## Chapter 3

## Morphological Characteristics and the Influence of Storage Temperature on Quality of Queen Pineapple cv. Ninhbinh

### 3.1 Abstract

Temperature and relative humidity are the two main factors that affect a pineapple's properties after harvest. The post-harvest morphological characteristics and properties of Queen pineapples cv. Ninhbinh planted in Vietnam are described here. The effects of different temperature conditions (9, 12, 15 and 20°C ± 1°C) at a high relative humidity (85 ± 2% RH) on the chilling injury index (CI), weight loss, total soluble solids (TSS) content, titratable acidity (TA), firmness, color, ethanol content and microbial growth of Queen pineapples were studied over a 30 day storage period. There were chilling injury symptoms and a high CI index for the fruit stored at 9°C, 85% RH by the 10th day of storage. Weight loss in the pineapples increased with increasing temperature while the level of firmness decreased. The lowest value in terms of weight loss and the highest value in terms of firmness were obtained for those fruit stored at 12°C. The TSS and TA values of the pineapples increased over a 5 to 15 day storage period but had decreased slightly by the end of the storage stage for all treatments. An increase in storage temperature at a high relative humidity also increased the production of ethanol within the fruit. At 9°C and 12°C storage conditions, there was no significant difference found in the TSS and TA values for the fruit, but there was a significant difference found in terms of weight loss, firmness and chilling injury index values. Lower levels of weight loss, higher firmness levels and a lighter flesh color were found in pineapples preserved at 12°C. The effect of storage temperature on the total aerobic count (TAC) and the amount of yeast, mold and bacteria on the surface of the pineapples during 30 days storage was also studied. The results showed that 12°C and 85% RH were the optimal conditions needed to preserve the pineapples while maintaining a good quality level.

**3.2 Introduction**

Agriculture, and in particular food production, are key sectors requiring intervention as a result of climate change. Global warming has affected the weather and rainfall patterns of many countries, and can be seen as one of the main causes of reductions in crop production levels (Robert, 2007). Vietnam as a country has a tropical climate, with high temperatures and high relative humidity levels. The relative humidity in Ninhbinh Province during the pineapple growing season can be as high as 89%, and in addition, the temperature is able to reach between 32.8 and 39.1°C in the hot/summer season (Vietnam General Statistics Office, 2010). With plenty of sunshine, high rainfall levels, high temperatures and a high relative humidity almost throughout the year, there are many challenges to be faced when handling pineapples after the harvest and then during storage. Moreover, the Queen pineapple in particular has a non-smooth skin which can split, allowing micro-organisms to develop. About 20% of pineapples in Vietnam do not reach the consumer due to insufficient or inadequate post-harvest care (VFVA, 2008).

During storage and preservation, the pineapple can easily be spoiled due to the occurrence of yeasty fermentation (*Saccaromyces cerevisae, Hanseniaspora valbyensis; Candida intermedia*) (Bartholomew *et al.*, 2002; Nigam, 2000), black rot (*Chalara paradoxa - Thielaviopsis paradoxa*) (Oliveira and Nascimento, 2009; Reyes *et al.*, 2004; Wijeratnam *et al.*, 2006), black spot (*Penicillium funiculosum; Fusarium subglutinanas*) (Petty *et al.*, 2006) and fruit rot (*Aspergillus flavus; Rhizopus oryzae; Hendersonula toruloidea*) (Bartholomew *et al.*, 2002). The amount of yeast, fungi and the total aerobic count of fresh harvested pineapples held in storage has previously been determined and reported-on in a number of studies (Antoniolli *et al.*, 2005; Sangsuwan *et al.*, 2008b). However, little research has been carried out into the amount of yeast and fungi, neither the total aerobic count, for the whole of the pineapple fruit, nor the relationship between microbial growth and ethanol production in the fruit have been taken.

In order to extend the post-harvest life of pineapples, optimal temperature storage is a popular method used. Storing pineapples at between 0 and 5°C extends the shelf-life for several weeks, but upon transfer to non-refrigerated conditions, the fruit does not ripen and severe chilling injury symptoms appear (Abdullah and Atan, 1983). The application of refrigeration for pineapples is limited due to the development of chilling injury symptoms at temperatures of below 13$^{o}$C (Brown, 1986). Furthermore, there have been several studies carried out in order to assess the optimal storage temperature for pineapples, including cvs. Smooth Cayenne, Queen type Mauritius, Cayenne type Kew, Honey type Nanglae and Hawaiian Gold (Hewajulige *et al.*, 2003; Ratanachinakorn *et al.*, 2003; Stewart *et al.*, 2002). At 10°C, blackheart symptoms have been observed in the flesh of Smooth Cayenne fruit (Stewart *et al.*, 2002), whilst almost no blackheart has been observed in the flesh of Queen fruit (Abdullah *et al.*, 1985). Refrigeration is the main tool used to slow undesirable quality changes and increase the shelf-life of freshly harvested pineapples Montero-Calderon *et al.*, 2008; Sangsuwan *et al.*, 2008b); however, little research has been carried out into the preservation of the Queen pineapple at a high relative humidity.

Therefore, the key aim of this study was to determine the changes in ethanol content, microbial growth and chilling injury index levels, and some other properties, of the Queen pineapple cv. Ninhbinh during its storage at different temperatures and at high relative humidity. The study also assessed the morphological characteristics and the physical and chemical properties of this particular pineapple cultivar.

## 3.3 Materials and Methods

### 3.3.1 Raw materials

Queen pineapple (*Ananas comosus* L.) cv. Ninhbinh planted at a private farm in Tam Diep district, Ninhbinh Province in Vietnam were used for this research. After between 140 and 145 days of full bloom in the main growing season (April and May, 2010 and 2011), a total of 200 pineapples with uniform and similar characteristics, and at maturity stage 2 (Figure 3.1), were selected in order to determine changes in their properties over a 30 day storage period . The pineapples' crown leaves were cut to a crown length of about 1 cm from the top of the fruit, after which they were washed with water and drained for 10 to 15 minutes. The fruits were then stored at different temperature conditions (9, 12, 15 and 20 ± 1°C) and at 85 ± 2% relative humidity (RH). After that, chilling injury index levels, weight loss, the total soluble solids (TSS) content, titratable acidity (TA), firmness, color, ethanol content and microbial growth levels on the five fruits per each replication were determined every 5 days during the 30 day storage period.

In total, 100 of the pineapples at maturity stage 3, those of a high quality, were chosen for an assessment of their morphological characteristics (including weight, length and maximum fruit diameter, plus the number of fruitlets) and other properties (such as pH, TA, flesh color, flesh firmness and TSS).


**Figure 3.1** Shell color score of Queen pineapple at maturity stages (stage 1: Green; stage 2: 10- 25% yellow; stage 3: 26-50% yellow; stage 4: 51-75% yellow and stage 5: 76-100% yellow).

**3.3.2 Analysis of morphological characteristics of pineapple fruits**

The weight of the pineapples was determined using a scale balance (± 0.01 g), model PB1502-S (Mettler-Toledo, Menlo Park, CA, USA). The fruit length and maximum diameter were measured using a digital Caliper 0-200mm (Mitutoyo Corporation, Japan).

**3.3.3 Determination of the chilling injury index (CI)**

The severity of chilling injury symptoms was evaluated after the pineapples were transferred from the cold room to an environment at room temperature. Chilling injury levels were measured based on the extent of surface browning of the pulp. The fruits were cut longitudinally in half and the CI levels determined; then rated on a scale from 1 to 5 based on the intensity of surface browning, as follows: 0 = no chilling injury symptoms, 1 = browning symptoms covered <10% of the surface area, 2 = browning symptoms covered 10 to 25% of the surface area, 3 = browning symptoms covered 26 to 50% of the surface area, 4 = browning symptoms covered 51 to 75% of the surface area, and 5 = browning symptoms covered 76 to 100% of the surface area. The average chilling injury index score was calculated for each group of fruit (Hu *et al.*, 2011; Phrutiya *et al.*, 2008).

**3.3.4 Analysis of physical properties of pineapple fruits**

The flesh color of the pineapples was measured on the cut-surface using a colorimeter, model CR-200 (Minolta, Osaka, Japan). Multiple readings were taken on the cut-surface of the fruits over six tests (in the basal, middle and upper positions). The L*, a* and b* values were used to calculate the hue angles, based on a formula described by McGuire (1992). The firmness of the pineapples' flesh was determined using a stable micro-systems TA-TXT2i texture analyzer (Texture Technologies Crop, UK). Slivers of 3 cm thickness were taken from the basal, middle and upper parts of each pineapple, then measured for their flesh texture. The maximum force (Newton) of each sliver was then recorded at 3 positions - inner, middle and outer positions.



**3.3.5 Analysis of physical, chemical properties of pineapple fruits**

Fresh cut pineapple pieces (50g) were minced and filtered, and then the juice was used to determine the pH, titratable acidity (TA) and total soluble solids (TSS) content at $25^{o}$C. The pH was measured directly using a pH meter, while TA was measured using 5 ml of juice titrated with a standardized 0.1 N NaOH up to pH 8.2 by an auto-titrator (Titroline easy, Schott, Japan), expressed as a percentage of citric acid (g citric acid/100g fresh weight). The TSS content (%) was determined using a digital refractometer (Pocket PR-101, ATAGO Company, Japan).

The ethanol content of the pineapples was determined based on a gas chromatographic analysis of the headspace Sangsuwan *et al.*, 2008b). Five grams of pineapple flesh was placed in a 10 mL amber glass bottle with a rubber cap, then incubated in a water bath at 60°C for 45 minutes. Headspace gas was drawn using a 1 mL syringe and injected into a TRACE GC gas chromatograph (Agilent 6890N, USA) equipped with a flame ionization detector. The temperatures of the oven, injector and detector were set at 150, 175 and 200°C respectively, and the column used was a 30 m x 0.25 mm i.d. x 0.25 μm capillary column. A retention time and a standard curve of absolute ethanol in water solutions (0-2400 mg $L^{-1}$) were both used for peak identification and quantification.

#### 3.3.6 Microbial analysis

Changes in the microbial population across the whole surface of each fruit during storage at different temperatures were determined in line with Parish *et al.* (2001) and Eni *et al.* (2010). One hundred milliliters of peptone buffer water was poured over the pineapples in each plastic bag, and then the fruit were massaged for 15 minutes from outside the plastic bag and then removed from the bags. One milliliter of appropriate dilutions of the wash buffer was spiral plated on to a plate that contained Potato-Dextrose Agar (PDA) nutrient. The plate was incubated at 35°C for 24 hours, after which the amount of yeast and mould and the total plate count (TPC), were assessed and recorded, yielding the number of colony forming units (CFU) per fruit.

#### 3.3.7 Statistical analysis

Data were initially evaluated using analysis of variance (ANOVA) and Duncan's Multiple Range Test (SPSS 16.0 software program).

### 3.4 Results and Discussion

### 3.4.1 Morphological characteristics of Queen pineapple

The main morphological characteristics of the Queen pineapple cv. Ninhbinh are summarized in Table 3.1. Compared with other pineapples in the same group, the weight of the Queen cv. Ninhbinh pineapple is similar to that of cv. Morris (Malaysia) and cv. Tripura (India), but heavier than that of cv. Phulae (Thailand) and cv. Formosa (Philippines) (Balito, 2011; Kongsuwan *et al.*, 2009; Sema, 2011). Its leaves are spiny, narrow and long; light green in color with a streak in the middle. Small and sharp thorns line the edges of the leaves throughout its entire length. Regarding its taste and odor, the cv. Ninhbinh pineapple is crispy, very sweet (18.22 ± 0.66%) and a little sour (0.81 ± 0.04%), and overall has an excellent flavor. These properties play an important role in the fruit's quality and its popularity among consumers.

### 3.4.2 Effect of storage temperature on chilling injury index

Chilling injury symptoms are one of the most significant causes of post-harvest loss among pineapples when stored at low temperature (Bartholomew *et al.*, 2002). The effect of different storage temperatures (9, 12, 15 and 20°C) and high relative humidity (85% RH) on the chilling injury index values of the study pineapples is presented in Figure 3.2. Drying, discoloration and browning symptoms associated with CI were observed in the pulp adjacent to the core of the pineapples stored at 9°C, 85% RH by the 10$^{th}$ day of storage, and the browning symptoms increased with increasing storage duration (Figure 3.3 and 3.4). By the 30$^{th}$ day in storage, the CI index of the fruits stored at 9°C, 85% RH reached its highest level, leading to discoloration and browning over 50% of the surface area. Having been exposed to storage temperatures of 9°C, tissue discoloration and darkening became more pronounced when the fruits were stored at ambient temperature. Zero CI was found in the fruit stored at 12°C, 15°C and 20°C during the 30 days storage, and the color of the flesh was still yellow and homogenous up until the end of storage (Figure 3.3).

**Table 3.1** Morphological characteristics and physical-chemical properties of pineapple fruits cv. Ninhbinh at maturity stage 3

| Characteristics/ Properties | Value |
|---|---|
| * *Morphological characteristics* | |
| Fruit weight (g) | 990.28 ± 69.02 |
| Fruit length (mm) | 142.07 ± 2.49 |
| Maximum fruit diameter (mm) | 105.39 ± 3.60 |
| Minimum fruitlet number/fruit | 99 |
| Maximum fruitlet number/fruit | 156 |
| Shape | Round and elongated |
| Leaf of crown color | Green |
| Shell color | Light yellow |
| * *Physical-chemical properties* | |
| TSS (%) | 18.22 ± 0.66 |
| TA (%) | 0.81 ± 0.04 |
| TSS/TA ratio | 22.49 ± 0.22 |
| pH | 4.03 ± 0.10 |
| Firmness (N) | 16.17 ± 0.30 |
| Flesh color | |
| *L** | 64.18 ± 0.41 |
| *a** | 3.55 ± 0.33 |
| *b** | 32.01 ± 0.65 |

Our results agree with those of Paull and Rohrbach (1985), Hewaiulige *et al.*, (2003) and Stewart *et al.*, (2002). Paull and Rohrbach (1985) found that pineapple cv. Smooth Cayenne stored for up to 5 weeks at 12°C began to show symptoms of CI within 2 days of being transferred to storage at 22°C. CI symptoms were also found on pineapple cv. Nang Lae stored at 8°C and 13°C and with a 85 to 90% RH when stored for 25 and 20 days respectively, but there was no CI found in the pineapples stored at 10°C over 30 days (Ratanachinakorn *et al.*, 2003).

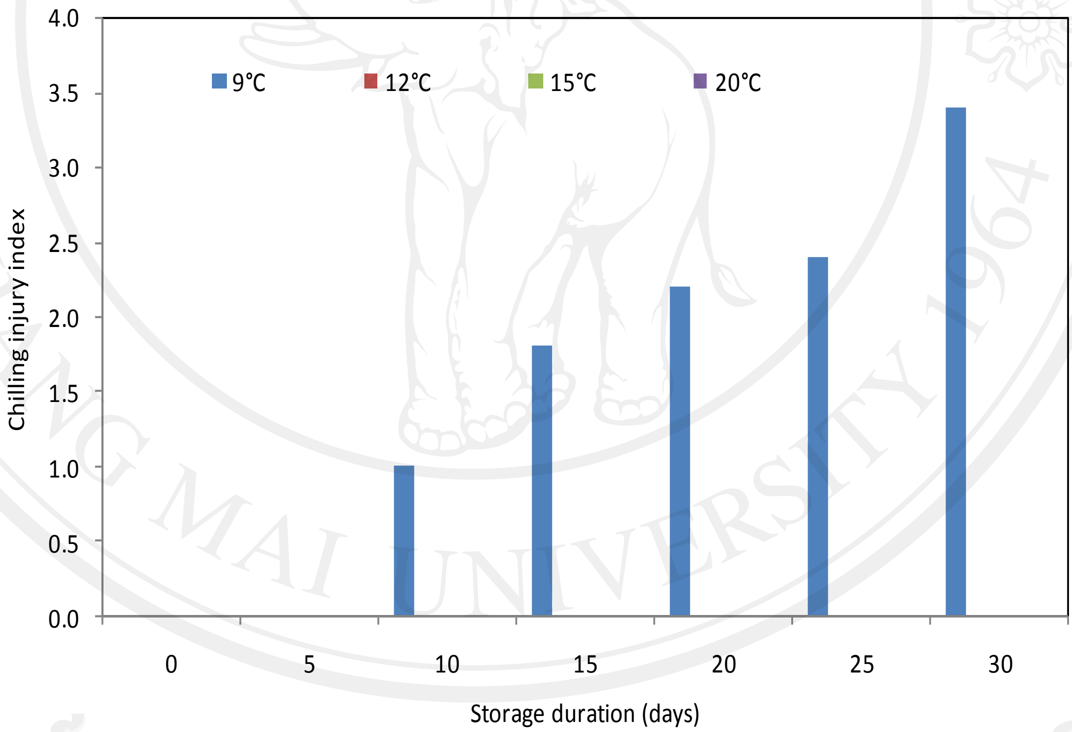



**Figure 3.2** Effect of storage temperature on chilling injury index of pineapple cv. Ninhbinh during 30 day storage at different temperatures at 85% RH.

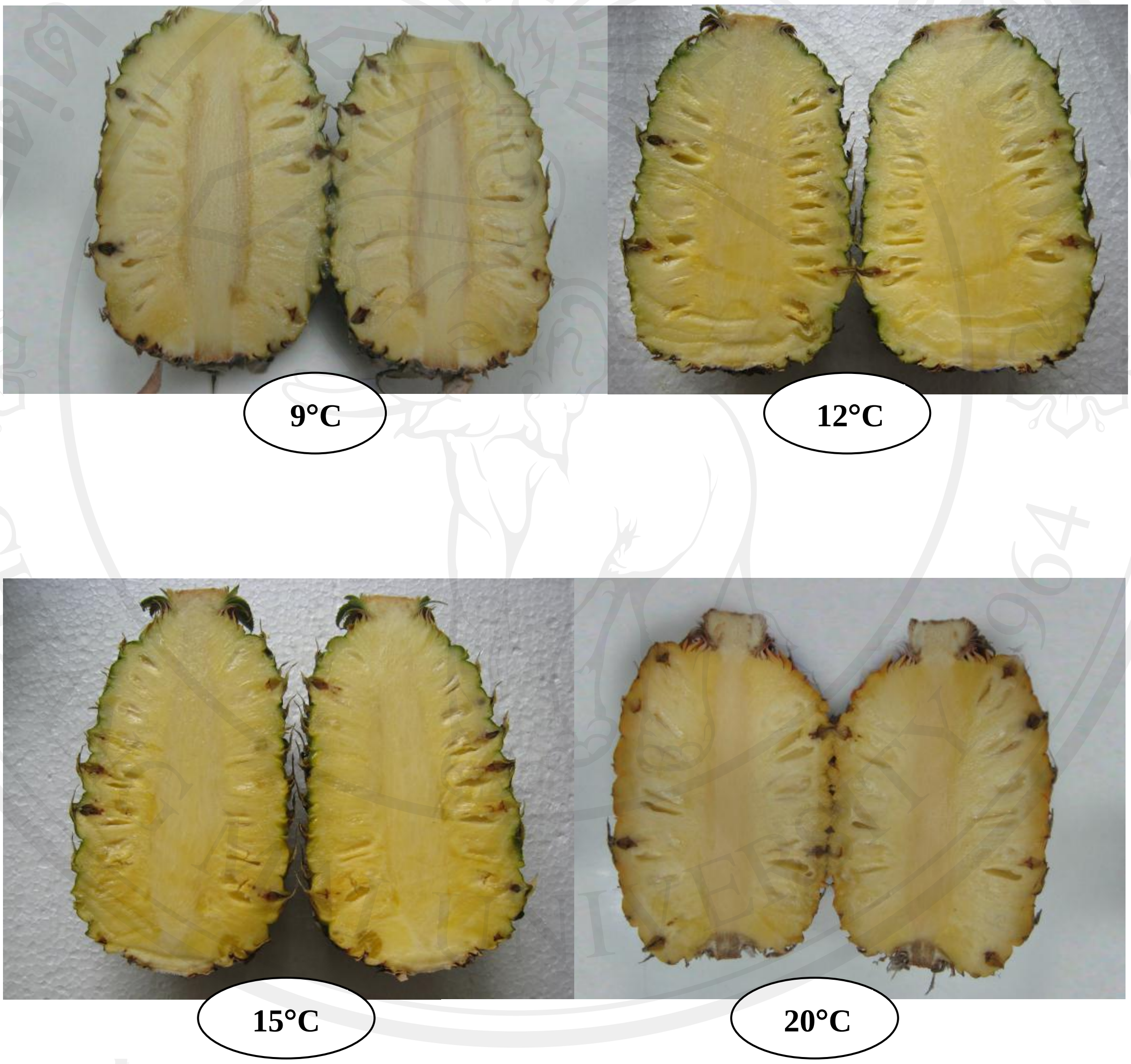



**Figure 3.3** Comparing the flesh color of pineapple fruit cv. Ninhbinh stored at different storage temperatures at the 10th day storage.

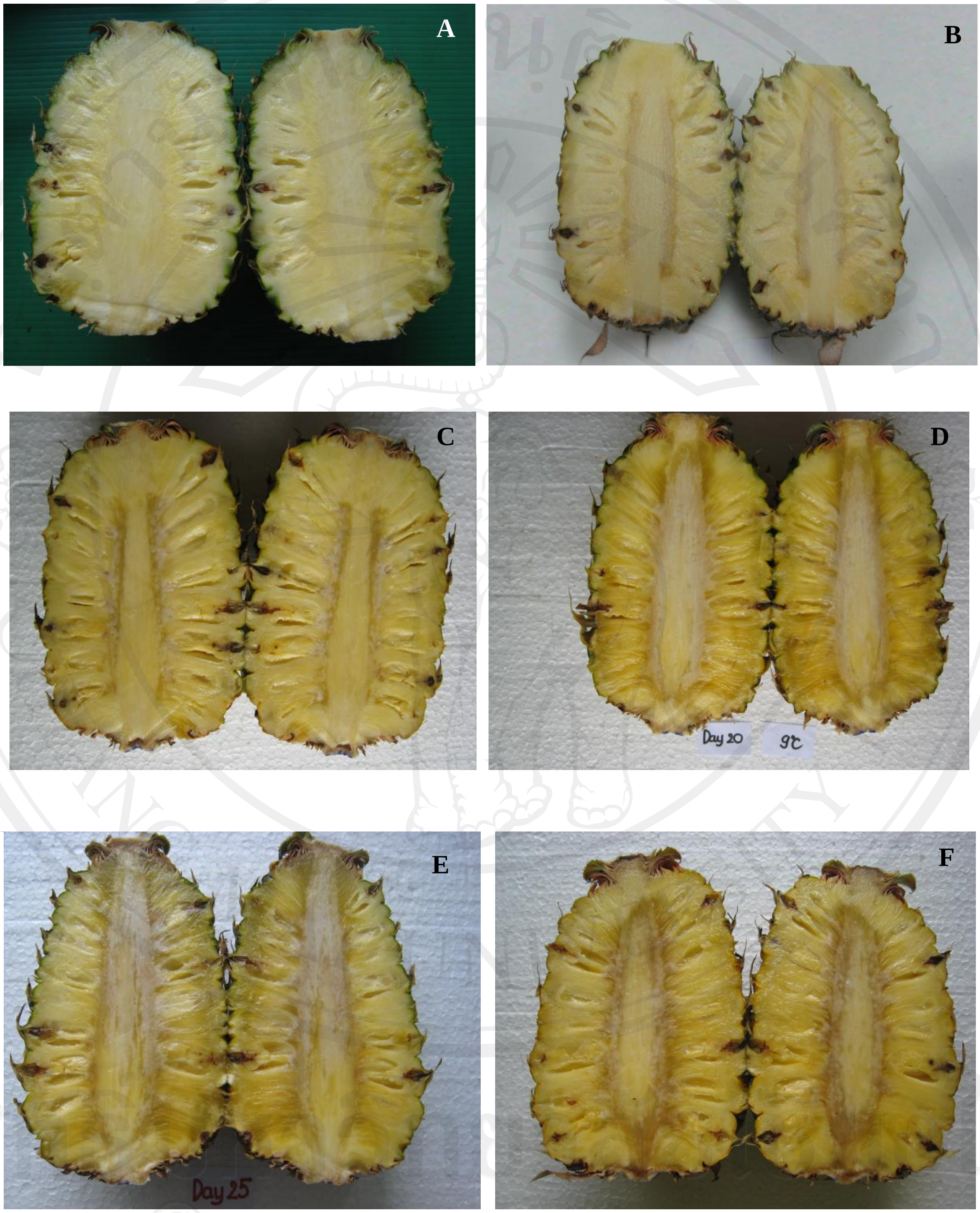



**Figure 3.4** Chilling injury symptoms of pineapple fruit cv. Ninhbinh stored at 9°C, 85% RH during storage duration: (A): day 5; (B): day 10; (C): day 15; (D): day 20; (E): day 25 and (F): day 30.

### 3.4.3 Effect of storage temperature on physical and chemical properties

The results of the study into changes in weight loss, total soluble solids content, titratable acidity, flesh firmness and flesh color of the cv. Ninhbinh pineapples during their storage at different temperatures and at 85% RH for 30 days, were investigated.

#### *3.4.3.1 Weight loss*

The change in weight loss of the pineapples at different storage temperatures is shown in Figure 3.5, revealing that weight loss increased with higher temperatures. Storage at lower temperatures effectively delayed weight loss among the pineapples, and when the storage temperature was increased from 9 to 20°C, weight loss increased significantly, from 17.89 to 20.68% respectively after 25 days of preservation. It was also found that when the storage period was prolonged, weight loss increased, with the maximum weight loss percentage recorded at the end of the storage period. Water loss was the most important factor in terms of weight loss among the pineapples, with relative moisture, the temperature of the product and the environment all playing an important role in terms of water loss (Wijeratnam *et al.*, 2006). In addition, internal activity within the fruit was enhanced at higher temperatures and resulted in a higher rate of transpiration (Wijeratnam *et al.*, 2006). As a result, pineapples stored at 20°C lost more water than those stored at lower temperatures; however, the smallest weight loss did not occur at the lowest temperature condition (9°C) but when the fruit were stored at 12°C, with values of 19.81 ± 0.1%. Higher weight loss values for pineapples stored at 9°C may be of concern in terms of the impacts of chilling injury, and according to Bartholomew *et al.*, (2002), CI symptoms not only include browning, dulling and discoloration in pineapples, but also drying and wilting.

#### *3.4.3.2 Total soluble solids (TSS) content and titratable acidity (TA)*

The TSS and TA values of the fruit increased between 5 and 15 days storage, but then had decreased by the end of the storage stage in all temperature conditions (Figure 3.6). The highest TSS value was found in the fruit preserved at 20°C after 5 days, this being 18.24 ± 0.13%. At the lower temperatures of 9°C and 12°C, there was no significant difference in the TSS and TA values during storage. Compared to some other pineapple cultivars such as cv. Comte de Paris in China (Hu *et al.*, 2011) and cv. Pattavia in Thailand (Phrutiya *et al.*, 2008), the study cv. Ninhbinh pineapples displayed a higher TSS value when stored at low temperatures. A 14% Brix value was reported for pineapple fruit cv. Pattavia when stored at 10°C/85% RH by the 28$^{th}$ day of storage (Phrutiya *et al.*, 2008). An increase or decrease in TSS and TA content might be related to the metabolic processes of the pineapples. During the early stages of storage, between 5 and 15 days, TSS and TA contents increased and reached their highest levels after the ripening process had set in. In contrast, sugar metabolism, respiration rate, ethanol procedure and microbial growth led to a decrease in TSS and TA values by the end of the storage period.

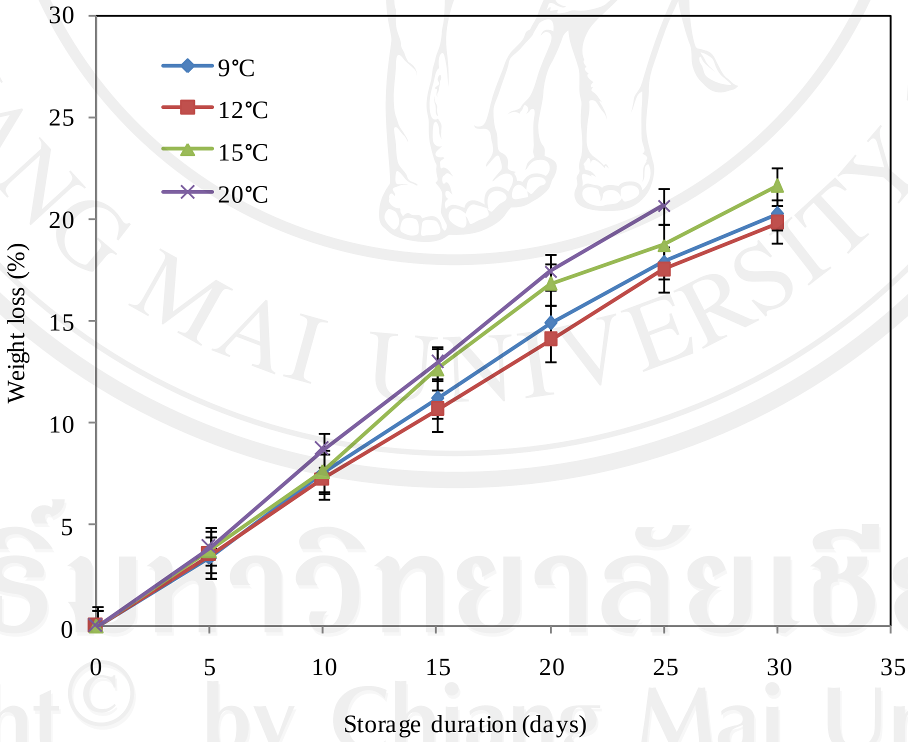
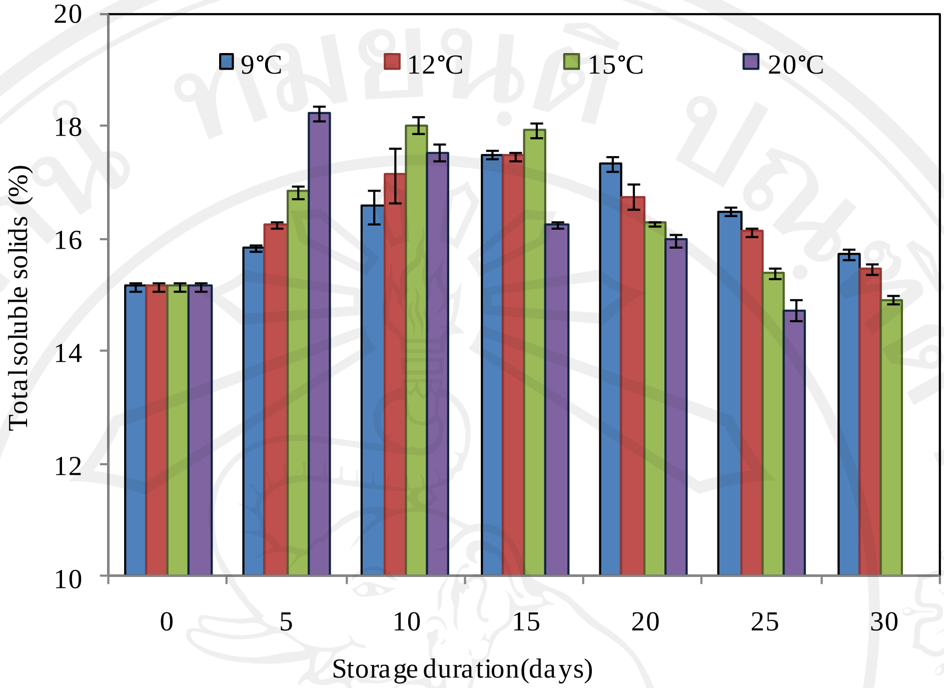



**Figure 3.5** Change of weight loss of pineapple fruit during 30 day storage at different temperatures at 85% RH.



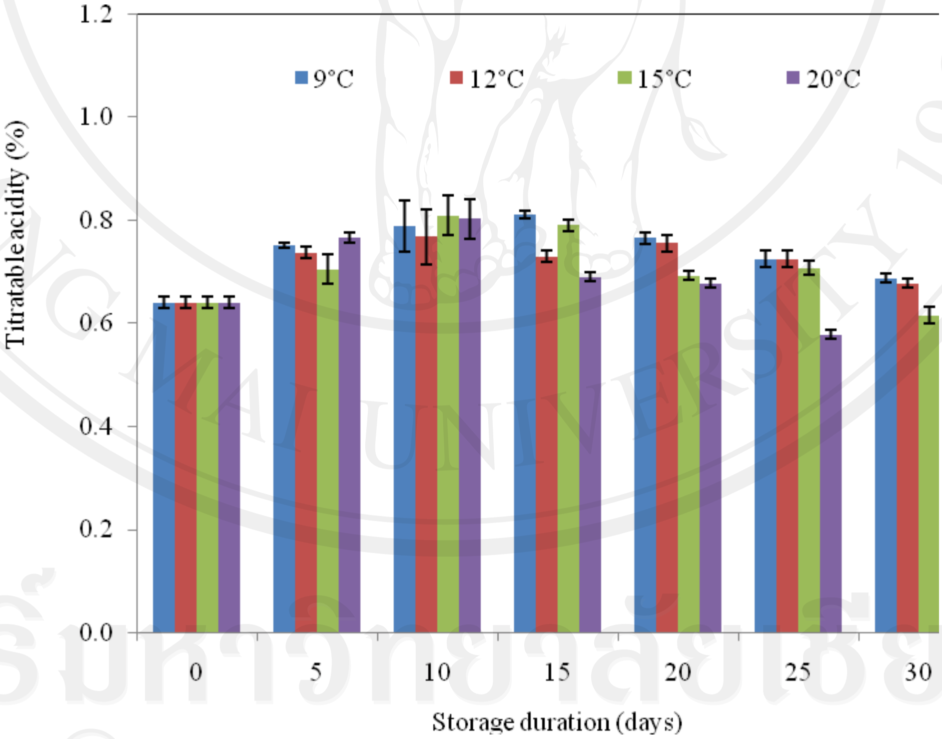




**Figure 3.6** Change of total soluble solids (a) and titrable acidity (b) of pineapple fruit during 30 day storage at different temperatures at 85 ± 2% RH.

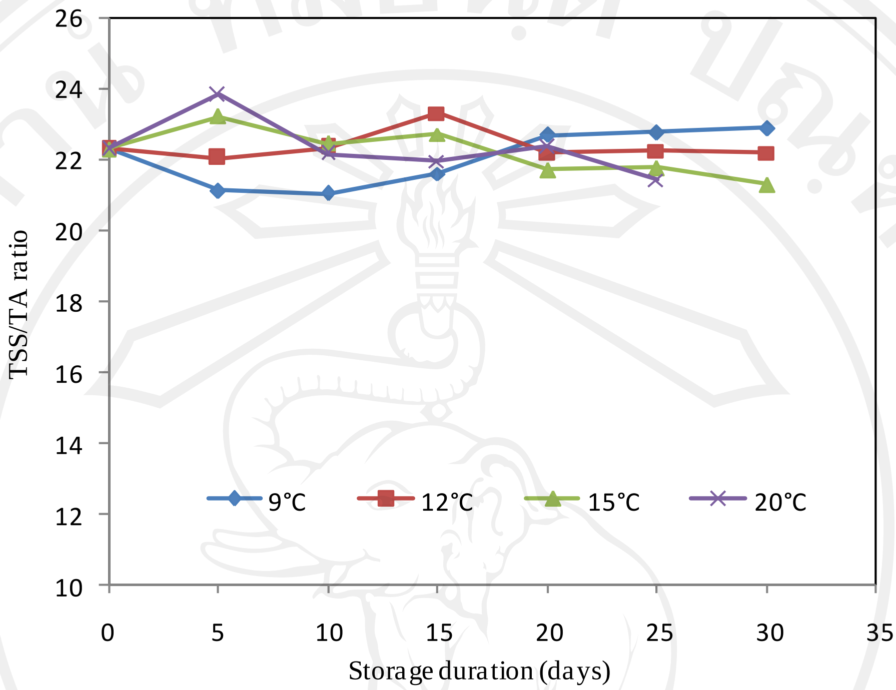



**Figure 3.7** Change of TSS/TA ratio of pineapple fruit during 30 day storage at different temperatures at 85% RH.

The total soluble solids (TSS) and titratable acidity (TA) contents are the factors that influence eating quality. The TSS/TA ratio is one of the most inportant value that shows the acceptance of customers. Smith (1988a,b) reported that TSS gave the highest average correlation with the eating quality among nine parameters tested. In fact, the highest values of TSS and TSS/TA raito were presented for the fruits stored at 20°C at 5$^{th}$ day, which were 18.24% and 28.8, respecively. At 9, 12 and 15°C, the highest TSS/TA ratio in the range 23.26-23.30 was obtained for fruits at the 20$^{th}$, 15$^{th}$ and 10$^{th}$ day storage, respectively. Gortner and Singleton (1965) pointed out that the fruit sample having TSS/TA ratio of 22-23 tend to the better received by comsumers than those below this average. These results suggested that there is the better acceptantcy for pineapple stored at 12, 15 and 20°C during the early stages of storage, between 5 and 15 days.

***3.4.3.3 Firmness***

In addition to total soluble solids content, firmness is also a critical quality attributed in terms of consumer acceptability for fruits and vegetables. Figure 3.8 illustrates the changes in flesh firmness found for the study pineapples when stored at 85 ± 2% RH and for different temperatures. Higher temperatures significantly affected flesh firmness characteristics. The initial firmness value of 16.33 (N) on the first day had dropped to 6.44, 7.43 and 6.47 (N) by the 30th day of storage at 9, 12 and 15°C respectively, and to 5.94 by the 25th day of storage at 20°C. According to Figure 3.8, storage at 12 ± 1°C reduced the change in firmness of the pineapples' flesh, and it was found to be the optimal temprature in terms of preserving the fruit. However, storing the pineapples at a lower tempertaure, here 9°C, did not delay any change in flesh firmness either.

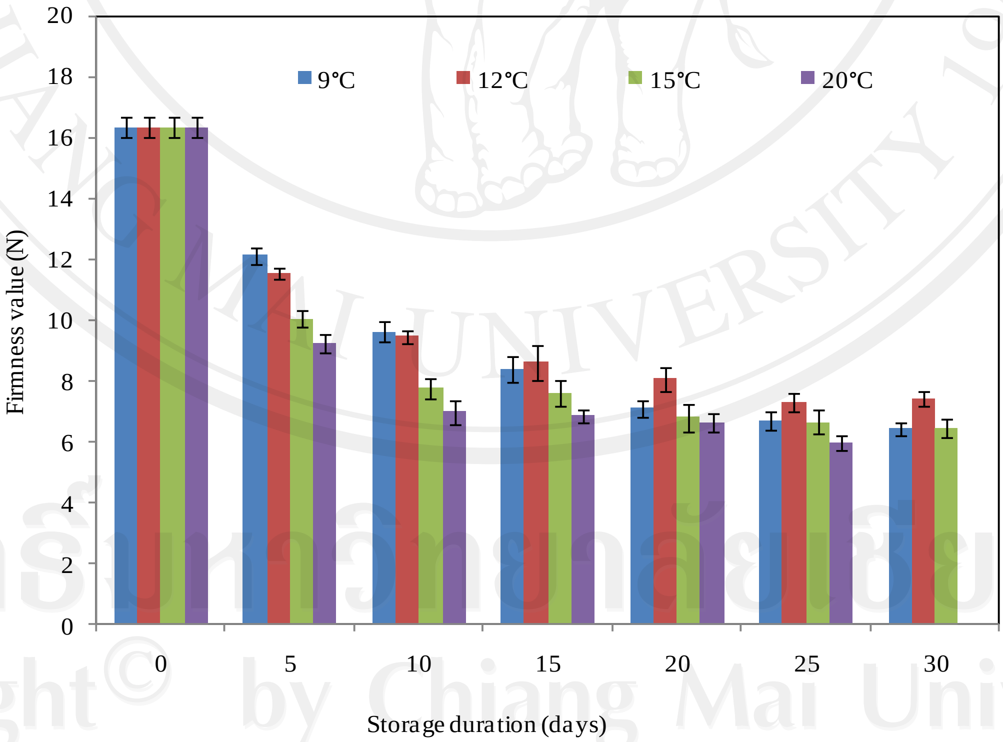



**Figure 3.8** Change of firmness of pineapple fruit during 30 day storage at different temperatures at 85% RH.

The firmness of the pineapples at 9°C began to diminish under 9.62 (N) and after a storage period of 10 days. The significant decrease in firmness for pineapples stored at 9°C may have been due to the appearance of chilling injury symptoms at low temperatures. A decrease in the firmness of the pineapples' flesh during storage was found for all treatments, and this result might have been as a direct result of cell wall modification during fruit ripening, that decreased the cells rigidity and eventually generated softness (Beaulier and Gorny, 2001). Moreover, a degradation in carbohydrates, as well as cell wall deterioration can lead to a decrease in firmness (Tucker and Seymour, 1993). Our results are in accordance with previously reported data on trends in the firmness of cv. Smooth Cayenne pineapples stored at 10°C (Acedo *et al.*, 2004).

***3.4.3.4 Flesh color***

Figure 3.9 shows the L* values and hue angle values for the pineapple flesh when maintained at 9, 12, 15 and 20°C, 85 ± 2% RH. The L* parameter describes the darkness to whiteness color, with a range of 0 to 100, while the hue angle parameter shows that the actual color altered from red-yellow to yellow and then yellowish-green when the hue angle was increased from 49° to 90° and then 135° respectively. The L* value of the pineapple flesh increased slightly during the early storage stage, and then decreased during the later stages (Figure 3.9a). The L* value of the pineapple flesh stored at 9 and 12°C decreased slightly, but then decreased dramatically at 15 and 20°C after 5 days in storage (Figure 3.9a). The decrease in L* values between the $5^{th}$ and $25^{th}$ days might be related to the pineapple flesh darkening in color as a result of the ripening process, which led to a yellowish color appearing. However, pineapple flesh stored at 9°C for 30 days had a lower L* value than that stored at 12°C over the same period.

The hue angle value or actual color of the pineapple flesh decreased with an increase in storage temperature and storage time (Figure 3.9b). The change in color of the pineapple flesh when stored at 20°C was faster than when stored at lower temperature conditions. By the end of the storage period, the color of the pineapple flesh stored at 15 and 20°C had turned an orange-yellow color, while the color of the pineapple flesh stored at 9 and 12°C retained a slightly yellow color. Similar flesh color results have been found for fresh-cut pineapple cvs. Josapine and Smooth Cayenne when stored at 10°C (Latifah *et al.*, 2011; Marrero and Kader, 2006).

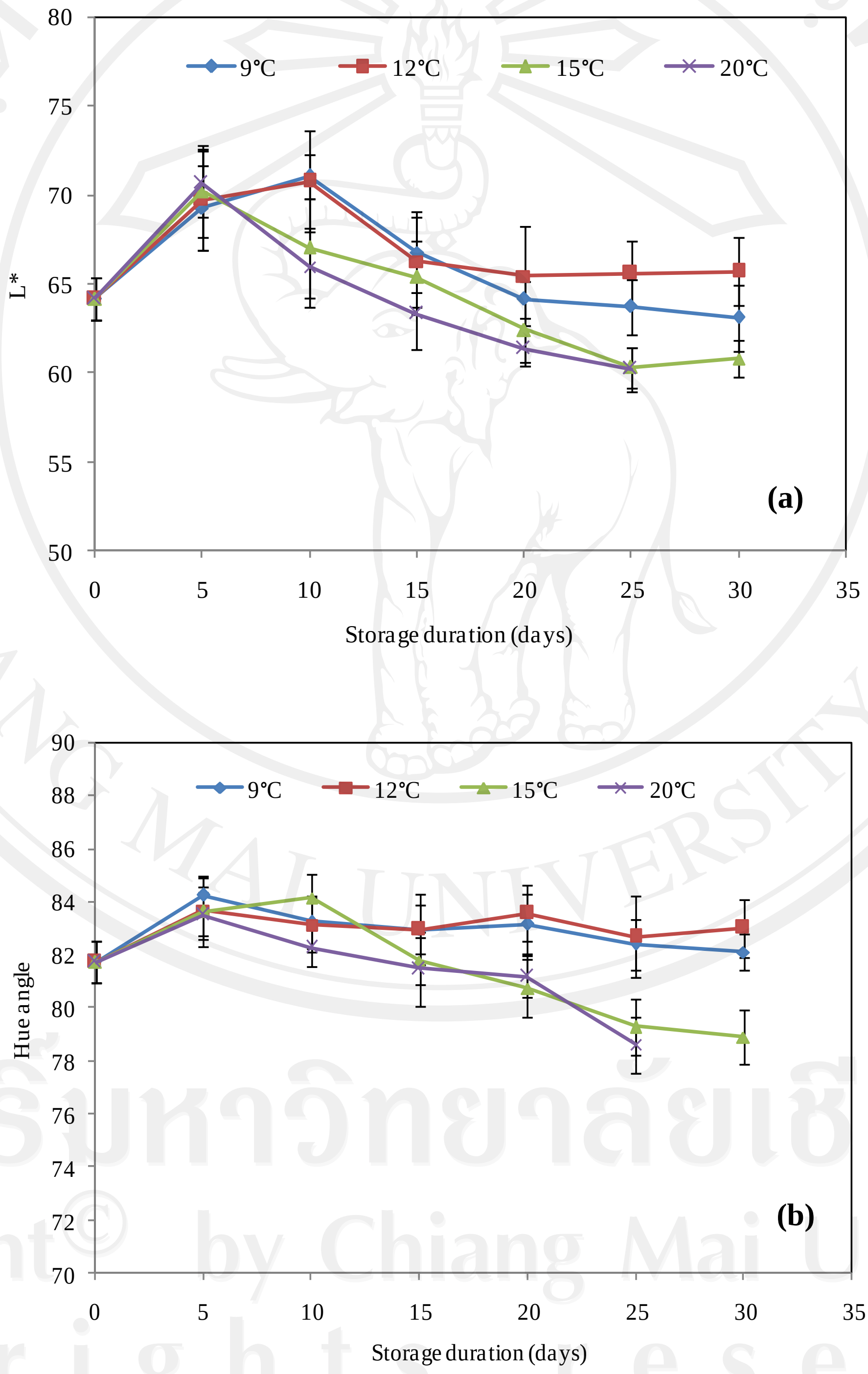



**Figure 3.9** Change of flesh color of pineapple fruit during 30 days storage at different temperatures combined 85% RH with a) L* and b) hue angle.

### *3.4.3.5 Ethanol content*

The statistical analysis shows that there was a significant difference in the mean ethanol content of the pineapples for all treatments. Ethanol levels among the pineapples increased with an increase in temperature and storage times (Figure 3.10), though the rate of increase in ethanol content among fruit kept at 15 and 20°C was more two times that of the fruit preserved at 9 and 12°C after 20 day storage. Maintaining the pineapples at 9 ± 1°C led to the lowest ethanol content in the fruit after 30 days (1.67 ± 0.054 µL $g^{-1}$). In contrast, at 20°C, the ethanol content of the fruit increased dramatically, from 0.026 to 3.07(µL/g) by the end of the storage period, and reached a maximum ethanol content level of 4.4 (µL $g^{-1}$) after 15 days in storage. No significant difference in ethanol content was found among pineapples stored at either 9 or 12°C.

With increasing in storage duration, an increase in the ethanol content of the fruit was linked to a decrease in total soluble solids content and an increase in fermentation due to microbial activities. The total soluble solids amount in the study pineapples was very high at the initial stage (16.25 to 17.53%) and the solids content acted as a nutrient source for microbial growth and an increase in fermentation and thus ethanol production.

### 3.4.4 Effect of storage temperature on microbial growth on pineapple fruit

The effect of storage temperature on the total aerobic count (TAC) of the pineapples after 30 days in storage is shown in Figure 3.11. The TAC of the fruit increased significantly with increasing storage temperature. On the first day, the TAC of the whole fruit was 3.6 x $10^6$ (CFU/fruit); however, after 5 days in storage, the TAC had increased dramatically to 14.4, 21.8, 43.1 and 54.5 x $10^6$ (CFU/fruit) at 9, 12, 15 and 20°C respectively. The highest TAC figure was 102.1 x $10^6$ (CFU/fruit) for fruit maintained at 20°C and after 20 days in storage. The rate of development of micro-organisms in those fruits stored at 15°C and 20°C was about two times higher than in fruit preserved at 9 and 12°C after 20 days in storage. There was a significant difference in the TAC of fruits preserved at 9°C and 12°C on the 15$^{th}$ day of storage, but no significant difference among these same fruits over the 10 last days. Our results are close to those given previously for the TAC of oranges stored at ambient temperature, or 1.23 x $10^6$ (CFU/fruit) (Parish *et al.*, 2001). In addition, the TAC of ten samples of different fruits tested in Sango Ota, Nigeria (carrots, runner beans, cucumbers, pineapples, green peppers, cabbages, spring onions, lettuces, water melons and apples) was reported to be in the range 3.8 x $10^6$ to 1.8 x $10^7$ (CFU/mL) (Eni *et al.*, 2010).

Bacteria, yeast and mould have an important impact on fruit quality after harvest, with fermentation and spoilage being common symptoms among pineapples in storage. As a result, here the amount of bacteria, yeast and mold in the pineapples was determined for all the storage treatments. A comparison of the amount of bacteria, yeast and mold (CFU/fruit) that developed on the surface of the pineapples during the 30 days in storage at different temperatures and at 85 ± 2% RH, is presented in Table 3.2. The results show that a low storage temperature is important when wishing to keep the yeast and mold populations low.

In this study, yeast attained the highest percentage figures in terms of the microbial population found in the fruit, followed by bacterium and mold. By the 25$^{th}$ day in storage, the amount of yeast had decreased from 64.1 to 28.2 x $10^6$ (CFU/fruit) at 20 and 9°C respectively. Similarly, the amount of mold had also decreased from 19.2 to 5.8 x $10^6$ (CFU/fruit) at 20 and 9°C respectively. These results shed light on the composition of and nutrient levels found in pineapples. A high total soluble solids content combined with high amounts of yeast provides ideal conditions for the development of fermentation and the production of ethanol, a result which can be explained as being due to the increase in ethanol content among pineapples kept in storage, especially at high temperatures.

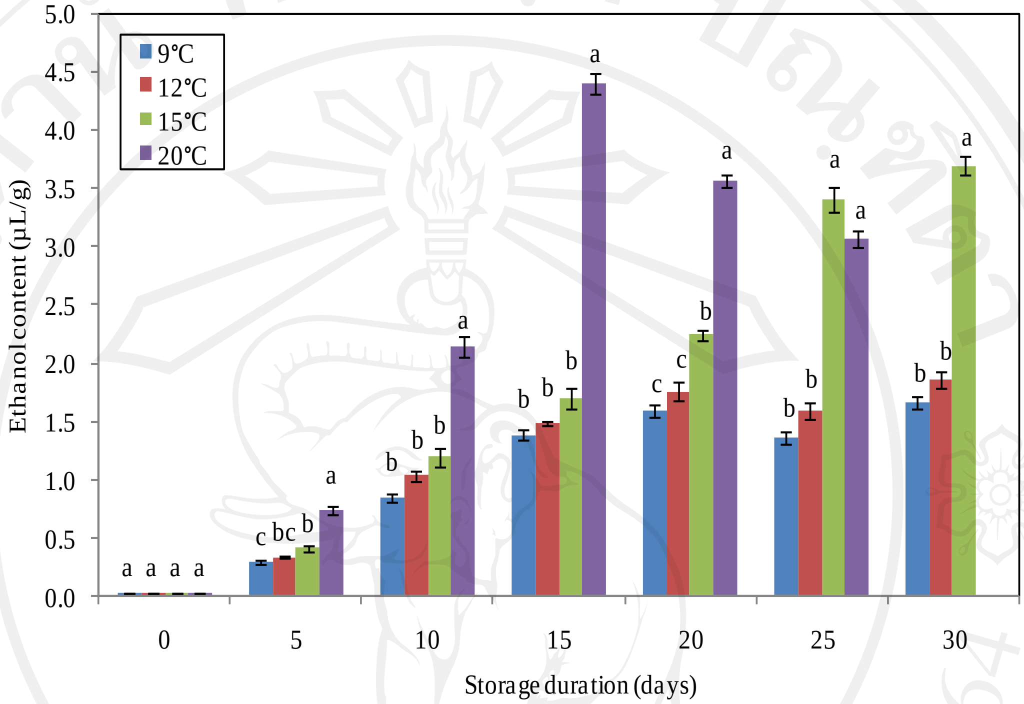



**Figure 3.10** Change of the ethanol content in flesh pineapple fruit during 30 day storage at different temperatures at 85% RH.

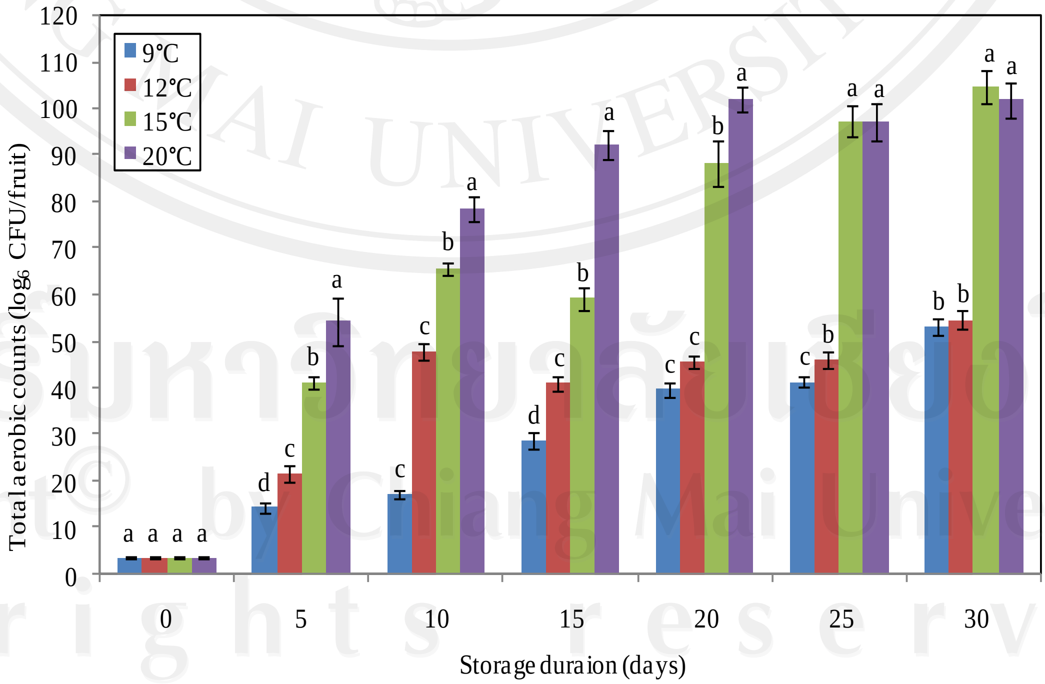



**Figure 3.11** Change of total aerobic counts on the surface of pineapple fruit during 30 day storage at different temperatures at 85% RH.

**Table 3.2** Change in number of yeast, bacterium and mold on the surface of Queen pineapple during 30 day storage at different temperatures

**(A) Yeast** (x$10^{6}$ CFU/fruit)

| Storage | Duration (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| Temp. | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| 9°C | $2.1^{a}$ | $2.4^{c}$ | $2.4^{d}$ | $12.9^{d}$ | $26.4^{c}$ | $28.2^{c}$ | $35.2^{b}$ |
| 12°C | $2.1^{a}$ | $5.0^{b}$ | $11.2^{c}$ | $24.9^{bc}$ | $27.8^{c}$ | $28.3^{c}$ | $32.7^{b}$ |
| 15°C | $2.1^{a}$ | $14.5^{a}$ | $25.8^{b}$ | $37.7^{b}$ | $53.9^{b}$ | $58.1^{b}$ | $62.8^{a}$ |
| 20°C | $2.1^{a}$ | $15.8^{c}$ | $44.9^{a}$ | $57.8^{a}$ | $65.7^{a}$ | $64.1^{a}$ | |

**(B) Bacteria** (x$10^{6}$ CFU/fruit)

| Storage | Duration (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| Temp. | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| 9°C | $7.1^{a}$ | $9.4^{d}$ | $12.4^{c}$ | $8.7^{c}$ | $7.8^{c}$ | $7.4^{d}$ | $7.7^{c}$ |
| 12°C | $7.1^{a}$ | $13.8^{c}$ | $32.6^{a}$ | $15.1^{b}$ | $10.6^{cb}$ | $10.5^{c}$ | $10.7^{bc}$ |
| 15°C | $7.1^{a}$ | $22.9^{b}$ | $23.4^{b}$ | $36.4^{a}$ | $30.2^{a}$ | $30.8^{a}$ | $30.5^{a}$ |
| 20°C | $7.1^{a}$ | $35.8^{a}$ | $29.9^{a}$ | $16.1^{a}$ | $15.3^{b}$ | $15.8^{b}$ | |

**(C) Mold** (x$10^{6}$ CFU/fruit)

| Storage | Duration (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| Temp. | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| 9°C | $1.6^{a}$ | $2.6^{c}$ | $2.6^{c}$ | $3.1^{c}$ | $4.3^{c}$ | $5.8^{b}$ | $7.3^{b}$ |
| 12°C | $1.6^{a}$ | $3.1^{b}$ | $4.2^{b}$ | $4.5^{b}$ | $7.3^{b}$ | $7.3^{b}$ | $8.4^{a}$ |
| 15°C | $1.6^{a}$ | $3.9^{a}$ | $4.8^{a}$ | $4.7^{b}$ | $4.2^{c}$ | $7.5^{b}$ | $8.5^{a}$ |
| 20°C | $1.6^{a}$ | $2.9^{bc}$ | $3.9^{bc}$ | $20.4^{a}$ | $22.3^{a}$ | $19.2^{a}$ | |

### 3.5 Conclusions

The influence of storage temperature on the ethanol content, microbial growth, chilling injury index, and physical and chemical properties of the Queen pineapple cv. Ninhbinh was studied here. It was found that storage temperature significantly affected the chilling injury symptoms, flesh color, flesh firmness, and ethanol production and microbial growth levels of the pineapples. The pineapples stored at 12°C at 85% RH, maintained their quality without signs of chilling injury until the end of storage, though such symptoms were observed in the pulp adjacent to the core of pineapples stored at 9°C by the $10^{th}$ day in storage. Moreover, lower weight loss and higher firmness levels, plus a lighter flesh color, were found in pineapples stored at 12°C than at other storage conditions. Storing the pineapples at 12°C also delayed the development of yeast, fungi and bacteria, as well as the production of ethanol in the fruit. Therefore, pineapples should be stored at 12°C and 85% RH in order to help extend their shelf-life.