CHAPTER 5 DISCUSSION

The application of different sanitizers is necessary for research and processing industries to maintain microbial quality and safety of fresh-cut produce during storage (prior to reaching the consumers). Broccoli is a perishable commodity with a shorter postharvest shelf-life, and it needs better and safer postharvest quality maintenance (Beltrán et al., 2005). The enhancement of shelf life of fresh-cut produce is very important as even a few days extension of shelf life could represent a significant economic advantage for food companies (Sipahi et al., 2013).

5.1 HW Treatments

The application of a thermal treatment including hot water and vapor heat treatments in suitable conditions reduced microbial populations and moreover, those samples could maintain quality during storage when compared to non-thermally treated samples. Similar results have been reported by Cantwell et al. (2001) and Koukounaras et al. (2009), who applied thermal treatments to fresh-cut green onions and rocket leaves, respectively. For broccoli in particularly, Lemoine et al. (2009) and (2010) reported an effective delay in yellowing and maintained chlorophyll content approximately 40% higher than the control when a hot air postharvest treatment was applied.

The HW treatments at 55 and 60 °C completely inhibited both food-borne pathogens (coliform and *Salmonella-Shigella* spp.) on day 1 of storage, while the HW treatment at 50 °C for 3 min reduced both food-borne pathogens by 1.0-1.2 log $CFU \cdot g^{-1}$ FW. A similarly result was found by Li et al. (2001), who reported that warm water (50 °C), with or without 20 $mg \cdot L^{-1}$ chlorine significantly reduced the initial population of mesophilic aerobic microflora in fresh-cut iceberg lettuce by 1.73-1.96 log $CFU \cdot g^{-1}$ FW. The population counts of both pathogens gradually increased throughout the storage time at 4 °C. These results suggest that both pathogens can survive and multiply at low temperature; similar results were found by Li et al. (2002) and Moreira et al. (2008). This study indicated that the HW treatments were more effective in controlling the growth of coliform compared with *Salmonella-Shigella* spp.

The L* value and hue angle of broccoli florets treated at 50 °C were similar to the control (dark green color appearance). However, physiological disorders, including tissue softening and scald-like symptoms occurred in broccoli florets treated with HW at 55 and 60 °C due to improper temperature and dipping time. HW treatments at 55 and 60 °C seemed to decrease the florets quality due to these temperatures causing tissue softening and scald-like symptoms on the broccoli florets. The results indicate that HW at 50 °C was the best treatment to maintain the green color of broccoli. Several reports had also shown that heat treatments such as HW or hot air delay the yellowing process and senescence of broccoli florets (Tian et al., 1996; Terai et al., 1999; Funamoto et al., 2002). Green color is important for the quality and shelf-life of broccoli florets. Green color is indicated by a* value.

Gas compositions inside the package were significantly different (P<0.05) depending on the degree Celsius of water temperature. The lowest levels of O_2 and the highest level of CO_2 were detected in the package of fresh-cut broccoli treated HW at 50 °C for 3 min. Thus, the increase of CO_2 and the decrease of O_2 gas levels in the package were the result of a higher respiration rate of HW treated broccoli florets. The development of the gas levels is mostly based on film permeability, surface area, storage temperature, cultivar, wounding, and respiration activity of the produce (Baur et al., 2004; Serrano et al., 2006). In this study, off-odor developing in the package of fresh-cut broccoli florets treated HW in high temperatures was also detected. In general, the off-odor is usually associated with the lead of aerobic respiration under reduction in O_2 and rise in CO_2 levels and the formation of ethanol and acetaldehyde; thus the occurrence of off-odor during storage period is indicated by the presence of living cells or microbial and microbial respiration in the packaging (Kader, 2002; Allende et al., 2004).

Toivonen et al. (2001) suggested that the gas levels of 3.1-4.2% O_2 was acceptable to maintain broccoli head quality, and CO_2 levels of about 15.0-15.6% affecting the development of off-odors can be found after 4 days of storage at 1°C. Fonseca et al. (2005) showed that 1-2 kPa O_2 and 15-20 kPa CO_2 in the atmosphere of packaging was effective maintaining the quality of shredded Galega kale and extending the storage life for 4-5 days at 20 °C. Serrano et al. (2006) reported that concentration of gas on No-P bags was about 4-12 kPa O_2 and at 4.5-6 kPa CO_2, the storage-life of broccoli heads extended up to 28 days at 1 °C, with no symptoms of bad aroma and off-flavors could be detected inside the bags after opening. Jia et al. (2009) reported that MAP with no holes packaging had gas levels of around 1-6% O_2 and 8-14% CO_2, which could maintain the quality of broccoli florets for 13 days at 4 °C.

The most important goals of the fresh-cut processed fruits and vegetables are keeping fresh-like quality and regarding the reduction of the microbial loads as it concerns decay and safety problems. This study confirmed that food-borne pathogens (coliforms and *Salmonella-Shigella* spp.) are found in broccoli florets originating in their general cultivation and handling. HW at either 55 °C or 60 °C for 3 min were the most effective treatments inhibiting coliforms and *Salmonella-Shigella* spp. loads in fresh-cut broccoli with 4 days of storage at 4 °C. However, at these temperatures, the tissue of the broccoli florets could not tolerate heat. The HW treatment at 55 °C for 3 min can be used to reduce food-borne pathogens on contamination and maintained the physical quality of fresh-cut broccoli florets for 7 days. These results suggest that HW at 50 °C for 3 min has potential to maintain the physical quality of broccoli florets. However, this treatment may not be sufficient to control food-borne pathogen infections.

Most reports refer to the efficacy of the HW, which can extend the shelf-life and maintain quality by delaying yellowing and maintaining chlorophyll content of fresh broccoli (Tian et al., 1996; Forney et al., 1998; Dong et al., 2004; Stringer et al., 2007). In fact, using higher temperature and longer dipping time of HW may induce physiological injury of fresh-cut broccoli. This study showed that HW at 50 °C for 3 min on fresh-cut broccoli florets increased in the microbial loads more than the control after 10 days of storage at 4 °C.

5.2 VH Treatment

After treatments, the broccoli florets treated VH at 90 °C for 15, 30 and 45 sec slightly reduced the coliforms and *Salmonella-Shigella* spp. loads of fresh-cut broccoli florets. However, this treatment was not sufficient to reduce pathogen loads. Therefore, these results suggest that tissue injuries of florets were detected possibly due to the increase of the exposure time of VH. Moreover, 3 days of storage showed that samples of VH treatment had increased symptoms of disorders that related to sensory evaluation (Figure 4.8; Table A25-27).

The exposure time in VH (90 °C) for 30 and 45 sec showed sharply decreased respiration rates on the first day of storage in comparison with the control. The decline in respiration rates of VH treated samples may be the result of a shocking duration of heat exposure in the plant. Similar results were found in fresh-cut lettuce treated with steamer jet-injection (Martín-Diana et al., 2007). The rate of respiration is often a good indication of the storage life of fresh-cut produce. In general, off-odor is always associated with the onset of aerobic respiration under lower O_2 and higher CO_2 levels; thus, the lack of off-odor indicates that no aerobic respiration has occurred. It has been reported that the off-flavor is mainly due to ethanol, aldehyde, ethyl acetate, volatile compounds, and ethylene associated compounds, which are basically responsible for off-odor during the storage period either by living cells or by microbes present in the packages (Kumar and Kim, 2010). Fresh-cut broccoli florets treated with VH for 30 and 45 sec resulted in reduced marketable life, and tissue damage from high temperature seemed to be a result of over cooking fresh-cut broccoli florets.

5.3 Ozonated Water Treatment

Ozonated water treatment for 15 min (1.50 ppm) reduced pathogen counts by 0.5 to 2.0 log $CFU \cdot g^{-1}$ FW on fresh-cut broccoli florets during storage. The present study showed that the efficiency of ozone for eliminating yeasts and molds was slightly greater than coliform counts (Figure 4.9B and D; Table A29 and A31). Hildebrand et al. (2008) reported that a concentration of continuous low ozone exposure (50 ppb) may have killed or restricted surface mycelium, which resulted in a reduced rate of lesion expansion by both *S. sclerotioru* and *B. cinerea* in carrots. This difference may be due to inherent differences in the cell envelope, which is a primary target of ozone activity (Yuk et al. 2007; Rong et al., 2010). These results suggest that the ozonated water treatments have a slightly significant effect on retarding microbial growth on fresh-cut broccoli florets. This is similar to previous studies that have shown less log reductions of pathogens by ozonated water when applied with green peppers, rocket leaves, and green leaf lettuce (Ketteringham et al., 2006; Martínez-Sánchez et al. 2006; Ölmez et al., 2009). Moor et al. (2000) found that ozone is in general more effective against Gram-negative than against Gram-positive bacteria and is ideal as a terminal disinfectant for food processing because of the lack of odor and residue. Application of ozone is being actively tested for its potential to improve the safety of fresh fruits and vegetables (Xu, 1999).

Hue angle of fresh-cut broccoli samples indicated green color and was correlated with chlorophyll content (Figure 4.11; Table A36), which is consistent with the fact that color green intensity should demonstrate higher chlorophyll content. Viña et al. (2007) reported that the hue value is a good indicator of chlorophyll content. This result indirectly shows that ozonated water treatment induced chlorophyll degradation.

A decrease in the amount of chlorophyll in fresh-cut broccoli florets was caused by these ozonated water treatments, thus an acceleration of the process of chlorophyll degradation during storage was similar to previous studies on tobacco (*Nicotiana tabacum* L.) (Saitanis et al., 2001). Moreover, the treated ozonated water samples had much lower chlorophyll content than the untreated. It is known that decreased chlorophyll contents results in a loss of nutrition values and quality, which results in limited shelf-life of product.

The growth of total bacteria and yeast and mold (as spoilage flora) and coliform (as a risk for food safety) in fresh-cut broccoli florets during 6 days at 4 °C was reduced by ozonated water treatment. However, the visual quality and color of all samples decreased during storage at 4 °C and appeared as a loss in freshness appearance and dark green color, which resulted in the reduction of the shelf-life of the products. The best condition of ozonated water treatment as a sanitizer to control microbial counts of fresh-cut broccoli florets was 15 min of ozonated water treatment (1.50 ppm). In conclusion, longer contact time of ozonated water showed the best results for reducing microbial loads, and the quality of ozonated water treated fresh-cut broccoli florets is acceptable for the consumer. However, microbial growth was not inhibited completely by ozonated water alone, which suggests a need for a combination with another disinfectants and/or agents to further reduce microbial populations.

5.4 SC Treatment

On total bacteria and yeast and mold counts, SC did not show clear effect on concentration (Figure 4.13A and C; Table A43 and A45). SC at 500 ppm resulted in consistently lower total bacteria load than that of the control. At higher concentrations of 750 to 1,000 ppm, the effect was erratic and during certain periods of storage, bacterial load did not differ much from that of the control. On yeast and mold counts, the SC treatments had comparable inhibitory effect.

Weight loss of SC-treated and untreated samples was not significantly different. At the end of storage, weight loss ranged from 0.16 to 0.29%. Furthermore, it can be seen from Figure 4.14A that after 3 days of storage negative weight loss values were noted, indicating that the florets gained weight possibly as a result of rehydration due to the humid condition inside the PVC clamshell box.

SC-treated florets had generally higher respiration rates than the control. Previously, Cruz et al. (2006) and Martínez-Sánchez et al. (2006) similarly observed respiratory increases in shredded carrots and rocket leaves treated with acidified SC solutions. However, in the present study, the florets treated with higher SC concentrations showed some tissue damage (color darkening or browning) that was more severe at 1,000 ppm than at 750 ppm SC.

Green floret color is one of the most important appearance attributes of broccoli, and after harvest, yellowing usually sets in due to chlorophyll breakdown (Fukasawa et al., 2010). Lemoine et al. (2008) showed that fresh broccoli has hue values of 126 to 128 which compare well with that obtained in the present study. SC could have a detrimental effect on green color retention as also obtained in fresh-cut rocket leaves (Martínez-Sánchez et al., 2006) and cilantro (Kim et al., 2007). However, chlorophyll analysis showed that treatment with 1,000 ppm did not change chlorophyll content in the broccoli florets.

In this study, I found that 500 ppm SC solution for 1 min was the most promising treatment to maintain quality and at the same time to reduce the microbial load on fresh-cut broccoli florets. Higher SC concentrations were detrimental to floret quality although they had greater effectiveness in reducing microbial contamination. While SC solutions have been reported to possess a strong antibacterial effect antibacterial activity can be greatly reduced by the presence of organic matter (Inatsu et al., 2007).

5.5 HW Combined SC Treatment

The reduction of microbial is a result of SC more than HW, because the low temperature (45 °C) and short time (1 min) might not be sufficient to inactivate the microbial. Previous research shows that HW treatments (50 °C for 2 min or 45 °C for 4 min) had great effectiveness for maintaining the quality and reducing the yellowing of harvested broccoli heads (Forney, 1995; Dong et al., 2004). The microbial reduction in this experiment is a result of the antimicrobial property of SC as referred to FDA (2010). SC was used for controlling microbial growth in fresh-cut produce i.e., apple and cilantro (Lu et al., 2007; Allende et al., 2009). Furthermore, washing the fresh-cut produce with clean water or disinfected solutions also help to remove the microbial loads that contaminate the plant surface. However, the influencing factors on the efficacy of disinfection depended on type, concentration, temperature of disinfectants, the ratio of disinfectant and water, dipping time, type of fresh produce and characteristics of plant surface (FDA, 2013). Moreover, the microbial load was increased consistently in all samples during storage period at 4 °C. These results suggest that microbes can survive and multiply at low temperature (Li et al., 2001; 2002; Moreira et al., 2008).

Fresh-cut operations including washing and sanitizing steps are important to guarantee food safety of the final products. HW treatment at 45 °C for 1 min combined with 300 ppm of SC solution for 1 min had efficiency to reduce some of the natural microorganisms counts (aerobic bacteria, coliforms and yeasts and molds) and it also maintained the antioxidant capacity without negative effect on their quality (florets color, chlorophyll content and ascorbic acid content) of fresh-cut broccoli during storage at 4 °C for 12 days. However, this treatment is not sufficient to reduce the microbial load much. Thus, it is necessary to use the other sanitizing step to ensure the microbiological quality of fresh-cut broccoli during storage.

Green florets color is the one of the most important appearance attributes for broccoli florets for customer preference (Fonseca et al., 2005). The main physiological disorder of broccoli is the yellowing of florets (Fukasawa et al., 2010). L* value indicates lightening and the increase in L* value is associated with yellowing of broccoli florets (Fonseca et al., 2005). HW combined with 300 ppm SC treatment did not suppress the yellowing or chlorophyll degradation in fresh-cut broccoli, but the slight decrease of chlorophyll might be due to the effect of storage at low temperature as described by Dong et al. (2004) while storage at ambient temperature accelerates yellowing due to chlorophyll breakdown of flowering buds (Kasim et al., 2007).

Several reports have also shown that heat treatments could delay yellowing process and senescence of broccoli florets (Tian et al., 1996; Terai et al., 1999; Funamoto et al., 2002). A thermal treatment at 45 °C for 1 min appears to be an effective, inexpensive and environmentally safe method that reduces microbial loads and maintains quality of fresh-cut broccoli florets. However, the thermal treatment at high temperature and longer contact time in fresh-cut broccoli florets could be harmful to produce quality by over cooking.

Phenolic compounds in vegetables are present in both soluble forms and combine with cell wall complexes. Thus, increased surface area of tissues in contact with hot water treatment, as well as high water temperatures and lengthy dipping times are all likely to cause disruption of cell walls and breakdown of phenolic compounds (Francisco et al., 2010). Moreover, results showed changes in flavonoid content during treatment to follow similar patterns such as phenolic content, suggesting that flavonoids contribute to the phenolic content of broccoli.

Total ascorbic acid (AsA), which is also called vitamin C, is a powerful natural antioxidant that promotes in the scavenging free radical. It is abundant in many fresh fruits and vegetables including broccoli (Koh et al., 2009). During storage oxidizing enzymes, e.g. ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT) and polyphenol oxidase (PPO) might help to reduce the ascorbic acid of the fruits and vegetables (Nath et al., 2011).

Senescence is considered to be associated with the defense system, including antioxidant enzymes and antioxidants. Antioxidant enzymes (SOD, CAT and POD) are considered to be important in the oxy-radical detoxification process in plant tissue

(Mittler, 2002). Toivonen and Sweeney (1997) reported that antioxidant protection offered by SOD and POD is important for the retention of green color in broccoli florets, and the increases in SOD, POD and CAT were likely responses to the increases in oxygen radical production in broccoli, which could subsequently lead to yellowing. Postharvest treatment could change the activity of antioxidant enzymes in vegetables and fruits. The H_2O_2 generated by SOD or other sources accumulated and thus CAT and APX activities were higher towards the end of the storage. This increase in APX activity could lead to a higher accumulation of DHA in treated samples. The combined treatment of the POD and CAT activities increased, which suggests a majority role of these enzymes to be detoxification of H_2O_2 in comparison to APX. The role of this antioxidant system may be critical in controlling yellowing in broccoli. It has been suggested that the increases in the activities of SOD, CAT and POD are generally a consequence of the system's ability to delay senescence (Toivonen and Sweeney, 1997). From the results of this study, The HW combined SC treatment leads to high activities of SOD, POD and CAT in broccoli florets during storage, as compared with the control. Therefore, HW might delay the senescence of broccoli florets by means of regulating the antioxidant enzymes system.