CHAPTER 4

Results and Discussion

Experiment 1 **Effects of season and harvesting time on senescence in pak-choi**

This study focuses on studying the changing physical and chemical changes after harvesting organic pak-choi during different periods of the day in each season. It is expected that the optimum harvesting time in each season will have the least effect on produce senescence. The organic pak-choi was harvested from a farmer's greenhouse at three particular times (morning, afternoon and evening) in three growing seasons (winter, summer and rainy). Then they were brought to the Royal Project packinghouse, located about 87 km from the farmer's plot in Muang district, Chiang Mai province where the vegetable was sorted for uniformity, trimmed, and packed in 25×40 cm perforated polyethylene bags with 18, 0.8-cm-diameter holes. Each bag contained a 300 g pak-choi sample. Subsequently, the samples were placed at an ordinary room condition (25-30 ºC, 49-70% RH). The data were recorded every day until senescence occurred (yellow leaf). The experimental design was 3×3 factorials in CRD with four replications. The first factor was growing season and the second was harvesting time. The results include the following parameters:

Weight loss

Only seasonal effects was observed on weight loss of organic pak-choi; harvest in summer resulted in the highest weight loss, 5.32±0.23% (Table 4.1). The average temperature was high (25.78 ºC) and the lowest relative humidity (56.13%) were recorded in the summer (Table 4.2). Loss of water was correlated with temperature and RH. Psychrometric charts present the relationship between temperature, RH and water vapor pressure. The water vapor pressure deficit is the difference between vapor pressure in the air and vapor pressure in the produce and determines the evaporation rate from the fresh commodity at the same temperature. When the produce temperature is not close to the air temperature, high RH cannot prevent water loss (Paull, 1999). Loss of water affects produce weight, visual quality, flavor and texture. Water content in plants has a direct effect on cell turgidity that is important for the texture of leafy vegetables such as 3% water loss in spinach results in a product unmarketable due to loss of texture (Sams, 1999). It is essential to assess the pak-choi handling system, since one of the major losses in weight results from wilting. At the retail market weight loss in summer (7.7%) is higher than winter (3.7%) (Xiangyang and Bagshaw, 2001).

Content of reducing sugar and total sugar

Seasonal environmental factors recorded in the greenhouse including min/max temperature, light intensity and relative humidity are presented in Table 4.2. At the harvesting date, seasonal and harvesting time did not effect on reducing sugar content. Only season affected total sugar content that was 7.85±0.48% in summer, 7.33±0.22% in winter and 6.15±0.48% in rainy season (Table 4.3). After 3-day storage, the study showed that season, harvesting time and their interaction affected reducing sugar and total sugar contents in pak-choi. Summer harvested pak-choi had the highest reducing and total sugar contents of 7.13±0.62% and 9.60±0.55%, respectively. Also, pak-choi harvested in the evening had the highest reducing and total sugar contents of 6.82±0.44% and 8.71±0.68%, respectively. A season by time of day interaction was observed; summer harvested pak-choi in the morning and evening had reducing sugar contents of 8.46±0.74% and 8.18±0.42%, respectively, higher than in the afternoon (4.74±0.84%). Pak-choi harvested in summer in the evening had the highest total sugar content of 11.67±0.71%. (Table 4.1)

A high sugar content was detected in the evening because plants accumulate sugar from photosynthesis and it was used as a substrate in the respiration process. In pak-choi leaf, sugar is the main energy substrate consisting of glucose and fructose (Xiangyang and Lianqing, 2000). Therefore, the plant has been conducting 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 (Clarkson *et al*., 2005). Broccoli which exposed to sun light for a full day before harvest at 18.00 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; Hasperué *et al*., 2014). Sugar content is also related to the quality of light received in each season. Radicchio grown in spring had a higher content of simple and total sugar than summer/fall, 55.9% and 39.4% respectively (Francke and Majkowska-Gadomska, 2008). In brussels 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). Additionally, the decrease in total soluble sugar was related to the chlorophyll degradation during leaf senescence. The degradation of chlorophyll occurs when the soluble sugar was used by 60%, and the sugar concentration increased during the senescence of leaves. The rate of sugar decline was the key determinant of leaf yellowing (Able *et al*., 2005).

Content of vitamin C

During the harvesting date, season and harvesting time affected vitamin C content. It was the highest 39.91±1.92 mg/100 g FW in rainy season. Pak-choi had got the highest vitamin C content 36.17±2.95 mg/100 g FW when harvested in the evening. Moreover, both factors were interacted on vitamin C content, pak-choi had got the highest vitamin C content 46.99±2.45 mg/100 g FW when harvested in the evening of rainy season (Table 4.3). This is because light intensity in summer and rainy season is higher than in winter leading to higher vitamin C production (Table 4.2). L-ascorbic acid (vitamin C) 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 (Lee and Kader, 2000). Thirty five pak-choi varieties planted in the rainy season had 48% higher ascorbic acid than in the dry season because the rainy season has a higher temperature and light intensity, and longer days than the dry season (Hanson *et al*., 2009). Furthermore, Makus and Lester (2004) suggested that vitamin C content in leafy mustard greens (*Brassica juncea* L.) could be enhanced by harvesting during the day characterized by high light intensity.

After 3-day storage, the study revealed that season and harvesting time affected vitamin C content but there was no interaction between these factors. Pak-choi harvested in summer and the rainy season had vitamin C contents, 39.88±3.72 and 35.19±2.03 mg/100 g FW, respectively, higher than harvested in winter. Pak-choi harvested in the afternoon and in the evening had vitamin C contents, 37.06±2.68 and 39.17±2.46 mg/100 g FW, higher than that harvested in the morning (Table 4.1). Normally, the factors affected vitamin C content in fruit and vegetables includes genotypic differences, preharvest climatic conditions, cultural practices, maturity and harvesting methods and postharvest handling procedures. Controlling of temperature is an important factor to maintain vitamin C content of fruit and vegetables. Loss of Vitamin C was induced at high temperature and longtime storage (Lee and Kader, 2000; Weston and Barth, 1997). However, this study showed that some treatments had vitamin C content after 3-day storage higher than at the beginning of storage (Table 4.1 and 4.3). This may be due to the entire plant (except roots) that included young and old leaves were used for the analysis of vitamin C content and. Asante *et al*. (2016) reported that vitamin C content in young leaves of *Verninia amygdalina* was more than in old leaves. While the research conducted by Mathiventhan and Ramiah (2015) reported that vitamin C content in the leaves of *Drega volubilis*, *Delonix elata* and *Murraya koenigii* stored at 4 ºC on day 4 of storage was higher than at the beginning of storage.

Content of glucosinolate

The study showed that season and harvesting time affected glucosinolate content at the beginning and after 3-day storage. During the harvesting date, pak-choi had the highest glucosinolate of 6.21±0.39 µmol/g FW in rainy season and the samples had the highest glucosinolate of 6.53±0.35 µmol/g FW when harvest in the morning. But both main factors did not interact on the glucosinolate content (Table 4.3). After 3-day storage, Pak-choi had the highest glucosinolate of 8.46±0.92 µmol/g FW in winter season and when it was harvested in the morning, 8.22±1.03 µmol/g FW. Moreover, interaction of season and harvesting time affected glucosinolate content; pak-choi harvested in the morning during winter had an extra high glucosinolate content of 12.16±1.03 µmol/g FW (Table 4.1).

Several reports suggested that crop genetics and environmental factors such as temperature, photoperiod and light quality during the period before harvest and their interaction affected glucosinolate concentration (Aires *et al*., 2011; Engelen-Eigles *et al*., 2006; Mithen *et al*., 2000; Verkerk *et al*., 2009). Thirty five varieties of pak-choi grown in the wet season had a 72% higher glucosinolate content than grown in the dry season. The wet season has higher rainfall, higher average temperatures and solar intensities and longer day lengths than the dry season (Hanson *et al*., 2009). Likewise, in the present study the highest light intensity occurred in the rainy season (Table 4.2). 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 the 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). In this experiment, senescent leaves which indicated by yellow leaf color, were less common in winter than summer and rainy season.

Content of Crude fiber

Only season affected crude fiber content at the beginning and after 3-day storage, season and harvesting time were interacted only at the beginning. Pak-choi harvested in summer and winter had got higher crude fiber content 16.05±0.66 and 15.33±0.34 g/ 100 g DW than the one harvested in rainy season 13.62±0.61 g/100 g DW.
And pak-choi harvested in the morning of summer had the highest crude fiber content (Table 4.3). After 3-day storage, pak-choi harvested in summer, winter and rainy season had crude fiber contents of 12.54±0.60, 16.61±0.46, and 17.38±0.87 g/100 g DW, respectively (Table 4.1). 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 (Aletor *et al*., 2002; Punna and Prachuri,

2004; Tendaj *et al*., 2013; Uusiku *et al*., 2010). This results may be the difference of plant maturity. Pak-choi samples were harvested at 27, 24 and 24 days after transplanted in summer, winter and rainy season respectively. Therefore, the vegetables grown and harvested in summer had high fiber content but was not different in winter. In addition, Tendaj *et al*. (2013) reported that in cabbage group, content of crude fiber depended on type and variety. The variety with longtime planting had higher dietary fiber than short time one. In addition, the seasonal effect on crude fiber may be the result of nutrient supply and substrate competition. Andarwulan *et al*. (2015) reported that 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).
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).

Leaf color change

At the beginning, only harvesting time affected leaf color, pak-choi harvested in the evening had the darkest green 40.48±0.53 SPAD unit (Table 4.3). But after 3-day storage, only season affected leaf color in pak-choi. The produce harvested in winter generated more green color, 32.20±0.34 SPAD units, than in summer, 26.52±0.68 SPAD units, and the rainy season 29.35±0.61 SPAD units (Table 4.1). 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 effect on chlorophyll synthesis or degradation are caused by day length and temperature. Pak-choi planted at 21 °C had the highest content of total chlorophyll followed by 18 °C and 25 °C (Mahmud *et al*., 1999). The seasonal variation in chlorophyll content may be due to differences in solar radiation. The highest total chlorophyll content of baby spinach occurred in August, when radiation was the smallest and the lowest total chlorophyll in June, when radiation was the highest (Bergquist *et al*., 2006). On the other than, the assessment of Xiangyang and Bagshaw (2001) suggested leaf yellowing of pak-choi was caused by high temperatures and delays through the handling system. Likewise, Yang *et al*. (2010) reported that pak-choi storage at 20 °C exhibited leaf yellowing after the third day while 4 °C inhibited leaf yellowing. This study showed that 2-3 outer leaves were partially yellow on the second day and fully yellow on the third day of storage. The storage temperature was the highest in rainy season 28.33±0.13 ºC follow by summer 26.97±0.18 ºC and winter 25.00±1.00 ºC (Table 4.4).

Respiration rate

The main factors affecting the respiration rate in pak-choi included season and harvesting time. The produce had the highest respiration rate, 99.50±10.52 mg CO_2/kg-h, in summer. The effect of harvesting time showed that pak-choi harvested in the afternoon had the highest respiration, 86.75±13.27 mg CO_2/kg-h. In addition, the interaction of both factors affected respiration, pak-choi harvested in summer in the afternoon had the highest respiration rate, 135.05±8.11 mg CO_2/kg-h. The respiration rate was increased by ethylene production because pak-choi harvested in the afternoon had the highest ethylene production (Table 4.1).

Respiration in vegetables changes carbohydrates, starches and sugars, to CO_2 and H_2O, with the releasing of heat energy (Løkke, 2012). The postharvest respiratory of fresh produce depends on the storage temperature. For each increase of 10 degree celsius above optimum, the rate of deterioration increases by two-to-threefold (Kader, 2002). 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 salad (*Diplotaxis tenuifolia* L.); the produce harvested in spring had a higher respiration rate [O_2 (RRO_2)], 6.95 mmol $O_2kg^{-1}h^{-1}$, than in early and late summer, 3.99 and 3.92 mmol $O_2kg^{-1}h^{-1}$, respectively (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 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 cutting. CO_2 production increased 2-4 fold when temperature increased 1-12 ºC (Martínez-Sánchez *et al*., 2008). Baby spinach was harvested at three times include 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).

Ethylene production rate

After 3-day storage, only season affected ethylene production rate of pak-choi. The lowest rate of ethylene production was 0.08±0.02 µl/g/h in winter, the highest rate was 1.11±0.20 and 0.83±0.06 µl/g-h in rainy season and summer, respectively (Table 4.1).

The rate of ethylene production of pak-choi on the last day storage decreased from the first day in winter and summer, as opposed to in the rainy season (Table B9). On the first two day, the outer leaves became senescence indicating that the leaves color were changed from green to yellow, and at the last stage the leaves were fully yellow and rotten. In many plant tissues, ethylene treatment results in rapid loss of chlorophyll, the green color in leaves and unripe fruit (Reid, 2002). Ethylene accelerated chlorophyll degradation and green leafy vegetable rapidly turned yellow without pigments synthesis (Siripanich, 2006). Moreover, at temperature over 25 ºC, bacterial growth and rotting may be accelerated (Reid, 2002). This study reveals that storage temperature in rainy season was the highest follow by summer and winter, respectively (Table 4.4).

In addition, we observed that the highest losses due to microbial infection occurred in rainy season. Likewise, Garrido *et al*. (2015) reported that in spring, baby spinach had higher losses due to *Pseudomonas* than in winter because leave had high water content and storage condition with high humidity.

Storage life and produce appearance

All treatments did not significantly differ with regard to shelf life (Table 4.5), as indicated by visual appearance: leave color or wilting. The sample was rejected when the outer leaves started turning from green to yellow. The leaves on the outside begin to yellow but the inner leaves remain green and the youngest leaves are light yellow (Figure 4.1). Yang *et al*. (2010) reported that pak-choi leave changed from green to yellow after the third day when storage at 20 ºC. This study, both of main factors did not affected shelf life. It was possible that high storage temperature (~24 - 29 ºC) dominated others, so the produce had short shelf life (Table 4.4).

The leave color relates to consumer perception of visual quality especially in leafy vegetables. In general, the environment at harvesting time including temperature, light intensity, relative humidity, etc., affects produce quality (Mahmud *et al*., 1999; Weston and Barth., 1997; Paull., 1999; Xiangyang and Bagshaw., 2001). In *Brassica* crops especially pak-choi, senescence most widely characterized by yellowing caused by a breakdown of chlorophyll pigments inside the tissues (Dixon, 2007). During leaf senescence, the chlorophyll is degraded, the final product derived from the decomposition of chlorophyll is nonfluorescent chlorophyll catabolites (NCCs) which are colorless, so green loss is an observed symptom of leaf decay (Sakuraba *et al*., 2012). Additionally, pak-choi newly harvested in the morning are easier to break than those harvested at other times of the day due to the produce's higher water content at this time; the leaves are soft and more susceptible to bruising. Water content of plants has a direct influence on cell turgor, particularly the texture of leaf vegetables (Sams, 1999; Thompson, 1996; Garrido *et al*., 2015).

Figure 4.1 The color of different growth stages of pak-choi leaves; the first two leaves on the left are senescent

Conclusion

After 3 days storage, it was revealed that season affected all parameters, whereas harvesting time only affected reducing and total sugar contents, glucosinolate, vitamin C, respiration rate. Nevertheless, the interaction of both factors affected reducing and total sugar contents, glucosinolate, and respiration rate. The shelf life did not significantly differ in each season.

The results of this experiment is useful to enhance postharvest handling of pak-choi at farm and packing house level. Rescheduling of planting dates in different season and appropriate harvesting time in a day can positively affect quality of organic pak-choi. For nutritional benefits, pak-choi generates the highest glucosinolate content when harvested in the morning. On the other hand, pak-choi harvested in the evening has the highest vitamin C content. Moreover, summer season may affect pak-choi quality due to the highest respiration rate and weight loss.

Table 4.1 Reducing sugar, total sugar, glucosinolate, vitamin C, crude fiber, leaf color, weight loss, respiration rate and ethylene production rate at 3 days after harvesting of organic pak-choi in different seasons and at different times

Season	Harvesting Time (HT)			Mean (season)
	Morning	Afternoon	Evening	
		Weight loss[1/] (%)		
Winter	2.23±0.74	2.08±0.47	3.54±0.58	2.61±0.37 B
Summer	5.57±0.10	5.29±0.63	5.11±0.39	5.32±0.23 A
Rainy	1.52±0.17	1.63±0.15	1.53±0.33	1.56±0.12 C
Mean (HT)	3.10±0.58	3.00±0.55	3.39±0.50	
		Reducing sugar[1/] (%)		
Winter	3.68±0.36 cd	5.20±0.60 bc	5.77±0.22 b	4.88±0.35 B
Summer	8.18±0.42 a	4.74±0.84 bcd	8.46±0.74 a	7.13±0.62 A
Rainy	3.50±0.52 d	3.25±0.35 d	6.25±0.35 b	4.33±0.46 B
Mean (HT)	5.12±0.69 B	4.40±0.41 B	6.82±0.44 A	
		Total sugar[1/] (%)		
Winter	5.79±0.56 de	7.49±0.16 c	7.35±0.37 c	6.88±0.31 B
Summer	9.04±0.57 b	8.10±0.47 bc	11.67±0.71 a	9.60±0.55 A
Rainy	5.70±0.65 de	5.38±0.50 e	7.12±0.30 cd	6.07±0.35 B
Mean (HT)	6.84±0.56 B	6.99±0.41 B	8.71±0.68 A	
		Vitamin C[1/] (mg/100g FW)		
Winter	21.05±1.24	28.07±1.01	30.26±2.19	26.46±1.44 B
Summer	27.00±6.47	45.62±4.84	47.02±1.92	39.88±3.72 A
Rainy	27.88±1.88	37.47±1.75	40.22±3.25	35.19±2.03 A
Mean (HT)	25.31±2.26 B	37.06±2.68 A	39.17±2.46 A	
		Glucosinolate[1/] (µmol/g FW)		
Winter	12.16±1.03 a	5.83±0.72 bc	7.38±0.75 b	8.46±0.92 A
Summer	5.61±1.02 bc	0.47±0.02 d	0.64±0.11 d	2.24±0.78 C
Rainy	6.90±1.21 b	6.54±0.51 bc	4.31±0.40 c	5.92±0.54 B
Mean (HT)	8.22±1.03 A	4.28±0.86 B	4.11±0.87 B	
		Crude fiber[1/](g/100g DW)		
Winter	16.82±0.71	16.10±0.96	16.91±0.87	16.61±0.46 A
Summer	13.88±1.43	12.10±0.83	11.63±0.52	12.54±0.60 B
Rainy	18.69±1.18	15.57±1.63	17.87±1.57	17.38±0.87 A
Mean (HT)	16.46±0.85	14.59±0.82	15.47±1.00	

Table 4.1 Reducing sugar, total sugar, glucosinolate, vitamin C, crude fiber, leaf color, weight loss, respiration rate and ethylene production rate at 3 days after harvesting of organic pak-choi in different seasons and at different times (continued)

Season	Harvesting Time (HT)			Mean (season)
	Morning	Afternoon	Evening	
		Leaf color[1/] (SPAD unit)		
Winter	30.45±1.19	32.50±0.27	33.65±0.84	32.20±0.34 A
Summer	25.58±2.06	27.60±2.42	26.38±2.46	26.52±0.68 BC
Rainy	28.53±1.30	26.75±3.30	32.78±2.29	29.35±0.61 AB
Mean (HT)	28.18±0.74	28.95±0.50	30.93±0.64	
		Respiration[1/] (mg CO_2/kg-h)		
Winter	16.52±1.04 d	39.92±4.28 cd	46.75±3.90 c	34.39±4.29 C
Summer	84.33±22.13 b	135.05±8.11 a	79.10±4.93 b	99.50±10.52 A
Rainy	65.43±6.99 bc	84.80±6.38 b	87.11±6.11 b	77.75±5.08 B
Mean (HT)	57.00±11.11 B	86.75±13.27 A	69.53±6.26 B	
		Ethylene[1/] (µl/g-h)		
Winter	0.06±0.02	0.15±0.01	0.02±0.02	0.08±0.02 B
Summer	0.77±0.12	0.83±0.13	0.89±0.05	0.83±0.06 A
Rainy	1.23±0.19	1.01±0.59	1.09±0.20	1.11±0.20 A
Mean (HT)	0.69±0.16	0.66±0.21	0.67±0.15	

Note: ANOVA of main factor and interaction effects: NS = not significant, * = significant ($p < 0.05$)
- Weight loss: Season = *, Time = NS, Season × Time = NS
- Reducing sugar: Season = *, Time = *, Season × Time = *
- Total sugar: Season = *, Time = *, Season × Time = *
- Vitamin C: Season = *, Time = *, Season × Time = NS
- Glucosinolate: Season = *, Time = *, Season × Time = *
- Crude fiber: Season = *, Time = NS, Season × Time = NS
- Leaf color change: Season = *, Time = NS, Season × Time = NS
- Respiration rate: Season = *, Time = *, Season × Time = *
- Ethylene production: Season = *, Time = *, Season × Time = *

[1/]Means followed by different letters in each characteristics were significantly different by DMRT at $\alpha = 0.05$ levels.

Table 4.4 The average of temperature, light intensity and relative humidity conditions in an organic pak-choi greenhouse during different seasons

Season	Temperature (ºC)			Light intensity	RH
	Max	Min	Mean	(lux)	(%)
Winter	33.76	17.71	24.17	2,764.15	79.28
Summer	38.27	15.08	25.78	4,558.52	56.13
Rainy	37.60	22.42	28.28	5,807.08	74.89

Table 4.3 Reducing sugar, total sugar, glucosinolate, vitamin C, crude fiber and leaf color at harvesting date of organic pak-choi harvested at different time and season

Season	Harvesting time (HT)			Mean (season)
	Morning	Afternoon	Evening	
		Reducing sugar (%)		
Winter	4.71±0.51	4.84±0.49	5.57±0.26	5.04±0.25
Summer	6.24±1.27	5.88±1.10	3.48±0.75	5.20±0.67
Rainy	3.96±0.70	3.59±0.52	3.80±1.43	3.78±0.51
Mean (HT)	4.97±0.54	4.77±0.48	4.28±0.57	
		Total sugar[1/] (%)		
Winter	7.21±0.54	7.77±0.31	7.01±0.15	7.33±0.22 AB
Summer	8.30±0.87	7.01±1.05	8.26±0.59	7.85±0.48 A
Rainy	5.33±1.00	6.16±0.70	6.96±0.82	6.15±0.48 B
Mean (HT)	6.95±0.57	6.98±0.44	7.41±0.36	
		Vitamin C[1/] (mg/100g FW)		
Winter	6.66±1.12 e	7.55±1.12 e	37.30±1.26 b	17.17±4.34 C
Summer	19.20±2.42 d	18.74±1.37 d	24.22±1.15 c	20.72±1.17 B
Rainy	34.34±1.28 b	38.40±2.26 b	46.99±2.45 a	39.91±1.92 A
Mean (HT)	20.06±3.53 B	21.56±3.94 B	36.17±2.95 A	
		Glucosinolate[1/] (µmol/g FW)		
Winter	6.65±0.64	3.39±0.15	2.91±0.46	4.31±0.56 B
Summer	6.39±0.66	4.30±0.61	3.58±0.26	4.76±0.46 B
Rainy	6.54±0.71	6.65±0.74	5.45±0.55	6.21±0.39 A
Mean (HT)	6.53±0.35 A	4.78±0.51 B	3.98±0.40 B	

Table 4.3 Reducing sugar, total sugar, glucosinolate, vitamin C, crude fiber and leaf color at harvesting date of organic pak-choi harvested at different time and season (continued)

Season	Harvesting time (HT)			Mean (season)
	Morning	Afternoon	Evening	
		Crude fiber[1/] (g/100g DW)		
Winter	14.77±0.79 abc	16.18±0.06 ab	15.03±0.50 abc	15.33±0.34 A
Summer	17.45±0.88 a	14.07±1.01 bc	16.64±0.91 ab	16.05±0.66 A
Rainy	13.23±1.30 c	14.91±0.86 abc	12.72±0.92 c	13.62±0.61 B
Mean (HT)	15.15±0.74	15.06±0.48	14.80±0.64	
		Leaf color[1/] (SPAD unit)		
Winter	36.98±0.23	37.85±0.36	39.93±0.84	38.25±0.47
Summer	37.53±0.57	38.38±0.84	40.05±1.15	38.65±0.56
Rainy	37.88±1.85	38.85±1.17	40.85±0.93	39.19±0.81
Mean (HT)	37.46±0.60 B	38.36±0.46 B	40.28±0.53 A	

Note: ANOVA of main factor and interaction effects: NS = not significant, * = significant ($p < 0.05$)
- Reducing sugar: Season = NS, Time = NS, Season × Time = NS
- Total sugar: Season = *, Time = NS, Season × Time = NS
- Vitamin C: Season = *, Time = *, Season × Time = *
- Glucosinolate: Season = *, Time = *, Season × Time = NS
- Crude fiber: Season = *, Time = NS, Season × Time = *
- Leaf color change: Season = NS, Time = *, Season × Time = NS

[1/]Means followed by different letters in each characteristics were significantly different by DMRT at $\alpha = 0.05$ levels.

Table 4.4 Storage conditions, temperature and relative humidity during different seasons

Season	Temperature (°C)			Relative humidity (%)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Winter	24.81±0.53	24.79±0.75	25.00±1.00	64.22±0.30	61.95±0.61	58.95±0.62
Summer	27.12±0.34	26.70±0.28	26.97±0.18	47.30±0.06	47.50±0.06	49.00±0.10
Rainy	29.06±0.12	29.22±0.05	28.33±0.13	70.04±0.03	70.42±0.03	67.84±0.04

Table 4.5 Storage life (days) at ambient temperature of pak-choi in different seasons and harvesting times

Season	Harvesting Time (HT)			Mean (season)
	Morning	Afternoon	Evening	
Winter	1.75±0.25	1.75±0.25	2.00±0.00	1.83±0.11
Summer	1.75±0.25	1.75±0.25	2.00±0.00	1.83±0.11
Rainy	1.75±0.25	1.75±0.25	1.75±0.25	1.75±0.13
Mean (HT)	1.75±0.13	1.75±0.13	1.92±0.08	

Note: ANOVA of main factor and interaction effects: NS= not significant, *=significant (P<0.05)
- Shelf life: Season=NS, Time =NS, Season × Time =NS

<u>Experiment 2</u> Effects of vacuum cooling on delay senescence of organic pak-choi in each season

The purpose of this experiment was to investigate the effects of vacuum cooling on quality changes of organic pak-choi grown in winter, summer and rainy season during storage. This results will lead to proper pak-choi handling. The produce was harvested from the farmer's greenhouse at 5.30-7.30 in three growing seasons (winter, summer and rainy). Then they were brought to the Royal Project packinghouse in Muang district, Chiang Mai province at 9.00. The produce was then graded, trimmed and packed into 25×40 cm perforated polyethylene bags with 18, 0.8 cm-diameter holes. Each bag contained a 300 g pak-choi sample. Quality of produce from two treatments were compared. For the first treatment, the produce was precooled using a vacuum cooling system. The operating parameters used for precooling were at a holding pressure of 6 mbar with a holding time of 5 min. This conditions estimated for final produce temperature was 5±1 ºC, minimum cycle time and low weight loss percentage. Subsequently, the produce was stored at 8 ºC. Samples without the vacuum cooling process (control) were also stored at 8 ºC immediately after harvest.

2.1 Effect of vacuum cooling on delay senescence of organic pak-choi in winter

The produce was precooled by vacuum cooling, the final bleed pressure was 6 mbar and 5 min soaktime. Pak-choi had an initial average temperature at its center of 16.10 ºC and final temperature of 6.0 ºC. The weight loss was 1.57%. The cycle time for precooling was about 16 min, with energy consumption of 2.20 kWh, and electrical expense of 0.13 Baht/kg (Table 4.6).

The relationship between temperature in a vacuum chamber and time, and pressure and time for reduction of pak-choi temperature are shown in Figure 4.2. At the first 8 min, the pressure was declined from 972 mbar to 18.7 mbar as the air temperature was slowly decreased while the temperature of pak-choi slightly changed. Later, during the period of 9-14 min, the pressure in the chamber was stable, the rate of temperature decreasing in the chamber and pak-choi was rapid. When the rapid evaporation of water in pak-choi started, the temperature of the vegetable dropped rapidly. The pressure in the vacuum chamber dropped from 972 to 6.1 mbar and reaching the reserved pressure of 6 mbar and which was held for 5 min. During this period the rate of pak-choi temperature decreased slightly until the final temperature of 6.0 ºC. The average of temperature decrease rate was 0.85 ºC per min. Weight loss caused by water evaporation from the vegetable during cooling process, depended on the holding time.

Table 4.6 Process parameters and measuring indices for vacuum cooling of pak-choi in perforated polyethylene bags, which were grown in winter

Process parameters	Value
Holding pressure (mbar)	6
Holding time (min)	5
Cycle time (min)	16
Initial chamber temperature(ºC)	23.80
Initial center temperature of pak-choi (ºC)	16.10
Final center temperature of pak-choi (ºC)	6.00
Weight loss (%)	1.57
Energy consumption (kWh)	2.20
Electrical expense (Baht/kg)	0.13

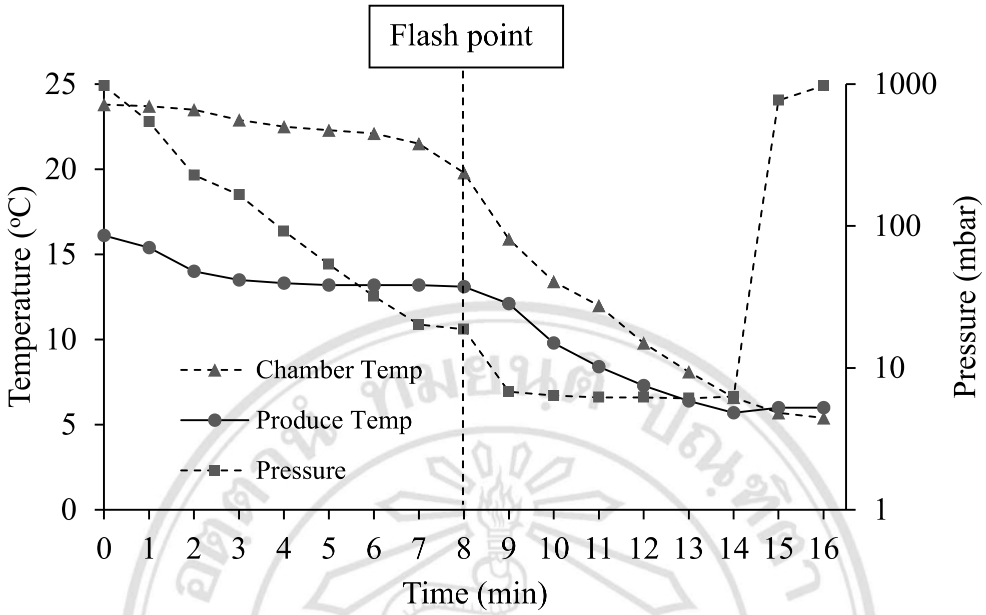

Figure 4.2 Temperature and pressure history in the vacuum chamber and cooling curve of pak-choi packed in perforated polyethylene bags during vacuum cooling at 6 mbar with 5 min holding time in winter

Weight loss

The average weight loss percentages of pak-choi with and without vacuum cooling treatments during storage are presented in Figure 4.3. Weight loss of pak-choi in all treatments did not significantly differ and slightly increased throughout the storage period. After 3 days storage, weight losses of vacuum cooled and non-vacuum cooled pak-choi were 0.70±0.11% and 0.75±0.09%, respectively (Table 4.10). This result was consistent with the study of Boonyakiat *et al*. (2007) and Boonyakiat *et al*. (2009), which reported that vacuum cooling did not affect weight loss in pak-choi, organic baby pak-choi, pointed cabbage, sweet basil, holy basil and coriander. In the current study, the vacuum cooling did not affect the weight loss of pak-choi. Because pak-choi was harvested in the early morning which low temperature, pak-choi had low metabolic level. Furthermore, the vegetable harvested in the morning has high turgidity. Water content in plant directly affects cell turgor that is important for the texture of leafy vegetables, e.g. 3% weight loss affects the texture of spinach (Sams, 1999).

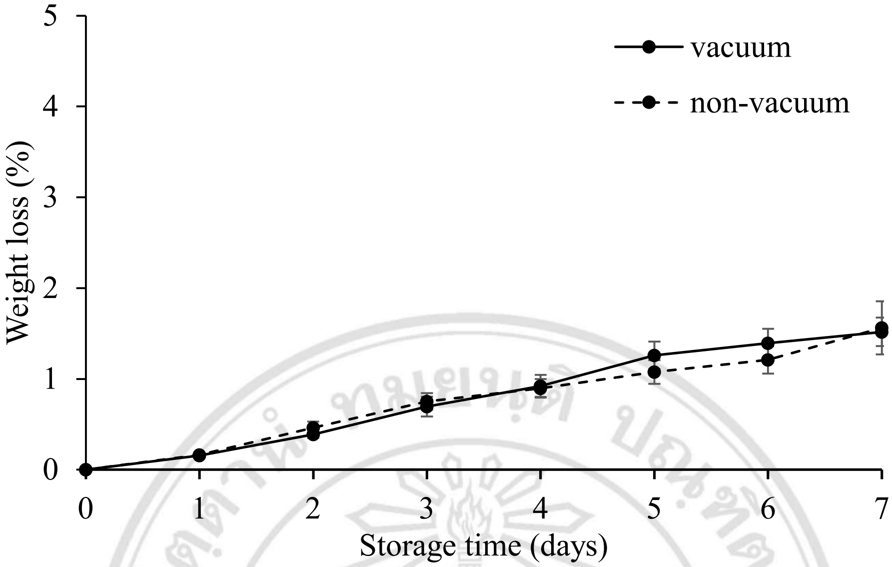

Figure 4.3 Weight loss of vacuum and non-vacuum cooling of winter harvested pak-choi strored at 8 ºC for 7 days

Content of reducing sugar and total sugar

Sugar is the essential energy source of vegetables and is also the main substrate for vegetable respiration. Therefore, reducing sugars are thought to be closely related to the physiological and biochemical properties of vegetables (Ding *et al*., 2016). In pak-choi leaves, sugar is the main energy substrate, consisting of glucose and fructose. The sugar content in the inner leaves was significantly higher than outer leaves. (Xiangyang and Lianqing, 2000). In the present study, the content of reducing and total sugar in vacuum and non-vacuum cooled pak-choi fluctuated during the period of storage (Figure 4.4 and 4.5). After 3-day storage, vacuum cooling did not significantly affect total sugar content, vacuum cooled pak-choi had total sugar content 7.21±0.52% and non-vacuum cooled pak-choi had total sugar content 8.17±0.29% (Table 4.10). While vacuum cooled pak-choi had significantly lower reducing sugar (4.35±0.43%) than non-vacuum cooled pak-choi (6.10±0.27%). That may be due to the different of the number of leaves in produce sample (this study used whole plant), many inner leaves had more sugar content.

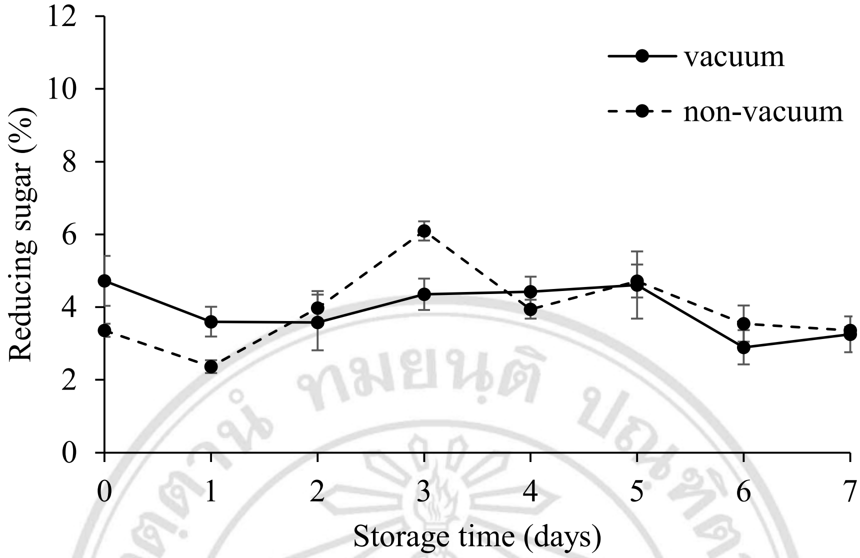

Figure 4.4 The content of reducing sugar in vacuum and non-vacuum cooling of winter harvested pak-choi stored at 8 ºC for 7 days

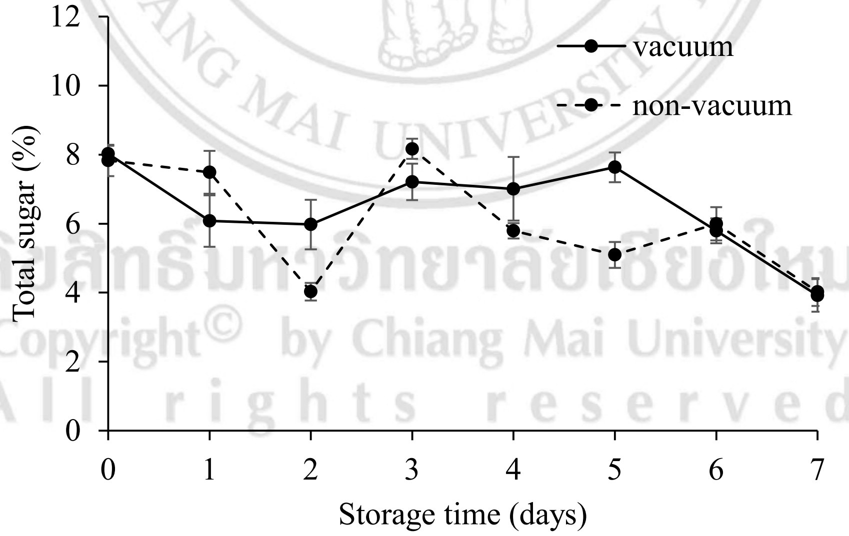

Figure 4.5 The content of total sugar in vacuum and non-vacuum cooling of winter harvested pak-choi strored at 8 ºC for 7 days

Content of vitamin C

The content of vitamin C fluctuated during the storage period. The content of vitamin C in vacuum cooled pak-choi was slightly higher than non-vacuum throughout the treatment (Figure 4.6). After storage for 3 days, there was no significant difference, vacuum cooled pak-choi had 31.14±2.31 and non-vacuum cooled had 29.39±5.33 mg/100g FW (Table 4.10). This result indicated that vacuum cooling had no effect on vitamin C content. Likewise, Chinnapun (2009) reported that the vacuum cooling process had no effect on the vitamin C content of pak-choi. Other research found that vacuum cooling also had no effect on vitamin C of Chinese cabbage (Kamon *et al.*, 2013), cos lettuce (Panyakham, 2011), and spinach (Hemrattrakun, 2009).

The reason that there was no difference in vitamin C content between the treatments may due to the low initial temperature of the pak-choi (16.2 ºC); the metabolism of the produce was low since it was harvested in cool, winter mornings. Additionally, the vacuum cooled and non-vacuum cooled pak-choi were stored at the same low temperature (8 ºC) which reduced enzyme activities and delayed the loss of vitamin C.

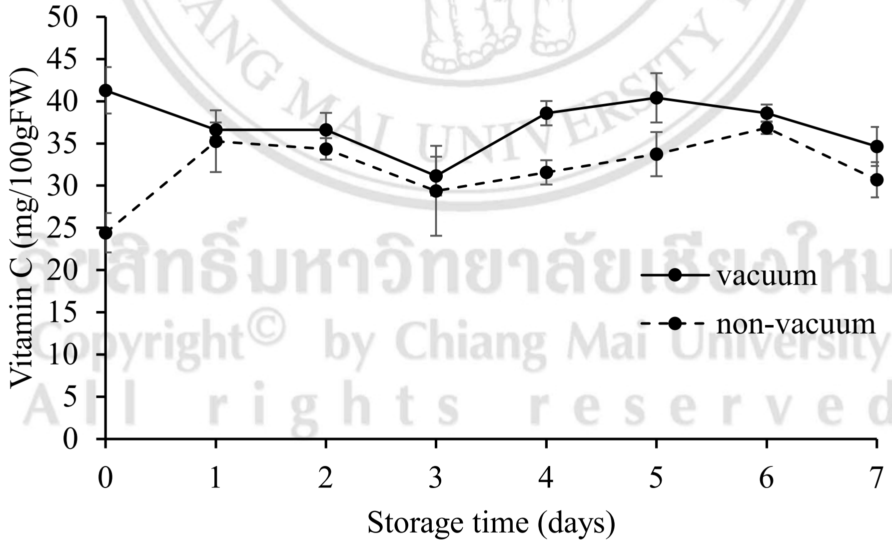

Figure 4.6 The content of vitamin C in vacuum and non-vacuum cooling of winter harvested pak-choi strored at 8 ºC for 7 days

Content of glucosinolate

The vacuum cooling had no effect on glucosinolate content in vacuum and non-vacuum cooled pak-choi when stored for 3 days. The glucosinolate content in vacuum cooled pak-choi was 9.63±1.12 µmol/g FW and non-vacuum cooled pak-choi was 10.29±0.95 µmol/g FW. Moreover, the glucosinolate content in both treatments were higher on the last day of storage than the beginning (Figure 4.7 and Table 4.10). According to Yang *et al*. (2010) when pak-choi was stored at 4 ºC, the total glucosinolate content was not different when stored for 3 days. Total glucosinolate increased between 5 and 7 days of storage and returned to the same level as at the beginning when stored for 9 days. In addition, the younger leaves of the rosette have much higher glucosinolate concentrations than the older leaves in *Arabidopsis thaliana*. During senescence, rosette and cauline leaves showed greatly reduced glucosinolate concentrations. This may be ascribed to export to other organs since the onset of senescence in rosette leaves coincides with bolting and the development of the inflorescences, fruits and seeds, which all accumulate high concentrations of glucosinolates (Brown *et al*., 2003). In our study, we used whole plants, which had both young and old leaves. Therefore, the amount of glucosinolate may vary with the number of young and old leaves of plants.

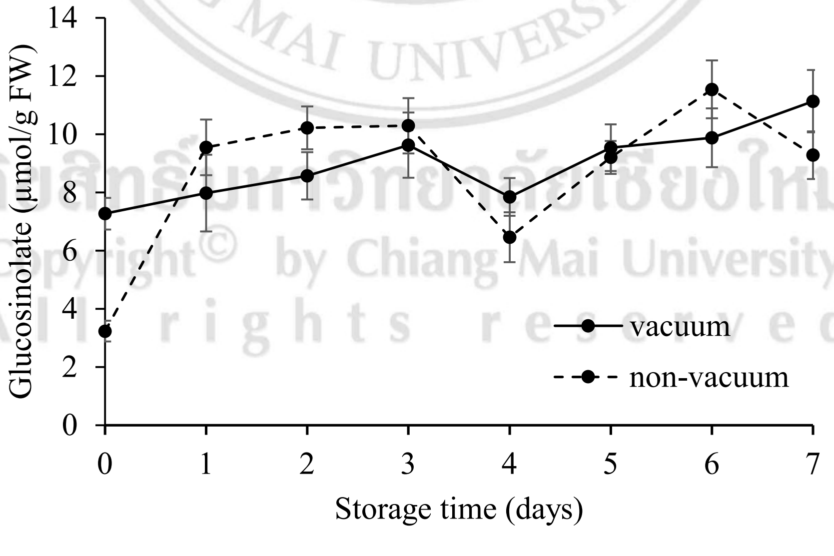

Figure 4.7 The content of glucosinolate in vacuum and non-vacuum cooling of winter harvested pak-choi stored at 8 ºC for 7 days

Content of crude fiber

Figure 4.8 showed a slight fluctuation of crude fiber content in both vacuum and non-vacuum cooled pak-choi. The content of crude fiber in vacuum and non-vacuum cooled pak-choi was not significantly different after 3 days of storage. The amount of crude fiber of vacuum and non-vacuum cooled pak-choi were 16.17±0.64 and 15.99±0.49 g/100 g DW, respectively (Table 4.10). Crude fiber content in vacuum cooled pak-choi had 16.63±0.45 g/100 g DW at the end of storage which was higher than at the beginning, 15.13±0.82 g/100 g DW (Table B10). Acho *et al*. (2015) found that five leafy vegetables; *Colocacia esculenta*, *Basella alba*, *Solanum melongena*, *Telinum triangulare*, and *Corchorus olitorius* were stored in a refrigerator at 4 ^{o}C for 15 days, the fiber contents slightly increased with refrigeration storage time but were not significantly different. Likewise, Niyomlao *et al*. (2000) reported that the fiber content of both hydro and non-hydrocooled chinese kale increased over time in storage. Increases in the total fiber of leafy vegetables may be due to increasing amounts of uronic acid in the insoluble fiber fraction (Acho *et al*., 2015; Marlett, 2000).

In non-vacuum pak-choi, the content of crude fiber at the end of storage was 15.19±0.82 g/100 g DW which was lower than the beginning of 17.63±0.48 g/100 g DW. Andarwulan *et al*. (2015) reported that dietary fiber related to the level of chlorophyll, as the chlorophyll level decreased the products of photosynthesis and the synthesis of other compounds, such as dietary fiber was decreased.

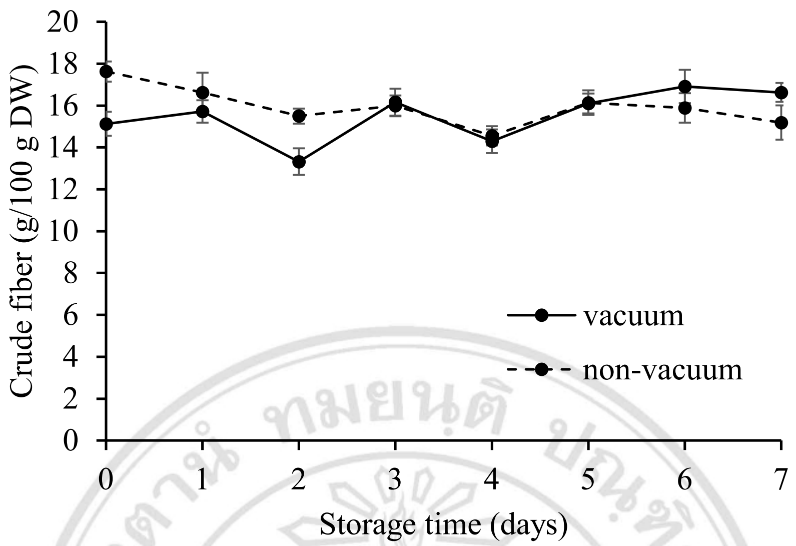

Figure 4.8 The content of crude fiber in vacuum and non-vacuum cooling of winter harvested pak-choi stored at 8 ºC for 7 days

Leaf color change

The leaf color in both vacuum and non-vacuum cooled pak-choi were not different during storage period. The level of chlorophyll in both trials tended to decreased. Vacuum cooled pak-choi had slightly higher chlorophyll than non-vacuum cooled pak-choi (Figure 4.9). After storage for 3 days, the chlorophyll content of vacuum cooled pak-choi was 37.53±0.70 SPAD units, and non-vacuum cooled was 37.08±0.22 SPAD units (Table 4.10). The results showed that vacuum cooling did not affect leaf color change in pak-choi when storage at 8 ºC. This is consistent with the research of Poonlarb and Boonyakiat (2015) who reported that vacuum cooling had no effect on the change of color in Chinese kale when storage at 4 ºC for 5 days, and the total chlorophyll tended to decrease with storage time. The same phenomenon occurred in organic chayote shoots which showed that vacuum cooling had no effect on the chlorophyll content. However, precooled organic chayote shoots had a better appearance than non-precooled produce (Poonlarp *et al*., 2012). Moreover, the vacuum cooling had no effect on leaf color change in cos lettuce and Chinese cabbage (Panyakham, 2011; Kamon *et al*., 2013). The color change is the first visual symptom of senescence in many vegetables. The catabolism of leaf pigments is strongly connected with storage conditions. Low temperature usually slows down all leaf metabolism preserving the quality of the produce (Ferrante *et al*., 2004).

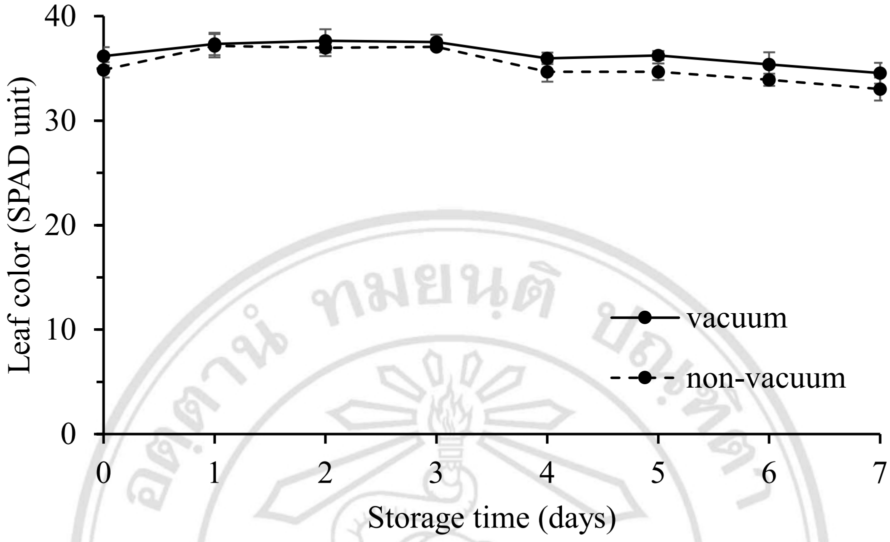

Figure 4.9 Leaf color change in vacuum and non-vacuum cooling of winter harvested pak-choi stored at 8 ºC for 7 days

Respiration rate

Figure 4.10 showed that the respiration rate in vacuum cooled pak-choi was lower than non-vacuum cooled produce and it was significantly different throughout the treatment. After storage for 3 days, the vacuum cooled pak-choi had lower respiration rates than non-vacuum cooled pak-choi which were 13.36±3.28 and 28.99±3.22 mg CO_2/kg-h, respectively (Table 4.10). The rate of deterioration after harvest is closely related to the respiration rate of the harvested vegetables. The important factor affecting the respiration rate is temperature. Precooling, especially vacuum cooling, rapidly 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). Similarly, Ding *et al*. (2016) reported that vacuum cooling was the best method for reducing the respiratory rate of harvested broccoli when compared with hydrocooling, room cooling and without precooling. Furthermore, baby spinach pre-cooled by hydrocooling and vacuum cooling before being stored for 11 days at 7 ºC showed the lowest respiration rate compared to room cooling and forced air cooling (Garido *et al*., 2015). However, these studies showed that an increased respiration rate in the last stage, at 5 and 6 days of storage, may be caused by the onset of decay by microorganisms (Fonseca *et al*., 2002).

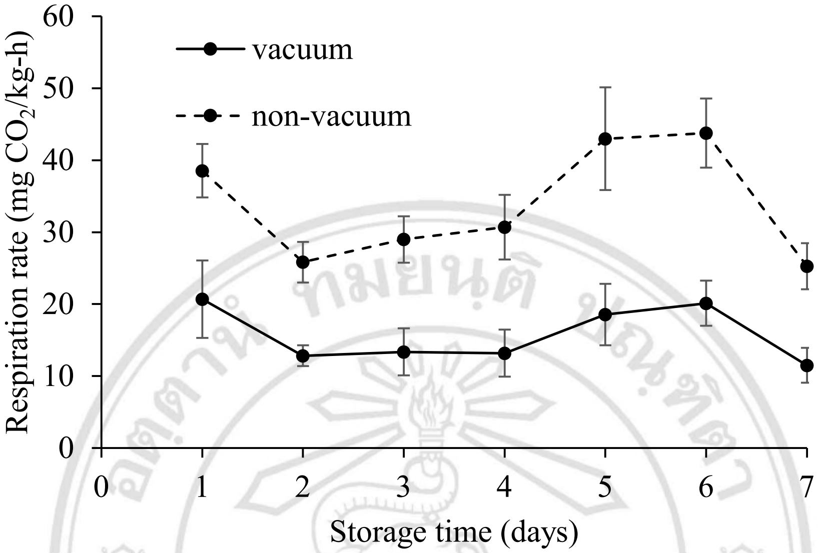

Figure 4.10 Respiration rate in vacuum and non-vacuum cooling of winter harvested pak-choi stored at 8 ºC for 7 days

Ethylene production rate

Ethylene production rate of pak-choi was slightly decreased during the trial period (Figure 4.11). The ethylene production rate was different in 1-2 days after storage, vacuum cooled pak-choi ethylene was lower than non-vacuum cooled, and after that the rate did not different (Table B10). At 3-day storage, the ethylene production rate did not significantly differ between vacuum cooled and non-vacuum cooled pak-choi which were 0.13±0.01 and 0.14±0.05 µl/g-h, respectively (Table 4.10). Pak-choi had a pattern of respiration like non-climacteric. Ethylene was well known to promote senescence in non-climacteric produce (Kader, 1985). At first 2 days vacuum cooling affected ethylene production rate because vacuum cooling is the rapid removal of field heat from fresh produce that reduced the rate of physiological change especially ethylene production (Borompichaichartkul *et al*., 2009).

Precooling methods can be employed in combination with other storage methods for maintaining fresh produce quality (Brosnan and Sun, 2001; Borompichaichartkul *et al*., 2009). Additionally, the storage conditions was previously shown to affect 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). In green tissues, ethylene commonly stimulates senescence, as indicated by loss of chlorophyll, loss of protein, and decay (Reid, 2002). Likewise, detached pak-choi leaves stored at 20ºC produced more ethylene than at 2 ºC and 10 ºC and ethylene evolution was related to leaf senescence, induction of yellowing (Able *et al*., 2005).

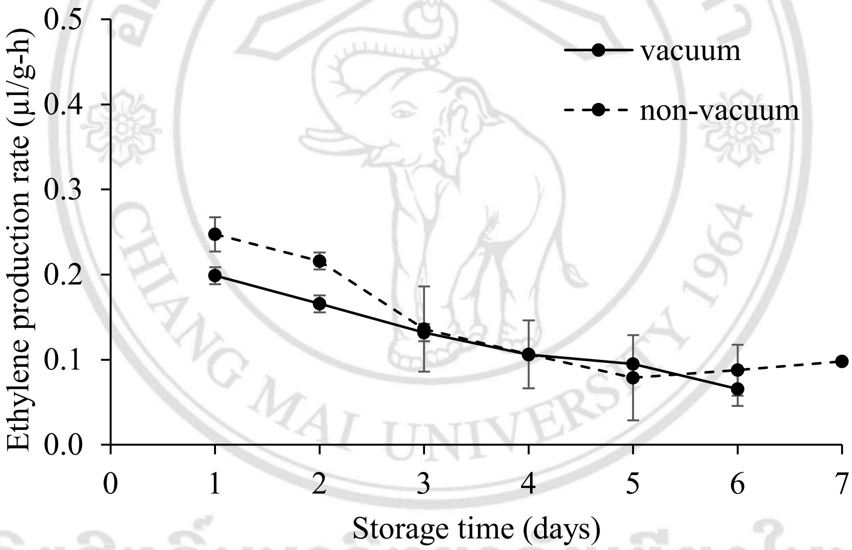

Figure 4.11 Ethylene production rate in vacuum and non-vacuum cooling of winter harvested pak-choi stored at 8 ºC for 7 days

Shelf life and produce appearances

In the present study vacuum cooling had a significant effect on pak-choi shelf life. The vacuum cooled pak-choi had a longer shelf life, 6.75±0.85 days, than non-vacuum cooled pak-choi, 3.50±0.29 days (Table 4.9). After harvest, the produce still metabolically active, the physiological and biochemical processes continue. Detrimentally affecting the physical and chemical qualities of pak-choi lowers its nutritive value. Precooling after harvest can maintain produce quality because at low temperature reduced metabolic processes such as respiration, dehydration and microbial activity. The positive effects of vacuum cooling on shelf life of some vegetables were reported as follows: Chinese cabbage, pak-choi, chinese kale, cauliflower, and broccoli. The vacuumcooled produce had a longer shelf life than non-vacuum cooled produce (Kamon *et al*., 2013; Chinnapun, 2009; Poonlarp and Boonyakiat, 2015; Alibas and Koksal, 2015; Ding *et al*., 2016).

The storage produce had a good appearance. The color change is the first visual symptom of senescence in many vegetables. The catabolism of leaf pigments is strongly connected with storage conditions (Ferrante *et al*., 2004). Xiangyang and Bagshaw (2001) suggested that leaf yellowing of pak-choi was caused by high temperatures. Low temperature usually slows down all leaf metabolism preserving the quality of the produce (Ferrante *et al*., 2004). This study, vacuum cooled pak-choi stayed greener than non-vacuum cooled pak-choi. Yellowing starts from the edge of leaf, then it expands into the leaf. Moreover, vacuum cooled and non-vacuum cooled pak-choi showed wilting symptom at the end of storage.

Conclusion

After storage for 3 days in winter, vacuum cooling affected on reducing sugar, respiration rate and shelf life. The reducing sugar content in vacuum cooled pak-choi had 4.35±0.43% lower than non-vacuum cooled pak-choi, 6.10±0.27% and the content of reducing sugar fluctuated during the storage period. The respiration rate of vacuum cooled pak-choi (13.36±3.28 mg CO_2/kg-h) was lower than non-vacuum cooled produce (28.99±3.22 mg CO_2/kg-h) and it was significantly different throughout the storage period. Consistent with shelf life, the vacuum cooled pak-choi had shelf life (6.75±0.85 days) longer than non-vacuum cooled pak-choi (3.50±0.29 days).

2.2 Effects of vacuum cooling on delay senescence of organic pak-choi in summer

The produce was precooled by vacuum cooling; final bleed pressure was 6 mbar and the soak time was 5 min. The initial temperature at the pak-choi center was 19.50 ºC, and the final temperature 5.0 ºC. The weight loss was 1.38%. The cycle time for precooling was 19 min, energy consumption 3.20 kWh, and electrical expense 0.17 Baht/kg (Table 4.7).

Table 4.7 Process parameters and measuring indices for vacuum cooling of pak-choi in perforated polyethylene bags, which were grown in summer

Process parameters	Value
Holding pressure (mbar)	6
Holding time (min)	5
Cycle time (min)	19
Initial chamber temperature(ºC)	24.60
Initial center temperature of pak-choi (ºC)	19.50
Final center temperature of pak-choi (ºC)	5.00
Weight loss (%)	1.38
Energy consumption (kWh)	3.20
Electrical expense (Baht/kg)	0.17

The relationship between temperature in a vacuum chamber and time, and pressure and time for reducing pak-choi temperature are shown in Figure 4.12. During the first 8 min, the pressure showed an abrupt change from 966.4 mbar to 17.8 mbar as the air temperature slowly decreased and the temperature of pak-choi slightly changed. Later, during the period of 9-15 min, the rate of reduction in pressure in the chamber began to slow down, and the rate of temperature reduction in the chamber and pak-choi rapidly decreased. During this period, the pressure in the chamber was reduced. When the rapid evaporation of water in pak-choi starts, the temperature of the vegetable dropped rapidly. The pressure in the vacuum chamber dropped from 966.4 to 6.0 mbar and reaching the reserved pressure of 6 mbar which was held for 5 min. During this period, the rate of decrease in temperature of pak-choi decreased slightly until a final temperature at 5.0 ºC was reached. The average of temperature decrease rate was 0.74 ºC per min.

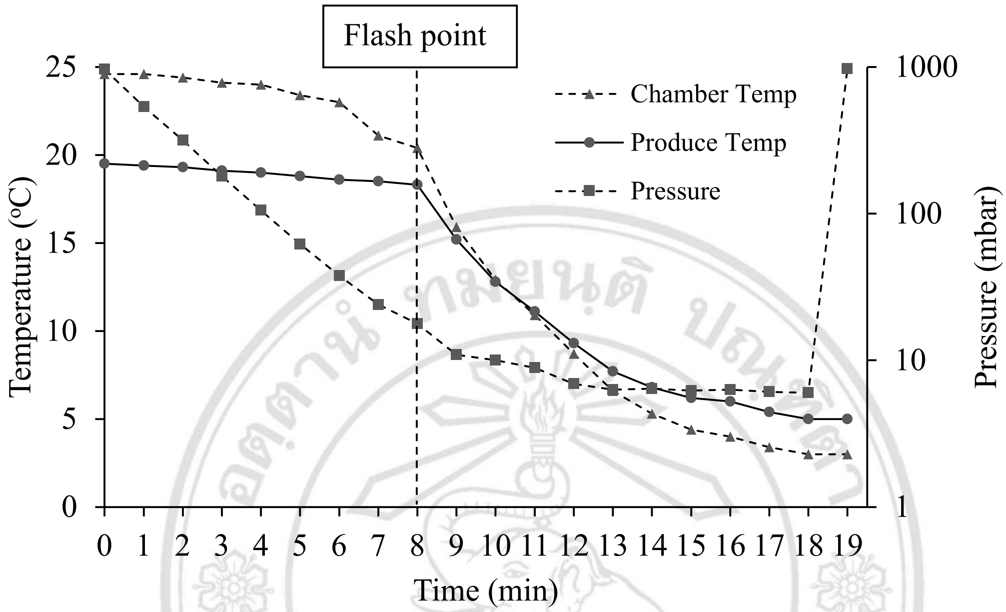

Figure 4.12 Temperature and pressure history in the vacuum chamber and cooling curve of pak-choi packed in perforated polyethylene bags during vacuum cooling at 6 mbar with 5 min holding time in summer

Weight Loss

After storage for 3 days, weight loss was not significantly different between vacuum and non-vacuum cooled pak-choi which were 1.75±0.20 and 1.93±0.26 %, respectively (Table 4.10). The weight loss continued to increase throughout the storage period (Figure 4.13). Correspondingly, vacuum cooling applied to some vegetables: organic pointed cabbage, organic baby pak-choi, organic baby carrot, sweet basil, holy basil, and coriander was found to have no effect on weight loss compared with non-vacuum cooling (Boonyakiat *et al*., 2009). In addition, pak-choi in this study was harvested in the morning with high turgidity. Water content in plant directly affects cell turgor that is important for the texture of leafy vegetables, e.g. 3% weight loss affects the texture of spinach (Sams, 1999).

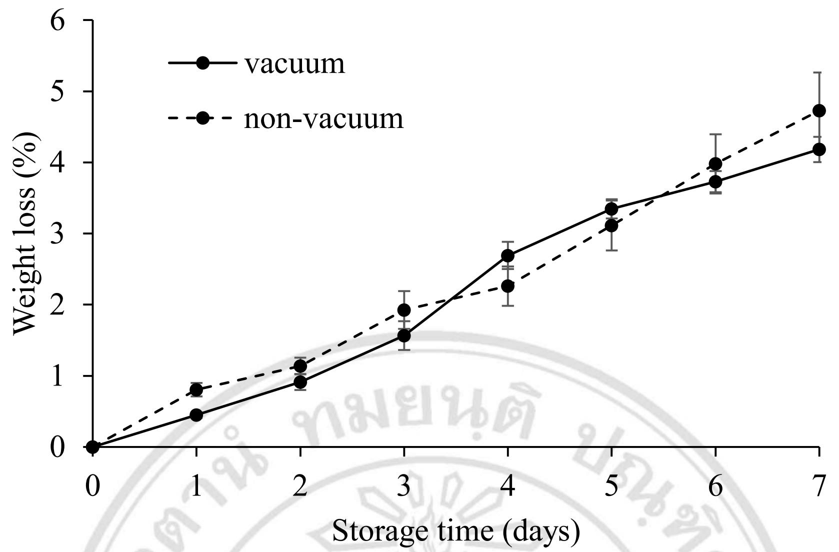

Figure 4.13 Weight loss of vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 ºC for 7 days

Content of reducing sugar and total sugar

The content of reducing and total sugar in pak-choi was not significantly different after storage for 3 days; the reducing sugar content in vacuum and non-vacuum cooled pak-choi was 5.75±0.21 and 6.29±0.76 %, respectively. Total sugar content in vacuum and non-vacuum cooled pak-choi were 8.65±0.13 and 9.15±0.34 %, respectively (Table 4.10). Likewise, Álvares *et al*. (2007) reported that hydrocooling did not affect total soluble sugar in parsley leaves during storage at 5 ºC, while the reducing sugar content was gradually elevated during storage. The content of reducing sugar rose from 5.01 to 20.71 g/kg leaf dry weight, a 4.1-fold increment in 144 hours. Barbosa *et al*. (2016) indicated that the soluble sugars levels varied according to relative water content. The present study indicated that the fresh weight of summer harvested pak-choi was rapidly lost during storage.

Additionally, the content of reducing and total sugar fluctuated throughout storage and the level at the end was higher than the initial level (Figure 4.14 and 4.15). That may be because this study used whole plant (except root) include younger and older leave. Sugars tend to be the highest in younger leaves growing close to the tip, and lowest in leaves towards the base of the stem, even though the leaves may look similar in size and appearance. Moreover, yellowing in pak-choi leaves is associated with a depletion of sugars (the main energy substrate). Increasing the initial leaf sugar level, or slowing the rate of sugar depletion, will directly increase shelf life (O'Hare *et al.*, 2001b).

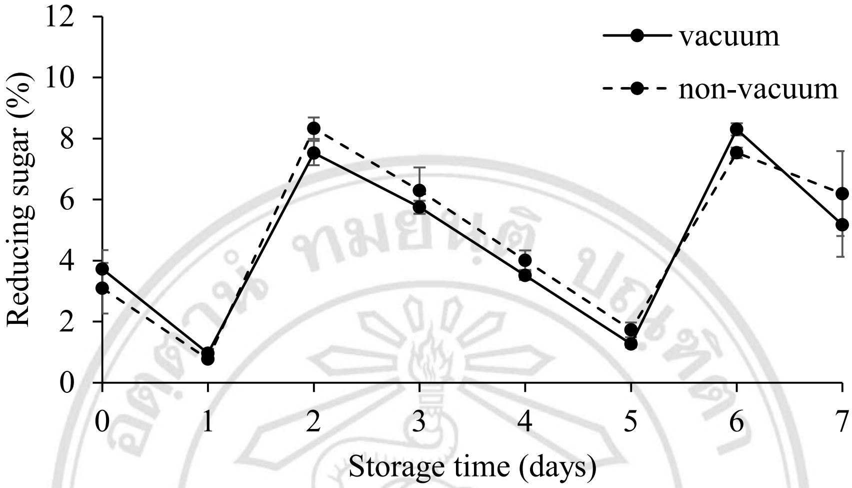

Figure 4.14 The content of reducing sugar in vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 ºC for 7 days

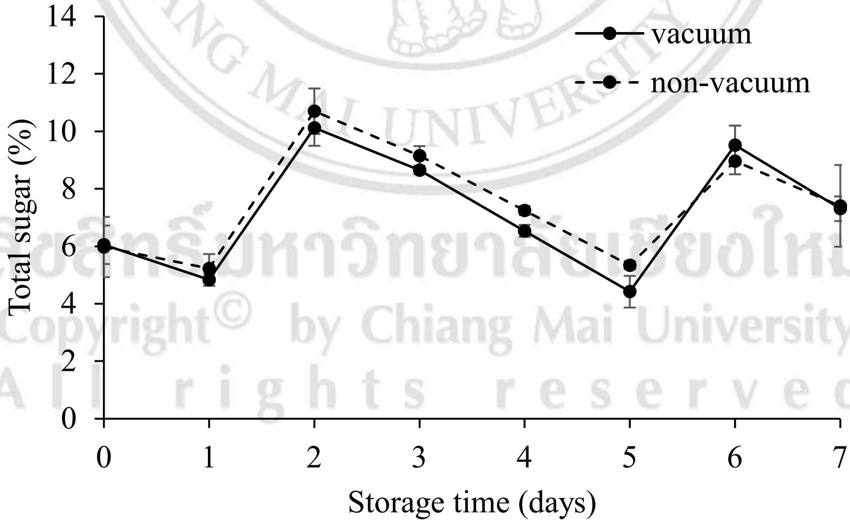

Figure 4.15 The content of total sugar in vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 ºC for 7 days

Content of vitamin C

The vitamin C content was not significantly affected by precooling. The vacuum and non-vacuum cooled pak-choi had 44.07±7.63 and 42.83±2.15 g/100 g FW, respectively, after storage at 8 ºC for 3 days (Table 4.10). This result indicated that vacuum cooling had no effect on vitamin C content. Likewise, Chinnapun (2009) reported that the vacuum cooling process had no effect on the vitamin C content of pak-choi. Other research had the same result: that vacuum cooling had no effect on vitamin C content of Chinese cabbage (Kamon *et al*., 2013), cos lettuce (Panyakham, 2011), and spinach (Hemrattrakun, 2009). It is noteworthy that in summer, the amount of vitamin C in both treatments varied more than in the other seasons, and at the end of storage had higher than an initial state (Figure 4.16). In this seasonal trial, water loss in pak-choi was very high and the loss continued throughout the storage period. This may be because the amount of water within the cells decreased, the concentration of dissolved substance inside the cells increased (Siripanich, 2006). On the other way, the increasing in total ascorbic acid content during storage of fruits and vegetables might be attributed to synthesis of ascorbic acid from monosaccharides, since in plants most synthesis starts with preformed D-glucose (Liao and Seib, 1988).

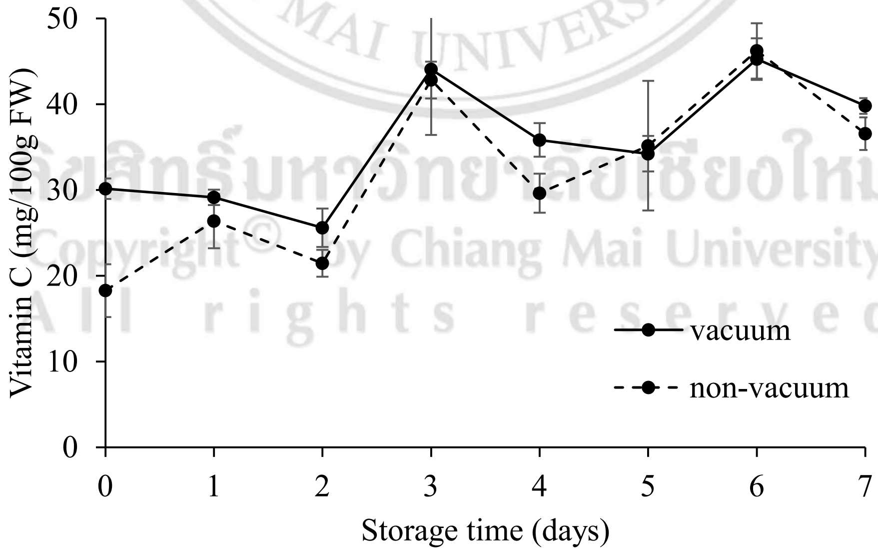

Figure 4.16 The content of vitamin C in vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 ºC for 7 days

Content of glucosinolate

After storage for 3 days, the glucosinolate content was not significant different between the vacuum and non-vacuum cooled pak-choi which were 2.25±0.19 and 2.72±0.51 µmol/g FW, respectively (Table 4.10). The content of glucosinolate was quite variable and tended to decrease during storage (Figure 4.17). According to Bérard and Chong (1985) reported that cabbage stored at 1±1 ºC in a common refrigerator, the glucosinolate content fluctuated or increased slowly during the first 122 days of storage; thereafter, it increased more rapidly, especially toward the end of the storage period, in coincidence with the increasing senescence of the cabbage heads. Moreover, the glucosinolate content related to water loss causes deterioration of plant tissue; the glucosinolate in cytoplasm is hydrolyzed by myrosinase (Barth and Jander, 2006; Mithen *et al*., 2000 and Wittstock and Burow. 2010). In the present study, in the summer season, vacuum and non-vacuum cooled pak-choi had high water loss up to 4.18±0.18 and 4.73±0.54%, respectively, by the end of storage (Figure 4.13 and Table B11).

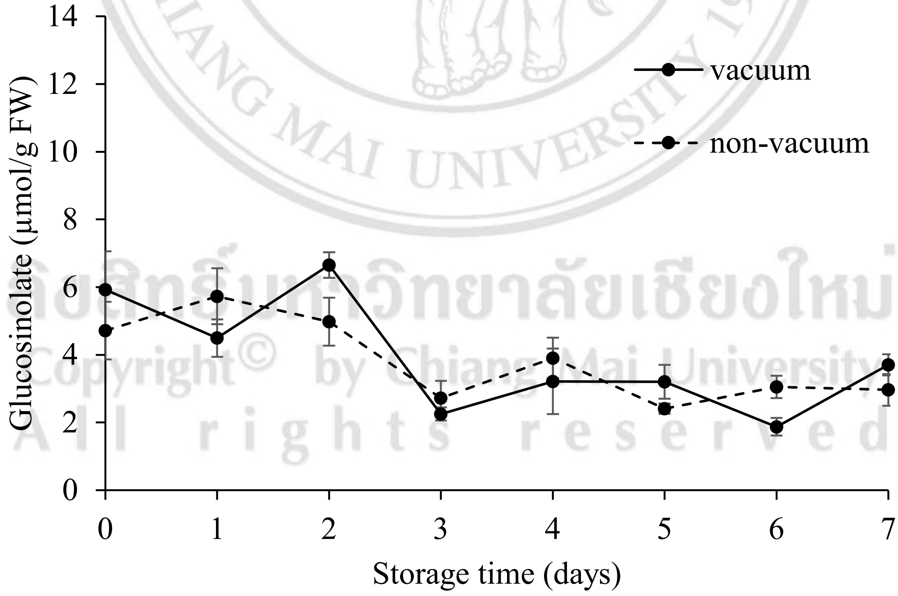

Figure 4.17 The content of glucosinolate in vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 ºC for 7 days

Content of crude fiber

Figure 4.18 showed that the content of crude fiber did not significantly differ and fluctuated much during the treatment period. After storage for 3 days, the vacuum cooled pak-choi had 16.62±0.60 g/100 g DW and non-vacuum cooled pak-choi had 16.55±1.17 g/100 g DW crude fiber content (Table 4.10). Likewise, the research of Acho *et al*. (2015) found that the content of total fiber in five leafy vegetables (*Colocacia esculenta*, *Basella alba*, *Solanum melongena*, *Telinum triangulare* and *Corchorus olitorius*) slightly increased with storage time, but were not significantly different in each vegetable when stored in a refrigerator at 4 ºC for 15 days. The increase of the total fiber may be due to increased amounts of uronic acid in the insoluble fiber fraction (Marlett, 2000). 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 (Aletor *et al*., 2002; Punna and Prachuri, 2004; Tendaj *et al*., 2013; Uusiku *et al*., 2010). The relation between vacuum cooling and crude fiber in vegetables need more knowledge.

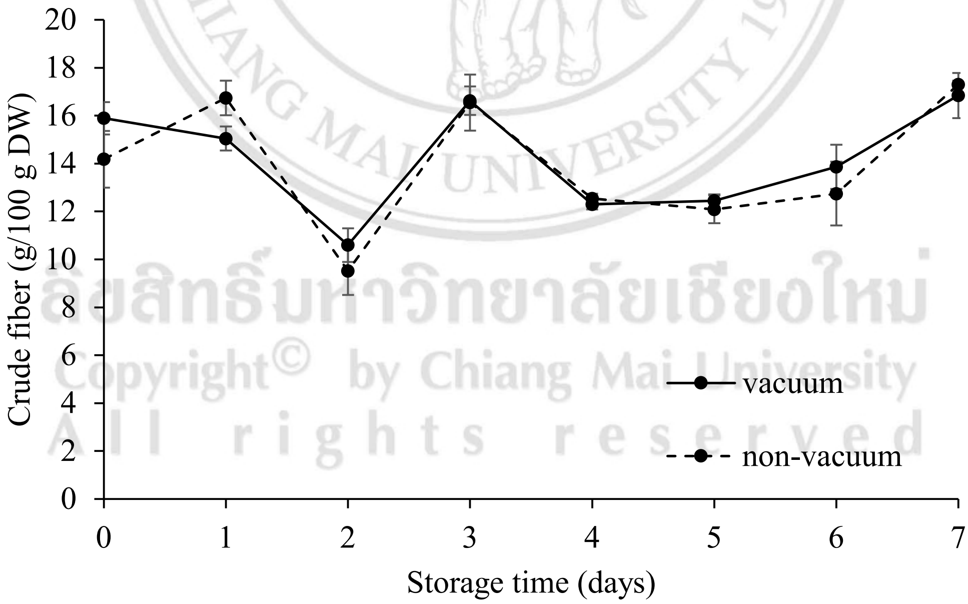

Figure 4.18 The content of crude fiber in vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 ºC for 7 days

Leaf color change

The results showed that vacuum cooling had no effect on the change of leaf color in organic pak-choi. The color value (SPAD unit) that indicates the chlorophyll content tended to slightly decrease throughout the storage period (Figure 4.19). After storage for 3 days, the color values of vacuum cooled and non-vacuum cooled pak-choi were 37.63±2.06 and 37.03±1.67 SPAD unit, respectively (Table 4.10). Similarly, Sirinanuwat *et al*. (2012) reported that in organic coriander vacuum cooled and stored at 5 ºC, the vacuum cooling had no effect on the change of chlorophyll and it tended to decrease with storage time which correlated with the decreased green color. The research of Poonlarb and Boonyakiat (2015) reported that vacuum cooling had no effect on the change of color in Chinese kale and the total chlorophyll tended to decrease with storage time. Additionally, Boonyakiat *et al*. (2007) reported that in pak-choi, lettuce and broccoli, vacuum cooled before storage, no significant difference in leaf color change was observed compared with non-vacuum cooled produce.

The chlorophyll degradation in fruit and vegetables depend on internal and external factors. At high temperature chlorophyll degradation was rapid while low temperature can delayed chlorophyll degradation (Heaton and Marangoni, 1996). In addition, the pattern of chlorophyll degradation depended on the plants' production environment where higher temperatures resulted subsequently in rapid degradation during storage (Mahmud *et al*., 1999). In the present study, pak-choi was harvested in the morning at low temperature of 17.33±0.18 ºC (Table A2) and transported immediately (1.50 hour, from farm to packing house). Then the produce was processed (trimming, packing and precooled) and stored at low temperature 8 ºC. All process (harvest to storage) spent short time 2-3 hour and low storage temperature can maintained green color of pak-choi leaves.

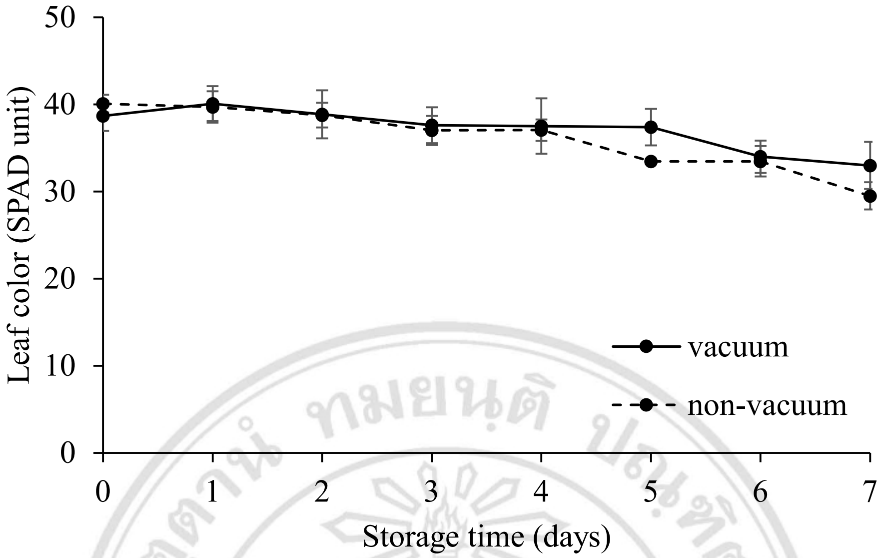

Figure 4.19 Leaf color change in vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 °C for 7 days

Respiration rate

The respiration rate of precooled pak-choi was lower than non-precooled throughout the storage period (Figure 4.20). After storage for 3 days, the respiratory rate was significantly different. The vacuum cooled pak-choi had a lower respiration rate than non-vacuum cooled of 11.81±2.91 and 23.95±0.83 mg CO_2/kg-h, respectively (Table 4.10). The vegetables harvested from the farmer plot when the temperature is high, are subject to physiological and other processes that cause rapid produce deterioration (Boonyakiat, 2015). Vacuum cooling can delay or slow down cellular metabolic processes in the produce, delay senescence and aging, maintain produce quality and lengthen shelf life (Brosnan and Sun, 2001). The results of this study agree with those of Ding *et al*. (2016) who reported that vacuum cooling significantly reduced the respiration rate of postharvest broccoli when compared with ice-water cooling, cold room cooling and without precooling.

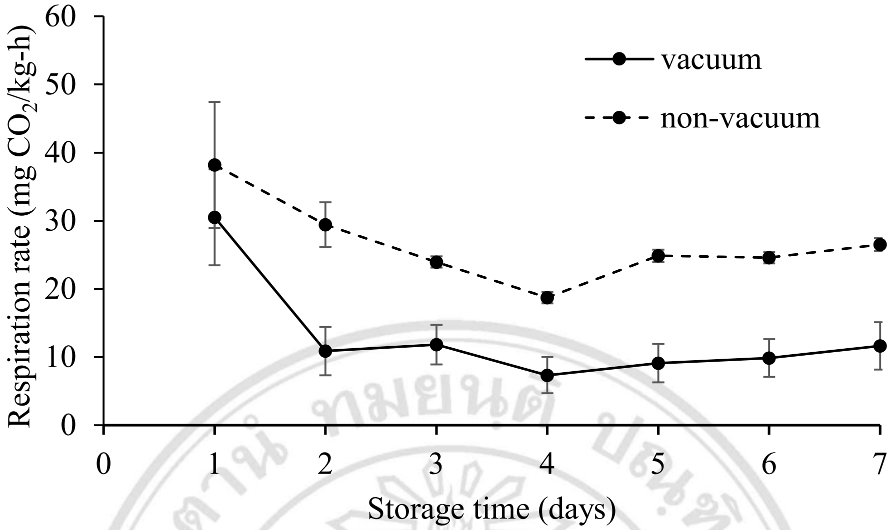

Figure 4.20 Respiration rate in vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 ºC for 7 days

Ethylene production rate

The ethylene production rate increased after storage for 2 days, then rapidly declined after that. The ethylene production rate did not significant differ throughout the storage period (Figure 4.21). The production rate of ethylene in vacuum and non-vacuum cooled pak-choi was 1.02±0.14 and 1.12±0.19 µl/g-h, respectively, after storage for 3 days (Table 4.10). In general, ethylene production rates increase with maturity at harvest and with physical injuries, disease incidence, increased temperatures up to 30 ºC, and water stress. In contrast, ethylene production rates in fresh horticultural crops are reduced by storage at low temperature, by reduced O_2 levels (<8%), and elevated CO_2 levels (>2%) around the commodity (Kader, 2002). Precooling methods can be used in combination with other storage methods for maintaining fresh produce quality (Borompichaichartkul *et al*., 2009).

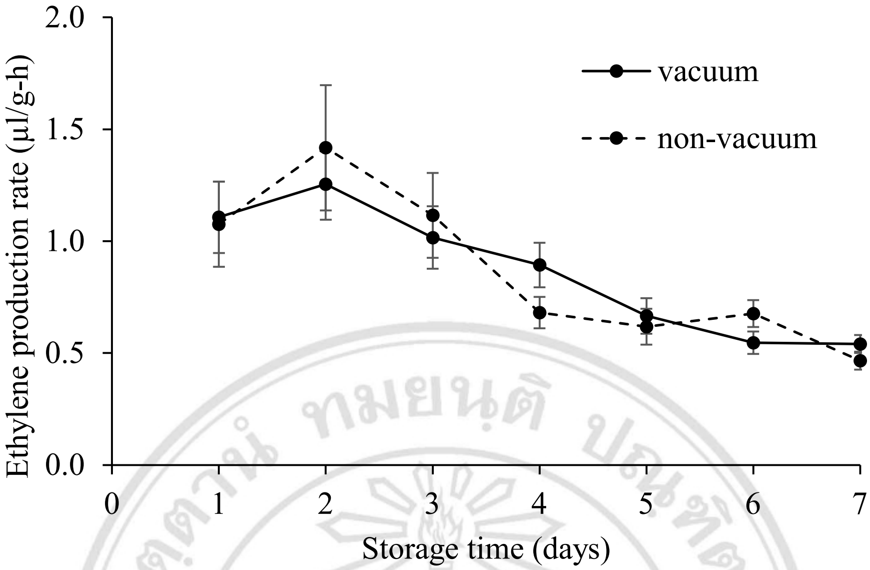

Figure 4.21 Ethylene production rate in vacuum and non-vacuum cooling of summer harvested pak-choi stored at 8 ºC for 7 days

Shelf life and produce appearances

Pak-choi that was precooled by vacuum cooling, then stored at 8 °C had longer shelf life, 6.25±0.95 days than non-vacuum cooling, 3.00±0.58 days (Table 4.9). Fresh vegetables are living tissues that are subject to continuous changes after harvest. Postharvest changes in fresh produce cannot stopped, but they can be slowed within certain limits (Kader, 2002). Vacuum cooling can delay the metabolism within the cell, maintain the quality of produce and prolong shelf life (Brosnan and Sun, 2001). Many research reported that vacuum cooling extended shelf life of pak-choi, coriander, chinese kale, and organic chayote shoots, longer than non-vacuum cooled (Chinnapun, 2009; Sirinanuwat *et al*., 2012; Poonlarp and Boonyakiat, 2015; Poonlarp *et al*., 2012).

The color change is the first visual symptom of senescence in many vegetables. The catabolism of leaf pigments is strongly connected with storage conditions (Ferrante *et al*., 2004). Xiangyang and Bagshaw (2001) suggested that leaf yellowing of pak-choi was caused by high temperatures. Low temperature usually slows down all leaf metabolism preserving the quality of the produce (Ferrante *et al*., 2004). In addition, wilting occurred in both treatments at the end of storage because weight loss more than

4% (Table B11). Water content in plant directly affects cell turgor that is important for the texture of leafy vegetables, e.g. 3% water loss in spinach results in a product unmarketable due to loss of texture (Sams, 1999).

Conclusion

In summer, vacuum cooling affected respiration rate and shelf life. As in winter, the respiration rate of precooled pak-choi was lower than non-precooled throughout the storage period. The shelf life of vacuum cooled pak-choi was 6.25±0.95 days and non-vacuum cooled pak-choi, 3.00±0.58 days.

2.3 Effects of vacuum cooling on delay senescence of organic pak-choi in rainy season

The produce that was precooled by vacuum cooling had a final bleed pressure of 6 mbar and soak time of 5 min. The initial temperature at the pak-choi center was 20.10 ºC, and the final temperature, 6.0 ºC. The weight loss was 1.35%. The cycle time for precooling was 20 min, energy consumption, 3.20 kWh and electrical expense, 0.17 Baht/kg (Table 4.8).

Table 4.8 Process parameters and measuring indices for vacuum cooling of pak-choi in perforated polyethylene bags, which were grown in rainy season

Process parameters	Value
Holding pressure (mbar)	6
Holding time (min)	5
Cycle time (min)	20
Initial chamber temperature(ºC)	24.00
Initial center temperature of pak-choi (ºC)	20.10
Final center temperature of pak-choi (ºC)	6.0
Weight loss (%)	1.35
Energy consumption (kWh)	3.20
Electrical expense (Baht/kg)	0.17

The relationship between temperature in vacuum chamber and time, and pressure and time for reduction of pak-choi temperature are shown in Figure 4.4. At the first 8 min, the pressure showed an abrupt change from 963.10 mbar to 19.10 mbar as the air temperature was slowly decreased and the temperature of the pak-choi slightly changed. Later, during the period of 9-19 min, the rate of decrease in pressure in the chamber began to slow down, and the rate of decrease in temperature in the chamber and pak-choi were rapidly decreased. During this period the pressure in the chamber was reduced. The rapid evaporation of water in pak-choi started, and the temperature of the vegetable dropped rapidly. The pressure in the vacuum chamber dropped from 963.1 to 6.0 mbar and reaching the reserve pressure of 6 mbar which was held for 5 min. During this period the rate of decrease in temperature of pak-choi slightly decreased until the final temperature of 6.0 ºC was reached. The average of temperature decrease rate was 0.68 ºC per min.

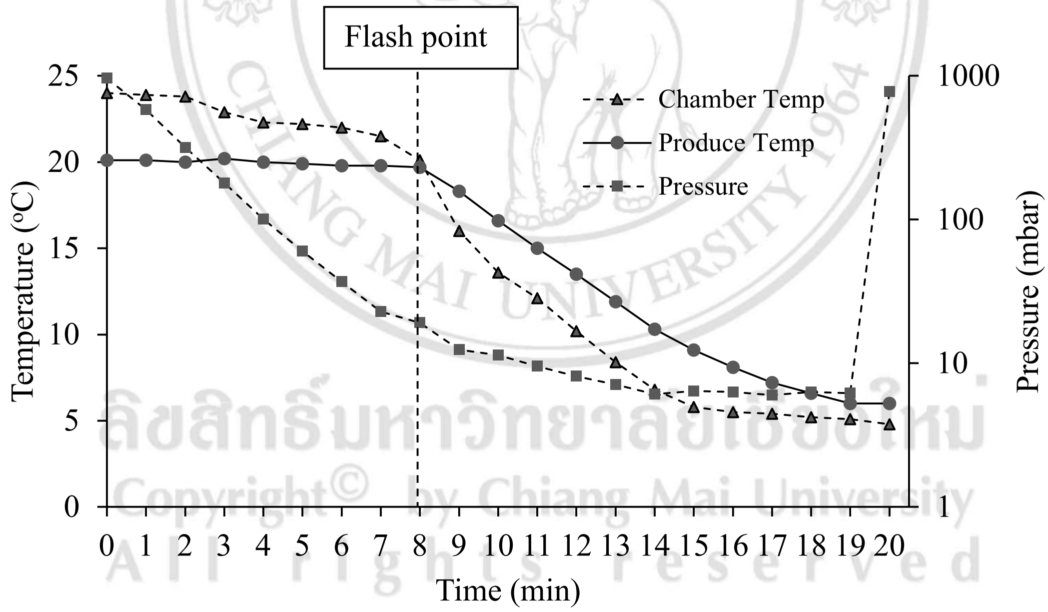

Figure 4.22 Temperature and pressure history in the vacuum chamber and cooling curve of pak-choi packed in perforated polyethylene bags during vacuum cooling at 6 mbar with 5 min holding time in rainy season

Weight loss

The weight loss percentage of vacuum cooled pak-choi was lower than non-vacuum cooled pak-choi and both showed a slightly increased weight loss throughout the storage period (Figure 4.23). After storage for 3 days, vacuum cooled pak-choi lost 0.53± 0.09% less than non-vacuum cooled pak-choi lost 0.86±0.12% (Table 4.10). The harvested produce has field heat, vital heat and heat from the surrounding environment. These factors result in the accumulation of heat and increase the temperature inside the produce. Temperature is an important factor influencing the quality and shelf life of vegetables. Because of the high temperature, it catalyzes various chemical reactions, increase respiration rate and water loss from the cells of the produce. The temperature of the produce, atmospheric conditions and the relative humidity of the storage condition have a great effect on the loss of water. Precooling and storage of the produce at low temperature is necessary. Precooling with the suitable method removes heat from the product prior to storage (Siripanich, 2006). Proper postharvest cooling can slow or inhibited water loss (Wilson *et al*., 2009).

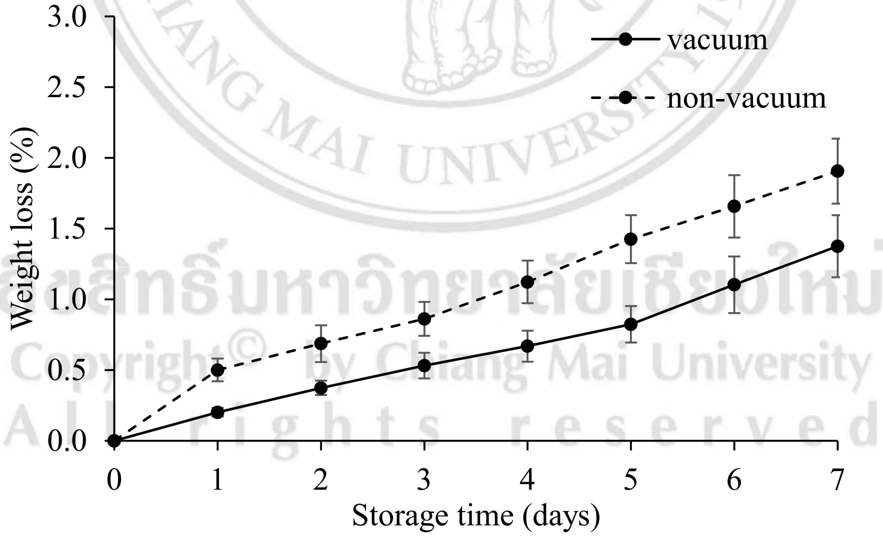

Figure 4.23 Weight loss of vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 °C for 7 days

Content of reducing sugar and total sugar

The content of reducing and total sugar fluctuated from beginning until 3 days of storage and tend to decrease after that (Figure 4.24 and 4.25). After storage for 3 days, reducing and total sugar content were not significantly different. Reducing sugar content in vacuum cooled pak-choi was 4.44± 0.86% and non-vacuum cooled was 3.97±0.62%. Total sugar content in vacuum and non-vacuum cooled pak-choi was 7.13±0.98 and 5.88±0.77%, respectively (Table 4.10). The vacuum cooling did not significantly affect the reducing and total sugar content in the pak-choi.

Sugar is the essential energy source of vegetables and is also the main substrate for vegetable respiration. Therefore, reducing sugars are thought to be closely related to the physiological and biochemical properties of vegetables (Ding *et al*., 2016). The decrease in total soluble sugar is related to the chlorophyll degradation during leaf senescence. The degradation of chlorophyll occurs when the soluble sugar is depleted by 60%, and the sugar concentration is decreased during the senescence of leaves (Able *et al*., 2005). The research of Ding *et al*. (2016) indicated that in broccoli precooled by vacuum cooling, ice-water cooling, and cold room treatment before storage at 5 ºC for 30 days, the content of reducing sugar declined during storage.

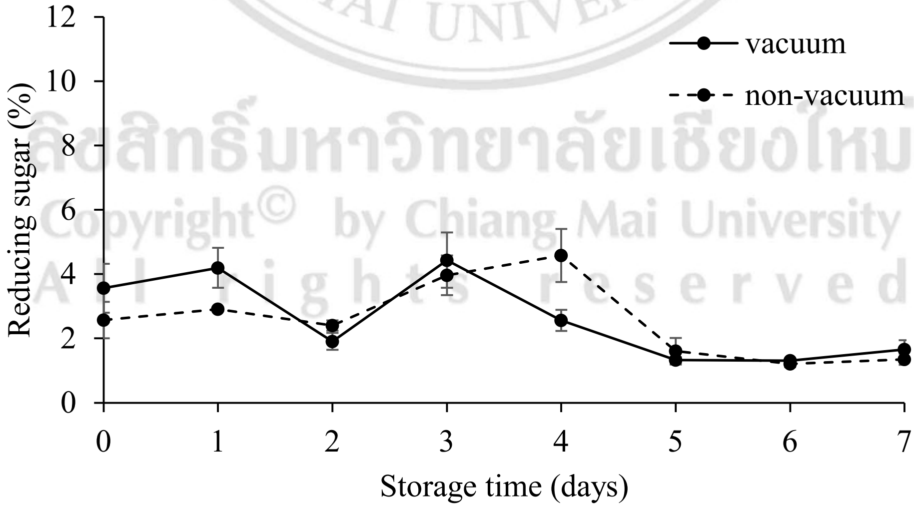

Figure 4.24 The content of reducing sugar in vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 ºC for 7 days

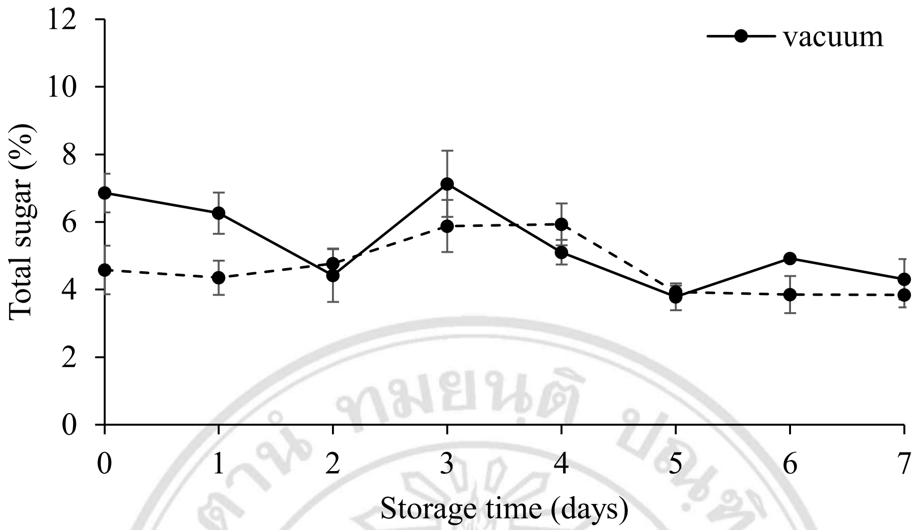

Figure 4.25 The content of total sugar in vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 ºC for 7 days

Content of vitamin C

In rainy season, the content of vitamin C in vacuum cooled pak-choi was more stable than non-vacumm cooled pak-choi (Figure 4.26). After storage for 3 days, the vitamin C content differed significantly; vacuum cooled pak-choi had vitamin C content 43.42±0.46 g/100 g FW was higher than non-vacuum cooled 35.65±1.75 g/100 g FW (Table 4.10). Temperature management after harvest is the most important factor to maintain the vitamin C of fruits and vegetables. The loss of vitamin C content is accelerated at higher temperatures and with longer storage durations. However, some chilling sensitive crops show more losses in vitamin C at lower temperatures. Conditions favorable to water loss after harvest result in a rapid loss of vitamin C especially in leafy vegetables. The retention of vitamin C is lowered by bruising, and other mechanical injuries, and by excessive trimming (Lee and Kader, 2000). These results are consistent with those of Boonyakiat *et al*. (2009) who reported that vacuum cooling had a significant effect on vitamin C content after storage for 5 days, baby pak-choi precooled using a final pressure of 6 mbar with a reserving time of 15 and 25 minutes had a higher vitamin C content than non-cooled produce. Likewise, He *et al*. (2004) reported that vacuum cooling lettuce before cold stored for 2 week had a positive effect on the ascorbic acid content. In addition, the research of Ding *et al*. (2016) showed that the broccoli precooled by vacuum cooling, ice-water cooling and cold room cooling had higher vitamin C content than non-precooled broccoli during storage for 30 days.

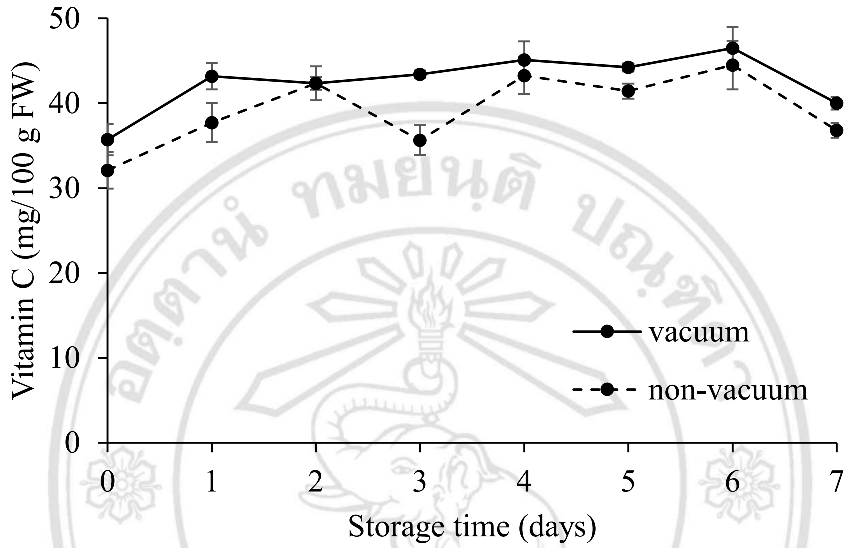

Figure 4.26 The content of vitamin C in vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 ºC for 7 days

Content of glucosinolate

The glucosinolate content in vacuum cooled pak-choi decreased in the first 3 days of storage, then it increased and reached the initial level at the end of storage. In non-vacuum cooled pak-choi, the glucosinolate content tended to decrease over the storage period although it increased after 3 days (Figure 4.27). At 3 days of storage, the glucosinolate content was not significantly different in vacuum and non-vacuum cooled pak-choi which were 4.71±0.96 and 3.96±0.56 µmol/g FW, respectively (Table 4.10). Yang *et al*. (2010) reported that low temperature was beneficial to maintain the glucosinolate content in pak-choi or even increase the content of total glucosinolate at the end of the experiment. Low temperature (~4 ºC) is optimal for maintaining glucosinolate content in broccoli, because these conditions maintain cellular integrity by preventing the mixing of glucosinolate with myrosinase (Jones *et al*., 2006; Rodrigues and Rosa, 1999). In present study, pak-choi was harvested in the morning and at low temperature, and spend 2-3 hour for transportation and process before storage at low temperature 8 ºC.

While the content of glucosinolate was significant different only at the end of storage, vacuum cooled pak-choi had 8.88±0.36 µmol/g FW of glucosinolate which was higher than non-vacuum cooled. Because vacuum cooled pak-choi had lower weight loss of 1.38±0.22% than non-vacuum cooled pak-choi of 1.91±0.23% (Table B12). The glucosinolate content related to water loss which causes deterioration of plant tissue, leading to the hydrolysis by myrosinase with glucosinolate in the cytoplasm (Barth and Jander, 2006; Mithen *et al*., 2000; Wittstock and Burow. 2010).

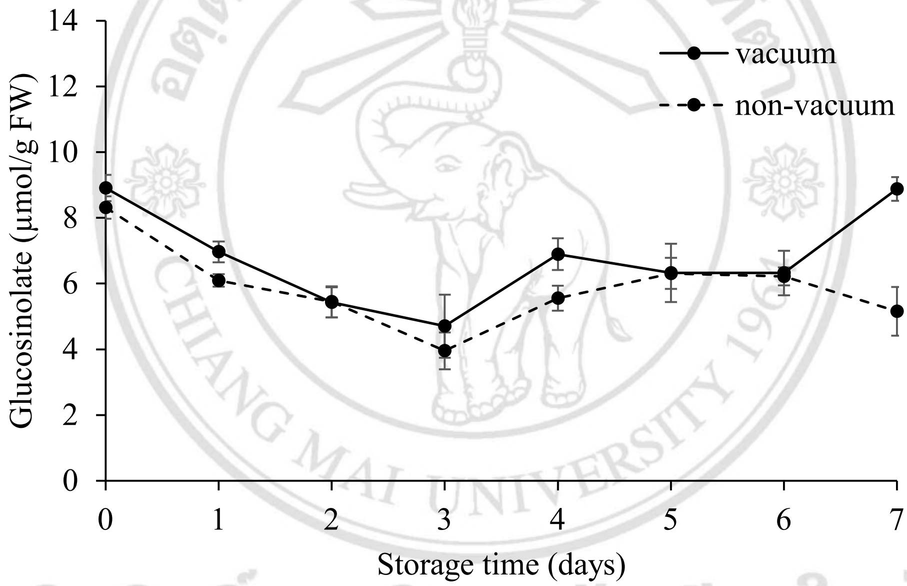

Figure 4.27 The content of glucosinolate in vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 ºC for 7 days

Content of crude fiber

Figure 4.28 showed experimental results in rainy season similar to winter season; the content of crude fiber slightly fluctuated. After storage for 3 days, the content of crude fiber did not significantly differ between the vacuum and non-vacuum cooled pak-choi, which was 15.80±0.67 and 14.67±1.09 g/100 g DW, respectively (Table 4.10). Likewise, Acho *et al*. (2015) reported that in five leafy vegetables (*Colocacia esculenta*, *Basella alba*, *Solanum melongena*, *Telinum triangulare* and *Corchorus olitorius*) stored in a refrigerator at 4 ºC for 15 days, the content of total fiber was not significant in each vegetable and slightly increased with refrigeration storage time. This may be due to increasing amounts of uronic acid in the insoluble fibre fraction (Marlett, 2000). 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 (Aletor *et al*., 2002; Punna and Prachuri, 2004; Tendaj *et al*., 2013; Uusiku *et al*., 2010).

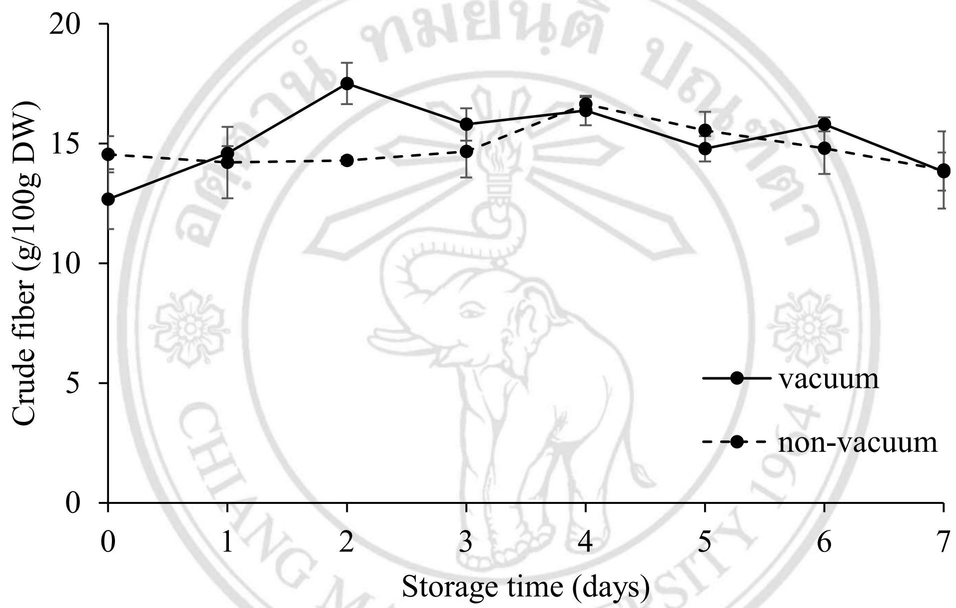

Figure 4.28 The content of crude fiber in vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 ºC for 7 days

Leaf color change

The leaf color of vacuum and non-vacuum cooled pak-choi tended to be stable throughout storage and not significantly different throughout storage (Figure 4.29). Leaf color change in vacuum cooled pak-choi was 39.40±0.26 SPAD unit and non-vacuum cooled was 40.50±1.56 SPAD unit after 3 days of storage (Table 4.10). The results showed that vacuum cooling did not affect leaf color of pak-choi and the changes of vacuum and non-vacuum cooled pak-choi were at the same direction during storage. Corresponding with Boonyakiat *et al*. (2007) and Boonyakiat *et al*. (2009) reported that the color change of pak-choi and baby pak-choi did not differ significantly between vacuum cooled and non-vacuum cooled produce before storage at 8-10 ºC for 4 days.

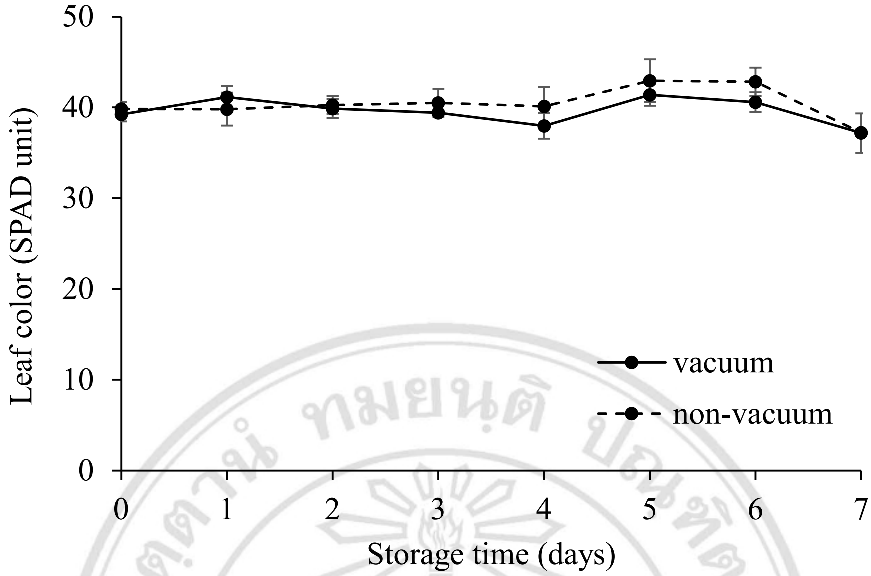

Figure 4.29 Leaf color change in vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 ºC for 7 days

Respiration rate

In rainy season, the respiration rate in vacuum cooled pak-choi was lower than non-vacuum cooled throughout the treatment. The respiration rates were significantly different at 3 days after storage, the vacuum cooled pak-choi respiration rate was 12.85±3.06 mg CO_2/kg-h and non-vacuum cooled pak-choi was 23.16±1.43 mg CO_2/kg-h (Table 4.10). The rate of respiration in vacuum cooled pak-choi was more consistent than non-vacuum cooled pak-choi. In non-vacuum cooled pak-choi the rate of respiration declined after harvest, before stabilizing (Figure 4.30).

The important factor affecting the respiration rate is temperature. Precooling especially vacuum cooling is a method that rapidly removes the field heat before storage, and will reduce 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 vacuum cooled and hydro cooled before stored at 7 ºC 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 respiration rate of harvested broccoli compared to hydro cooling, room cooling and broccoli without precooling.

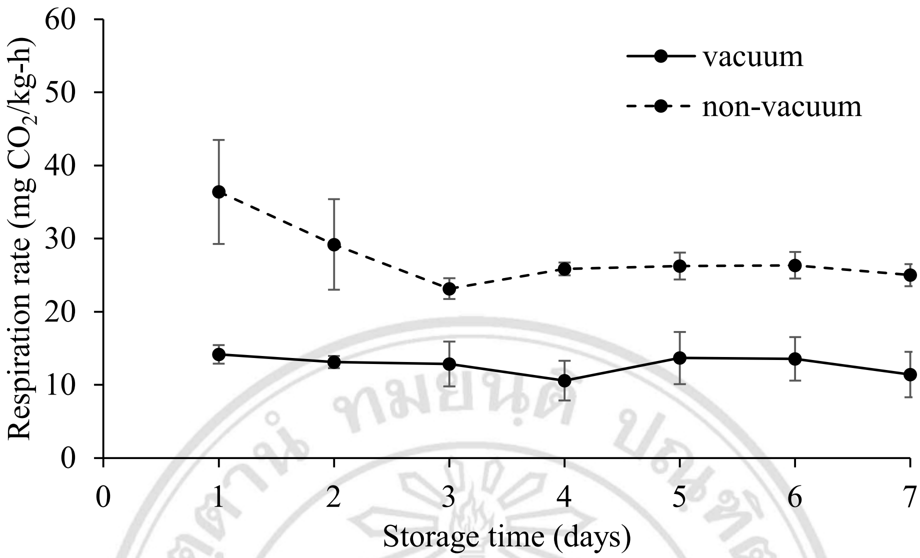

Figure 4.30 Respiration rate in vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 ºC for 7 days

Ethylene production rate

Figure 4.31 showed that the ethylene production rate in vacuum and non-vacuum cooled pak-choi tended to slightly decrease during the storage period. There was no significant difference on day 3 of storage, the ethylene production rate in vacuum cooled produce was 0.38±0.05 µl/g-h and non-vacuum cooled was 0.37±0.08 µl/g-h (Table 4.10). Proper precooling preserves product quality by: (1) inhibiting the growth of decay producing microorganisms; (2) restricting enzymatic and respiratory activity; (3) inhibiting water loss; and (4) reducing ethylene production. Precooling methods can be employed in combination with other storage methods for maintaining fresh produce quality (Borompichaichartkul *et al*., 2009). While pak-choi was harvested in rainy season in the morning and stored at room temperature (experiment 1) had more ethylene production rate 2-3 times (1.23±0.19 µl/g/h) than non-vacuum cooled pak-choi stored at 8 ºC on day 3 of storage (Table 4.1 and 4.10).

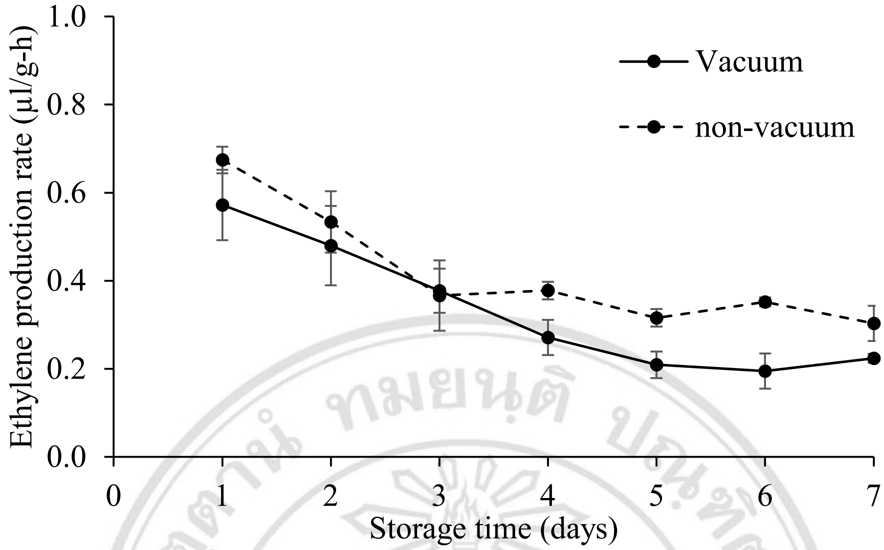

Figure 4.31 Ethylene production rate in vacuum and non-vacuum cooling of rainy season harvested pak-choi stored at 8 ºC for 7 days

Shelf life and produce appearances

According to our results, the vacuum cooled pak-choi had longer shelf life (6.75±0.48 days) than non-vacuum cooled (4.15±0.75 days) (Table 4.9). Fresh vegetables are living tissues that are subject to continuous change after harvest. The changes in fresh produce after harvest cannot be stopped, but they can be slowed down within certain limits. Temperature management is the most effective tool for extending the shelf life of fresh horticultural commodities. It begins with the rapid removal of field heat by using one of a number of precooling methods (Kader, 2002). Many research reported that the vacuum cooling can maintain the quality of produce and prolong shelf life in Chinese cabbage, pak-choi, Chinese kale, cauliflower, and broccoli (Kamon *et al*., 2013; Chinnapun, 2009; Poonlarp and Boonyakiat, 2015; Alibas and Koksal, 2015; Ding *et al*., 2016).

In this experiment, the leaves of pak-choi remained green and there were no differences between two treatments throughout storage (Figure 4.29). In *Brassica* crops especially pak-choi, senescence is most widely characterized by yellowing caused by a breakdown of chlorophyll pigments inside the tissues (Dixon, 2007). Xiangyang and Bagshaw (2001) suggested that leaf yellowing of pak-choi was caused by high temperatures. Low temperature usually slows down all leaf metabolism preserving the quality of the produce (Ferrante *et al*., 2004). In addition, this study showed that the leaves yellowing starts to appear from the edge of the leaf. In naturally senescing leaves, senescence occurs in a coordinated manner at the whole-leaf level started from the tip and margins towards the base of the leaf (Guiboileau *et al*., 2010)

Conclusions

In the rainy season, the vacuum cooling affected vitamin C content, respiration rate and shelf life. The contents of vitamin C were significantly different, the vitamin C content in vacuum and non-vacuum cooled pak-choi was 43.42±0.46 g/100 g FW and 35.65±1.75 g/100 g FW, respectively after 3 days storage and the content of vitamin C in vacuum cooled pak-choi was more stable than in non-vacuum cooled. Likewise in winter and summer, the respiration rate of precooled pak-choi was lower than non-precooled throughout storage period. The shelf life tend to be difference, vacuum cooled pak-choi was 6.75±0.48 days and non-vacuum cooled was 4.75±0.75 days (Table 4.9).

Table 4.9 Effects of vacuum and non-vacuum cooling on shelf life of pak-choi harvested in the winter, summer and rainy season

Treatments	Shelf life (days)		
	Winter	Summer	Rainy
Vacuum cooling	6.75±0.85 a	6.25±0.95 a	6.75±0.48 a
Non-vacuum cooling	3.50±0.29 b	3.00±0.58 b	4.75±0.75 a
2-tail Sig.	0.011	0.026	0.066

Note: Overall means with different letters in the same column are significantly different at $P \leq 0.05$ by T-test.

Table 4.10 Effects of vacuum and non-vacuum cooling on organic pak-choi after storage for 3 days in winter, summer and rainy season

Treatments	Weight loss (%)	Reducing sugar (%)	Total sugar (%)	Glucosinolate (µmol/g FW)	Vitamin C (mg/100 g FW)	Crude fiber (g/100 g DW)	Leaf color (SPAD unit)	Respiration rate (mg CO_2/kg-h)	Ethylene production rate (µl/g-h)
Winter									
Vacuum	0.70±0.11 a	4.35±0.43 b	7.21±0.52 a	9.63±1.12 a	31.14±2.31 a	16.17±0.64 a	37.53±0.70 a	13.36±3.28 b	0.13±0.01 a
Non-vacuum	0.75±0.09 a	6.10±0.27 a	8.17±0.29 a	10.29±0.95 a	29.39±5.33 a	15.99±0.49 a	37.08±0.22 a	28.99±3.22 a	0.14±0.05 a
2-tail Sig.	0.710	0.014	0.162	0.665	0.772	0.834	0.578	0.027	0.934
Summer									
Vacuum	1.57±0.20 a	5.75±0.21 a	8.65±0.13 a	2.25±0.19 a	44.07±7.63 a	16.62±0.60 a	37.63±2.06 a	11.81±2.91 b	1.02±0.14 a
Non-vacuum	1.93±0.26 a	6.29±0.76 a	9.15±0.34 a	2.72±0.51 a	42.83±2.15 a	16.55±1.17 a	37.03±1.67 a	23.95±0.83 a	1.12±0.19 a
2-tail Sig.	0.326	0.519	0.219	0.423	0.883	0.957	0.829	0.006	0.691
Rainy									
Vacuum	0.53±0.09 a	4.44±0.86 a	7.13±0.98 a	4.71±0.96 a	43.42±0.46 a	15.80±0.67 a	39.40±0.26 a	12.85±3.06 b	0.38±0.05 a
Non-vacuum	0.86±0.12 a	3.97±0.62 a	5.88±0.77 a	3.96±0.56 a	35.65±1.75 b	14.67±1.09 a	40.50±1.56 a	23.16±1.43 a	0.37±0.08 a
2-tail Sig.	0.066	0.673	0.356	0.523	0.018	0.413	0.514	0.02	0.915

Note: Overall means with different letters in the same column are significantly different at $P \leq 0.05$ by T-test