with the control, during storage for 12 days at 4 °C. This result suggested that HW combined with SC treatment is also an effective method to maintain glucosinolate content.

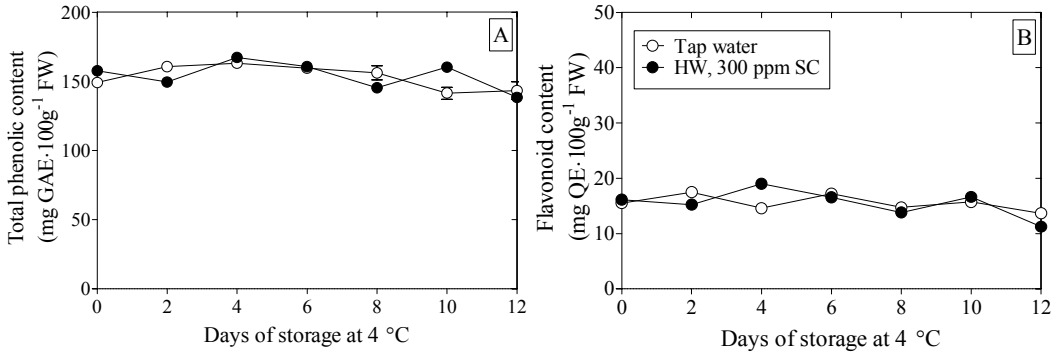

Figure 4.25 Changes in total phenolic (A) and flavonoid (B) contents of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

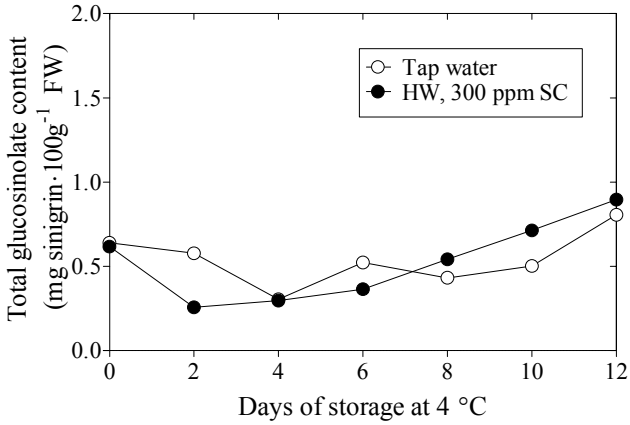

Figure 4.26 Changes in total glucosinolate content of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.3.5 Effect of HW Combined with SC on Antioxidant Capacity

Antioxidant capacity expressed as a percentage of DPPH inhibition was determined using the DPPH$^{\bullet}$. The combined treatment showed efficacy in maintaining the antioxidant capacity of fresh-cut broccoli after 2 days of combined treatment in comparison to the control. The average antioxidant capacity of treated broccoli ranged from 25.26 to 23.04 %DPPH inhibition, whereas the control had 27.86 to 20.71% DPPH inhibitions (Figure 4.27; Table A88). Then, antioxidant activity decreased in both the control and treated samples, but treated ones kept significantly (P<0.05) higher values (range from 8 to 23%) than the control during storage. Heat treatments can affect the antioxidant activity in fruits and vegetables during postharvest storage, including strawberry (Vicente et al., 2006). It was shown that heating conducts an increase in the antioxidant activity of fruits. The result indicated that HW combined with SC treatment has the potential to delay the decrease of antioxidant capacity in fresh-cut broccoli. According to the obtained results, a slight decline in the initial antioxidant activity was observed in both the control and treated samples at the end of storage. These findings indicate that HW at 45 °C combined with 300 ppm of SC treatment maintained the nutritional value of fresh-cut broccoli by maintaining the content of total antioxidant capacity.

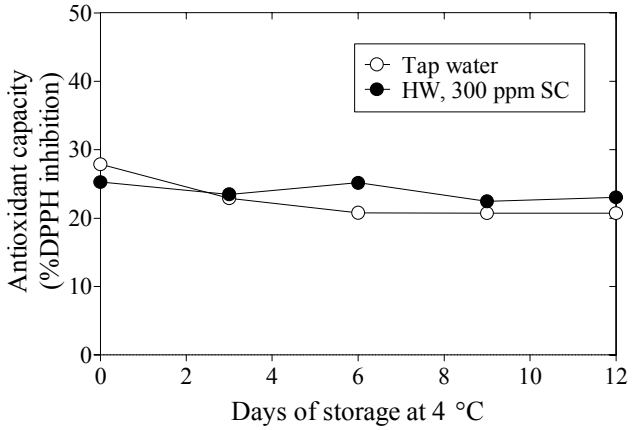

Figure 4.27 Changes in antioxidant capacity by %DPPH inhibition of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.4 Experiment IV: The Effects of Hot Water Combined with Sodium Chlorite on the Change of Antioxidant Activities in Fresh-Cut Broccoli Florets during Storage

4.4.1 Effect of HW Combined with SC on Chlorophyllase Activity

The chlorophyllase (Chlase) activity in the control sample was slightly higher than in treated sample. In the initially study, Chlase activities in the control and treated samples were approximately 1.05 and 0.73 units·mg^{-1} protein, respectively (Figure 4.28; Table A89). Chlase activities of both the control and treated samples slightly decreased from the initial day until day 4, and it increased again thereafter to the initial level at the end of the storage at 4 °C. Broccoli florets washed with tap water (control) showed higher activity of Chlase compared to HW combined with 300 ppm SC, which increased after day 6 to a level higher than the control. This indicated that HW combined with 300 ppm SC suppressed the activity of Chlase and also delayed the increasing of activity.

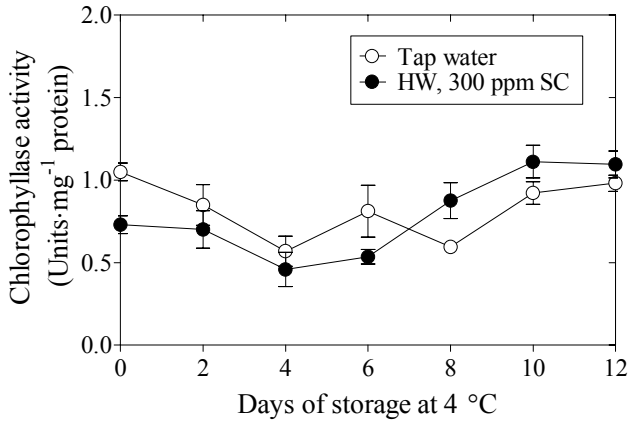

Figure 4.28 Changes in chlorophyllase (Chlase) activity of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.

4.4.2 Effect of HW Combined with SC on Catalase Activity

Levels of H_2O_2 must be controlled to avoid toxicity. Catalase (CAT) and peroxidase (POD) are the primary H_2O_2-scavenging enzymes in plant cells. In this study, CAT activity increased in both the control and treated samples during period storage at 4 °C. In treated samples, CAT activity increased and reached values similar to the control. CAT activities of fresh-cut broccoli florets increased from 0.54 to 3.11 units·mg^{-1} protein in the control florets and 0.52 to 3.59 units·mg^{-1} protein in treated samples (Figure 4.29A; Table A90). This study showed no significant (P>0.05) differences of CAT activity immediately after treatment between the control and treated samples. However, after 4 and 8 days of storage a significant (P<0.05) increase in the activity of the control was observed. The increasing of CAT activity in the control broccoli suggests a high accumulation of H_2O_2, which probably leads to an increase in catalase synthesis necessary to eliminate excess H_2O_2. Instead, in treated samples, the increasing in CAT activity was detected after 10 days of storage, indicating a delay in the accumulation of H_2O_2, which in turn suggests lower tissue damage due mainly to the delay in the senescence process.

4.4.3 Effect of HW Combined with SC on Peroxidase Activity

In this study, POD activities of both the control and treated samples increased thereafter to initial levels at the end of the storage at 4 °C. In treated samples, POD activity increased and reached values similar to the control. POD activities of fresh-cut broccoli florets increased from 3.64 to 10.16 units·mg^{-1} protein in the control florets and 2.90 to 17.07 units·mg^{-1} protein in treated samples (Figure 4.29B; Table A91). Broccoli florets washed with tap water (control) showed higher activity of Chlase compared with HW combined with 300 ppm SC and increased after day 6 to a level higher than the control. This indicated that HW combined with 300 ppm SC suppressed the activity of Chlase and also delayed the increasing of activity. Thus, POD was the efficient scavenging enzyme in decreasing the cellular levels of H_2O_2 in plant cell. Moreover, the increase of POD activity can contribute to the resistance enhancement since POD participates in many other cell processes involved in the plant defense reaction. The roles that POD can play in cell wall toughening and in the production of secondary metabolites and its simultaneous oxidant and antioxidant capabilities make it an important factor in the integrated defense response of plants (Li and Yi, 2012).

4.4.4 Effect of HW Combined with SC on Superoxide Dismutase Activity

Superoxide dismutase (SOD) catalyzes the dismutation reaction of $O_2^{-\bullet}$ into H_2O_2 and O_2, being the first line of defense from damage caused by oxygen radicals. At the initial day after treatment, SOD activities of fresh-cut broccoli florets in the control and treated sample were 12.53 and 13.98 units·mg^{-1} protein, respectively (Figure 4.29C; Table A92). After 6 days of storage, SOD activity slightly increased in both of the control and treated samples (within the range of 18.38 and 15.65 units·mg^{-1} protein) and maintained higher levels until day 8. At the end of storage, SOD activity decreased in both treatments and decreased again to initial level at the end of the storage at 4 °C. Higher SOD activities can increase tolerance to different stress factors. It was detected that plants with an increased tolerance to heat stress have a raise of SOD activity (Almeselmani et al., 2006). In broccoli, correlation between antioxidant enzymes and yellowing was observed. Broccoli maintained its green floret color during the longer postharvest storage and showed higher SOD activity (Toivonen and Sweeney, 1997). This study indicated that increased SOD activity in treated samples could support to delay the accumulation of superoxide radicals during storage.

4.4.5 Effect of HW Combined with SC on Ascorbate Peroxidase Activity

An outstanding enzyme consuming ascorbate is ascorbate peroxidase (APX), which catalyzes the reduction of hydrogen peroxide (H_2O_2) to water with the simultaneous oxidation of ascorbate. APX activity slightly increased in both the control and treated samples and maintained higher levels during 6 days of storage at 4 °C (Figure 4.29D; Table A93). At the initial day of storage, the average APX activities of the control and treated samples were 2.80 and 2.11 units·mg^{-1} protein, respectively. After 6 days of storage, both the control and treated samples declined from 3.30 to 2.61 and 3.87 to 2.47 units·mg^{-1} protein, respectively. In this study, treated samples showed a significantly (P<0.05) higher APX activity of about 46% after 2 days of combined treatment. As it was mentioned, a higher APX activity could support to protect against reactive oxygen species (ROS) generated under stress situations. Treated samples could maintain lower levels of H_2O_2 and thus contribute to tissue integrity in stages of senescence. Also, an increase in APX activity was detected in wheat (Almeselmani et al., 2006), strawberries (Vicente et al., 2006), and broccoli (Shigenaga et al., 2005) subjected to postharvest heat treatments.

4.4.6 Effect of HW Combined with SC on Hydrogen Peroxide Content

In the present study, increases in hydrogen peroxide (H_2O_2) content were observed during period storage at 4 °C. Significant differences in the H_2O_2 accumulation were found for the different treatment (Figure 4.33; Table A94). H_2O_2 content of the control slightly increased from the initial day until day 8 (within the range of 23.52 to 26.51 mg H_2O_2 100·g^{-1} FW) and it sharply increased to 40.05 mg H_2O_2 100·g^{-1} FW at the end of storage. Also, H_2O_2 content of the treated florets sharply increased from the initial day until day 4 (within the range of 11.40 to 38.59 mg H_2O_2 100·g^{-1} FW) and it remained almost constant throughout of day 10 of storage (within the range of 33.80 to 40.51 mg H_2O_2 100·g^{-1} FW). After that it gradually decreased to 30.94 mg H_2O_2 100·g^{-1} FW until the end of storage. This result indicated that the increase in H_2O_2 could explain the lack of visible damage during the 12 days of storage.

4.4.7 Effect of HW Combined with SC on Glutathione Reductase Activity

GR activities of both the control and treated samples of fresh-cut broccoli florets showed the same trend during storage at 4 °C. However, the GR activities in treated samples were higher than those in the control (Figure 4.29F; Table A95). There was a significant difference (P<0.05) in the GR activities between the control and treated samples at the end of storage (12 days). From the initial day until day 8, the GR activities of all samples remained nearly constant at 0.027 to 0.036 units·mg^{-1} protein in the control, and 0.027 to 0.042 units·mg^{-1} protein in treated samples. After that, GR activity of the control and treated samples sharply increased to 0.053 and 0.070 units·mg^{-1} protein, respectively, and then decreased slightly at the end of the storage at 4 °C (Figure 4.29F; Table A95). Moreover, HW combined with SC treated florets had generally slightly higher GR activity than the control. Heat treatment might suppress senescence in broccoli florets because it might enhance the action of the ascorbate-glutathione cycle. HW combined with SC treatment maintains the GR activity for the first 8 days.

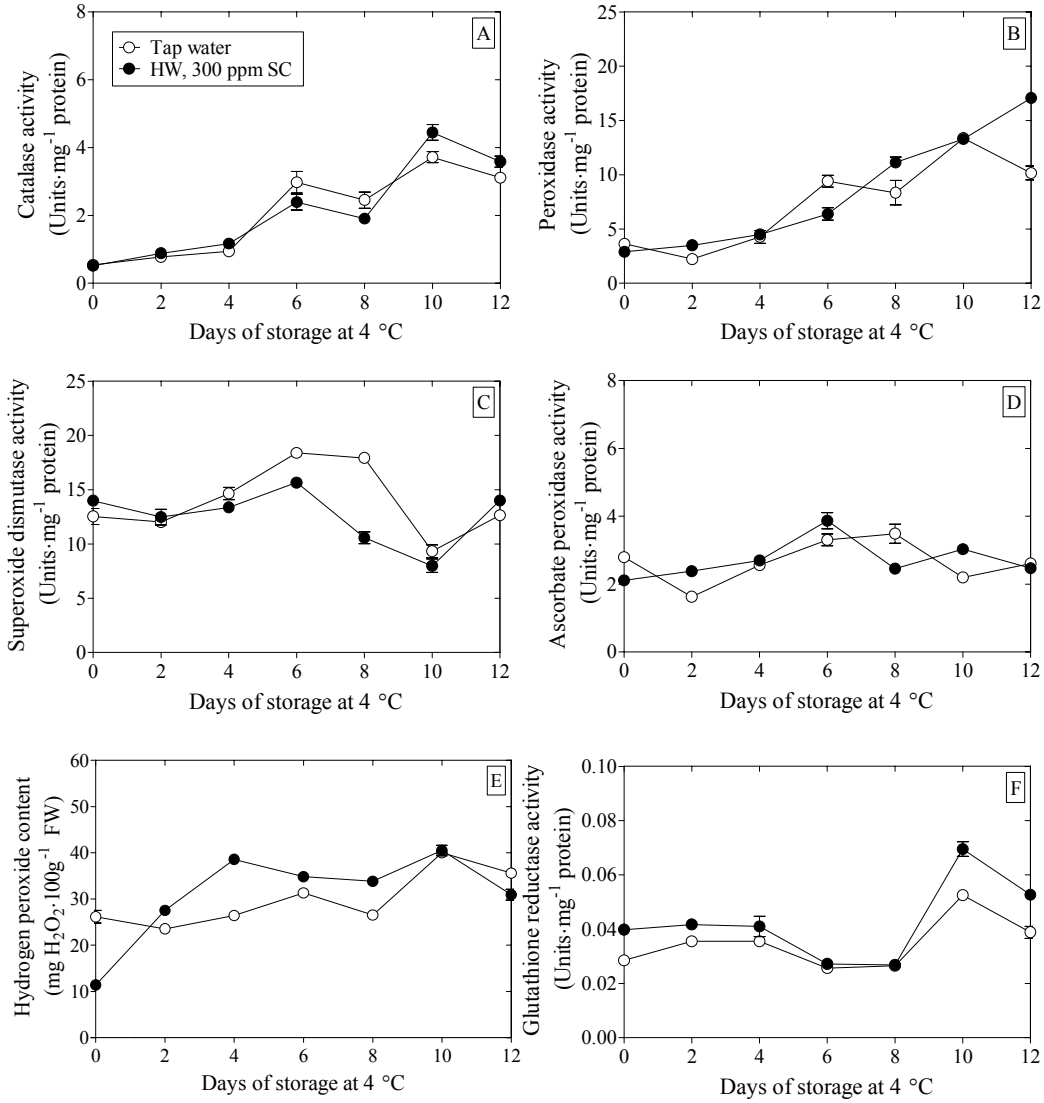

Figure 4.29 Changes in catalase (CAT) activity (A), peroxidase (POD) activity (B), superoxide dismutase (SOD) activity (C), ascorbate peroxidase (APX) activity (D), hydrogen peroxide (H_2O_2) content (E), and glutathione reductase (GR) (F) activity of fresh-cut broccoli florets with tap water (control) and treated with hot water (HW) at 45 °C for 1 min and 300 ppm of sodium chlorite (SC) solutions for 1 min. Florets were stored at 4 °C for 12 days. Vertical bars represent the mean ± standard error.