Research Article

Effect of Exogenous Methyl Jasmonate on Chilling Injury and Quality of Pineapple (*Ananas comosus* L.) cv. Pattavia

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Abstract

This experiment was carried out to determine the effect of methyl jasmonate (MeJA) on chilling injuries and quality of pulp in pineapple (*Ananas comosus* L. cv. Pattavia). The effect of exogenous MeJA on quality and storage life of pineapple cv. Pattaviawas was investigated. Pineapples were treated with 0 (control), 10^{-3}, 10^{-4} and 10^{-5}M MeJA and then stored at 10 °C, 85% relative humidity. Pineapple treated with MeJA significantly reduced chilling injuries and percentage of fruit weight loss compared to the control treatment. Percentage of weight loss of pineapple non-treated with MeJA was ranged from 13.73-19.64%, while that of treated pineapple ranged from 3.81-11.99%. After storage for 21 days, the browning symptoms associated with chilling injuries were observed in the pulp adjacent to the core of the pineapple fruit. Chilling injuries were found to be associated with electrolyte leakage, chilling injury index and total phenolic content, which had increased during storage. The value of electrolyte leakage, chilling injury index and total phenolic content from pulp tissue treated with MeJA was lower than with non-treated fruits. Total soluble solids (TSS), titratable acidity (TA), total sugars and reducing sugars of treated pineapple were not significantly different from that of the control pineapple.

Key Words: Pineapple; Methyl jasmonate; Postharvest quality; Chilling injury; Phenolic content

Introduction

Chilling injuries (CI) impose a limitation on extended storage for many horticultural crops, especially those of tropical or subtropical origin. One of the most significant reasons for postharvest loss is due to pineapple susceptibility to chilling injury when stored below 13 °C (Dull, 1971). Chilling induced symptoms were observed in the pulp adjacent to the core, although the critical temperature at which chilling injury symptoms occurred varied, depending upon the type of produce. Chilling injury in tropical fruits is characterized by browning of the skin, greater firmness in texture, and off-flavor in the fruit. The temperature that induces chilling injury differs with fruit species, and the skin is generally more sensitive to low temperatures than the fruit flesh (Mitra, 1997). It was observed that post harvest life of the pineapple is limited due to its susceptibility to chilling temperatures. It is assumed that the browning symptom often appears around the vascular strands in fruits, probably as a result of the action of the enzyme polyphenol oxidase. This enzyme may cause the leakage of phenolic compound from the vacuole after chilling.

Jasmonic acid (JA) and its volatiles derivative methyl jasmonate (MeJA), are collectively called jasmonates (Mueller et al., 1993; Creelman and Mullct, 1997). Jasmonates are fatty acid derivative with a 12-carbon backbone that plays a role in plant development and plant defense against pests. Jasmonate main activities as plant growth regulators includes inhibition of seed germination and callus growth and promotion of' leaf and fruit senescence, root forming and petiole abscission. Moreover, Jasmonates are plant stress hormones that play a prominent role in signaling plant defense (Creelman and Mullet, 1997). JA and MeJA were shown to induce increased production of the secondary metabolites. The level of JA in plants varies as a function of tissue and cell and development stage (Gonzalez-Aguilar et al. 2000).

Recently, it has been observed that MeJA treatment could be used to reduce deterioration and the development of chilling injury symptoms of zucchini, mango, avocado, and papaya fruit (Wang and Buta, 1994; Meir et al., 1998; Gonzalez-Aguilar et al., 2000; Gonzalez-Aguilar et al., 2003). It was reported that MeJA can also be applied, as a post-harvest treatment, to suppress grey mould rot diseases caused by *Botrytis cinerea*, *Penicillium digitatum*, and *Alternaria alternata* in strawberries, grapefruit, and papaya fruit, respectively (Wang and Buta, 1994; Meir et al., 1998; Gonzalez-Aguilar et al., 2000; Gonzalez-Aguilar et al., 2003; Moline et al., 1997; Droby et al., 1999) It has also been observed that MeJA treatment maintains higher organic acid in radishes and zucchini when stored at 5 °C and improves skin color of mangos during storage at 20°C (Wang, 1998; Gonzalez-Aguilar et al., 2001). However, the study on the effect of MeJA treatment on the quality of pineapple has not been investigated.

This study investigated the effect of exogenous MeJA on browning symptoms and quality of pineapple cv. Pattavia, a popular pineapple cultivar in Thailand, to prolong and maintain the fruit's quality during storage.

Material and methods

Plant material

Pineapples cv. Pattavia were harvested at commercial maturity from the plantation in Phetchaburi province. They were immediately transported to the laboratory of the Faculty of Animal Sciences and Agricultural Technology at Silpakorn University by truck. In the laboratory, the uniformity of the fruit was determined to select the fruits which had the same size and color. The fruits were then dipped in 200 ppm hydrochloride solution for 3 minutes to control fruit rot disease. The fruits were then air-dried at ambient temperature. The fruits were dipped with 10^{-3}, 10^{-4} and 10^{-5}M methyl jasmonate solution for 5 minutes and then stored at 10 °C and 85% relative humidity. The fruits were randomly chosen from each treatment at 7 days interval to determine the change of pineapple fruit quality.

Weight loss

The harvested pineapple fruits were immediately weighed before storage. Fruits were weight at room temperature (25 °C) and at 7 day intervals. Weight loss of each sample was calculated as a percentage with respect to the initial weight.

Chilling injury Index

The severity of chilling injuries was evaluated after pineapples were transferred from the cold room to room temperature. The degree of chilling injury was measured by the extent of surface browning of the pulp. They were rated on a scale from 1 to 5, based on the intensity of surface browning; Score 1 = no chilling injury symptoms; Score 2 = browning symptoms covered 1-25% of the surface area; Score 3 = browning symptoms covered 26-50% of the surface area; Score 4 = browning symptom covered 51-75% surface area; Score 5 = browning symptoms covered 76-100% of the surface area.

Electrolyte leakage

Electrolyte leakage was determined based on the method of McCollum and McDonald (1991). Twelve disks, approximately 3 grams from pulp, were excised with a cock bored and then rinsed with 0.4 M mannital. These tissues were then placed in 50 ml of 0.4 M mannital, and incubated at 25 ˚C for 3 hours (data A). The Electrical conductivity of the solution was measured at room temperature using a conductivity meter (Model EcoScan CON6, EUTECH). Total electrolyte was determined on the same samples after they were autoclaved for 30 min at 121 ˚C and cooled to room temperature (data B). Leakage data was expressed as a percentage of total electrolyte reading.

Total soluble solids and titratable acidity

The determination of titratable acidity (TA) and total soluble solid (TSS) was carried out using the method of A.O.A.C (1990). TSS was measured by using a hand refractometer. TA was determined by titrating the pulp extract with 0.1N sodium hydroxide (NaOH) using phenolphthalein as an indicator and expressed as percent citric acid.

Total sugars and reducing sugars

Determination of total sugars and reducing sugars extracted one gram sample of pulp with 20 ml of 50% ethanol and then incubated in the oven at 60 ˚C for 2 hours. One ml of the supernatant liquid was mixed with 0.5 ml of 0.1N hydrochloric acid and boiled for 15 min. This mixture was then mixed with 0.5 ml of 0.1N sodium hydroxide. One ml of the supernatant liquid was then taken for quantifying the total sugar using Somogy-Nelson's method (Hodge and Hofreiter, 1962) and reducing sugar using the method of Plews (1970).

Total phenolic content

Total phenolic content was determined using the method of Ketsa and Atantee (1998). Five grams of pulp were homogenized with 12 mL of 80% ethanol for 1 minute. The homogenized mixture was later centrifuged at 4,400 x g for 20 min. One ml of the supernatant liquid was mixed with 8 mL of 10% Folin-Ciocaltea reagent and 10 mL of 7.5% sodium carbonate, and then allowed to settle for 2 hours. The absorbance of the sample solution was measured with a spectrophotometer (Model Libra S22, Biochrom) at 765 nm. A standard curve of gallic acid was used for quantifying the total phenolic content.

Statistical analysis

Completely randomized design (CRD) was used throughout the whole experiment with three replications. All the data was analyzed with one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests. Differences at $p = 0.05$ were considered as statistically significant.

Results

Weight loss

Weight loss of pineapple increased during storage of the fruits at 10 ˚C (Figure 1). Weight loss is a parameter which can be used to evaluate the quality of fruit. The loss of weight for each treatments of pineapple treated with MeJA was slightly lower than that of the non-treated (control), with a statistical difference between the treated and non-treated. But this, however, had no statistically significant difference among the treatments of MeJA.

It was found that during storage, weight loss was drastically increased after 7 days of storage. The weight loss was later remained at the same rate during storage. Percentage of weight loss of pineapple not treated with MeJA was between 13.73-19.64%, while pineapple treated with MeJA was between 3.81-11.99%.

Chilling injury development and phenolic content

Chilling injury (CI) index and electrolyte leakage of the pulp in pineapple increased during storage. Browning symptoms associated with CI were observed in the pulp adjacent to the core. Chilling injury index and changes in the electrolyte leakage of the pulp are

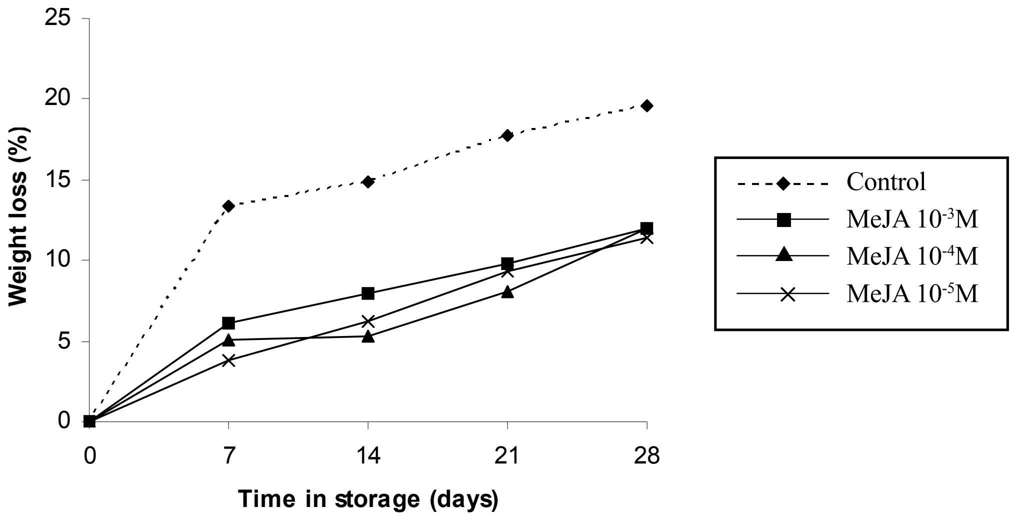

Figure 1 Weight loss in pineapple treated with 0 (control), 10^{-5}, 10^{-4} and 10^{-3} M MeJA and then stored at 10 ˚C. Each data point represents the mean of three replications.

presented in Figures 2A and 2B, respectively. After 21 days of storage at 10 ˚C, the pulp treated and non-treated showed browning symptom, but it appeared lower (25% of surface area) for those with the MeJA treatment. CI index of pineapple treated with 10^{-3}, 10^{-4} and 10^{-5}M MeJA was lower than that of the non-treated, and this was statistically significant at 21 and 28 days of storage. At 21 and 28 days of storage, the level of chilling injury index of pulp in pineapple was not higher than 25% of the surface area. However, a pineapple treated with 10^{-5}M MeJA had slightly more severe browning symptom than the other treatments. MeJA treatment resulted in a reduction of CI symptom.

Electrolyte leakage was significantly higher in non-treated than in the treated pineapple. Changes in the electrolyte leakage of pulp were used to assess the development of chilling injuries. At 21 days of storage, electrolyte leakage of pineapple non-treated with MeJA had increased to 90.91%, while pineapple treated with 10^{-3}, 10^{-4} and 10^{-5} M MeJA had lower electrolyte leakage at 72.61%, 73.23%, 74.59%, respectively.

Changes in the total phenolic content of pulp were used to assess the development of browning symptoms associated with CI. Total phenolic content increased with the duration of storage in all treatment (Figures 3). The total phenolics content of both non-treated and treated pineapple increased during storage at 10 ˚C. However, total phenolic content of pineapple treating with MeJA was lower than that of the non-treated fruits with statistical significance at 21 and 28 days of storage. However, there was no statistical difference among treatments with various levels of MeJA.

Change of total soluble solids and titratable acidity

Total soluble solids (TSS) and titratable acidity (TA) were analyzed and the result is shown in Figures 4A and 4B. There was no statistical difference of the level of TSS of pineapple treated with 0, 10^{-3}, 10^{-4} and 10^{-5} M of MeJA. During storage, the value of TSS varied between 13.5-15 °Brix. Initially, the level of TSS had increased but it had declined afterwards. The changes in TA in pineapple prior and after storage were similar to those of TSS. The level of TA was about 0.42-0.90%. There was a slight difference in the level of TA in pineapple treated with MeJA and those non-treated. However, there was no statistically significant difference among these treatments.

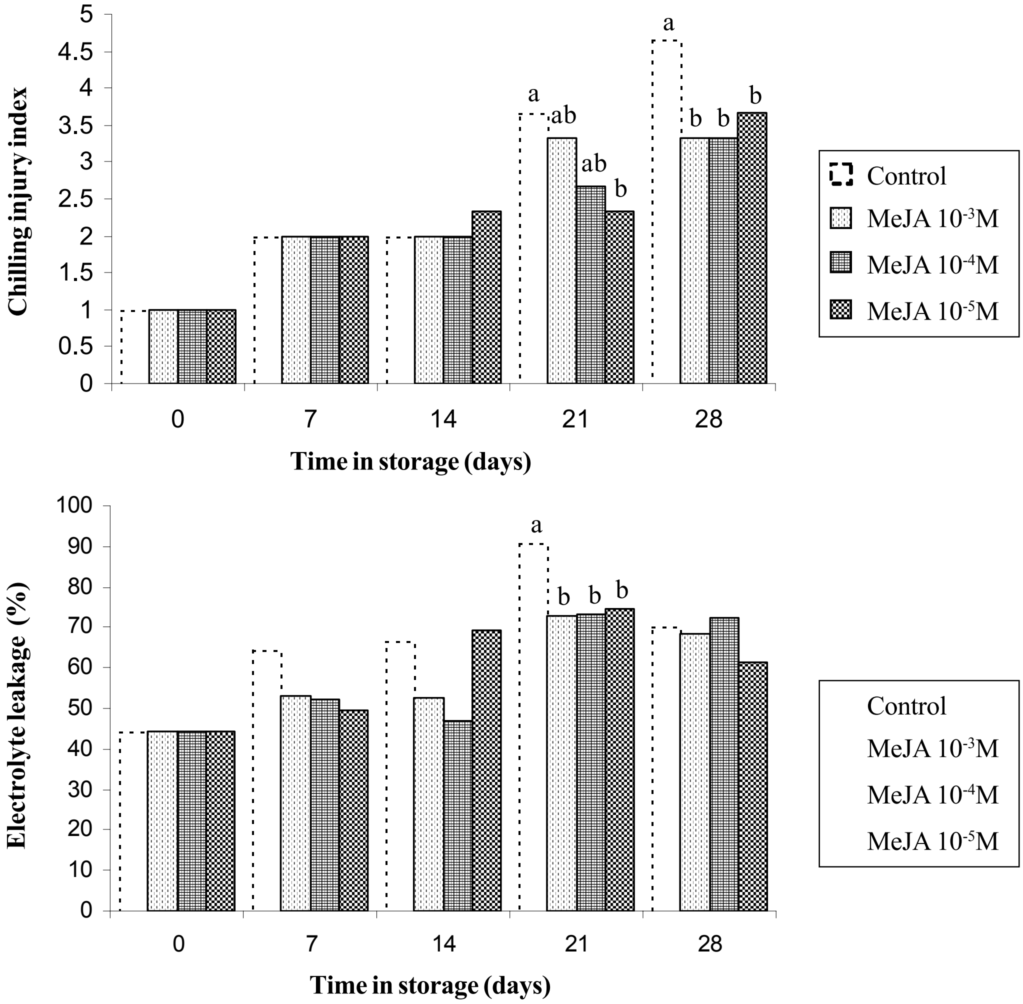

Figure 2 Chilling injury index and percentage of electrolyte leakage in pulp of pineapple treated with 0 (control), 10^{-5}, 10^{-4} and 10^{-3} M MeJA and then stored at 10 °C. A: Chilling injury index; B: Percentage of electrolyte leakage of pulp. Each data point represents the mean of three replications.

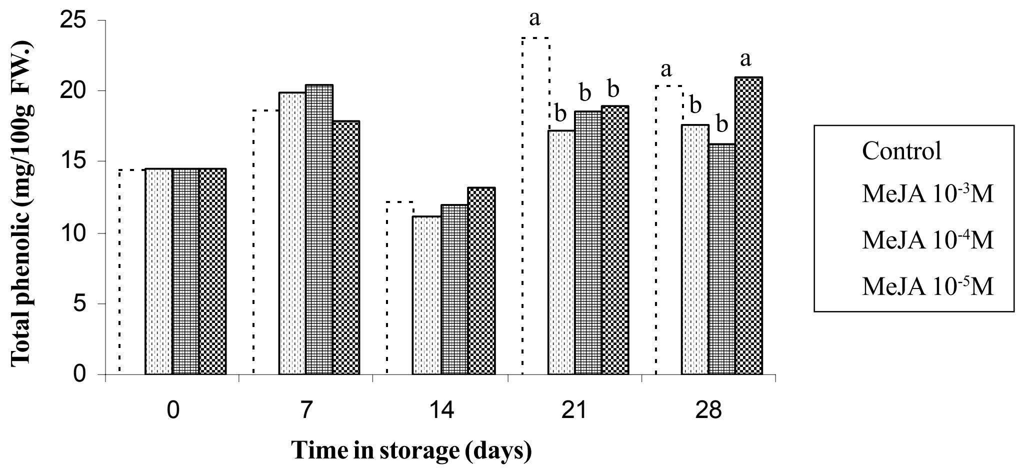

Figure 3 Total phenolic content in pulp of pineapple treated with 0 (control), 10^{-5}, 10^{-4} and 10^{-3} M MeJA and then stored at 10 °C. Each data point represents the mean of three replications.

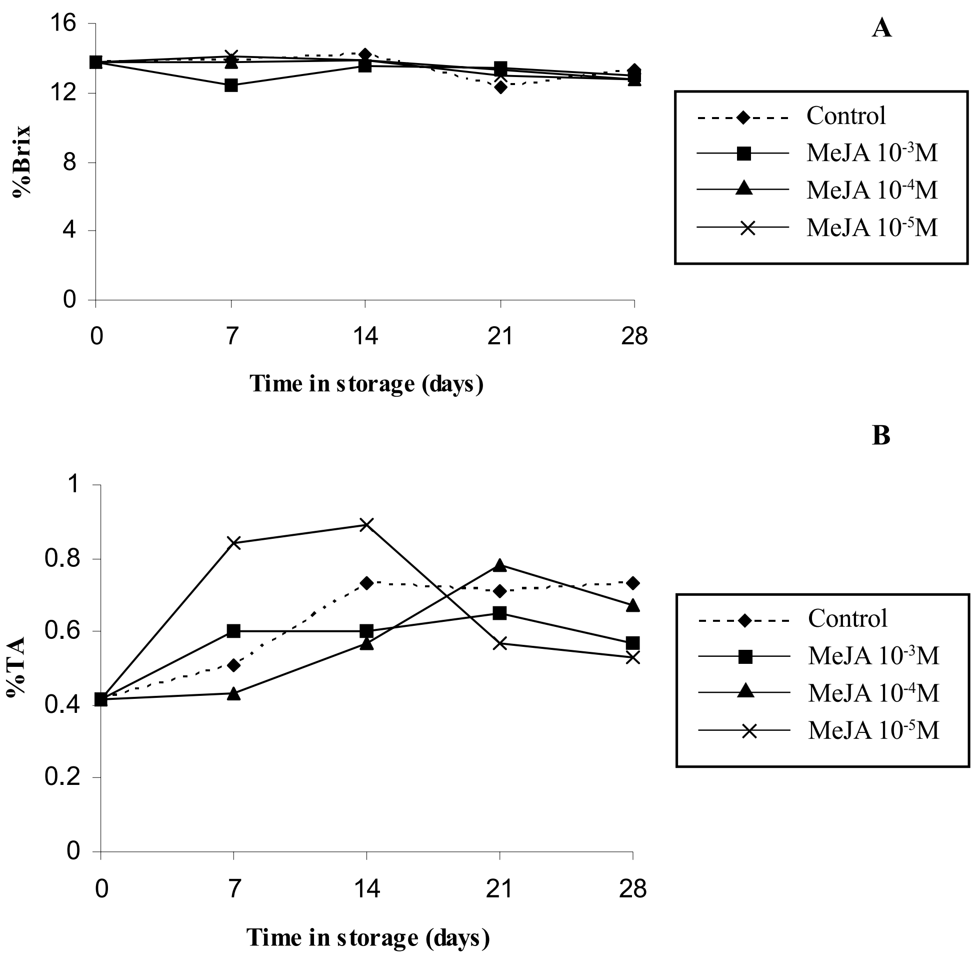

Figure 4 Total soluble solid and titratable acidity in pulp of pineapple treated with 0 (control), 10^{-5}, 10^{-4} and 10^{-3} M MeJA and then stored at 10 °C. A: Percentage of Brix; B: Percentage of titratable acidity. Each data point represents the mean of three replications.

Change of total sugar and reducing sugar

Change of total sugars and reducing sugars was analyzed and the result is shown in Figures 5A and 5B. Total sugars and reducing sugars of both non-treated and treated pineapple increased during storage. There was no statistical difference on the level of total sugars and reducing sugars of pineapple treating with 0, 10^{-3}, 10^{-4} and 10^{-5} M MeJA. The level of total sugar had increased during storage. During storage, the level of total sugars was about 4.72-10.58 mg/g FW and reducing sugars was about 1.61-6.25 mg/g FW. At 28 days of storage, pineapple had maximum total sugars and reducing sugars.

Discussion

Chilling injury (CI) occurs when tropical or sub-tropical fruits have been exposed to low temperature for a certain period of time. In this study, we investigated the effect of methyl jasmonate (MeJA) in preventing chilling injuries and improving the quality of pulp in pineapples. It was reported that in pineapple, CI symptom started developing and could be detected when the fruit was stored at lower than 13 °C (Dull, 1971). The abnormality was caused by physiological changes in cell membrane where the liquid-crystalline state had been changed to solid gel state. The rigidity of the cell membrane accelerated

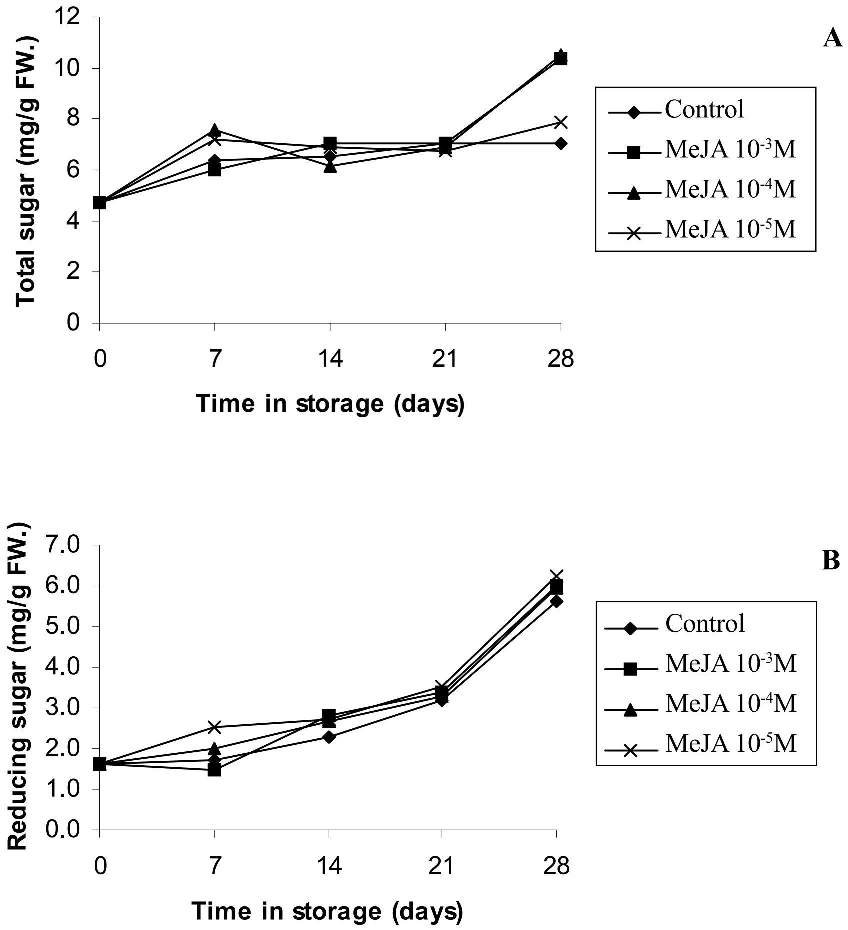

Figure 5 Total sugar and reducing sugar in pulp of pineapple treated with 0 (control), 10^{-5}, 10^{-4} and 10^{-3} M MeJA and then stored at 10 °C. A: Total sugar; B: Reducing sugar. Each data point represents the mean of three replications experiments.

the leakage of cell content and caused electrolyte leakage from the cell (Lyons, 1973; Paull, 1994), causing of weight loss and browning in pineapples. Permeability can be assessed by measuring the rate of leakage of these solutes which have passed through the membrane. (Wang, 1990)

In the present study, CI symptoms in pineapple were detected after storage of the fruits for 21 days. The symptom development was followed by measuring the CI index and calculating the electrolyte leakage during stored of pineapple fruits. Nukuntornprakit (2004) used both parameters to study the effect of low temperature on CI in pineapples and found that pineapples (cv. Pattavia) was resistant to CI symptoms development when the fruits were stored at 10 °C for 3 weeks. After treating the pineapple fruits with MeJA at the concentration of 10^{-3}, 10^{-4}, and 10^{-5} M, it was found that the value of CI index and electrolyte leakage was reduced, with statistical differences from those of the non-treated control when the fruits were assessed at 21 and 28 days after storage. This demonstrated that treating the pineapple fruits with MeJA effectively reduced CI symptom in pineapple. As this research is a preliminary study, the pineapple treated with MeJA still had CI symptoms, which may not be acceptable to the consumer although the CI symptoms have been reduced as a result of the MeJA treatment. More study should be carried out to find optimal MeJA concentrations which can reduce CI symptoms acceptable to the market.

CI was visually detected when the browning symptom occurred at the core of the pineapple fruits during storage. This browning discoloration occurred as a result of physiological disorder, possibly from the accumulation of phenolic compounds and polyphenol oxidase in the fruit (Paull and Rohrbach, 1985). In this study, total phenolic content had increased during storage and this occurred simultaneously with the appearance of the browning symptom in the fruit. Pineapple fruits treated with MeJA had lower total phenolic content than those of the non-treated control, with statistical differences when the fruits were assessed at 21 days of storage. This may indicate that MeJA may play a role in retarding the development of browning symptom in pineapple. Vamos-Vigyazo (1981) and Walker and Ferrar (1998) reported that polyphenol oxidase had played a role in catalyzing the discoloration process, with the browning pigment as the oxidation of phenolic substrates by polyphenol oxidase is thought to be the major cause of the brown discoloration of many fruits and vegetables during handling, storage and processing.

Quality of pineapple fruit during storage can be assessed by measuring weight loss. The result showed that weight loss of the pineapple fruits during storage had escalated. This may be a result of stress initiated from low temperature which in turn activates the respiration of the fruit. In this study, pineapple treated with MeJA had a lower weight loss than the non-treated control, with statistical differences. However, it was found that the concentrations of the MeJA had no effect on weight loss of the pineapple fruits. This was possibly because MeJA treatment had reduced the severity of CI symptom. The CI symptom is an indicator which reflects the malfunction of the membrane permeability. This membrane malfunction has caused the leakage of water and electrolytes from the cell. The end result is the loss of weight in the pineapple fruits.

During storage, the value of TSS ranged from 13.5-15 °Brix and the value of TA varied between 0.42-0.90 % of citric acid, regardless of whether the pineapple fruits were treated with MeJA or not. Marrero and Kader (2006) reported that the consumer was prepared to accept the pineapple with the lowest value of TSS at 12 °Brix and the highest value of TA at 1%. In this study, the value of TSS has not fluctuated. This is because the pineapple is a non climacteric fruit which can be harvested when they are demanded for consumption. Nonetheless, the value of TA, which initially had increased, declined during storage. The reduction of the level of acidity in the pineapple fruits is possibly due to the utilization of malic acid and citric acid as a substrate during the respiration process (Dull, 1971).

In this study, the total sugars and reducing sugars had increased. This is possibly due to the fact that most fruits have stored the energy in the form of sucrose, the non-reducing sugars. Subsequently, this sucrose was transformed to glucose and fructose. This observation was also consolidated by Bantaotook (2002) who had conducted the study of pineapple quality using high performance liquid chromatography (HPLC) techniques.

Conclusion

Pineapple stored at low temperature had developed CI symptoms at 21 days of storage as shown by CI index, electrolyte leakage and total phenolic content. It was found that MeJA played a role to reducing CI symptoms of pulp tissue. Treating pineapple with MeJA was found to result in lower weight loss; one of the parameter of pineapple fruit's quality. MeJA had no effect on TSS, TA, reducing sugar and total sugar. MeJA reduced the CI symptoms and maintained quality of pineapple fruits during storage at low tempurature.

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