Effect of Exogenous Methyl Jasmonate on the Internal Browning of Pineapple Fruit (*Ananas comosus* L.) cv. Pattavia

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Abstract

Internal browning in pineapples (*Ananas comosus* L.) cv. Pattavia was studied in non-treated (control) fruit and fruit treated with 0.01, 0.1 and 1 mM methyl jasmonate (MeJA) after being stored at 10°C and 85% relative humidity (RH). The parameters, such as browning index, phenolic content, polyphenol oxidase (PPO) and peroxidase (POD) activity, were assessed. The browning symptom was initially mild, but rapidly increased at 21 days of storage which could be observed with the naked eye. Non-treated (control) pineapple had a browning index that was above 50% of the surface area while those treated with MeJA showed delayed browning of pulp in pineapple and had less damage. The phenolic content, PPO and POD activity were initially low, but steadily increased until 28 days of storage. The pineapple fruit treated with 0.1mM MeJA had the lowest PPO and POD activity as compared with non-treated fruit and those treated with 0.01 and 1 mM MeJA. The result showed significantly that MeJA treatments were able to delay internal browning. The accumulation of phenolic content, PPO and POD activity corresponded to changes in the colour of the pulp in pineapples. Therefore, MeJA can play a role in the reduction of internal browning in pineapples.

Keywords: Methyl jasmonate, Polyphenol oxidase and Peroxidase

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Introduction

Pineapple (*Ananas comosus* L.) is a tropical fruit of high commercial value in the fruit market. Pineapple has symptoms of internal browning which is induced by exposure to low temperature. Browning symptoms are a physiological disorder which is characteristic of symptoms which occur at the core of the pineapple fruits during storage (Paull and Rohrbach, 1985). The development of internal browning of fresh pineapple subsequent to cold storage imposes a severe limitation in the marketing of fresh fruit. The manifestation of this condition is a result of chilling injury of the tissue during cold storage. The development of effective methods to alleviate browning has been widely reported, and these include heat treatment, waxing, atmosphere control and application of 1-methylcyclopropene (Rohrbach and Paull, 1982; Selvarajah and Herath, 1997; Selvarajah et al., 2001). They have been tested as alternatives to prevent internal browning without success.

The biochemical pathway of browning development during low temperature storage of pineapple fruits has not been clearly documented. In other species, similar internal damage has been widely linked to stress-induced polyphenoloxidase (PPO, EC 1.10.3.2 or 1.14.18.1) and peroxidase (POD, EC. 1.11.1.7) (Mayer, 1987; Teisson, 1972). PPO are a group of copper-containing enzymes (Robb, 1984) catalyzing oxidation of polyphenolic compounds in the presence of molecular oxygen which are responsible for enzymatic browning reactions occurring during harvesting, handling, processing and storage of many fruits (Sheptovitsky and Brudwig, 1996). PPO is present in a latent form in many plant species, and is tightly bound to the chloroplast membrane. The latent form of PPO is often activated during ripening, senescence or stress conditions when the membrane is damaged, which results in an increase of PPO activity (Mayer, 1987). Similarly, the relationship between POD and browning of fruits and vegetable has been widely reported.

Jasmonic acid (JA) and its volatile derivative methyl jasmonate (MeJA), are collectively called jasmonates (Mueller et al., 1993; Creelman and Mullet, 1997). Jasmonates are fatty acid derivatives with a 12-carbon backbone that plays a role in plant development and plant defense against pests. The main activity of Jasmonates as plant growth regulators include 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 Mullct, 1997). JA and MeJA were shown to induce increasing secondary metabolite production. In addition to a role in plant defense, JA also appears to play a role in plant development. The level of JA in plants varies as a function of tissue, cell and development stage (Gonzalez-Aguilar et al., 2000). Recently, it has been observed that MeJA treatment can be used to reduce deterioration and the development of chilling injury symptoms of zucchinis, mangos, avocados, and papaya fruit (Wang and Buta, 1994; Meir et al., 1998; Gonzalez-Aguilar et al., 2000; Gonzalez-Aguilar et al., 2003). It has also been observed that MeJA treatment maintains the skin color of mangos during storage at 20 °C (Gonzalez-Aguilar et al., 2001). The objective of the present work was to study the effect of MeJA levels on some characteristics of internal browning of fresh pineapple (*Ananas comosus* L.).

This study investigated the effect of exogenous MeJA on browning symptoms by measurement of the change of phenolic compound, polyphenol oxidase and peroxidase with interval to

determine the change in fruit quality by browning development during low temperature storage of pineapple.

Material and methods

1. Plant material

Pineapples (cv. Pattavia) were harvested at commercial maturity from a plantation in Phetchaburi province. They were immediately transported to the laboratory of the Faculty of Animal Science and Agricultural Technology, Silpakorn University. In the laboratory, the uniformity of the fruit was tested to select fruits which had the same size and color. The fruits were then dipped in 200 ppm Hydrochloride solution for 3 minutes to suppress fruit rot disease. The fruits were air-dried at ambient temperature. The fruits were then treated with 0.01, 0.1 and 1 mM 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 day intervals to determine the change on the internal browning.

2. Browning index

The severity of browning symptoms was evaluated after the fruits were transferred from the cold room to room temperature. The degree of browning was measured by the extent of surface browning of the pulp. They were scored from 1 to 5, based on the intensity of surface browning i.e. Score 1 = no chilling injury symptoms; Score 2 = browning symptoms cover 1-25% of surface area; Score 3 = browning symptoms cover 26-50% of surface area; Score 4 = browning symptoms cover 51-75% of surface area and Score 5 = browning symptoms cover 76-100% of surface area.

3. 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 rpm 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.

4. Polyphenol oxidase and peroxidase extraction and enzyme activity

PPO and POD extraction was carried out at 4°C and the activity was determined using the modified method from De Oliveira Lima et al. (1999). One gram of pulp was extracted into 10 ml of 0.05M sodium phosphate buffer pH 6.2 and centrifuged at 4,400 rpm for 60 min. The supernatant was used for PPO and POD activity

PPO and POD activity were modified from the method of Flurkey and Jen (1978). The PPO activity was determined in a reaction mixture consisting of 0.25 ml of enzyme extract, 2 ml of 0.2M sodium phosphate buffer pH 6.5 and 0.25 ml of 0.25M catechol and the absorbance was measured with a spectrophotometer (Model Libra S22, Biochrom) at 420 nm every 30 seconds for 3 min. The POD activity was determined in a reaction mixture consisting of 0.1 ml of enzyme extract, 2.4 ml of 0.2M sodium acetate buffer pH 6.0 which contained 0.1% hydrogen peroxide and 0.5% Guaiacol and then the absorbance was measured with a spectrophotometer at 470 nm every 30 seconds for 5 min. The enzyme activity was defined as the change in absorbance per milligram of protein per minute.

Protein content was measured using the method described by Bradford (1976). One ml extracted enzyme (PPO or POD) was added with 4 ml Coomassie brilliant blue G-250. The samples were measured with a spectrophotometer at 595 nm and protein concentration was determined based on a standard curve of bovine serum albumin.

5. Statistical analysis

The completely randomized design (CRD) was used throughout the whole experiment with three replications. All statistical analysis was performed with SAS. 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

1. Development of tissue browning

The internal browning symptom occurred at the core of the pineapple fruits during storage. The non-treated pineapple (control) and MeJA-treated pineapple showed internal browning discoloration after 7 days of storage. This internal browning symptom was initially mild, but rapidly increased at 21 days of storage. Browning symptoms in pineapple treated with MeJA were lower than the control. At 21 days of storage, there was a significant difference in the browning index between MeJA treatments and control, but no significant difference among those treated with different concentration of MeJA. At 28 days, internal browning symptoms in the control pineapple appeared on over 50% of the surface area, which could be observed with the naked eye. At 21 and 28 days of storage, the level of browning index of pulp in pineapple treated with MeJA was not above 25% of the surface area. The control pineapple showed a significantly higher level of pulp browning ($P<0.05$) than that treated with MeJA. Those treated with 0.1 mM MeJA show less discoloration than other treatments through the storage (Figure 1). Thus MeJA resulted in a reduction of internal browning symptom in the pulp of pineapple.

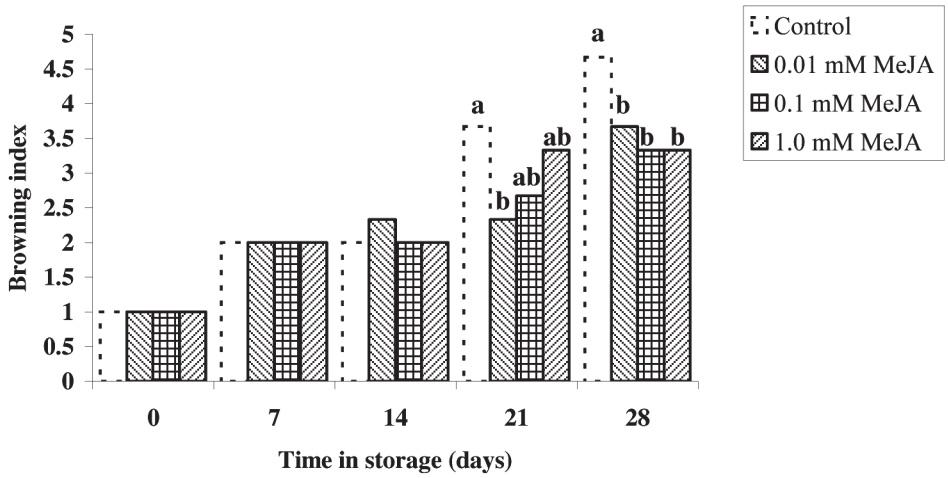

Figure 1. The browning index of pineapple pulp treated with 0 (control), 0.01, 0.1 and 1.0 mM MeJA and then stored at 10°C and 85% relative humidity. Mean separation is shown on each column by F-test at $p \leq 0.05$.

2. Total phenolic content and development of PPO and POD activity

Changes in the total phenolic content of pulp were used to relate the development of internal browning symptoms associated with chilling injury. The total phenolic content of both treated and non-treated pineapple fruits increased during storage at 10°C. During 21 day of storage, the total phenolic level in the pulp of non-treated pineapple fruits increased more rapidly than that in the MeJA-treated ones, and after that it steadily decreased.

At 21 and 28 days of storage, total phenolic content of all MeJA-treated groups (except for the group treated with 0.01 mM MeJA at 28 days) was significantly lower than the non-treated fruits. However, there was no statistical difference among pineapples treated with various levels of MeJA (Figure 2). The total phenolic content correlated with the degree of internal browning and electrolyte leakage in the pulp of the pineapple.

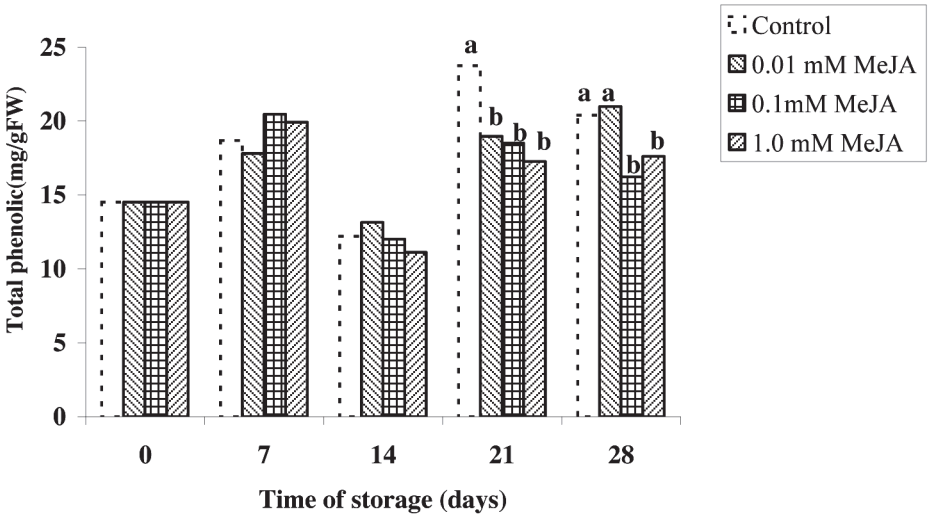

Figure 2. Total phenolic content of pineapple pulp treated with 0 (control), 0.01, 0.1 and 1.0 mM MeJA and then stored at 10°C and 85% relative humidity. Mean separation is shown on each column by F-test at $p \leq 0.05$.

Changes in PPO and POD activity during storage of fruit pulp dipped with MeJA 0.01, 0.1 and 1.0 mM MeJA and the control storage are shown in Figures 3 and 4, respectively. PPO and POD activity in the pulp of the non-treated pineapple stored at 10 ?C increased and rapidly increased at 21 days until the last day of the storage. At 21 days, the pineapples treated with 0.1 and 1.0 mM MeJA showed significantly lower activity of PPO compared to non-treated fruits. However, those treated with 0.01 mM MeJA showed higher PPO activity than the non-treated fruits. At 28 days, only those treated with 0.01 mM MeJA showed lower PPO activity compared with the control fruits.

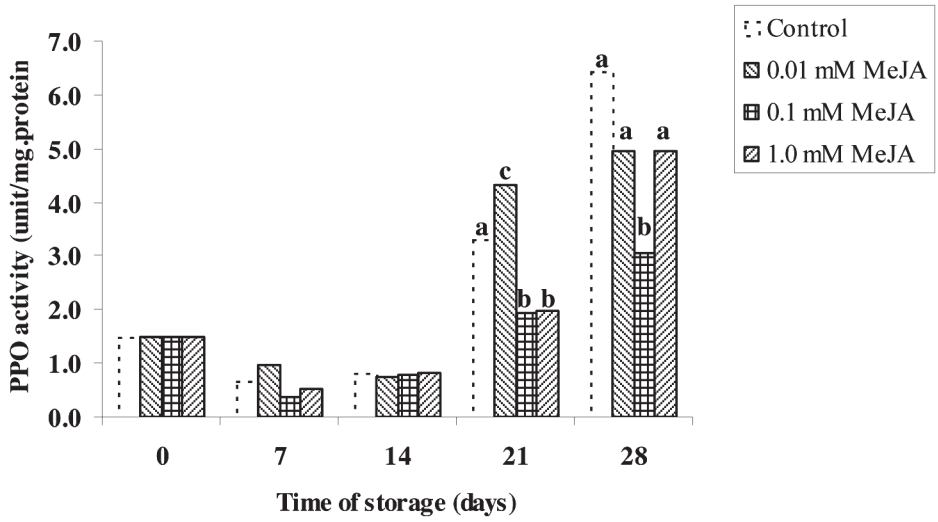

Figure 3. Changes of polyphenol oxidase activity of pineapple pulp treated with 0 (control), 0.1, 0.01 and 1 mM MeJA and then stored at 10°C and 85% relative humidity. Mean separation is shown on each column by F-test at p≤0.05.

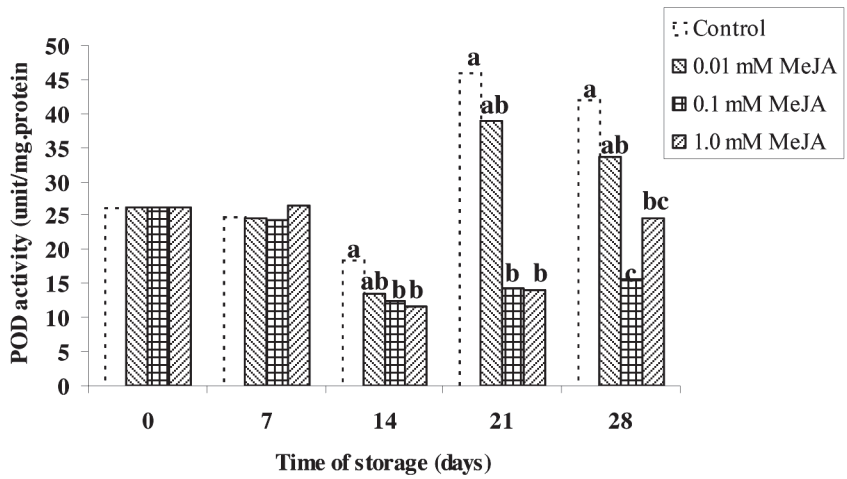

Figure 4. Changes of peroxidase activity of pineapple pulp treated with 0 (control), 0.1, 0.01 and 1 mM MeJA and then stored at 10°C and 85% relative humidity. Mean separation is shown on each column by F-test at p≤0.05.

POD activity of fruits, similarly to PPO activity, also increased during storage. At 7 days, there was no significant difference in POD activity between MeJA treatment and control. POD activity of both the control and the MeJA-treated group decreased slowly and then rapidly increased at 21 days of storage. At 21 and 28 days, POD activity of pineapples treated with various concentrations of MeJA was significantly lower than the control. At 14 and 21 days, there was no difference statistically between fruits treated with 0.1 and 1 mM MeJA, but the difference was clearly observed after 28 days. The pineapple fruits treated with 0.1 mM MeJA showed the lowest activity of POD after 28 days of storage. Therefore, MeJA treatments delayed PPO and POD activity in pineapple when compared to control.

Discussion

The internal browning symptoms occur at the core of pineapple fruits during storage as a result of stress from storage at low temperature, which causes a symptom called "chilling injury". Most internal browning symptoms were found to develop after 3 weeks of storage at 0-13°C, which was consistent with previous reports (Dull, 1971; Paull and Rorhbach, 1985; Smith, 1983).

Fruits with chilling injuries had an abnormality in the cell membrane. This abnormality occurred when the liquid-crystalline state had been changed to a solid gel state. The rigidity of the cell membrane had accelerated the leakage of the cell content and caused the cell damage (Lyons, 1973; Paull, 1994), which was a consequence of the accumulation of phenolic compound, polyphenol oxidase (PPO), and peroxidase (POD) in the cell (Paull and Rohrbach, 1985).

In this study, the change of pulp color was used to assess the development of browning injury by observing the value of browning index, phenolic compound, polyphenol oxidase (PPO) and peroxidase (POD). Pineapple treated with 0.1 mM MeJA had the lowest browning index as compared to non-treated fruits. Gonzalez-Aguilar et al. (2001) reported that MeJA could reduce CI and enhance color development of 'Kent' mangoes.

The loss of electrolytes from cells causes damage to the cell membrane. The exposed PPO, POD and phenolic substrate then reacts with O2 and results in the polymerization of the polyphenols to form browning pigments (Sveine et al., 1967). It is thus possible that MeJA at the optimal concentration may play a role in inhibiting either PPO, POD or both enzymes, resulting in the reduction in internal browning and enzymatic browning. In the previous study, it was found that treatments with MeJA reduced the increase of peroxidase (EC 1.11.1.7; POD) activity, which alleviated the decline in the degree of fatty acid unsaturation and the ratio of linolenic (18:3) to linoleic acid (18:2) in strawberry leaves under water stress (Wang, 1999). The POD activity of fruits also increased during storage. Pineapples treated with MeJA could retard POD activity.

This experiment showed that pineapple fruits treated with 0.1mM MeJA had the lowest POD activity as compared with non-treated fruits. It has been suggested that POD is involved in the oxidation of phenolics, and results in colour changes in fruit and vegetables (Lin et al., 1988). The mechanisms, in which MeJA alleviates the browning symptom in pineapple, should be investigated further.

PPO is a key enzyme for enzymatic browning in many fruits (Mayer, 1987). It is a terminal oxidase enzyme occurring widely in plants, which catalyzes oxidation of phenolics, resulting in tissue

browning in fruits and vegetables (Macheix et al., 1990). The increase of PPO activity was related to the degree of pulp browning. The change of PPO activity is possibly due to the fact that most development of browning pigments and pitting has been associated with the stimulation of enzymatic activities, such as polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), peroxidase (POD) and total phenolic compound content (Tan and Lam, 1980). Browning symptom occurred so dramatically in pineapple tissue after tissue damage, or senescence. This was caused by the oxidation of phenols catalyzed by Polyphenol oxidase (PPO). This was followed by chemical reactions that convert the oxidative products into the brown polymer known as melanin (Burton and Noble, 1993).

In this experiment, the increased activity of the browning enzyme (Figure 3 and Figure 4) and total phenolic content (Figure 2) on the stored pineapple coincided with the browning symptom in pineapples. The total phenolic increased during storage was in association with PPO and POD activity (Figure 3 and 4). PPO activity in the pulp of treated and non-treated pineapple fruits also increased during storage, more so in the non-treated pineapple than in treated fruits.

Thus, it is quite clear that PPO and POD enzymes have played a role in browning symptom in pineapple in this study and by treating the pineapple fruits with MeJA, the browning symptom can be retarded and the shelf life of the pineapple can be extended.

Conclusion

Pineapples treated with MeJA had reduced browning symptoms, when phenolic content, PPO and POD activities were measured. The internal browning symptom was expressed after 21 days of storage, which was associated with the increased phenolic content, PPO and POD activities of pineapple. MeJA could delay the browning symptoms in pineapples held in storage at low temperature, in which the activity of the enzymes was retarded. Thus, dipping pineapple fruit in 0.1 mM MeJA for 5 min has the potential to inhibit the enzyme activity. This can extend the shelf life and improve the quality of the pineapple fruits during storage.

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