CHAPTER 2 LITERATURE REVIEW

2.1 Broccoli

Broccoli (*Brassica oleracea* var. *italica*) is a compact, rapidly-developing floral vegetable, that is usually harvested when the flowering heads are immature. Broccoli, therefore, refers to the young shoots that develop in spring on some species of the genus Brassica (*brocco* is Italian for shoot). Sprouting broccoli is believed to be the ancestor of the present-day quick-growing cauliflowers. The usual types of sprouting broccoli first develop a central head of green color, and a number of smaller auxiliary stalks bear smaller heads, which are sold in bunches. Sprouting broccoli is a vegetable of the highest quality. Broccoli is also a popular vegetable of commercial importance in other parts of the world (Rangavajhyala et al., 1998).

Fresh fruits and vegetables are essential components of the human diet and have important nutritional, dietary antioxidant and health benefits associated with their consumption. Diet rich cruciferous family vegetables are recommended for decreasing the risk of developing human cancer (Rangavajhyala et al., 1998). Broccoli is one of these families that has rich nutritional value because of numerous bioactive compounds and health-promoting properties such as vitamins, antioxidants and anti-carcinogenic substances. Consumption of cruciferous vegetables is more strongly associated with cancer protection than vegetable consumption in general. Crucifers contain many bioactive components including flavonoids such as quercetin, minerals and vitamin C. Among the most studied bioactive compounds in crucifers associated with cancer protection are glucosinolates (Finley, 2005). An additional advantage of broccoli is its tendency to accumulate heavy metals. Consumption of 150 g of broccoli per day satisfies an adult's requirements for vitamins E, A, B_1 and C, and boosts the immune system (Borowski et al., 2008). Broccoli is a highly perishable vegetable that senescences quickly after harvest and thus its postharvest life is quite short due to yellowing, softening, water loss, decay and off-odor incidences (Forney et al., 2003). Yellowing in broccoli is considered a major determinant of shelf-life and the green color of broccoli is an important quality feature for consumers (Toivonen and Sweeney, 1997). As the broccoli florets mature, they become more susceptible to yellow florets; it is assumed that this is because of the senescence process in natural plants. (Tian et al.,

1995; Toivonan and Beveridge, 2005). The loss of green color in broccoli florets occurs due to chlorophyll breakdown, and this is stimulated by exogenously applied and endogenously produced ethylene (Sabir, 2012).

2.2 Fresh-cut Produce

Fresh-cut or minimally processed broccoli is a popular vegetable. Consumers demand that these types of products maintain fresh qualities with regard to appearance, taste, flavor, nutrition, and convenience. Food safety has become a very important issue in fresh and fresh-cut produce for consumption (Sapers, 2001; Garcia and Barrett, 2002).

Many synonyms are used for the term “fresh-cut”, including “minimally processed”, “lightly processed”, “ready to eat”, “partially processed”, and “fresh-cut” fruits and vegetables (Cantwell and Suslow, 2002). There are various definitions of fresh-cut products such as:

“Fresh-cut produce is defined as any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state. Regardless of commodity, it has been trimmed, peeled, washed and cut into 100% usable product that is subsequently bagged or prepackaged to offer consumers high nutrition, convenience and value while still maintaining freshness” (IFPA and PMA, 1999).

“The USDA and FDA definitions for “fresh” and “minimally-processed” fruits and vegetables imply that fresh-cut (pre-cut) products have been freshly-cut, washed, packaged and maintained with refrigeration” (Beaulieu and Gorny, 2004).

“Fresh-cut produce, defined as “any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state,” offers the flavor, nutritional value, and freshness of fresh produce along with added convenience (Morris and Brady, 2005).

Processing of fresh-cut produce requires washing, sorting, trimming, peeling, slicing, or chopping that does not affect the “fresh-like” quality of fruits and vegetables. However, the shelf life is often greatly diminished because of undesirable physiological changes caused by wounding, water loss, etc. Loss of cellular integrity at the cut surface of the fruits and vegetables causes microbial contamination and quality loss that makes products to be unmarketable due to undesirable appearance and accelerated respiration and ethylene production. Various approaches have been developed to control undesirable physiological changes and extend shelf life of fresh-cut fruits and vegetables.

Fresh-cut processing involves the well-considered wounding of fruit and vegetable tissues to provide a product that requires less handling by the consumer. Fresh-cut products are usually washed, packaged, and maintained with refrigeration. Cutting removes the natural protection of the epidermis and destroys the internal compartmenting that keeps cells separate. The disruption of tissue and cell integrity that results from fresh-cut processing decreases produce’s shelf life through physiological and biochemical changes similar to those seen in tissues of fruits and vegetables that are not fresh-cut (Lamikanra, 2005).

Wound severity in cut produce may also be affected by cultivar preharvest crop management, physiological maturity, and degree of cutting-induced injury. The extent of physical damage that results from cutting fruit tissue affects the intensity of physiological stress, microbial growth, and product shelf-life. Inner tissue exposure facilitates contamination by the epithelial microflora. It also increases respiration rates, and decreases enzymes and substrates, total moisture loss, and overall sensory quality. Very sharp cutting tools limit the number of injured below the actual cut. Fruit pieces prepared with sharp cutting tools retained marketable visual qualities longer than the same fruit processed with dull cutters. Pre- and postharvest conditions, tissue anatomy, cell-to-cell adhesion, cell turgor, and cell wall strength are factors contributing to the bruising response, and the magnitude of these factors seems to be affected by ripening and variety (Lamikanra, 2005).

2.2.1 Production of Fresh-cut Produce

Fresh-cut fruit preparation is more difficult because processors have to select the ripening stage that may lead to a compromise between flavor and texture. Defining the best storage and conditioning procedures for fruit destined for fresh-cut processing is difficult (Cantwell and Suslow, 2002). Like fresh-cut vegetable processing, fresh-cut fruit processing should be done under clean and low temperature conditions. Fruits have to be peeled and sectioned into small pieces by a slice cutter or by hand. Some commodity slices may be washed in sanitizer before drying and packing in containers that control gas exchanges to help to maintain quality of fresh-cut products. Controlling low temperature and sanitization and/or using treatments such as an antimicrobial and anti-browning agent can delay over-ripening.

Fresh-cut processing includes peeling, trimming and cutting fresh produce to specific sizes (Figure 2.1). After thorough washing and cutting, fresh-cut broccoli is followed by cooling to 5 °C to prevent color and flavor loss. Consumer purchases are based primarily on external characteristics such as visual appearance and texture. An important component of visual appearance is color, and loss of green color is a major limiting factor in the shelf life of broccoli. Fresh-cut produce must also be safe, wholesome and nutritional (James and Ngarmsak, 2011).

Mechanical damage is one of the most important factors that reduce product quality. The mechanical stress can be separated into two general types: mechanical perturbation and physical wounding (Lamikanra, 2005). Cutting and shredding should be performed with the sharpest possible knives or blades made from stainless steel (Allende et al., 2006).

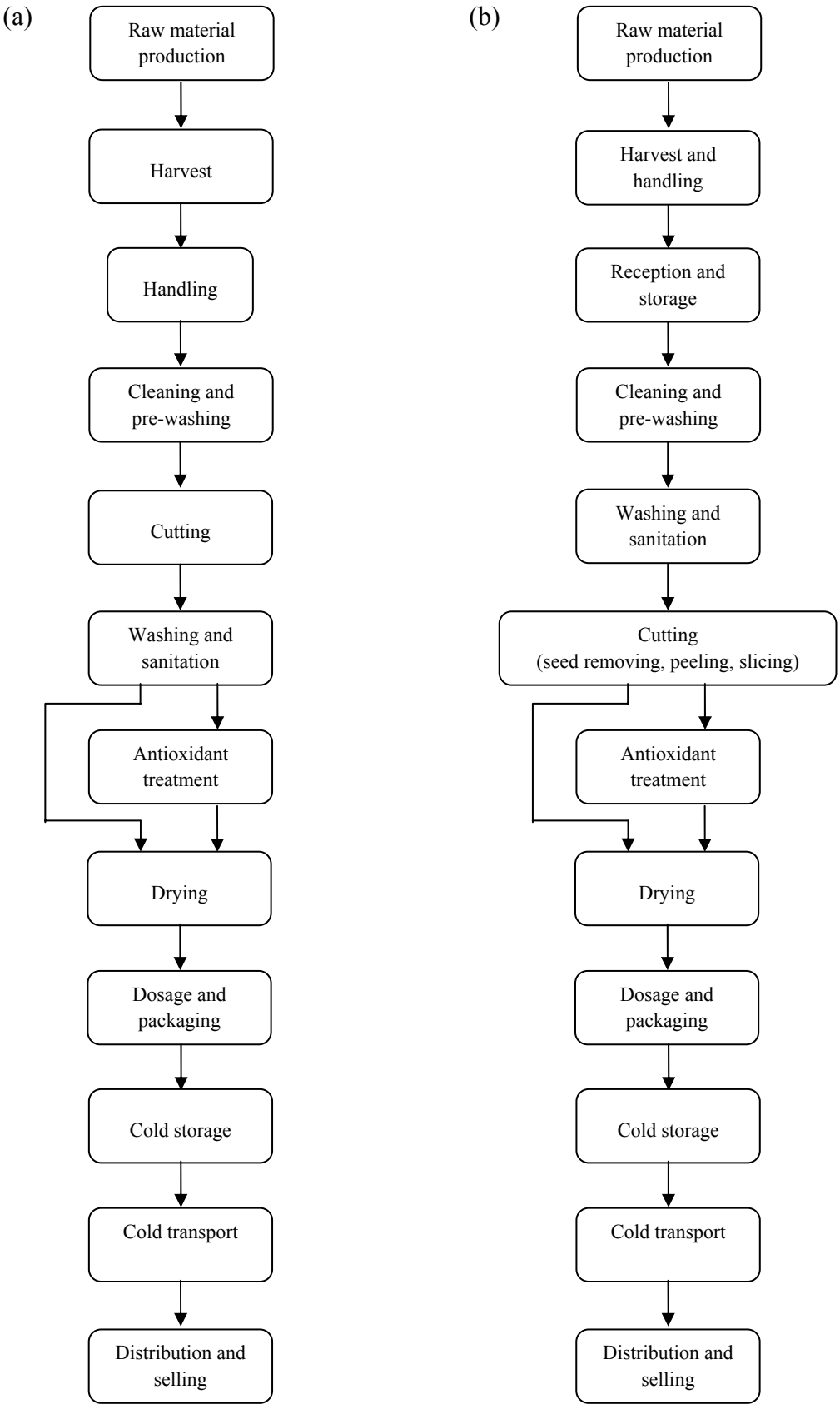

Figure 2.1 General diagram flows of processing operation for vegetables (a) and fruits (b) (Nicola et al., 2009)

2.2.2 Microbial Contamination of Fresh-cut Produce

The natural microbial contamination of raw fruits and vegetables is usually non pathogenic for humans and may be present at the time of consumption (Ramos et al., 2013). Moreover, it is highly dependent on the conditions of cultivation, harvesting, handling, transport, and postharvest storage, as well as marketing conditions (Sela and Fallik, 2009).

In fresh-cut preparation, the surface of produce is exposed to air and potential contamination with bacteria, yeasts and molds. In fresh-cut or minimally processed fruits and vegetables, these microorganisms were found inside broken cells or cells adjacent to broken tissue and accumulation of moisture, and the exudates from the cut surface provide favorable conditions for microbial growth (Ahvenainen, 1996; Watada et al., 1996). Therefore, although fresh-cut fruits and vegetables are washed with chlorine solution, microbials can survive when they are located within cells or areas not penetrated by the sanitizer or disinfectant (Watada et al., 1996).

According to the microbiological criteria recommended by the German Society for Hygiene and Microbiology, to load aerobic mesophilic microorganisms on mixed =, packaged salads at the consumer level should not exceed 7.7 log (5×10^7) $CFU \cdot g^{-1}$. Additionally, the use-by-date should not exceed 6 days, and the directions for use should advise to keep the product below 6 °C (Baur et al., 2004).

Fresh-cut fruits and vegetables have been associated with outbreaks of food borne disease in many countries. Contamination of microbial may occur by cultivation and operation or preparation of fresh-cut produce. Moreover, unsafe water used for rinsing the raw vegetables and sprinkling to keep them fresh is also a source of contamination (Johannessen et al., 2002). Microbial population generally increased on broccoli florets after the washing process (Zhuang et al., 2003).

The control of these contamination sources can enhance the successful management of microbial safety risk in the fresh-cut industry. Three types of microbes are present on the surface of fresh-cut produce (Nicola et al., 2009):

1. Useful microbes such as some lactic acid bacteria, which should not be removed or killed.
2. Spoilage microbes such as pectinolytic Gram negative bacteria belonging to *Pseudomonadaceae* or *Enterobacteriaceae* and yeasts with fermentative metabolism like *Saccharomyces* spp. These are found on fruit, and should be minimized during processing because they reduce shelf life.
3. Pathogens such as *Escherichia coli*, *Salmonella* spp., *Clostridium botulinum*, *Listeria monocytogenes* and *Staphylococcus aureus*; these are considered food-borne disease outbreaks.

Many fruits and vegetables support almost optimum conditions for the survival and growth of microbial species. The internal tissues are rich in many nutrients and vegetables have a near neutral pH. The structure of cell walls is mainly composed of polysaccharides cellulose, hemicelluloses, and pectin. Starches are the principal energy storage polymer in plants. Microbial utilization by using extracellular lytic enzymes breaks down substances such as these polymers to release water and other elements within the cell of plants for use as nutrients for their growth. Fungi in particular produce an abundance of extracellular pectinases and hemicellulases that are important factors for fungal spoilage (Saranraj et al., 2012).

2.2.2.1 Common Microbial Quality

The loads and types of microbial found in fresh and fresh-cut produces were conducted following microbiological methods used for identification and enumeration. The microbiological quality and safety of the fresh-cut produce is similar to other food products that have been frequently evaluated in terms of “total plate count” (TPC), “standard plate count” (SPC) or more accurately “aerobic mesophillic count” (AMC). AMC considers the growth rate of bacteria at 30-37 °C that are present with food, and does not give information about the presence of specific spoilage and/or pathogenic microorganism. The initial loads of AMC at harvest differ widely in fresh fruits and vegetables by considering several factors such as geographical region, cultivar and environmental factors. Moreover, AMC is used as a parameter to evaluate the microbiological quality of several food products and also generally increases during storage, especially if the product is exposed to an inappropriate temperature. Therefore, an AMC population is correlated to the products shelf life under specific storage and

handling conditions. AMC determination provides information regarding the number of bacteria even at storage temperature abuse (above refrigeration temperature). Knowledge about the bacteria populations that can grow at low temperatures during storage is useful for assessing the microbial quality and predictability of shelf-life period (Sela and Fallik, 2009).

2.2.2.2 Type of Spoilage Microorganisms

Microbial decay can be a major source of spoilage of fresh-cut produce. Microbial spoilage including off-flavor (e.g., fermented aroma, sour taste) formation, slimy surface, wetness and soft rot, discoloration, and visual microbial growth has been used as a main or exclusive objective criterion to determine shelf life of fresh-cut products (Brackett, 1994; Saranraj et al., 2012).

Pseudomonas sp.

Pseudomonas sp. are the most common and important spoilage microorganisms of refrigerated fresh-cut fruits and vegetables. *Pseudomonas* sp. have Gram-negative rod and strict aerobe. They generally require a high water activity for growth (0.95 or higher) and are inhibited by pH values less than 5.4 (Doyle, 2007). They are able to utilize a wide variety of organic compounds and produce acids oxidatively from glucose and maltose. Some Pseudomonas species produce pyoverdine or fluorescein that are water soluble, fluorescent pigments and can be observed in spoiled food under ultraviolet light. They are usually yellow-green but may appear blue or orange depending on the species and environmental factors. Pseudomonas produce catalase, oxidase, and enzymes that catalyze proteolytic and lipolytic reactions that cause formation of sulfides and trimethylamine (off-odors) and by forming biofilms (slime) on surfaces contribute to spoilage of refrigerated fresh animal products, and pectinolytic enzymes can cause soft rot of fleshy vegetables (Doyle, 2007; Saranraj et al., 2012).

Erwinia sp.

Erwinia sp. is another common Gram-negative spoilage microbe that causes soft rot of vegetables in storage at ambient temperatures (Doyle, 2007). *Erwinia*, a genus within the family Enterobacteiaceae, have small rods and facultative anaerobes. Their optimum growth temperature is 30 °C, and they can ferment sugar anaerobically to form acids. *Erwinia* cause rapid necrosis, progressive tissue maceration called “soft-rot” occlusion

of vessel elements called "vascular wilt," and hypertrophy leading a gall or tumor formation in plant tissues. *Erwinia* sp. are the major single cause of microbial spoilage of whole vegetables (Liao and Wells, 1987; Saranraj et al., 2012).

Enterobacteriaceae

The family Enterobacteriaceae is a large family of Gram-negative stains and rod-shaped bacteria such as *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Proteus*, *Erwinia*, and others (www.en.wikipedia.org/wiki/Enterobacteriaceae). They are facultative anaerobes, fermenting sugars to produce lactic acid and various other end products. The different genera with a variety of ecological niches, including plants, insects, animals and humans, may contaminate fresh-cut products on the farm and during processing (Heard, 2002).

Yeasts and molds

Yeasts of the genera *Saccharomyces*, *Candida*, *Torulopsis* and *Hansenula* have been associated with fermentation of fruits. Yeasts can cause quality loss of fresh-cut products and mixed salad. Yeasts have a slightly higher growth rate than molds, are well known for their beneficial ferment sugars into alcohols, and are responsible for off-flavors and off-odors (Saranraj et al., 2012). Yeast, a facultative microorganism, can grow with or without oxygen (Doyle, 2007).

Molds are fungi that cover surfaces as fluffy mycelia and usually produce masses of asexual, or sometimes sexual, spores (Saranraj et al., 2012). Molds are tolerant of acidic conditions and low water activity and are involved in spoilage of fruits and vegetables (Doyle, 2007). These pathogens are commonly members of the class *Ascomycetes* and the associated Fungi. Mold spoilage of fresh produce, especially fresh fruit, is caused by species of *Penicillium*, *Phytophthora*, *Alternaria*, *Botrytis*, *Fusarium*, *Cladosporium*, *Phoma*, *Trichoderma*, *Aspergillus*, *Alternaria*, *Rhizopus*, *Aureobasidium* and *Colletotrichum.* The symptoms include visible growth, rots and discoloration, such as blue mold rot, gray mold rot, botrytis rot, and brown rot (Saranraj et al., 2012).

2.2.2.3 Type of Food-borne Pathogens

The most concerning in fresh-cut produce involves several pathogenic organisms include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Shigella* spp., *Salmonella* spp. and hepatitis A virus (FDA, 2013). These organisms have all been implicated in foodborne disease outbreaks associated with the consumption of contaminated fresh produces (Singh et al., 2002; Sela and Fallik, 2009). Fresh produce can be contaminated with pathogenic bacteria during production from irrigation water, manure fertilizers and during food processing by contaminated equipment and food handlers (Martínez-Sánchez et al., 2006; Mahmoud, 2010).

Coliform bacteria

Coliform bacteria are a commonly used bacterial indicator of sanitary quality foods and water, especially freshly processed vegetables or fresh-cut produce (Nguyen-The and Carlin, 2000). Coliforms are found in faces of animals and humans, and also in soil and on plants. These are facultative anaerobic, Gram-negative, rods shaped, non-spore forming bacteria that can ferment lactose with the production of acid and gas formation within 48 hrs when grown in lactose broth at 35-37 °C (www.en.wikipedia.org/wiki/ Coliform_bacteria).

Escherichia coli

While the importance of *Escherichia coli* (*E. coli*) strains as a food-borne pathogen, the enterohemorrhagic (EHEC) stain designated *E. coli* O157:H7 is particularly recognized as an important emerging food-borne pathogen (Kim et al., 2007). *Escherichia coli* are the species associated with fecal contamination and are naturally found in the intestines of humans and warm-blooded animals. The presence of these bacteria poses a serious threat to public health with outbreaks arising from food and water that has been contaminated by human or animal feces or sewage (Saranraj et al., 2012).

***Salmonella* sp.**

The genus *Salmonella* is a member of the *Enterobacteriaceae* family, and is a rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameter of around 0.8 to 1.5 μm, and has peritrichous flagella (flagella that are all around the cell body). They are facultative anaerobes that can grow in a temperature range of 5-45 °C with optimum temperature of 35-37 °C. They are able to grow at low

pH and are generally sensitive to increased concentrations of salt (Bhunia, 2008). They are also chemoorganotrophs, which obtain their energy from oxidation and reduction reactions using organic sources. *Salmonella* belongs to the same family as *Escherichia*. Most subspecies of *Salmonella* produce hydrogen sulfide, which can readily be detected by growing them on media containing ferrous sulfate, as in the ripple sugar iron test (TSI) (www.en.wikipedia.org/wiki/Salmonella). Within the genus *Salmonella,* differentiation into species is based on antigenic differences. There are currently over 2370 serovars recognized; however, only 200 are known to cause disease in humans, including *Salmonella typhi*, the causative agent in the disease typhoid (Heard, 2002). Food-borne disease caused by nontyphoid serovars of Salmonella includes gastroenteritis and enterocolitis, with symptoms appearing from 8-72 h after food consumption. Salmonellae have been isolated from fresh produce, and fruits and vegetables have been linked to outbreaks of salmonellosis. Fresh produce may become contaminated with salmonellae either from sewage and contaminated water or from handling by infected workers (Heard, 2002). Salmonellae do not grow in foods at less than 7 °C and, therefore, should not pose a risk to public health in fresh-cut products, provided they are maintained at refrigeration temperatures (Heard, 2002).

Shigella sp.

Shigella is a genus of Gram-negative, facultative anaerobic, nonspore-forming, non-motile, rod-shaped bacteria closely related to *Salmonella* (www.en.wikipedia.org/wiki/ Shigella). The genus is divided into four species: *Shigella dysenteriae, Shigella boydii, Shigella sonnei* and *Shigella flexneri*. All species can cause shigellosis or bacillary dysentery in humans at low doses of infection (FDA, 2013). Outbreaks of shigellosis are generally transmitted from person-to-person but may also occur by consumption of contaminated water and foods, including foods such as fruits or vegetables that have received little or no heat treatment (FDA, 2013). Thus, fresh produce can become contaminated through the use of contaminated irrigation water, the use of raw sewage as fertilizer, insect transfer or human contact (Beuchat, 1998). Processed fruits and vegetables have been implicated in a number of outbreaks of shigellosis. Salad vegetables, cantaloupe and potato salad are examples of the associated products (Heard, 2002).

Staphylococcus aureus

Staphylococcus aureus is the third most common cause of confirmed food poisoning in the world, and the illness is due to the ingestion of preformed enterotoxins produced in foods (Saranraj et al., 2012). *S. aureus* is a facultative anaerobic Gram-positive coccal bacterium also known as "golden staph". *S. aureus* appears as grape-like clusters when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates (www.en.wikipedia.org/wiki/ Staphylococcus_aureus).

2.2.3 Thai Agricultural Commodity and Food Standard (TACFS-9016, 2007)

Current Criteria and Standards

To minimize foodborne disease from being transmitted through fresh produce, it is necessary to prevent initial contamination of these products and to control the potential amplification of pathogens in them throughout the production and distribution chain. Intervention strategies currently being applied in the fresh produce industry are Good Agricultural Practices (GAPs) in the field and packinghouses (FDA/USDA/CDC, 1998) and Good Manufacturing Practices (GMPs) used by food processors; GAPs address agricultural activities, including preplanting, planting, harvest, and postharvest practices that are designed to reduce microbial risks (Scientific Criteria and Performance Standards to Control Hazards in Produce and Related Products// In: Scientific Criteria to Ensure Safe Food, 2003 page 202). Several guidance documents that address GAPs have been developed and widely disseminated by government agencies, growers, shippers, processor trade associations, and academia (IFPA, 2001). Some of these publications include the Voluntary Food Safety Guidelines for Fresh Produce, published by the International Fresh Cut Produce Association (IFPA) and the Western Growers Association (IFPA, 1997); and the Food and Drug Administration (FDA) guidance document, Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (FDA/USDA/CDC, 1998).

Establishing and implementing microbiological criteria is a part of Microbiological Risk Management (MRM) and is referred to in Section 8.2 of the Principles and Guidelines for the Conduct of Microbiological Risk Management (CAC/GL 63-2007). In addition, revision of microbiological criteria should be considered in response to revision of other

MRM Metrics and also in response to emerging issues or changes in the following, but not limited to:

- Taxonomy, prevalence or distribution for the selected microorganism;
- The incidence of disease including attribution to specific foods;
- Traits of microorganisms (e.g. anti-microbial resistance, virulence);
- The suitability of an indicator organism;
- Available analytical methods/tests/appropriateness of test;
- Food/ingredients/technology/process of food production;
- Food safety control system;
- Population(s) at risk;
- Consumer behavior or dietary intake pattern of the food concerned;
- Understanding/knowledge of risk;
- Trend analysis results; and
- Required level of assurance.

Table 2.1 *E. coli* levels as an indicator of process hygiene for minimally processed or fresh-cut fruits and vegetables (EC, 2005)

Fresh-cut fruits and vegetables (ready to eat)	Level normally achieved using HACCP and good hygienic practice (GHP)	Maximum acceptable level
E. coli	100 CFU·g^{-1}	1,000 CFU·g^{-1}

Table 2.2 Regulation (EC) No. 2073/2005 on Microbiological Criteria for Foodstuffs (EC, 2005)

Food category	Micro-organisms/ their toxins, metabolites	Sampling-plan (1)		Limits (2)		Storage where the criterion applies
		n	c	m	M	
Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes	*Listeria monocytogenes*	10	0	Absence in 25 g		Products placed on the market during their shelf-life

Food category	Micro-organisms/ their toxins, metabolites	Sampling-plan (1)		Limits (2)		Storage where the criterion applies
		n	c	m	M	
Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	*Listeria monocytogenes*	5(a)	0	100 CFU·g^{-1} (b)		Products placed on the market during their shelf-life
		5(a)	0	Absence in 25 g (<0.04 CFU·g^{-1}) (b)		Before the food has left the immediate control of the food business operator, who has produced it
Minced meat, meat preparations and meat products intended to be eaten raw and cooked	*Salmonella* sp.	5	0	Absence in 25 g (raw)		
		5	0	Absence in 10 g (cooked)		

(1) n = number of units comprising the sample; c = number of sample units giving values over m or between m and M.
(2) For points m = M. m = level normally achieved using HACCP and good hygienic practice (GHP); M = Maximum acceptable level.
(a) National governments should provide or support the provision of guidance on how samples should be collected and handled, and the degree to which compositing samples can be employed.
(b) Absence in a 25-g analysis unit. This criterion is based on the use of ISO 11290-1 method. Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated (e.g., based on ISO 16140).

2.2.4 Physiological and Biochemical Changes of Fresh-cut Produce

Compared to whole stalks, broccoli florets appear to represent a different set of physiological conditions because of the large relative increases in cut surface. The processing of florets causes a wound induced increase in the rates of both respiration and ethylene production compared to whole stalks (Rushing, 1990), resulting in physiological and chemical changes that cause rapid deterioration in fresh-cut produce. Wounding plant tissues enhances rate of ethylene production and respiration rate and may accelerate deterioration and senescence in plant tissues (Siddiq et al., 2012). Increased ethylene production induces softening in banana and kiwifruit, and chlorophyll loss in spinach (Abe and Watada, 1991). From wounds, ethylene plays a role in membrane lipid degradation by increasing enzymatic degradation, particularly lipoxygenase. Damage to membrane systems causes a loss of lipid and increased permeability and production of both desirable and undesirable volatiles (Saltveit, 1997). In addition, the cutting and abrasion of tissue rapidly produces a wound signal that is thought to be responsible for phenolic compounds and the induction of wound healing (Figure 2.2).

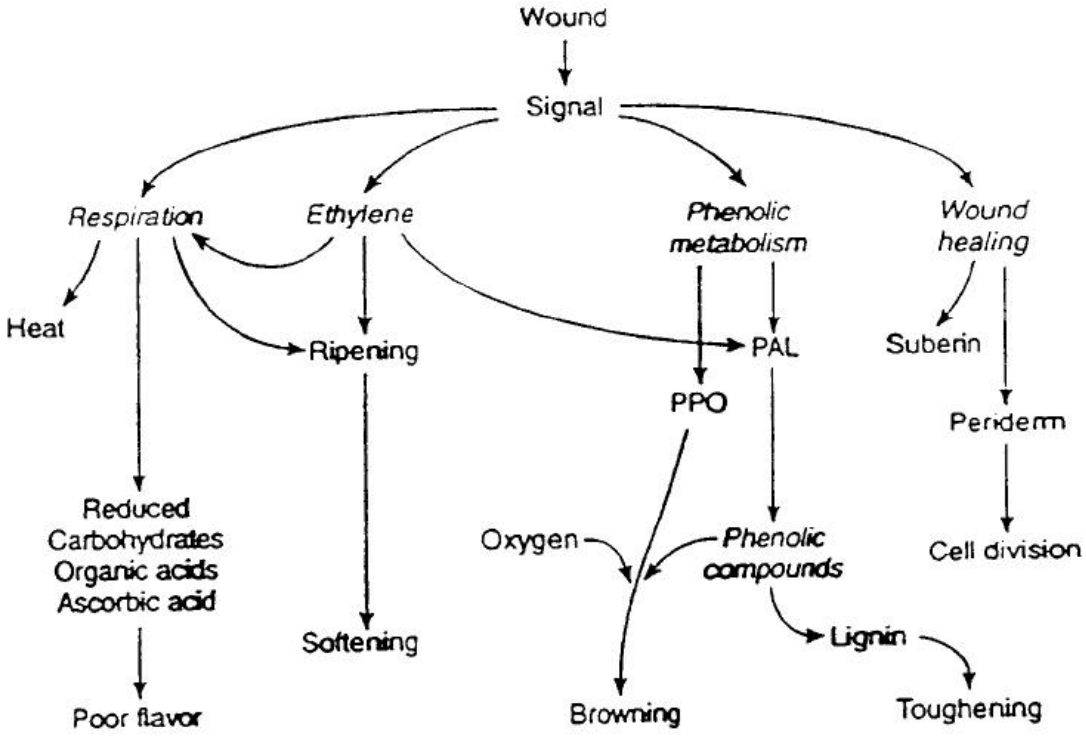

Figure 2.2 The interrelationship among the many effects of wounding on physiological processes in fresh-cut produces (Saltveit, 1997)

2.2.4.1 Respiration and Ethylene Production

Respiration plays a major role in the post-harvest physiology of fresh fruits because the process of respiration involves combining O_2 in the air with organic molecules in the tissue (usually a sugar) to form various intermediate compounds and eventually CO_2 and water. In general, the storage life of commodities varies inversely with the rate of respiration. This is because respiration supplies compounds that determine the rate of metabolic processes directly related to quality parameters, such as firmness, sugar content, aroma, flavor, etc. Respiration plays a major role in the postharvest life of fresh commodities because it reflects the metabolic activity of the tissue that also includes the loss of substrate, the synthesis of new compounds, and the release of heat energy (Saltveit, 2004).

Respiration is the oxidative breakdown of complex substrate molecules normally present in plant cells, such as starch, sugars, and organic acids, to simpler molecules such as CO_2 and H_2O. Concomitant with this catabolic reaction is the production of energy and intermediate molecules that are required to sustain the numberless of metabolic reaction essential for the maintenance of cellular organization and membrane integrity of living cells (Kader and Saltveit, 2003). The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate), NADH (reduced nicotinamide adenine dinucleotide) and PPi (pyrophosphate), and is a primary function of respiration. The overall process of aerobic respiration involves regeneration of ATP from ADP (adenosine diphosphate) and Pi (inorganic phosphate) with the release of CO_2 and H_2O. The summary equation for cellular respiration is written as follows:

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + 686\ \text{kcal/mole}$$

During respiration, plant cells use O_2 from the atmosphere and food in the cell to produce energy and CO_2. If the O_2 concentration within the tissue falls below about 2% or if the CO_2 concentration raises about 5%, the predominant respiratory reactions within the tissue could change from aerobic to anaerobic. The tissue will then undergo fermentation with the production of compounds that give the product and undesirable flavor and aroma (Saltveit, 2003).

2.2.5 Plant Responses to Stresses

When plants are exposed to stressful environmental conditions, the production of reactive oxygen species (ROS) increases and can cause significant damage to the cells. Antioxidant defenses, which can detoxify ROS, are present in plants. Most of the physiological disorders and metabolic abnormalities in plants are caused through the production of deleterious free radicals/reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radical ($\bullet O^-_2$), hydrogen peroxide (H_2O_2), hydroxyl ion (OH^-) and free hydroxyl radical (•OH), which are invariably produced in mitochondria, endoplasmic reticulum, micro-bodies, plasma membranes and chloroplasts during normal metabolism but more so on exposure to stresses (Singh, 2009). The ROS detoxification process in plants is essential for the protection of plant cells and their organelles against the toxic effect of these species. The differences in subcellular localization and biochemical properties of antioxidant enzymes and the distinct responses in gene expression, in addition to the presence of non-enzymatic mechanisms, result in a versatile and flexible antioxidant system able to control the optimum ROS levels. The ROS detoxification systems include enzymatic and non-enzymatic antioxidant components (Caverzan et al., 2012). The non-enzymatic antioxidants involved include ascorbic acid, chlorophyll, carotenoids, phenolic compounds (phenolic acid and flavonoids) and glucosinolates. Enzymatic antioxidants comprise catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX) and gluthione reductase (GR).

2.2.5.1 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is one of the major reactive oxygen species (ROS) in plant tissues. It is produced in chloroplasts and mitochondria via electron transport, where oxygen is reduced to superoxide, which is further dismuted into H_2O_2 spontaneously or catalyzed by superoxide dismutase (SOD). When plants are subject to environment stress, it accumulates and leads to oxidative damage. Accumulating evidence suggests that H_2O_2 is a key signaling molecule involved in plant response to both biotic and abiotic stresses, such as pathogen attacks, extreme temperatures, drought, excessive radiation, ozone and wounding. Therefore it is frequently important to determine hydrogen peroxide concentration in plant tissue.

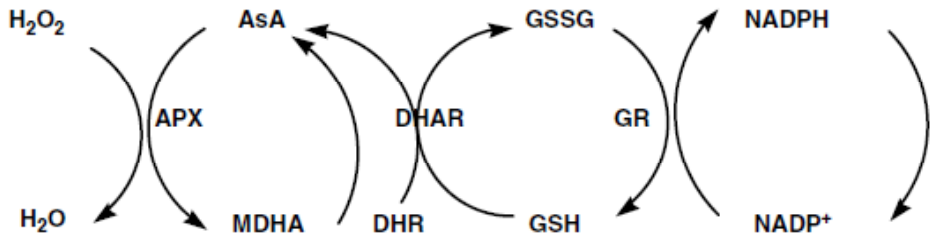

Figure 2.3 Ascorbate-glutathione cycle (Halliwell-Asada pathway) of H_2O_2 scavenging. AsA, ascorbate; APX, ascorbate peroxidase; MDHAR, mondehydroascorbate reductase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GSH, glutathione (Quan et al., 2008)

Ascorbate is present in chloroplasts, cytosol, and vacuole and apoplastic spaces of leaf cells in high concentration. It is the most important antioxidant in plants, with a fundamental role in the removal of H_2O_2. The ascorbate/glutathione cycle is the most important H_2O_2 detoxifying system in the chloroplasts. But it also has been an identifying system in the cytosol, peroxisomes, and mitochondria. Two enzymes are involved in the regeneration of reduced ascorbate, namely MDHAR, which uses NAD(P)H directly to recycle ascorbate, and dehydroascorbate reductase (DHAR). Monodehydroascorbate is reduced directly to ascorbate using electrons derived from the photosynthetic electron transport chain (ETC) as follows in Figure 2.3.

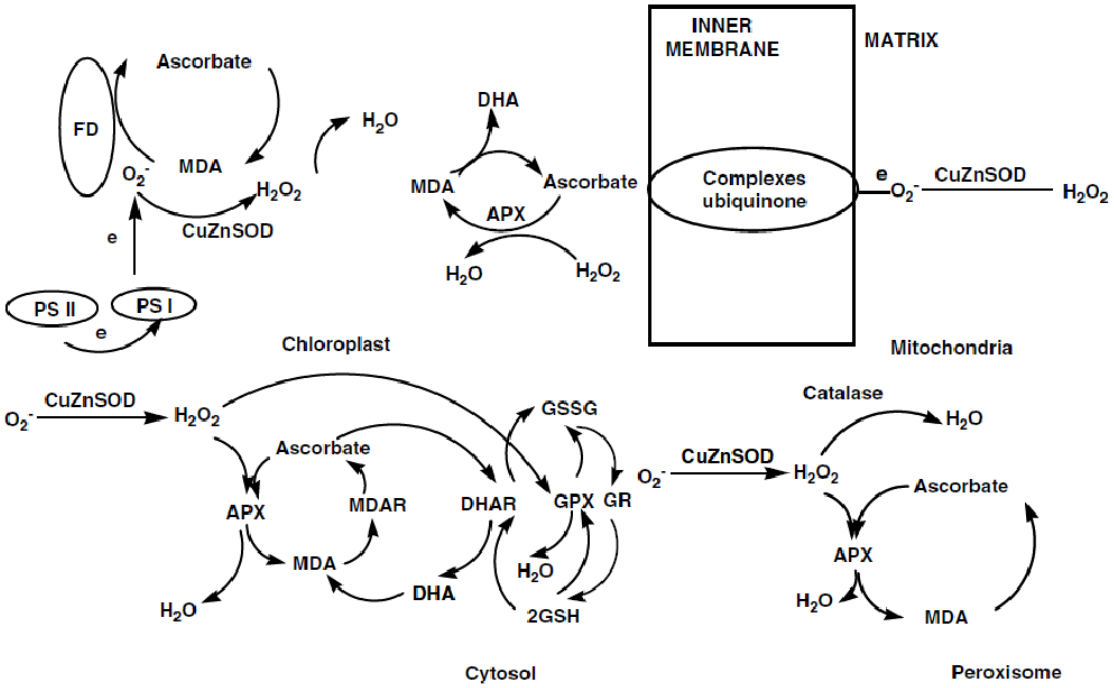

Figure 2.4 Localization of reactive oxygen species (ROS) scavenging pathways in plant cells (Quan et al., 2008)

Localization of reactive oxygen species (ROS) scavenging pathways in plant cells including chloroplast, peroxisome, cytosol and mitochondria. A transmission electron micrograph of a portion of a plant cell is used to demonstrate the relative volumes of the different cellular compartments and their physical separation. The enzymatic pathways responsible for ROS detoxification are shown in Figure 2.4. The water–water cycle detoxifies O_2- and H_2O_2. H_2O_2 distributes in peroxisomes, mitochondria, chloroplast and cytosol. Catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and other components of the ascorbate-glutathione cycle are also present in mitochondria and peroxisomes. Glutathione peroxidase (GPX) is involved in H_2O_2 removal in the cytosol. H_2O_2 can easily diffuse through membranes, and antioxidants such as glutathione and ascorbic acid (reduced or oxidized) can be transported between the different compartments (Mittler et al., 2004; Quan et al., 2008).

2.2.5.2 Non-Enzymatic Antioxidant Scavenging Systems in Fruits and Vegetables

A. Ascorbic Acid

Ascorbic acid (AsA) is one of the most important compounds for human nutrition present in fruits and vegetables. The role of AsA in disease prevention has been associated with its capacity to neutralize ROS. AsA and its first oxidation product dehydroascorbic acid (DHA) can be considered vitamin C. AsA is a water soluble carbohydrate-derived compound showing antioxidant and acidic properties due to the structure formula presence in Figure 2.5. Plants synthesize AsA via a pathway that uses L-galactose as a precursor (Vicente et al., 2009).

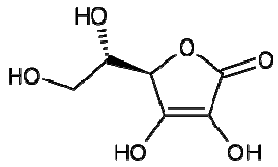

Figure 2.5 Structure of ascorbic acid, a main antioxidant present in fruits and vegetables (Vicente et al., 2009)

AsA is highly susceptible to oxidation, either directly or through the enzyme ascorbate oxidase catalyzing the oxidation of AsA to DHA, with the concomitant reduction of molecular oxygen to water. AsA can even be oxidized during eating, while food is being chewed. However, it is important to consider that the first breakdown product of AsA, DHA, still has vitamin C activity and all activity is lost if oxidation proceeds

beyond this stage. When vegetables are cooked before eating, high losses of vitamin C can occur. For instance, starchy vegetables may lose between 40% and 80% of their vitamin C during cooking because of leaching and oxidation. Loss of vitamin C can be reduced by steaming or by placing the vegetables directly into boiling water. Freezing reduces vitamin C slightly, but at the end of long-term frozen storage (12 months), a significant decrease (33 to 55%) in vitamin C can occur (Vicente et al., 2009).

B. Chlorophyll

Broccoli senesces rapidly, and the florets turn yellow at room temperature after harvest. The yellowing is an important indicator of quality deterioration in harvested broccoli and occurs with chlorophyll (Chl) breakdown. The breakdown of Chl is the main visible symptom of broccoli senescence, therefore it is important to investigate Chl catabolism and develop treatment to maintain Chl to prolong the shelf-life of broccoli.

In the degradation pathway proposed in Figure 2.6, the first step in Chl *a* degradation starts with the removal of phyton chain and the formation of chlorophyllide *a* (Chllide *a*) by chlorophyllase (Chlase), and the form of Chlide *a* retains a green color (Amir-Shapira et al., 1987). In the second step, the acidic removal of the Mg^{2+} form Chlide *a* to produce pheophorbide *a* (Pheide *a*) is induced by Mg-dechelatase (Langmeier et al., 1993) or Mg-dechelating substances (Shioi et al., 1996), and the Pheide *a* through the loss of green color. After that, Pheide *a* derived from chlorophyll (Chl) is converted into a primary fluorescent Chl catabolite (pFCC) in a two-step reaction by Pheide *a* oxygenase (PAO) and red Chl catabolite reductase (RCCR), in which the green pigments disappear or become colorless. The pFCC is modified in several steps, and nonfluorescent Chl catabolites are stored in the vacuole as final Chl catabolites (Hörtensteiner, 2006). In addition, Chl-degrading peroxidase or Chl oxidase are suggested to also be involved in Chl degradation as the first-step enzymes that oxidize Chl *a* and form $C13^2$-hydroxychlorophyll *a* ($C13^2$-OHChl *a*) (Fukasawa et al., 2010).

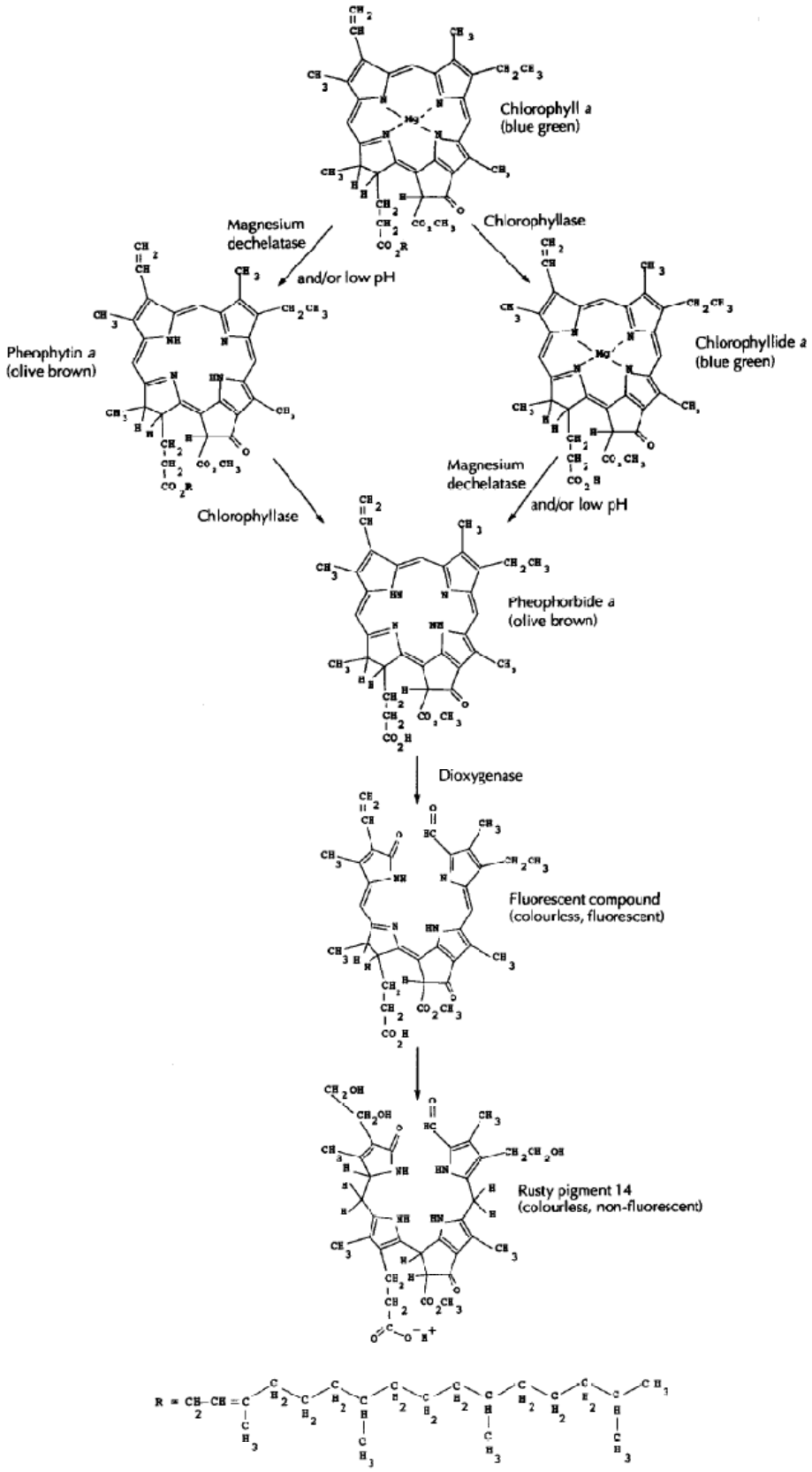

Figure 2.6 Proposed mechanism for the degradation of chlorophylls in plant tissues (Heaton and Marangoni, 1996)

C. Carotenoids

Fruits and vegetables are the main sources of carotenoids in human diet. The presence of conjugated double bonds in carotenoids has a main role in determining their antioxidant properties. Carotenoids are lipid-soluble, secondary plant metabolites in vegetables known to be essential in the human diet and are reported to confer various positive health-promoting effects when consumed (Farnham and Kopsell, 2009). Cruciferous vegetables contain high concentrations of carotenoids, which are believed to be chemopreventive and associated with a decreased risk of various human cancers in epidemiological studies (Higdon et al., 2007).

D. Phenolic Compounds

Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. Many of the phenolics have been shown to contain high levels of antioxidant activities. Phenolic compounds contribute to the overall antioxidant activities of plants mainly due to their redox properties. Generally, the mechanisms of phenolic compounds for antioxidant activity neutralize lipid free radicals and prevent decomposition of hydroperoxides into free radical (Javanmardi et al., 2003; Rohman et al., 2010). This group a great of compounds is derived from the aromatic amino acids, phenylalanine and tyrosine. Their main functions are acting as deterrents of potential predators or antimicrobials, protecting against UV-radiation and contributing to the pigmentation of fruits and flowers. Phenolic compounds can contribute to the astringency and bitter taste of some products. In general, they also accumulate in the peel more than in the pulp of fruits. The general characteristic of the compounds within this group is to have aromatic rings with variable degrees of hydroxylation. Phenolic compounds are easily oxidized to quinones. A large number of phenolic compounds have been identified in plants. They are subdivided into different subclasses, such as phenolic acid, flavonoids and other compounds (e.g., lignans, stibenes, tannins, coumarins and lignin) (Vicente et al., 2009).

1) Phenolic acid

Phenolic acids include derivatives of benzoic and cinnamic acid (Figure 2.7). The most common benzoic acid derivatives are *p*-hydroxybenzoic, vanillic, syringic and gallic acid, while common cinnamic acid derivatives include *p*-coumaric, caffeic, ferulic and sinapic acid. The derivatives differ in the degree of hydroxylation and methoxylation of the aromatic ring. The contribution of each of the phenolic compounds to the antioxidant capacity depends on their structure. For instance, the number of hydroxyls present in the molecule can increase the antioxidant capacity (Vicente et al., 2009).

Benzoic acid Cinnamic acid

Figure 2.7 Structures of the major classes of phenolic acids present in fruits and vegetables (Vicente et al., 2009)

2) Flavonoid

Flavonoids represent a large group of phenolic compounds with two aromatic rings in their structure that are associated together by a 3C-oxygenated heterocycle. Phenolic compounds are usually present as glycosides, which reduce their activity against free radicals and increase their solubility. At the cellular level, they are compartmentalized in the vacuoles. There are different classes of flavonoids such as (Vicente et al., 2009):

a) Flavones and flavanols
b) Flavanones, flavanols
c) Isoflavones
d) Proanthocyanidins
e) Anthocyanidins

One of the best described flavonoids, quercetin is a member of class of flavonoids called flavonols and is found in abundance in broccoli, curly kale, leek, cherry tomato, apple, green and black tea, black grapes and blueberry (Lakhanpal and Rai, 2007).

Flavonoids can be divided into various classes on the basis of their molecular structures as shown in Figure 2.8 (Vicente et al., 2009).

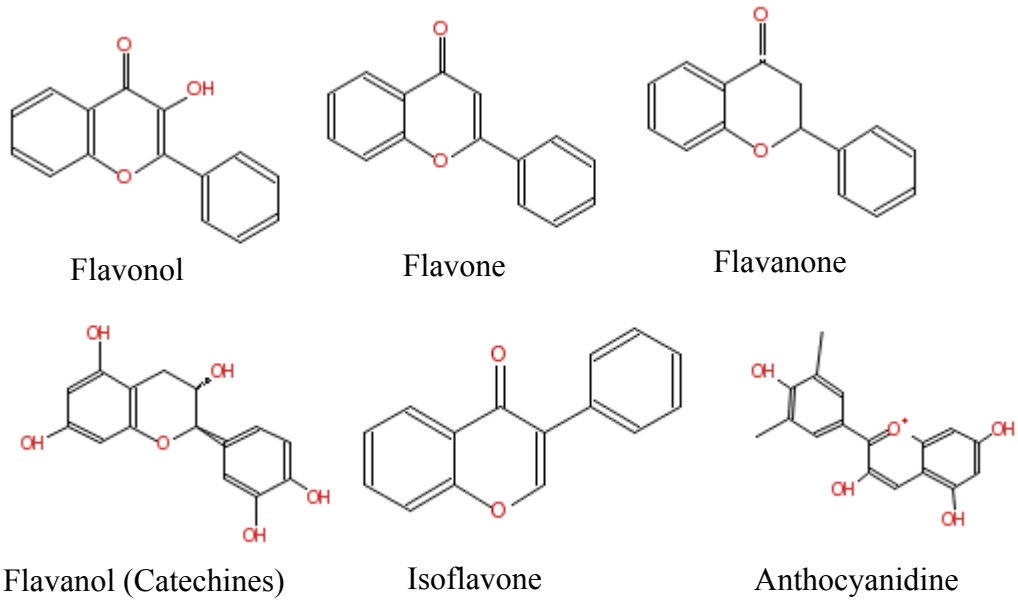

Figure 2.8 Structures of the major classes of flavonoids present in fruits and vegetables (Lakhanpal and Rai, 2007)

E. Glucosinolate Compounds

Broccoli has been proposed as a bioactive compound associated with cancer pretection, based on its glucosinolates (Finley, 2005). Glucosinolates are particularly abundant in Brassicaceae, an important group of cultivated plants in the world (Rosa and Rodrigues, 2001). Glucosinolate (thioglucosides) compounds are sulfur-containing compounds present in *Brassica* vegetables. A generalized structure of glucosinolate is shown in Figure 2.9. In broccoli, the mean content is approximately 1 $mmol \cdot g^{-1}$ fresh weight (a typical portion of vegetables) (Paolini et al., 2004). The glucosinolates are anions and occur in plants mostly as potassium salts. Breakdown products of glucosinolates possess important sensory properties such as odor and flavor, and they may induce physiological changes in humans, including carcinogenesis inhibition and goiter formation. At high intake levels, certain glucosinolates are associated with toxic effects, especially goiter development (Rangavajhyala et al., 1998).

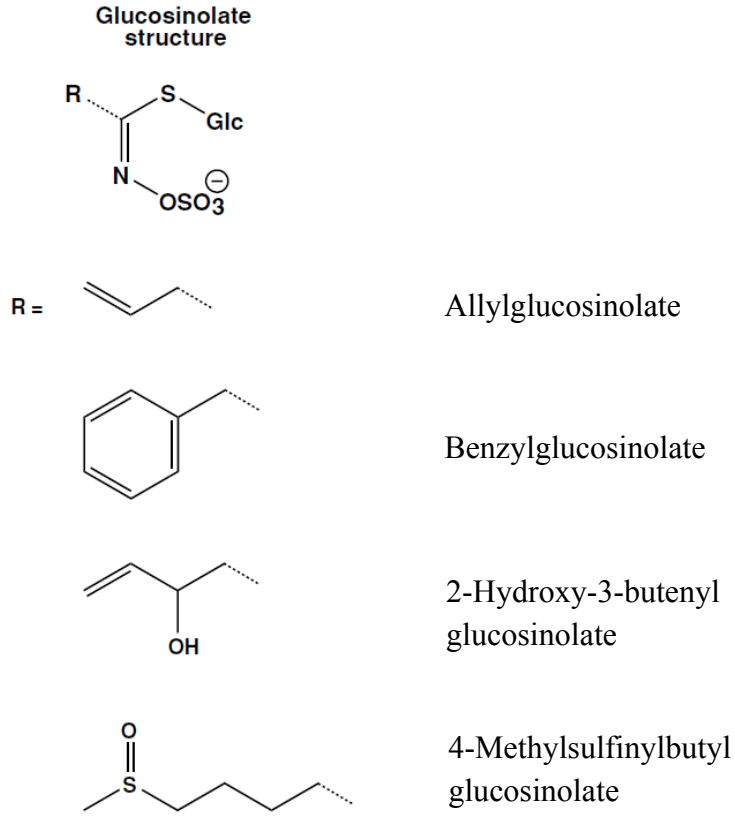

Figure 2.9 Chemical structures of glucosinolates. The common structure is shown, as well as examples of some specific glucosinolates cited in the text that show typical variation in the structure of the side chain (Halkier and Gershenzon, 2006)

Every glucosinolate contains a central carbon atom that is bonded via a sulfur atom to the glycone group, and via a nitrogen atom to a sulfonated oxime group. In addition, the central carbon is bonded to a side group; different glucosinolates have different side groups. Sulfur is an essential nutrient required for growth, and is primarily used to synthesize cysteine and methionine. The sulfur-containing amino acids play pivotal roles in the structural and catalytic functions of proteins. Sulfur nutrition is important in the species within the order Brassicales (e.g., white cabbage, broccoli, cauliflowers, and capers) for the synthesis of anticarcinogenic glucosinolated compounds (Vicente et al., 2009).

The breakdown of glucosinolates, a group of thiocyanate compounds found in cruciferous plants, is catalysed by dietary or microbial myrosinase. This hydrolysis releases a range of breakdown products among which are the isothiocyanates, which have been implicated in the cancer-protective effects of cruciferous vegetables

(Rouzaud et al., 2003). Glucosinolates are degraded upon plant damage to a variety of hydrolysis products that are responsible for virtually all of the biological activities of this compound class (Halkier and Gershenzon, 2006). The process begins with myrosinase-catalyzed hydrolysis of the thioglucoside linkage, leading to the formation of glucose and an unstable aglycone. Depending on the structure of the side chain and the presence of additional proteins and cofactors, the aglycone then rearranges to form different products, including isothiocyanates, nitriles, and thiocyanate (Halkier and Gershenzon, 2006). The chemical structures of glusocinolates are similar in all the plants in which they are present (>3000 crucifer species). Their basic structure consists of a ß-D-thioglucose group, a sulfonated oxime group, and a side chain derived from methionine, phenylalanine, trytophane, or branched-chain amino acids (designated as R in Figure 2.10) (Keck and Finley, 2004). The sulfate group of a glucosinolate molecule is strongly acidic, and plants accumulate glucosinolate by sequestering them as potassium salts in plant vacuoles. The potent odor and taste of glucosinolate has resulted in a proposed role of glucosinolate in herbivore and microbial defense (Keck and Finley, 2004).

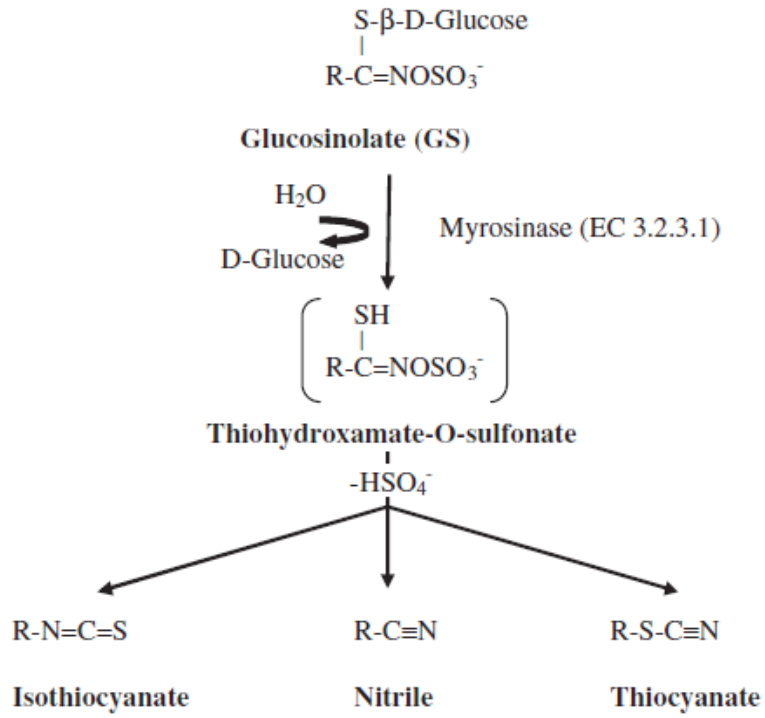

Figure 2.10 Bioactivation of glucosinolates. Hydrolysis of glucosinolates by the endogenous enzyme myrosinase, to form glucosinolate hydrolysis products: nitriles, isothiocyanates, and thiocyanates (Keck and Finley, 2004)

Glucosinolates are not bioactive in the animal that consumes them until they have been enzymatically hydrolysed to an associated iso-thiocyanate (Rouzaud et al., 2003) by the endogenous myrosinase enzyme that is released by disruption of the plant cell through harvesting, processing or mastication. The hydrolysis products of common glucosinolates are shown in Figure 2.8 (Keck and Finley, 2004).

2.2.5.3 Enzymatic Antioxidant Scavenging Systems in Fruits and Vegetables

A. Catalase

Catalase (CAT) was the first antioxidant enzyme to be discovered and characterized. Catalase is a common enzyme found in nearly all living organisms that and functions to catalyze the reduction of H_2O_2 to water and molecular oxygen. It is localized in mitochondria and peroxisomes, and is absent in chloroplast (Kuk et al., 2003)

Hydrogen peroxide (H_2O_2) is an important signal molecule involved in plant development and environmental responses. Changes in H_2O_2 availability can result from increased production or decreased metabolism. While plants contain several types of H_2O_2-metabolizing proteins, catalases are highly active enzymes that do not require cellular reductants as they primarily catalyse a dismutase reaction (Mhamdi et al., 2010). Catalase is one of the most active catalysts produced by nature. At high concentrations of substrate, catalase decomposes toxic H_2O_2 at an extremely rapid rate using the "catalatic" reaction in which H_2O_2 acts as both acceptor and donor of hydrogen molecules (Scandalios et al., 1997).

The catalase reaction is as follows:

$$2H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2$$

Plants subjected to various chemical and environmental oxidative stresses that accumulate H_2O_2 in these tissues frequently demonstrate a decrease in CAT activity. Therefore, reducing the CAT decrease in stressed plants is essential to overcome stress damage, particularly when CAT activity becomes a limiting factor in the scavenging of active oxygen species produced in the oxidative stresses (Shim et al., 2003).

A. Peroxidase

Peroxidase (POD) is a present enzyme in animals, plants and microorganisms. They are an iron heme protein that catalyses the reduction of H_2O_2 with a concurrent oxidation of a substrate, mostly located in cell wall and involved in oxidation of phenol compounds towards the synthesis of lignin (Kuk et al., 2003).

In general terms, the majority of reactions catalysed by the classical plant peroxidases can be represented as follows, where RH and $R^{\bullet}$ represent a reducing substrate and its oxidized radical product, respectively (Veitch, 2004).

The peroxidase reaction is as follows:

$$H_2O_2 + 2RH \xrightarrow{POD} 2\,H_2O + 2\,R^{\bullet}$$

The plant peroxidase superfamily can be separated into 3 main classes based on their structural properties according to the Welinder System (Welinder, 1992).

Class I: peroxidases include the yeast cytochrome C peroxidase, the ascorbate peroxidases and the catalase-peroxidases. These are not glycosylated and they lack signal peptides, calcium ion content and disulfide bridges.

Class II: are secreted fungal peroxidases. They have signal peptides used for secretion through the endoplasmic reticulum, two calcium ions, carbohydrate content and four disulfide bridges.

Class III: comprises all of the plant secretary peroxidases. They have two conserved calcium ions, one *N*-terminal signal peptide, four conserved disulfide bridges located in different positions to those in the fungal peroxidases and an extra helical region which plays a role in access to the heme edge. Examples of class III peroxidases are the heme horseradish, peanut and barley peroxidases. All peroxidases have conserved arginine and histidine residues adjacent to the heme-binding site.

Plant peroxidases are believed to be involved in many physiological and biological processes, including the cross-linking of molecules in cell wall, auxin oxidation, oxidation of cinnamyl alcohols prior to the their polymerization during lignin and suberin formation, and responses to biotic and abiotic stresses (Kawano, 2003).

B. Superoxide Dismutase

Superoxide dismutase (SOD), a class of metal-containing proteins, accelerates the dismutation reaction of superoxide radical ($^{\bullet}O^{-}_{2}$) into H_2O_2 and molecular oxygen (Apel and Hirt, 2004). SOD removes singlet oxygen, prevents formation of hydroxyl radicals and has been implicated as an essential defense against potent toxicity of oxygen. There are three different types of SOD according to their catalytic metal ions: the Fe-SOD, Mn- SOD, and Cu/Zn-SOD. Fe-SOD is localized in the chloroplast, Mn-SOD is localized in the mitochondria and peroxisome, and Cu/Zn-SOD is localized in the cytosol, chloroplast, peroxisome and cell wall (Alscher et al., 2002).

The superoxide dismutase reaction is as follows:

$$^{\bullet}O^{-}_{2} \xrightarrow{SOD} O_2$$

$$O_2 + 2H^{+} \xrightarrow{SOD} H_2O_2$$

The beneficial effects of normal levels of SOD were reported to be deleterious effects imposed by greater than normal levels of these enzymes. Among the proposals advanced to account for the harmful effects of too much SOD are (Liochev and Fridovich, 2007):

(a) Low levels of O^{-}_{2} may exert a beneficial effect by terminating radical propagated chain reactions. Thus, if $L^{\bullet}$ is a lipid radical that reacts with O_2 to yield the corresponding chain-propagation peroxyl radical ($LOO^{\bullet}$), it will react with O^{-}_{2} plus a proton to yield the relatively unreactive hydroperoxide (LOOH).

(b) Overproduction of an ordinarily abundant metallo-SOD must be in competition with the biosynthesis of other metalloenzymes. Thus hyperproduction of Cu,ZnSOD would limit availability of Cu(II) required for the formation of cytochrome oxidase.

(c) SODs may exert activities unrelated to the dismutation of O^{-}_{2} and those extraneous activities may be deleterious at hyperlevels of the SOD. Thus the Cu,ZnSOD acts as a cysteine oxidase, a nonspecific peroxidase and as a reductant: superoxide oxidoreductase.

(d) Last is the view that overproduction of SOD necessarily causes more production of H_2O_2.

C. Ascorbate Peroxidase

A major hydrogen peroxide detoxifying system in plant cells in the ascorbate-glutathione cycle, ascorbate peroxidase (APX) enzymes play a key role catalyzing the conversion of H_2O_2 into H_2O, using ascorbate as a specific electron donor (Caverzan et al., 2012). The importance of APX is a key enzyme regulating ROS levels acting in different subcellular compartments (chloroplasts, cytosol, mitochondria and peroxisomes). The expression of APX encoding genes is differentially modulated by several abiotic stresses in different plant species. APX isoforms play important and direct roles as protective elements against adverse environmental conditions. The diverse effects of the knockdown or knockout of different APX genes on the plant growth, physiology and antioxidant metabolism indicate that APX may also regulate redox signaling pathways involved in plant development (Caverzan et al., 2012).

Expression of APX genes can be activated by specific factors such as pathogen attack, mechanical pressure, injury, UV-B radiation, water deficiency, salt stress, excess excitation energy, excessively high or low temperature, atmospheric pollution (e.g. sulphur dioxide, nitrogen oxide and dioxide or ozone), excess metal ions, deficiency of some mineral salts (e.g. phosphates), and herbicides (Dabrowska et al., 2007).

The ascorbate peroxidase reaction is as follows:

$$\text{Ascorbate} + H_2O_2 \xrightarrow{\text{APX}} \text{Dehydroascorbate} + H_2O$$

Ascorbate, the electron donor to which the enzymes show high affinity, is the substrate utilized in the reduction of hydrogen peroxide. APXs are extremely sensitive to the ascorbate concentration. Detoxification of H_2O_2 by APX is followed by a set of reactions catalyzed by monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). Together these reactions comprise one of the most important antioxidant systems in plants – the ascorbate-glutathione or Halliwell-Asada cycle. In this cycle, ascorbate and glutathione act as reducing substrates for scavenging H_2O_2, and are ultimately recycled at the expense of ATP and NAD(P)H. The ascorbate-glutathione cycle plays a critical role in chloroplast redox protection. These organelles are devoid of catalases and are a major source of superoxide and H_2O_2 as a consequence of the highly energetic reactions that take place

there during photosynthetic activity. A series of reports have convincingly demonstrated the activity of ascorbate-glutathione cycle enzymes in the mitochondria of plant cells, confirming that this cycle also plays an important role in protecting this organelle against the harmful effects of the ROS that are regularly produced in respiratory chain reactions (Dabrowska et al., 2007).

D. Glutathione Reductase

Glutathione reductase (GR) is a potential enzyme of the enzymatic antioxidant system, which sustains the reduced status glutathione via ascorbate-glutathione pathway and plays a vital role in maintenance of sulfhydryl (-SH) group and acts as a substrate for glutathione-*S*-transferases. GR has been characterized and has been used in transgenics to provide the plants with tolerance against the oxidative stress (Yousuf et al., 2012).

Glutathione reductase (GR), which converts oxidized glutathione (GSSG) to reduced glutathione (GSH) using NADPH, is ubiquitous in living organisms. It is necessary for maintaining a high ratio of GSH/GSSG in the plant cells and accelerating the H_2O_2 scavenging pathway in plants particularly under stress conditions (Smith et al., 1989).

The glutathione reductase reaction is as follows:

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2\,GSH + NADP^+$$

Glutathione reductase has been purified and characterized from different sources such as bacteria, fungi, plants and human. The molecular weight of plant GRs ranges from 60 to 190 kDa. GR has been mainly localized in chloroplasts, mitochondria and the cytosol. Generally, more than 80% of its activity in photosynthetic tissues was reported to be chloroplastic isoforms (Pang and Wang, 2010). GR plays an essential central role in cell defense against reactive oxygen metabolites by efficiently maintaining the cellular reduced GSH pool through catalyzing the reduction of GSSG to GSH with the accompanying oxidation of NADPH (Gill et al., 2013).

Studies regarding the GR have shown an increased GR activity in various plant species under different types of abiotic stresses. From studies using transgenic plants, it has been proved that GR plays a prominent role in conferring resistance to oxidative stress

caused by drought, ozone, heavy metals, high light, salinity, cold stress, etc. (Yousuf et al., 2012).

2.3 Treatments to Extend Shelf-life and Enhance Safety of Fresh-cut Produces

Microorganisms play an important role in the shelf-life of fresh-cut fruits and vegetables (Moreira et al., 2008). Fresh-cut fruits and vegetables generally specify a washing or sanitizing step to remove dirt, pesticide residues and microorganisms responsible for quality loss and decay (Sapers, 2006). Most research has focused on the efficacy of washing and sanitizing treatments for assuring the microbial safety of fresh-cut produce by using physical and chemical methods (Gil et al., 2009). Generally, the efficacy of sanitizer is dependent on the types of vegetable, the characteristics of the produce surfaces (cracks, crevices, hydrophobic tendency and texture) and the tissue location (inner and outer leaves) (Kondo et al., 2006). The microorganisms can be found in tissue damage and also accumulate access to nutrients for their metabolism. In addition, microorganisms can survive in the cells or areas that have not been penetrated by washing treatment (Moreira et al., 2008). The combination of chemical and physical methods for sanitizing fresh-cut produce has potential applications to the fresh-cut industry to control microbial and maintain quality during storage (Allende et al., 2006). Therefore, there is much interest in developing safer and more effective sanitizers for fruits and vegetables.

2.3.1 Heat Treatment

Hot water plays an important role in the sanitation of food products before processing. Some food products are treated with hot water to eliminate insects and to inactivate microorganisms and enzymes (FAO, 2003). Heat treatment was effective in improving postharvest quality for a variety of fresh-cut fruits and vegetables and horticultural products (Lemoine et al., 2010). For broccoli, the loss of green florets color is a major limitation to shelf life. Heat treatments such as hot water, hot air (Funamoto et al., 2002; 2006) and vapor heat treatment are able to maintain the postharvest quality and are especially effective to reduce yellowing and delay chlorophyll loss in broccoli florets. Funamoto et al. (2002) reported that broccoli was kept in circulating hot air at 50 °C for 1 or 2 h, followed by storage at 15 °C in dark and was kept in polyethylene film bags

with the top folded over and placed in a corrugated cardboard box. Chlorophyll (Chl) contents of broccoli treated at 50 °C for 2 h showed almost no change after 4 days of storage at 15 °C. Chlorophyllase activity of heat-treated broccoli for 2 h decreased during storage before the occurrence of yellowing. Chl oxidase activity of heat-treated broccoli for 2 h remained unchanged. These results indicate that heat treatment could reduce Chl degradation due to the suppression of Chl degrading enzyme activities.

The cost of a typical hot water technology commercial system is significantly less than that of a commercial vapor heat treatment system (Fallik, 2004). HW is applied at temperatures between 43 and 53 °C for periods of several minutes up to 2 h for quarantine treatments. The temperature and exposure time that is most beneficial for fresh harvested quality depend on cultivar, fruit maturity, fruit size and condition during the growing season (Fallik, 2004). Heat treatments can also be used to inhibit ripening processes or to induce resistance to chilling injury (CI) and external skin damage during storage, thus extending storability and marketing (Fallik, 2004).

The advantages of hot water treatment are that it results in a reduction of the yellowing of broccoli florets by suppression of the activities of chlorophyll (Chl) degradation enzymes, such as chlorophyllase (Chlase) and Chl-degrading peroxidase (Funamoto et al., 2002; 2003). In addition, hot water treatment prevented rot development, killed skin-borne decay-causing agents (Fallik, 2004). Pre-storage heat treatments (immersion in a hot water bath) at 50 °C for 10-25 min, at 52.5 °C for 7.5-12.5 min and at 55 °C for 1-5 min inhibited postharvest anthocyanin synthesis in white asparagus spears stored for 6 days at 2.5°C and one more day at 25 °C and the initial white spear color was retained. Heat-treated spears kept at 50 °C for 10-15 min and 55 °C for 3-5 min had excellent overall appearance. The most effective treatment to inhibit anthocyanin synthesis and to avoid loss of overall spear appearance was a heat treatment at 55 °C for 3 or 2 min (Siomos et al., 2005).

Mode of Action of Heat Treatment

Microorganisms are able to grow over a wide range of temperatures, but have a typical optimum range. The optimum and limiting temperatures for an organism are a reflection of the temperature range of its enzyme systems, which in turn are determined by their three-dimensional protein structures. Once the optimum value is passed, the loss of

activity caused by denaturation of enzymes causes the rate of growth to fall away sharply, and continued increase in temperature leads to microbial death. The mode of action of hot water against fungal and microbial infection is probably due to direct effects on the pathogen (cell damage) and indirect effects on the fruit or vegetable host (induction of resistance mechanisms) (Margosan et al., 1997 site by Barry-Ryan, 2012). Some bacteria alter their cell membrane in response to heat by increasing the ratio of *trans* to *cis* fatty acids in the membrane. This structural change is thought to decrease fluidity caused by increasing temperatures. The reported data regarding heat-induced sublethal injury might be due to different times and temperature regimes used. The use of mild heat treatment inhibits pathogens by the induction of defense mechanisms in the outer layers of the epicarp (Barry-Ryan, 2012). Moist heat treatment causes destruction of microorganisms by denaturation of macromolecules, primarily proteins. Destruction of cells by lysis may also play a role. While "sterility" implies the destruction of free-living organisms that may grow within a sample, sterilization does not necessarily entail destruction of infectious matter.
(www.en.wikipedia.org/wiki/Moist_heat_sterilization#Action_on_micro-organisms)

2.3.2 Ozone Treatment

Ozone (O_3), the tri-atomic form of oxygen (O_2), is a strong antimicrobial agent and the United Stated Food and Drug Administration (US FDA) has classified it as "generally recognized as safe" (GRAS), and it can be directly applied to food products (FDA, 2012).

Ozone is one of the most potent disinfectant agents, due to it being a powerful oxidizer that has a broad spectrum of antimicrobial activity (Alexandre et al., 2011). The required O_3 concentrations and the disinfection rates depend on the type of microorganism, extent of microbiological contamination, temperature, pH, turbidity and presence of ozone-oxidisable substances (Alexandre et al., 2011). Ozone is also highly unstable in water and decomposes to oxygen in a very short time. Less than half the O_3 activity remains after 20 min in pure water and the activity may only have a residual of 2-3 min in more complex, potable water. In postharvest packing water or fresh-cut processing water with suspended soil and organic matter, the half-life of ozone activity may be less than 1 min. Moreover, lower water temperatures extend the half-life of O_3 (Suslow, 2004).

Mode of Action of Ozone Treatment

The ozone molecule acts as dipole with electrophilic and nucleophilic properties. Organic and inorganic compounds in aqueous solutions react with ozone in one of two pathways (Staehelin and Hoigné, 1985 site by Khadre et al., 2001):

(a) Direct reaction of organic compound (M) with molecular ozone.

$$O_3 + M \longrightarrow M_{ox}$$

(b) Decomposition of ozone in water into a radical (for example, OH) that reacts with the compound (M).

$$O_3 \xrightarrow{OH^-} OH \xrightarrow{M} M_{ox}$$

Molecular ozone reactions are selective and limited to unsaturated aromatic and aliphatic compounds. Ozone oxidizes these compounds through cycle-addition to double bonds. Oxidation of sulfhydryl groups, which are abundant in microbial enzymes, may explain rapid inactivation of microorganisms and bacterial spores by O_3 (Khadre et al., 2001). Reactions of O_3 alter the function and activity of organelles and cellular components. Kim et al. (1999) suggested that the double bonds of unsaturated lipids in the cell membrane of bacteria were the principle site of the O_3 reaction, leading to cellular lysis and leakage. The targeting of sulfhydryl groups in enzymes by ozone can lead to cell death. Moreover, O_3 can also damage nucleic acids, which could affect both cellular function and reproduction of pathogenic species (Kim et al., 1999).

Ozonated water is reported to have 1.5 times the oxidizing potential of chlorine and 3,000 times the potential of hypochlorous acid (Gil et al., 2010). Moreover, O_3 has been reported to reduce microbial populations, extending the shelf life without negative effects on sensory quality of fresh-cut produces such as broccoli (Forney et al., 2003; Kumar and Kim, 2010), cilantro (Wang et al., 2004), celery (Zhang et al., 2005), lettuce (Beltrán et al., 2005; Selma et al., 2007; Ölmez and Akbas, 2009), and cantaloupe (Selma et al. 2008). The first step in the use of O_3 is to know the O_3 concentration to apply. In general, fungi are more resistant to O_3 than bacteria. However, O_3 doses that kill spores of postharvest fungal pathogens vary widely, and depend on the fungal species, spore morphology, substrate, moisture status of the species, length of exposure, and O_3 doses (Sela and Fallik, 2009). Kumar and Kim (2010) demonstrated that

washing fresh-cut broccoli with ozonated water at 2 ppm for 180 sec reduced total aerobic and coliform counts. However, the effectiveness of O_3 as a disinfectant depends on the application methods, O_3 concentration, exposure or contact times, types of pathogenic and spoilage microorganisms, levels of initial inoculums, and sensitivity of fresh produce to O_3 (Liew and Prange, 1994). Most O_3 studies with fresh produce have focused on antimicrobial efficacy; however, little information exists regarding its effect on the nutritional constituents and sensory quality of fresh and fresh-cut produce (Rico et al., 2007; Ölmez and Kretzchmar, 2009), including fresh-cut broccoli. Forney et al. (2003) reported that the application of O_3 gas at 0.7 ppm delayed the yellowing of fresh-cut broccoli. Also, symptoms will occur if produce is exposed to excessively high concentrations and long time periods that cause oxidative stress by ozone (Forney et al., 2007; Hildebrand et al., 2008).

2.3.3 Sodium Chlorite Solution

Chlorinated water is usually applied to reduce microbial populations by approximately 1-2 log $CFU \cdot g^{-1}$. 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) [CAS No. 7758-19-2] is marketed in two forms, as a solid characterized by approximately 80% SC and as an aqueous solution. SC is soluble in water, insoluble in non-polar solvents, and sparingly soluble in polar solvents (Merck, 2001). Aqueous solutions are colorless to greenish yellow and exhibit a slight chlorine-like odor. While the chlorite ion is stable in aqueous solution, under acidic conditions, chlorite forms a semi-stable intermediate, chlorous acid ($HClO_2$). Chlorous acid disintegrates to chlorine dioxide (ClO_2), which further degrades to chlorous (ClO_2^-) and ultimately chloride (Cl^-) is formed. The extent of each of the degradation pathways and thus the proportion of each of the oxy-chlorine species depends on part of the pH of the solution. Other factors such as temperature and alkalinity of the water also affect the composition of the oxy-chlorine constituents. At a pH in the range of 2.3-3.2, chlorous acid (5 to 35%) is the main active ingredient produced by the reaction and is in equilibrium with H^+ and the chlorite ion (ClO_2^-) (65 to 95%) (Rao, 2007).

$$NaClO_2 + H^+ \longleftrightarrow HClO_2$$

Mode of Action of Sodium Chlorite Treatment

SC is approved by the FDA as a "secondary direct food addition permitted in food for human consumption", specifically as an antimicrobial treatment. Moreover, based on the inadequate availability of information, the International Agency for Research on Cancer determined that SC is not classifiable as human carcinogenicity (IARC, 1991). The antibacterial activity of SC is attributed to the oxidative effect of chlorous acid ($HClO_2$), which is the metastable oxychlorine species (Inatsu et al., 2007). Once formed, chlorous acid gradually decomposes to form chlorate ion – chlorine dioxide – chloride ion. The mode of action of SC derives from the uncharged chlorous acid ($HClO_2$) (AMS, 2013), which is able to penetrate bacterial cell walls and disrupt protein synthesis by virtue of its reaction with sulfhydryl, sulfide, and disulfide containing amino acid and nucleotides. Chlorous acid kills microorganisms by direct action on the cell membrane and by the oxidation of cell constituents (Castillo et al., 1999).

Commercially, SC is manufactured *via* chemical or electrochemical reduction of sodium chlorate to chlorine dioxide gas (IARC, 1991; ATSDR, 2004). Chlorine dioxide dissolved in aqueous sodium hydroxide is subsequently reduced with hydrogen peroxide (H_2O_2) to form SC. The resulting solution contains SC at concentrations ranging from 30 to 50% and is dried to a solid state by a sodium chlorite content of ca. 80% or diluted to an aqueous solution. More specifically, sodium chlorate, which is obtained by electrolytic oxidation of sodium chloride, is reduced electrochemically in the presence of hydrochloric acid to chlorine dioxide and chlorine, with sodium chloride formed as a by-product (sodium chloride is recycled to form sodium chlorate) (Rao, 2007).

Alternatively, chlorine dioxide is obtained by reacting together sodium chlorate, hydrogen peroxide, and sulfuric acid as in the following (Rao, 2007):

$$2NaClO_3 + H_2O_2 + 2H_2SO_4 \longrightarrow 2ClO_2 + 2NaHSO_4 + O_2 + 2H_2O$$

Subsequently, chlorine dioxide is absorbed in a solution of sodium hydroxide and hydrogen peroxide to form a sodium chlorite solution as in the following (Rao, 2007):

$$2ClO_2 + 2NaOH + H_2O_2 \longrightarrow 2NaClO_2 + O_2 + 2H_2O$$

Sulfuric acid can be used to neutralize any excess alkali, and the resulting SC solution can be drum-dried to form solid SC. The dried product is adjusted to contain at least 79% of SC by addition of sodium chloride, sodium sulphate, or sodium carbonate. Instead of producing a solid product, the resultant SC solution also can be diluted to a 25% solution (Rao, 2007). SC solution was described as a sanitizer and is an 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).

A summary of chemical and physical properties for SC and chlorous acid is provided in Table 2.3 (AMS, 2013).

Table 2.3 Properties of sodium chlorite (solid and aqueous) and chlorous acid (AMS, 2013)

Property	Description
Color	White crystalline solid (80% technical grade) Solution: colorless to light green
Density/Specific gravity	Crystal: 2.468 g/cm^3 Bulk, Packed, 80% Technical grade: 1.176 25% aqueous solution: 1.21 g/ml
Dissociation constants	pK_a of chlorous acid ($HClO_2$) = 1.72 at 25 °C
Hydrolysis	Sodium chlorite reacts with hydrogen ions to form chlorous acid (pK_a = 1.72 at 25 °C)
Melting or crystallization points (liquids)	25% aqueous sodium chlorite solution: -8 °C
Melting point or range (solids)	180-200 °C

Property	Description
Molecular weight	Sodium chlorite (solid): 90.44 g/mole Chlorous acid: 68.46 g/mole
Odor	Slight chlorinous (i.e., chlorine-like) odor
Oxidation stability (air)	Stable to air oxidation
Photolysis	80% technical grade is stable to photolysis; Photolysis of sodium chlorite solutions produces chlorine dioxide
Physical state	White crystalline solid, slightly hygroscopic (80% technical)
Solubility in organic solvents	Sodium chlorite: Insoluble in non-polar organic solvents; sparingly soluble in polar solvents
Solubility in water	Sodium chlorite: 64 g per 100 g water
Thermal stability	Sodium chlorite decomposes at 180-200 °C (i.e., melting point)
Vapor pressure	25% aqueous solution: 21.085 mm Hg at 25 °C
Viscosity (liquids)	25% aqueous solution: 1.851 cps at 25 °C

Specific gravity = ratio of the density of a substance compared to the density of a reference substance (e.g., water).
CPS = centipoises (one hundredth of a poise), unit of measure for viscosity.

2.4 Storage of Fresh-Cut Processing

Cut and packaged produce provides consumers with speed and ease of preparation. However, cut produce can be expected to behave differently from intact produce during storage due to its response to wounding and damage to the skin. Possible results of slicing produce may include increased ethylene production and respiration rates, accelerated senescence, and enzymatic browning (Morris and Brady, 2005).

Freshly harvested broccoli has a very high respiration rate and is perishable. Storage temperature has a significant effect on the keeping quality of fresh-cut broccoli products. Therefore, broccoli must be cooled immediately after harvest to rapidly lower the respiration rate and must be kept at low temperature for maximum shelf life. Broccoli should be hydrocooled or packed in ice immediately after harvest and kept at 0 °C to maintain good marketable condition, fresh green color, and vitamin C content. Florets were categorized as marketable or unmarketable based on yellowing and flower opening (Rushing, 1990). Senescence and the associated loss of chlorophyll in broccoli florets are highly depending on temperature, RH, and storage atmosphere. The recommended conditions are storage with sufficient air circulation and spacing between containers; broccoli should keep at a satisfactory quality for 10 to 14 days at 10 °C (32 °F) and relative humidity of 95 to 100% (Nunes and Emond, 2003). Longer storage is undesirable because leaves may become discolored, buds may become yellow and drop off, and tissues may soften (Hemphill, 2010).