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# Lysophosphatidylethanolamine Treatment Delays Leaf Senescence and Improve Fruit Storability in Melon (*Cucumis melo* L.)

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**Abstract.** The influence of lysophosphatidylethanolamine (LPE) on anti-senescence of melon leaves and the change in fruit quality during the storage at low temperature were studied. In most of the crops, freshness of leaves is important factor for characteristics of fruits, such as sugar contents, color, and firmness. Melon (*Cucumis melo* L. cv. Prince) plants were sprayed with LPE at 5 and 3 weeks before commercial harvest. In upper part, LPE treatment showed slight high number of fresh leaf compared to no treatment (None). However, in lower part, LPE resulted in apparent inhibition effect on senescence, showing that lower side of melon plant kept fresh upon LPE application up to about 30%. The SSC of melon treated with LPE was similar to that of fruit from None at harvest. There was no change in soluble solids content (SSC) for all treatment during the storage at 7°C. There were no significant differences in firmness of mesocarp from melons given different treatments at harvest. The firmness of mesocarp from melon treated with LPE was higher than none after 2 weeks storage. The electrolyte leakage means for melon treated with LPE did not differ significantly from the means at initial storage after 2 weeks storage among the treatments. None increased 57% from its initial electrolyte leakage during storage. These results suggest that the application of LPE may have potential to inhibit senescence of leaves and maintain fruit quality during the storage in melon.

***Additional key words*:** electrolyte leakage, firmness, phospholipid, soluble solids content

## Introduction

Lipids are known to play important role in biological membrane structure, energy source, and metabolic pathway. Recent studies suggest that lipids and their metabolites play roles in cellular signal pathways, which contain signal transduction, cell activation/proliferation, and hormonal regulation (Cowan, 2006; Divecha and Irvine, 1995; Ryu et al., 1997). Lysophospholipids, particularly classified from lipids group, have interesting actives in plant cells. Concentration of lysophospholipids changes during chilling (Welti et al., 2002) and during cell expansion (Lee et al., 2003; Scherer, 2002) even though they present in membranes a little amount. Lysophosphatidylethanolamine (LPE) is a minor lipid present in all cell membranes and is formed from phosphatidylethanolamine (PE), a parent phospholipid, by the action of phospholipase $A_2$ ($PLA_2$).

Previous studies showed that LPE, natural phospholipid, can accelerate ripening and prolong the shelf life of tomato fruit (Farag and Palta, 1993a), and enhancing ethylene production in the fruit tissues (Farag and Palta, 1989). LPE also can retard senescence in attached and detached leaves and fruit of tomato (Farag and Palta, 1993b). In other studies, LPE application has been found to extend vase-life of cut flowers (Kaur and Palta, 1997). LPE inhibited the activity of phospholipase D (PLD), a membrane degrading enzyme, of which active is increased during senescence (Ryu et al., 1997). More recently, it was observed that LPE increased marketable yield by extending shelf life of green pepper (Hong and Chung, 2006). Hong (2006) demonstrated that LPE can enhance the activity of ACC oxidase in mature green tomato fruit, and also reported that the influence of LPE treatment on fruit was dependent on the stage of ripening. LPE stimulated ripening in a mature green fruit (ready to ripen), while in a ripened fruits, it inhibited ethylene production and maintained fruit firm thereby prolong shelf life. It is reported that LPE can also accelerate color development and promote shelf life of cranberries (Ozgen et al., 2004), and increase fruit qualities of Thompson seedless grapes, in such as soluble solids contents (SSC), titratable acidity (TA), firmness, and size (Hong et al., 2007). Along with these results, it shows that LPE can accelerate ripening of fruits and also

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has potential to protect senescence.

As shown above, LPE has been found to play various physical roles in plants, which was interest to study the effect of LPE on plant and fruit quality of melon. The commercial value of melon is related to SSC, size, and storability which are induced from healthy state of plant. To maintain plant fresh, contributing to fruit quality, is thus most important issues to growers. Grower limits or controls watering for near harvesting time to increase quality. Leaf senescence often limits yield and quality of melon fruit. It was reported that LPE can mitigate stresses of plants from biotic and abiotic factors. Therefore in this current study, the effects of LPE application at preharvest on leaf senescence, fruit quality, and storability in melon fruit were investigated.

## Materials and Methods

### Plant Materials and Application

The study was performed in green house, located at Andong-si, Gyeongsangbuk-do, Korea. Experimental plots were established in commercial melon (cv. Prince) beds. Plots were sprayed by commercial air blaster sprayer which is equipped as green house facility with height of 2.4 m. Every plot was sprayed with 1,000 mL solutions, and separated by 5 m buffer area. To evaluate fairly, for all treatments, even row against treated one was selected carefully in the center of green house. The spray solution included LPE (10 $mg \cdot L^{-1}$) developed as a water soluble product which contains emulsifying agent such as tween 80, glycerol, and fatty acid salt. LPE derived from egg lecithin was used in experiments and was produced from Doosan Glonet, Iksan-si, Jeollabuk-do, Korea. To investigate the effects of sub-materials for resolving LPE on this experiment, another application was made for the formulation without LPE as a mock.

Experimental design was a randomized complete block with 14 treatments. Experimental observation was performed with 1 plant sited in the middle among 3 treated plants. As same as treated plots, 1 plant sited in the middle among 3 plants was selected for the untreated (None) and the mock (Mock) controls. Spray application were made two times on August 4 and 18, 2011, of which times were 5 and 3 weeks before harvest (WBH). Fruit were harvested on September 8, 2011.

### Quality Measurements

Leaf observations are carried out at 3 WBH ($2^{nd}$ application) and harvest time. Each melon plant had 12 leaves on the upper part of plant and 12 leaves on lower part. Leaves were graded into four qualities according to the degree of rot, disease, wilt or senescence showing area: Fresh, above 90% healthy; Good, 90-70% healthy; Lack, 70-30% healthy; Bad, below 30% healthy. To remove individual bias, the leaves were graded by three researchers without treatment identification.

For soluble solids content (SSC) and titratable acidity (TA) analyses at 20°C, samples removed from cold storage were kept at room temperature until adjustment and were homogenized in a blender. A refractometer and an autotitrator were then used to estimate SSC and TA, respectively. TA was measured by titrating the sample to pH 8.2 using 0.1 $mol \cdot L^{-1}$ NaOH. Acidity was expressed as an acid meq factor of 0.065.

Weight loss was calculated as the percent difference in weight for each fruit from the day of harvest to 1 or 2 weeks storage. Firmness of whole-fruit equatorial hypodermal mesocarp tissue was measured using a 4.5 cm × 3 mm diameter V-tip gauge (Chatillon force gauge; Lindy Electric, NY, USA) after peeled epidermis of fruit and expressed as mean force in Newtons (N).

Electrolyte leakage was determined on three mesocarp disks (10 mm diameter × 1 mm thick) from three different locations. Disks were placed in 30 mL of 0.4 $mol \cdot L^{-1}$ mannitol and incubated at 30°C for 6 h. Twenty milliliters of suspension was taken and conductivity measured using a conductance meter (YSI model 32, Yellow Spring, Ohio, USA). Total electrolytes were determined on the same sample disk that those were thawed after freezing at -20°C for 24 h. Electrolyte leakage was expressed as a percentage of total electrolytes.

### Statistical Analysis

A Randomized Complete Block Design with three replicates of four units each (from each location) was followed in this experiment. Analyses of Variance (ANOVA) were performed using the Statistical Analysis Systems computer package (SAS Institute, 2004, Cary, NC, USA). Differences between treatment means were compared by the Duncan's multiple range test at $p = 0.05$.

## Results and Discussion

The leaf is a major photosynthetic organ in plants. Leaf development initially requires an input of nutrients from the plant. The payoff for this investment occurs as the leaf is converted from a nutrient sink to a nutrient source, when photosynthetic competence is achieved. After a productive photosynthetic period, leaf cells enter the senescent phase (Bleecker and Patterson, 1997). The leaf of melon becomes foliage brown as grown to harvest season. In this study, LPE showed the ability of maintaining leaf health of melon in green house (Table 1). All of leaves in each treatment were kept fresh until $2^{nd}$ application, 3 WBH. Symptom of disease on leaves was not shown within experimental plots but

**Table 1.** Effect of LPE on leaf senescence 3 weeks before harvest and at harvest. Leaf condition was observed at 3 weeks before harvest (2nd application) and harvest time. Melon (cv. Prince) plants grown in green house were foliar-sprayed with 10 mg·L$^{-1}$ LPE on August 4 and 18, 2011, of which times were 5 and 3 weeks before harvest (WBH). Fruit were harvested on September 8, 2011.

| Location | Treatment[y] | No. of leaf[z] | | | |
|---|---|---|---|---|---|
| | | Fresh | Good | Lack | Bad |
| 3 weeks before harvest | | | | | |
| Upper | None | 12.0 a[x] | 0.0 a | 0.0 a | 0.0 a |
| | Mock | 12.0 a | 0.0 a | 0.0 a | 0.0 a |
| | LPE | 12.0 a | 0.0 a | 0.0 a | 0.0 a |
| Lower | None | 12.0 a | 0.0 a | 0.0 a | 0.0 a |
| | Mock | 11.7 a | 0.3 a | 0.0 a | 0.0 a |
| | LPE | 12.0 a | 0.0 a | 0.0 a | 0.0 a |
| At Harvest | | | | | |
| Upper | None | 10.0 a | 0.7 a | 1.0 a | 0.3 a |
| | Mock | 10.0 a | 0.7 a | 0.3 a | 0.9 a |
| | LPE | 11.7 b | 0.3 a | 0.0 a | 0.0 a |
| Lower | None | 3.1 b | 0.4 a | 0.7 a | 7.7 a |
| | Mock | 4.0 b | 0.1 a | 1.0 a | 6.7 a |
| | LPE | 7.9 a | 0.6 a | 1.0 a | 2.4 b |

[z]Number of leaf with the same grade of leaf status according to symptom: Fresh (above 90% healthy area), Good (90-70% healthy area), Lack (70-30% healthy area), Bad (below 30% healthy area).
[y]None, no treatment; Mock, emulsifying agent (tween 80, glycerol, and fatty acid salt); LPE, LPE plus emulsifying agent.
[x]Means within day and column with different letters are statistically different at $p$ = 0.05 according to Duncan's multiple range test.

**Table 2.** Change in fruit quality of melon fruit applied with LPE during the storage at 7°C. Melon (cv. Prince) plants grown in green house were foliar-sprayed with 10 mg·L$^{-1}$ LPE on August 4 and 18, 2011, of which times were 5 and 3 weeks before harvest (WBH). Fruit were harvested on September 8, 2011.

| Treatment[z] | SSC[y] (%) | TA[x] (%) | Firmness (N) | Wt[w] loss (%) | EL[v] (%) |
|---|---|---|---|---|---|
| At harvest | | | | | |
| None | 12.4 a[u] | 0.14 a | 19.4 ab | 0 | 20.1 a |
| Mock | 12.2 a | 0.14 a | 18.9 b | 0 | 19.0 a |
| LPE | 12.8 a | 0.13 b | 20.2 a | 0 | 19.3 a |
| After 1 week storage | | | | | |
| None | 12.3 a | 0.14 a | 16.8 b | 2.2 a | 17.2 a |
| Mock | 12.6 a | 0.14 a | 17.2 ab | 1.9 a | 19.0 a |
| LPE | 12.9 a | 0.13 b | 18.5 a | 1.7 a | 18.2 a |
| After 2 week storage | | | | | |
| None | 12.4 a | 0.13 a | 10.3 b | 3.2 a | 31.6 a |
| Mock | 12.5 a | 0.13 a | 7.4 b | 3.9 a | 31.1 a |
| LPE | 13.0 a | 0.12 b | 12.7 a | 2.6 b | 22.0 b |

[z]None, no treatment; Mock, emulsifying agent (tween 80, glycerol, and fatty acid salt); LPE, LPE plus emulsifying agent.
[y]Soluble solids content.
[x]Titratable acid.
[w]Weight.
[v]Electrolyte leakage.
[u]Means within day and column with different letters are statistically different at $p$ = 0.05 according to Duncan's multiple range test.

senescence of leaves was presented resulting in fallen leaves at fruit harvest time. Count investigation was performed with two parts of plant, which are the upper part and the lower part of fruit, since leaves of upper part are younger than those of lower part. Leaves located in upper part are generated at the middle of growth, while the lowers are generated at the beginning of growth. As shown in table 1, difference in the degree of leaf senescence between None and Mock was not significant in upper location, and LPE treatment showed slight high number of fresh leaf compared to other treatments. However, LPE resulted in apparent inhibition effect on senescence of leaves in lower part, showing that lower side of melon plant kept fresh upon LPE application up to about 30% (4/12 leaves), and number of bad leaf treated with LPE was statistically lower than None. There was no difference in senescence between None and Mock in lower location.

The SSC of melon treated with LPE was similar with that of fruit from None and Mock at harvest. There was no change in SSC for all treatment during the storage at 7°C (Table 2). TA generally decreased for melon from None and Mock during storage, but TA of melon treated with LPE was maintained during the storage. Melon treated with LPE had lower TA than None and Mock at harvest. There were no significant differences in firmness of mesocarp from melons given different treatments at harvest (Table 2). After 2 weeks of storage, the firmness of mesocarp from melon treated with LPE was higher than that of any other control. Melon treated with LPE showed slight lower weight loss compared to None and Mock at 2 weeks storage. After 2 weeks storage, only the electrolyte leakage means for melon treated with LPE did not differ significantly from the means at initial storage. Electrolyte leakage of None and Mock increased 57 and 63% from their initial means during storage, respectively.

Significant difference between experimental plots was observed (Table 1). LPE treated plots had more fresh leaves than the control treatments. Although environmental practices varied through all experimental plots, LPE had a positive effect on leaf conditions. Farag and Palta (1993a) reported earlier that exogenous LPE showed the apparent leaf senescence

delaying effect. These authors demonstrated that exogenous LPE reduced catabolic respiration (measured as $CO_2$ evolved) and reduced electrolyte leakage in attached and detached leaflets of tomato. The results of the present investigation and those reported by Farag and Palta (1993a) would seem to indicate that the senescence delaying effect of LPE arises as a consequence of improvement of fruit quality. Grower had limited watering at the end period of growth for quality enhancement in commercial culture. Considering this drought condition, LPE not only may have effect of health enhancement but also have the effect of mitigation against drought stress.

The results showed that LPE application had no effect on sugar content of melon fruits (Table 2). This unexpected result might due to limitation of water as well as nutrition provided through water line. As mentioned above, grower used to limit water and nutrition at the end period of growth because watering at that time could cause cracking of fruit and over weight growth resulting in loss of quality. In general, sugars are produced at leaf and translocated to the other parts of plant (Giaquinta, 1978). It has been found that sugar accumulation in leaf stimulate senescence of leaf (Wingler and Roitsch, 2008). From our results that LPE kept leaf fresh, sugars produced were thought to be more translocated into fruit in treated than untreated, however actual values did not reflect the expectation. In this experiment, why values of sugar content had no difference between plots is presumed that plant could not produce sufficient sugar under the limitation of nutrition by the watering restriction. Analysis of sugar metabolism related enzyme and sugar content in pre-senescent leaves are recommended. Maintaining leaf health until harvest is related to fruit qualities such as color development, sugar contents, disease resistance, and storability. Result of the present study showing effect on leaf condition suggests that an application of LPE has the potential to maintain melon plant health during culture, followed by increase in storability. It is expected that LPE, natural phospholipid, has potentials for its effect on leaves and can play a positive role in eco-friendly agriculture.

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