CHAPTER 2

Literature Review

2.1 Pak-choi

Pak-choi (*Brassica rapa* var. *chinensis*) belongs to the family Brassicaceae (previously the Cruciferae); it is a non-heading Chinese cabbage. Pak-choi evolved in China, and its cultivation was recorded as far back as the 5th century AD. It is widely grown in southern and central China, and Taiwan. This group is a relatively new introduction in Japan where it is still referred to as 'Chinese vegetables'. It was introduced into southeastern Asia in the Malacca Straits Settlement in the 15th century. It is now widely cultivated in the Philippines and Malaysia, and to a lesser extent in Indonesia and Thailand. In recent years, it has gained popularity in North America, Australia and Europe. Pak-choi is an erect biennial herb, cultivated as an annual. In the vegetative stage, it is glabrous, dull green and 150-300 mm tall, and in the generative stage reaches 700 mm. The leave are arranged spirally, not forming a compact head but spreading, in groups of 15-30. Petioles are enlarged, flattened, 15-40 mm wide and 5-10 mm thick, growing in an upright manner forming a sub cylindrical bundle. Each white, greenish-white to green leaf blade is orbicular to obovate, 70-200 × 70-200 mm. Stem leaves are entire, tender, blistering, shiny green to dark green and auriculate-clasping (Dixon, 2007).

Pak-choi is rich in vitamins A and C (USDA, 2016). Moreover, pak-choi is a rich source of glucosinolates (GSs) that can reduce the activity of carcinogens, and the risk of cancer and coronary heart diseases (Chen *et al*., 2008; Higdon *et al*., 2007 and Verkerk *et al*., 2009). The report of Zhu *et al.* (2013) suggested that pak-choi had high levels of beneficial glucosinolates when it was harvested at 20-25 days after transplanting.

2.2 Organic production system

The definition of an organic production system used by the International Federation of Organic Agriculture Movement (IFOAM, 2017) is “Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved.”

The Royal Project Foundation has implemented “The Organic Vegetable Program” since 2002. The organic vegetables from farmers in the Royal Project areas are certified by A.C.T. (IFOAM Accredited) and Organic Thailand standards. Pak-choi is one of many vegetables that are produced organically. In 2016, pak-choi represented 26% of the total organically produced vegetables. Pak-choi is favored by farmers because of its short production time, 40-45 days including the seedling stage, and high yield/unit area, 250-300 kg for one greenhouse (180 square meters).

2.3 Senescence of leafy green vegetables

Senescence is the process whereby plant cells age and finally die, or, in other words, that plant cells have an ‘expiration date’ (Løkke, 2012). This process is a natural change in plant cells which causes breakdown and unavoidable cell death (King and O'Donoghue, 1995). Senescence is part of the plant’s survival and development program, as leaf senescence aims at remobilization of leaf nutrients, such as nitrogen and carbohydrates. In naturally senescing leaves, senescence occurs in a coordinated manner at the whole-leaf level starting from the tip and margins towards the base of the leaf (Guiboileau *et al*., 2010). Leaf senescence is the last step of leaf development (Lim *et al*., 2007). There are several studies that describe the characteristics of the senescence of green leafy vegetables including the following:

2.3.1 Chlorophyll degradation

In green leafy vegetables, the chlorophyll degradation is an obvious visual change during senescence (Løkke, 2012). In *Brassica* crops especially pak-choi, senescence is most widely characterized by yellowing caused by a breakdown of chlorophyll pigments inside the tissues. In the last stage, the tissues die and become necrotic. This stage is characterized by dehydration, complete color loss and abscission (Dixon, 2007). Able *et al*. (2005) reported that the chlorophyll degradation was related to ethylene production, the pak-choi leaves stored at 20°C produced ethylene causing the leaves to turn from green to yellow. During leaf senescence, the chlorophyll is degraded, the final product derived from the decomposition of chlorophyll is nonfluorescent chlorophyll catabolites (NCCs) which are colorless (Sakuraba *et al*., 2012).

The chlorophyll breakdown is related to the reuse of proteins in the plant, and protein degradation during senescence (Hörtensteiner, 2006). Matile *et al*., 1997 and Ferente *et al*., 2004 reported that chlorophyll is degraded by chlorophyllase enzymes when the membrane deteriorates. The chlorophyllase is located in the membrane envelop of chloroplasts, so chlorophyllase activity is correlated with disruption of membranes. The chlorophyllase catalyzes the first step in the catabolic pathway of chlorophyll. The last products of chlorophyll degradation are NCCs. The age of the plant affects the leaf yellowing rate. The storage of 7 types of Brassica at 20 °C indicated that a leaf's age affected its yellowing rate; old leaves have a faster yellowing rate and shorter shelf life than young leaves (O'Hare *et al*., 2001a). The rate of degradation depends on many factors such as hormones; cytokinin, gibberellin, ethylene and ABA, and lack of water and light. Gibberellin and light can delay chlorophyll degradation while ethylene, ABA and lack of water induce senescence. Moreover, chlorophyll degrades slowly in young tissue as compared to aging tissue (Boonyakiat, 1997).

2.3.2 The changes of sugar

Plants accumulate sugar from photosynthesis and it was used as a substrate in the respiration process. In pak-choi leaves, glucose and fructose are the main energy substrates (Xiangyang and Lianqing, 2000). The postharvest study of detached pak-choi leaves during storage at 2, 10 and 20 °C, it was found that at 20 °C, ethylene evolution occurs concurrently with yellowing with a rapid decline in sugar concentration immediately prior to yellowing. The decrease in total soluble sugar was related to the chlorophyll degradation during leaf senescence. The degradation of chlorophyll occurs when 60% of the soluble sugar was used, and the sugar concentration declined during the senescence of leaves. The rate of sugar decline was the key determinant of leaf yellowing (Able *et al*., 2005).

2.3.3 The changes of vitamin C

Factors affecting vitamin C content in fruits and vegetables include genotypic differences, preharvest climatic conditions and cultural practices, maturity and harvesting methods, and postharvest handling procedures (Lee and Kader, 2000). After harvest, leafy vegetables lose vitamin C, but it is not clear whether it is caused by temperature or water loss. Kale, collards, turnip greens, spinach, cabbage, and snap beans exposed to conditions favorable for water loss have more rapid loss of vitamin C. However, wilting is much less important than temperature with the loss of vitamin C in kale. (Ezell and Wilcox, 1959; Paull, 1999). After harvest, temperature management is the most important factor to maintain vitamin C in fruits and vegetables (Lee and Kader, 2000). The lower storage temperature can maintain the stability of vitamin C levels, the leave of rocket salad was stored at 4 ºC had higher total vitamin C content than in 15 °C (Kim and Ishii, 2007). While two African leafy vegetables, *Cassia tora* and *Corchorus tridens*, stored under different temperature conditions, i.e. room storage (20 °C), refrigerated storage (4 °C) and frozen storage (-18 °C). The degradation of ascorbic acid was highest as a result of frozen storage, followed by room temperature storage (Prabhu and Barrett, 2009). In addition, the lettuce precooled by vacuum cooling before cold stored for 2 week had a positive effect on the ascorbic acid content (He *et al*., 2004). Likewise, baby pak-choi was precooled by vacuum cooling before storage for 5 days had a higher vitamin C content than non-precooled produce (Boonyakiat *et al*., 2009).

2.3.4 The changes of glucosinolate

Glucosinolate (GS) is a secondary metabolite, a group of thioglucosides in plants of the Brassicales order (Wittstock and Burow. 2010). There are over 100 different glucosinolates that can be classified into three groups: the aliphatic group having an alkyl or alkenyl side-chain (sinigrin, progoitrin), the aromatic group (gluconasturtiin) and the indolyl group (glucobrassicin, neoglucobrassicin) (Mithen *et al*., 2000).

Several reports suggested that crop genetics and environmental factors such as temperature, photoperiod and light quality during the period before harvest and their interaction affect glucosinolate concentration (Aires *et al*., 2011; Engelen-Eigles *et al*., 2006; Mithen *et al*., 2000 and Verkerk *et al*., 2009). Thirty five varieties of pak-choi grown in the wet season had 72% higher glucosinolate content than grown in the dry season. The wet season has higher rainfall, average temperatures, solar intensity and longer daylength, than the dry season (Hanson *et al*., 2009). Five botanical groups of *B. oleracea* had high concentrations of total and indole glucosinolates that were associated with cultivation at higher temperature and photosynthetic photon flux (PPF) as well as to longer day length (Charron *et al*., 2005). Whereas total glucosinolate content in kale sown in the fall was lower than spring and summer, because in the fall there was increased activity of myrosinase which is a hydrolytic enzyme for glucosinolates (Velasco *et al*., 2007).

In addition, glucosinolate content was also associated with senescence because senescent leaves have high myrosinase activity (Barth and Jander, 2006). When the plant tissue is damaged the glucosinolate in cytoplasm is hydrolyzed by myrosinase. Moreover, the glucosinolate content is related to water loss which causes deterioration of plant tissue (Barth and Jander, 2006; Mithen *et al*., 2000 and Wittstock and Burow. 2010). In broccoli, the glucoraphanin (aliphatic glucosinolate) concentration decreased when the broccoli heads deteriorated. The postharvest handling conditions affects the glucosinolate content and the visual quality of produce (Rangkadilok *et al*., 2002 and Winkler *et al*., 2007)

2.3.5 The changes of crude fiber

The total dietary fiber in vegetables varies due to plant maturity, season, fertilizer or chemical used, plant cultivars or varieties, geographical location and the method used for analysis (Uusiku *et al*., 2010; Aletor *et al*., 2002; Punna and Prachuri, 2004; Tendaj *et al*., 2013). The seasonal effect on crude fiber may be the result of nutrient supply and substrate competition. In *Brassica rapa* var. *narinosa*, the content of crude fiber grown in mid August and harvested in mid September was higher than grown in late August and harvested in early October (Kalisz *et al*., 2013). Waterleaf (*Talinum triangulare* (Jacq.) Willd) cultivated in the dry season (May-July) had significantly lower total dietary fiber than in the rainy season (February-April). These results are related to water stress in plants; during the dry season limited rainfall and high light intensity causes a lower chlorophyll content than in the rainy season, because of the low supply of nutrients, and substrate competition. The substrate for complex compounds like dietary fiber may decrease causing low levels of this substance in the dry season (Anderwulan *et al*., 2015).

After harvest, the formation of fiber cells or lignin can occur for example, in asparagus, cauliflower, broccoli, okra, onions and five leafy vegetables *(Basella alba*, *Colocasia esculenta*, *Corchorus olitorius*, *Solanum melongena* and *Talinum triangulare*) consumed in Southern Côte d'ivoire (Acho *et al*., 2015; Kay, 1991; Marlett, 2000; Rodríguez *et al*., 2006 and Siripanich, 2006). The increase of fiber content is due to the increase of uronic acid in the insoluble fiber fraction (Marlett, 2000).

2.3.6 Respiration rate

Harvested vegetables and fruits are still living, and carry out metabolic activities and respiration processes. The shelf life of produce with a high respiration rates tends to be shorter than those with low rate. It is an important parameter in postharvest quality. Respiration in vegetables changes carbohydrates, starches and sugars, to CO_2 and H_2O, with the releasing of heat energy. Oxygen (O_2) is used in this process, and carbon dioxide (CO_2) is produced. (Kader, 2002 and Løkke, 2012). Respiration is controlled by enzyme activities which depend on temperature. When temperature increases by 10 °C respiration increases by 2-3 fold up to 25-30 °C. Respiration rate is reduced and cell death occurs if the temperature exceeds 30 °C. So temperature is a main factor effecting decay and relates to shelf life (Finger *et al*., 1999; Løkke, 2012). At high temperatures, enzymes may be denatured and the respiration rate is decreased. Physiological disorders may also occur if temperatures are too low for respiration. (Fonseca *et al*., 2002). Pre-cooling beneficial to removes the field heat before storage and reduces the respiration rate as a way to maintain the quality of vegetables (Kader, 2002; Brosnan and Sun, 2001). The research of Garido *et al*. (2015) reported that baby spinach which was vacuum cooled and hydro cooled before stored at 7 °C showed the lowest respiration rate compared to room cooling and forced air cooling. While Ding *et al*. (2016) reported that vacuum cooling was the best method for reducing the respiratory rate of harvested broccoli compared to hydro cooling, room cooling and broccoli without precooling.

2.3.7 Ethylene production

Generally, ethylene production rates increase with maturity at harvest and with physical injuries, disease incidence, increased temperature up to 30 ºC, and water stress. On the other hand, ethylene production rates by fresh horticultural crops are reduced by storage at low temperature, by reducing O_2 levels (less than 8%), and elevated CO_2 levels (more than 2%) around the commodity (Kader, 2002). The storage conditions affected ethylene production; pak-choi stored at 20 ºC had ethylene production from leaves that rapidly increased for 4 days, but did not increase if stored at a low temperature (4 ºC) for 8 days (Xiangyang and Lianqing, 2000). Reducing ethylene levels around produce delays the senescence of fruit and vegetables and therefore has the potential to reduce the need for refrigeration during transport and storage, which would result in substantial energy savinGlucosinolate. Pak-choi, broccoli, mint and green bean were stored at 0, 5, 10, and 20 °C in an atmosphere containing 0.001, 0.01, 0.1, and 1.0 µL L^{-1} ethylene. The postharvest life of pak-choi, broccoli, mint and green bean increased as the temperature and ethylene concentration decreased (Li *et al*., 2017). In many plant tissue, ethylene treatment results in rapid loss of chlorophyll, the green color in leaves and unripe fruit (Reid, 2002). Likewise, detached pak-choi leaves stored at 2, 10 and 20 ºC, ethylene evolution occurs concurrently with yellowing at 20 ºC. As temperature was lowered, ethylene production decline slowed or became negligible (Able *et al*., 2005).

King and O’Donoghue (1995) summarized the overall effects of senescence on fruit and vegetables in Table 2.1. The changes that take place during senescence can be seen both at a physical level (from a consumer’s point of view) and a physiological level (what happens in the plant).

Table 2.1 Physical and physiological changes accompanying the senescence of fruit and vegetables

Physical changes:	
Color	Loss of green color
Texture	Softening Wilting Drying
Loss of resistance to pathogens	Development of infections Lesions
Physiological changes:	
Cellular	Loss of chlorophyll, disassembly of chloroplast structure Degradation of cell walls Altered membrane composition Loss of cellular compartmentalization, release of vacuolar contents
Composition	Altered sugar content, switch to alternative substrates for respiration Net loss of RNA Increased protease activity, net loss of protein Altered amino acid content Altered plant hormones; ethylene, cytokinin and abscisic acid

Note: Modified from King and O’Donoghue (1995).

The factors affecting senescence

Senescence is a complicated process and interacts with internal factors in plants and the environment (Boonyakiat, 1997).

Internal factors

1) Genetic. The senescence of *Arabidopsis* leaves involves the expression of more than 800 genes, collectively termed senescence-associated genes (SAGs group), that relate to the senescence period (Buchanan-Wollaston *et al.*, 2003 and Lim *et al*., 2003).
2) Plant hormones such as ethylene, cytokinin and abscisic acid (ABA). Ethylene and ABA induce senescence while cytokinin, auxin and gibberellin inhibit senescence. However, in many cases, such hormones may have the opposite effect on the tissue type, growth and development stage and interact with other hormones. (Boonyakiat, 2013). Able *et al.* (2005) reported that storage at 20 ºC, caused increased ethylene production and color change from green to yellow in the leaves of pak-choi.
3) Endogenous signals induce leaf senescence. It is a reproductive signal from oval and fruit to branches which is not clear but does not involve in plant hormones (Boonyakiat, 2013).
4) The water condition in leaves is an important factor in the senescence process of leaves. Leaf senescence occurs when loss of water is more rapid than normal, and is related to ABA. ABA increases in senescence and induces *SAG113* expression. *SAG113* expression is suppressed when the water loss ratio in the leaf is reduced, which in turn, leads to delayed leaf senescence. Guard cell movement responds to the ABA decrease (Zhang *et al.*, 2012). Reducing water loss can delay senescence because the cell has more water which inhibits ethylene synthesis or may affect the interaction of ABA and ethylene (Boonyakiat, 2013).

External factors

1) Environmental temperatures have a direct impact on the metabolic processes of the produce. The leafy vegetables will rapidly senescence at high temperatures and slowly at low temperatures, except the produce sensitive on chilling injury.

The respiration rate increases when temperature is increased and decreases or stops when the temperature is higher than optimum (Boonyakiat, 2013). Respiration in vegetables changes carbohydrates, starches and sugars, to CO_2 and H_2O, with the releasing of heat energy. Respiration is controlled by enzyme activities which depend on temperature. When temperature increases by 10 ºC respiration increases by 2-3 fold up to 25-30 ºC and the rate is reduced and cell death occurs if the temperature higher than 30 ºC. (Finger *et al.*, 1999; Løkke, 2012). At high temperatures, enzymes may be denatured and the respiration rate is decreased. Physiological disorders may also occur if temperatures are too low for respiration. (Fonseca *et al.*, 2002). There were seasonal effects on the respiration rate of wild rocket; the produce harvested in spring had a higher respiration rate than in early and late summer (Seefeldt *et al.*, 2012). In addition, seasonal effects on respiration were observed in four baby leaf crops, salad rocket, wild rocket, mizuna and watercress, harvested in two cuttings between February and March. The second cutting was made about 20-30 days after the first harvest.

2) Atmosphere control during storage or transport is an important external factor to the control of the senescence rate of vegetables, followed by temperature. Lower oxygen and higher carbon dioxide conditions can delay yellowing in leafy vegetables by reducing the ethylene synthesis of vegetables and also reduce the efficiency of the ethylene as well (Boonyakiat, 2013). Beaudry (1999) reported that the rate of chlorophyll destruction could controlled by low O_2 and elevated CO_2. Likewise, Schouten *et al.* (2009) found that the decay of chlorophyllide was affected by the gas composition in storage, and the effect of low O_2 and high CO_2 only occurred at high temperatures.
3) Light can delay senescence of leafy vegetables. But the effect of light is relatively minor on postharvest produce because most produce is kept in the dark. In case of certain harvested vegetables, the light may prolong green coloration when the vegetables receive a suitable light intensity (Boonyakiat, 2013).

2.4 The effect of season and harvesting time on the quality of vegetables

Effective control of the quality of the produce throughout the handling process, from harvesting to the consumer is essential. Quality control begins in the field by choosing the suitable time to harvest for the highest quality (Kader, 2001). Effects of season and harvesting time on the quality of vegetables have been discussed by numerous authors and include:

Chlorophyll is the green pigment in the chloroplast that enables photosynthesis in plants (Løkke, 2012). Plants need suitable light and temperature for chlorophyll synthesis and photosynthesis. Seasonal effects on chlorophyll synthesis resulting in degradation are caused by day length and temperature. Pak-choi planted at 21 ºC had the highest content of total chlorophyll followed by 18 ºC and 25 ºC (Mahmud *et al*., 1999). The seasonal effects on chlorophyll depend on differences in solar radiation. The highest total chlorophyll content of baby spinach occurred in August when radiation was lowest. The lowest total chlorophyll occurred in June when radiation was highest (Bergquist *et al*., 2006). On the other hand, the assessment of Xiangyang and Bagshaw (2001) suggested that leaf yellowing of pak-choi was caused by high temperatures and delays through the handling system.

The total dietary fiber in vegetables varies due to plant maturity, season, fertilizer or chemical used, plant cultivars or varieties, geographical location and the method used for analysis (Uusiku *et al*., 2010; Aletor *et al*., 2002; Punna and Prachuri, 2004; Tendaj *et al*., 2013). The seasonal effect on crude fiber may be the result of nutrient supply and substrate competition. Waterleaf (*Talinum triangulare* (Jacq.) Willd) cultivated in the dry season (May-July) had significantly lower total dietary fiber than in the rainy season (February-April) (Andarwulan *et al*., 2015). In Chinese flat cabbage (*Brassica rapa* var. *narinosa*), the content of crude fiber grown in mid August and harvested in mid September was higher than grown in late August and harvested in early October (Kalisz *et al*., 2013). These results are related to water stress in plants; during the dry season limited rainfall and high light intensity causes a lower chlorophyll content than in the rainy season, because of the low supply of nutrients, and substrate competition. The substrate for complex compounds like dietary fiber may decrease causing low levels of this substance in the dry season (Andarwulan *et al*., 2015).

L-ascorbic acid is synthesized from sugars provided through photosynthesis in plants. Normally, low light intensity during the growing period results in a low content of vitamin C in plant tissue. In contrast, high light intensity during the growing period results in a higher content of vitamin C. In addition, temperature influences the composition of plant tissues during growth and development. So optimal temperature control is an important factor for maintaining vitamin C content in fruits and vegetables. Thirty five pak-choi varieties planted in the rainy season had 48% higher ascorbic acid than in the dry season because in rainy season has higher rainfall, higher average temperatures and solar intensities and longer daylength than the dry season (Hanson *et al.*, 2009). Likewise, *Brassica rapa* var. *narinosa* cultivated in central Europe had a higher vitamin C content when grown in mid August and harvested in mid September than when grown in late August and harvested in early October. Because in second schedule had higher light intensity (Kalisz *et al*., 2013). Acikgoz (2016) reported that pak-choi planted in late autumn-early winter had higher ascorbic acid than in late winter-early spring because late winter-early spring had limited light due to clouding and low light intensity that reduced ascorbic acid content in plant tissue. Furthermore, Makus and Lester (2004) suggested that the amount of vitamin C in leafy mustard greens (*Brassica juncea* L.) increased when harvested during daylight hours because of high light intensity.

Plants accumulate sugar from photosynthesis and it is used as a substrate in the respiration process. In pak-choi leaves, sugar is the main energy substrate consisting of glucose and fructose (Xiangyang and Lianqing, 2000). Therefore, the plant has conducted photosynthesis during the day, resulting in a high sugar content. Lipton (1987) and Clarkson *et al*. (2005) suggested that leafy vegetables should be harvested in late afternoon, when the energy substrate level is high. Likewise, harvesting some baby salad leaves at the end of day is associated with accumulation of sucrose following daily photosynthesis and can extend shelf life. Broccoli which received sun light for a full day before harvest at 6 PM had a higher starch level than when harvested at sunrise. The conversion of starch to sugar fraction in broccoli contributes to maintenance of the sugar level (King and Morris, 1994; Hasperué *et al*., 2011 and Hasperué *et al*., 2014). Sugar content is also related to the quality of light received in each season. Radicchio grown in spring had higher content of simple and total sugar than summer/fall (Francke and Majkowska-Gadomska, 2008). In Brussel sprouts, the planting dates (every 10th of April, May, June and July) affected the content of sugar, it increased with later planting dates. (Mirecki, 2006).

Several reports suggested that crop genetics and environmental factors such as temperature, photoperiod and light quality during the period before harvest and their interaction affect glucosinolate concentration (Aires *et al*., 2011; Engelen-Eigles *et al*., 2006; Mithen *et al*., 2000 and Verkerk *et al*., 2009). Thirty five varieties of pak-choi grown in the wet season had 72% higher glucosinolate content than grown in the dry season. The wet season has higher rainfall, higher average temperatures and solar intensities and longer daylength than the dry season (Hanson *et al*., 2009). Five botanical groups of *Brassica oleracea* had high concentrations of total and indole glucosinolates that were associated with cultivation at higher temperatures and photosynthetic photon fluxes (PPF) as well as to longer daylength (Charron *et al*., 2005). Whereas total glucosinolate content in kale sown in fall was lower than spring and summer, because in the fall there was increased activity of myrosinase which is a hydrolytic enzyme for glucosinolates (Velasco *et al*., 2007). In addition, glucosinolate content was also associated with senescence because senescent leaves have high myrosinase activity (Barth and Jander, 2006).

Respiration in vegetables changes carbohydrates, starches and sugars, to CO_2 and H_2O, with the releasing of heat energy. Respiration is controlled by enzyme activities which depend on temperature. When temperature increases by 10 ºC, respiration increases by 2-3 fold up to 25-30 ºC. Respiration rate is reduced and cell death occurs if the temperature exceeds 30 ºC. So temperature is a main factor effecting decay and relates to shelf life (Finger *et al*., 1999; Løkke, 2012). At high temperatures, enzymes may be denatured and the respiration rate is decreased. Physiological disorders may also occur if temperatures are too low for respiration. (Fonseca *et al*., 2002). Many research reported that effects of the respiration rate on leafy vegetables such as Seefeldt *et al*. (2012) reported that the wild rocket harvested in spring had higher respiration rate than in early and late summer. In addition, seasonal effects on respiration were observed in four baby leaf crops, salad rocket, wild rocket, mizuna and watercress, harvested in two cuttings between February and March. The second cutting was made about 20-30 days after the first cutting. The respiration rates of mizuna and watercress were higher in the second cutting, while salad rocket and wild rocket exhibited slight differences in respiration between the first and second cuttings. (Martínez-Sánchez *et al*., 2008). Baby spinach was harvested at 8.30, 13.00 and 17.30. In spring, the highest respiration rate occurred at 17.30. But no differences in respiration among harvest times were observed in winter (Garrido *et al*., 2015).

2.5 Precooling

Vegetable losses occur rapidly at high temperatures. Vegetables are living tissue, physiological processes and disease occur after harvest. The vegetable need to use substrates in their respiration in order to gain the energy to survive, this process occurs slowly at low temperature. Controlling vegetable temperature and reducing the time that the vegetable is under an unsuitable temperature, is the way to maintain the quality of vegetables for a longer shelf life. Postharvest temperature management starts with planning time for harvesting vegetables. Precooling is reducing the temperature of vegetables after harvest, before storage, precooling should be done immediately after the vegetable is harvested. The purposes of precooling are to remove the heat accumulated from field (field heat) during harvest, reduce the rate of dehydration, respiratory rate and loss of water to extend shelf life. Cooling methods include room cooling, forced-air cooling, hydro cooling, ice packing and vacuum cooling. (Brosnan and Sun, 2001; Boonyakiat, 2015; Boonyakiat and Ratanapanon 2005; Thompson *et al*., 2002).

2.5.1 Room cooling

This method is low cost and easy to design but utilizes a large space and is the slowest to reduce produce temperature. The produce is kept in a cooling room at approximately 3 ºC, air cooling is used to reduce produce temperature. But the temperature should not be too low because chilling injury could be occur. The cool room should have good air circulation (~200-400 ft^3/min), a powerful refrigerant and low ceiling. For the best results, the produce container should be stacked with adequate spaces for air to flow easily and pass through the produce containers (Boonyakiat and Ratanapanon, 2005; Thompson *et al*., 2002).

2.5.2 Forced-air cooling

This method is faster than room cooling because it causes cold air to move through, rather than around the produce containers. It reduces temperature by cold air passing into a long and narrow tunnel. This cooling method applies cool air through the container for direct removal of heat from the product. The air flows from the high pressure side to the low pressure side and the heat from the produce is removed. However, the airflow may be slow or fast depending on the resistance. The total resistance depends on the volume and stacking of containers; if they are stacked transversely to the airflow and in many rows, more resistance occurs. This affects the pressure difference between the flows of air in and out. Moreover, the resistance also depends on the type of produce. The air circulation should be reduced or stopped when the produce cools down to the desired temperature, continued high air circulation would cause water loss. The limitations of this method is that it takes more time than hydrocooling and vacuum cooling and causes too more water loss in some produce. Force-air cooling should utilize containers suitable for airflow and stable for cold air (Boonyakiat and Ratanapanon, 2005; Thompson *et al*., 2002).

2.5.3 Hydrocooling

This method can be reduce temperature 15 times faster than room cooling, avoids produce water loss, maintains better produce texture and freshness, and can be used for large or small produce volumes. The limitations of hydrocooling are that the produce, packages and packing material must be tolerant of wetting, and some disease can develop when the produce is wet. Moreover it uses more energy than other methods and is limited to certain types of containers. Hydrocooling can use either an immersion or shower system to bring produces in contact with the cold water. The water for hydrocooling should include a low concentration of chlorine for sanitation and to protect the produce from some diseases. Caution is necessary in hydrocooling to ensure that the water contacts all the produce surfaces, and its temperature is not be too low to avoid produce damage (Boonyakiat and Ratanapanon, 2005; Thompson *et al*., 2002).

2.5.4 Ice packing

This method packs vegetables with ice. Ice packing has been used for a long time and is most beneficial with unrefrigerated vegetables that are transported by truck. It creates more humidity in the package or container and can reduce vegetables weight loss. The temperature is reduced faster when the ice contacts the vegetables. The ice packing can be done in many ways such as top icing, and liquid icing or ice-injection cooling. With top icing, the ice is packed on the top of containers. Ice-injection cooling uses water mixed with ice which is injected in the container. The weaknesses of ice packing are more ice but less vegetables in the container, and the water from the ice melting can affect to other produce (Boonyakiat, 2015; Thompson *et al*., 2002). The research of Boonyakiat and Chuamuangphan (2007a) and Boonyakiat and Chuamuangphan (2007b) found that pak-choi and broccoli precooled by packing with ice at the ratio of vegetables to ice of 1:1 had the best for quality and shelf life.

2.5.5 Vacuum cooling

Vacuum cooling has long been widely used as a rapid cooling method for fruits, vegetables and cut flowers (Brosnan and Sun, 2001). Vacuum cooling is the fastest and most stable method for reducing heat in produce. The produce cool down rapidly compared to other precooling and is popular with leafy vegetables (Boonyakiat and Ratanapanon, 2005). Vacuum cooling is achieved by the latent heat of vaporization rather than conduction. At normal air pressure of 760 mm Hg water will boil at 100 ºC. As air pressure is reduced, the boiling point of water is reduced, and at 4.6 mm Hg, water boils at 0 ºC (Thompson, 1996). The vegetable are placed in a closed vacuum chamber and the air pressure is reduced. This decrease in pressure causes the boiling point of the water drop to a temperature close to 0 °C. The evaporation of water is due to the boiling of water so that vaporization heat of vegetables is removed and the temperature of the vegetables is thereby reduced (Boonyakiat and Ratanapanon, 2005).

The speed and effectiveness of this cooling is related to the ratio between the produce volume and its surface area, so it is especially suitable for leafy vegetables (Thompson, 1996). This method is clean and quick but requires a strong structure to accommodate the pressure, high cost and the user must have expertise. So vacuum cooling is commonly used with large quantities of produce to be cost effective (Boonyakiat and Ratanapanon, 2005).

The components of vacuum cooling systems (Poonlarp *et al*., 2012; Wang and Sun, 2001)

The vacuum cooling system includes a vacuum chamber, vacuum pumping system, condenser, refrigerator, and automatic control and operation system. The details of the vacuum cooling system are presented in Figure 2.1.

1) Vacuum chamber used to enclose the produce must be a strong structure to accommodate the pressure. The size of the room depend on the size of the vacuum pump and the produce volume. During the cooling process, the door of the chamber is hermetically sealed; any leakage of air into the vacuum cooler increases the load of the vacuum pumping system.
2) The vacuum pump is designed to reduce the pressure in the vacuum chamber from the atmospheric pressure to the saturation pressure at the initial temperature of vegetables. The size of vacuum pump must be matched to the size of the vacuum chamber to enable an adequate pressure reduction rate.
3) The cooling system includes:
    - Evaporator: The chamber has a low pressure and a lot of water vapor when the cooling is running. The evaporator is used to catch the mist in the air conversion to ice for reduction the pressure in the vacuum room because the vacuum pump can not completely remove the mist to outside.
    - Condenser: It condenses the refrigerant from gas to liquid by cooling.
    - Compressor: It pump and compress the refrigerant from the evaporator sent to the condenser before sending the refrigerant back into the evaporator.
4) Condensate, it is the condensed water from the evaporator.

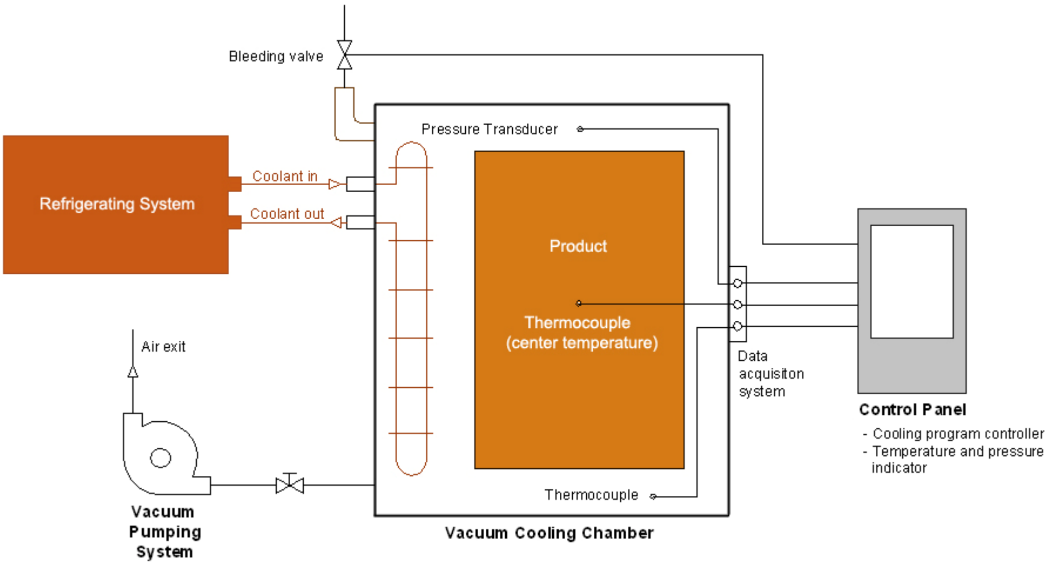

Figure 2.1 The schematic diagram of a vacuum cooler (Poonlarp *et al*., 2012)

The procedure of vacuum cooling (Sun and Zheng, 2006)

1) The vegetables are placed in the vacuum chamber then closed the chamber.
2) The air is evacuated out of the chamber by the vacuum pump. The vacuum pressure in the vacuum chamber is rapidly decreased.
3) The total pressure in the vacuum chamber is reduced from atmospheric to the set final pressure. With the reduction of pressure in vacuum chamber, the time at the beginning of boiling which is called the "flash point" that affects the change of water to gas, the temperature of the vegetables is rapidly reduced.
4) A lot of mist occurs inside the vacuum chamber, it sticks to the evaporator and becomes to ice.
5) The temperature decrease continuously until the specified period is reached or the product temperature drops as desired.
6) At the end of the process, the vacuum pump stops running and the ventilation valve of the chamber open to allow air flow into the chamber then the vegetables are moved to cold room storage.

The effect of vacuum cooling on the quality of vegetables

When harvesting vegetables from the field, there are physiological processes. High temperatures cause the rapid deterioration of vegetables. Preventing deterioration of vegetables is associated with proper temperature management; controlling vegetable temperatures and reducing the time that the vegetables are under an unsuitable temperature. It is a way to maintain the quality of vegetables for longer periods (Boonyakiat and Ratanapanon, 2005). Vacuum cooling can be used to shorten the processing time, extend product shelf life, and improve product quality and safety (Sun and Zheng, 2006). Previous studies had reported the good effect of vacuum cooling on some vegetables. The research of Kamon *et al*. (2013); Poonlarp *et al*. (2012) and Sirinanuwat *et al*. (2012) showed Chinese cabbages, organic chayote shoots, and organic coriander pre-cooled by vacuum cooling had a better appearance, longer shelf life, and lower weight loss than non-vacuum cooled produce. In addition, Poonlarp and Boonyakiat (2015) reported the chinese kale precooled under vacuum cooling with the best active packaging (M1) had a storage life of 14 days compared to the normal storage life of 3 days with non-precooled Chinese kale packaged in perforated polyethylene. Moreover, Alibas and Koksal (2015) reported that vacuum precooling was the best method for the storage of the cauliflowers under controlled atmosphere and room conditions in terms of quality parameters compared with forced-air, and high and low flow hydro precooling. The vacuum precooling method showed the lowest weight loss and deterioration rate of the cauliflower heads and highest hardness. Likewise, Ding *et al*. (2016) reported that vacuum cooling was the most effective method for extending the postharvest shelf life of broccoli in terms of cooling rate, respiration rate, chlorophyll, vitamin C, reducing sugar and sensory properties.