





Article

Configuration of Strawberry Yield, Nutritional and Functional Traits in Response to LPE Application in a Two-Year Study

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Abstract: Lysophosphatidylethanolamine (LPE) is a promising natural lysophospholipid which can be employed as a growth regulator for horticultural purposes. The present research was accomplished to investigate the effects of LPE (0 or 10 ppm) on the yield and quality of “Savana” strawberry plants grown during two consecutive cultivation cycles (I (2020–2021); II (2021–2022)). Plants cultivated in year I and treated with LPE revealed the highest total yield (838.3 g plant⁻¹), marketable yield (735.4 g plant⁻¹) and average marketable fruit weight (39.8 g plant⁻¹). Fruits from year II plants treated with LPE had the highest total phenolics concentration (491.4 mg 100 g⁻¹ dw). LPE significantly enhanced strawberry antioxidant activity, firmness, soluble solids content, ascorbic acid and anthocyanins by 5.2%, 7.6%, 15.3%, 13.8% and 19.7%, respectively, compared with the control. Although LPE application significantly reduced fruit dry matter, yellowness and lightness by 7.2%, 30.1% and 14.6%, respectively, it significantly increased, in year II, anthocyanins, discarded production, fruit lightness and dry matter. Overall, our findings also revealed that, even under sub-optimal growing conditions (year II), LPE application increased important productive and qualitative strawberry parameters.

Keywords: *Fragaria × ananassa*; lysophosphatidylethanolamine; foliar application; protected environment; production; fruit quality



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1. Introduction

The strawberry (*Fragaria × ananassa*) is a perennial plant of the *Rosaceae* family extensively cultivated in many regions of the world. The strawberry is the most important grape-like fruit, and it is appreciated by consumers for its flavour and for its nutritional and nutraceutical characteristics [1,2]. In Italy, Sicily is the leading region for early strawberry production, with the strawberry area in Trapani Province covering around 200 hectares [3]. In Sicily, strawberries are usually cultivated in a protected environment using a long cycle system, from September to May [4]. Traditional strawberry cultivation techniques have a high environmental impact since the productive cycle is long and requires significant quantities of plastic materials, nutrients and pesticides [5]. The heavy use of these chemical products has a negative impact on human health, on the ecosystem and on climate change. Consequently, in recent years, there has been an increasing interest in developing and testing new agronomic tools and schemes characterized by a low ecological impact [6–9]. In this scenario, the application of natural products, such as biostimulants and other natural extracts, is of high interest [10–14]. The modulation of plant physiological processes via bioactive compounds is a long-used horticultural practice [15]. Indeed, plant growth regulators have been widely adopted by farmers to increase plant stress tolerance, reduce crop alteration morphology and modulate fruit ripening [16].

Natural lysophospholipids such as lysophosphatidylethanolamine (LPE) represent a promising class of natural products. LPE is part of cell membranes, and it can be employed

as a growth regulator [15]. The first report of LPE application effects dates back to 1989, when Farag and Palta [17,18] underlined the increase in ethylene production in cranberry fruits. Furthermore, Özgen and Palta [19] found that LPE supply enhances cranberry fruit quality. Moreover, Farag and Palta [20,21] reported the effects of LPE on tomato, apple and cranberry fruits. Recently, there has been an upsurge in interest in the use of LPE supply as a tool to improve the quality of vegetables and fruits. Thus, a number of studies have been carried out to enhance the pre- and post-harvest quality of horticultural crops. LPE benefits comprise delayed leaf aging [15], ripening stimulation, colour development acceleration, and an increased shelf-life for fruits and cut flowers [22–30]. Nowadays, LPE is approved by the Environmental Protection Agency (EPA) and applied for agricultural purposes [15]. However, there are contradictory reports on LPE effects [15]. Moreover, since its real mechanism of action is not completely known, it would be useful to establish specific protocols for different growing conditions. In this regard, a specific study was conducted on strawberry plants cultivated during two consecutive growing cycles to appraise LPE application effects on yield and fruit quality.

2. Materials and Methods

2.1. Plant Materials and Trial Conditions

The experiments were carried out for two cultivation years (2020–2021 and 2021–2022) in an experimental field of the Department of Agricultural, Food and Forestry Sciences (SAAF) of the University of Palermo, located near Marsala (TP). Strawberry plants cv. “Savana” (*Fragaria × ananassa*) were transplanted at a density of 8 plants m^{-2} inside multiple tunnels covered with 0.05 mm thick polyethylene film (PE). The winter planting system usually adopted in Sicily [31] was followed. In both years, plants were transplanted on September 15th and the cycle was concluded on May 31st. The soil of the experimental site—derived from the “Sciare”, a typical type of soil of the Marsala area, which has an alkaline pH (8.5)—is composed of ~70% sand and 8.8% limestone and is rich in calcium carbonate (K_2O , 650 ppm), phosphorous (70 ppm), iron oxides (20 $mg\ kg^{-1}$), nitrogen (1.9 $g\ kg^{-1}$) and potassium (0.35 meq 100 g^{-1}). Powdered sulphur treatments were carried out to control powdery mildew, while launches of *Phytoseiulus persimilis* were arranged to control *Tetranychus urticae*. Regarding the fertilization ($kg\ ha^{-1}$), 190 of N, 155 of P_2O_5 , 295 of K_2O , 60 of Fe-chelate, 180 of CaO and 40 of MgO were administered during the whole cycle, taking into consideration the soil endowments. Before each year’s transplanting, the soil was solarized in the summer period using 0.05 mm thick green PE film and brought to field capacity through irrigation. The same film was maintained during the whole cultivation cycle as mulching. During the cultivation cycles, 4200 and 4000 $m^3\ ha^{-1}$ were supplied to the experimental fields in 2020/2021 and 2021/2022, respectively. Daily maximum and minimum temperature values for both years were recorded at the site using a data logger placed inside the tunnels (Figure 1).

2.2. Trial Set-Up and Experimental Design

Starting twenty days after transplanting, the lysophosphatidylethanolamine (LPE) treatment was administered via foliar spray every seven days (34 applications in total). The LPE (Merck Life Science, Darmstadt, Germany) was supplied at a rate of 10 ppm. For each foliar application, 100 $mL\ m^{-2}$ of solution was used. Control plants received only water. Three replications for each treatment (LPE and control), consisting of 20 plants each, were planned. A total of 240 plants were arranged in a randomized complete block design (RCBD). The experiment was repeated for two consecutive growing cycles (2020–2021 and 2021–2022).

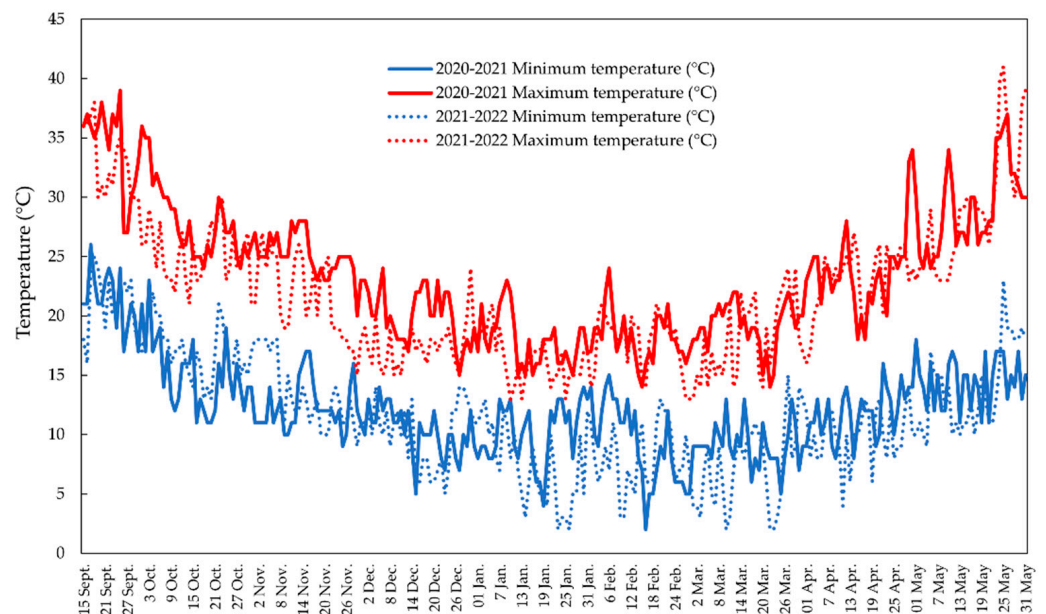


Figure 1. Maximum and minimum temperatures recorded inside the tunnel during the 2020–2021 and 2021–2022 strawberry growing cycles (September–May).

2.3. Yield Parameters

Yield parameters were recorded weekly for all plants from November to May. The first harvest was accomplished 60 (2020–2021) and 64 (2021–2022) days after transplanting. Total yield (TY), marketable yield (MY), number of marketable fruits per plant (NMF) and average marketable fruit weight (AMFW) were assessed. Marketable fruits were those not affected by *Botrytis* or misshapen, and with a weight >25 g. The discarded production (DP) was also calculated and presented as a percentage. Total yield and marketable yield were presented as g plant^{-1} , whereas AMFW was expressed as g.

2.4. Fruit Quality Parameters

Qualitative parameters were determined using a sample of 10 fruits per replicate and sampling started on the 4th harvest. Dry matter values were determined by drying 300 g of fresh sample in a ventilated oven (Thermo Scientific Heratherm OGS750, Thermo Fisher Scientific, Monza, Italy), setting the temperature at 105 °C. Dry matter data were expressed as a percentage. Fruit ascorbic acid content (AA) was determined manually by squeezing the fruit with a juicer, after which the measurement was accomplished using a refractometer (RQflex10 Reflectoquant of Sigma-Aldrich, Saint Louis, MO, USA) and Reflectoquant ascorbic acid test strips. Results were expressed as $\text{mg } 100 \text{ g}^{-1}$ fresh weight (fw). For the detection of total phenolic concentration in fruits (TP), the method of Slinkard and Singleton [32] was followed. The parameter was expressed as $\text{mg } 100 \text{ g}^{-1}$ of dry weight (dw). Rabino and Mancinelli's [33] method was followed to measure the anthocyanin content of fruits. The value was reported as $\text{mg of Cya-3-glucoside equivalent per } 100 \text{ g}^{-1} \text{ dw}$. For the determination of fruit firmness, the measurement was performed twice using a digital penetrometer (Trsnc, Forli, Italy) at two different points in the equatorial part of the fruit, testing the resistance of the berries to compression. A 6 mm diameter stainless steel cylindrical probe was used for the measurement. Values were reported in Newtons (N). To determine the soluble solids content (SSC), filtered strawberry juice was used, the parameter was measured with a digital refractometer (MTD-045nD, Three-In-One Enterprises Co., Ltd., New Taipei, Taiwan) and the data were expressed as °Brix. Strawberry fruit colour measurements were performed in two different areas of the peel using a digital colorimeter (Chroma-meter CR-400, Minolta Corporation, Ltd., Osaka, Japan), thereby determining the CIELab parameters: a^* , b^* and L^* , indicating redness, yellowness and lightness, respectively. Titratable acidity (TA) was measured following the method reported by Han et al. [34].

Briefly, using a pipette, 10 g of juice was transferred to a beaker, 50 mL of distilled water was added and the solution was titrated with 0.1 M NaOH. The equivalent point of titration was measured with a pH meter until pH 8.1. Values were expressed as percentage of citric acid. The radical-scavenging activity of the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) of the strawberry samples was evaluated as radical-scavenging capacity (RSA), using the DPPH method [35,36]. The values were reported as percentage of inhibition (antioxidant capacity).

2.5. Statistical Analysis

All statistical analyses were conducted using SPSS software v. 28.0 (StatSoft, Inc., Chicago, IL, USA) and considering LPE supply and year as the main factors. The influence of the two factors was assessed by a two-way analysis of variance (ANOVA). Separation of means was performed with Tukey's HSD test ($p \leq 0.05$). Before the analysis of variance was performed, data expressed as percentages were subjected to arcsin ($\emptyset = \arcsin(p/100)^{1/2}$) transformation. Principal components analysis (PCA) was also performed on the overall dataset. For screening the optimal number of principal components (PCs), factors with eigenvalues greater than 1.0 were considered.

3. Results

As shown in Figure 2A–C, statistical analysis for TY, MY and AMFW underlined a significant interaction between the LPE supply and the year of cultivation.

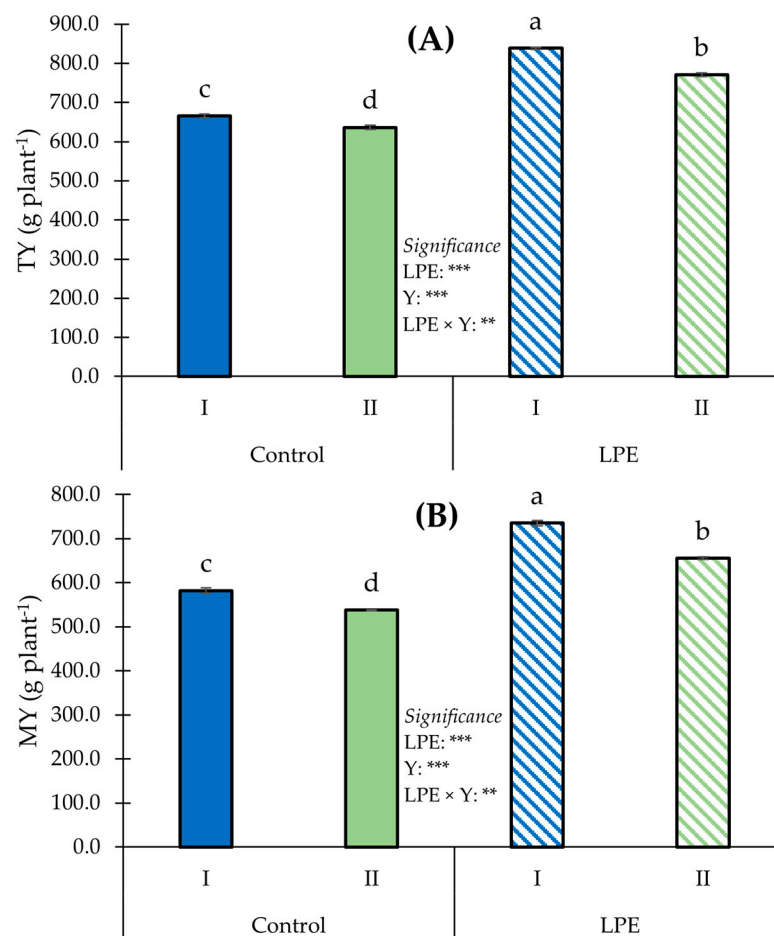


Figure 2. Cont.

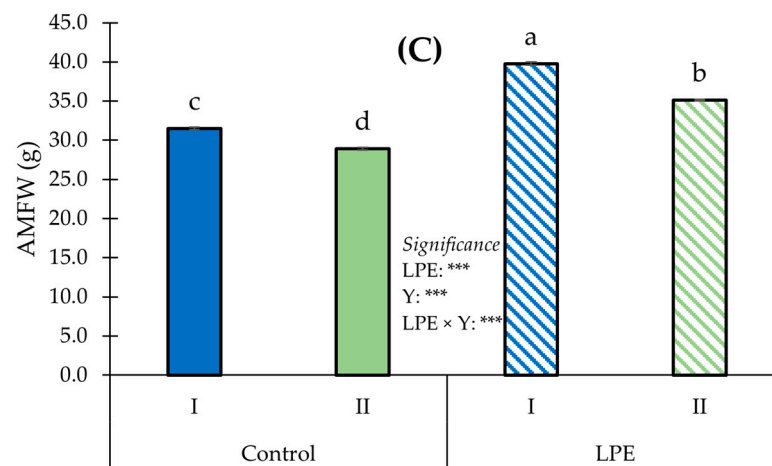


Figure 2. Total yield (TY) (A), marketable yield (MY) (B) and average marketable fruit weight (AMFW) (C) of strawberry plants in response to lysophosphatidylethanolamine (LPE) application and year of cultivation (Y). Different letters indicate means that are statistically dissimilar according to the Tukey HSD test ($p \leq 0.05$). **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$. Bars indicate the standard error. I = 2020–2021 growing period; II = 2021–2022 growing period. Data are the mean of all harvests, starting from the 4th harvest.

Plants cultivated during the 2020–2021 growing period and treated with LPE revealed the highest TY values, followed by those cultivated in 2021–2022 and treated with LPE. The lowest TY value was recorded in control plants cultivated during 2021–2022 (Figure 2A). The same trend established for the TY was followed by the MY and the AMFW.

As shown in Table 1, the NMF and the DP were not significantly influenced by the interaction of the two main factors.

Table 1. Number of marketable fruits (NMF) and discarded production percentage (DP) of strawberry plants in response to lysophosphatidylethanolamine (LPE) application and year (Y).

| Treatments | NMF (Fruits Plant ⁻¹) | | DP (%) | |
|---------------------|-----------------------------------|---|--------|---|
| <i>LPE</i> | | | | |
| Control | 18.5 | A | 14.0 | a |
| LPE | 18.6 | A | 13.6 | a |
| <i>Year</i> | | | | |
| I | 18.5 | A | 12.4 | b |
| II | 18.6 | A | 15.2 | a |
| <i>Significance</i> | | | | |
| LPE | NS | | NS | |
| Y | NS | | *** | |
| LPE × Y | NS | | NS | |

Different letters indicate means that are statistically dissimilar according to the Tukey HSD test ($p \leq 0.05$). NS: not significant; ***: significant at $p \leq 0.001$. I = 2020–2021 growing period; II = 2021–2022 growing period. Data are the mean of all harvests, starting from the 4th harvest.

The LPE supply and the year did not significantly affect the NMF values. Regardless of the year, LPE had no significant effect on the DP. In contrast, the year meaningfully influenced the DP; in the second trial year (2021–2022), a significant increase in the DP was recorded.

ANOVA analysis for dry matter revealed a not significant interaction between the LPE supply and the year (Figure 3).

Regardless of the year, the LPE application significantly decreased strawberry dry matter content (−6.7%). On the other hand, strawberry fruits from plants cultivated during the 2021–2022 growing period had higher dry matter percentage values (+5.1%) than those cultivated in the first year (Figure 3).

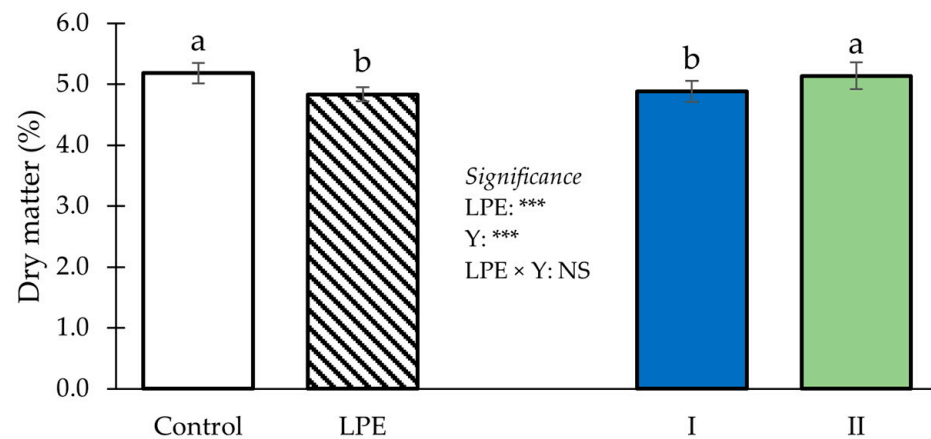


Figure 3. Dry matter percentage of strawberry fruits in response to lysophosphatidylethanolamine (LPE) application and year of cultivation (Y). Different letters indicate means that are statistically dissimilar according to the Tukey HSD test ($p \leq 0.05$). NS: not significant; ***: significant at $p \leq 0.001$. Bars indicate the standard error. I = 2020–2021 growing period; II = 2021–2022 growing period. Data are the mean of all harvests, starting from the 4th harvest.

For AA and anthocyanin concentrations in the fruits, the statistical analysis did not show a significant influence of the LPE × Y interaction (Figure 4A,B).

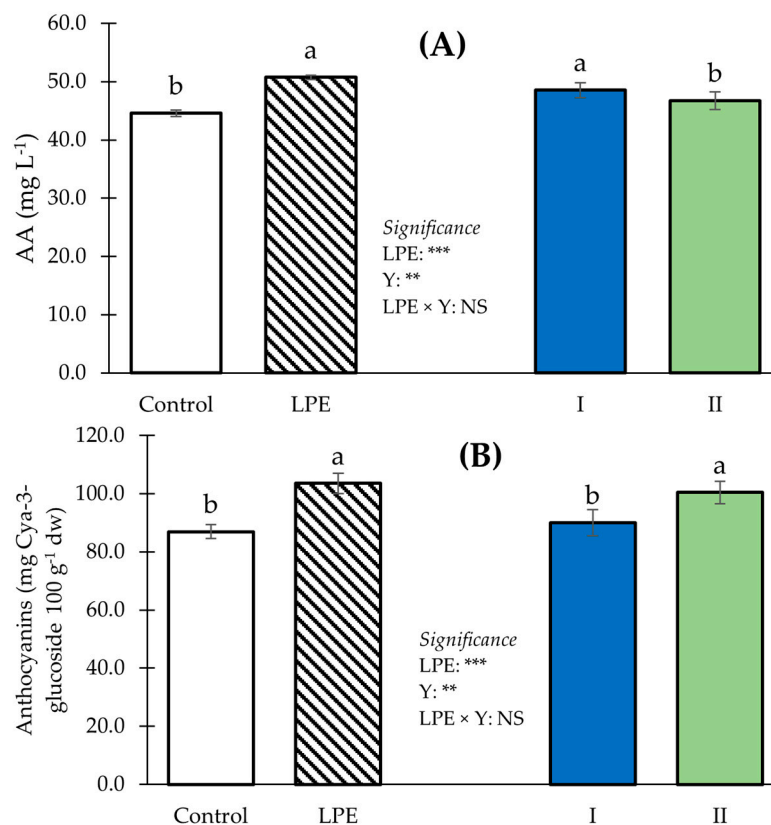


Figure 4. Ascorbic acid (AA) (A) and anthocyanin (B) content of strawberry fruit in response to lysophosphatidylethanolamine (LPE) application and year of cultivation (Y). Different letters indicate means that are statistically dissimilar according to the Tukey HSD test ($p \leq 0.05$). NS: not significant; ***: significant at $p \leq 0.001$; **: significant at $p \leq 0.01$. Bars indicate the standard error. I = 2020–2021 growing period; II = 2021–2022 growing period. Data are the mean of all harvests, starting from the 4th harvest.

As shown in Figure 4A, LPE treatments boosted the AA concentration in the fruits by a significant 13.8%. When averaged over LPE treatments, fruits from plants cultivated in year I (2020–2021) have a higher (+3.9%) AA than those from plants grown in year II (Figure 4A). When averaged over years, the highest anthocyanin concentration was recorded in fruits from plots treated with LPE (+19.1%). On the other hand, regardless of the LPE application, fruits harvested from plants grown during the second year revealed the highest anthocyanin concentrations (Figure 4B).

Regarding the TP concentration in the fruits, ANOVA revealed a significant interaction between the two main factors (Figure 5).

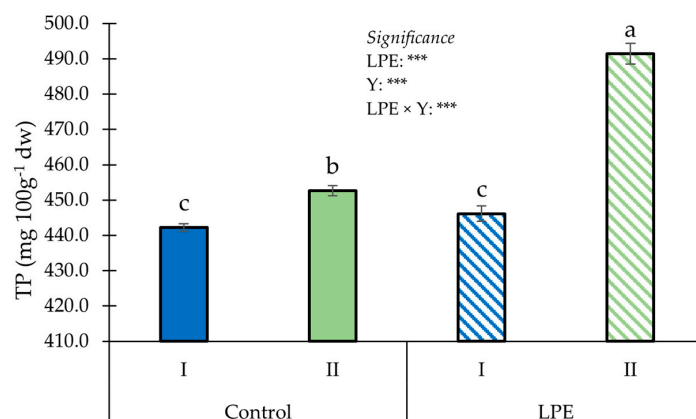


Figure 5. Total phenols (TP) of strawberry fruits in response to lysophosphatidylethanolamine (LPE) application and year of cultivation (Y). Different letters indicate means that are statistically dissimilar according to the Tukey HSD test ($p \leq 0.05$). ***: significant at $p \leq 0.001$. Bars indicate the standard error. I = 2020–2021 growing period; II = 2021–2022 growing period. Data are the mean of all harvests, starting from the 4th harvest.

The highest TP values were recorded in fruits from plants cultivated in year II (2021–2022) treated with LPE, followed by those harvested from control plants grown in the same period. The lowest values were found in fruits cultivated during the 2020–2021 cultivation period, whether treated with LPE or not (Figure 5).

The colour parameter a^* was significantly influenced by the interaction LPE \times Y (Figure 6). The highest values were measured in fruits from plants treated with LPE, followed by control plants grown in the second year.

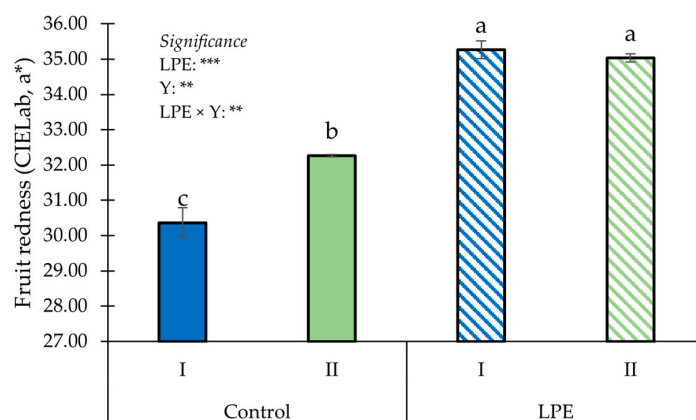


Figure 6. Fruit redness—CIELab parameter a^* —of strawberry fruits in response to lysophosphatidylethanolamine (LPE) application and year of cultivation (Y). Different letters indicate means that are statistically dissimilar according to the Tukey HSD test ($p \leq 0.05$). ***: significant at $p \leq 0.001$; **: significant at $p \leq 0.01$. Bars indicate the standard error. I = 2020–2021 growing period; II = 2021–2022 growing period. Data are the mean of all harvests, starting from the 4th harvest.

As reported in Table 2, the statistical analysis for antioxidant activity, TA, firmness, b^* , L^* and SSC did not underline any significant effect of the interaction between the main experimental factors.

Table 2. Antioxidant capacity, titratable acidity (TA), firmness, CIELab parameters (b^* and L^*), and soluble solids content (SSC) of strawberry fruits in response to lysophosphatidylethanolamine (LPE) application and year of cultivation (Y).

| Treatments | Antioxidant Capacity (% Inhibition) | TA (% Citric Acid) | Firmness (N) | b^* | L^* | SSC ($^{\circ}$ Brix) |
|---------------------|-------------------------------------|--------------------|--------------|---------|---------|------------------------|
| <i>LPE</i> | | | | | | |
| Control | 81.9 b | 0.52 a | 6.3 b | 33.37 a | 41.15 a | 7.6 b |
| LPE | 86.2 a | 0.50 a | 6.8 a | 23.30 b | 35.03 b | 8.8 a |
| <i>Year</i> | | | | | | |
| I | 84.9 a | 0.53 a | 6.5 a | 28.67 a | 38.68 a | 8.2 a |
| II | 83.2 a | 0.48 a | 6.6 a | 28.00 a | 37.50 b | 8.1 a |
| <i>Significance</i> | | | | | | |
| LPE | * | NS | *** | *** | *** | *** |
| Y | NS | NS | NS | NS | ** | NS |
| LPE \times Y | NS | NS | NS | NS | NS | NS |

Different letters indicate means that are statistically dissimilar according to the Tukey HSD test ($p \leq 0.05$). NS: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$. I = 2020–2021 growing period; II = 2021–2022 growing period. Data are the mean of all harvests, starting from the 4th harvest.

Regardless of the year, LPE applications significantly increased the antioxidant capacity, the firmness and the SSC of the fruits, whereas they meaningfully decreased the b^* and L^* parameters and had no significant effect on the TA (Table 2). On the other hand, the year of cultivation had no significant influence on the antioxidant capacity, TA, firmness, b^* parameter and SSC, although it had a relevant effect on the L^* parameter. In this regard, fruits from plants cultivated in the first trial year revealed higher lightness values than those harvested from plants grown in the second (Table 2).

The PCA for all productive and qualitative parameters revealed two principal components (PC) with eigenvalues higher than 1 (Table 3). Altogether, the two components explained 96.4% of the total variance.

Table 3. Eigenvalues, variance and cumulative percentages of total variance of the two principal components (PCs) for all 16 variables.

| Variables | PC1 | PC2 |
|-------------------------|--------|--------|
| TY | 0.955 | −0.282 |
| MY | 0.914 | −0.399 |
| NMF | 0.342 | 0.922 |
| AMFW | 0.901 | −0.427 |
| DP | −0.168 | 0.961 |
| AA | 0.969 | −0.228 |
| TP | 0.528 | 0.802 |
| Anthocyanins | 0.824 | 0.554 |
| Antioxidant capacity | 0.882 | −0.213 |
| TA | −0.273 | −0.956 |
| Firmness | 0.990 | 0.122 |
| a^* | 0.922 | 0.186 |
| b^* | −0.988 | −0.082 |
| L^* | −0.971 | −0.218 |
| SSC | 0.999 | −0.019 |
| Dry matter | −0.836 | 0.516 |
| Eigenvalue | 10.900 | 4.525 |
| Variance cumulative (%) | 68.123 | 96.401 |
| Variance | 68.123 | 28.278 |

In particular, PC1 was mainly positively correlated with TY, MY, AMFW, AA, anthocyanins, antioxidant capacity, firmness, a^* and SSC, whereas it was mostly negatively correlated with b^* , L^* and dry matter. On the other hand, PC2 was largely positively correlated with NMF, DP and TP and mainly negatively correlated with TA (Table 3).

LPE application and year of cultivation determined a clear separation, with the control on the left side of the plot and the LPE on the right side (Figure 7).

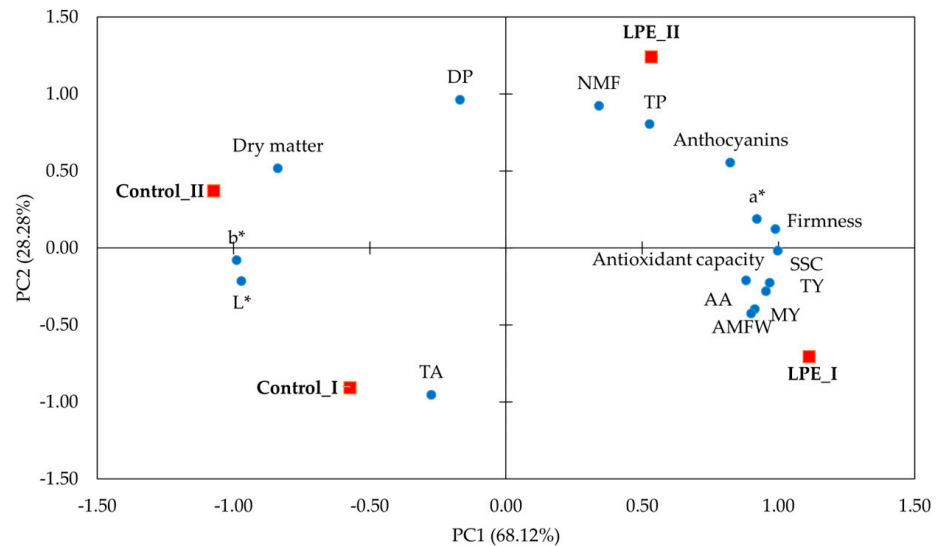


Figure 7. Loading plots of principal components analysis (PCA) of strawberry plant productive and qualitative traits as affected by the combination of LPE (control or LPE) and year of cultivation (I: 2020–2021 or II: 2021–2022).

Moreover, the two cultivation years were separated into top (year II) and bottom (year I) of the plot. Each combination of the two main factors occupied a specific quadrant of the plot. The combination Control_II was positioned in the upper left quadrant with the variables dry matter and DP, while the combination LPE_II was placed in the upper right quadrant with NMF, TP, anthocyanins, a^* and firmness. The bottom-left side was occupied by the combination Control_I, with TA, L^* and b^* , while the combination LPE_I was located in the bottom-right quadrant with AA, antioxidant capacity, SSC, TY, MY and AMFW (Figure 7).

4. Discussion

The need for reducing the environmental impact of horticultural systems while preserving crop yield and quality has generated an increasing interest in developing eco-sustainable products, such as natural lipids. Among them, LPE is certainly one of the most widely used due to its recognized benefits in terms of yield and quality [15]. Simultaneously, it is well known that plant performance can be affected significantly by weather conditions, which is why a two-year study was conducted to evaluate the response of strawberry plants to LPE.

Our study revealed that LPE applications significantly increased strawberry yield parameters. These results are in contrast with those of Çeler et al. [37], who, studying the effects of LPE applications on strawberry plants, found no significant variation in yield parameters. However, our findings could be explained by the LPE function of molecular chaperone, enhancing the proper folding of citrate synthase and protecting it from heat denaturation [38]. Since this enzyme is involved in processes which are fundamental for proper plant metabolic pathways functioning such as the Krebs cycle, the yield increase may be related to an increase in physiological traits of treated strawberry plants. On the other side, our experiment showed that plants cultivated during the 2021–2022 period had lower yield parameters than those grown in the first year (Figure 2A–C). These results could

be linked to the different weather conditions (Figure 1). Indeed, in the 2021–2022 cycle, especially from January to mid-April, daily minimum temperatures were lower than those of 2020–2021. Considering that these are the months into which most of the production is concentrated, the relevant variation in yield traits could be related to the colder temperatures recorded during the 2021–2022 growing period. The research underlined that the DP was significantly influenced by the cultivation year (Table 1). Plants grown during the 2021–2022 period had the highest DP values. As previously shown, the first growing period was warmer than the second one; consequently, we may suppose that the higher DP values recorded in year II were related to the stressful climatic conditions recorded.

Ascorbic acid, also known as vitamin C, is an important constituent of plants. It has vital functions in photosynthesis and in cell growth [39]. In our study, LPE treatments significantly increased the AA concentration in the fruits. As stated by Cowan [40], LPE may function as a signalling molecule, impacting the membrane transport process, ionic balance homeostasis and cell turgor. Consequently, the observed increase in AA concentration in the fruits can be attributed to an improved physiological status of the plant. Moreover, since the AA biosynthesis is also related to the activity of nucleotide sugars [39], and considering that the LPE application protects α -glucosidase (enzyme involved in sugar metabolism) from thermal degradation [38], we may suppose that the LPE supply promoted the natural biosynthesis of AA in strawberry plants. Regarding the effect of the year, our study pointed out that fruits from plants cultivated during the 2020–2021 growing period had the highest AA concentration. These results are in line with those obtained by Ergin et al. [41], who observed an increase in ascorbic acid concentration in heat-stressed plants. The observed effects could be related to an increase in reactive oxygen species production prompted by the high temperatures recorded during year II. Indeed, since AA is an electrons donator, it is biosynthesized by plants for reducing the oxidative stress damage [42]. Moreover, as reported by Smirnoff and Wheeler [39], ascorbate peroxidase rapidly responds to the photo-oxidative stress that plants may experience during hot periods.

Anthocyanins are pigments belonging to the flavonoids family that can be found in the vacuoles of plants at the surface of the fruit, also known as the outer epidermal peel, and are responsible for the red colour of many fruits and vegetables [34,43]. Anthocyanins are important for human health since they have functions in decreasing the incidence of many diseases such as cancer and other chronic disfunctions [43]. Our findings revealed that LPE supply significantly increased anthocyanin concentration in the fruits. This is in line with Özgen et al. [27], who, studying the effects of LPE on the development of cranberry fruits, found an anthocyanin increase in fruits from LPE-treated plants. Moreover, our data are in accordance with those of Hong et al. [26], who reported an enhanced concentration of anthocyanins in LPE-treated grape berry fruits. These results may be related to the capacity of LPE to modulate the ethylene production which is involved in the anthocyanins biosynthesis [21]. On the other hand, our results revealed that fruits from plants grown during the colder growing period (2021–2022) had the highest anthocyanin concentration. These outcomes are coherent with those stated by Matsushita et al. [44], who found a decrease in anthocyanin concentration in strawberry fruits exposed to high temperatures. Moreover, since anthocyanins are related to an increase in environmental stress resistance [45], we may speculate that the higher anthocyanin concentration recorded in fruits from the 2021–2022 period is a plant defence reaction to cold distress conditions.

Phenols are secondary metabolites biosynthesized by plants, which possess several important properties [46]. Although they are not nutrients, they have important health benefits and are components that play an important role in plant responses to stressful situations, since they contribute to the redox homeostasis in cells [46]. In our study, LPE significantly enhanced the total phenols concentration in strawberry fruits. These findings are in line with those of Özgen et al. [47], who, studying the effects of LPE application on sweet cherry plants, found a significant increase in total phenols in fruits from LPE-treated plants. Since the biosynthesis of phenols is modulated by ethylene activity [48], and considering that LPE is effective in ethylene modulation in plant tissues [15], we may hypothesize that

the enhanced phenols concentrations in fruits from LPE-treated plants could be attributed to increased ethylene production. Our study also pointed out that total phenols were higher in fruits from plants cultivated during 2021–2022. These results are coherent with those of Rivero et al. [49], who, testing the resistance to extreme temperatures in tomato and watermelon plants, reported an increase in phenolics in chilled plants. Moreover, our findings agree with those of Król et al. [50], who, investigating the effect of cold stress on phenolic compounds in grapevine, found an upsurge of phenols concentration in chilled plants. Since phenolics participate in plant responses to environmental stresses [51], and considering that the year II cultivation period was more stressful than year I, the higher content of phenolics compounds is connected to a plant defence mechanism.

Colour is an important quality trait for fruits and vegetables, especially for a berry-like plant, such as strawberry. Our data on redness (a^*) revealed that LPE significantly increased the redness of the fruits; moreover, our data underlined that the reddest strawberry fruits were those harvested from plants grown during the coldest year (II). As reported by Wesche-Ebeling and Montgomery [52] and Wang and Zheng [53], the main factor affecting the red colour of strawberry fruits is the presence of anthocyanins. Consequently, the redness data could be related to the anthocyanin concentration in the fruits. Moreover, LPE application significantly decreased the yellowness and lightness of the strawberry fruits. These data are coherent with those of Özgen et al. [47] relating to the sweet cherry.

The data highlighted that LPE application significantly increased the antioxidant capacity of fruits. These findings are in line with those of Özgen et al. [47], who found that LPE meaningfully enhanced the antioxidant activity of the sweet cherry. The higher antioxidant activity of the LPE-treated plants is due to the ascorbic acid, total phenols and anthocyanins concentration in the fruits, as these are scavenging compounds [54].

In our work, firmness was significantly increased by LPE application. This is in line with the findings of Farag and Palta [21], who found an increase in cranberry fruit firmness after LPE application. Moreover, our results are coherent with those of Hong et al. [26] and Altwies et al. [55], who found that LPE application increased the firmness of grape berry and tomato, respectively. However, our findings are in contrast with those of Amaro et al. [56], who found no significant effect of LPE on cantaloupe fruit firmness. The LPE action on the firmness of strawberry fruits could be linked to a reduction in polygalacturonase activity, an enzyme involved in the maturation of fruits [57,58].

Our study revealed that LPE treatment increased the fruit SSC. These data tie well with those of Hong et al. [26], who found a significant SSC increase in grape berry. Moreover, our findings are in accordance with those of Özgen et al. [47], who revealed an upsurge of SSC in LPE-treated sweet cherry plants. As previously reported by Kern et al. [38], this effect could be linked to the LPE participation in sugar metabolism.

Principal components analysis showed a comprehensive structure for assessing the influences of LPE application and year on strawberry yield and quality traits. The treatments combinations were clearly divided via the PCA. Nonetheless, the PCA recognized that many variables were affected by the LPE \times year interaction, since only these combinations were clustered in the negative side of PC1.

5. Conclusions

In the contemporary era, the development and use of environmentally sustainable products that can improve qualitative and quantitative aspects of production is crucial. Natural lysophospholipids, such as LPE, are natural products approved by the Environmental Protection Agency for agricultural use. Our results showed that the application of LPE significantly increased yield and quality traits, such as ascorbic acid, anthocyanin, total phenolics, firmness and soluble solids content of fruits. In addition, LPE application improved the visual quality of fruits by increasing their colorimetric parameters. Moreover, our data revealed that year of cultivation may have a relevant effect on strawberry yield and quality. This result turns out to be very important, in view of the increasingly evident climate changes we are facing. Interestingly, our results showed also that the use

of LPE was able to improve some productive and qualitative strawberry parameters by interactively acting with the growing year. Specifically, it was noted that LPE treatments improved yield traits (TY, MY and AMFW), total phenolics and fruit redness, even under non-favourable growing conditions (year II). In conclusion, our results showed that the foliar application of LPE at a dose of 10 ppm is an effective and environmentally sustainable strategy to efficiently modulate the yield and quality of strawberry plants grown in a protected environment under different climatic conditions.

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