



Full Length Article

Influence of Storage Temperature on Ethanol Content, Microbial Growth and other Properties of Queen Pineapple Fruit

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Abstract

Temperature and relative humidity are the two main factors that affect a pineapple's properties after harvest. The post-harvest physico-chemical characteristics Queen pineapples cv. Ninh Binh planted in Vietnam were studied. The pineapple is crispy, very sweet, a little sour and has an excellent flavor. The effects of different storage temperature (9, 12, 15 and 20°C ± 1°C: 85 ± 2% RH) on ethanol content, microbial growth, chilling injury index (CI), weight loss, total soluble solids (TSS) content, titratable acidity (TA), firmness and color of Queen pineapples were studied over a 30 days storage period. An increase in storage temperature also increased the production of ethanol within the fruit. 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 the 30 days storage period was also studied. Fruit surface TACs were generally in the range of 10⁵ to 10⁶ colony forming units (CFU). There were chilling injury symptoms and a higher CI index for the fruit stored at 9°C, 85% RH by the 10th day of storage. 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 for all treatments. The lowest value in terms of weight loss, the highest value in terms of firmness and a lighter flesh color were obtained for those fruit stored at 12°C. The results showed that 12°C: 85% RH were the optimal conditions needed to store the pineapples, while maintaining a good quality level up to 30 days. © 2013 Friends Science Publishers

Keywords: Ethanol content; Physic-chemical properties; 'Queen' pineapple; Storage temperature

Introduction

The pineapple (*Ananas comosus* (L.) Merr.) is a member of the Bromeliaceae family, and is the third most important tropical fruit in the world after banana and citrus fruit (Bartholomew *et al.*, 2002) in terms of production. The main pineapple-producing countries are Brazil, Thailand, Philippines, Vietnam, Mexico, China, Nigeria, Indonesia, and Columbia (Chen *et al.*, 2011). In Vietnam, the pineapple is the second most important fruit in terms of area planted and production levels, after the banana, and is also considered one of the most important fruit in terms of processing. At present the total area under pineapple in Vietnam has increased slightly from 36500 ha in 2000 to 39900 ha in 2010, with production increased dramatically from 291,000 to 502,700 tons over the same period. Of the many pineapple cultivars in Vietnam, cv. Ninh Binh is one of the most attractive and has set market attention due to its excellent texture, taste and flavor. Moreover, the fruit is small - weighing around 750 to 1100 g, has a sweet taste and a crispy texture and thick skin. The skin is usually yellow or greenish-yellow in color when it ripens.

Vietnam as a country has a tropical climate, with high temperatures and high relative humidity levels. The relative humidity in Ninh Binh province during the pineapple growing season can be as high as 89%, and in addition, the temperature can reach between 32.8 and 39.1°C in the summer season. 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. During storage and preservation, the pineapple can easily be spoiled due to the occurrence of yeasty fermentation (*Saccharomyces spp.*; *Saccaromyces cerevisiae*, *Hanseniaspora valbyensis*; *Candida intermedia*) (Nigam, 2000; Bartholomew *et al.*, 2002), black rot (*Chalara paradoxa* - *Thielaviopsis paradoxa*) (Reyes *et al.*, 2004; Wijeratnam *et al.*, 2006; Oliveira and Nascimento, 2009), black spot (*Penicillium funiculosum*; *Fusarium subglutinans*) (Petty *et al.*, 2006) and fruit rot (*Aspergillus*

flavus; *Rhizopus oryzae*; *Hendersonula toruloidea*) (Bartholomew *et al.*, 2002). The amount of yeast and fungi and the total aerobic count of freshly harvested pineapples hold in storage has previously been determined and reported-on in a number of studies (Antoniolli *et al.*, 2005; Sangsuwan *et al.*, 2008). However, little research has been carried out to into load of yeast and fungi, or the total aerobic count, for the whole of the pineapple fruit, nor the relationship between microbial growth and ethanol production in the fruit.

In order to extend the post-harvest life of pineapples, optimal low temperature storage is a popular method used. Storing pineapples at 0–5°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°C (Brown, 1986). Furthermore, there have been several studies carried out in order to assess the optimal storage temperature for pineapples, including cv. Smooth Cayenne, cv. Queen type Mauritius, cv. Cayenne type Kew, cv. Honey type Nanglae and Hawaiian Gold (Stewart *et al.*, 2002; Hewajulige *et al.*, 2003; Ratanachinakorn *et al.*, 2003). At 10°C, blackheart symptoms have been observed in the flesh of Smooth Cayenne fruit (Stewart *et al.*, 2002), whilst almost no blackheart were observed in the flesh of Queen fruit (Abdullah *et al.*, 1985). It is summarized that 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.*, 2008); Response of different varieties varies towards low temperature and studies were mainly to be undertaken for optimizing storage temperature for 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. Ninh Binh 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.

Materials and Methods

Raw Materials

Queen pineapples (*Ananas comosus* L.) cv. Ninh Binh planted at a private farm in Tam Diep district, Ninh Binh province in Vietnam were used for this research. After about 140 and 145 days of full bloom, a total of 200 pineapples with uniform and similar characteristics, and at maturity stage 2 (Fig. 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 min. The fruit 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 fruit were determined every 5 days during the 30 days storage period. Five fruit were selected for each replication.

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

Analysis of Physical Characteristics of the Pineapples

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 0-200 mm (Mitutoyo Corporation, Japan).

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 Corp, UK). Slices 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.

Biochemical Properties of the Pineapples

Fresh cut pineapple pieces (50 g) were minced and filtered, and then the juice was used to determine the pH, titratable acidity (TA) and TSS content at 25°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/100 g fresh weight). The TSS content (°Brix) was determined using a digital refractometer (Pocket PR-101, ATAGO Company, Japan) (Joomwong, 2006).

The ethanol content of the pineapples was determined based on a gas chromatographic analysis of the headspace (Sangsuwan *et al.*, 2008). 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 min. 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 × 0.25 mm i.d. × 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.

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 25 to 50% of the surface area, 4 = browning symptoms covered 50 to 75% of the surface area, and 5 = browning symptoms covered 75 to 100% of the surface area. The average chilling injury index score was calculated for each group of fruit (Phrutiya *et al.*, 2008; Hu *et al.*, 2011).

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 min 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 h, 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.

Statistical Analysis

All experiments were conducted by triplicate determinations. Statistics on a completely randomized design were determined using the one-way analysis of variance (ANOVA) procedure in the SPSS software (version 16.0) and Tukey's-*b* multiple range test was used to analyze the significant differences at 95% confidential of each variable.

Results and Discussion

Morphological Characteristics and Properties of Vietnamese Pineapple cv. Ninh Binh

The main morphological characteristics of the Vietnamese pineapple cv. Ninh Binh are summarized in

Table 1. Compared with other pineapples in the same group, the weight of the Queen cv. Ninh Binh 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) (Kongsuwan *et al.*, 2009; Balito, 2011; 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. Ninh Binh pineapple is crispy, very sweet ($18.22 \pm 0.66\%$) and a little sour ($0.81 \pm 0.04\%$), and overall has an excellent flavor. These properties play an important role in the fruit's quality and its popularity among consumers.

Effect of Storage Temperature on the Ethanol Content

The statistical analysis showed that there was a significant difference in the mean ethanol content of the pineapples cv. Ninh Binh stored at different storage temperatures (9, 12, 15 and 20°C) and high relative humidity (85% RH). The storage life of the pineapples stored at 20°C was prolonged to 25 days, while it was enhanced up to 30 days for the pineapple stored at 9, 12 and 15°C. Ethanol levels among the pineapples increased with an increase in temperature and storage times (Fig. 2), though the rate of increase in ethanol content among fruit kept at 15°C and 20°C was more two times that of the fruit preserved at 9°C and 12°C after 20 days storage. Storing the pineapples at $9 \pm 1^\circ\text{C}$ led to the lowest ethanol content in the fruit after 30 days ($1.67 \pm 0.054 \mu\text{L g}^{-1}$). In contrast, at 20°C, the ethanol content of the fruit increased dramatically, from 0.026 to $3.07 \mu\text{L g}^{-1}$ by the end of the storage period, and reached a maximum ethanol content level of $4.4 \mu\text{L g}^{-1}$ after 15 days in storage. No significant difference in ethanol content was found among pineapples stored at either 9°C or 12°C.

With increasing in storage duration, an increase in the ethanol content of the fruit might relate 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.

Our results on the ethanol production are similar to that reported by Karaoulanis and Dilley (1993) who found that the ethanol content of ripening apples increased during storage at room temperature. Maintaining fresh-cut 'Gold' pineapple at 5°C also produced high ethanol content, which was $46 \mu\text{L g}^{-1}$ after 20 days storage. Besides, effect of storage conditions on ethanol content of fruit was reported by authors, but most of them presented on the ethanol formation from fruit pulp, fruit waste or fruit wine (Obire *et al.*, 2008; King *et al.*, 2012; Togarepi *et al.*, 2012).



Fig. 1: Shell colour score of Queen pineapples 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

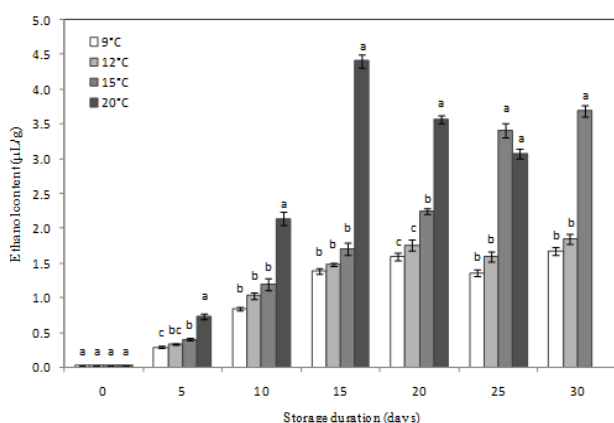


Fig. 2: Change of the ethanol content in flesh of pineapple during 30 days storage at different temperatures at 85 ± 2% RH

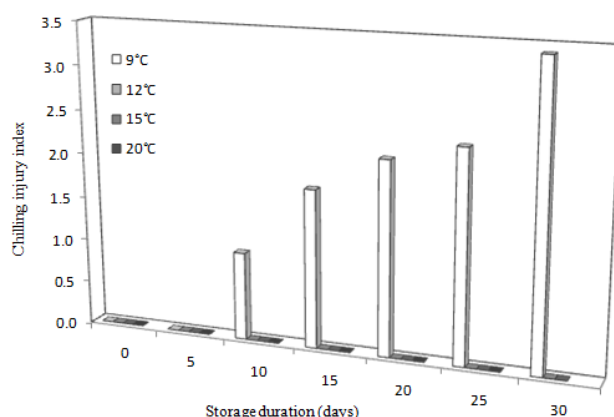


Fig. 3: Effect of storage temperature on chilling injury index of Queen pineapples during 30 days storage at different temperatures at 85 ± 2% RH

Table 1: Morphological characteristics, physical and physical-chemical properties of Queen pineapples cv. Ninh Binh

Characteristics/Properties	Value
<i>* Morphological characteristics</i>	
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
<i>* Properties</i>	
TSS (%)	18.22 ± 0.66
TA (%)	0.81 ± 0.04
pH	4.03 ± 0.10
Firmness (N)	16.17 ± 0.30
Flesh colour	
L*	64.18 ± 0.41
a*	3.55 ± 0.33
b*	32.01 ± 0.65

Effect of Storage Temperature on the Chilling Injury Index

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 Fig. 3. 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 10th day of storage, and the browning symptoms increased with increased storage duration (Fig. 4). By the 30th day in storage, the CI index of the fruits stored at 9°C; 85% RH reached its highest level, which was 3.4/5.0, 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 fruit were stored at ambient temperature. No 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. 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). Our results agree with those of Paull and Rohrbach (1985), Hewajulige *et al.* (2003) and Stewart *et al.* (2002). In their study, Paull and Rohrbach (1985) found that pineapple cv. Smooth Cayenne stored for up to five weeks at 12°C began to show symptoms of CI within two 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).



Fig. 4: Chilling injury symptoms of Queen pineapples cv. Ninh Binh 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

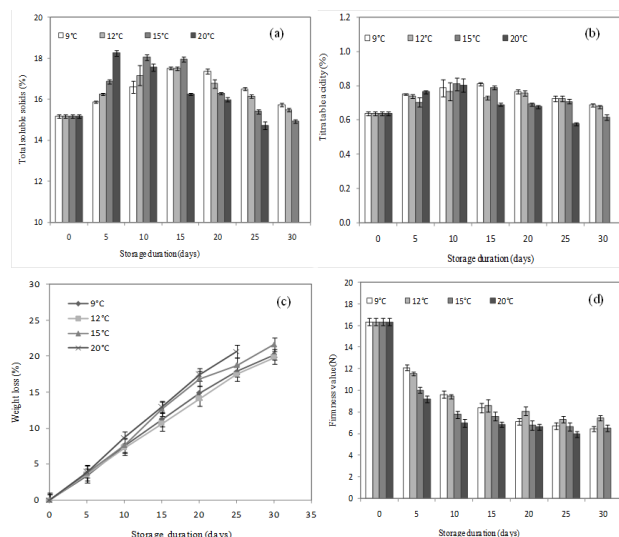


Fig. 5: Change of weight loss, TSS, TA and firmness value of Queen pineapples during 30 days storage at different temperatures at 85% RH

Effect of Storage Temperature on Physical and Chemical Properties

The results regarding changes in weight loss, total soluble solids content, titratable acidity, flesh firmness and flesh color of the pineapples during their storage at different temperatures at 85% RH for 30 days, are presented in Fig. 5. The TSS and TA values of the fruit increased between 5 and

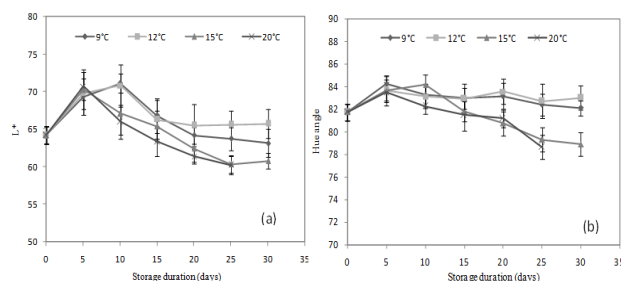


Fig. 6: Change of flesh colour of Queen pineapples during 30 days storage at different temperatures at 85 ± 2% RH with a) L* and b) hue angle

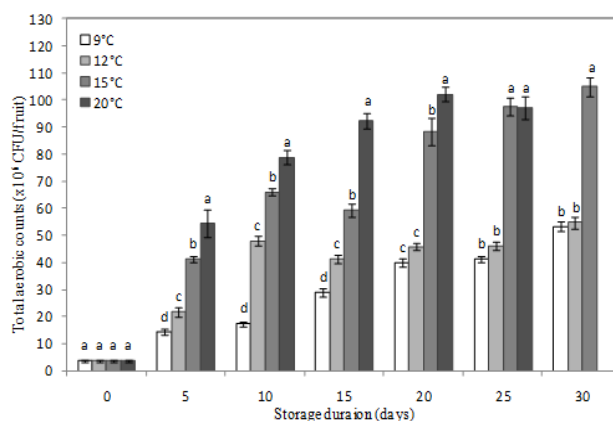


Fig. 7: Change of total aerobic counts on the surface of Queen pineapples during 30 days storage at different temperatures at 85 ± 2% RH

15 days storage, but then decreased by the end of the storage stage in all temperature conditions (Fig. 5a, b). The highest TSS value ($18.24 \pm 0.13\%$) was found in the fruit kept at 20°C after 5 days. 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. Ninh Binh 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 28th 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.

The change in weight loss of the pineapples at different storage temperatures is shown in Fig. 5c, revealing that weight loss increased with increase in temperature. Storage at lower temperatures effectively decreased weight

Table 2: Change in number of bacteria, yeast and mold on the surface of Queen pineapples during 30 days storage at different temperatures at $85 \pm 2\%$ RH

Storage duration (days)	Number of bacterium, yeast and mold ($\times 10^6$ cfu/fruit)								
	9°C			12°C			15°C		
	Bacteria	Yeast	Mold	Bacteria	Yeast	Mold	Bacteria	Yeast	Mold
0	7.1 ^a	2.1 ^a	1.6 ^a	7.1 ^a	2.1 ^a	1.6 ^a	7.1 ^a	2.1 ^a	1.6 ^a
5	9.4 ^d	2.4 ^c	2.6 ^a	13.8 ^c	5.0 ^b	3.1 ^a	22.9 ^b	14.5 ^a	3.9 ^a
10	12.4 ^c	2.4 ^d	2.6 ^a	32.6 ^a	11.2 ^c	4.2 ^a	23.4 ^b	25.8 ^b	4.8 ^a
15	8.7 ^c	12.9 ^d	3.1 ^d	15.1 ^b	24.9 ^{bc}	4.5 ^c	36.4 ^a	37.7 ^b	4.7 ^b
20	7.8 ^c	26.4 ^c	4.3 ^c	10.6 ^{cb}	27.8 ^c	7.3 ^b	30.2 ^a	53.9 ^b	4.2 ^c
25	7.4 ^d	28.2 ^c	5.8 ^b	10.5 ^{bc}	28.3 ^c	7.3 ^b	30.8 ^a	58.1 ^b	7.5 ^b
30	7.7 ^c	35.2 ^b	7.3 ^b	10.7 ^{bc}	32.7 ^b	8.4 ^b	30.5 ^a	62.8 ^a	8.5 ^b

Means sharing same letter do not differ significantly

loss among the pineapples, and when the storage temperature was increased from 9°C to 20°C, weight loss increased significantly, from 17.89 to 20.68% respectively after 25 days storage. 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, the temperature of the product and the environmental condition play 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 conditions (9°C) but when the fruit were stored at 12°C, with values of $19.81 \pm 0.1\%$. 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.

In addition to total soluble solids content, firmness is also a critical quality attribute in terms of consumer acceptability for fruit and vegetables. Fig. 5d illustrates the changes in flesh firmness for the pineapples when stored at $85 \pm 2\%$ 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 5d, storage at $12 \pm 1^\circ\text{C}$ reduced the change in firmness of the pineapples' flesh, and was found to be the optimal temperature in terms of preserving the fruit. However, storing the pineapples at a lower temperature (9°C) did not delay any changes in flesh firmness either. The firmness of the pineapples at 9°C began to diminish under 9.62(N) after a storage period of 10 days. The significant decrease in firmness for pineapples stored at 9°C may have been due to the chilling injury at low temperatures. A decrease in the firmness of the pineapples' flesh during storage was found for all treatments, and this

might have been as a direct result of cell wall modification during fruit ripening, that in turn 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 (Acedo *et al.*, 2004)..

Fig. 6 shows the L^* values and hue angle values for the pineapple flesh when maintained at 9, 12, 15 and 20°C/ $85 \pm 2\%$ 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, then decreased during the later stages (Fig. 6a, b). The L^* value of the pineapple flesh stored at 9°C and 12°C decreased slightly, but then decreased dramatically at 15°C and 20°C after 5 days in storage (Fig. 6a). The decrease in L^* values between the 5th and 25th 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 duration (Fig. 6b). 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°C and 20°C had turned an orange-yellow, while the color of the pineapple flesh stored at 9°C and 12°C retained a slightly yellow. Similar flesh color results have been found for fresh-cut pineapple cv. Josapine and Smooth Cayenne when stored at 10°C (Marrero and Kader, 2006; Latifah *et al.*, 2011).

Effect of Storage Temperature on Microbial Growth of Pineapple Surface

The effect of storage temperature on the total aerobic count (TAC) of the pineapple surfaces after 30 days in storage is shown in Fig. 7. The TAC of the fruit increased

significantly with increased storage temperature. On the first day, the TAC of all the fruit was 3.6×10^6 (CFU/fruit); however, after 5 days in storage, the TAC had increased dramatically to 14.4, 21.8, 43.1 and 54.5×10^6 (CFU/fruit) at 9°C, 12°C, 15°C and 20°C respectively. The highest TAC figure was 102.1×10^6 (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°C and 12°C after 20 days in storage. There was a significant difference in the TAC of fruits stored at 9°C and 12°C on the 15th day of storage, but this difference was not significant over the 10 last days. Our results are close to those previously reported for the TAC of oranges stored at ambient temperature, or 1.23×10^6 (CFU/fruit) (Parish *et al.*, 2001). In addition, the TAC of ten 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 3.8×10^6 to 1.8×10^7 (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. In the experiment, 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 $85 \pm 2\%$ RH, is presented in Table 2. The results show that a low storage temperature is important to keep the yeast and mold populations low. In this study, yeast attained the highest percentage in terms of the microbial population found in the fruit, followed by bacterium and mold. By the 25th day in storage, the amount of yeast had decreased from 64.1×10^6 (CFU/fruit) at 20°C to 28.2×10^6 (CFU/fruit) at 9°C. Similarly, the amount of mold had also decreased from 19.2 to 5.8×10^6 (CFU/fruit) at 20°C and 9°C respectively. This represents composition of nutrient levels found in pineapples. A high total soluble solids content combined with high amounts of yeast provides ideal conditions for the development of fruit fermentation, especially at high temperatures.

Conclusion

The influence of storage temperature on the ethanol content, microbial growth, chilling injury index, and physical and chemical properties of the Queen pineapple cv. Ninh Binh was studied. 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 and 85% RH, maintained their quality without showing 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 10th 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, cv. Queen pineapple should be stored at 12°C; 85% RH in order to help extend its shelf-life.

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