CHAPTER 1 INTRODUCTION

1.1 Research Background

Broccoli (*Brassica oleracea* L.) is a plant in the mustard family (Brassicaceae), which is known for cruciferous vegetables such as cabbage, cauliflower and kale. Broccoli is a popular commercial vegetable and is becoming a popular vegetable in other parts of the world (Moreno et al., 2006; Herr and Büchler, 2010; Latté et al., 2011). The fresh-cut vegetables that are easy to prepare and ready to use are one of the fastest-growing product categories due to a rising consumer demand for healthy and convenient food (Herr and Büchler, 2010). Broccoli is highly perishable when held in air, but controlled and modified atmospheres have proved to be able to maintain quality and extend the shelf-life of fresh broccoli (Soliva-Fortuny and Martin-Belloso, 2003; Jones et al., 2006). However, fresh-cut processing, which includes unit operations such as peeling, trimming or cutting, alters the integrity of the commodity’s tissues (Saltveit et al., 2003).

Contamination sources of fresh-cut fruits and vegetables include raw materials and contact with processing equipments. The microorganisms that exist on the surfaces of raw, whole produce appear to be the major source of microbial contamination and consequent spoilage of fresh-cut fruits and vegetables (Saranraj et al., 2012). Several disinfectant chemicals or sanitizing treatments have been used to reduce the initial microbial loads on fresh produces destined for fresh-cut processing (FDA, 2001). Sanitization of produce plays an important role in the preservation of food quality and safety of consumption. The control of spoilage microbial and food-borne pathogens can be done by several methods, such as physical treatments; heat treatment, irradiation and modified atmosphere packaging (Ukuku, 2006; Erkan et al., 2008; Montero-Calderon et al., 2008; Wen et al., 2008), chemical treatments (sanitizing agents and antimicrobial compounds) (Lee et al., 2003; Baur et al., 2005; Ayala-Zavala et al., 2008) and biological treatments (antagonists and plant extracts) (Bautista-Banos et al., 2000; Bautista-Banos et al., 2003; Ponce et al., 2008). In particular, sanitizing agents are highly effective in killing microorganisms and leaves less chemical residues such as chlorine, sodium hypochlorite, ozone, hydrogen peroxide, organic acids and etc. (Sapers, 2003).

Behrsing et al. (2000) showed that the dipping broccoli florets in a 100 $mg{\cdot}L^{-1}$ free chlorine solution for 2 min at 4 °C and 25 °C reduced *E. coli* cells by approximately 2.4 log $CFU{\cdot}g^{-1}$. Zhang et al. (2005) reported fresh-cut celery dipped in 0.18 ppm of ozonated water for 5 min reduced the microbial population to 1.69 log $CFU{\cdot}g^{-1}$. Use of the ozonated water and chlorinated water during washing processes of shredded iceberg lettuce minimized the microbial contamination but had less effectiveness in prolonging the shelf life of product (Baur et al., 2004). Ukuku (2006) found that washing cantaloupe fruit with hot water at the temperature of 96 °C for 2 min reduced *Salmonella* sp. contamination on peel to 4.91 log $CFU{\cdot}cm^{-2}$. Efficacy of sanitary treatments increases when the integrated treatments are used. Yuk et al. (2007) reported that 3 ppm ozone was able to reduce the population of *E.coli* 0157:H7 and *L. monocytogenese* on Enoki mushroom but its efficacy was increased when ozone was combined with 1% citric acid. Manuwong et al. (2007) also showed that sodium hypochlorite (NaOCl) treatment at 200 ppm was the best treatment to reduce *Salmonella* sp. in peel of pineapple to about 2.72 log $CFU{\cdot}cm^{-2}$, while use of hot water treatment at 50 and 60 °C significantly retarded *Salmonella* sp. in peel of pineapple to 0.77 and 0.99 log $CFU{\cdot}cm^{-2}$, respectively. However, pineapple fruit treated with warm sodium hypochlorite solution (50 °C, 200 ppm NaOCl) for 3 min was able to reduce *Salmonella* sp. in peel and flesh of fresh-cut pineapple more than use of NaOCl or hot water treatment alone. Washing fresh-cut carrot with warm chlorinated water (200 ppm) resulted in 0.23 log $CFU{\cdot}g^{-1}$ reduction (Klaiber et al., 2005). The microbial population in lettuce washed in chlorinated water (100 $\mu g{\cdot}g^{-1}$ FW) at 47 °C for 3 min reduced approximately 3 log $CFU{\cdot}g^{-1}$ (Delaquis et al., 1999). Warm water (50 °C), with or without 20 ppm chlorine, and chlorinated water at 20 °C significantly reduced the initial population of mesophilic aerobic microflora in fresh-cut iceberg lettuce by 1.73-1.96 log $CFU{\cdot}g^{-1}$ (Li et al., 2001). These results indicate that the combined treatments enhance the efficacy of microbial decontamination more than using a single treatment.

Fruits and vegetables are known as the major source of bioactive compounds and antioxidants, which can reduce the free radicals in human cells. Most bioactive compounds usually studied in fruit and vegetables are chlorophyll (Yamauchi et al., 1997; Funamoto et al., 2002), carotenoids (Müller, 1997; Singh et al., 2007; Sikora et al., 2008), flavanoids (Gil et al., 1998; Vallejo et al., 2002; Singh et al., 2007), glucosinolate (Vallejo et al., 2002; Schreiner et al., 2006) and vitamin C or ascorbic acid (Gil et al., 1998; Vallejo et al., 2002; Singh et al., 2007; Sikora et al., 2008). Moreover, many enzymatic activities in plant tissues are related to the elimination of reactive oxygen species (ROS) that cause cell damage such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione (GHS) and glutathione reductase (GR) (Chanjirakul et al., 2006; Zielinski et al., 2007; Wanga and Ballington, 2007; Erkan et al., 2008). Several studies have shown that sanitary treatments do not affect the microbial growth only but also affected quality, antioxidant activities or bioactive compounds in fresh produces. Ruiz-Cruz et al. (2007) reported that shredded carrot sanitized with sodium hypochlorite had higher levels of carotene and antioxidant capacity than non-sanitized shredded carrot. Wang et al. (2004) found that use of ozonated water for controlling total aerobic bacteria and total enterobacteriaceae in cilantro leaves did not affect the chlorophyll contents compared with non-ozonated water treatment. Beltrán et al. (2005) observed vitamin C content of fresh-cut lettuce that was treated with 20 ppm ozonated water. The results revealed that no differences of vitamin C content were observed between the ozone-treated and non-ozone-treated fresh-cut lettuce. Hot water treatment at 47 °C for 7.5 min and hot air treatment at 50 °C for 2-3 h inhibited the yellowing of broccoli florets by retarding the chlorophyll degrading due to the effect of heat treatment strongly retarding the activities of ACC oxidase, a ethylene production associated enzyme, and suppressing the activities of chlorophyll degrading enzymes (Tian et al., 1996; Terai et al., 1999; Funamoto et al., 2002).

At the present, the effect of sanitary treatments on the development of antioxidant properties (antioxidant activities and bioactive compounds) in fresh-cut broccoli florets have not been reported yet and there are only a few papers reporting about suitable sanitary treatments for microbial controlling in fresh-cut broccoli florets. Thus, the hypothesis of this study was that the sanitary treatments may affect the antioxidant properties of fresh-cut broccoli florets and also had the aim to find out the best solution of sanitary treatments for minimizing the microbial contamination and maintaining the quality of fresh-cut broccoli. The knowledge from this experiment may help to clarify the relationship between sanitary treatments and changes of antioxidant properties in fresh-cut broccoli florets.

1.2 Objectives of the Research

1.2.1 To study the effects of sanitary treatments (hot water (HW), vapor heat (VH), ozonated water and sodium chlorite (SC) solution) for controlling of microbial growth and maintaining qualities in fresh-cut broccoli floret during cold storage.

1.2.2 To study the effects of combined sanitary treatments for controlling or minimizing microbial growth and maintaining qualities in fresh-cut broccoli floret during cold storage.

1.2.3 To study the combined effects of sanitary treatments on preserving of bioactive compounds and antioxidant activities in fresh-cut broccoli floret during cold storage.

1.3 Scopes of Study

1.3.1 Broccoli (*Brassica oleracea* L.) heads were harvested from The Royal Project Foundation, Chiang Mai province, Thailand

1.3.2 Study on the effects of sanitary treatments on microbial growth and visual quality in fresh-cut broccoli florets during cold storage. Fresh-cut broccoli florets were treated in sanitary treatments as followed:

Experiment 1: Hot water (HW) treatment at 25 (control), 50, 55, and 60 °C for 3 min

Experiment 2: Vapor heat (VH) treatment at 25 (control) and 90 °C for 15, 30, and 45 sec

Experiment 3: Ozonated water (2,500 $mg{\cdot}h^{-1}$ ozone production rate) for 5, 10, and 15 min, the concentration of ozone dissolved in the water were 0.56, 1.00, and 1.50 ppm, respectively

Experiment 4: Sodium chlorite (SC) solutions at 0 (control), 500, 750 and 1000 ppm for 1 min

The design of the experiments was to reduce microbial growth and maintain visual qualities in fresh-cut broccoli florets during storage at 4 °C.

1.3.3 Study on the combined effects of hot water (HW) treatment at 45 °C for 1 min, followed by treated with sodium chlorite (SC) solutions at 100 and 300 ppm for 1 min on microbial growth and visual quality in fresh-cut broccoli florets during storage. The design of the experiment was to reduce microbial growth and maintain visual qualities in fresh-cut broccoli florets during storage at 4 °C.

1.3.4 Study on the combined effects of hot water (HW) treatment at 45 °C for 1 min, followed by treated with sodium chlorite (SC) solutions at 100 and 300 ppm for 1 min on bioactive compounds (ascorbic acid, dehydroascorbic acid, total chlorophyll, carotenoids, total phenolic, flavonoid, glucosinolate, antioxidant activity ($DPPH^{\bullet}$)) and change in antioxidant activities (chlorophyllase, catalase, peroxidase, superoxide dismutase, ascrobate peroxidase, hydrogen peroxide and glutathione reductase) in fresh-cut broccoli floret during cold storage. The design of the experiment was to maintain bioactive compounds and antioxidant activities in fresh-cut broccoli florets during storage at 4 °C.

1.4 Expected Benefits

This research will expand knowledge and understanding of the microbiology and quality properties changes of fresh-cut broccoli florets when treated with sanitary treatments during cold storage. Moreover, this information might lead to development of processes and effectiveness of sanitary treatments to control or minimize changes in fresh-cut broccoli florets products. Further applications of the best sanitary treatments may be beneficial for safety on microbial and also prolong the shelf life of fresh-cut broccoli florets in the food industry.