Use of Lysophosphatidylethanolamine (LPE), a Natural Lipid, to Accelerate Ripening and Enhance Shelf Life of Cranberry Fruit

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Abstract

Recent studies suggest that a natural lipid, lysophosphatidylethanolamine (LPE), can accelerate fruit ripening, while at the same time promote shelf life. LPE is commercially derived from egg and soy lecithin. We studied the influence of LPE on anthocyanin accumulation and storage quality of cranberry fruit (*Vaccinium macrocarpon* Ait. 'Stevens'). For this purpose 2 m x 1 m plots were established in commercial cranberry beds at two different locations. Plots were sprayed with LPE at about 4 weeks before commercial harvest. The spray solution consisted of LPE (200 mg L^{-1}), ethanol (5% v/v), and a nonionic surfactant Sylgard (0.05% v/v). Fruit samples were removed at two weeks after spray application and at final harvest to determine the changes in fruit color. Plots were finally wet harvested with a machine along with the scheduled harvest and stored in commercial cold storage. Marketable fruit were counted and weighed at one and two months after cold storage to determine effect of LPE on shelf life of cranberries. In general, applications of LPE resulted in 13-28% increase in fruit anthocyanin contents, compared with control. LPE treatments also resulted in 6-12% increase in marketable fruit in cold storage, compare with control. This influence of LPE on fruit quality was more apparent after one month of storage. 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.

INTRODUCTION

Cranberries (*Vaccinium macrocarpon* Ait.) are a native species to Wisconsin (Eck, 1990), and this state leads the nation in total production in the US (USDA-NSS Ag. Stat., 2000). Most of the fresh cranberries are sold around Thanksgiving and Christmas holidays with the remainder of the berries being processed, primarily as juice. The commercial value of cranberries is directly related to anthocyanin development of the fruit. Growers are paid a bonus by the processor for improved fruit color. Also, berries with poor color are graded out during fresh pack. Thus, undercolored fruit naturally bring a lower economic return to the grower. 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 has always been of high interest to cranberry growers for both fresh and processing markets.

Ethephon, an ethylene releasing compound, was sold as Ethrel to enhance color in cranberries. Several early studies showed that spray application of Ethrel is effective in improving fruit color (Bramlage et al., 1972; Devlin and Demoranville, 1970; Eck, 1972; Rigby et al., 1972). However field application of Ethrel has inconsistent results (Shawa, 1979), later found to be due to lack of penetration of Ethrel across this thick cranberry cuticle (Farag et al., 1992). After the ALAR scare, the Ethrel label was withdrawn from cranberries. Some studies have shown that the insecticide malathion (Devlin et al., 1969; Eck, 1968) and the herbicide dichlobenil (Devlin and Demoranville, 1968) can induce fruit color in cranberries when applied as a spray. However, these compounds are not labeled for use on cranberries for color enhancement. Furthermore, growers are reluctant

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to use these products because of environmental concerns. Thus, currently no product is available to enhance color in cranberries.

The value of fresh market cranberries is also related to quality and shelf life of the berries besides the fruit color. This is especially true for Wisconsin-grown berries where harvest is done more effectively in flooded beds by water harvester. 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).

Studies from our laboratory suggest that lysophosphatidylethanolamine (LPE) 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, 1989; Hong et al., 2001). Enhanced ethylene production is an indication of stimulation of fruit ripening and consequently improved color. 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 is prolonged to six days, compared with three days for the control (Kaur and Palta, 1997). Also, LPE inhibits 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 enhancing shelf life of cranberry fruit. Ethanol has been used in cranberries to enhance transport of ethephon (Farag et al., 1992). In our studies we investigated the effect of ethanol on the efficacy of LPE.

MATERIALS AND METHODS

Experimental plots were established in commercial 'Stevens' cranberry beds at two different locations near Wisconsin Rapids in central Wisconsin in 1999. Spray application was performed on 24 Sept. Plots, 2 m x 1 m in size, were sprayed with 1000 mL solutions by a hand sprayer when the top berries on the canopy are in the blush stage, about four weeks before final harvest. Spray solution included LPE (200 mg L^{-1}) and Sylgard 309, a silicone-based (Abbott Laboratories, Abbott Park, IL) nonionic surfactant (0.05% v/v). Ethanol was included in the spray solution to enhance penetration 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 a sonicator (Sonic Dismembrator model 550; Fisher Scientific, Pittsburgh Pa.). Experimental design was a randomized block design with four replications for each treatment at each location.

Fruit samples were hand-raked from a part of the plot at two and four weeks after applications to determine the changes in fruit anthocyanin content. From each plot at each sampling time two separate samples of 100 g each were used to determine anthocyanin contents. For quantification of total anthocyanin a procedure similar to Fuleki and Francis (1968) was used. For this purpose, 100 g (CrW) of whole berries were homogenized for five minutes in 100 ml of extracting solution (95% ethanol/1.5 mol 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 two ml slurry (SV) were diluted with 25 ml in a 50 ml centrifuge tube with extracting solution (dilution volume DV=27 ml). The anthocyanin was allowed to diffuse into the solution overnight. The samples were then stirred vigorously and centrifuged (Beckman J2-21M; Beckman Instruments Inc., Irvine CA) at 6870 g_n for 10 minutes and the supernatant was used to measure the absorbance (OD) at 535 nm (Beckman DU 50 spectrophotometer; Beckman Instruments Inc., Irvine CA). The total anthocyanin (TA) was calculated using the following equation of Fuleki and Francis (1968):

$$TA\ (mg/100\ g) = OD \times DV \times 100 \times TEV/SV \times CrW \times 98.2.$$

Plots were wet-harvested with a Getsinger-style commercial fresh fruit harvester (Paul's Machine and Tool, Warrens, Wis.) at about four weeks after spray applications. 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 one and two months of cold storage. Fruit were graded according to the industry standards. Fruit showing rot, mechanical injury, disease, or flesh softness were counted as non-marketable. To remove individual bias, the fruit samples were graded by four researchers without treatment identification.

RESULTS

In this experiment all possible combinations of LPE, Sylgard and ethanol were applied for treatment comparisons. In general, treatments containing LPE gave higher anthocyanin content than the respective control (Tables 1 and 2). Untreated control fruit had similar values of anthocyanin as ethanol, Sylgard or a combination of ethanol+ Sylgard. Two weeks after application sampling differences between LPE and the control were only significant at South Yellow River. However, at four weeks after treatment all the LPE- treated plots had significantly higher anthocyanin content than the control. Overall, a combination of LPE with ethanol and Sylgard improved anthocyanin content by 16 and 19% over ethanol and Sylgard alone at two weeks after application. Similar increases were observed at four weeks after application as 17 and 21%, respectively. There were no significant differences between ethanol and Sylgard alone.

LPE-treated fruit also showed more marketable fruit after one and two months of cold storage (Tables 1 and 2). Ethanol was able to give some of the desirable effects on shelf life, but was not effective in accelerating color development. Particularly, the combination of LPE and ethanol showed the best effect on fruit quality after one and two month of cold storage. Sylgard alone showed no effect on either color enhancement or shelf life (Table 1 and 2). The combination of LPE+ethanol+Sylgard had 12 and 6% more marketable fruit than control after one and two months of cold storage.

DISCUSSION

Results of the present study suggest that LPE has the potential to enhance anthocyanin production in cranberries. Neither the surfactant or ethanol had any effect on fruit color, but a combination of LPE, surfactant and ethanol was generally most effective in enhancing fruit color (Table 1). Our results are consistent with previous results where acceleration of color development with LPE was found in tomato fruit with LPE (Farag and Palta, 1993a). In that study tomatoes grown for processing and fresh market were treated with LPE as a preharvest spray similar to cranberries in the present study. We do not know the exact mechanism by which LPE mediates color development. 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 the fruit tissue (Farag and Palta, 1989; Hong et al., 2001). Thus, it appears that LPE application may be enhancing ethylene production in cranberries, which in turn accelerates color development. Results of the present study also suggest that a preharvest application of LPE has the potential to increase cranberry fruit shelf life during storage (Table 3). Although overall LPE had a significant effect on fruit quality during storage (Table 2) this effect was more prominent at one month after storage (Table 3). This suggests as fruit rot increases (less marketable fruit) during storage, the differences among treatments disappear. A major action of LPE on longer shelf life may be due to its known effect on membrane integrity. Reduced leakage of electrolytes in LPE-treated leaves (Farag and Palta, 1993b), flowers (Kaur and Palta, 1997), and post-harvest fruit (Farag and Palta, 1993a) suggest that LPE may protect membrane integrity during senescence. LPE was also shown to be a strong inhibitor of phosholipase D, an enzyme known to cause membrane lipid degradation during senescence (Ryu and Wang, 1995). Another explanation to enhanced keeping quality in cold storage by LPE treatment may be unique characteristics of cranberries. It has been found that ripe (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 and thicker

cuticle (Ozgen et al., 2002). Thus, it is possible that LPE-treated fruit stores better because LPE enhances anthocyanin production in the fruit.

Interestingly, we also found that application of ethanol alone improved shelf life of cranberries (Table 2). 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, as our cranberry fruit were wet-harvested according to the commercial practice, it is unlikely that a sterilizing influence of ethanol would last in cold storage. Future studies might explore the possibilities of using ethanol for this purpose.

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Tables

Table 1. The effect of LPE on anthocyanin production and shelf life of 'Stevens' cranberry fruit in 1999 at two different locations.

Treatments	Anthocyanin mg (100 g FW)$^{-1}$ 2 week		4 week		Marketable Fruit (%) 1 Month		2 Month	
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
LS	23.3	ab	38.1	ab	84.4	b	79.8	ab
E	22.3	ab	32.9	bc	83.7	b	76.4	bc
L+E	24.4	ab	39.1	a	85.6	ab	80.1	ab
S+E	21.7	ab	28.7	c	82.0	b	75.8	bc
L+S+E	24.6	a	40.4	a	90.0	a	84.0	a
N. Yellow River								
C	25.6	dc	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
E	25.6	bcd	33.7	b	72.4	ab	53.8	abc
L+E	29.2	a	41.4	a	78.2	a	62.5	a
S+E	24.2	d	30.7	b	71.4	ab	53.7	abc
L+S+E	28.5	ab	42.2	a	75.8	ab	55.9	abc

C, water: S, Sylgard (0.05 % v/v); E, Ethanol (5% v/v); L, LPE (200 mg L^{-1})

The values shown are averaged of 4 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.

Table 2. The effect of LPE on anthocyanin production and shelf life of 'Stevens' cranberry fruit at two different locations.

Treatments	Color	P value	Quality	P value
L	**	<0.0001	**	0.0002
S	NS	0.7144	NS	0.8355
E	NS	0.7033	*	0.0328
Time	**	<0.0001	**	<0.0001

S, Sylgard (0.05% v/v); E, Ethanol (5% v/v); L, LPE (200 mg L^{-1})

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

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 E, each with two levels.

Table 3. The effect of LPE on anthocyanin production and shelf life of 'Stevens' cranberry fruit in 1999 at two locations.

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

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