Journal of Food Science and Agricultural Technology (2019) 5 (Spcl. Iss.): 212-219

Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal

Published online: http://rs.mfu.ac.th/ojs/index.php/jfat

Original Research Article

Effect of harvesting season, maturity stage and storage temperature on internal browning and postharvest quality of 'Phulae' pineapple

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ARTICLE INFO

Article history:
Received 31 July 2018
Received in revised form 31 December 2018
Accepted 08 January 2019

Keywords:
Internal Browning
Maturity Stage
Phulae pineapple
Quality
Storage Temperature

ABSTRACT

The effect of harvesting season, maturity stage and storage temperature on internal browning (IB) and postharvest quality of pineapple 'Phulae' (*Ananas comosus* L. Merr) were investigated. Fruits were harvested at mature green and ¼ ripe stage in different seasons (rainy and winter) and stored at 10°C and 20°C with 85-90% RH for 35 days. Postharvest quality parameters including IB incidence, peel and flesh color, weight loss, total soluble solid (TSS), titratable acidity (TA), TSS/TA ratio, vitamin C content, total phenolic (TP) content and antioxidant activities using ferric reducing antioxidant power (FRAP) assays were analyzed weekly. Fruit harvested in rainy and winter and then stored at 10°C had longer storage life than those kept at 20°C. The mature green fruit had the severity of IB higher than fruit harvested at ¼ ripe stage. At 10°C, the pineapple harvested in rainy season had more IB than the pineapple harvested in winter season. The IB incidence found in fruit harvested in both seasons became more severe when the samples were kept at 10°C followed by transfer to a room temperature (25ºC ± 2ºC). The color changes of peel (hue angle) and flesh (L*) of the pineapple in both harvesting seasons stored at 10°C and 20°C decreased during storage, especially on the day at IB symptom occurred. Weight loss of the pineapple harvested during the rains was significantly higher than the winter in both storage temperatures. At mature green stage, there were no significant difference between harvesting seasons on TSS content, but TA content, TP contents and FRAP activity increased significantly during storage. Fruit harvested in the winter had significantly higher vitamin C content than fruit harvested in the rainy season. The harvesting season, maturity stage and storage temperature affected to IB development, storage life and postharvest quality of pineapple.

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Published by School of Agro-Industry, Mae Fah Luang University

ISSN: 2408-1736

INTRODUCTION

'Phulae' pineapple (*Ananas comosus* L. Merr) refers to the Queen pineapple variety. This cultivar is an economic crop and a Geographical Indication (GI) of Nang Lae district, Chiang Rai province, Thailand (Kongsuwan *et al*., 2009). It can be grown all year round. The fruit has a cylindrical shape. The size of this variety is smaller than other varieties with a rather thick peel. The young fruit has green color on the peel. When it is ripe, the peel becomes orange-yellow color. The flesh of Phulae pineapple has pale-yellow color, crispy texture, sweet smell, and the sweet taste. Moreover, the core of this variety is edible with crispy texture (Kongsuwan *et al*., 2009). It has been considered to be a good source of the bioactive compounds such as citric acid, antioxidants and it also contains high amount of bromelain and dietary fiber (Ketnawa *et al*., 2011) which provides some health benefits. Many preharvest factors have influenced on the postharvest qualities of crops including the temperature of growing season, light condition, amount of rainfall and irrigation, mineral nutrition, and maturity at the time of harvest. All these factors can be some direct or indirect effects on the postharvest qualities, storage life and susceptibility of crops to disorder (Wang, 1997). Water stress during the growing season affects the size of the harvested plant organ and leads to soft or dehydrated fruit that is easy to damage and decay during storage (Galindo *et al*., 2014). Light, temperature and rainfall conditions during the growing season affecting sugars accumulation in the fruit before harvest (Hewett, 2006). Moreover, maturity at harvest time is the most important factor that determines postharvest life and final qualities such as appearance, texture, flavor and nutritional values of fruits and vegetables (El-Ramady *et al*., 2015). Immature fruits were highly susceptible to wilt, mechanical damage and inferior flavor when ripe. Overripe fruits were soft and powdery with insipid flavor soon after harvest (Kader & Rolle, 2004). Currently, the demand to consume fresh pineapple is increasing rapidly. Cold storage was one effective method due to reduced respiration rate, ethylene production and various chemical changes. The commercial storage temperature of Phulae pineapple was 10-13°C (Sari *et al*., 2016). However, pineapple fruits were susceptible to low temperature and cold storage associated with physiological disorders (Yahia *et al*., 2011). For example, internal browning (IB) was the most common physiological disorder of pineapple fruit that is induced by exposure to low temperature after harvest (Hong *et al*., 2013). IB symptom starts from a small grey translucent zone begin at the base of the fruitlet near the fruit core after that the core become darkens. When the symptom is more severe, the internal fruit tissue turns to brown and black. IB is thought to occur from increased polyphenol oxidase activity (Rohrbach & Johnson, 2003). The symptoms of chilling injury, especially IB could be observed during cold storage of pineapple fruit. IB not only results in considerable consumer dissatisfaction but leads to significant wastage during storage of pineapple fruit. The previous information regarding preharvest factors affected postharvest qualities and pineapple fruits were susceptible to IB during low temperature storage. Therefore, the objectives of this study were to study the harvesting season (rainy and winter), maturity stage (mature green and ¼ ripening stage) and storage temperatures (10°C and 20°C) on internal browning development, postharvest qualities, total phenolic (TP) contents and antioxidant capacity by ferric reducing antioxidant power (FRAP) assays of 'Phulae' pineapple.

MATERIALS AND METHODS

Raw Materials and Experimental Design

Pineapple (*Ananas comosus* L.) cv. 'Phulae' was harvested at mature green (180 days after induce flowering) and ¼ ripening (190 days after induce flowering) stage from the same orchard in Nang-Lae District, Chiang Rai province, Thailand. The flowering induction, fruit development and harvesting period of Phulae pineapple in winter and rainy season were shown in Figure 1. Fruits were selected for uniformity as the same size approximate 250-300 g/fruit and color, and no defect or disease. The selected fruits in each season were randomly divided into two groups and placed into the corrugated box size 28×44×20 cm (10 fruits per box). The first group was stored at 10°C with 85-90% relative humidity and the second group was stored at 20°C. The incidence of IB and postharvest qualities were subjected to be measured every 7 days up to 35 days. After 35 days, the samples in both groups were moved to 25°C for 2 days for IB observation.

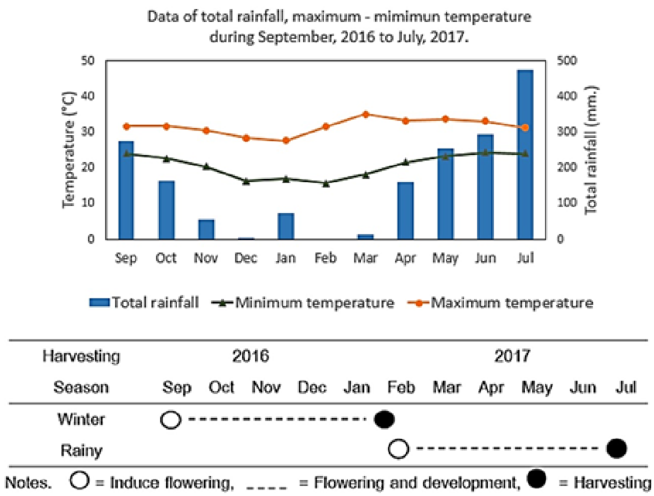

Figure 1. Total rainfall (mm.), maximum and minimum temperature (°C) during induce flowering, fruit development and harvesting of 'Phulae' pineapple in winter and rainy season in year 2016 and 2017

Color Properties and storage life

Peel and flesh color were measured on top, central, and basal part of the fruit by the colorimeter (Konica Minolta cr-10, Japan) using the CIE system (L*, a*, and b*) and the color values were expressed as L* (lightness) for flesh and hue value for peel. Storage life was evaluated by the visual appearance of pineapple fruit. The acceptable storage life of fruit has no spoilage on the fruit and no IB symptom showed on the fruit flesh.

Internal Browning (IB)

Fruit were cut in half longitude and were evaluated the severity of IB by scored subjectively according to the estimated area of the flesh fruit near the core with browning: 0 points (no browning symptom), 1 points (10-25% browning of surface area), 2 points (26-50% browning of surface area), 3 points (51-75% browning of surface area) and 4 points (>75 % browning of surface area) as shown in Figure 2.

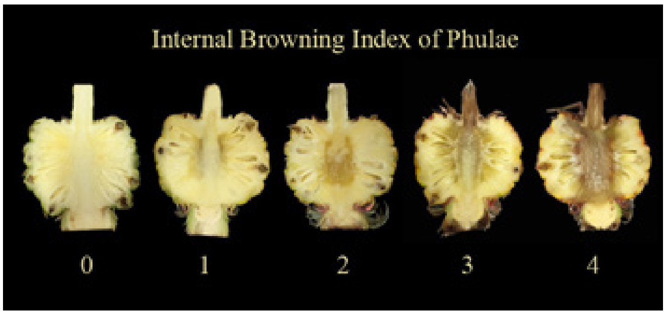

Figure 2. The Internal Browning Index of 'Phulae' pineapple. Internal browning level was given by scores of surface area browning: 0 points (no browning symptom), 1 points (10-25% browning of surface area), 2 points (26-50% browning of surface area), 3 points (51-75% browning of surface area) and 4 points (>75 % browning of surface area).

Physical and Physicochemical Analysis

Fruit were weighed individual and the results were expressed as the percentage of weight loss. Total soluble solids (TSS) was determined by using a hand digital refractometer PAL-1 (ATAGO, Japan). Titratable acidity (TA) was determined by titrimetric method, with 0.1 N NaOH and phenolphthalein as an indicator. The results were expressed as the percentage of citric acid (meq. Citric acid = 0.064). From the results of TSS and TA were used to calculate as the TSS/TA ratio.

Vitamin C Content

Vitamin C content was determined by using spectrophotometer according to the procedure of Roe et al., (1948) with some modifications. Two grams of flesh sample were homogenized with 10 ml of 5% metaphosphoric acid solution then centrifuged at 12,000×g, 4°C for 15 min. The supernatant was filtered through Whatman filter paper No.4 and the supernatant was collected. The absorbance was measured at 540 nm using a spectrophotometer (Thermo Fisher Scientific Genesys 20 (Model 4001/4Cat 4001-03). A standard curve was prepared by using a standard solution of ascorbic acid and the results were expressed as mg ascorbic acid equivalents AA g^{-1}FW.

Sample Extraction for Total Phenolic Content and Antioxidant activity

Five grams of pineapple tissues were homogenized with 20 ml of 95% methanol then centrifuged at 15,000×g, 4°C for 15 min. The supernatant was filtered through Whatman filter paper No.4 and collected supernatant for analyzing the total phenolic content and antioxidant activities.

Total Phenolic Content

Total phenolic (TP) content was determined by using the Folin-Ciocalteu assay according to ISO 14502-1 (2005) and using gallic acid as a standard. Extracted sample 250 µl, 1250 µl of 10% Folin-Ciocalteu reagent and 7.5% of Na_2CO_3 were mixed and incubated the mixture at room temperature for 60 minutes. The absorbance was measured at 765 nm using a spectrophotometer (Thermo Fisher Scientific Genesys 20 (Model 4001/4Cat 4001-03)). The results were expressed as mg gallic acid equivalents GAE g^{-1} FW.

Antioxidant activity by Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power (FRAP) assay was evaluated followed the method described by Benzie & Strain (1999) with some modifications. Extracted sample 400 µl and 2.6 ml of FRAP solution were mixed. The active mixture was incubated at 37°C for 30 minutes and measured the absorbance at 595 nm using spectrophotometer (Thermo Fisher Scientific Genesys 20 (Model 4001/4Cat 4001-03)). The results were expressed as micromole of ferrous equivalents Fe (II) g^{-1} FW.

Statistical analysis

Statistics on a completely randomized design by analysis of variance (ANOVA) using the Statistical Analysis System (SPSS, version 20, Inc., USA). The significant difference ($P \leq 0.05$) among means was determined by Duncan's Multiple Range Test (DMRT). All experiments were conducted by 5 replicates determinations.

RESULTS AND DISCUSSION

Color properties

The color was indicated for the quality of food and it influences consumer acceptance (Crisosto *et al*., 2003). The development of color on the peel is a parameter used to determine the harvest maturity (Thompson, 2003). At harvesting time, the hue value of the pineapple was harvested in rainy season especially at mature green stage was significantly higher than the value in the pineapple harvested in winter season (Figure 3), the result might be caused by the cooler seasons. The yellow color of the winter pineapple developed better than the rainy pineapple (Hassan *et al*., 2011). During fruit development in the winter season, the decreasing of temperature at night caused a rapid color change of the pineapple fruit (Joomwong & Sornsrivichai, 2005). The hue value in both rainy and winter pineapple tended to decrease during storage, which related to the ripening process. The peel color changes from green to yellow in result to decrease in chlorophyll and start increasing in carotenoids (Tareen *et al*., 2012). In addition, storage temperature affect the storage life, the result showed that the pineapple stored at 20°C had a shorter storage life than the pineapple stored at 10°C. At 10°C, the pineapple harvested at mature green and ¼ ripe stage in both rainy and winter season had storage life 21 days while at 20°C, the pineapple harvested at mature green stage in both rainy and winter season had storage life 14 days. For the pineapple harvested at ¼ ripe stage in winter season had storage life 14 days but in rainy season had storage life lower than 7 days. It can be explained that the pineapple plant may receive a lot of rainfall before harvest leading to susceptible to deterioration.

The changes of lightness (L*) value on flesh were observed (Figure 4). At 10°C, the pineapple harvested at mature green and ¼ ripe stage in winter season had the L* value significantly higher than rainy season. The L* value of both harvesting seasons significantly decreased after day 21. The pineapple harvested in winter season had higher L* value meant the flesh of the winter pineapple was lighter than the rainy pineapple. Similarly to Joomwong (2006) found that the yellow color of pineapple flesh in the winter season was lighter than in rainy season. In addition, the L* value of both seasons decreased during storage indicated that flesh was intense yellow in color. However, at 20°C, the L* value of pineapple harvested at mature green stage was not significantly different between harvesting seasons and during storage. The L* value of pineapple harvested at ¼ ripe stage in winter season had significantly higher than rainy season but not significantly different during storage.

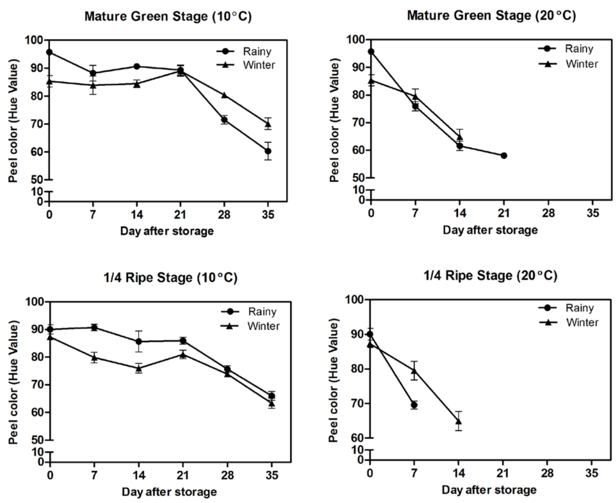

Figure 3. Change in hue value of peel color of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n = 5).

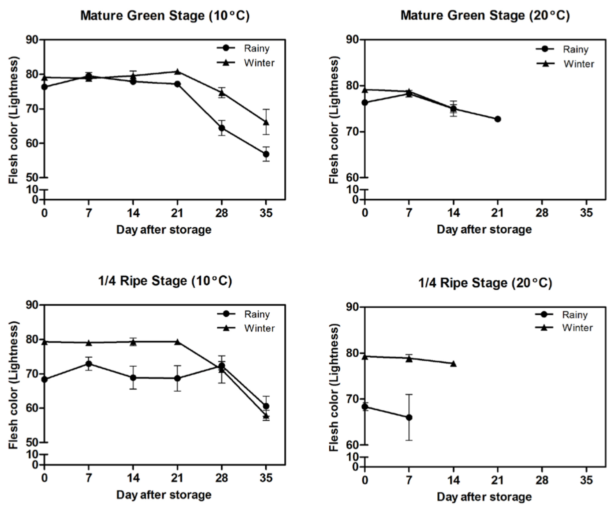

Figure 4. Change in lightness (flesh color) of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n = 5).

Internal browning incidence (IB)

IB, endogenous browning or black heart, is a physiological disorder of pineapple fruit caused by chilling injury. This disorder is very important in the marketing of fresh fruit when cold storage has been applied for extending shelf life (Rohrbach & Johnson, 2003). The IB severity score of Phulae pineapple increased during storage (Figure 5). The pineapple harvested at mature green stage, fruits harvested in the rainy season had significantly higher in the severity of IB than the pineapple harvested in winter season whereas the pineapple harvested at ¼ ripe stage, fruits harvested in the rainy season had lower in the severity of IB symptoms than the pineapple harvested in winter season when store at 10°C. The different response in this study might be due to FRAP value because the pineapple harvested at mature green stage in the rainy season had FRAP value higher than harvested in winter season and the pineapple harvested at ¼ ripe stage in the winter season had FRAP value higher than fruit harvested in rainy season. In addition, the IB severity was observed on day 21 of storage which related to the decrease of lightness in flesh. The IB symptom became more severe when moved to hold at 25ºC for 2 days (2SL). However, the fruit stored at 20°C showed no IB symptoms form the temperature stress but the IB symptoms at 20°C caused from the senescence, it showed dry and wilt at the peel and spoilage (Figure 6 and Figure 7). Furthermore, the pineapple harvested in rainy season showed flesh translucence symptom at harvest time (data not showed). It might result from the temperture during rainy pineapple development. During fruit development, the temperature in rainy season was higher than the temperature in winter season. Flesh translucency can occur in the green stage of fruit, it is found more frequently during high temperature periods (Green, 1963).

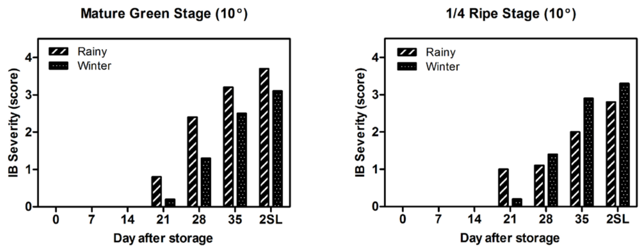

Figure 5. The IB severity of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C. Data presents as mean ± SE (n = 5).

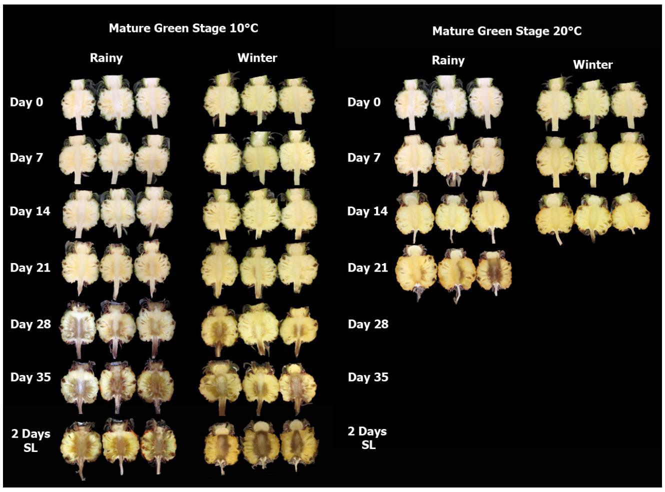

Figure 6. The IB incidence of Phulae pineapple fruits harvested in rainy and winter season at mature green stage and stored at 10°C and 20°C.

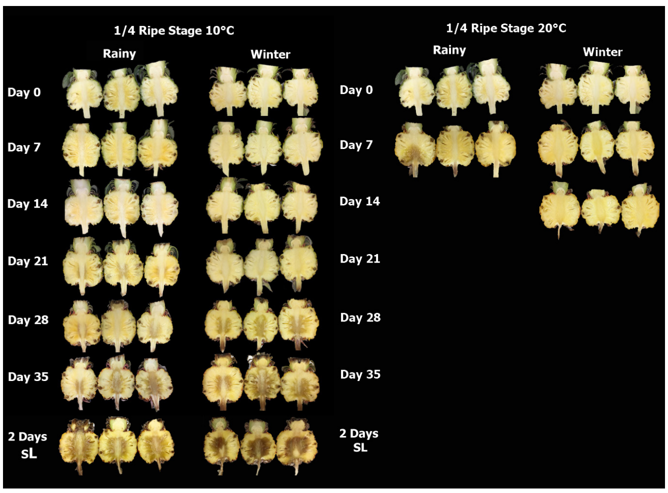

Figure 7. The IB incidence of Phulae pineapple harvested in rainy and winter season at ¼ ripening stage and stored at 10°C and 20°C.

Physical and physicochemical analysis

Water loss affects shelf life and nutritional quality. Weight loss percentage tended to increase during storage (Figure 8). The pineapple harvested at ¼ ripe stage had higher weight loss than the pineapple harvested at green mature stage.

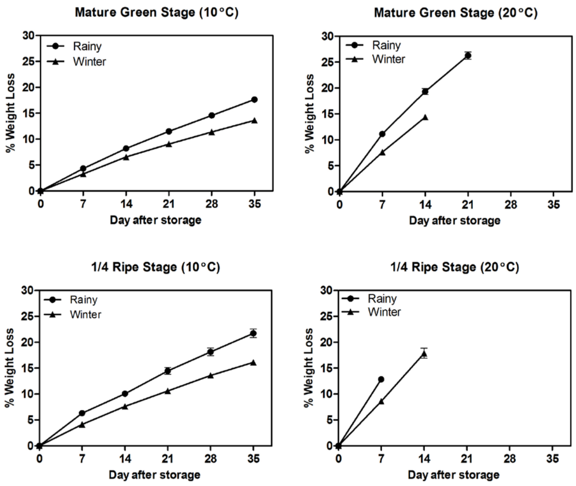

Figure 8. Change in weight loss percentage of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n = 5).

At harvesting time, total soluble solid in pineapple varied a great deal depending upon maturity, season and cultural practices (Hassan *et al*., 2011). The fruit harvested in rainy season had higher TSS than the fruit harvested in winter season. The TSS slightly increased on day 7 then gradually changed during storage (Figure 9). Increase in sugar content of pineapple was very small and that increase might be due to loss of moisture (Wijesinghe & Sarananda, 2002). However, TSS did not significantly change during storage. After harvest, there is only small decline in sugar or soluble solid content. High storage temperature enhances the decrease in sugar content (Paull & Rohrbach, 1982). At harvesting time, the fruit harvested at mature green stage in rainy season had higher TA than the fruit harvested in the winter season while the fruit harvested at ¼ ripe stage in winter season had higher TA than the fruit harvested in rainy season (Figure 10). According to Paull & Chen (2003) found that acid levels were higher during the cool season. TA of pineapple tended to slightly increase at the first week of storage after that it slightly decreased at the end of storage. The decrease of TA might be due to organic acids in fruit that would be used as substrates in the respiration which usually decreased during maturity (Hong *et al*., 2013). The changes of TSS/TA ratio tended to decrease at the first week of storage and subsequently constant through the end of storage (Figure 11), In contrast, changes of TSS/TA rather low and stable throughout storage period in Trad Sri Thong pineapple (Buanong & Wongs-Aree, 2012).

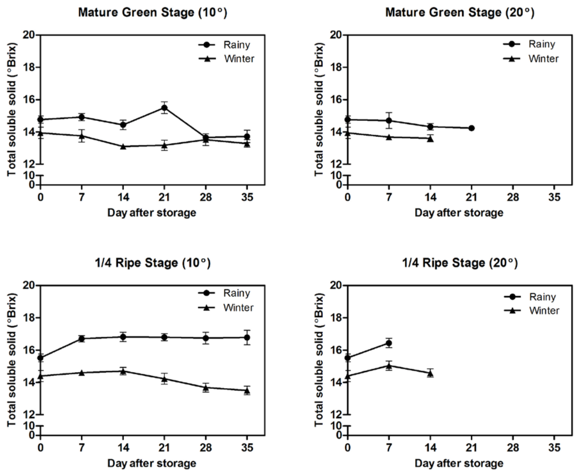

Figure 9. Change in total soluble solid (TSS) of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n = 5).

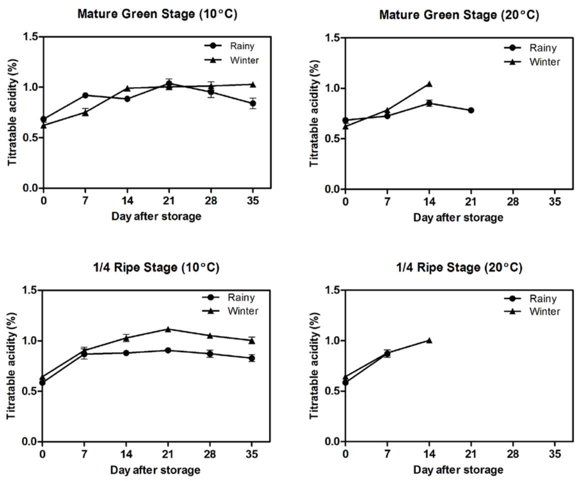

Figure 10. Change in titratable acidity (TA) of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n = 5).

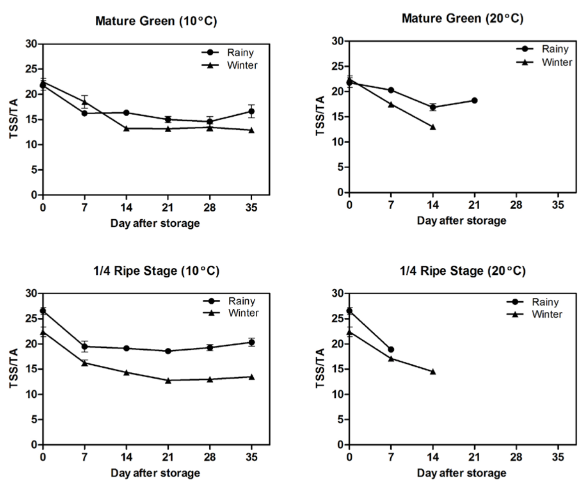

Figure 11. Change in TSS/TA ratio of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n= 5).

Vitamin C content

The vitamin C content in the fruit after harvest depends on the amount of solar radiation received by the fruit while it still attached with the mother plant (Paull, 1993). If the plant received higher intensity of light during the growing season, the vitamin C content in plant tissues was greater (Lee & Kader, 2000). According to Sevillano *et al*., (2009). The ascorbic acids are able to detoxify the reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) during chilling-induced oxidative stress. For the change of vitamin C content was showed in Figure 12, the pineapple harvested in winter season had higher vitamin C content than the pineapple harvested in rainy season. The lower of vitamin C content in the pineapple harvested in the rainy season might be affected by the amount of rainfall during fruit development. In this study, vitamin C content tended to slightly increase during 7 days of storage then decreased during storage. The decrease of vitamin C content during storage might cause from the water loss. Furthermore, the level of the vitamin C content in fruit at harvesting time has been negatively related to the severity of internal browning symptoms associated with postharvest chilling injury (Paull & Rohrbach, 1982). From this study, at mature green stage, the incidence of IB showed more severity in the pineapple was harvested in rainy season than the pineapple harvested in winter season. This result might be due to the vitamin C content in rainy fruit had lower than winter fruit at harvest. However, the significant difference among harvesting seasons in vitamin C content was not found in fruit harvested at ¼ ripe stage at harvesting time.

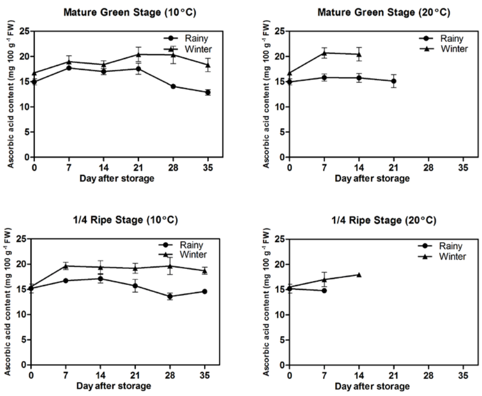

Figure 12. Change in vitamin C content of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n = 5).

Total phenolic content and antioxidant activity

Non-enzymatic antioxidants such as glutathione, ascorbate and phenolic compounds play a role in scavenging excessive reactive oxygen species (ROS) in plant (Phonyiam *et al*., 2016). Fruits and vegetables, the phenolic compound is widely distributed and contributes to antioxidant intake (Hossain & Rahman, 2011). TP contents are very important plant constituents exhibiting antioxidant activity by inactivating lipid free radicals, or by preventing the decomposition of hydroperoxides into free radicals (Samatha *et al*., 2012). At harvest, the pineapple harvested at mature green in rainy season had TP contents higher than winter season while harvested at ¼ ripe stage in winter season was higher in TP contents than fruit in rainy season. After that fruit harvested at mature green and ¼ ripe stage in both storage temperature had TP contents in rainy season higher than winter season (Figure 13). The changes of TP contents mostly increased during storage. However, no significantly different between harvesting seasons. According to Howard *et al*. (2003), they reported that the growing season could influence phenolic contents in blueberries. Nevertheless, the result contrasted to the study by Lima *et al*., (2005) in mature acerola fruits (*Malpighia emarginata* D.C.). They found that acerola fruits harvested in the dryer season showed higher phenolic contents than the fruits harvested in the rainy season. The phenolic compounds such as p-coumaric acid, ferulic acid, and caffeic acid were increased higher in fruit showed IB symptom (van Lelyveld & de bruyn, 1977).

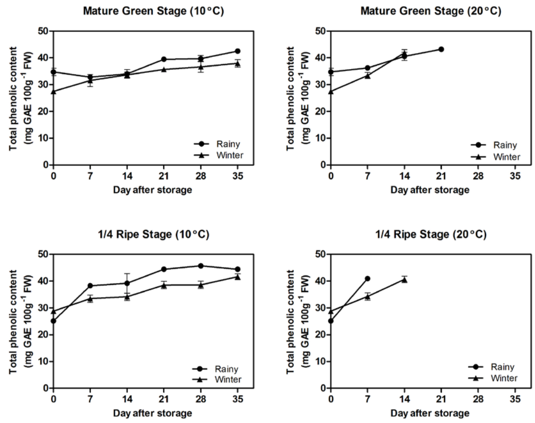

Figure 13. Change in total phenolic content of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n = 5).

Antioxidant activity by FRAP assay has been widely used to determine the antioxidant activity of natural products (Prabhune *et al*., 2013). The pineapple harvested at mature green stage in rainy season had higher FRAP value than the pineapple harvested in winter season while the pineapple harvested at ¼ ripening stage in winter season had higher FRAP value than the pineapple harvested in rainy season (Figure 14). The changes of FRAP value in pineapple stored at 10°C was significantly increased and then decreased at the end of storage. The antioxidant capacity of chilled pineapple fruit coincided with the timing of IB appearance during 10 °C storage (Luengwilai *et al*., 2018). The increases of FRAP activities in Phulae pineapple corresponded to higher severity of IB (Phonyiam *et al*., 2016). The antioxidant capacity could alleviate CI and would correlate with plant tissues showing IB (Sevillano *et al*., 2009). Plant cells synthesized more antioxidant to balance ROS (Nukuntornprakit *et al*., 2015). Therefore, high antioxidant activity should have high efficiency to eliminate free radicals in the plant cell.

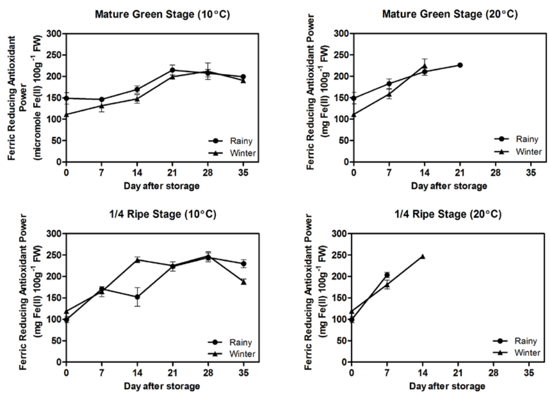

Figure 14. Change in total phenolic content of Phulae pineapple fruit harvested in rainy and winter season at mature green stage and ¼ ripening stage and stored at 10°C and 20°C. Data presents as mean ± SE (n = 5).

CONCLUSIONS

Harvesting season, maturity stage and storage temperature affected IB incidence. The fruit that was stored at 10°C induced the IB symptom but the fruit that stored at 20°C was not showed the IB symptom. The fruit that was harvested at mature green stage during the rainy season had a higher severity of IB compared to that in winter season; on the other hand the fruit harvested at ¼ ripening stage showed the opposite trend to the above. The presence of IB symptoms affected to postharvest qualities. The pineapple that showed higher severity of IB tended to have lower vitamin C and higher TP and antioxidant activity.

ACKNOWLEDGEMENTS

The authors would like to thank National Research Council of Thailand (NRCT) and Mae Fah Luang University for the financial support of this study.

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