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EVALUATION OF FREEZING TEMPERATURES AND RIPENESS LEVELS ON THE QUALITY CHARACTERISTICS OF FROZEN PINEAPPLE FRUITS

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ABSTRACT

Freezing is considered an effective means to preserve fruits without using any chemical preservatives. In this study, the effects of ripeness levels and two freezing temperatures (-25ºC and -86ºC) on the frozen pineapples were evaluated. The faster freezing rate (0.4 ºC/min) was obtained by the -86ºC freezing system to achieve the core temperature of -18ºC in frozen pineapple in ~0.65 h compared to that at -25ºC with respective to freezing rate of 0.09 ºC/min and freezing time of ~3 h. Freezing pineapple fruits at -86ºC was found to reduce the drip loss, vitamin C, and structure loss compared to that at -25ºC. Ripeness levels were found to cause significant impacts on the quality of frozen pineapples. The increase in ripeness level (level 1 to level 4) was found to cause the increment in freezing time, drip loss, and vitamin C loss. Meanwhile, frozen pineapple fruits the green maturity (ripeness level 1) were observed with lower structure retention compared to riper pineapple (level 2 to level 4). The total soluble solids (TSS) and titratable acidity of frozen pineapples were found to be insignificantly affected by the freezing temperatures and ripeness levels. The freezing system at -86ºC for frozen pineapple was found to promote better performance in maintaining the quality of frozen pineapple products.

Keywords: Freezing, frozen pineapple, ripeness level, fruit quality

INTRODUCTION

Pineapple (*Ananas comosus* [L.] Merr.), which is in the family *Bromeliaceae*, is grown in subtropical regions and widely consumed in the world due to its attractive flavor and taste **(Dellacassa *et al.*, 2017)**. The top world producers of pineapple are listed as Costa Rica, Philippines, Brazil, India, Thailand, and Vietnam **(Kaewtathip & Charoenrein, 2012; Mohd Ali *et al.*, 2020).** Pineapple comprises a high content of sugars, organic acids, vitamins, dietary fibers, and minerals **(Begoña de Ancos *et al.*, 2016)**. However, the high moisture content of pineapple fresh flesh is a considerable factor that limits its shelf-life, leading to high demand for preservative techniques **(Zzaman *et al.*, 2021).** Therefore, pineapple, a perishable fruit, has gained the attention of researchers to find a suitable approach for extending the shelf-life of pineapple as well as meeting the demand of the product's availability in the off-season.

Freezing is one of the effective approaches to preserve fruits as it mostly retains the original state of fruits, color, flavor, and nutritional values **(Chauhan *et al.*, 2009)**. The freezing process has been reported to hamper chemical and cellular metabolism reactions, limit the growth of microorganisms and the rate of deterioration, and maintain the acceptability of fruit products **(Hui *et al.*, 2004; Sutariya & Sunkesula, 2021)**. However, the high water content of pineapple is considered the most concerning issue in the freezing process due to the formation of ice crystals which caused the changes in visual appearance, textural, flavor, and taste of fruit after thawing **(Sirijariyawat & Charoenrein, 2012)**. The quality changes in terms of pH, total soluble solids content (TSS), acidity, and organic acids are commonly observed during the freezing process **(Chauhan *et al.*, 2009)**. Frozen fruit qualities are dependent on the fruit type, maturity at harvest, pretreatment methods, freezing systems as well as freezing temperature **(Begóna De Ancos *et al.*, 2006)**. Mango, cantaloupe, and pineapple fruits showed a decrease in firmness and drip loss after freezing **(Sirijariyawat & Charoenrein, 2012)**. Apple was reported to experience a severe change compared to mango fruit according to texture, color, TSS, water content, pH, and titratable acidity **(Chassagne-Berces *et al.*, 2010)**. Drip loss in frozen *Prunus mume* fruit at -20ºC was considerably higher than that at -50ºC **(Chung *et al.*, 2013).**

Vietnam is one of the top producers which contributes to the large segment of pineapple production. The perishability of pineapple during storage is also a concerning issue that requires researchers to provide an effective means for extending shelf-life as well as remaining its acceptability to consumers. Freezing is the most applied method for preserving fruit, however, the difference in freezing temperature of each freezing system might noticeably affect the quality of frozen fruits **(Chassagne-Berces et al., 2010).** A low freezing temperature system was observed with a faster freezing rate which formed small ice crystals in frozen fruits, less causing impacts on the quality of frozen fruits. Meanwhile, a slower freezing rate of high freezing temperature system might induce larger ice crystals, deteriorating the textural structure of frozen fruits **(Chassagne-Berces et al., 2009, 2010).** Besides, the ripeness levels were also reported to significantly cause the influence on the quality of frozen fruit due to the variations in such chemical constituents and textural characteristics as soluble solids, titratable acidity, firmness, total pectin, etc. **(Rimkeeree & Charoenrein, 2014)**. Up to date, the influences of freezing process and ripeness levels on the quality of frozen pineapple are scarcely reported. Therefore, this study attempted to investigate the effect of freezing temperatures on the pineapple characteristics including texture, vitamin C, total soluble solids, titratable acidity, and drip loss after thawing. The influence of ripeness levels of pineapple fruits on the quality of frozen products was also evaluated. The expected result could possibly provide a suitable freezing temperature to maintain the fruit quality of pineapple as well as extend the shelf-life of pineapple fruit with an acceptable level to consumers. Besides, suitable ripeness levels for harvesting to be prepared for freezing storage will be chosen to ensure the quality of frozen pineapples that are less affected by the freezing process.

MATERIAL AND METHODS

Materials

Pineapples were collected from the local market, Vi Thanh city, Hau Giang province. Pineapples were selected at four maturity stages and cleaned by tap water to remove impurity particles. The maturity stages were visually observed via the peel color with the help of a digital colorimeter (CR-400 Konica Minolta, Konica Minolta, Inc., Japan). The color measurement according to the CIELAB system was preliminarily measured for the green mature pineapple (green peel) and fully ripe pineapple (dark yellow peel). The color change (ΔE) of pineapple peel from green to dark yellow was recorded. The ΔE value was then divided into 4 ranges which were defined as 4 maturity stages as followed **(Rimkeeree & Charoenrein, 2014):**

Ripeness level 1: green maturity, 0-25% yellowish
Ripeness level 2: less ripe maturity, 25-50% yellowish

Ripeness level 3: ripe maturity, 50-75% yellow
Ripeness level 4: fully ripe maturity, 75-100% dark yellow
Each pineapple was later allocated to the suitable ripeness level upon its measured ΔE value.
2,6-Dichlorophenolindophenol (DCPIP) and ascorbic acid were supplied from Merck KGaA (Darmstadt, Germany). Other analytical chemicals were purchased from

Freezing process

Pineapples were sampled in a cylindrical shape (21 mm in diameter and a thickness of 15 mm). Samples (500 g) were firstly packaged in vacuum-sealed PA packaging (Hoang Loc Ltd., Can Tho City, Vietnam). The packaged samples were subjected to the -25ºC chest freezer and -86ºC ultra low temperature chest freezer (Qingdao Carebios Biological Technology Co.,Ltd, China) using hydrofluorocarbons (HFC) refrigerant and SECOP compressor to decrease the temperature. The freezing time was determined when the sample core reached -18ºC by using a thermometer (OEMTOOLS 25245, OEMTOOLS^{TM}, Memphis, TN 38118, USA). The freezing rate was determined by the decrease in the temperature of the pineapple over time.

Textural measurement

Textural characteristics of pineapple samples were characterized by using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Godalming, UK). Pineapple cylinders (21 mm × 15 mm) were positioned in the testing area. The compression test was conducted with a loading force of 25 kg equipped with a probe (HDP/BSK blade cutter) at a compression speed of 2 mm/s until reaching a 50% strain. The results of the compression test were the average of ten measurements **(Chassagne-Berces *et al.*, 2010).**

Vitamin C content determination

The vitamin C content was measured following the titrimetric method using 2,6-Dichlorophenolindophenol (DCPIP), complying AOAC 967.21 with modifications **(AOAC, 2006).** Five grams of sample were ground with 20 mL of 1% HCl. Oxalic acid (1%) was included in the mixture to get the final volume of 100 mL and allowed to stand for 10 min. The mixture was filtered, and the filtrate (10 mL) was then titrated with 0.001 N DCPIP solution until the appearance of persistable pink color for 30 sec. The mixture comprising 1% oxalic acid (8 mL) and 1% HCl (2 mL) served as a blank sample. The vitamin C content was governed as followed:

$$\text{Vitamin C} = \frac{(X-B)*F*V*100}{m*Y} \text{ (mg/100 g)}$$

Where: X is the titrated DCPIP volume, B is the titrated DCPIP volume of blank sample, F is the mg vitamin C with respect to 1.0 mL DCPIP, V is the volume of the initial pineapple sample, Y is the volume of the test sample and m is the mass of the solid sample.

Drip loss measurement

A weighed pineapple samples were subjected to the freezing process until reaching the core temperature of -18ºC. Then, the samples were thawed at 8ºC. The drip loss (D) was calculated as followed:

$$D = \frac{m_1 \times (1 - W_1) - m_2 \times (1 - W_2)}{m_1 \times (1 - W_1)} \times 100$$

Where: D: drip loss (%), m_1: weight of pineapple before freezing (g), m_2: weight of pineapple after thawing (g), W_1: moisture content of pineapple before freezing, W_2: moisture content of pineapple after thawing **(Chung *et al.*, 2013).**

Total soluble solids and titratable acidity determination

The total soluble solids (TSS) of pineapple were determined by using a refractometer (Atago Co., Ltd., Japan). The titratable acidity was measured by using a titration method. Briefly, 10 g of the sample was mashed with 50 mL distilled water for 1 h. The mixture was filtered followed by the titration step with 0.1 N NaOH solution with the help of 1% phenolphthalein as a color indicator. The titration process was stopped until the appearance of perishable pink color for 30 sec.

Statistical analysis

Each experiment was carried out in thrice replicates. SPSS software (ver. 20.0, IBM Corp., Armonk, Newyork, USA) was used for the evaluation of one-way analysis of variance (ANOVA). Tukey HSD test was utilized to show the difference in mean values at the significance level of 5% ($p<0.05$%)

RESULTS AND DISCUSSION

Effect of ripeness levels and freezing temperatures on freezing time and freezing rate of frozen pineapple fruits

Figure 1 presents the effect of ripeness levels and freezing temperature on the freezing time. Obviously, lowering the freezing temperature shortened the freezing time in this study. The core temperature of frozen pineapple reached -18°C when applying a freezing temperature of -86°C for 0.65 h, whereas it required an extended time (~3 h) to reach the designated core temperature of frozen pineapple. Similar findings were reported in previous studies **(Chassagne-Berces *et al.*, 2010; Chung *et al.*, 2013).** The ripeness levels showed significant impacts on the freezing time. The freezing time was prolonged when pineapple was riper. This was indicated by the higher TSS in ripe fruit **(Sirijariyawat & Charoenrein, 2012)**. The more ripening fruit is reported to have the higher TSS content due to the physicochemical conversions during the ripening process **(Ntsoane *et al.*, 2019; Shamsudin *et al.*, 2007).** It was reported that the higher TSS in mango fruits resulted in a lower freezing point compared to those in pineapple, apple, and cantaloupe fruits. Meanwhile, the lower freezing point was reported to have a longer freezing time **(Sirijariyawat & Charoenrein, 2012).** Besides, other factors than the TSS also contribute to the variations of the freezing point at different maturity stages such as types of sugar, acid content, or other chemical constituents in pineapple fruits **(Jie *et al.*, 2003). Bonat Celli *et al.* (2016)** also reported that the performance of the freezing process was partially influenced by the textural characteristics and chemical compositions of frozen fruit.

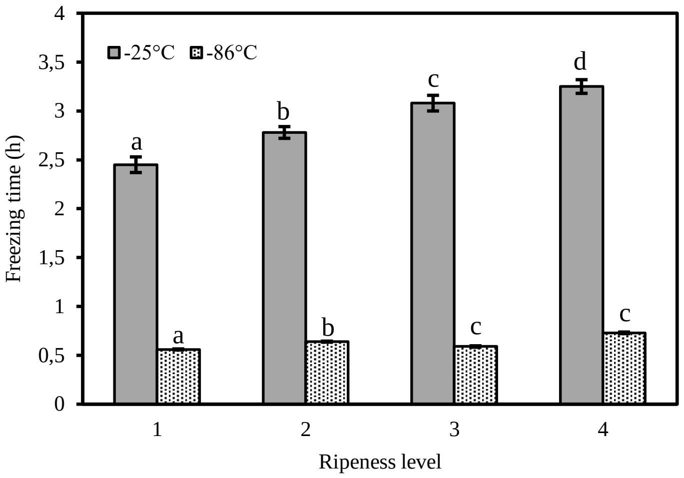

Figure 1 Effect of ripeness levels and freezing temperatures on freezing time of pineapple. Superscripts (a, b, c, d) indicate significant differences ($p < 0.05$) in ripeness levels of each freezing temperature

The freezing rate is a key factor for the preservation of food products as it significantly changes the physicochemical properties of raw materials. Similarly, the -86ºC freezing system was found to accelerate the freezing rate (~0.4 ºC/min) to reach the final core temperature of -18ºC in frozen pineapple compared to the -25ºC freezing system (~ 0.09 ºC/min). It was reported that a faster freezing rate was found to better preserve the physicochemical properties of frozen pineapple. The fast-freezing rate induces a large number of small ice crystals, whereas a slow freezing rate leads to fewer ice crystals but of large size which mainly damages the cell tissues and deteriorates the textural characteristics of frozen fruits **(Chassagne-Berces *et al.*, 2010). Laohasongkram *et al.* (1995)** indicated that the thermal conductivity of fruit also contributed to the difference in freezing rate. In this study, pineapple fruits at different maturity stages could possibly reveal different thermal conductivities which discriminated freezing rates. In summary, the freezing temperature at -86ºC was found to be a suitable parameter in the freezing process but the implementation and maintenance costs for the -86ºC freezing system could be a concerning issue.

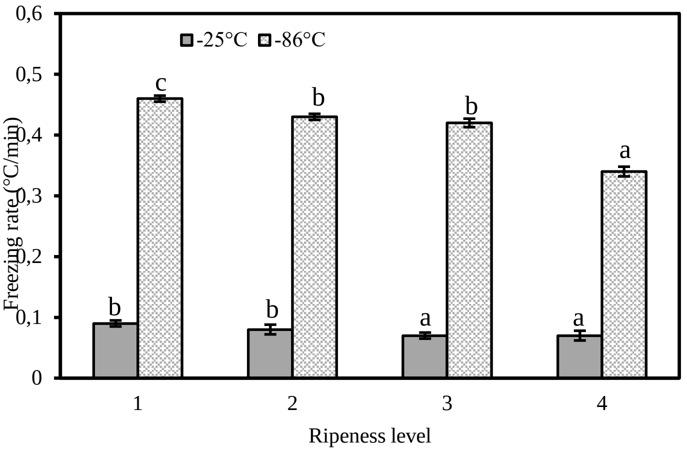

Figure 2 Effect of ripeness levels and freezing temperatures on freezing rate of pineapple fruits. Superscripts (a, b, c, d) show significant differences ($p < 0.05$) in ripeness levels of each freezing temperature

The freezing rate is a key factor for the preservation of food products as it significantly changes the physicochemical properties of raw materials. Similarly, the -86ºC freezing system was found to accelerate the freezing rate (~0.4 ºC/min) to reach the final core temperature of -18ºC in frozen pineapple compared to the -25ºC freezing system (~ 0.09 ºC/min). It was reported that a faster freezing rate was found to better preserve the physicochemical properties of frozen pineapple. The fast-freezing rate mainly induces small ice crystals, whereas a slow freezing rate leads to creation of large size ice crystals which mainly damages the cell tissues and deteriorates the textural characteristics of frozen fruits **(Chassagne-Berces *et al.*, 2010). Laohasongkram *et al.* (1995)** indicated that the thermal conductivity of fruit also contributed to the difference in freezing rate. In this study, pineapple fruits at different maturity stages could possibly reveal different thermal conductivities which discriminated freezing rates. In summary, the freezing temperature at -86ºC was found to be a suitable parameter in the freezing process but the implementation and maintenance costs for the -86ºC freezing system could be a concerning issue.

Effect of ripeness levels and freezing temperatures on structure retention of frozen pineapple fruits

The textural characteristic of frozen/thawed fruit is also considered a key factor contributing to the quality of fruit products. The effects of freezing temperature and ripeness level on the structure retention of frozen pineapples are presented in Figure 3. The freezing temperature of -86ºC was found to better maintain the structure of pineapple compared to the freezing temperature of -25ºC, retaining approximately 50% and 30%, respectively. As previously discussed, freezing fruit resulted in significant changes in the texture of fruits as compared to fresh fruit which was due to the disruption or damage of the cell structure via ice crystal formation **(Charoenrein & Owcharoen, 2016; Rincon & Kerr, 2010)**. The freezing process caused the formation of ice crystals from the water content in the internal structure of samples which seriously disrupted the cellular integrity, leading to the reduction of turgor pressure and the firmness of values of the samples **(Sirijariyawat & Charoenrein, 2012).** Therefore, an appropriate freezing approach should be carefully selected to minimize the negative effect of the freezing process on the textural characteristics of fruit samples. The -86ºC freezing system induced a faster freezing rate which promoted the formation of small ice crystals, less affecting the structure **(Chassagne-Berces *et al.*, 2010).** On the contrary, the slow freezing rate was found to cause large ice crystals which remarkably deteriorate the textural characteristic of frozen fruit **(Chassagne-Berces *et al.*, 2009). Ramallo & Mascheroni (2010)** also reported that the loss in firmness of pineapple was noticeably caused by the ice crystal formation during freezing at -31.5ºC.

The ripeness level showed significant differences among the analyzed groups ($p < 0.05$). Frozen pineapple at the ripeness level 1 experienced a severe loss in the structure as this ripeness level had the highest firmness due to less maturity. It was implied that fresh fruit with the higher firmness appeared to have the higher textural deterioration **(Chassagne-Berces *et al.*, 2010; Sousa *et al.*, 2007).** The result was consistent with previously reported studies **(Carbonell *et al.*, 2006; Charoenrein & Owcharoen, 2016).** The ripe Granny Smith experienced two times higher firmness loss than that of the unripe Granny Smith after freezing at -80ºC **(Chassagne-Berces *et al.*, 2010).** However, the ripeness of pineapple at levels 2, 3, and 4 was insignificantly different in the structure degradation. Thus, a higher ripeness level, in further study, should be conducted to observe the noticeable change in the textural characteristic of frozen pineapple.

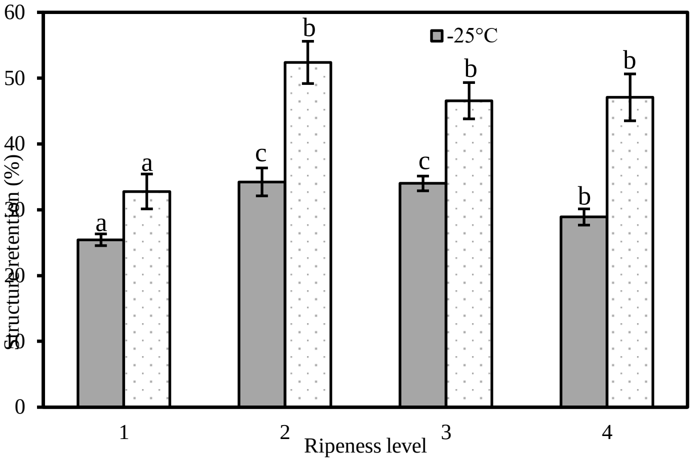

Figure 3 Effect of ripeness levels and freezing temperatures on structure retention of pineapple fruits. Superscripts (a, b, c) indicate significant differences ($p < 0.05$) in ripeness levels of each freezing temperature

Effect of ripeness levels and freezing temperatures on drip loss of frozen pineapple fruits

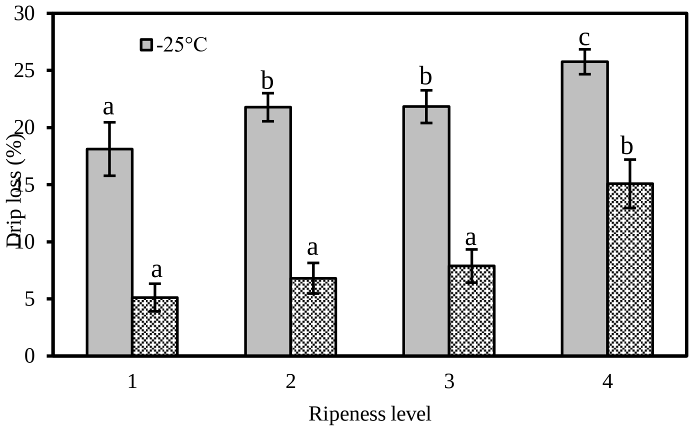

Figure 4 Effect of ripeness levels and freezing temperatures on drip loss of pineapple fruits. Superscripts (a, b, c) present significant differences ($p < 0.05$) in ripeness levels of each freezing temperature

In frozen fruit, drip loss after thawing is considered an essential variable affecting the quality of frozen fruit. Figure 4 depicts the variations in drip loss of frozen pineapple at different ripeness levels and subjected to different freezing temperatures. Generally, high drip loss was induced by a slow freezing rate of -25ºC freezing operation, whereas a fast-freezing rate from the -86ºC freezing system was found to alleviate the drip loss after thawing. The drip loss of pineapple fruits subjected to the -86°C freezing system was around 5-15%, whereas it reached greater than 25% when pineapples were stored in the -25°C freezing system. This was also attributed to the ice crystal sizes during freezing as above discussed. The formation of small ice crystals from the -86ºC freezing system was found to slightly affect the cell structure of plant tissue, leading to less leakage of solute from the interior to the exterior of the plant tissues **(Sirijariyawat & Charoenrein, 2012).** Drip loss from thawed fruit was also explained by the reduction in turgidity of fresh fruit as a result of an imbalance between continuous water loss by transpiration and less water uptake **(Bonat Celli *et al.*, 2016).** The drip loss of the fruits frozen at -20ºC and -50ºC was 2.6 ± 0.6% and 1.1 ± 0.4%, respectively, reported by **Carbonell *et al.* (2006)**. In terms of ripeness level, the riper stage of pineapple fruit induced more drip loss in pineapple fruits. Specifically, the -86ºC frozen pineapple at ripeness level 1 showed a drip loss of 5.12 ± 1.21%, whereas it increased to 15.08 ± 2.12% at the ripeness level 4. The drip loss of the thawed frozen Nam Dok Mai and Chok Anan mangoes was observed to increase with the increasing ripening stage **(Rimkeeree & Charoenrein, 2014).** This was explained that the fully ripe fruit had lower firmness than partially ripe fruit, contributing to the increase in the drip loss after thawing.

Effect of ripeness levels and freezing temperatures on vitamin C retention of frozen pineapple fruits

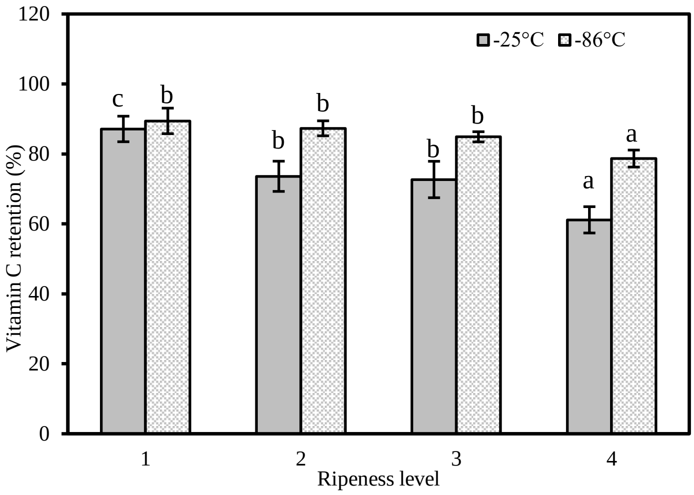

Figure 5 Effect of ripeness level and freezing temperature on vitamin C retention of pineapple fruits. Small letters (a, b, c) present significant differences ($p < 0.05$) in ripeness levels of each freezing temperature

Frozen-thawed pineapple fruits have been found to experience a noticeable amount of vitamin C. In this study, the effects of ripeness level and freezing temperature on the vitamin C retention of frozen-thawed pineapples are illustrated in Figure 5. Similarly, the fast-freezing rate of -86ºC freezing system was found to attenuate the vitamin C loss compared to that of -25ºC. Besides, increasing the ripeness level also contributed to a higher reduction in the percentage of vitamin C retention. Vitamin C loss was previously reported to be highly correlated to the drip loss of frozen-thawed fruit **(Bonat Celli *et al.*, 2016). Cano *et al.* (1993)** noted that the drip loss of frozen-thawed fruits facilitated the entrainment of vitamin C in which water played a role as a carrier. Thus, it is not considered a true degradation process of vitamin C during freezing**. Sahari *et al.* (2004)** reported that the least vitamin C loss (8.9%) was obtained when Iranian strawberries were subjected to the freezing system of -24ºC compared to that of -12ºC (64.5%). In this study, pineapples with the ripeness level 4 showed the lowest vitamin C retention (78.67 ± 2.43% at -86ºC and 61.15 ± 3.76% at -25ºC) compared to the others. This result confirmed the positive correlation between drip loss and vitamin C loss when pineapples at different ripeness levels were subjected to different freezing temperatures.

Effect of ripeness levels and freezing temperatures on the titratable acidity and total soluble solids of frozen pineapple fruits

The freezing process has been found to cause considerable impacts on the titratable acidity and TSS of frozen pineapple compared to the fresh one, shown in Table 1. In this study, different ripeness levels exhibited variations in the total acid and TSS content. The riper pineapple showed a higher TSS but lower total acid content which was due to the physicochemical changes during the ripening process **(Sivakumar *et al.*, 2011)**. In terms of titratable acidity, frozen pineapple at -25°C showed a significant reduction ($p < 0.05$) at the ripeness level 1 and 2, shown in Table 1. This was compatible with previous studies that the loss of organic acids was due to some diffusion of these acids into the surrounding medium when occurring drip loss after thawing **(Rincon & Kerr, 2010; Tovar *et al.*, 2001).** Normally, the freezing process was found to facilitate the leaking of soluble sugars in plant tissues due to the formation of ice crystals which disrupted cell walls and helped release soluble solids **(Chassagne-Berces *et al.*, 2010)**. In this study, the TSS of frozen-thawed pineapple, however, was insignificantly different in comparison with fresh pineapples ($p > 0.05$). The formation of ice crystals during freezing in these studied conditions was not adequate to cause the significant impacts to entrain the soluble solids from plant cells. It was also implied that the diffusion of water in plant cells was considerably faster than that of soluble solids such as sucrose **(Rincon & Kerr, 2010).** This might mitigate the influence of the freezing process on the TSS content.

Table 1 Effects of ripeness level and freezing temperature on titratable acidity and TSS of frozen pineapple

Ripeness level	Titratable acidity (%)			TSS (%)		
	Fresh pineapple	**Freezing at -25ºC**	**Freezing at -86ºC**	**Fresh pineapple**	**Freezing at -25ºC**	**Freezing at -86ºC**
1	0.72 ± 0.04^{aB}	0.54 ± 0.02^{bA}	0.62 ± 0.04^{bB}	13.50 ± 0.98^{aA}	12.60 ± 0.32^{aA}	14.00 ± 1.20^{aA}
2	0.64 ± 0.05^{aB}	0.46 ± 0.06^{aA}	0.58 ± 0.05^{bB}	15.71 ± 0.12^{bA}	14.20 ± 1.21^{aA}	16.18 ± 1.45^{abA}
3	0.55 ± 0.03^{bA}	0.51 ± 0.04^{aA}	0.51 ± 0.07^{bA}	16.99 ± 0.21^{cA}	16.60 ± 0.37^{bA}	17.00 ± 0.39^{bA}
4	0.49 ± 0.04^{bA}	0.45 ± 0.05^{aA}	0.46 ± 0.08^{bA}	18.31 ± 0.45^{dA}	18.06 ± 0.24^{cA}	18.72 ± 0.53^{cA}

Data were expressed as mean ± standard deviation. Superscripts (a, b, c) presented significant differences ($p < 0.05$) within the same column. Capital letters (A, B, C) indicates significant ($p < 0.05$) differences within the same row among analyzed group of titratable acidity and TSS.

CONCLUSION

In this study, the effects of freezing temperature and ripeness level on the frozen pineapple characteristic were successfully evaluated. Freezing pineapple at -86ºC was found to promote a faster freezing rate and shorten the freezing time compared to that at -25ºC. The freezing system at -86ºC showed better performance in the retention of textural characteristics and vitamin C while attenuating the drip loss of frozen-thawed pineapples. The ripeness level also caused significant impacts on the quality of frozen pineapples. More ripening pineapple was observed with the increase in freezing time, drip loss, and vitamin C loss, whereas the structure was better maintained as compared to less mature pineapple. The titratable acidity and TSS, in this study, were less affected by freezing temperature and ripeness level. In summary, the freezing system at -86ºC was a considerably suitable approach to preserve the good quality of frozen pineapple. However, the implementation and maintenance costs, which should be taken into consideration, must be very high to ensure effective operation.

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