International Food Research Journal 25(4): 1403-1409 (August 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Physiological and physical responses of packaged minimally processed *Pinakbet* vegetables to 1-methylcyclopropene (1-MCP) pre-cutting treatment

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

[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]*Laguna State Polytechnic University- Los Baños, 4030, College Laguna*

Article history

Received: 5 April 2017
Received in revised form: 2 June 2017
Accepted: 11 June 2017

Keywords

Pinakbet mix vegetables
1-methylcyclopropene
Pre-cutting treatment
Ethylene
Minimally processed vegetables

Abstract

The physiological and physical responses of minimally processed *pinakbet* vegetables (MPPV) to 1-MCP pre-cutting treatment were evaluated to determine its possible use as a tool in maintaining the storage quality. *Pinakbet* mix vegetables consisting of string beans, okra, bitter gourd, eggplant and squash were exposed to 3ppm 1-MCP prior to cutting, followed by packaging in PET trays overwrapped with PVC cling film and storage at 8-10°C. Physical quality indices such as browning of the cut edges, color changes, firmness and overall visual quality were determined for each vegetable in the pack. Physiological changes were also measured as changes in concentration of headspace ethylene, carbon dioxide and oxygen. The pre-cutting application of 3 ppm 1-MCP suppressed the biosynthesis of ethylene as shown by decreasing headspace ethylene levels which correlates with the decreased headspace oxygen concentrations. However, it did not affect the respiration rate as headspace carbon dioxide was maintained close to 1%. The pre-cutting application of 3 ppm 1-MCP to MPPV maintained storage quality of vegetables as shown by suppressed development of brown spots in string beans. The formation of white blush in squash was also significantly alleviated and firmness was retained. Discoloration of eggplant was also lessened as shown by L^*,a^* and b^* values. No effect on okra and bitter gourd were observed. Pre-cutting 1-MCP application can be potential tool in maintaining storage quality of minimally processed *pinakbet* vegetable mix. To the best of the authors' knowledge, this is the first report on the application of 1-MCP on *pinakbet* vegetable mix to test its efficacy in maintaining its storage life.

Introduction

A number of minimally processed products are presently available in the Philippine market. There had been a marked upward trend for these products especially for fruits and vegetables, such as papaya, durian, watermelon and mango, either alone or in combination with some other fruits. There is also gaining recognition for fresh-cut vegetable ingredients for dishes such as chopsuey, *pinakbet*, kare-kare and sinigang.

Exposure to stress induced during handling and preparation marks the main difference of fresh-cut products from the whole ones. According to Siddiqui *et al.* (2011), the processes used for preparing fresh-cut products cause mechanical injury in the tissues, altering its physiology and increasing deterioration.

A lot of physico-chemical and biochemical changes in fruits and vegetables during minimal processing are induced by the elevated ethylene production. Ethylene is known to be the ripening hormone in plants. According to Watada *et al.* (1990), ethylene production speeds up ripening, thus resulting to a difference in a physiological age between intact and sliced tissue. Similar to ethylene, 1-MCP is a low molecular weight, unsaturated hydrocarbon gas that readily diffuses through plant tissues. When exposed to 1-MCP, plant tissues lose the capacity to respond to ethylene due to the interaction between 1-MCP and ethylene receptors (Sisler and Serek, 2003). The application of 1-MCP fresh-cut processing systems has been conducted in three ways: 1) treatment of freshly harvested crop before longer-term storage after which the product is processed, 2) treatment of whole product just before processing, or 3) treatment of fresh-cut product immediately after processing (Toivonen, 2008).

The study on 1-MCP application to fruits and vegetables in the local market poses a significant impact in the minimal processing industry, the fact that no protocol could be generalized based on single study alone and since results deviate from

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

commodity, cultivar, application and place. Benefits for Philippine agriculture and business sector can be drawn upon the standardized methods and condition of applications, such that shelf-life of minimally processed products can be extended, thus increasing the selling period without compromising the quality.

This study focused on one of the most common form of minimally processed vegetable mixes in the Philippines, the *Pinakbet* mix, which is composed mainly of tropical vegetables. *Pinakbet* is a popular Filipino dish, and so these vegetable mixes are sold widespread in both wet markets and grocery stores. Having a limited shelf-life ranging from 1 day at ambient and 3 days in refrigerated storage, *pinakbet* mix is a good candidate to test 1-MCP as a possible tool to maintain its storage quality.

Materials and Methods

Procurement and sample preparation

The vegetables constituting the Minimally Processed *Pinakbet* Vegetables (MPPV) were string beans, bitter gourd, squash, okra and eggplant, procured in San Pablo City, Laguna, the day prior to processing. The vegetables were washed and disinfected with chlorinated water (200 ppm). Inedible portions were removed and the vegetables were cut into desired sizes, excess water was shook off using a salad spinner. The eggplant slices were treated with 1.25% ascorbic acid as anti-browning agent. The sliced vegetables were then packed in PET tray and overwrapped in PVC cling film (Figure 1).

A beaker containing the weighed amount of 1-MCP powder (AnSIP™, 0.43% 1-MCP) was placed inside the chamber containing the whole vegetables. The 1-MCP gas was generated upon adding water to the powder afterwhich the chamber was immediately closed and stored for 3 hours at 8-10°C. The vegetables where then minimally processed and packaged accordingly.

Measurement of headspace gas concentrations (ethylene, oxygen, carbon dioxide)

Headspace gas was withdrawn from the package using a 1 mL syringe. The headspace ethylene level was determined using gas chromatograph (Shimadzu GC-SA) equipped with flame ionization detector (GC- FID) with the following settings: injection port temperature- 120°C, column temperature – 100°C, column length - 2.0 m, inner diameter: 3.0 mm, and gas flow rate: 35 mL/min. For headspace O_2 and CO_2, a gas chromatograph (Shimadzu GC-SA) with thermal conductivity detector (GC-TCD) was used with the following settings: injection port temperature – 90°C, column temperature – 50°C, gas pressure - 1.25 kg/cm^2. Headspace gas concentrations were calculated as:

Figure 1. Preparation of MPPV (a) removal of inedible portion (b) cutting (c) cutting of eggplant under water (d) eggplant dipped in 1.25% ascorbic acid (e) drying of vegetables using a salad spinner (f) packaging of plastic tray overwrapped with PVC cling wrap (g) final product: minimally processed pinakbet vegetables (MPPV).

%Ethylene= (peak height of sample)/(peak height of standard) X 1 ppm (std)

%Oxygen =(peak height of sample)/(peak height of standard) X 15% (std)

%Carbon dioxide=(peak height of sample)/(peak height of standard) X 1% (std)

Visual quality rating (VQR)

The overall acceptability of the sample was measured using VQR. The following scores with corresponding description applied: 9,8 (excellent , field fresh); 7,6 (good with minor defects); 5,4 (fair with moderate defects); 3 (poor with serious defects/ limit of saleability); 2 (limit of edibility); 1 (non-edible under usual conditions). This VQR scale is established and is being used by the Postharvest Horticulture Training and Research Center (PHTRC), UP Los Banos.

Browning of the surface

Browning of the surface was evaluated as follows: 1 (slightly noticeable), 2 (up to 10% of the surface discoloured), 3 (11-25% of the surface discoloured), 4 (26-50% of the surface discoloured), 5 (>50% of the surface discoloured).

Formation of white blush

Particularly in squash, formation of white blush was evaluated as follows: 1 (white blush absent), 2 (slight, up to 10% of the surface), 3 (moderate 11-60% of the surface), 4 (severe 61-100% of the surface).

Color evaluation

Color values (L^*, a^*, b^*) were monitored using Konika Minolta Color Reader CR-10. L^*-value stands for lightness, a^* value represents the red/green opponent along the a^*-axis. Green is at negative a^* values while red is at positive a^* values. The b^* value represents the yellow/blue opponent where blue is at negative b^* values and yellow is at positive b^* values.

Firmness measurement

A hand-held penetrometer was used to measure the firmness of each commodity. Pointed tip plunger was used. The values were reported in kg force.

Interpretation of results

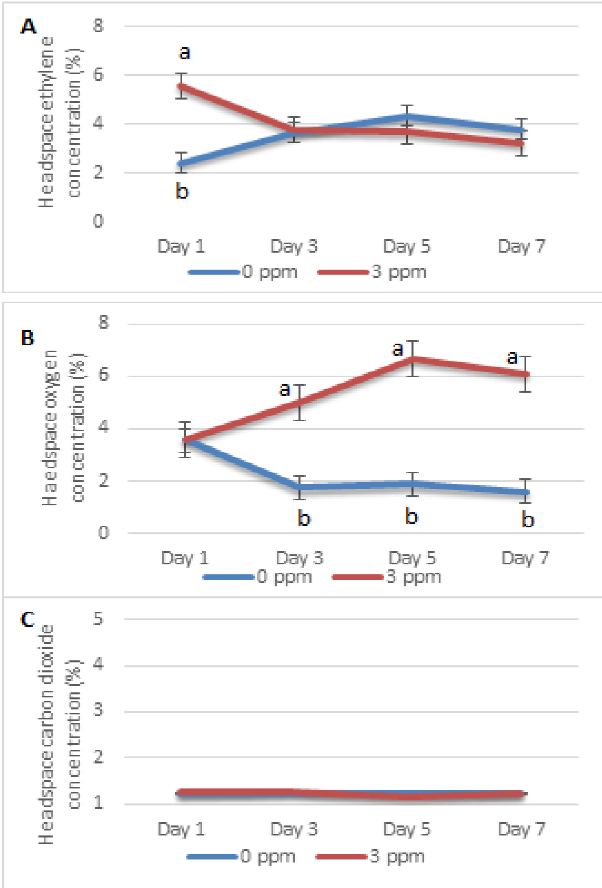
Data were analyzed statistically for significant differences using LSD at 95% confidence level. SAS software was used.

Results and Discussion

Headspace gas concentrations

Headspaces gas concentrations (ethylene, oxygen and carbon dioxide) were measured to determine the physiological changes taking place in the MPPV packs during storage as affected by 1-MCP application. MPPV packs with 3 ppm 1-MCP pre-cutting treatment were shown to have higher ethylene production compared to the control on day 1 (Figure 2). However, during storage, the ethylene of the treated packs declined while that of the control increased.

The trend for the two treatments varied specifically for oxygen and ethylene, wherein, elevated oxygen concentration was observed for the treated package while decreasing trend was observed for the control. The subsequent change in the oxygen concentration is coupled with the inverse trend for ethylene. The inverse relationship between oxygen and ethylene throughout storage follows the relationship between ethylene and oxygen in the ethylene biosynthetic pathway. Since oxygen is a pre-requisite in the anabolic pathway of ethylene, specifically for the ACC oxidase catalyzed reaction, the decreasing trend of oxygen implies its consumption for ethylene synthesis (control package having higher ethylene production, lower for 1-MCP treated). The pre-cutting application of 3 ppm 1-MCP possibly have taken its effect by inhibiting the synthesis of ethylene in MPPV.



Values with different letter symbols are significantly different at 5% level based on LSD

Figure 2. Headspace gas concentrations in MPPV during storage at 8-10°C (a) % ethylene (b) % oxygen (c) % carbon dioxide

Blankenship (2001), described the process of ethylene attaching to the receptor as a key fitting in a lock, with ethylene as the key and the receptor as the lock. When ethylene attaches to the receptor, ethylene unlocks and opens a door cascading different events leading to senescence. In contrast, 1-MCP blocks ethylene binding to its receptors. The fruit/vegetable may still produce some ethylene but does not respond. 1-MCP is also able to attach to the ethylene receptor. It also acts as a key that goes into the lock due to structural similarities with ethylene. However, unlike ethylene, 1-MCP is not capable of triggering ethylene responses inside the cell. When 1-MCP has already saturated the receptors present in the cells, ethylene is no longer able to bind, which results to inhibition of ethylene responses.

Cutting or wounding of tissues trigger the production of ethylene. When 1-MCP is applied prior to wounding, 1-MCP occupies the receptors ahead of ethylene. This results to accumulation of the ethylene produced after wounding. This explains the observed effectiveness of pre-cutting treatment of 1-MCP in controlling ethylene levels as well as ethylene-induced responses. Following the established mechanism of 1-MCP and ethylene action, the proposed rationale behind the increase in ethylene of the pre-cutting treated packs was the saturation of cells with 1-MCP prior to the production of ethylene as triggered by minimal processing activities. As inhibitor, the presence of 1-MCP before wounding secures the receptor cell slots for ethylene binding, disabling the latter to bind into the receptor sites. Since the commodities continued to respire at postharvest, there is continuous production of ethylene which supposed to bind with the ethylene receptors. However, 1-MCP already occupied the receptors thus; ethylene accumulates at the headspace of the packaging. Compared with the post-cutting application,

The production of carbon dioxide within the package represents the respiration rate of the commodity. No significant differences were observed between the treatments which may be due to the nature of the vegetables which is non-climacteric, having relatively low respiration rates. The CO_2 levels were maintained at about 1.2% throughout storage.

The pre-cutting application of 1-MCP to MPPV was proven to be effective. The combined effect of cold storage and 1-MCP treatment did not only suppress the production of ethylene, rather it keeps the oxygen level below 8% but not lower than 2%, creating the ideal MAP to maintain the quality of the produce. According to Kendra (2010), reduction of oxygen concentration below 8% along with increased carbon dioxide level above 1% decreased the onset of ripening.

String bean

The progress of shriveling of string beans was observed throughout storage but with no significant difference between the two treatments. However, the visual rating of fresh-cut string beans in the control packages significantly dropped on the 5th day of storage along with the observed formation of brown spots on the skin (Figure 3). The presence of brown spots however, were not observed in the 1-MCP treated packages. Similar results were observed by Cho *et al.* (2008) on 'Thoroughbred' and 'Carlo' green beans. Their study showed that application of 0.5 µL/L 1-MCP and storage at 7°C was effective in delaying color change, brown spots incidents and water soaking in both cultivars. The formation of

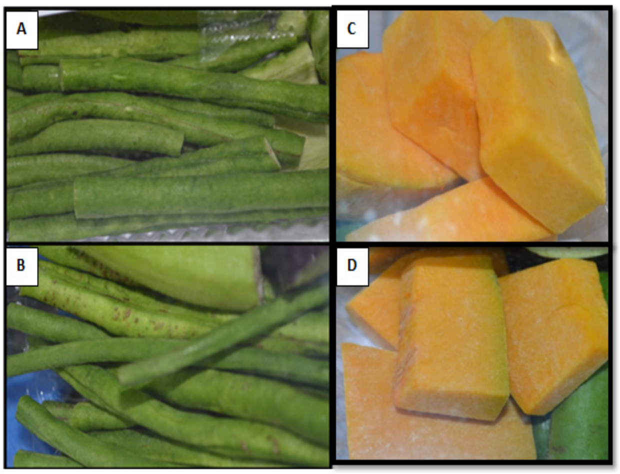


Figure 3. Brown spots and white blush observed in MPPV vegetables during storage at 8-10°C: (a) string beans treated with 1-MCP, (b) string beans without 1-MCP treatment, (c) squash treated with 1-MCP, (d) squash without 1-MCP treatment.

brown spots which was hypothetically referred to as chilling injury disorder was reduced in 1-MCP treated beans, thus alleviating symptoms of senescence and chilling injury.

Okra

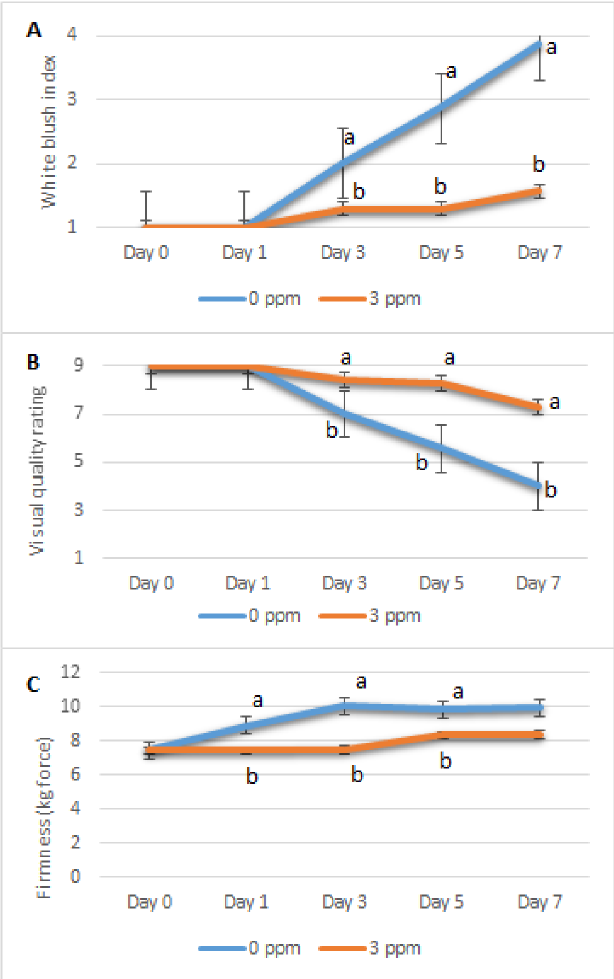
The quality parameters of okra such as shriveling and browning of the cut edges (data not shown) did not significantly vary for the two treatments. After seven days of storage, the VQR of okra was rated 5. The physical appearance deemed it still saleable.

Bitter gourd

Throughout the experiment, fresh-cut bitter gourd had the best keeping quality among all the vegetables in the MPPV. Consistent with the result in okra, the application of 1-MCP did not significantly affect the quality of bitter gourd. After seven days of storage, fresh-cut bitter gourd scored 6 for its visual quality rating (data not shown).

Squash

The most obvious physical change observed in squash was the formation of white blush (Figure 3). Subjecting the squash to lower temperature under modified atmosphere packaging caused tissue desiccation, evidently seen as white blush on the surface. The same observation was noted by Simões *et al.* (2010) in carrots and Vitti *et al.* (2004) in beets. The formation of white blush in squash was more pronounced in the control which commenced on Day 3 and rapidly increased during storage (Figure 4). This led to a more rapid deterioration of squash without 1-MCP treatment which greatly determined its visual quality rating. On the other hand, 1-MCP



Values with different letter symbols are significantly different at 5% level based on LSD

Figure 4. Physical changes in squash during storage at 8-10°C. (a) development of white blush, (b) visual quality rating, (c) firmness.

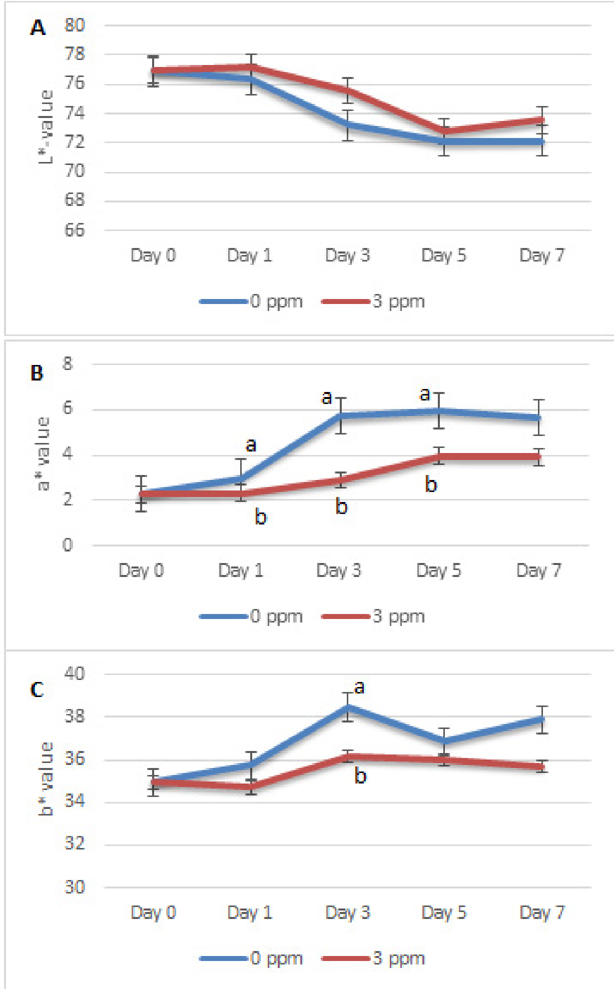


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Figure 5. Color values of eggplant in MPPV during storage at 8-10°C. (a) L^*value, (b) a^*value, (c) b^* value.

treatment effectively delayed development of white blush in squash starting at Day 3 resulting to higher visual quality scores.

Firmness is another attribute wherein 1-MCP significantly affected the quality of minimally processed squash in MPPV. Significant difference in firmness was observed on Day 3 onwards, with the treated squash having lower firmness than the control. The 1-MCP pre-cutting application has shown to have a negative effect on firmness. Mechanical wounding may promote an increase in ethylene production that can initiate physiological responses like softening related to cell wall degrading enzymes (Vilas-Boas and Kader 2006). Decline in firmness is a manifestation of cell wall breakdown mainly brought about by cell wall degrading enzymes such polygalacturonases (PG) which degrade the pectins.

Effects of 1-MCP on firmness highly varied. The mode of application, as to whether the application is pre-cutting or post-cutting had shown to be a factor in previous studies. Jeong *et al.* (2004a) observed that tomatoes post-treated with 1 µL/L 1-MCP at 5°C for 24 hours and stored at 5°C have retained firmness compared to untreated. No benefit was observed for the pre-cutting treatment. Jeong *et al.* (2004b), in another study, found that the treatment with 1-MCP of the entire cantaloupe muskmelon immediately before cutting into cubes had no effect on firmness or quality retention of cubes. On the other hand, Ergun *et al.* (2006) noted that minimally processed papaya had improved firmness retention when intact fruit was treated with 1-MCP. Likewise, Mao *et al.* (2007) obtained higher firmness in fresh-cut kiwi with pre-cutting 1-MCP treatment. Vilas-Boas and Kader (2006) reported that banana did not respond when whole fruit were treated before cutting, however, a post-cutting application reduced softening of banana slices.

Eggplant

The most prominent physical change observed in eggplant is the browning discoloration. Browning in fruits and vegetables is one of the most important factors which hinders the storage life of minimally processed fruits and vegetables. This is enzymatic in nature, involving phenylammonia lyase (PAL) which synthesizes phenolics, which in turn are oxidized by polyphenol oxidase (PPO) to brown polymers which contribute to tissue browning(Siddiqui *et al*., 2007). Minimal processing involves interaction of various physiological processes including wound-induced respiration and greater browning potential due to damage of tissues that allows contact of enzymes and substrates at the tissue surface (Calderon-Lopez *et al.*, 2005).

Fresh-cut eggplant with 1-MCP had a lesser extent of discoloration based on its L^*,a^*,b^* values (Figure 4). Discoloration as measured by L^*a^*,b^* values indicated that the extent of browning in control is higher than in the treated sample. L^*-value, which indicates the degree of lightness, decreased for both treatments with a steeper decline in the control. On Day 3 onwards, significant difference in a^*-value existed between the two treatments. Yellowing of the pulp as measured by b^*-value increased during storage, with a higher value for the control.

Similar results were obtained by Massolo *et al.* (2011) in eggplant treated with 1-MCP at concentration 1µL/L for 12 hours at 20°C. Fruit calyxes of treated eggplant were greener and have reduced damage. Budu and Joyce(2003) were also able to observe higher retention of L-values in pineapples with pre-cutting 1-MCP treatment. In 'Carabao' mangoes however, 1-MCP did not elicit positive effects on alleviation of browning as shown by the L-values (Castillo-Israel *et al.*, 2015). Thus the effect of 1-MCP on browning seem to vary among species. This may be due to the difference in the nature of tissues, the activities of browning-related enzymes and the amounts of substrate phenolics innate in the commodity.

Conclusion

The pre-cutting application of 1-MCP to MPPV was shown to be effective in controlling physiological processes in the vegetables such as ethylene production. Decreased headspace ethylene concentrations were observed in the packs of treated samples while increasing trend was observed in the control. This goes along with the inverse trend for oxygen. Results implied that there was an inhibition of ethylene biosynthesis in treated packs. The pre-cutting 1-MCP treatment has also shown to maintain the storage quality of individual vegetables in the MPPV mix. Suppressed formation of brown spots and alleviation of white blush was observed in string beans and squash, respectively. Firmness in squash however, was not alleviated. Discoloration of cut surface area was lessened in treated eggplant as shown by L^*,a^* and b^* values. For okra and bitter gourd, 1-MCP did elicit significant effects on their physical properties during storage. This is the first study reported on the possible application of 1-MCP as a pre-cutting treatment to maintain the keeping quality of minimally processed *pinakbet* vegetables.

Acknowledgements

The authors would like to thank the UP System Emerging Interdisciplinary Research (EIDR) Program (OVPAA-EIDR P-19-612-102) for providing funds for this research. The author is also deeply grateful to Romel Felismino for the kind assistance.

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