A Postharvest Dip Treatment with Lysophosphatidylethanolamine, a Natural Phospholipid, May Retard Senescence and Improve the Shelf Life of Banana Fruit

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Abstract. Banana is one of the most consumed tropical fruits around the world. It is marketed nearly all year-around and has a relatively short shelf life. Fruits are harvested mature green and treated with ethylene to stimulate ripening before distribution and sale. The fruits generally ripen within 4–5 days after ethylene treatment and sold primarily at yellow stage of ripening. After turning yellow the fruit becomes unsuitable for marketing in 1–3 days. Thus 1–2 days of improvement in the fruit shelf life could enhance the market value of banana. Previous studies conducted in our laboratory have demonstrated that both pre- and postharvest application of lysophosphatidylethanolamine (LPE) can retard aging and improve shelf life of various fruits. The objective of this study was to investigate the possibility of improving shelf life of banana fruit by a postharvest dip in LPE. For this purpose, whole fruits obtained from the market at ripeness stage of 2.5 (about 75% green) were dipped in solution of 500 ppm LPE for 30 minutes and observed for a period of five days at room temperature. Each treatment was applied to 50 uniform fruits. From each hand bought, an equal number of fruits were separated for LPE and control treatments to reduce the variability. Five days after dip treatment, the fruit treated with LPE were firmer and thicker as compared with the control. Starch breakdown was also delayed in the LPE-treated fruits. LPE treatment slowed the development of brown spots on the peel tissue. Both peel and pulp tissue of LPE-treated fruits had lower ion leakage compared with the control. Fruit peel slices treated with LPE for 3 hours also showed a decrease in ion leakage and respiration rate at 24 hours after treatment. Results of the present study indicate that a postharvest dip treatment with LPE may improve shelf life of banana fruit by 1–2 days. These results suggest that LPE may improve shelf life by maintaining membrane integrity, reducing respiration, and slowing the breakdown of starch and cell walls during ripening and senescence of banana fruit tissue.

Banana fruit (Musa sp.); ‘Cavendish’ variety is one of the most important grown and widely consumed fruit in the world. It is also the most frequently purchased fruit in western countries (Americafruit, 2001). Commercially, bananas are harvested mature green, and shipped refrigerated to the importing countries. Upon arrival to their destination, the green bananas are stored in modified atmospheric rooms at the distribution centers where they are treated with ethylene gas before marketing (Ahmed et al., 2006). This gas treatment is known to provide uniformly ripened fruit within few days (Reid, 1992). Fruit peel color and firmness are commonly used indexes of banana marketability (Kader, 1992).

The peel color of banana fruit is the primary criterion for fruit ripening (Kader, 1992; Medlicott et al., 1992; Seymour et al., 1993). Seven distinct visual color stages have been distinguished as banana fruit ripens where the peel color changes from dark green (stage 1) to yellow (stage 5) followed by the appearance of dark brown spots (stage 7) when about 20% of the fruit surface is covered with brown spots and fruit is considered unmarketable (Tapre and Jain, 2012). These seven color stages are presented in a color chart that is now used for quality evaluation by the industry. Fruits are considered marketable as long as the peel color is mostly yellow with very little brown spots up to stage 6. Ripened banana fruit goes from marketable to unmarketable within 1–3 d. Thus, increasing the shelf life of the banana fruit by just a couple days would add significant commercial value.

Banana is classified as a climacteric fruit (Rhodes, 1970). In this fruit, series of physiological and biochemical changes are known to lead to the development of soft edible fruits (Prasanna et al., 2007; Tapre and Jain, 2012). Some of the primary changes during this ripening process include an increase in membrane permeability (ion leakage), loss of fruit pulp firmness, decrease in starch, increase in sugar, decrease of peel thickness as well as changes in color and aroma (Baur and Workman, 1964; Goldstein and Wick, 1969; Ludford, 1987; Seymour et al., 1993; Wade and Bishop, 1978).

Generally, fruits such as bananas are delicate and have a short shelf life after ripening (Kader, 1992). Historically, many postharvest treatments have been investigated to increase the shelf life of banana fruit; however, only with limited success (Kader, 1992). In several studies, a postharvest treatment with 1-methycycloprenone (1-MCP) has been tried to improve shelf life of banana fruit (Golding et al., 1998; Harris et al., 2000). In many fruit, gas treatment with 1-MCP has been found to block ethylene receptors and prevent ethylene effects on fruit tissues and delay ripening (Serek et al., 1994, 1996; Siiter and Serek, 1997). Although treatment with 1-MCP maintained fruit firmness, it also inhibited peel color changes and aroma and flavor development (Botrel et al., 2002; Jiang et al., 1999; Jiang et al., 2002; Lohani et al., 2004; Pelayo et al., 2003). Because of these undesirable effects, 1-MCP treatment is not considered commercially suitable for extending the shelf life of banana fruit (Golding et al., 1998; Pelayo et al., 2003; Watkins, 2006). More recently effect of preharvest and postharvest application of calcium has been investigated. However these treatments did not have any significant effect on the shelf life (green-life) of banana fruit.

Previous studies conducted in our laboratory have shown that both pre- and postharvest application of LPE is able to reduce senescence and promote shelf life of fruits, cut flowers, and leaf tissue (Farag and Palta, 1991a, 1991b; Farag and Palta, 1993a; Kaur and Palta, 1997; Ozgen et al., 2005; Ryu et al., 1997; Snider et al., 2003). Several studies have verified and provided additional evidence in support of the positive effect of LPE on the shelf life of red pepper, tomato, and grape fruits (Hong, 2006; Hong and Chung, 2006; Hong et al., 2007). There is additional evidence that phospholipids and lysophospholipids such as LPE are signaling molecules that can regulate plant growth and development.


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development (Chapman, 1998; Cowan, 2006a, 2006b; Laxalt and Munnik, 2002; Meijer and Munnik, 2003). The exact mechanism by which LPE can delay senescence and improve shelf life is not known. However, LPE has been found to inhibit the activity of phospholipase D (PLD), in highly specific manner (Ryu et al., 1997). PLD is known to be activated during ethylene-induced senescence (Bao et al., 2007; Fan et al., 1997; Ryu and Wang, 1995; Wang, 2001, 2002) and this activation leads to membrane breakdown. Thus it appears that LPE may be enhancing shelf life by preserving membrane health during senescence of fruit.

At present there is no commercially viable product available to improve shelf life of banana fruit. The present study was conducted to determine the potential use of a postharvest dip treatment of LPE to improve shelf life of banana fruit.

**Materials and Methods**

**Plant materials**

Dessert Bananas Cavendish cultivar (Chiquita® no. 4011) at ripeness stage about 2.5 (peel about 75% green) were purchased from a local supermarket in Madison, WI. The fruit hands were washed with bleach (0.5%) for 2 min and rinsed three times with distilled water then pat dried. The fruits were sorted for uniformity of weight and shape. To minimize variability, equal numbers of uniform fruits were separated from each hand and divided among the LPE and control (water) treatments.

**Whole intact fruit experiments**

LPE solution preparation and dip treatment of fruits. LPE derived from soy lecithin was obtained from Doosan Serdary Research Laboratories, Englewood, NJ. To prepare dip solution, LPE was suspended in distilled water by sonication as described by Ozgen et al. (2005) using (Sonic dismembrator Model 550; Fisher Scientific, Pittsburgh, PA). Fruits were dipped in solution of various concentration of LPE for 30 min and the control fruits were dipped in distilled water. Fruits were stored at room temperature and various measurements and observations were made at different time periods.

**Visual observations on fruit marketability.** Fruits were dipped in various concentration of LPE (0, 200, and 500 ppm) for 30 min and observed for color changes for 7 d. Three replicates per each treatment were used and each replication contained 20 fruits. When about 20% of the fruit surface was covered with brown spots, the fruit was considered unmarketable.

**Fruit weight loss, fruit thickness loss, fruit firmness, and ion leakage.** Fifty fruits were dipped in LPE (500 ppm) or distilled water. Each fruit was weighed at the start of the experiment and again at the end of experiment (5 d after treatment). Weight loss was determined as percent of the initial weight. For each fruit, thickness was measured at the midpoint (lengthwise) using digital caliper at the start of the experiment and again at the same place 5 d after treatment. A reduction in the fruit thickness was calculated as % changes in the fruit thickness as compared with the initial reading. Fruit pulp firmness was determined for 50 fruits using Penetrometer (Model FT-011, QA Supplies Norfolk, VA) equipped with a probe (tip diameter 10 mm). This instrument gives force of resistance (kg/mm²). The data were expressed as force needed for penetration in newtons (N) by multiplying the reading by 9.8066 (Dadzie and Orchard, 1997). For this purpose, the pressure required for the probe to penetrate to 7 mm depth into the fruit pulp was measured. Three readings were taken from each fruit at three separate locations (right, middle, and left).

**Ion leakage from the peel tissue was measured using a standard procedure (Fan and Sokorai, 2005) with a slight modification.** Briefly, the fruits were transversely sliced at the midpoint (1 cm thick) and the peel was separated. The peel was then cut into 2-mm slices and put into tubes containing 20 mL of distilled water. Initial electrolyte leakage was measured (C1) by a conductivity meter (YSI Model 32, Yellow Springs Instrument, Inc., Yellow Springs, OH) after shaking the tubes on a gyratory shaker at 250 ppm at room temperature (23 ± 2 °C) for 15 min. The samples were then autoclaved at 121 °C for 15 min and conductivity was measured again (C2). The percent of ion leakage (IL %) was calculated for all values using this equation:

\[ \text{IL} \% = \frac{C1 - C2}{C1} \times 100 \]

Total soluble solids, ion leakage from peel and pulp tissue, starch iodine test, and respiration rate. In a separate experiment, 50 fruits were dipped in LPE 500 ppm solution and distilled water as a control treatment. Three fruits were sampled from each treatment every 7 d to determine total soluble solids (TSS) and ion leakage. TSS were measured by blending 30 g of fruit pulp tissue (from the middle portion of the fruit) with 90 mL distilled water. The water was extracted by filtering the slurry. TSS were estimated by placing 1–2 drops of the filtrate on the prism of a digital refractometer (Model PR-32α, Atago Co. Ltd, Japan). The recorded value was multiplied by three to correct for sample dilution (Dadzie and Orchard, 1997). Ion leakage was measured using the procedure described above.

**Starch degradation (conversion of starch into sugar) in the fruits was visually evaluated by staining sections of fruit with iodine-potassium iodide (Blankenship et al., 1993; Dadzie and Orchard, 1997).** For this purpose, 2-cm thick transverse slices were removed and stained on one side of the cut for 5 s in iodine solution (containing 10 g potassium iodide and 2.5 g iodine per liter). From each treatment (LPE 500 ppm, distilled water) 16 fruits were randomly sampled at 5 d after treatment. Stained cut surfaces with dark blue color indicated high starch (not breakdown into free sugars) (Blankenship et al., 1993; Dadzie and Orchard, 1997).

For measuring respiration rate, three fruits were placed in a 2-L airtight container for one hour. A 10 mL gas sample was withdrawn from the headspace of the container to quantify CO₂ concentration. An infrared gas analyzer equipped with an automatic sampler (LI-COR LI-820, Lincoln, NE) was used to determine CO₂ concentration. Three independent air samples were taken per chamber daily and respiration rate were expressed as mL CO₂/kg/h on fresh weight basis. Air samples of known concentration (3 ppm CO₂) were routinely used as an internal standard. For calculation purposes, the free headspace of each chamber was estimated by subtracting the fruit volume from the total volume of each chamber. Each experiment was repeated at least three times.

**Experiments with fruit peel slices**

Fruit (at 2.5 ripening stage) peel slices (5 mm thick) were dipped in 100 ppm LPE for 3 h to investigate the influence of LPE on respiration rate and ion leakage. The slices used for each treatment were separated from same fruit and divided between two treatments (LPE and control) to reduce the variability among fruits. One day after treatment, ion leakage and respiration rates were measured using the same procedure as for whole fruits with slight modification. For the ion leakage about 10 g of peel slices were used for each replication and three replications were used for each treatment. For respiration rate the peel slices (about 10 g) were placed in a 50-mL air tight glass container for 1 h and 1-mL gas sample was withdrawn from the head space.

**Statistical analysis**

Data were analyzed by one-way analysis of variance in PROC GLM procedure of Statistical Analysis System [SAS version 9.2 for Windows, SAS Institute Inc., Cary, NC (SAS® 2009)]. Mean comparisons were performed using Duncan’s multiple range test to determine the significance differences at \( P \leq 0.05 \).

**Results**

**Whole intact fruit experiments.** Banana fruits at stage 2.5 (about 75% green) were dipped in various concentrations of LPE (0, 200, and 500 ppm) and observed for color changes for 7 d. At 5 and 6 d after dip treatment, significantly higher numbers of fruits remained marketable for 500 ppm LPE treatment as compared with 200 ppm treatment or control. For example, at day 6 after dip treatment only 21.4% fruits remained marketable for control and 47.6% for 200 ppm LPE. Whereas 64.1% fruits dipped in 500 ppm LPE remained marketable at the same time (Table 1). Repeated experiments showed that the concentration of 500 ppm LPE gave consistent results. So that for all subsequent studies the concentration of LPE 500 ppm was used.
Table 1. Effect of dip treatment in lysophosphatidylethanolamine (LPE) on banana fruit marketability.

| Treatment          | Day after dip treatment |
|--------------------|-------------------------|
|                    | 4 | 5 | 6 | 7            |
| Control            | 83.1 + 0.75 b          | 46.8 + 0.30 c | 21.4 + 1.89 c | 19.5 + 1.80 b |
| LPE 200 ppm        | 93.6 + 0.37 a          | 56.1 + 1.28 b | 47.6 + 2.07 b | 39.6 + 1.61 a  |
| LPE 500 ppm        | 96.0 + 2.02 a          | 72.9 + 2.26 b | 64.1 + 0.68 a | 43.7 + 1.58 a  |

*Banana fruits were dipped in 200, 500 ppm LPE or water (control) for 30 min and stored at room temperature for 7 d. Data for marketability were recorded from 20 fruits/replication and three replications/treatment (n = 3) starting at day 4.

*Mean values within a column that have different letter are significantly different at P ≤ 0.05 as determined by Duncan’s multiple range test (n = 3).

LPE treatment had no significant effect on fruit weight loss during ripening while it had a significantly positive effect on fruit firmness and fruit thickness at day 5 after treatment (Table 2). LPE-treated fruits had almost the same percent of fruit weight loss (6.40%) as the control (6.47%) after 5 d from the treatment. Loss of fruit thickness was about 1% less in the LPE-treated fruits as compared with the control and this difference was significant (Table 2). LPE-treated fruit firmness was about 12% higher as compared with control fruits. The average firmness of LPE-treated fruits was 93 N as compared with 82 N for control fruits (Table 2).

The electrolyte leakage from the peel tissue was significantly 3% higher in the control peel tissue samples as compared with the LPE-treated fruits. On average the ion leakage of the LPE-treated fruits was 35.9% compared with 38.78% for the control (Table 2).

Discussion

Our results show that a dip treatment of banana fruit in a 500 ppm LPE solution extended the shelf life. At 6 d after treatment, LPE treatment resulted in nearly three times more marketable fruit (64.1%) as compared with the control (21.4%) (Table 1). The LPE treatment appears to add about 2 d of shelf life. For example, 7 d after treatment 43.7% of LPE-treated fruits were marketable and this was similar to 46.8% marketable fruit in the control at day 5 after treatment (Table 1). These results are consistent with previous studies where LPE was found to improve shelf life of tomato, cranberry, red pepper, and grape fruits (Farag and Palta, 1993b; Hong, 2006; Hong and Chung, 2006; Hong et al., 2007; Ozgen et al., 2005). Once ripening is initiated, banana fruits have a relatively short shelf life. Therefore, improving shelf life by couple more days can add significant commercial value. The results of the present study show that LPE may have potential commercial value for improving shelf life of banana fruits by about 1–2 d.

It is important to point out that although LPE treatment improved shelf life, this treatment does not appear to have an undesired effect on fruit marketability and visual quality. Many studies have investigated the potential of a postharvest treatment with 1-MCP to improve the shelf life of banana fruits (Botrel et al., 2002; Jiang et al., 1999; Jiang et al., 2002; Pelayo et al., 2003; Zhang et al., 2006). 1-MCP treatment of banana fruits after ethylene treatment has shown to maintain fruit firmness, but delayed color changes as well as aroma development. Thus, 1-MCP treatment negatively affected the marketability of the banana fruits (Golding et al., 1998; Golding et al., 1999; Pelayo et al., 2003; Watkins, 2006).

Changes in cell membrane properties leading to enhanced ion leakage has been long recognized as a cellular event during ripening of climacteric fruits such as banana (Baur and Workman, 1964; Bugaud et al., 2013; Fernandes et al., 1979). Our results show that a dip treatment of whole banana fruit in LPE decreased ion leakage from both peel and pulp tissues (Tables 2 and 3). A significant decrease in this leakage was consistently observed from days 1–7 (Table 3).
been proposed as a mechanism by which LPE can decrease ion leakage from fruit, leaf, and flower tissue (Ryu et al., 1997). Therefore, LPE may be maintaining fruit firmness and delay the activity of cell wall hydrolytic enzymes in this fruit (Ali et al., 2004). An inhibition of the activity of PG by LPE (Farag and Palta, 1991a, 1991b; Farag and Palta, 1993a). Our results show that LPE-treated fruits had higher firmness of the pulp tissue at 5 d after treatment (Table 2). Fruit softening is known to result from alteration in structure and composition of cell wall, partial or complete breakdown of cell wall polysaccharides; such as pectin and cellulose, as well as hydrolysis of starch and other storage polysaccharides (Fuchs et al., 1980; Selvaraj and Kumar, 1989; Selvaraj et al., 1989; Tucker, 1993; Tucker and Grierson, 1987). Consistent with this, we found higher starch and lower sugar contents in the pulp of LPE-treated fruits (Table 3; Fig. 2). Therefore, higher fruit firmness in LPE-treated fruits could be, in part, due to slower breakdown of starch into sugar. Cell wall degrading enzymes such as polygalacturonases (PGs), pectin methyl esterases, pectate lyases, and rhamnogalacturonases are known to be involved in fruit tissue softening (Prasanna et al., 2007). Previous work on tomato fruit showed an in vitro inhibition of the activity of PG by LPE (Farag and Palta, 1992). Although several cell wall hydrolytic enzymes are known to be involved in the softening of banana fruit, polygalacturonase is known to be the major softening enzymes in this fruit (Ali et al., 2004). An inhibition of the activity of polygalacturonase by LPE was reported in tomato fruit tissue (Farag and Palta, 1992). These published reports, taken together with the results of the present study (Table 3; Fig. 2), suggest that LPE may be maintaining fruit firmness by reducing cell wall breakdown during ripening or by regulating the activities of some of these hydrolytic enzymes. This action of LPE treatment in reducing softening and that of reducing starch breakdown have been also reported for the treatment of fruits with auxin (Lohani et al., 2004). Exogenous application of indol-3-acetic acid has been shown to delay starch degradation and sugar formation in banana (Purgatto et al., 2001) and delay the activity of cell wall hydrolytic enzymes.
enzymes (Lohani et al., 2004). Therefore, LPE action also appears to be, in part, similar to auxin.

In conclusion, banana is a perishable fruit with very short shelf life. So far no post-harvest treatment is available to extend the shelf life of this fruit without undesirable effect on ripening and fruit marketability. Our results suggest that LPE has a potential to extend the shelf life of banana fruit by about 1–2 d without negative impact on fruit marketability.

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