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Responses of ready-to-eat salad vegetable mixes prepared with different lettuce varieties to 1-methylcyclopropene (1-MCP) post-cutting treatment

[1,3]Marquez, K.L.D., [1*]Castillo-Israel, K.A.T., [2]Serrano, E.P., [1]Gandia, J.B.L. and [2]Absulio, W.L.

[1]*Institute of Food Science and Technology, College of Agriculture, University of the Philippines Los Baños, 4031, College Laguna*
[2]*Postharvest Horticulture Training and Research Center, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños, 4031, College Laguna*
[3]*President Ramon Magsaysay State University Candelaria Campus, Sinabacan,2212 Candelaria, Zambales*

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Abstract

A post-cutting treatment of 1-methylcyclopropene (1-MCP) was applied to ready-to eat salad vegetable mixes (RTE-SVM) consisting of lettuce, carrots and cucumber to determine its potential in maintaining the storage quality. The responses of different varieties of lettuce were also compared. Minimally-processed lettuce (Corelle, Romaine, and Iceberg varieties), Japanese cucumber, and carrots were prepared and packed in PET trays then sealed with PVC cling wrap film. 1-MCP gas was injected in the packs to have a final concentration of 1ppm and 3ppm inside the RTE-SVM packs. The packs were stored in a cold room (5-8°C) until they became unacceptable as a whole, but the shelf life and limit of marketability of the packs were set when the packs reached an average VQR of 6 (Fair, moderate defects). Headspace gas analyses (oxygen, carbon dioxide, and ethylene) and visual evaluation (visual quality rating, lettuce wilting and yellowing, carrots discoloration, and cucumber water-soaking) were performed. The RTE-SVM prepared with Corelle lettuce has the best response to the 1-MCP treatment, having the highest average VQR, lowest incidence of wilting and yellowing and carrots discoloration when treated with 3 ppm 1-MCP. The RTE-SVM packs prepared with Iceberg and Romaine lettuce, on the other hand, can be treated with 1ppm 1-MCP to reduce the incidence of lettuce wilting and yellowing and carrots discoloration in the packs. Furthermore, it was determined that the RTE-SVM prepared with Corelle, Romaine, and Iceberg lettuce can be stored for 8, 10, and 5 days, respectively, at 5-8°C before reaching the limit of marketability.

Introduction

Minimally-processed produce, which are also called ready-to-use, fresh-cut or pre-cut produce, are fresh fruits or vegetables that have been prepared and packaged to provide convenient and safe ready-to-eat products for consumers (Nicola and Fontana, 2014). Similarly, the International Fresh-Cut Produce Association (IFPA) defines the fresh-cut produce as "any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state". Fresh and raw vegetables and fruits are subjected to minimal process operations such as cutting, trimming, shredding, peeling, washing, decontamination, dipping, rinsing and packaging (Nicola and Fontana, 2014).

Fresh-cut products are a convenient way to supply consumers with washed, bite-sized, and packaged fresh fruit and vegetables that allow consumers to eat healthy on the run and to save time on food preparation. However, since they remain in a fresh state, fresh-cuts are characterized by living tissues that undergo or are susceptible to enzymatic activity, texture changes, undesirable volatile compound production and microbial contamination, which reduce the shelf life of the product (Nicola and Fontana, 2014). As a result of processing, tissue damage is encountered by minimally-processed fruits and vegetables, which contributes to rapid deterioration of their components and subsequently, shorter shelf-life compared to their whole and unprocessed counterparts (Martin-Diana *et al*., 2007). Moreover, deterioration in fresh-cut produce due to physiological ageing, biochemical changes and microbial spoilage may result in degradation of the color, texture and flavor of the produce (Ahvenainen, 1996).

In addition to the changes in quality, ethylene production is also enhanced by wounding during processing, and the accumulation of this gas within the packages of fresh-cut produce can be detrimental to their quality and shelf-life (Yahia, 2011). Ethylene

*Corresponding author.
Email: kcisrael@up.edu.ph

is responsible for triggering many ripening and stress and wound responses, such as enhanced activities of browning enzymes, chlorophyllase, cell wall degrading enzymes, as well as increased production of secondary metabolites, and lignin (Martin-Belloso and Soliva-Fortuny, 2011), which further make the shelf-life of the fresh-cut produce shorter and their appearance more undesirable for consumers. The effects of ethylene can therefore be reduced by exclusion and/or removal of ethylene from packages and treatment with 1-MCP to block ethylene action (Yahia, 2011).

1-methylcyclopropene (1-MCP or C4H6) is an ethylene action inhibitor that interacts with ethylene receptors and thereby prevents ethylene-dependent responses in many horticultural commodities (Siddiq, 2012). Application of 1-MCP was found to delay softening and darkening of fresh-cut 'Kent' and 'Keitt' mango and 'Fuyu' persimmons, while it is reported to decrease ethylene production and delay softening of fresh-cut 'Hayward' kiwifruit (Vilas-Boas and Kader, 2007). Treatment with 1-MCP was also reported to inhibit chlorophyll degradation, yellowing, flowering process and visual quality deterioration in fresh-cut broccoli raab (Cefola *et al*., 2010).

Due to the nature of fresh-cut produce, it is important to prolong its shelf-life and nutrient content that is comparable to the intact produce until it reaches the consumers. It is known that deterioration can be retarded through storage at low temperature, proper packaging, and by slowing down the rate of respiration and ethylene production of the produce. It is therefore possible to prolong the shelf-life of packed fresh-cut produce such as salad vegetable mixes through the use of ethylene antagonists such as 1-MCP. This study aimed to assess 1-MCP's potential as a tool in maintaining the storage quality of ready-to-eat salad vegetable mixes prepared with different lettuce varieties.

Materials and Methods

Preparation of the ready-to-eat salad vegetable mix (RTE-SVM)

The different lettuce varieties used, as well as the Japanese cucumber, were purchased from a local farm at Tagaytay City, Cavite, Philippines while the carrots were purchased from Batong Malake Public Market at Los Baños, Laguna, Philippines. The plant materials were washed and prepared based on the protocol developed by Flor *et al*. (2007). The lettuce, cucumber and carrots were washed thoroughly with water prior to processing to remove adhering dirt and reduce the initial microbial load of the commodities. The commodities were then soaked in 15 L of tap water containing 1.48 mL liquid detergent for 10 minutes before rinsing the carrots and cucumber thrice with running tap water. The lettuce, however, was only rinsed twice with tap water to avoid damaging the leaves. The carrots and cucumber were then peeled and cut into cylinders having a length of approximately 2.5 inches. The core of the cucumber was removed to delay the water-soaking of the fruit. Afterwards, the cucumber and carrots were cut into sticks. Lettuce of different varieties were cut into appropriate sizes. The cut lettuce, carrots and cucumber were then sanitized by soaking at 200 ppm chlorinated water for two minutes and were spin-dried manually. The spin-dried commodities were then weighed and packed in trays which were sealed using PVC cling wrap film. Each pack contains approximately 40 g cucumber, 40 g carrots and 20 g lettuce.

After sealing the packs using PVC cling wrap, some of the packs were injected with 1-MCP to have different concentrations of the gas in the headspace. The 1-MCP stock solution (500 ppm) was prepared by weighing and transferring 0.0054 g 1-MCP (4.12%) into a 200 mL volumetric flask containing distilled water to dissolve the powder. The gas was allowed to evolve for at least 30 minutes before withdrawing appropriate amounts of gas to be injected into the salad packs. The volume of gas withdrawn from the flask was replaced with an equal volume of water to maintain the concentration of the gas inside the flask.

Using the total volume of the tray, the volume of the gas to be injected on each pack was calculated. To have a final volume of 1 ppm 1-MCP inside the salad pack, 1 mL of 500 ppm 1-MCP was injected to the packs while 3 mL of 500 ppm 1-MCP was injected to have a final concentration of 3ppm inside the packs. The injection point was sealed afterwards with adhesive tape to prevent gas leaks from the pack.

Three different lettuce varieties (Corelle, Romaine, and Iceberg) were used to prepare the salad. Treatments with 1ppm and 3ppm 1-MCP were prepared for each lettuce variety. Packs without 1-MCP treatment were considered as the control. All of the packs were stored at 5-8°C. Gas levels (O_2, CO_2, and C_2H_4) inside each pack were monitored every other day using gas chromatography while visual evaluations were performed on a daily basis.

Headspace gas (ethylene, CO_2, O_2) analyses

The carbon dioxide and oxygen levels inside each salad pack were analyzed using a gas chromatograph equipped with thermal conductivity detector (GC-TCD; Shimadzu GC-SA) operated with the injection port temperature at 90°C, column temperature at 50°C, and the gas pressure was at 1.25kg/cm^2. On the other hand, the ethylene levels inside each pack were analyzed using a gas chromatograph equipped with flame ionization detector (GC-FID; Shimadzu GC-2014) operated with the injection port temperature at 120°C, column temperature at 100°C, having a column length of 2.0 m with an inner diameter of 3.0mm, and a gas flow rate of 35 mL/min.

Lettuce wilting and yellowing

The degree of wilting and yellowing of the lettuce inside the packs were observed and evaluated by visual inspection separately using the following scale: 1 – none; 2 – 1-10% wilting/browning; 3 – 11-25% wilting/browning; 4 – 26-50% wilting/browning; 5 – >50% wilting/browning.

Carrot discoloration

The degree of discoloration of the carrots inside each pack wasalso observed and evaluated by visual inspection using the following scale: 1 – none; 2 – 1-10% discoloration; 3 – 11-25% discoloration; 4 – 26-50% discoloration; 5 – 50% discoloration.

Cucumber water-soaking

The degree of water-soaking of the cucumbers inside each pack was alsoobserved and evaluated by visual inspection using the following scale: 1 – none; 2 – 1-10% water-soaking; 3 – 11-25% water-soaking; 4 – 26-50% water-soaking; 5- >50% water-soaking.

Visual quality rating (VQR)

The VQR of the RTE-SVM packs were observed and evaluated using the following scale: 9,8 – excellent, field fresh; 7 – good, minor defects; 6 – fair, moderate defects, limit of marketability; 5,4 – poor, serious defects; 3,2 – limit of edibility; 1 – non-edible under usual conditions.

To properly determine the maximum period by which the packs can be kept in cold storage, the gas and visual analyses were performed until poor, serious defects (VQR = 4-5) were observed on the samples used for the visual assessment, or when the packs became unacceptable as a whole. However, the shelf life and limit of marketability of the packs were decided to be set when the average VQR of the packs reach a VQR of 6 (Fair, moderate defects), since the RTE-SVM packs are intended to be sold at supermarkets with strict requirements for quality.

Statistical analysis

The experiment was carried out using a completely randomized design (CRD). Statistical analysis of the data gathered were done through the use of SAS program using ANOVA Least Significant Difference (LSD).

Results and discussion

RTE-SVM packs prepared with Corelle lettuce

The RTE-SVM packs, both treated and control, prepared with Corelle lettuce exhibited severe cucumber water-soaking after 13 days of storage at 5-8°C. Although the lettuce and carrots were still acceptable, the water-soaking of the cucumbers made the RTE-SVM packs highly unacceptable as a whole after 13 days.

The average O2levels in the control packs was not significantly different from those treated with 1-MCP during day 1, but was significantly different with the 1ppm treatment during the termination day (day 13). Likewise, the O_2 levels inside the packs injected with 3ppm 1-MCP had very similar trend with the control, with the latter being slightly lower than the former (Figure 1A). The 1ppm treatment, on the other hand, had incurred the lowest O_2 levels on most points, except on day 13 where the oxygen levels increased. However, the low O_2 levels in the 1 ppm treatment were not significantly different from the other treatments. Furthermore, the O_2 levels inside packs should ideally decrease during storage due to the modified atmosphere created by the cling wrap. In modified atmosphere packaging, the gas composition within the package is modified through the respiration of the vegetable tissue, which leads to the consumption of oxygen from the headspace and evolution of carbon dioxide until the creation of an equilibrium-modified atmosphere that depends on both temperature changes and gas permeability of the packaging film (Del Nobile *et al*., 2008).

Significant differences in the CO_2 levels in the headspace were observed during the start (days 1 and 3) and end (day 13) of storage, while no significant difference was observed between the CO_2 levels of the samples during day 6 to day 10 of storage. The CO_2 levels inside the control steadily increased starting day 3 (Figure 1B) while in the 1-MCP-treated samples, CO_2 generally decreased until day 8, and then increased until the last day of storage (day 13). These results suggest that the normal trend in CO_2 levels in a modified atmosphere condition was exhibited in the control, which was not observed in the 1-MCP treatments, showing a positive effect of 1-MCP on decreasing respiration rates. 1-MCP has been reported to decrease the respiration rate of Chinese kale (Sun *et al*.,2012), fresh-cut banana (Vilas-Boas and Kader, 2006), Chinese chive scapes

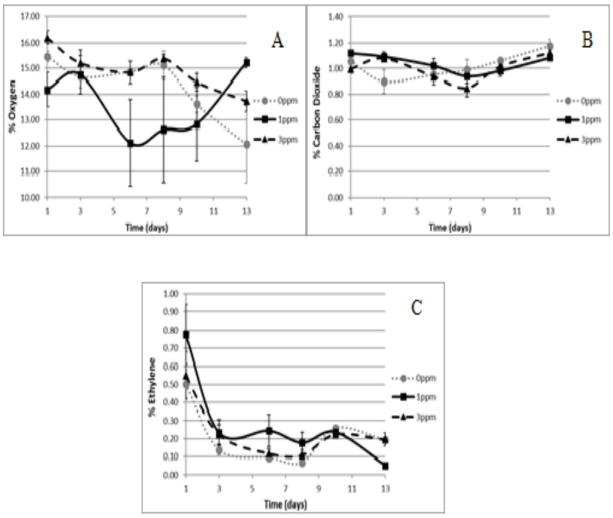

Figure 1. Oxygen (A), carbon dioxide (B), and ethylene (C) concentrations in the headspace of the RTE-SVM packs prepared with Corelle lettuce and treated with different 1-MCP concentrations.

(Wu *et al.*, 2009), and delay the onset of carbon dioxide and ethylene climacteric peaks in various avocado cultivars (Hershkovitz *et al*., 2005). Treatment with 1-MCP together with the use of micro-perforated film packaging was also found out to inhibit respiration and ethylene production of 'Laiyang' pear (Li *et al*., 2013).

The ethylene production patterns inside the packs also followed a similar trend as in CO2 concentration, where there is a noticeable increase in ethylene concentration in the headspace during day 10 (Figure 1C). It is possible that this increase triggered the increase in respiration rate, leading to an increase in CO_2 concentration in the headspace of the treated packs (Figure 1B). Furthermore, the ethylene levels in the control packs were mostly lower than those observed in the treated packs. Since 1-MCP is designed to bind to ethylene receptors (Rees *et al*., 2012), it is possible that 1-MCP treatments led to the accumulation of ethylene in the headspace, causing an increase in ethylene concentration for the treated samples. For the control samples, however, it is possible that ethylene binds with vegetable tissues; therefore, less ethylene was accumulated and detected in the headspace compared to the treated samples. During postharvest storage, ethylene can induce negative effects such as senescence, over-ripening, accelerated quality loss, increased fruit pathogen susceptibility and physiological disorders (Han *et al*., 2015). Treatment with 1-MCP inhibits these responses by suppressing ethylene production in several commodities like bittermelon (Han *et al*.,2015) and Chinese kale (Sun *et al*., 2012).

Significant differences in wilting indices (WI) of the Corelle lettuce in the treated and untreated packs were observed only on days 7, 8, and 9. The average WI of the packs treated with 3 ppm 1-MCP were consistently lower than the other two (Figure 2A), suggesting that treatment with 3 ppm 1-MCP helps delay the wilting of Corelle lettuce in the RTE-SVM packs. Wilting is a consequence of high respiration rate, where loss of moisture during respiration results to loss of turgidity of the cells.

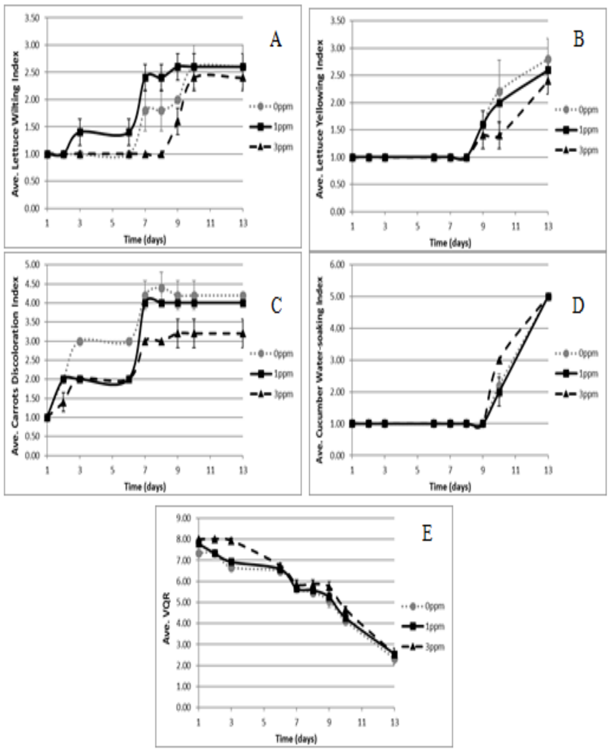

Figure 2. Lettuce wilting (A) and yellowing (B) indices, as well as carrots discoloration indices (C), cucumber water-soaking indices (D), and average visual quality rating (E) in the RTE-SVM packs prepared with Corelle lettuce and treated with different 1-MCP concentrations.

The yellowing index (YI) of the lettuce in the RTE-SVM 3ppm treatment received the lowest score among the three (Figure 2B). Wang *et al.* (2014) explained that the treatment with 1-MCP could contribute to leaf yellowing inhibition by maintaining leaf capacity for carbon assimilation, inhibiting the tricarboxylic acid cycle, inducing biosynthesis of gibberellic acid and by reducing the levels of reactive oxygen species in Tsai Tai leaves during cold storage. However, Gómez-Lobato *et al*. (2012) clarified that although 1-MCP markedly delayed degreening and reduction of chlorophyll content in broccoli, the action of 1-MCP is selective in some of the genes-encoding enzymes related to chlorophyll catabolism and did not have a clear effect on the expression of chlorophyllase-related genes.

The discoloration indices (DI) of the carrots in the RTE-SVM packs were also noted. Carrots discoloration, which has been used to indicate the

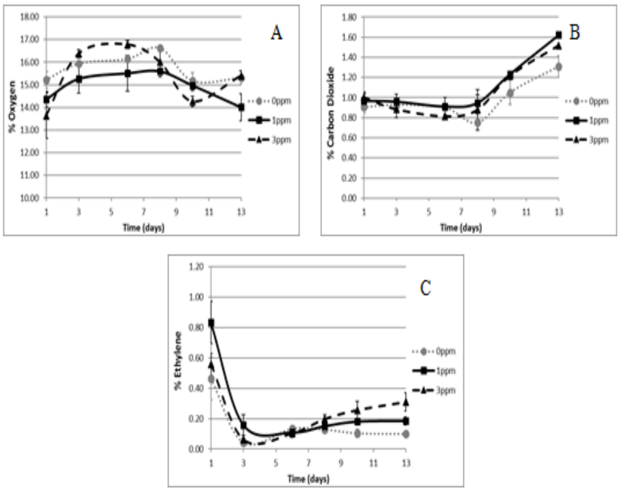

Figure 3. Oxygen (A), carbon dioxide (B), and ethylene (C) concentrations in the headspace of the RTE-SVM packs prepared with Romaine lettuce and treated with different 1-MCP concentrations.

storability of minimally-processed carrots (Lavelli *et al*., 2006), is characterized by the loss of the orange color as well as whitening of the carrot surface. It was observed that significant differences between the treatments exist during days 2 to 8 (Figure 2C). During days 3 to 6, there was a significant difference between the average DI of the treated and untreated samples. During days 2 and 7, on the other hand, the average DI of the carrots in the 3ppm RTE-SVM was significantly different from those untreated and the 1 ppm treatment, but the latter two were not significantly different from each other. Although there were no significant differences observed during the other days, it was noticed that the DI of the carrots in the 3 ppm treatment were always lower (if not equal) than the other two treatments. This may suggest that treatment with 3 ppm 1-MCP was effective in inhibiting the discoloration of the carrots packed together with cucumber and Corelle lettuce.

Surface discoloration in carrots can be reversible or irreversible. Whitening of the outer layers is caused by the reversible surface dehydration of cut carrot surfaces, while the formation of lignin results to an irreversible change in surface color (Lavelli *et al*., 2006). Although modified atmosphere packaging was known to prevent dehydration, it is possible that in the experiment, the carrot discoloration was caused by the surface dehydration during cutting and prolonged cold storage.

The water-soaking of the cucumbers on the treated RTE-SVM packs were also determined, though it was observed that there was no significant difference between the water-soaking incidence in the samples. Water-soaking on all samples was observed on day 10 of storage at 5-8°C, while severe water-soaking was observed on day 13 (Figure 2D), making the packs highly unacceptable on this day.

The average VQR of the RTE-SVM packs were also calculated by determining the mean of the individual VQR of the carrots, lettuce, and cucumbers in each pack. Significant differences between the average VQR of the samples were only observed during days 1 to 3, with the treated samples having a significantly different VQR than the untreated samples. Although no significant difference between the samples were observed afterwards, the packs treated with 3 ppm 1-MCP always had the highest average VQR throughout the experiment (Figure 2E), which may indicate that treatment with 3 ppm 1-MCP results in higher acceptability of the RTE-SVM packs prepared with Corelle lettuce compared to treatments with the control and 1ppm 1-MCP treatment. This coincides with the previous quality indices determined, where treatment with 3 ppm 1-MCP always gave the best rating for the different component commodities inside the packs.

RTE-SVM packs prepared with Romaine lettuce

Similar to the RTE-SVM packs prepared with Corelle lettuce, all of the RTE-SVM packs prepared with Romaine lettuce exhibited severe cucumber water-soaking after 13 days of storage at 5-8°C. Although the lettuce and carrots were still acceptable, the water-soaking of the cucumbers made the RTE-SVM packs highly unacceptable as a whole on this day.

A significant difference between the average O2concentration of the 1ppm treatment was only observed on day 8, and no significant difference was observed during the other days. Furthermore, the O2levels in the headspace did not follow the ideal decreasing trend due to atmosphere modification (Figure 3A), which may indicate that 1-MCP treatment has no significant effect on the O_2 levels of the RTE-SVM packs prepared with Romaine lettuce.

A significant difference in the CO_2 levels was observed only on the last day (day 13) of storage at 5-8°C, where the average CO_2 levels of the 1ppm RTE-SVM packs are significantly different from the control. The desirable increase in CO_2 levels in the headspace was also observed on almost all samples after day 6, with the samples treated with 1ppm 1-MCP having the highest CO_2 concentration among the three (Figure 3B). This may indicate that the respiration rate started to increase after day 6.

Ethylene concentrations in the headspace were also determined and significant differences between the samples were observed only on the last days of storage (days 10 and 13) at 5-8°C, with the control samples always being significantly different from

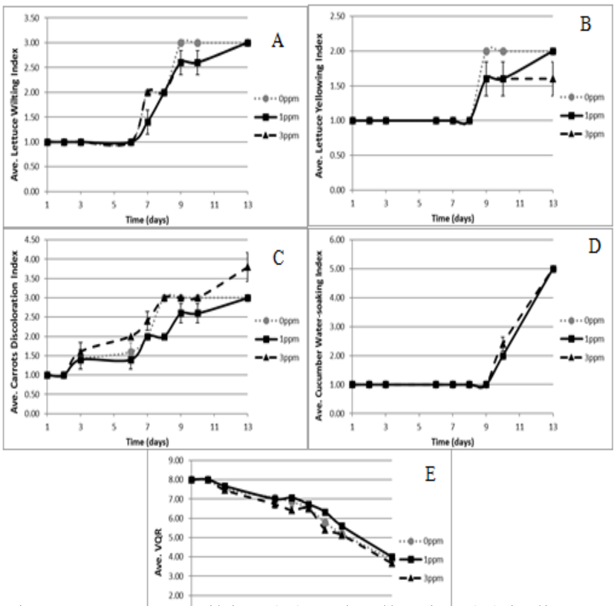

Figure 4. Lettuce wilting (A) and yellowing (B) indices, as well as carrots discoloration indices (C), cucumber water-soaking indices (D), and average visual quality rating (E) in the RTE-SVM packs prepared with Romaine lettuce and treated with different 1-MCP concentrations.

the samples treated with 3ppm 1-MCP (Figure 3C). Also, there was a noticeable decrease in ethylene concentrations for all samples during day 3, which was followed by an increase in ethylene concentrations for all samples treated with 1-MCP. Since 1-MCP is designed to bind to ethylene receptors (Rees *et al.*, 2012), it was possible that 1-MCP treatments led to accumulation of ethylene in the headspace which was observed after day 7, causing an increase in ethylene concentration. For the control samples, however, it was possible that ethylene binds with vegetable tissues, therefore, less ethylene was accumulated and detected in the headspace compared to the treated samples.

Most of the wilting indices were not significantly different from each other, except on day 7 where the WI of the Romaine lettuce with 1 ppm 1-MCP was significantly different from the other two treatments (0 and 3 ppm). Wilting became noticeable after day 6, which coincides with the increase in respiration rate (in terms of CO_2 produced). Furthermore, the average WI of the Romaine lettuce in the packs treated with 1ppm 1-MCP hadthe lowest scores among the three (Figure 4A), which may indicate that treatment with 1ppm 1-MCP may influence the delay of wilting of Romaine lettuce packed with carrots and cucumber. The same was observed in basil leaves (Hassan and Mahfouz, 2010), where a lower concentration (0.2 g m^{-3}) of 1-MCP resulted to a higher weight loss compared with the higher concentration applied(0.6 g m^{-3} 1-MCP). This implies that higher concentrations of 1-MCP does not equate to greater effectivity. Responses of commodities to 1-MCP vary depending on the species.

In terms of the average yellowing indices of the Romaine lettuce in the RTE-SVM packs, no significant differences were found between the samples, and it can be seen from Figure 4B that all the Romaine lettuce from each RTE-SVM pack increased in yellowing during Day 9, with the control packs exhibiting a higher degree of yellowing compared to the treated samples. The Romaine lettuce in the RTE-SVM packs injected with 3 ppm 1-MCP, on the other hand, had the lowest yellowing indices.

Significant differences between the discoloration indices of the carrots in the treated and untreated RTE-SVM packs only existed during days 8 and 13. However, the carrots in the 1 ppm RTE-SVM packs had the lowest scores and those treated with 3 ppm 1-MCP suffered the highest carrots discoloration (Figure 4C). With this, the effects of the 1-MCP treatment were unclear on the carrots packed with Romaine lettuce and cucumber, but it is possible that the optimum 1-MCP concentration for inhibiting carrots discoloration is 1ppm.

Similar to the packs prepared with Corelle lettuce, the water-soaking of the cucumbers packed with Romaine lettuce and carrots started to manifest during Day 10, which then became severe during Day 13 (Figure 4D). 1-MCP treatment has no effect on the water-soaking of the cucumbers packed together with carrots and Romaine lettuce.

Significant differences between the VQR of the samples were only observed during Day 7, 9, and 10, and a decreasing trend was observed for the VQR of the samples (Figure 4E). The average VQR of the 1 ppm RTE-SVM packs had the highest scores among the three treatments, which may indicate that 1 ppm was the optimum 1-MCP concentration that should be applied to RTE-SVM packs prepared with Romaine lettuce to attain the optimum VQR.

RTE-SVM packs prepared with Iceberg lettuce

Unlike the RTE-SVM packs prepared with Corelle and Romaine lettuce, the packs prepared with Iceberg lettuce became highly unacceptable after only 8 days of storage at 5-8°C regardless of the 1-MCP concentration applied. The severe discoloration observed in the Iceberg lettuce signaled the termination of the experiment.

The average O_2 levels in the control packs were generally significantly different from those of the 1-MCP treated packs, with the former almost always lower than the latter (Figure 5A). The treated samples had a minor decrease in O_2 levels, while control

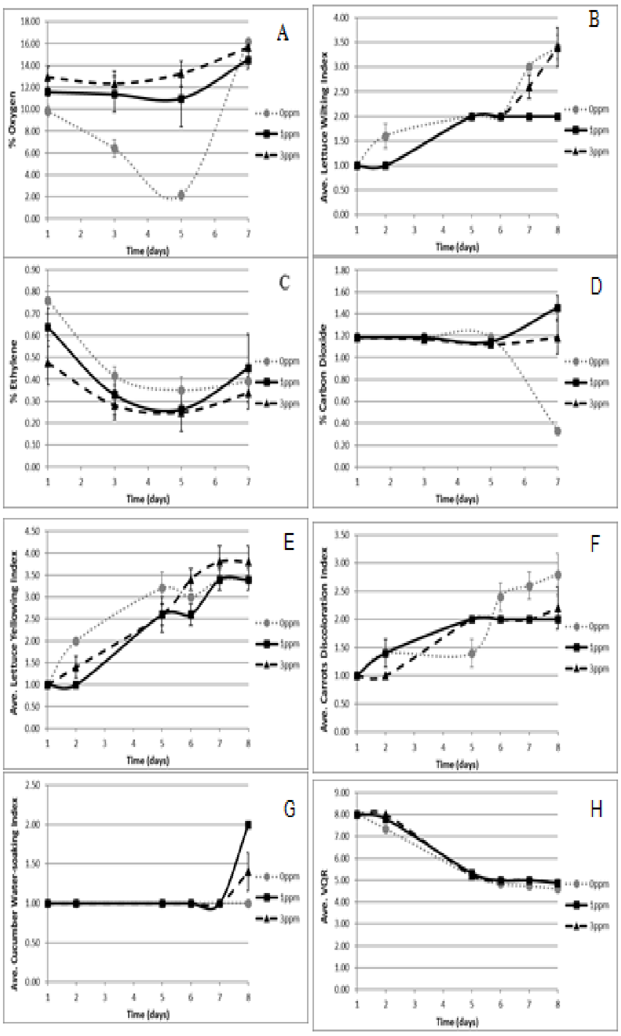

Figure 5. Oxygen concentration (A), lettuce wilting (B), ethylene concentration (C), carbon dioxide concentration (D), lettuce yellowing (E) carrots discoloration (F), cucumber water-soaking (G), and average visual quality rating (H) in the RTE-SVM packs prepared with Iceberg lettuce and treated with different 1-MCP concentrations.

samples exhibited a dramatic decrease in O_2 until Day 5. This indicates lower respiration rate (in terms of O_2 consumption) in the 1-MCP treatments.

A significant difference in CO_2 concentration was observed between the treated and untreated samples during the last day of storage (Day 7) at 5-8°C, where the treated samples exhibit the desirable increase in CO_2 in the headspace (Figure 5B). The control samples, however, exhibited a further decrease in CO_2 levels on Day 7. These results indicate a favorable response of the RTE-SVM to 1-MCP treatment in terms of lowering respiration rate.

The ethylene concentrations of the untreated RTE-SVM pack were significantly different from that of the 3 ppm treatment during Day 1. However, no significant difference was observed between the treatments onwards. The ethylene concentrations in all treatments followed the same trend. It decreased until Day 5 but increased again in Day 7 (Figure 5C). It is possible that by binding to the ethylene receptors, 1-MCP prevented the ethylene responses which include increased (autocatalytic) ethylene production (Martin-Belloso and Soliva-Fortuny, 2011), which explained the lower ethylene concentrations observed for the treated samples.

Significant differences were found between the wilting indices of the Iceberg lettuce during Days 2, 7, and 8. It was observed that the Iceberg lettuce in the control packs had higher wilting than the treated packs, with the Iceberg lettuce treated with 1ppm 1-MCP having the lowest wilting index at the end of the storage period (Figure 5D). With this, it can be said that treatment with 1ppm 1-MCP retards wilting of the Iceberg lettuce packed with cucumber and carrots.

The average yellowing/browning indices of the Iceberg lettuce inside the RTE-SVM packs injected with different 1-MCP concentrations were also determined and it was found out that the said indices of the samples differ significantly during Days 2 and 6. Iceberg lettuce yellowing/ browning was higher for the control packs until Day 5, which was then surpassed by the high occurrence of yellowing/ browning in the Iceberg lettuce in the packs treated with 3 ppm 1-MCP during the last days of storage (Figure 5E). Because of this, it can be assumed that the treatment was not effective in preventing the Iceberg lettuce discoloration if it was to be stored beyond five days at 5-8°C.

The 1-MCP-treated samples had higher carrots discoloration than the control during the first five days of storage (Figure 5F). Beyond those days, however, the carrots in the control packs had higher discoloration compared to those in the treated packs. Unlike in the Iceberg lettuce discoloration, the 1-MCP treatment could be beneficial in preventing the carrots discoloration if it were to be stored beyond five days at 5-8°C, since no further discoloration was observed beyond this storage period compared with the untreated carrots..

Water-soaking of the cucumbers inthe untreated RTE-SVM packs and those treated with 3 ppm 1-MCP were significantly different from that of the RTE-SVM packed with 1 ppm 1-MCP, with the latter being higher than the former (Figure 5G). It can be recalled that ethylene concentration in the headspace of the packs increased during the Day 7 of storage. Since water-soaking is one of the responses of cucumber to ethylene exposure, it is possible that the increase in ethylene during Day 7 triggered the onset of water-soaking during Day 8. However, because water-soaking was observed only on the samples treated with 1-MCP and not on the untreated samples, it can be said that 1-MCP influences the development of water-soaking in cucumbers packed together with carrots and Iceberg lettuce.

The average VQR of the RTE-SVM packs prepared with Iceberg lettuce were also calculated by determining the mean of the individual VQR of the carrots, lettuce, and cucumbers in each pack. Significant differences between the VQRs of the packs were observed during Days 2, 6, and 7 of storage at 5-8°C. The treated and control samples varied significantly while the 1ppm and 3 ppm samples did not significantly vary from each other. The average VQR of the untreated samples are usually lower than the VQR of the treated samples (Figure 5H). This means that treatment with 1-MCP leads to higher average VQR of the RTE-SVM packed with Iceberg lettuce.

In the RTE-SVM packs prepared with Corelle and Romaine lettuce, the main cause of deterioration and decrease in acceptability was the water-soaking of the cucumbers. Water-soaking is a response to many postharvest stresses, including chilling, exogenous ethylene exposure, and wounding (Hurr *et al.*, 2013). Although cucumbers are chilling sensitive and are injured if held at temperatures of less than 10°C for more than 3 days, modified atmosphere packaging, as in done in the experiment, increases the chilling tolerance of cucumber fruits (Karakas and Yildiz, 2007). Since water-soaking is an ethylene-induced disorder observed in some members of the Cucurbitaceae including cucumber (Hurr *et al.,* 2013), it was assumed that treatment with 1-MCP will minimize the occurrence of the disorder. This was observed in fresh-cut 'Carabao' mango slices where a delay in development of water-soaking was observed (Castillo-Israel *et al.*, 2015). 1-MCP also caused less water soaking in fresh-cut 'Sinta' papaya (Relox *et al*., 2015). However, the 1-MCP treatments performed in the experiment did not have an effect on the water-soaking of cucumber, and even hastened the disorder in the case of the RTE-SVM packs prepared with Iceberg lettuce. This result corroborates with the findings of Nilsson (2005) that cucumbers will probably show little benefit from 1-MCP treatment unless exogenous ethylene is present.

On the other hand, the main cause of deterioration of the RTE-SVM packs prepared with Iceberg lettuce was the tissue discoloration (e.g. russet spotting, rusty brown discoloration, pink rib, etc.), which was associated with the production and perception of the plant hormone ethylene, and the induction of increased phenylpropanoid metabolism as determined by Saltveit (2004). Since discoloration developed faster in the Iceberg lettuce, it can be assumed that the said lettuce variety has higher phenolic content than the other two varieties. However, Llorach *et al.* (2008) revealed that the total phenols for Romaine lettuce (63.5 mg/100g lettuce) was higher than the total phenols for Iceberg lettuce (18.2 mg/100g lettuce). It is therefore possible that Iceberg lettuce is more ethylene-sensitive than the other two varieties, and the effect of ethylene is the increase in phenolic compounds after wounding, making the lettuce tissue prone to discoloration. Pre-cutting treatment with 1-MCP was determined to reduce the occurrence of russet spotting of ribs as well as cut-edge browning (Saltveit, 2004), but if the application was made after cutting (as in the experiment), the increase in phenolic compounds was not affected (Paliyath *et al*., 2008).

It can also be noticed that all of the RTE-SVM packs, regardless of the lettuce variety used, had the highest ethylene concentration on Day 1, but maintained a lower level on the succeeding days of storage at 5-8°C. This may be because although wounding stimulates ethylene synthesis in many plant tissues, wound-induced ethylene production from lettuce has very low rates compared with other species, and they return to non-stressed control levels within 24 h (Saltveit, 2004).

Gas analysis results also revealed that the RTE-SVM packs mostly did not reflect the ideal conditions in a modified atmosphere packaging, where the O_2 levels usually decrease and the CO_2 concentrations increase. This might be due to the un-hermetic nature of the sealing of the RTE-SVM packs and gas permeability of the packaging material. However, based on the results of most visual evaluations (average VQR, lettuce wilting and yellowing, and carrots discoloration), it was found out that the RTE-SVM packs prepared with Corelle lettuce responded favorably to treatment with 3ppm 1-MCP. On the other hand, the RTE-SVM packs prepared with Romaine and Iceberg lettuce responded positively well with 1 ppm 1-MCP.

Conclusion

The RTE-SVM prepared with Corelle lettuce responded favorably to 3 ppm 1-MCP based on the results of visual evaluation, and high average VQR, lower incidence of Corelle lettuce wilting and yellowing and carrots discoloration.Levels of gases (ethylene, O_2 and CO_2) inside the packs were generally not affected by 1-MCP treatment. The RTE-SVM packs prepared with Iceberg and Romaine lettuce, on the other hand, can be treated with 1ppm 1-MCP to reduce the incidence of lettuce wilting and yellowing and carrots discoloration in the packs. The expected shelf-life of the packs was set when the average VQR of the packs reached a VQR of 6 (fair, moderate defects, limit of marketability) to meet the strict requirements of the target market. Therefore, based on the results of the calculated average VQR, the shelf lives of the RTE-SVM packs prepared with Corelle, Romaine, and Iceberg lettuce were 8, 10, and 5 days, respectively when stored at 5-8^{o}C.

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