The individual influences of high CO$_2$ on chilling injury suppression of ‘Merah Delima’ papaya fruit

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Abstract. ‘Merah Delima’ papaya is chilling-sensitive products, and injured when stored below threshold-temperature. Modified Atmosphere Packaging (MAP) is the promising method to reduce chilling injury (CI) because it creates gas composition with low O$_2$ and high CO$_2$ inside the packed product. Too high CO$_2$ concentration inside the packed one causes physiological damage of fruit. This study was carried out to investigate the individual effect of high CO$_2$ in suppressing CI in ‘Merah Delima’ papaya fruit. Papaya was stored at 10 °C for 16 days under high CO$_2$ concentration of 4%, 6% CO$_2$ with 21% O$_2$, Balance N$_2$) and ambient air as the control. After storage at 10 °C, fruits were transferred to ambient air at temperature 25 °C for 1 week to evaluate the progress of CI symptoms. Quality parameters including weight loss, firmness as physical indices, skin colour as sensory evaluation and electrolyte leakage and malondialdehyde (MDA) content as CI indices were evaluated. The result of study showed that individual high CO$_2$ concentration reduced development of CI in papaya fruit compared to ambient air. However, too high CO$_2$ concentration did not give beneficial effect on CI suppression of ‘Merah Delima’ papaya fruit

1. Introduction

‘Merah Delima’ papaya fruit is specific cultivar produces in some regencies in West Sumatra province, and being intensified its development. The characteristics of this fruit are orange red flesh, sweet taste and chewy flesh. However, papaya is perishable fruit after harvesting. The lack of postharvest handling reduces the quality and shorten the self-life. Therefore, application of postharvest technology is required to maintain the quality and minimize the losses during storage and distribution chain.
In actual distribution chain, it usually transports without any post-harvest treatment. The fruits are just loaded on the truck in package and controlling temperature. These condition increase the losses of the fruit. It has been reported that the amount of postharvest losses was estimated around 30% of total production [1; 2]. This condition inhibited the expansion of ‘Merah Delima’ papaya fruit not only in domestic but also export market.

Low-temperature storage is common technology used for maintaining the quality of fresh product during storage and distribution chain. Storage at low temperature reduces respiration rate, ethylene production, and physiological activities [3]. However, papaya is chilling-sensitive fruit, and injured when stored at below threshold temperature [4]. Chilling injury (CI) is physiology injury occurring on the sensitive produces when stored at temperature below 5o-15oC, but above freezing temperature [4]. Modified Atmosphere Packaging (MAP) is the promising method that used to alleviate chilling injury in sensitive produces. It has been reported that MAP reduced CI in persimmon [5], mango [6], melon [7] and peach [8]. This technology creates the atmosphere with low O2 and high CO2 inside the packed. However, the response of chilling-sensitive products to low O2 and high CO2 is quite different among commodities [9]. Exposure of fresh produce to levels above its CO2 limit may cause physiological damage, and storage under the lower O2 limit induces fermentation, leading to reduced aroma biosynthesis and the possibility of off-flavors [10]. Therefore, the response determination of fruit to low O2 and high CO2 plays the main role in development MAP for alleviating CI in chilling sensitive commodity.

In previous study, investigation on the individual and combined effect of low O2 and high CO2 in supressing CI in cucumber fruit was successful [11]. The effect of relative humidity on CI suppression was also evaluated [12]. The optimal design of MAP in reducing CI in cucumber was also developed [13]. In addition, we also determined the favourable condition for transportation of persimmon fruit for export market [14]. However, as for ‘Merah Delima’ papaya fruit, there was no information available on the response to low O2 and high CO2 concentration. This study is focused on investigating the individual effect of high CO2 in supressing CI in ‘Merah Delima’ papaya fruit. Thus, the aim of study was to determine the individual effect of high CO2 in suppressing the development of CI symptoms. Fruit was stored at 10 °C for 16 days under high CO2 concentration, and then after storage, fruit was transferred to room with temperature of (25 °C) to evaluate the progress CI by measuring the ion leakage and malonaldehyde (MDA) content which are indicator of cell membrane for CI [15].

2. Materials and Methods

2.1. Plant materials and storage conditions
“Merah Delima” papaya (Carica papaya L.) at commercial maturity were harvested in Tanah Datar Regency, West Sumatra Province, Indonesia and transported immediately to laboratory. Fruits were sorted and selected on the basis of uniform size and absence of visual defects, and placed into an acrylic chamber (30x30x40 cm) for each gas composition tested. The chamber was then placed in an incubator (Memmert, Germany) at temperature 10 °C for 16 days under high CO2 concentration: 1) 4% CO2 with 21% O2; 2) 6% CO2 with 21% O2 (Balance N2); and 3) ambient air as control based on information of recommended gas composition for storing papaya fruits [16]. Gas was flushed into the chambers continuously from standard gas bottles with a flow rate of 50 mL/min. The gas compositions during storage were monitored by a zirconia O2 sensor (MC-86, Ijima Electronic, Japan) and a solid state CO2 probe (GMP221, Vaisala, Finland). After storage at 10 °C, fruits were transferred to room temperature (25 °C) under ambient air for 1 week to evaluate the progress of CI symptoms. Fruits were taken randomly to evaluate of weight loss, skin colour, CI index ion leakage and malondialdehyde (MDA) content after storage at 10 °C and the subsequent storage under ambient air at 25 °C.
2.2. Weight loss
The weight of papaya fruit from each experimental condition was measured immediately after arrival at the laboratory and then after removal from refrigerated storage, and the subsequent storage under ambient air at 25 °C. Weight loss of each fruit in the chamber was calculated as a percentage of initial fresh weight.

2.3. Skin colour
Skin colour was measured after storage at 10 °C, and subsequent storage at 25 °C using a lobivon colour measurement (Model: RM200, China) yielding parameters $L^*$, $a^*$ and $b^*$. Skin colour measurement were made at the base, middle, and bottom of the fruit. The results were expressed as the chroma ($C^*$) and hue angle ($h^*$), calculated as follows:

$$C^* = \left( a^2 + b^2 \right)^{0.5}$$  
$$h^* = \tan^{-1} \frac{b^*}{a^*}$$  

2.4. CI Index
CI index was evaluated by visual observation of fruit based on surface area scale of surface pitting and watery darkening: 0 (no signs of surface pitting and dark watery patches); 1 (<25%); 2 (25-50%); 3 (51-75%) and 4 (>75%). CI index between 0 and 4 calculated follow [17]:

$$\text{CI Index} = \frac{\sum (\text{CI Scale}) \times (\text{number of fruit at CI level})}{\text{Total of fruit}}$$  

2.5. Ion leakage
Ion leakage was assessed using a method described by Saltveit [18] with some modifications. Mesocarps of papaya fruit (11 mm diameter) were excised with a stainless steel cork-borer to produce 4 mm thick discs. The discs were soaked into fresh deionized water for 1 min to remove the excess ion on the tissue surface. This treatment was replicated 3 times, after that, the discs were blotted to dry by spreading onto absorbent paper to remove free water present on the surface. Then, 3 selected discs were placed into 50 mL centrifuge tubes with 20 mL of 0.2 M mannitol. The tubes were shaken at 100 cycles/min in a water bath incubator (WNB 14 Shaking, Memmert, Germany). Electric conductivity was measured with a conductivity meter at 1 h after addition of the mannitol solution. The tubes were then frozen, thawed, incubated for 10 min, and allowed to cool to room temperature. Total conductivity was measured after an additional 0.5 h of shaking. Individual conductivity readings were converted to percentage of total conductivity.

2.6. MDA content
MDA was determined according to the method of Hodges et al. [19] with some modifications. Mesocarp tissue (1 g) of papaya fruit was homogenised in 10 mL of 80% (v/v) ethanol along with 0.5 g inert sand using a mortar and pestle, followed by centrifugation at 3000 × g (SL 8, Thermo Scientific, USA) for 10 min. A 2 mL aliquot of the appropriately diluted sample was added either to 2 mL of 0.65% thiobarbituric acid (TBA) solution containing 20% (w/v) trichloroacetic acid (TCA) and 0.01% butylatedhydroxytoluene (BHT) or to a solution containing 20% (w/v) TCA and 0.01% BHT. The samples were then vortexed (V 3 S000, Ika) vigorously, boiled for 25 min, cooled in an ice bath immediately and centrifuged at 3,000 × g for 10 min. Absorbances at 532 nm, 440 nm and 600 nm were recorded using a spectrophotometer (UV1600, Shimadzu, Japan). The MDA equivalents were calculated by the following equations:

$$\left[ A_{532_{\text{TBA}}} - A_{600_{\text{TBA}}} \right] - \left( A_{532_{\text{TBA}}} - A_{600_{\text{TBA}}} \right) = A$$  

(4)
\[
[(A_{440+TBA} - A_{600+TBA}) \times 0.0571] = B \tag{5}
\]

MDA equivalents (nmol mL\(^{-1}\)) = \left( (A - B)/157000 \right) \times 10^6 \tag{6}

2.7. Statistical analysis

The design was completely randomised with 3 replications (3 fruits per test). Statistical significance was determined by subjecting the mean values to analysis of variance and means were compared by Tukey’s test at the 5% level of significance using R 3.5.1 (R Foundation).

3. Results and Discussion

3.1. Weight loss

High CO\(_2\) concentration significantly reduced the weight loss of fruit compared to control (P < 0.05), however, significant difference was not found among of the fruit treated with high CO\(_2\) concentration (P < 0.05), and the minimum of weight loss was observed on fruit stored under 4% CO\(_2\) (Figure 1). This result was corresponded to our previous study which high CO\(_2\) reduced the weight loss cucumber fruit after chilling at 5 °C for 5 days [11]. Martin and Resende [20] also reported that the mass loss of Golden papaya was smaller under control atmosphere after 20 days in the low O\(_2\) and high CO\(_2\). The damage of membrane integrity is associated with development of CI during storage at low temperature [21].

![Figure 1.](image)

(a) Weight loss and (b) the chroma of Merah Delima’ papaya fruit after storage at 10 °C for 16 days under high CO\(_2\) concentration of 4% and 6%, with 21% O\(_2\) (Balance N\(_2\)) and ambient air as control followed by 25 °C under ambient air for 7 days. Vertical bars represent standard error of the means (n=3).

3.2. Skin colour

The chorma and hue values were decreased during period of storage at 25 °C for all gas composition tested (Figure 1b and 2a). However, significant difference was not found among fruit stored under all gas composition tested. From our previous study found that high CO\(_2\) concentration did not gave significant effect to preserve the skin colour of cucumber fruit, even though combined with low O\(_2\), only individual low O\(_2\) retarded development of skin colour [11]. Cia et al also reported that greatest decreased value in hue angle in MAP, and decreased in chorma could be indicative of CI [5].
3.3. CI Index

CI indexes as surface pitting and watery darkening were appeared on the day 17 on the fruit treated with 4% of CO₂ and ambient air (control), while for fruit treated with 6% of CO₂, the symptoms of CI were appeared on the day 18, and then the increasing of CI was supressed until the day 20 for all treatments (Figure 2b). After day 20, CI index was increased significantly for all treatment, which the higher of CI index value was shown on the fruit treated under 6% of CO₂ on the end of storage. The development of CI symptoms was more developed after fruit transferred to warm temperature. As reported to persimmon fruit, the number of fruit softening which is CI symptoms in persimmon increased after fruit moved to 24.5 °C [14], and using MAP reduced the softening of fruit comparing to control.

![Figure 2](image_url)

Figure 2. (a) The Hue, (b) CI index, (c) Ion leakage, and (d) MDA content of ‘Merah Delima’ papaya fruit after storage at 10 °C for 16 days under high CO₂ concentration 4%, 6% with 21% O₂ (Balance N₂) and ambient air as control followed by 25 °C under ambient air for 7 days. Vertical bars represent standard error of the means (n=3).

3.4. Ion Leakage

Ion leakage of papaya fruit increased during period of storage at 25 °C. However, significant difference was not found among all gas composition tested (P<0.05) (Figure 2c). Electrolyte leakage has been used
as an indicator of cell membrane damage caused by CI. Wilson [22] reported that the rate of ion leakage from chilling sensitive product increase at chilling temperature. From our study, significant higher in ion leakage value was shown on the fruit stored under 6% CO₂ compared to 4% and control on the day 9, after that the ion leakage decreased gradually until the end of storage. It indicated that high CO₂ accelerated the damage of cell membrane because of CI. It has been reported that ion leakage of cucumber increased significantly on the fruit stored under individual CO₂ concentration after chilling at 5 °C for 5 days, but after removed to warm temperature the ion leakage decreased because of interposed warming.

3.5. MDA Content
The MDA content of papaya increased during period of storage at 25°C. Significant difference (P<0.05) in MDA content was found between 4% CO₂ with 6% CO₂ and control (Figure 2d). However, significant (P<0.05) was not found between 6% CO₂ and control fruit. The higher of MDA content value was observed on the fruit under ambient air (control) on the end of storage. MDA content was measured as a preceding indicator for CI, because CI induces the accumulation of reactive oxygen species (ROS) which cause oxidative damage to the cell membrane lipid, leading to increase MDA [23]. It has been reported that the MDA content was higher in cucumber fruit as a response of CI on the fruit stored under high CO₂ [11]. High CO₂ concentration accelerated the accumulation of MDA content, resulting enhanced the cell membrane damage, however, the mechanism is unclear. It may cause the high CO₂ showed higher concentration of H₂O₂ [24] which is used for measuring of ROS production generated by the electron transport chain [25].

4. Conclusion
In this study we investigated the individual effect of high CO₂ on CI suppression in ‘Merah Delima’ papaya. Based on our study, too high concentration of CO₂ did not give the beneficial effect in suppressing on CI in ‘Merah Delima’ papaya fruit. High CO₂ increased the ion leakage rapidly and accumulated the MDA content resulting in enhanced cell membrane damage. Determination of the maximum limit of CO₂ concentration is very important in successful MAP design. In this study we suggest that using MAP with high CO₂ permeability is more effective for preserving the quality of ‘Merah Delima’ papaya during storage at low temperature.

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