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Lysophosphatidylethanolamine Accelerates Color Development and Promotes Shelf Life of Cranberries

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***Abstract*. Highly colored cranberries are desired for both fresh and juice markets. Berries accumulate more color when allowed to stay on the vines longer. However, early fall frosts often force growers to harvest before the fruit has reached its optimal color. This is especially true for the berries under the canopy. No product is currently available for grower to accelerate the color development in cranberries. Result from recent studies suggests that a natural lipid, lysophosphatidylethanolamine (LPE), can accelerate color production in fruit and, at the same time, promote shelf life. LPE is a natural lipid and is commercially derived from egg and soy lecithin. The influence of LPE on anthocyanin accumulation and storage quality of cranberry fruit (*Vaccinium macrocarpon* Ait. 'Stevens') was studied. Cranberry plants were sprayed with LPE at about 4 weeks before commercial harvest at multiple locations. Experiments were conducted in 1997, 1998 and 1999. Fruit samples were taken at 2 and 4 weeks after spray application to determine the changes in the fruit color. Plots were wet harvested using a standard commercial method and stored in a commercial cold storage facility. Marketable fruit were evaluated at 1 and 2 months after cold storage to determine effect of LPE on shelf life of cranberries. In general, a preharvest application of LPE resulted in a 9% to 27% increase in fruit anthocyanin concentration compared to the control. LPE treatments also resulted in 8% to 12% increase in marketable fruit compared to the control following cold storage. Influence of LPE on fruit quality was more apparent after 1 month of storage. These results are consistent with the observed effects of LPE on tomatoes. Interestingly ethanol application also enhanced storage quality. Our results suggest that a preharvest application of LPE may have the potential to enhance color and prolong shelf life of cranberry fruit.**

The commercial value of cranberries is directly related to anthocyanin development in the fruit since growers are paid a bonus by the processor for better fruit color. Also, berries with poor color are rejected for the fresh market. Berries accumulate more color when allowed to stay on the vines longer. However, early fall frosts often force growers to harvest before the fruit has reached its optimal color. This is especially true for the berries under the canopy. Thus, acceleration of color is important to cranberry growers. In addition to the fruit color, the value of fresh-market cranberries is also related to quality and shelf life of the berries. This is especially true for Wisconsin-grown berries where harvest is done more efficiently in flooded beds. Water-raked berries deteriorate much more rapidly in storage and have a shorter shelf life than dry-raked berries (Bergman, 1922; Ceponis and Stretch, 1983; Chaney, 1940). Recently we have found that fully colored berries store better than the white and blush colored berries (Ozgen et al., 2002). Thus, improving fruit color can be expected to enhance growers' profit by improving shelf life.

Several early studies showed that spray application of ethephon (Ethrel) was effective in improving fruit color (Bramlage et al., 1972; Devlin and Demoranville, 1970; Eck, 1972; Rigby et al. 1972). However field application of Ethrel had inconsistent results (Shawa, 1979), which were later explained by the lack of penetration of Ethrel across thick cranberry cuticle (Farag et al., 1992). Some studies have shown that the insecticide malathion (Devlin et al., 1969; Eck, 1968), and herbicide dichlobenil (Devlin and Demoranville, 1968) when applied as spray can induce fruit color in cranberries. However, at present these compounds, including Ethrel are not labeled for use on cranberries for color enhancement. Furthermore, growers are reluctant to use these products because of environmental concerns. Thus, currently no product is available to enhance color in cranberries.

Recent studies from our laboratory indicate that lysophosphatidylethanolamine (LPE), natural phospholipids (derived from egg and soy lecithin), can accelerate ripening and prolong shelf life of tomato fruit (Farag and Palta, 1993a). LPE application has been found to enhance ethylene production in the fruit tissues (Farag and Palta, 1989a; Hong et al., 2001). More recently Hong et al. (2002) demonstrated that LPE can enhance the activity of ACC oxidase in mature green (ready to ripen) tomato fruit. Taken together these studies show that LPE can enhance ACC oxidase activity and enhance fruit ripening. Also, LPE has been found to retard senescence in attached and detached leaves and fruit of tomato (Farag and Palta, 1993b). In another study, the vase-life of LPE-treated cut flowers was extended by three days compare to the control (Kaur and Palta, 1997). LPE inhibited the activity of phospholipase D (PLD), a membrane degrading enzyme, whose activity is increased during plant senescence (Ryu et al., 1997). These results suggest a specific role of LPE in both ripening and storage quality of fruit. In the present study we investigated the use of LPE for accelerating ripening and prolonging shelf life of cranberry fruit.

Materials and Methods

Field trials were conducted over a three years period. For this purpose experimental plots were established in commercial cranberry ('Stevens') beds at seven locations near Wisconsin Rapids in central Wisconsin. Plots, 2 × 1 m in size, were sprayed with 1000 mL solutions by a hand sprayer when the top berries on the canopy were in the blush stage about 4 weeks before final harvest. Each experimental plot was separated by 2 × 1-m guard area to prevent mixing of the treatments during harvesting. To facilitate machine harvest plots were laid out in a row following the length of the bed. The width of harvester exceeded the plot (2m). The spray solution included LPE (200 $mg \cdot L^{-1}$), and Sylgard 309 (Abbott Laboratories, Abbott Park, Ill.) a silicone based nonionic surfactant (0.05% v/v). Ethanol (5% v/v) was included in the spray solution to enhance penetration of solution into the fruit (Farag et al., 1989). LPE derived from egg lecithin was used in our experiments and was obtained either from Avanti Lipids, Alabaster Ala., or from Doosan Serdary Research Lab., Englewood, N.J.. LPE was suspended in water by using a sonicator (sonic dismembrator model 550; Fisher Scientific, Pittsburgh Pa.). Experimental design was a randomized complete block with four replications for each treatment at each location.

Experiment 1: Effect of LPE on fruit anthocyanin concentration. Experiments were conducted at East Nekoosa and Yellow River in 1997, East Nekoosa and Mather in 1998, and in Yellow River in 1999. Applications were made on 25, 19, and 24 Sept. 1997, 1998, and 1999 respectively. Fruit samples were hand raked from a part of the plot at 2 weeks after applications to determine the changes in fruit anthocyanin concentration. Additional samples

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were obtained at harvest. Plots were wet harvested with a Getsinger style commercial fresh fruit harvester (Paul's Machine and Tool, Warrens, Wis.) at about 4 weeks after spray applications. Samples were taken from mechanically harvested fruit for the measurement of anthocyanin concentration. Duplicate samples from each plot at each sampling time were used to quantify anthocyanin. concentration based on the procedure developed by Fuleki and Francis (1968). For this purpose, 100 g (CrW) of whole berries were homogenized for five minutes in 100 mL of extracting solution (95% ethanol/1.5 N HCl, 85:15, v/v) at the highest speed using a ten-speed blender. The total volume of the extract (TEV) was 175 mL. Duplicate samples of 2 mL slurry (SV) were diluted with 25 mL extracting solution in a 50 mL centrifuge tube (dilution volume DV = 27 mL). The anthocyanin was allowed to diffuse into the solution at room temperature, kept in dark overnight. The samples were then stirred vigorously and centrifuged (J2-21M; Beckman Instruments Inc., Irvine, Calif.) at 6870 g_n for 10 min and the supernatant was used to measure the absorbance (OD) at 535 nm (DU 50 spectrophotometer; Beckman Instruments Inc.). The total anthocyanin was calculated using the equation of Fuleki and Francis (1968): TAcy (mg/100 g) = OD × DV × 100 × TEV/SV × CrW × 98.2.

Experiment 2: Effect of LPE on fruit shelf life. Spray application were made on two separate fields at the Yellow River marsh on 24 Sept. 1999. The plots were wet harvested using a commercial Getsinger style fresh fruit harvester on 26 Oct. Total amount of fruit harvested from each plot was about 3000 g. This fruit was transferred to thin mesh onion bags, laid in a single layer in wooden crates and stored in a commercial cold storage room maintained at about 3 °C with 90% relative humidity. Two subsamples (500 g each) were drawn from each bag and evaluated for marketable quality after 1 and 2 months of cold storage. Fruit were graded according to the industry standards. Fruit showing rot, mechanical injury, disease, or flesh softness were counted as nonmarketable. To remove individual bias, the fruit samples were graded by four researchers without treatment identification.

Statistical analysis. Both experiments were designed as randomized complete blocks with four replications. Data were analyzed using a general linear mixed model (MIXED) procedure of the SAS Statistical Software (SAS Institute, Inc., Cary, N.C.). Blocks, locations and years are modeled as random effects and treatments are modeled as a fixed effect. Blocks are nested within locations and years. Treatments were compared by pair-wise comparisons using least significant difference (LSD) method at $p < 0.05$ and 0.01 levels. Estimated means of each treatment were over all locations and blocks.

Results

Experiment 1: Effect of LPE on fruit color. Anthocyanin concentration at the time of harvest varied among seasons as well as among locations within year (Table 1). In general, LPE enhanced color development of cranberry fruit in all three seasons of the study (Tables 1 and 2). The LPE together with ethanol and Sylgard consistently produced the highest anthocyanin concentration, although the difference between the treatments was not always significant (Table 1). The extent of color enhancement by LPE varied depending on year and location. Taken together, all the data from three seasons and total of seven different locations showed significant differences among the treatments (Table 2). Pairwise comparison showed that there were no differences between the ethanol alone and Sylgard alone treatments for the anthocyanin concentration (Tables 1 and 2). A combination of LPE+Sylgard and ethanol resulted in cranberries with a higher anthocyanin concentration than fruit treated with the ethanol or Sylgard alone. Fruit harvested 4 weeks after application had a significantly higher anthocyanin concentration than fruit harvested 2 weeks after application (Table 1).

Table 1. The effect of LPE on anthocyanin production of 'Stevens' cranberries in 1997, 1998, and 1999 with seven different locations and two different sampling times. Anthocyanin content of berries were measured 2 and 4 weeks (at harvest) after application. The values shown are averaged of four replications. All means were compared by Fisher's protected LSD ($P < 0.05$); within each column and each location means followed by the same letter do not differ significantly.

Seasons and		Anthocyanin mg/100 g fresh wt)	
Locations	Treatments[z]	2 weeks	4 weeks
1997			
E. Nekoosa	S	16.7 a	19.6 b
	S+EtOH	13.9 ab	18.3 b
	L+S+EtOH	19.6 a	23.9 a
Yellow River	S	18.2 b	22.0 ab
	S+EtOH	18.4 b	20.4 b
	L+S+EtOH	22.2 a	25.6 a
1998			
E. Nekoosa	S	13.6 a	23.1 b
	S+EtOH	15.2 a	23.4 b
	L+S+EtOH	16.2 a	26.9 a
S. Mather	S	21.0 ab	37.5 ab
	S+EtOH	20.0 b	35.8 b
	L+S+EtOH	24.5 a	40.7 a
N. Mather	S	22.3 a	35.8 a
	S+EtOH	21.3 a	34.3 a
	L+S+EtOH	25.3 a	39.6 a
1999			
S. Yellow River	S	24.5 b	33.3 b
	S+EtOH	24.9 b	32.9 b
	L+S+EtOH	28.5 a	42.2 a
N. Yellow River	S	22.0 a	32.8 b
	S+EtOH	21.7 a	28.7 b
	L+S+EtOH	24.6 a	40.4 a

[z]S = Sylgard (0.05 % v/v), EtOH = ethanol (5% v/v); L = LPE (200 mg·L^{-1}).

Table 2. The effect of LPE on anthocyanin production of 'Stevens' cranberries in 1997, 1998, and 1999 with seven different locations and two different sampling times. Data were analyzed using a general linear mixed model (MIXED) procedure of the SAS statistical software. Blocks, locations and years are modeled as random effects and treatments are modeled as fixed effect. Blocks are nested within locations and years. Treatments were compared pairwise by least significant difference (LSD) method at $p < 0.05$ and 0.01 levels. Estimated means of each treatment over all locations and blocks.

Source	*P* value		
Year	<0.0001**		
Location	<0.0001**		
Treatment	<0.0001**		
Year × treatment	0.2824^{NS}		
Location × treatment	0.0875^{NS}		
Sampling time	<0.0001**		
Sampling time × treatment	0.1391^{NS}		
Pairwise comparisons	2 weeks	4 weeks	Overall
Treatment[z]			
EtOH–S	0.6393^{NS}	0.1100^{NS}	0.0907^{NS}
EtOH–S+L+EtOH	0.0001**	<0.0001**	<0.0001**
S–S+L+EtOH	0.0006**	<0.0001**	<0.0001**

General linear mixed model	Color	
estimated means	2 weeks	4 weeks
S	19.3	27.7
S+EtOH	19.8	29.2
S+L+EtOH	23.0	34.2
SE	1.2	1.2

[z]S = Sylgard (0.05 % v/v), EtOH = ethanol (5% v/v), L = LPE (200 mg·L^{-1}).
^{NS,**}Nonsignificant or significant at $P < 0.01$.

In 1997, LPE with surfactant and ethanol improved fruit anthocyanin by about 20% compared to the control in both East Nekoosa and Yellow River (Table 1). The same effect was observed after the 2 and 4 week samplings. Although this season was not a good coloring year for the cranberries, fruit treated with LPE had increased fruit color compared to control treatments. In 1998, overall fruit anthocyanin development particularly in Mather was high (Table 1). At the 2 week sampling differences between treatments were only significant at South Mather. Although the mean anthocyanin of LPE treated fruit was higher than the control, this increase was not significant at North Mather or East Nekoosa. However, at 4 week sampling treated LPE fruit had significantly greater anthocyanin at East Nekoosa. In 1999, 2 weeks after application, anthocyanin differences between LPE and the control were only significant at South Yellow River. However, at 4 weeks after treatment all the LPE treated plots had significantly higher anthocyanin than the control. This increase in anthocyanin by LPE treatment was >20% at both the locations.

Overall a combination of LPE with ethanol and Sylgard improved anthocyanin by 16% and 19% over Sylgard alone and Sylgard plus ethanol at 2 weeks after application. Similar increases were observed at 4 weeks after application as 17% and 23% respectively (Table 2, estimated means). There was no significant difference between Sylgard alone and Slygard plus ethanol.

Experiment 2. In this experiment all possible combinations of LPE, Sylgard and ethanol were applied for treatment comparisons. In general, treatments containing LPE gave higher anthocyanin than the respective control (Tables 3–5). Untreated control fruit had similar values of anthocyanin as ethanol, Sylgard or a combination of ethanol+Sylgard. LPE in combination with Sylgard or ethanol enhanced anthocyanin development significantly as compared to untreated control. Overall, the color enhancement by LPE was more pronounced at 4 weeks after application as compared to 2 weeks after application (Table 5).

LPE treated fruit also showed a higher percentage of marketable fruit after 1 and 2 months of cold storage (Tables 3 and 5). Ethanol was able to improve shelf life, over control at one location, but was not effective in accelerating color development at either location. In particularly, the combination of LPE and ethanol had the greatest effect on fruit quality after 1 and 2 months of cold storage. Sylgard alone showed no effect on either color enhancement or shelf life (Table 4). The combination of LPE+ethanol+Sylgard had 12% and 8% more marketable fruit than the control after 1 and 2 months of cold storage respectively (Table 5).

Discussion

Although environmental and cultural practices varied over the three years and between the three experimental locations, LPE had a positive effect on color. Weather conditions such as number of cool nights and sunny days, field conditions such as high canopy density and cultural practices affect the anthocyanin accumulation of cranberries (Eck, 1990) and this was seen in these experiments (Tables 1 and 3). In general overall fruit color was poor in 1997 but good in 1998 and 1999 (Table 1). Furthermore, neither the surfactant nor ethanol had any effect on fruit color, but a combination of LPE, surfactant and ethanol was generally most effective in enhancing fruit color (Tables 1–3).

Our results are consistent with previous results where acceleration of color development with LPE was grown for the processing and fresh markets found in tomato fruit (Farag and Palta, 1993a), despite the fact that these fruit

Table 3. The effect of LPE on anthocyanin production and shelf life of 'Stevens' cranberries in 1999 at two different locations. The values shown are averages of four replications. All means were compared by Fisher's protected LSD ($P < 0.05$); within each column and each location means followed by the same letter do not differ significantly.

	Anthocyanin mg (100 g fresh wt)		Marketable fruit (%)	
Treatments[z]	2 weeks	4 weeks	1 Month	2 Months
S. Yellow River				
C	21.5 b	29.3 c	76.2 c	72.5 c
L	24.2 ab	38.7 a	82.3 b	75.2 bc
S	22 ab	32.8 bc	84.1 b	74.6 bc
L+S	23.3 ab	38.1 ab	84.4 b	79.8 ab
EtOH	22.3 ab	32.9 bc	83.7 b	76.4 bc
L+EtOH	24.4 ab	39.1 a	85.6 ab	80.1 ab
S+EtOH	21.7 ab	28.7 c	82.0 b	75.8 bc
L+S+EtOH	24.6 a	40.4 a	90.0 a	84.0 a
N. Yellow River				
C	25.6 cd	35.2 b	71.8 ab	56.3 abc
L	28.2 abc	41 a	75.2 ab	60.5 ab
S	24.5 d	33.3 b	69.3 b	50.8 c
L+S	28.8 a	42.9 a	74.4 ab	53.5 bc
EtOH	25.6 bcd	33.7 b	72.4 ab	53.8 abc
L+EtOH	29.2 a	41.4 a	78.2 a	62.5 a
S+EtOH	24.2 d	30.7 b	71.4 ab	53.7 abc
L+S+EtOH	28.5 ab	42.2 a	75.8 ab	55.9 abc

[z]C = water, S = Sylgard (0.05 % v/v), EtOH = ethanol (5% v/v), L, LPE (200 mg·L^{-1}).

Table 4. The effect of LPE on anthocyanin production and shelf life of 'Stevens' cranberries in 1999 at two different locations. Data were analyzed using a general linear mixed model (MIXED) procedure of the SAS Statistical Software. Blocks and locations are modeled as random effects and treatments are modeled as fixed effect. Blocks are nested within locations. The treatments consist of the factorial combinations of three factors, L, S, and EtOH, each with two levels.

Source	Color	*P* value	Marketable fruit	*P* value
LPE	**	<0.0001	**	0.0002
Sylgard	NS	0.7144	NS	0.8355
Ethanol	NS	0.7033	*	0.0328
Time	**	<0.0001	**	<0.0001

NS,*,**Nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

Table 5. The effect of LPE on anthocyanin production and shelf life of 'Stevens' cranberries in 1999 at two locations. Data were analyzed using a general linear mixed model (MIXED) procedure of the SAS Statistical Software. Blocks and locations are modeled as random effects and treatments are modeled as fixed effect. Blocks are nested within locations. Estimated means of each treatment over all locations and blocks.

Source	*p* value
Color	
(2 weeks)	0.0001**
(4 weeks)	<0.0001**
Quality	
(1 month)	0.0066**
(2 month)	0.0603NS

General linear mixed model	Anthocyanin mg (100 g fresh wt)		Marketable fruit (%)	
estimated means[z]	2 weeks	4 weeks	1 month	2 months
C	23.5	32.3	74.0	64.7
S	23.3	33.1	76.7	62.7
EtOH	24.0	33.3	78.0	65.1
S+EtOH	23.3	30.8	76.7	64.7
L	26.2	39.9	78.7	67.9
L+ EtOH	26.8	40.2	81.9	71.3
L+S	26.1	40.5	79.4	66.6
L+S+EtOH	26.5	41.3	82.9	69.9
SE	1.1	1.2	2.5	4.5

[z]C = water, S = Sylgard (0.05 % v/v), EtOH = ethanol (5% v/v), L, LPE (200 mg·L^{-1}).
NS,*,**Nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

accumulate different pigments during ripening. We do not know the exact mechanism by which LPE mediates fruit ripening. However, a previous study from our laboratory showed that the onset of color is associated with a distinct rise in ethylene production by the fruit (Abdallah and Palta, 1989). LPE has been found to enhance ethylene production in fruit tissue (Farag and Palta, 1989a; Hong et al., 2001). Consistent with these results Hong et al. (2002) reported an increase in the activity of ACC oxidase in mature green tomato fruit tissue. Thus, it appears that LPE application may be enhancing ethylene production in cranberries, which in turn accelerates fruit ripening.

Results of the present study also suggest that a preharvest application of LPE has the potential to increase cranberry fruit shelf life during storage (Tables 3–5). Although overall LPE had a significant effect on fruit quality during storage (Table 4) this effect was more prominent at 1 month after storage (Table 5). This suggests as fruit rot increases (less marketable fruit) during storage, the differences among treatments narrowed. Reduced leakage of electrolytes in LPE-treated leaves (Farag and Palta, 1993b), flowers (Kaur and Palta, 1997), and postharvest fruit (Farag and Palta, 1993a) suggest that LPE might protect membrane integrity during senescence. LPE was also shown to be a strong inhibitor of phosholipase D (Ryu et al., 1997), an enzyme known to cause membrane lipid degradation during senescence (Ryu and Wang, 1995). Thus, it is possible that a preharvest application of LPE enhanced storage quality by improving membrane health of the fruit.

Another explanation for LPE-enhanced keeping quality in cold storage may be unique characteristics of cranberries. It has been found that ripened (dark red) cranberry fruit store better than the less colored fruit (Ceponis and Stretch, 1983; Ozgen et al., 2002). This was reported to be due to lower respiration of dark red fruit as compared to blush and light red fruit (Ozgen et al., 2002). Thus, it is possible that LPE treated fruit stores better because LPE enhances anthocyanin production in the fruit. Furthermore, it is also known that with increase in color cranberry cuticle thickness and strength increases (Farag and Palta, 1989b; Ozgen et al., 2002). In Wisconsin, cranberries are wet harvested by flooding the fields. The harvester removes the fruit from the plants by a combing action during which fruit are subjected to a mechanical stress. It is possible that more colored berries having thicker and stronger cuticle would suffer from less mechanical stress during harvest. This in turn will maintain better storage quality.

Interestingly, we also found that application of ethanol alone may improve shelf life of cranberries (Table 4). There is some evidence that ethanol application can retard senescence (Wu et al., 1992). One can also envision that ethanol could surface sterilize the fruit. However, since our cranberry fruit were wet harvested according to the commercial practice, it is unlikely that the sterilizing influence of ethanol would last in cold storage. Future studies might explore the possibility of using ethanol for this purpose.

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