

Effect of Season and Harvesting Time on Quality of Organic Pak Choi (*Brassica rapa* var. *chinensis*)

P. Yusuk¹, S. Thumdee¹, P. Poonlarp^{2,3} and D. Boonyakiat^{1,3,*}

¹ Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

² Division of Food Engineering, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand

³ Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok 10400, Thailand

* Corresponding author Email: danai.b@cmu.ac.th

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ABSTRACT

This research was aimed at study the effects of season and harvesting time on the quality of organic Pak choi (*Brassica rapa* var. *chinensis*). Pak choi was harvested from a farmer's greenhouse at different times of the day: morning (05:00 am–08:00 am), afternoon (12:00 pm–2:00 pm) and evening (4:30 pm–6:30 pm) in the winter, summer and rainy season. Season affected on total sugar, glucosinolate, vitamin C and crude fiber content while harvesting time affected on glucosinolate, vitamin C content and leaf color. After 3-day storage at ambient temperature, it was revealed that season affected all parameters, whereas harvesting time only affected reducing and total sugar contents, glucosinolate, vitamin C and respiration rate. Nevertheless, the interaction of both factors affected sugar contents, glucosinolate and respiration rate. This study suggested that rescheduling planting dates depending on the season and selecting a suitable time in the day for harvest can positively affect the quality of organic Pak choi. For nutritional benefits, Pak choi has the highest glucosinolate content when it is harvested in the morning. On the other hand, the produce harvested in the evening has the highest vitamin C content. The summer season may affect the quality due to the highest respiration rate and weight loss at that time.

Keywords: Temperature, sugar, glucosinolate, vitamin C, crude fiber, respiration, ethylene

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INTRODUCTION

Pak choi belongs to the Brassicaceae family (previously Cruciferae). Pak choi was evolved in China, and its cultivation was recorded as far back as the 5th century AD. It is widely cultivated in the Philippines, Malaysia, Indonesia and Thailand. In recent years, its popularity has increased in North America, Australia and Europe (Welbaum, 2015; Dixon, 2007). Brassica vegetables are a rich source of glucosinolates (GSs) that can reduce the activity of carcinogens, and risk of cancer and coronary heart diseases (Chen *et al.*, 2008; Higdon

et al., 2007; Verkerk *et al.*, 2009). Zhu *et al.* (2013) reported that Pak choi harvested at 20–25 days after transplanting had high levels of beneficial GSs.

Pak choi is a favored vegetable among farmers because of its short crop duration (harvesting time at 40–45 days after sowing). On the other hand, Pak choi leaves rapidly become yellow indicating senescence within 2–3 days after harvest (Boonyakiat *et al.*, 2008). The change of color relates to consumer perception of visual quality especially in leafy vegetables. An investigation of Thai farmers' practices showed that each farmer used a different time in a day from sunrise to sunset to harvest organic

Pak choi. 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). This study aimed to determine the effect of season and harvesting time on the quality of organic Pak choi to enhance postharvest handling of the crop.

MATERIALS AND METHODS

Plant Materials

Organic Pak choi samples were grown under the organic standards of ACT (IFOAM Accredited). They were cultivated in a farmer's greenhouse in Muang Ang village, Chom Thong district, Chiang Mai province, Thailand. Fifteen-day-old Pak choi seedlings were transplanted. Then 24–27 days after

transplanting, organic Pak choi was harvested from the planting plot 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 similarity, trimmed, and packed in 25 × 40 cm perforated polyethylene bags with 18, 0.8 cm diam. holes. Each bag contained 300 g Pak choi samples. Subsequently, the samples were placed at ambient temperature (24–30°C) (Table 1). The samples were randomly collected at harvesting date and after 3-day storage to analyze physico-chemical qualities. The samples were kept by frozen and freeze-dry before glucosinolate analyzing and were oven-dried at 55–60°C for 2–3 days before sugar and crude fiber analyzing.

Table 1 Storage conditions, temperature and relative humidity, during different seasons

| Season | Average Temperature (°C) ± SD | | | Average Relative Humidity (%) ± SD | | |
|--------|-------------------------------|--------------|--------------|------------------------------------|--------------|--------------|
| | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 |
| Winter | 24.81 ± 2.58 | 24.79 ± 3.68 | 25.00 ± 4.89 | 64.22 ± 1.48 | 61.95 ± 2.98 | 58.95 ± 3.02 |
| Summer | 27.12 ± 1.64 | 26.70 ± 1.39 | 26.97 ± 0.90 | 47.30 ± 0.28 | 47.50 ± 0.29 | 49.00 ± 0.50 |
| Rainy | 29.06 ± 0.60 | 29.22 ± 0.25 | 28.33 ± 0.64 | 70.04 ± 0.15 | 70.42 ± 0.13 | 67.84 ± 0.17 |

Experimental Design

An experimental design was (3 × 3) factorial in CRD was used with four replications. The factor were growing season and harvesting time as follows:

Growing seasons: winter (November-December), summer (February-March) and rainy season (June-July).

Harvesting times: morning (5:00 am–8:00 am), afternoon (12:00 pm–2:00 pm) and evening (4:30 pm–6:30 pm)

Data Collection

Physico-chemical qualities changes of the produce were determined at the harvesting date and after 3-day storage as follows:

Reducing sugar content was determined based on the technique of James (1995). About 3 g of oven-dried sample was weighed, and 50 ml of distilled water were added. The solution was warmed in a water bath at 55°C for 10 min. Then it was filtered and made up to 100 ml with distilled water. 1 ml of the solution was pipetted and 1 ml alkaline 3,5-dinitrosalicylic acid (DNS) and 2 ml distilled water were added. The solution was boiled in a water bath at 100°C for 5 min, then cooled and adjusted to a volume of 25 ml with distilled water. The absorbance of the sample solution was read at a wavelength of 540 nm using a spectrophotometer (Genesys 20, Thermo Scientific, USA).

Total sugar content was determined by the James's method (1995). About 1 g of oven-dried sample was weighed and added to 10 ml of 1.5 M H_2SO_4 . The sample solution was boiled in a water bath at 100°C for 20 min, cooled and 12 ml of 10% NaOH were added. Then it was filtered and made up to 100 ml with distilled water. One ml of the solution was pipetted and 1 ml DNS and 2 ml distilled water were added. The sample solution was boiled in a water bath at 100°C for 5 min then cooled and adjusted to a volume of 25 ml with distilled water. Absorbance of the solution was read at 540 nm using a spectrophotometer (Genesys 20, Thermo Scientific, USA).

Ascorbic acid content was analyzed by the indophenol titration method (Ranganna, 1986). About 10 g of ground Pak choi sample was weighed then 0.4% oxalic acid was added and made up to a volume of 100 ml. The sample solution was filtered with Whatman® No.1 paper. 10 ml of the filtered sample solution was titrated with 0.04% 2,6-dichlorophenol indophenol until the end point. The volume of 2,6-dichlorophenol indophenol solution at the end point was used to calculate ascorbic acid content.

Glucosinolate (GS) content was measured according to the method of Chen *et al.* (2008). A 0.25 g freeze-dried powder sample of Pak choi was preheated for 5 min in 75°C water bath. Then 4 ml of 70% boiling methanol were added, and extraction was conducted at 75°C in a water bath for 10 min. For internal standardization, 200 ml of 5 mM sinigrin were added to one of the duplicates before extraction. 1 ml of 0.4 M barium acetate was rapidly added to the preheated sample and the test tube was vortexed for several seconds. The sample was centrifugated at 4,000 rpm for 10 min at room temperature. The supernatant was collected and the pellet was re-extracted twice with 3 ml of 70% boiling methanol. The three supernatants were combined and made up to 10 ml with 70% methanol as a sample extract. Five ml of the sample extract was loaded onto activated DEAE Sephadex A25 in a vacuum processor and allowed to desulfate overnight

with arylsulfatase. The resultant desulfo-GS was eluted with 2.5 ml of ultrapure water and stored at -20°C prior to separation and analysis by HPLC (LC-10AT pump, CTO-10A column oven, SCL-10A VP system controller, and SPD-M10A VP Diode Array Detector, Shimadzu, Japan).

Crude fiber content was determined according to the method of A.O.A.C. (2005). One g of oven-dried Pak choi sample was placed in a fiber bag, boiled in 0.128 M H_2SO_4 for 30 min and washed with warm water. The sample was re-boiled in 0.313 M NaOH for 30 min and washed with warm water. Then the sample was dried at 105°C for 8 hr and cooled in a desiccator. The dry sample was weighed, put in a crucible, burned at 600°C for 4 hr and cooled in a desiccator. The ash was weighed to calculate crude fiber content.

Leaf color was measured every day using a chlorophyll meter (SPAD 502 Plus, Konica Minolta Sensing, Inc., Japan)

Respiration rate was determined by keeping Pak choi samples in plastic boxes connected with a flow board at ambient temperature. CO_2 in the flow was detected by a bridge analyzer (Model 900151, Bridge Analyzers, Inc., USA). The CO_2 reading was calculated for respiration rate.

Ethylene production rate was determined by packing Pak choi samples in sealed plastic boxes at ambient temperature for 2 hr. 1 ml of gas in the plastic box was collected and injected into a gas chromatography (GC) machine (Gow-Mac Instrument Co., USA) under N_2 carrier gas, with a 100°C column oven and 50°C injector. Ethylene production rate was calculated using the peak reading sample data and ethylene standard, sample weight, time use and volume of the plastic box.

Statistical Analysis

Effects of main factors and their interaction were analyzed by ANOVA. DMRT was used for mean comparisons. Relationships between the data were determined by the Pearson correlation.

RESULTS AND DISCUSSION

Reducing and Total Sugar Contents

Seasonal environmental factors recorded in the greenhouse including min/max temperature, light intensity and relative humidity are presented in Table 4. At the harvesting date, season and harvesting time did not affect on reducing sugar content. While season affected on total sugar content. It was higher (7.85%) in summer than in rainy season (6.15%) (Table 2). After 3-day storage, the study showed that season, harvesting time and their interaction

affected reducing and total sugar contents in Pak choi. Pak choi harvested in summer had the highest reducing and total sugar contents of 7.13% and 9.60%, respectively. Also, Pak choi harvested in the evening had the highest reducing and total sugar contents of 6.82% and 8.71%, respectively. A season by time of day interaction was observed. Pak choi harvested in summer in the morning and evening had higher reducing sugar contents of 8.18% and 8.46%, respectively, than in the afternoon (4.74%). Pak choi harvested in summer in the evening had the highest total sugar content of 11.67% (Table 3).

Table 2 Reducing sugar, total sugar, glucosinolate, vitamin c, crude fiber and leaf color (SPAD unit) at harvesting date of organic Pak choi harvested at different time and season

| Harvesting season | Harvesting time | | | Mean (season) |
|--|--------------------|--------------------|--------------------|--------------------|
| | Morning | Noon | Evening | |
| Reducing sugar (%) | | | | |
| Winter | 4.71 | 4.84 | 5.57 | 5.04 |
| Summer | 6.24 | 5.88 | 3.48 | 5.20 |
| Rainy | 3.96 | 3.59 | 3.80 | 3.78 |
| Mean (harvesting time) | 4.97 | 4.77 | 4.28 | |
| Total sugar ^{1/} (%) at harvest | | | | |
| Winter | 7.21 | 7.77 | 7.01 | 7.33 ^{AB} |
| Summer | 8.30 | 7.01 | 8.26 | 7.85 ^A |
| Rainy | 5.33 | 6.16 | 6.96 | 6.15 ^B |
| Mean (harvesting time) | 6.95 | 6.98 | 7.41 | |
| Glucosinolate ^{1/} (μmol/g FW) | | | | |
| Winter | 6.65 | 3.39 | 2.91 | 4.31 ^B |
| Summer | 6.39 | 4.30 | 3.58 | 4.76 ^B |
| Rainy | 7.19 | 7.31 | 5.99 | 6.83 ^A |
| Mean (harvesting time) | 6.74 ^A | 5.00 ^B | 4.16 ^B | |
| Vitamin C ^{1/} (mg/100 g FW) | | | | |
| Winter | 6.66 ^e | 7.55 ^e | 37.30 ^b | 17.17 ^C |
| Summer | 19.20 ^d | 18.74 ^d | 24.22 ^c | 20.72 ^B |
| Rainy | 34.34 ^b | 38.40 ^b | 46.99 ^a | 39.91 ^A |
| Mean (harvesting time) | 20.06 ^B | 21.56 ^B | 36.17 ^A | |

Table 2 (Continue)

| Harvesting season | Harvesting time | | | Mean (season) |
|--------------------------------------|----------------------|----------------------|----------------------|--------------------|
| | Morning | Noon | Evening | |
| Crude fiber ^{1/} (g/100 g) | | | | |
| Winter | 14.77 ^{abc} | 16.18 ^{ab} | 15.03 ^{abc} | 15.33 ^A |
| Summer | 17.45 ^a | 14.07 ^{bc} | 16.64 ^{ab} | 16.05 ^A |
| Rainy | 13.23 ^c | 14.91 ^{abc} | 12.72 ^c | 13.62 ^B |
| Mean (harvesting time) | 15.15 | 15.06 | 14.80 | |
| Leaf color ^{1/} (SPAD unit) | | | | |
| Winter | 36.98 | 37.85 | 39.93 | 38.25 |
| Summer | 37.53 | 38.38 | 40.05 | 38.65 |
| Rainy | 37.88 | 38.85 | 40.85 | 39.19 |
| Mean (harvesting time) | 37.46 ^B | 38.36 ^B | 40.28 ^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

Glucosinolate: Season = *, Time = *, Season × Time = NS

Vitamin C: Season = *, Time = *, Season × Time = *

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 3 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

| Harvesting season | Harvesting Times | | | Mean (season) |
|----------------------------------|--------------------|---------------------|-------------------|-------------------|
| | Morning | Afternoon | Evening | |
| Reducing sugar ^{1/} (%) | | | | |
| Winter | 3.68 ^{cd} | 5.20 ^{bc} | 5.77 ^b | 4.88 ^B |
| Summer | 8.18 ^a | 4.74 ^{bcd} | 8.46 ^a | 7.13 ^A |
| Rainy | 3.50 ^d | 3.25 ^d | 6.25 ^b | 4.33 ^B |
| Mean (harvesting time) | 5.12 ^B | 4.40 ^B | 6.82 ^A | |

Table 3 (Continue)

| Harvesting season | Harvesting Times | | | Mean (season) |
|---|--------------------|--------------------|--------------------|---------------------|
| | Morning | Afternoon | Evening | |
| Total sugar ^{1/} (%) | | | | |
| Winter | 5.79 ^{de} | 7.49 ^c | 7.35 ^c | 6.88 ^B |
| Summer | 9.04 ^b | 8.10 ^{bc} | 11.67 ^a | 9.60 ^A |
| Rainy | 5.70 ^{de} | 5.38 ^e | 7.12 ^{cd} | 6.07 ^B |
| Mean (harvesting time) | 6.84 ^B | 6.99 ^B | 8.71 ^A | |
| Glucosinolate ^{1/} (μmol/g FW) | | | | |
| Winter | 12.16 ^a | 5.83 ^{bc} | 7.38 ^b | 8.46 ^A |
| Summer | 5.61 ^{bc} | 0.47 ^d | 0.64 ^d | 2.24 ^C |
| Rainy | 7.59 ^b | 7.18 ^b | 4.73 ^c | 6.50 ^B |
| Mean (harvesting time) | 8.45 ^A | 4.50 ^B | 4.25 ^B | |
| Vitamin C ^{1/} (mg/100 g FW) | | | | |
| Winter | 21.05 | 28.07 | 30.26 | 26.46 ^B |
| Summer | 27.00 | 45.62 | 47.02 | 39.88 ^A |
| Rainy | 27.88 | 37.47 | 40.22 | 35.19 ^A |
| Mean (harvesting time) | 25.31 ^B | 37.06 ^A | 39.17 ^A | |
| Crude fiber ^{1/} (g/100 g) | | | | |
| Winter | 16.82 | 16.10 | 16.91 | 16.61 ^A |
| Summer | 13.88 | 12.10 | 11.63 | 12.54 ^B |
| Rainy | 18.69 | 15.57 | 17.87 | 17.38 ^A |
| Mean (harvesting time) | 16.46 | 14.59 | 15.47 | |
| Leaf color ^{1/} (SPAD unit) | | | | |
| Winter | 30.45 | 32.50 | 33.65 | 32.20 ^A |
| Summer | 25.58 | 27.60 | 26.38 | 26.52 ^{BC} |
| Rainy | 28.53 | 26.75 | 32.78 | 29.35 ^{AB} |
| Mean (harvesting time) | 28.18 | 28.95 | 30.93 | |
| Weight loss ^{1/} (%) | | | | |
| Winter | 2.23 | 2.08 | 3.54 | 2.61 ^B |
| Summer | 5.57 | 5.29 | 5.11 | 5.32 ^A |
| Rainy | 1.52 | 1.63 | 1.53 | 1.56 ^C |
| Mean (harvesting time) | 3.10 | 3.00 | 3.39 | |

Table 3 (Continue)

| Harvesting season | Harvesting Times | | | Mean (season) |
|---|---------------------|---------------------|---------------------|---------------------|
| | Morning | Afternoon | Evening | |
| Respiration ^{1/} (mg CO ₂ /kg/hr) | | | | |
| Winter | 16.52 ^f | 39.92 ^{ef} | 46.75 ^{de} | 34.39 ^C |
| Summer | 99.74 ^b | 135.05 ^a | 79.11 ^{bc} | 105.08 ^A |
| Rainy | 65.43 ^{cd} | 84.80 ^{bc} | 87.12 ^{bc} | 77.75 ^B |
| Mean (harvesting time) | 57.00 ^B | 86.75 ^A | 69.53 ^B | |
| Ethylene ^{1/} (µl/g/hr) | | | | |
| Winter | 0.06 | 0.15 | 0.02 | 0.08 ^B |
| Summer | 0.77 | 0.83 | 0.89 | 0.83 ^A |
| Rainy | 1.23 | 1.01 | 1.09 | 1.11 ^A |
| Mean (harvesting time) | 0.69 | 0.66 | 0.67 | |

Note: ANOVA of main factor and interaction effects: NS = not significant, * = significant (P < 0.05)

Reducing sugar: Season = *, Time = *, Season × Time = *

Total sugar: Season = *, Time = *, Season × Time = *

Glucosinolate: Season = *, Time = *, Season × Time = *

Vitamin C: Season = *, Time = *, Season × Time = NS

Crude fiber: Season = *, Time = NS, Season × Time = NS

Leaf color change: Season = *, Time = NS, Season × Time = NS

Weight loss: 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

A high sugar content was detected in the evening of summer because plants accumulate sugar from photosynthesis. The plant has been conducting photosynthesis during the longer day in summer, resulting in a higher sugar content. In Pak choi leaf, sugar is the main energy substrate (Xiangyang and Lianqing, 2000). 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 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; 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).

Glucosinolate

The study showed that season and harvesting time affected GS content at the beginning and after 3-day storage. During the harvesting date, Pak choi had the highest GS of 6.83 $\mu\text{mol/g}$ FW in rainy season and the samples had the highest GS of 6.74 $\mu\text{mol/g}$ FW when harvest in the morning. Both main factors did not have interaction effect on the GS content (Table 2). After 3-day storage, Pak choi had the highest GS of 8.46 $\mu\text{mol/g}$ FW in winter season and when it was harvested in the morning, 8.45 $\mu\text{mol/g}$ FW. Moreover, there was a season by harvesting time interaction effect on the GS content; Pak choi harvested in the morning during winter had an extra high GS content of 12.16 $\mu\text{mol/g}$ FW (Table 3).

Several reports suggested that crop genetics and environmental factors such as temperature,

photoperiod and light quality during the period before harvest and their interaction effect on GS 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 GS 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). Five botanical groups of *B. oleracea* had high concentrations of total and indole GSs that were associated with cultivation at higher temperatures and photosynthetic photon fluxes (PPF) as well as to longer day lengths (Charron *et al.*, 2005). Whereas total GS 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 GSs (Velasco *et al.*, 2007). In addition, GS 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.

Table 4 Temperature, light intensity and RH conditions in an organic Pak choi greenhouse during different seasons

| Season | Temperature ($^{\circ}\text{C}$) | | | Light intensity (lux) | RH (%) |
|--------|------------------------------------|-------|-------|-----------------------|--------|
| | Max | Min | Mean | | |
| 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 |

Vitamin C

During the harvesting date, season and harvesting time affected on vitamin C content and there was interaction effect of these factors. Vitamin C content of Pak choi had got the highest (46.99

mg/100 g FW) when harvested in the evening of rainy season (Table 2). In this experiment, light intensity in summer and rainy season was higher than in winter leading to higher vitamin C production (Table 4). 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 (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 higher vitamin C contents, 39.88 and 35.19 mg/100 g FW, respectively, than harvested in winter. Pak choi harvested in the afternoon and in the evening had higher vitamin C contents, 37.06 and 39.17 mg/100 g FW, than that harvested in the morning (Table 3). Normally, the factors affected on vitamin C content in fruit and vegetables include genotypes, preharvest climatic conditions, cultural practices, maturity, harvesting methods and postharvest handling procedures. Temperature is an important factor to maintain vitamin C content of fruit and vegetables. Loss of vitamin C is induced at high temperature and longtime storage (Lee and Kader, 2000; Weston and Barth, 1997). In this study, pak choi in some treatments especially that was harvested at the morning and afternoon time in winter and stored at lower temperature (Table 4) had vitamin C content after 3-day storage higher than at the beginning of storage (Table 2 and 3). This result agree with the research conducted by Mathiventhan and Ramiah (2015) which reported that vitamin C content in the leaves of *Dregea volubilis*, *Delonix elata* and *Murraya*

koenigii on day 4 of storage was higher than at the beginning when stored at lower temperature (4°C).

Crude Fiber

Only season affected crude fiber content at the beginning and after 3-day storage. These was seasonal and harvesting time interaction effect only at the beginning. Pak choi harvested in summer and winter had higher crude fiber content (16.05 and 15.33 g/100 g) than the one harvested in rainy season (13.62 g/100 g). And Pak choi harvested in the morning of summer had the highest crude fiber content (Table 2). After 3-day storage, Pak choi harvested in summer, winter and rainy season had crude fiber contents of 12.54, 16.61 and 17.38 g/100 g FW respectively (Table 3). The total dietary fiber in vegetables varies due to plant maturity, season, fertilizer or chemical used (Aletor *et al.*, 2002; Punna and Prachuri, 2004; Tendaj *et al.*, 2013; Uusiku *et al.*, 2010). Pak choi in this experiment was harvested at 27, 24 and 24 days after transplant in summer, winter and rainy season, respectively. However, the vegetables grown and harvested in summer had high fiber content and did not differ from in winter. The season affecting on crude fiber may be the result of nutrient supply and sugar substrate partitioning in each season. Waterleaf (*Talinum triangulare* (Jacq.) Willd) cultivated in May-July had significantly lower total dietary fiber than in February-April (Tendaj *et al.*, 2013). The content of crude fiber of *Brassica rapa* var. *narinosa* 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

At the beginning, only harvesting time affected on leaf color. Pak choi harvested in the evening had the darkest green (40.28 SPAD unit) (Table 2). However, after 3-day storage, only season affected leaf color in Pak choi. The produce harvested in winter generated more green color, 32.20 SPAD units, than in summer, 26.52 SPAD units, and the rainy season 29.35 SPAD units (Table 3). Chlorophyll is the green pigment in the chloroplast that enables photosynthesis in

plants (Løkke, 2012). Plants need suitable light and temperature for chlorophyll synthesis. Therefore the harvesting time effect on chlorophyll content may be caused by solar radiation and the season effect on chlorophyll content may be caused by storage temperature. Mahmud *et al.* (1999) reported that Pak choi planted at 21°C had the highest content of total chlorophyll followed by at 18°C and 25°C. The seasonal effects on chlorophyll also depend on 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). The assessment of Xiangyang and Bagshaw (2001) suggested leaf yellowing of Pak choi was caused by high temperatures. 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 highest in rainy season $28.33 \pm 0.64^\circ\text{C}$ following by summer $26.97 \pm 0.90^\circ\text{C}$ and winter $25.00 \pm 4.89^\circ\text{C}$ (Table 1).

Weight Loss

Only seasonal effect was observed on weight loss of organic Pak choi; harvest in summer resulted in the highest weight loss, 5.32% (Table 3). High average temperature (25.78°C) and lowest relative humidity (56.17%) were recorded in the summer (Table 4). 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% weight loss effects on the texture of spinach (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).

Respiration Rate

The main factors affecting the respiration rate in Pak choi included season and harvesting time. The produce had the highest respiration rate, 105.08 mg CO₂/kg/hr, in the summer. The effect of harvesting time showed that Pak choi harvested in the afternoon had the highest respiration, 86.75 mg CO₂/kg/hr. In addition, the interaction of both factors affected respiration, Pak choi harvested in summer in the afternoon had the highest respiration rate, 135.05 mg CO₂/kg/hr. The respiration rate was increased by ethylene production because Pak choi harvested in the afternoon had the highest ethylene production (Table 3).

Respiration in vegetables changes carbohydrates, starches and sugars, to CO₂ and H₂O releasing 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; the produce harvested in spring had a higher respiration rate [O₂ (RRO₂)], 6.95 mmol O₂/kg/hr, than in early and late summer, 3.99 and 3.92 mmol O₂/kg/hr, 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. 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. CO₂ production increased 2–4 fold when temperature increased 1–12°C (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 on ethylene production rate of Pak choi. The lowest rate of ethylene production was 0.08 µl/g/hr in winter, the highest rate was 1.11 and 0.83 µl/g/hr in rainy season and summer respectively (Table 3). The rate of ethylene production of Pak choi on the last day storage decreased from first day in winter and summer, as opposed to in the rainy season (data not showed). On the second day of storage, the outer leaves of Pak choi in rainy season started to senescence which was indicated by leaf color changing from green to yellow, and at 3 days of storage 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 synthesis any pigments (Siripanich, 2006). Moreover, at temperature over 25°C, 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 1). 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.

CONCLUSION

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 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. This information can be used for planning of Pak choi senescence delay.

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