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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation　Development of Coated Paper Containing 1-Methylcyclopropene (1-MCP) Inclusion Complex in α-Cyclodextrin as a New Functional Packaging (新規機能性包装材としての1-メチルシクロプロペン包接α-シクロデキストリン含有塗布紙の開発)

学位論文要約：
Dissertation Summary

Fruit losses during postharvest processing is a critical issue. In 2011, Food Agriculture Organization (FAO) estimated that 32 percent of all fruit produced in the world was lost or wasted; which was equivalent to approximately 1.3 billion tons of fruit are globally wasted or lost per year during shipping and distribution process (Lipinski et al., 2016). During shipping and distribution process, decreasing fruit quality is greatly impacted during postharvest handling of fruit by ripening, decaying and senescence because of ethylene action. Ethylene is a plant growth hormone that promotes and triggers ripening process on fruits including physiological changes in fruit color, texture, and flavor. It is produced from biosynthesis pathway of S-adenosyl-L-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Hamilton et al., 1990; Aizat et al., 2013; Mworia et al., 2010; Adams & Yang, 1979). Ethylene is key rule on the ripening process in the most of climacteric fruits includes banana, apple, pear, melon, and tomato (Barry & Giovannoni, 2007). Moreover, the ripening process on fruit frequently occur during long shipment and unloading treatment. Environmental conditions such as temperature and humidity, which dramatically changes during unloading container also promoted the decreasing fruit quality. Therefore, an additional function of fruit packaging for such aspects as prolonged shelf life and enhanced consumer convenience will be worthwhile during postharvest processing and distribution handling. 1-Methylcyclopropene (1-MCP) which originally discovered by (Sisler & Serek, 1997) is the last great generation of ethylene antagonist agent. By synthesis reaction between phenyllithium solution and 3-chloro-2-methylpropene, 1-MCP become a superior and get much. It had been also reported as the ethylene antagonist agent to delay ripening process on fruit and vegetable by blocking the ethylene receptor (Blankenship & Dole, 2003; Watkins, 2006). In many countries, 1-MCP has been extensively used to preserve fruit and vegetables since it was registered for food application (Watkins, 2006). 1-MCP has been successfully encapsulated into α-cyclodextrin (α-CD) as wall material through molecular encapsulation process (Neoh et al., Y 2007) and many studies have shown the promising effects of 1-MCP in delaying ripening and prolonging the shelf life of fruit and vegetables (Blankenship & Dole, 2003). The main purpose of this study was to develop a coated paper containing 1-methylcyclopropene (1-MCP) inclusion complex in α-cyclodextrin as functional packaging.

1-MCP was encapsulated into α-CD as wall material through molecular encapsulation technique which followed the method reported by Neoh et al. (2007). However, a cooling system was used as part of the encapsulation system to remove impurities. The cooling system was used at −25 °C during gas transfer to the encapsulation bottle to

remove the remaining impurities. N_2 was injected gradually into the reaction bottle using a glass syringe to obtain atmospheric pressure. After transferring 1-MCP gas from the reaction bottle to the encapsulation bottle, α-CD solution was incubated at 15 °C for 8 h. At the end of the encapsulation process, α-CD solution containing the precipitate of the inclusion complex powder was centrifuged at 3000 rpm for 15 min to separate the solution and inclusion complex powder. The supernatant was dried in a freeze dryer (Eyela FDU 1200; Eyela Co., Ltd., Tokyo, Japan) for 24 h before further experiment. The effect of humidity and temperature storage on the release rate of 1-MCP in an α-CD inclusion complex powder were investigated using a dynamic vapour sorption (DVS) system at 40, 50, and 60 °C with stepwise humidity changes (20% RH for 2 h to 40, 50, 60, and 80% RH for 2 h, respectively). The release rate constant of 1-MCP from inclusion complex powders increased linearly with moisture concentration at 40, 50, and 60% RH. The release rate constant of 1-MCP from the inclusion complex powders was well correlated with a first-order release rate equation:

$$\frac{M_t}{M_\infty} = 1 - exp[-(kt)] \qquad (1)$$

Where M_t is the integrated release of 1-MCP [g/m^2.g-powder] under the stepwise humidity change from 20% RH to 40, 50, 60, and 80% RH; and M_∞ is the maximum released amount of 1-MCP [g/m^2.g-powder].

Figure 1 shows the effect of the stepwise humidity change on the release flux of 1-MCP from the inclusion complex powders using DVS. In this figure, the release fluxes of 1-MCP were plotted from 20% RH for 2 h and humidity was raised to 40, 50, 60, and 80% RH for 2 h. The release fluxes were stable and almost zero at 20% RH before the stepwise RH change. This finding might be because of the high glass transition temperature of α-CD.

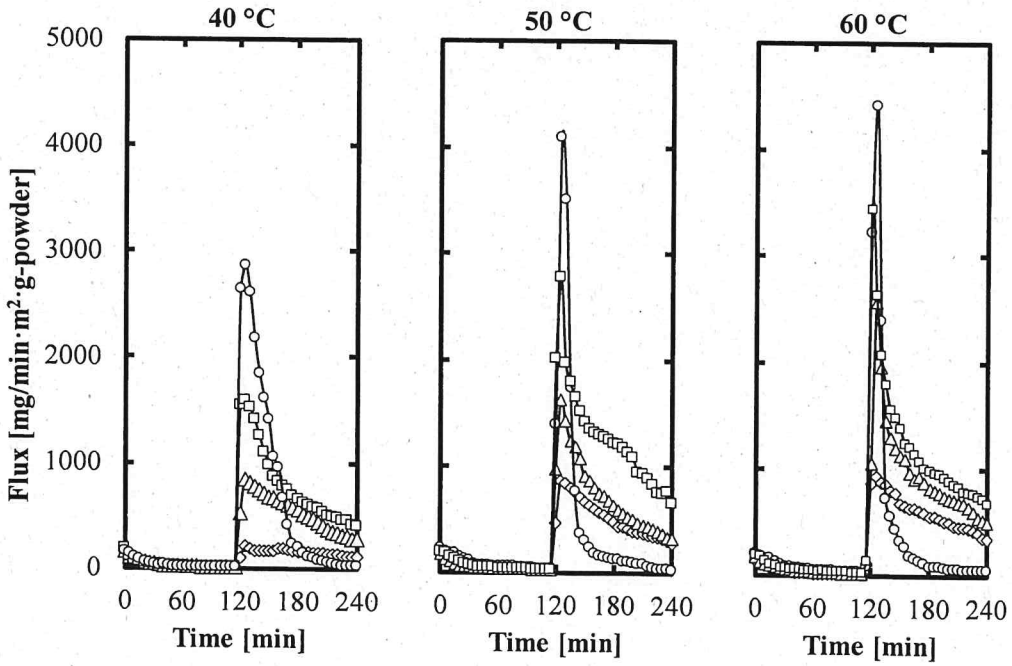

Figure 1. The release profile of 1-MCP gas from the inclusion complex powders under stepwise humidity changes of 40% RH (◇), 50% RH (△), 60% RH (□), and 80% RH (○) at different temperatures. Error bars (± standard error) are not shown because the value was small less than 0.03%.

The release flux at 40% RH and 40°C was very low. The release fluxes of 1-MCP increased significantly until the maximum point was reached shortly after the stepwise RH change and decreased gradually with the elapsed time. The release fluxes increased with the higher RH change. However, the release fluxes at 80% RH decreased significantly at 40 and 50°C to near zero. The highest release rate constant of 1-MCP was 7.5×10^{-2} s^{-1} for 80% RH at 60 °C. The activation energy of 1-MCP release at 80% RH was 32.9 kJ/mol. These findings indicated that the release of 1-MCP from the inclusion complex powders was influenced by moisture absorption. Furthermore, temperature also affected the release of 1-MCP during the experiments.

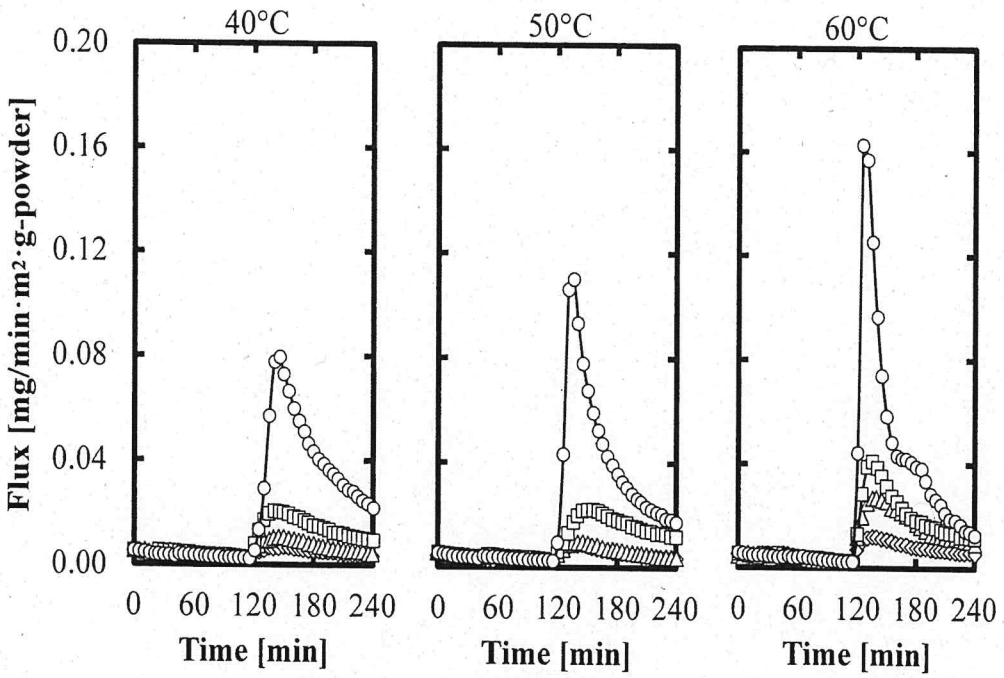

Figure 2. Dynamic release profiles of 1-MCP gas from coated paper under stepwise humidity changes of 40% RH (◇), 50% RH (△), 60% RH (□), and 80% RH (○) at 40, 50, and 60°C.

Development a 1-MCP controlled release system on coated paper was proposed to improve the release and inhibit the collapse behaviour of 1-MCP inclusion complex powder. 1-MCP coated paper was produced using shellac as the coating material. The formation of 1-MCP coated paper was done by dissolving a weight of 1-MCP inclusion complexes powder into 0.5 mL of shellac solution (35 %wt. in ethanol) and the mixture was stirred using a vortex machine at room temperature for 1 min to produce a 1-MCP slurry, which was then poured onto 100 cm2 of white paper. The effect of humidity and temperature on the release of 1-MCP from the coated paper was also investigated using a dynamic sorption system (DVS). In this study, the Avrami equation was used to simulate the release kinetic of 1-MCP from the coated paper. Originally, the Avrami equation was a mathematical model for analysis of crystallization (Avrami, 1940; Korsmeyer et al., 1983). However, as reported in some studies, it could be used to investigate the release behavior of encapsulated flavor (Neoh et al., 2007; Soottitantawat et al., 2004).

$$R = 1 - exp[-(kt)^n] \qquad (2)$$

Where R is the 1-MCP release ratio [–], defined as the ratio of the release amount of 1-MCP, M_t [g/m2.g-powder], to the total release amount of 1-MCP, M_∞ [g/m2.g-powder], in the coated paper state at time t after stepwise humidity change; k is the 1-MCP release rate constant [s−1]; t is the release time [s]; and n is the parameter of release mechanism [–]. Equation (2) can be rewritten as follows:

$$\frac{M_t}{M_\infty} = 1 - exp[-(kt)^n] \quad (3)$$

Figure 2 shows the release characteristic of 1-MCP subjected to stepwise humidity changes at constant temperature. For the first two hours, the humidity was set at a low 20% RH, which was then changed to 40, 50, 60, and 80% for 2 h, respectively. The release flux of 1-MCP was low and stable at 20% RH. However, after the humidity was changed to 40, 50, 60, and 80% RH, the release flux of 1-MCP increased to maximum for shortly, and afterwards decreased gradually under all conditions. Kurek et al. (2014) studied the effect of humidity on the release of carvacrol in coating system-based chitosan films. They reported that humidity strongly increased the release of carvacrol and also increased the diffusivity in the coating system. Different storage temperatures also affected the release flux of 1-MCP from the coated paper. In this study, the storage temperatures were fixed at 40, 50, and 60°C.

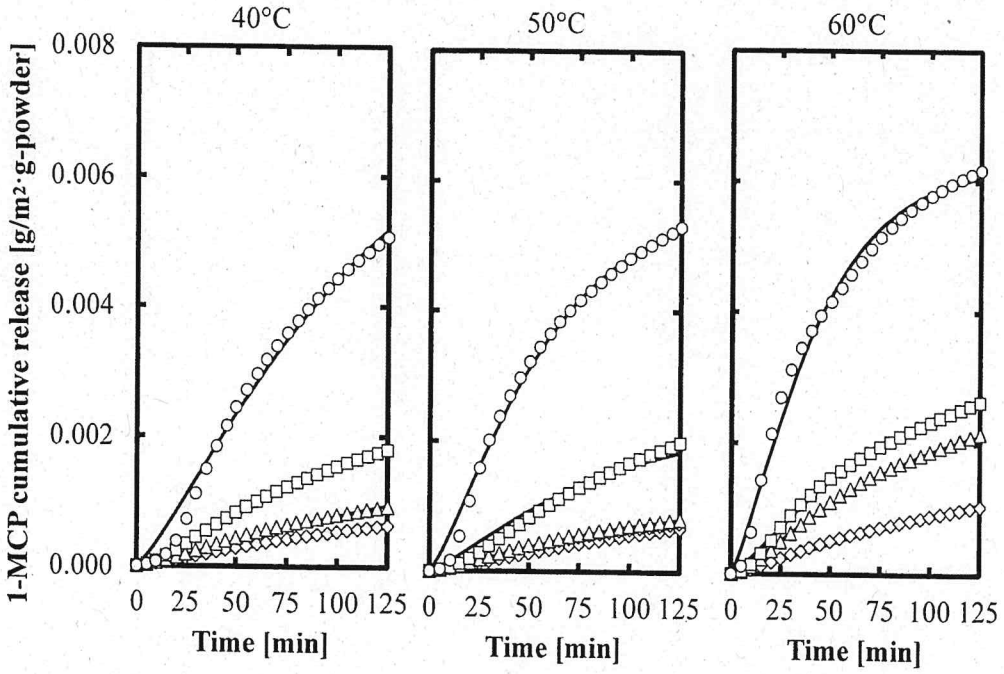

Figure 3. Cumulative release behavior of 1-MCP gas from coated paper under stepwise humidity changes of 40% RH (◇), 50% RH (△), 60% RH (□), and 80% RH (○) at 40, 50, and 60°C.

As shown in Figure 2, the different storage temperatures from 40 to 60°C significantly affected the increase in 1-MCP release flux from the coated paper under all humidity conditions (40–80% RH). A similar finding about temperature effect on the release of ethylene from a cyclodextrin inclusion complex was obtained by Ho et al. (2011a). They found that increasing the storage temperature accelerated the release of ethylene gas from a cyclodextrin inclusion complex. Chalier et al. (2009) investigated the effect of storage temperature on the release of an active agent from soy

protein coated paper. They found that increasing the storage temperature affected the properties of mass transfer in the surface layer of the soy protein. Furthermore, the glass transition temperature of the matrix also affected the release of flavor and the stability of the powder (Chalier et al., 2009; Bhandari & Howes, 1999; Carolina et al., 2007). Therefore, the effect of storage temperature on the release behavior of 1-MCP from coated paper might be explained by structural changes in the shellac layer as a function of external conditions.

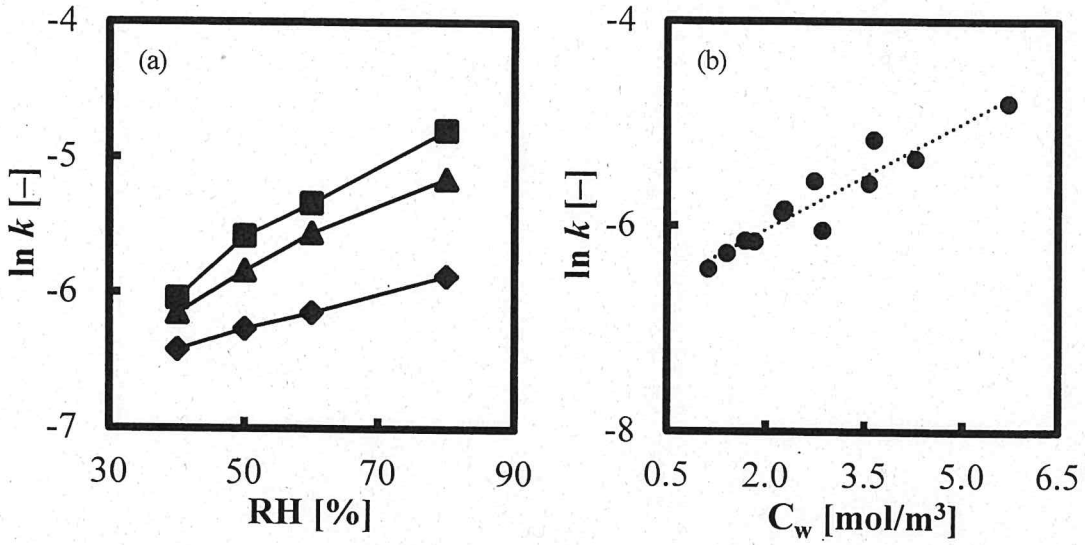

Figure 4a. Release rates of 1-MCP from coated paper under different humidity conditions at 40°C (◆), 50°C (▲), and 60°C (■). **Figure 4b.** Correlation of the moisture concentration and the release rate constant of 1-MCP from coated paper at various storage temperatures.

Figure 3 illustrates the cumulative release of 1-MCP from coated paper to obtain the release rate constant. In this figure, the release flux of 1-MCP was integrated with the release time to obtain the cumulative release of 1-MCP from the coated paper. The solid line represents the line calculated using Avrami's equation (Eq. 3). These lines could be simulated under all conditions with the same release mechanism number, n=1.26. At 80% RH for all temperature conditions (40, 50, and 60°C), the initial release time-courses could not be fitted well with the Avrami equation because the increasing release flux of 1-MCP from coated paper was rapid after the humidity changes under all temperature conditions.

The release rate constants of 1-MCP from coated paper at different humidity levels were analyzed by plotting ln k, obtained from Eq. 3, against the humidity condition. Figure 4a shows the plots of release rate constant of 1-MCP against different humidity conditions. The release rate constant of 1-MCP increased linearly according to humidity condition at constant temperature storage. This release mechanism of 1-MCP from coated paper is closely related to external resistance of release such as under the humidity condition mentioned above. This finding was different compared with the release rate constant of 1-MCP from an inclusion complex (Ho et al., 2011b). For the inclusion complex, the release of 1-MCP was represented as a diffusion release mechanism. This difference in release mechanism was caused by the coating process of the 1-MCP inclusion complex powder with shellac on the paper as functional packaging. Furthermore, different humidity and temperature conditions also affected the moisture concentration of 1-MCP, which in

turn also affected the release rate constant of 1-MCP from the coated paper.

Figure 4b confirms the relationship between the release rate constant of 1-MCP with moisture concentration. A higher moisture concentration increased the release rate constant of 1-MCP from the coated paper. Soottitantawat et al. (2004) obtained a similar result with the effect of water activity on the release rate of encapsulated flavor. They reported that the release rate of flavor from a matrix increased with higher moisture on the particle surface. The moisture concentration in this study ranged from 1.12 to 5.7 mol/m3 based on the humidity condition at a certain temperature storage. The linearity of the relationship with moisture concentration Cw was obtained using equation $\ln k = 0.35Cw - 6.7$ with $R = 0.89$. These results compared with the inclusion complex results (data not shown) showed better linearity. This relationship is assumed to result from the coating of the 1-MCP inclusion complex powder with shellac, which improved the stability and release rate of 1-MCP.

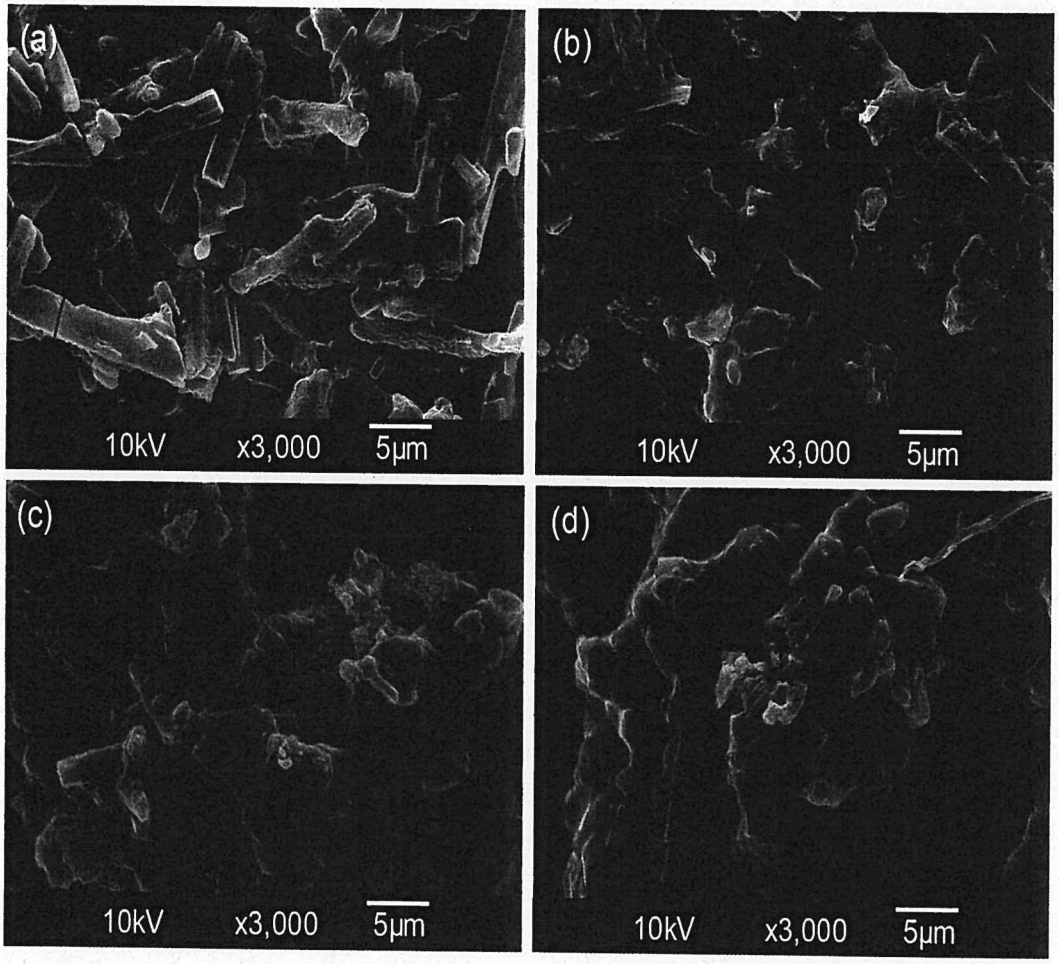

Figure 5. Microstructures observed under SEM of (a), initial 1-MCP coated paper before treatment; 1-MCP coated papers after treatment with humidity at 80% RH: (b), at 40°C; (c), at 50°C; (d) at 60°C.

At the self-bonding of the cellulose fibers, 1-MCP inclusion complex powder could be coated with 13% wt. of shellac solution as coating material. Cellulose is hydrophilic in that it can absorb moisture. However, this problem can be solved by a coating process that controls the release of 1-MCP and the moisture absorption rate from the upper layer of the coated paper. Figure 5 shows the structure and morphology of 1-MCP coated paper after humidity at 80% RH (at

3000-times magnification). At low humidity of 40–60% RH and at all temperature conditions, the fine particles of the 1-MCP inclusion complex powder with shellac coating were clearly seen. However, the particles of the 1-MCP inclusion complex powder changed to aggregated particles at 80% RH, especially at 60°C. This aggregation indicated the collapse phenomenon, in which moisture penetrated the 1-MCP inclusion complex powder through the shell wall of the shellac and changed the structure of the 1-MCP inclusion complex powder. Kurek et al. (2014) reported a similar finding, in which water molecules affected the increasing of gas permeability and the changing of the plasticizing structure of carvacrol release-based chitosan coating and films. Yamamoto et al. (2012) also reported the effect of moisture absorption on the release of d-limonene encapsulated in cyclodextrin powder. They found that moisture absorption changed and cracked the surface of the cyclodextrin and the fine particles aggregated.

The effectiveness of 1-MCP coated paper had been applied in apple during storage time. The effect of 1-MCP coated paper on apple storage was evaluated by measuring the ethylene production rate, flesh firmness, and titratable acidity (TA) of apple. Initially, apple were stored at 4°C for initial 15 days. Thereafter, apples were transferred to 20°C storage temperature for another 15 days. After this treatment, the first analysis of quality of apple was conducted immediately. The remain apples were kept stored at 20°C for another 15 days to evaluate the shelf life of apple, and the physicochemical properties of apples were also measured as the second analysis.

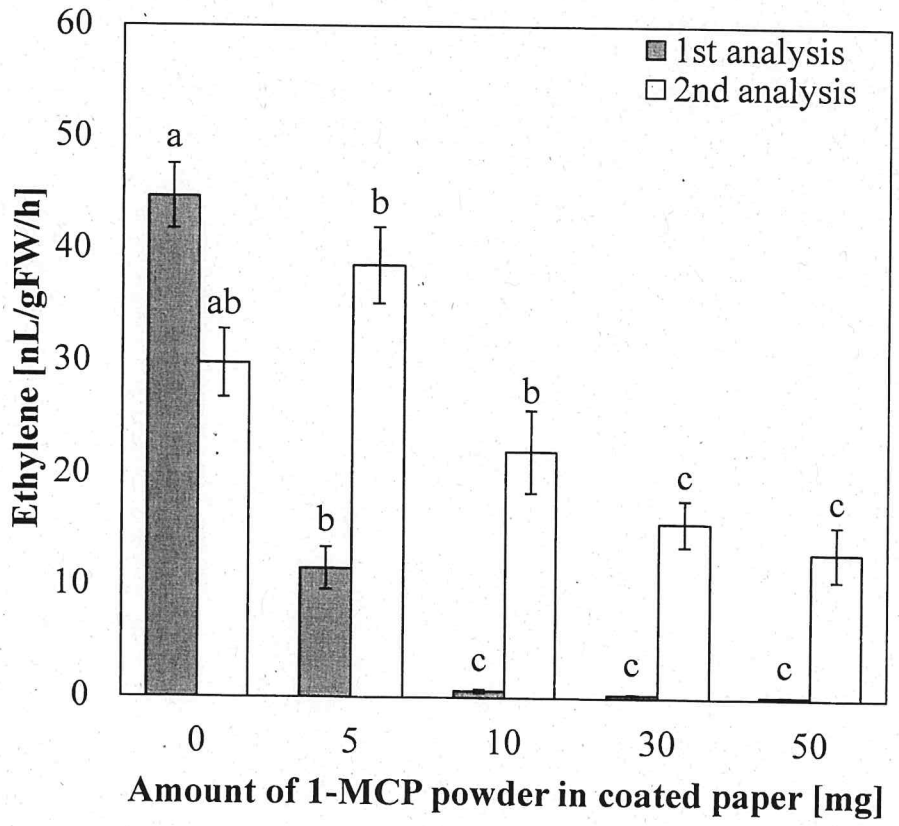

Figure 6. Ethylene production rate of apple after treatment with 1-MCP coated paper. Vertical bars represent the standard error SE (n=10). Different letters indicated significant differences under the treatment of 1-MCP coated paper in the same analysis time at $p<0.05$ using Tukey's test.

Figure 6 shows ethylene production rates of apple after treatment with 1-MCP coated paper. There was evidence to indicate that 1-MCP was effective to inhibit the ethylene production rate on apple as parameter of ripening process. As can be seen, during storage time at 4°C, the release of ethylene from apples with treatment of 1-MCP coated paper were less than the control apples. This results showed that apple with treatment of 1-MCP coated paper at 4°C produced low level of ethylene production rate. Furthermore, as reported in previous studies, low temperature storage was ineffective on the ethylene production because of the induction of expression of the ethylene biosynthetic enzymes (Tatsuki et al., 2011; Larrigaudiere et al., 1997; Tian et al., 2002). Therefore, during storage at low temperature 4°C, ethylene receptor did not active and produced low ethylene (Blankenship & Dole, 2003). However, after the storage temperature change, the release rate of ethylene for all samples increased steadily with time until reach the maximum level and then these rate decreased.

There was significant difference ($p<0.05$) within apples with 1-MCP coated paper treatment and the control of apples on ethylene production rate at the first analyses. Low ethylene production rate of 0.22 nL/g FW/h could be observed at 50 mg of 1-MCP inclusion complex powder. On the other hand, for the control apples without treatment of 1-MCP coated paper, the ethylene production rate was 44.7 nL/g FW/h. This suppression indicated that 1-MCP could be released from coated paper by moisture from apple and inhibited the ethylene production during storage time. In the second analysis, the ethylene production rates of apple with the treatment of 1-MCP coated paper were 13.0, 15.6, 22.0, 38.6 nL/g FW/h for 50, 30, 10 and 5 mg of 1-MCP inclusion complex powder, respectively. These rates increased in the 2nd analysis in comparison to the 1st analysis. However, for the control apple, the ethylene production rate decreased to 29.8 nL/g FW/h from 44.7 nL/g FW/h. These results indicated the effect of 1-MCP on the ethylene production rate and confirmed that ethylene production rate of apple depended on the amount of 1-MCP inclusion complex powder in coated paper. Larrigaudiere et al. (1997) observed the effect of cold storage on the ethylene biosynthesis of apple. They also found that high temperature storage stimulated the increasing of ethylene biosynthesis enzyme to produce more ethylene.

Thus, this study can be concluded that 1-MCP inclusion complex in α-CD has a potential as an active compound to delay the ripening on fruit. Furthermore, the release of 1-MCP could be controlled by coating paper with shellac solution and promising to produce a new functional packaging for fruit.

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