

Research Paper

Light-Emitting Diodes improve yield, quality and inhibitory effects on digestive enzymes of strawberry

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ABSTRACT

Strawberries are a widely consumed fruit that are increasingly popular due to the perceived health benefits associated with their consumption. Fruit quality is highly dependent on the growing environment where light is one of the most significant environmental factors influencing plant physiology and metabolism. In the present work we sought to test the hypothesis that manipulation of the light environment in a commercial growing environment would influence fruit yield and quality. Fruit were grown with supplemental light-emitting diodes in the red (623 nm), far-red (727 nm) and blue (470 nm) regions of the spectrum at three different densities. The majority of light treatments resulted in increased fruit yield. All treatments also significantly enhanced contents of anthocyanins and polyphenols. Furthermore fruit exhibited enhanced antioxidant activity. Individual strawberry sugars showed differences depending on sampling date whereas Brix, acidity and ascorbic acid was not affected by the LED lights. Strawberry fruit extracts from all treatments exhibited the capacity to inhibit the digestive enzymes pancreatic lipase and α -amylase activity in vitro, extract from fruit grown under supplemental lighting had a greater inhibitory capacity. These data suggest that strawberry fruit grown in the presence of supplemental light may impart health benefits via enhanced functional compounds and by limiting calorific assimilation. The findings of this study provide the first evidence that the use of light-emitting diodes increase the inhibitory effects of polyphenols on digestive enzymes in strawberry.

1. Introduction

Environmental factors such as CO₂, light, nutrients, temperature and water have an important influence on plants. Among of these, light is one of the most important factors, playing an important role on plant physiology (Yeh et al., 2009). Plants are characterized by their ability to respond to different wavelengths since they have different photoreceptors, phytochromes for red and far red light (Franklin and Quail, 2010), cryptochromes, phototropins and zeitlupes for blue light (Demarsy and Fankhauser, 2009; Lin and Shalitin, 2003) and UVR8 for ultraviolet b light (Rizzini et al., 2011). Use of Light-Emitting Diodes (LED) technology as supplementary light has become usual in different crops (Dlugosz-Grochowska et al. 2016; Tewolde et al. 2016; Piovene et al., 2015), due to advances in recent years that have allowed reduced cost since they are more efficient than conventional light (Goto et al., 2014). The peak wavelength of LED used in horticulture ranges from blue (450 nm) to far red (730 nm) (Yeh and Chung, 2009). This technology is used

mainly in northern countries (Särkkä et al., 2017), which are characterized by low sunlight conditions, resulting in a decrease in photosynthesis and hence decreased yields (Petridis et al., 2018). Therefore the first benefit that we can cite regarding the use of LED light in agriculture is the increase in yield (Lu et al., 2012; Chen et al., 2017), but this is not the only benefit, other benefits that have been described are control of diseases (Schuerger and Brown, 1994), reduction of nitrate content on plants (Bian et al., 2016) and increase of functional compounds such as anthocyanins (Heo et al., 2012), xanthophylls, β -carotene (Li and Kubota, 2009), carotenoids (Samuolienė et al., 2013), antioxidant properties (Samuolienė et al., 2012), phenolic compounds (Choi et al., 2015) and vitamin C (Bliznikas et al., 2012) on vegetables and fruits. Functional compounds in fruits and vegetables are an important issue, especially since their role in preventing diseases has been described (Limberaki et al., 2012; Lobo et al., 2010), for that reason many studies have been performed in order to study the effect of different agronomy techniques on functional compounds. The plant

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response to light is very specific, it varies between crops and even cultivars (Mizuno et al., 2011), could be influenced by a variety of factors (Ouzounis et al., 2015) and is still poorly studied. Previous studies on LED lights used during strawberry growing reported differences in content of functional compounds, thus have been described an increase of anthocyanins (Choi et al. 2013; Kadomura-Ishikawa et al., 2013 and Zhang et al. 2018), phenolic compounds (Choi et al., 2015) and flavonoids concentration (Piovene et al., 2015). As well differences in some parameter related with organoleptic quality have been described, for example an increase in content of fructose (Choi et al. 2013), organic acids (Choi et al., 2015) and color (Nadalini et al., 2017). However, indications regarding the most suitable wavelength are contradictory, and distinct results can be found in the scientific literature (Choi et al. 2013; Kadomura-Ishikawa et al., 2013; Choi et al., 2015; Nadalini et al., 2017 and Zhang et al. 2018). Another significant limitation of these studies is the performance in experimental glasshouses and/or growth chambers with a lack of testing in a commercial glasshouse.

There have been reports that strawberry inhibit some digestive enzymes such as α -amylase and pancreatic lipase (McDougall and Stewart, 2005) that are implicated on metabolism of sugar and triglycerides, therefore intake of strawberry can contribute to decrease digestion of sugar and triglycerides. There are no previous studies about the use of agronomic techniques to achieve increase capacity of strawberry to inhibit digestive enzymes. This study is the first evidence of use supplemental lights to improve strawberry capacity to inhibit α -amylase and pancreatic lipase.

The main objective of this work was to study how manipulation of light quality can influence strawberry chemistry. The results of this research will aid in identifying the most suitable wavelength to enhance strawberry functional compounds and will be useful in designing technologies that can be employed within a commercial setting. Our study was performed in two stages, one first stage was conducted in an experimental glasshouse at The James Hutton Institute (Dundee, UK), the aim was to test whether lighting system had a significant effect on fruit phytochemical. Once the hypothesis was validated, the second stage was conducted on a commercial farm at PJ Stirling (Arbortah, UK) to confirm the system's effectiveness in a commercial farm and assess its feasibility for implementation.

2. Material and methods

2.1. Plant material and cultivation

Strawberry plants (*Fragaria* \times *ananassa* 'Malling centenary'), were planted in plastic containers containing coir as substrate in the spring and autumn cycle. Fertilization was as follows: KNO_3 at the electrical conductivity level of 1.6 dS m^{-1} , MAP at 1.6 dS m^{-1} , MgSO_4 and NPK at 1.6 dS m^{-1} , Iron, and MnSO_4 at 1.8 dS m^{-1} . Two trials were performed, the first at The James Hutton Institute ($56^\circ 27' \text{ N}$; $3^\circ 04' \text{ W}$) in spring and the second at PJ Stirling ($56^\circ 35' \text{ N}$; $2^\circ 32' \text{ W}$) in autumn. The length of the growing season was 97 and 105 days for the first and the second trial, the growing season for the first trial extended from 10 February to 18 May, and from 1 September to 14 December for the second trial. The first trial aim was tested if lighting system had significant effect on fruit phytochemical, once the hypothesis was tested, the second trial was carried out in a commercial farm in order to corroborate that the system is effectiveness in a commercial farm and can be implemented. Weather data for the two growing season is shown in tables S1 and S2 (Supplementary materials).

2.2. LED lighting

Light treatments used were far red (727 nm), red (623 nm) and blue (470 nm) at a density of 20 lights per lineal meter in the first trial and 20, 10 and 5 lights per lineal meter in the second trial.

2.3. Sampling and extract preparation

Sampling was performed once in the first trial in order to test the hypothesis that supplemental light improve functional compounds and seven times in the second trial in order to observe that results was consistent along the cycle. Most of production in the second trial was concentrated in the specific timeframe choose (seven weeks). Strawberry fruit samples were harvested at commercial ripeness and snap-freezing immediately in liquid nitrogen, frozen and lyophilized by freeze-drying for seven days, then samples were milled in a handheld coffee grinder and extracted with 50% methanol containing 0.1% formic acid.

2.4. Total phenolic content

Total phenolic content were estimated according to the Folin-Ciocalteu method described by Singleton and Rossi (1965) with some modifications. Briefly, 50 μl of strawberry extract were added to 96-well microplates, followed by 50 μl of 10% of the Folin-Ciocalteu's reagent (Sigma F9252) and 100 μl of 13% sodium carbonate, the microplate was covered and left in the dark for 60 min in order to complete the reaction. Absorbance was measured at 750 nm with a spectrophotometer microplate reader. Results were expressed as trolox equivalents per gram of fresh weight.

2.5. Anthocyanin assay

Anthocyanin content was determined according to the pH differential method (Cheng and Breen, 1991), briefly 20 μl of strawberry extract were added into 96-well microplates and 20 μl either the HCl/KCl buffer at pH 1.0 or sodium acetate buffer at pH 4.5 were added, absorbance was measured at 510 nm and 700 nm with a spectrophotometer microplate reader. Results were calculated as follows: $A = (A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5}$ and expressed as mg of cyaniding-3-glucoside per kg of fresh weight.

2.6. Antioxidant activity

Antioxidant activity was determined by the Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996), briefly FRAP reagent was prepared by mixing 20 ml of acetate buffer pH 3.6, 2 ml TPTZ solution (10 mM 2,4,6-Tris(2-pyridyl)-s-triazine in 40 mM HCl) and 2 ml of ferric chloride. In a 96-well microplate 20 μl of strawberry extract and 180 μl of FRAP reagent were added, and absorbance was measured at 593 nm with a spectrophotometer microplate reader. Results were expressed as mmol Fe^{2+} per kg of fresh weight.

2.7. Total polyphenols

Total polyphenols was determined as described by Stevanato et al. (2004), briefly 145 μl of phosphate buffer, 20 μl of hydrogen peroxide 20 mM, 20 μl of 4-aminophenazone 30 mM, 10 μl of diluted strawberry sample solution and finally 5 μl of 100 U/ml horse radish peroxidase were added to 96-well microplates, samples were incubated for 5 min at room temperature, absorbance was measured at 500 nm and results were expressed as mg of catechin equivalents per g of fresh weight.

2.8. Total soluble solid, acidity and Brix/acidity ratio

Sugars are the major soluble solids in fruit juice, hence sugar content was determined as Total Soluble Solid. The determination was carried out in strawberry juice and was expressed as $^\circ\text{Brix}$, for that was used a portable ATAGO PR-101 digital refractometer. Titratable acidity was determined on 5 ml of strawberry juice titrated with 0.1 N sodium hydroxide until a of pH 8.1 was reached, results were expressed as% malic acid. The $^\circ\text{Brix}/\text{acidity}$ ratio was calculated as $^\circ\text{Brix}$ divided by acidity.

2.9. Soluble sugars

Simple sugars analyses (glucose, fructose and sucrose) were performed as described by [Viola et al. 2007](#), briefly samples were extracted with 80% ethanol during 1 h at 80 °C, centrifuged, supernatants were collected and sampled extracted again, ethanol was evaporated by speed-vac and resuspend in distilled water, 1000-fold samples into distilled water was used for sugars quantification by high-performance liquid chromatography-Pulsed Amperometric Detection (HPAEC-PAD; Dionex) (HPLC-PAD; Dione) on a CarboPac PA-20 column (3 × 150 mm) fitted with a (3 × 30 mm) guard column. Separation of sugars was achieved over 15 min in 10 mM NaOH at a flow rate of 0.5 mL min⁻¹. Between each injection the column was regenerated in 100 mM NaOH (0.5 mL min⁻¹, 5 min) and then reequilibrated for 10 min in 10 mM NaOH. Glucose, fructose and sucrose were expressed as mg per gram of fresh weight. Calculations of sugars for the two growing season are included as supplementary material.

2.10. AsA quantification

Quantification of ascorbic acid was performed as follow, 50 mg of lyophilized sample was diluted in 1 ml of 5 % metaphosphoric acid containing 5 mM tris (2-carboxyethyl) phosphine hydrochloride (TCEP), homogenized, and then centrifuged (16,000 g, 1 °C, 5 min). Quantification was performed by HPLC analysis as described by [Hancock et al. \(2000\)](#). Briefly 20 µl of supernatant were applied to a 300 × 7.8 mm ID Coregel 64H ion exclusion column (Interaction Chromatography, San Jose, CA, USA) with a 4 × 3 mm ID carbo-*H*+ guard cartridge (Phenomenex, Macclesfield, UK) the temperature was adjusted at 50 °C. The mobile phase was 8 mM H₂SO₄ that flowed at 0.6 ml min⁻¹ and AsA was detected at 245 nm using a Gynkotech UVD 340S diode array detector (Dionex, Camberley, UK).

2.11. Yield

Yield was recorded in the second trial, for that, strawberries at commercial ripeness were picked up two times per week.

2.12. Lipase assay

A lipase assay was performed using the turbidimetric method described by [Vogel and Zieve \(1963\)](#) and modified by [Wilcox et al. \(2014\)](#), briefly olive oil was removed fatty acids, passing through aluminum oxide. The olive oil substrate solution was made up of 4 ml of 1% v/v olive oil free from fatty acids in acetone added to a heated solution (70 °C) of 100 ml 0.05 M Tris buffer (pH 8.3) containing 0.35% sodium deoxycholate. This solution was heated at 70 °C and homogenized for 10 min and cooled to room temperature. The enzyme solution was prepared by adding 1.29 mg/ml lipase and 18 µg/ml colipase to UPW. The strawberry samples with enzyme solution were incubated at 37 °C for 15 min, after the incubation the substrate solution was added and absorbance was measured at 405 nm. The results are expressed as percentage of lipase inhibition.

2.13. α-amylase assay

An α-amylase assay was carried out as described by [Nwosu et al. \(2011\)](#), briefly synthetic saliva buffer was made with 100 mM HEPES (pH 6.8) containing 300 mg/l of CaCl₂. α-amylase was dissolved in synthetic saliva buffer at 380 mg/l. Starch solution was made by dissolving 1% (w/v) soluble potato starch in synthetic saliva buffer, then was gelatinized for 10 min at 90 °C. The control assay/sample assay included 800 µL of synthetic saliva buffer, 100 µL of α-amylase, 100 µL of UPW/ sample extract and 500 µL of starch. The results are expressed as percentage of α-amylase inhibition.

2.14. Statistical analysis

Analysis of variance (ANOVA) was performed using the Statistix software version 9. Results were expressed at the $p \leq 0.05$ level of significance using the Fisher's least significant difference (LSD) test.

3. Results

Overall the results showed that red, far red and blue LED lights were effective in enhancing anthocyanins, total phenolic content, antioxidant activity and total polyphenol contents in strawberry fruit, the majority of treatments resulted in increased fruit yield, individual sugars showed differences depending on sampling date, Total Soluble Solids, acidity and ascorbic acid were not affected by LED light and extract from strawberry grown under LED light showed higher inhibitory activity against α-amylase and lipase. These results are detailed below.

3.1. Anthocyanins

The results obtained in this study showed that red, far red and blue LED light treatments were effective in enhancing anthocyanins in strawberry fruit ([Table 1](#)). There were significant differences between treatments and control for all samplings with the exception of the third sampling of the second trial. In terms of light density beneficial impacts could still be observed at a lighting density one quarter of that used in the first trial with lights at a density of 5 LEDs per growing meter being sufficient.

3.2. Total phenolic content

In general total phenolic content (TPC) was higher for all strawberry fruit grown under LED light treatments compared with control. In detail the results obtained in the first trial showed higher content in strawberry grown under red and far red light and lower content in strawberry grown under blue light compared with the control. In the second trial it is remarkable that results were higher for most of treatment and densities although first, third, fourth and seventh sampling did not show statistical differences ([Table 2](#)). The reduction of light density did not decrease the TPC.

3.3. Antioxidant activity

The strawberry antioxidant activity was affected by light spectra treatments. The results showed in general higher antioxidant activity in strawberry grown under blue, red and far red light treatment compared with the control ([Table 3](#)). The results obtained on the first trial showed higher content in strawberry grown under red and far red light treatment and lower content in strawberry grown under blue light treatment compared with the control. Regarding the results obtained in the second trial, the antioxidant activity varied depending on sampling date, although the effect of LED light was observed on all samplings but not at all densities, the results were more consistent for second, fourth and fifth samplings ([Table 3](#)). Regarding light density, antioxidant activity was not significantly affected by it. Finally the antioxidant activity showed a trend towards a decrease during the season, since it was higher for those samples collected at the beginning of the season than those that were collected at the end of the season.

3.4. Total polyphenols

The results of total polyphenols of strawberry as influenced by the light spectral treatments are shown in [Table 4](#). The total polyphenols in the first trial showed that strawberry grown under red and far red light treatment was significantly higher than those grown under blue light treatment and control. Overall the results in the second trial showed higher content for strawberry grown under the three LED light

Table 1

Anthocyanins content in strawberry (mg of cyaniding-3-glucoside per kg of fresh weight) grown under three different LED light treatments. Different letters indicate significant differences at $p \leq 0.05$.

LED light treatment	Lights per lineal m	First trial		Second trial					
		1st sampling	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling
Red	20	1.94 b	5.99 c	4.20 ab	5.78 a	4.26 c	4.95 bcd	4.64 ab	2.99 ef
	10	–	6.68 ab	5.05 ab	5.78 a	4.86 abc	4.56 cde	3.82 c	3.72 cd
	5	–	6.61 b	5.45 a	6.25 a	5.34 a	5.13 bc	4.17 abc	3.91 bcd
Far red	20	2.41 a	5.11 d	4.44 ab	5.32 a	4.92 ab	5.54 ab	4.87 a	2.97 ef
	10	–	7.17 a	3.47 b	5.44 a	4.93 ab	4.98 bcd	4.74 ab	4.88 a
	5	–	6.30 bc	4.62 ab	6.83 a	5.22 a	4.42 de	4.38 abc	3.92 bcd
Blue	20	1.14 c	6.46 bc	3.76 ab	5.40 a	4.23 c	5.84 a	4.79 a	4.31 b
	10	–	5.97 c	4.20 ab	6.43 a	4.78 abc	4.99 bcd	3.99 bc	3.43 de
	5	–	5.22 d	4.39 ab	6.54 a	5.07 ab	5.52ab	4.52 abc	4.04 bc
Control	0	0.95 c	4.19 e	3.38 b	5.90 a	4.43 bc	4.09 e	4.03 bc	2.75 f

Table 2

TPC in strawberry (trolox equivalents per gram of fresh weight) grown under three different LED light treatments. Different letters indicate significant differences at $p \leq 0.05$.

LED light treatment	Lights per lineal m	First trial		Second trial					
		1st sampling	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling
Red	20	10.20 a	3.34 bcd	3.10 d	3.78 cd	3.73 e	3.57 abcd	3.87 ab	3.91 abc
	10	–	2.59 cd	3.71 a	3.73 cd	4.38 d	3.25 bcd	3.41bc	3.35 d
	5	–	3.44 bcd	3.29 c	4.24 b	4.43 d	3.73 a	3.36 bc	3.89 abc
Far red	20	7.90 b	3.24 bcd	3.31 c	3.25 f	4.63 cd	3.61 abc	4.07 a	3.98 ab
	10	–	2.47 d	3.53 b	3.67 de	3.65 e	3.23 cd	3.70 abc	3.55 cd
	5	–	3.19 bcd	3.49 b	4.67 a	4.96 b	3.29 abcd	3.33 bc	3.58 bcd
Blue	20	5.87 c	3.48 bc	3.08 de	3.52 def	4.61 cd	3.71 ab	3.36 bc	3.91abc
	10	–	6.38 a	2.93 ef	3.38 ef	3.84 e	3.37 abcd	3.40 bc	3.66 bcd
	5	–	3.66 b	3.64 ab	4.12 b	5.42 a	3.57 abcd	3.76 abc	4.17 a
Control	0	6.25 c	3.17 bcd	2.91 f	4.02 bc	4.79 bc	3.11 d	3.23 c	3.67 bcd

Table 3

Antioxidant activity in strawberry (mmol Fe²⁺ per kg of fresh weight) grown under three different LED light treatments. Different letters indicate significant differences at $p \leq 0.05$.

LED light treatment	Lights per lineal m	First trial		Second trial					
		1st sampling	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling
Red	20	10.19 a	9.87 a	7.39 de	8.21 bc	7.80 c	6.23 c	6.87 ab	8.19 a
	10	–	6.95 f	9.42 a	7.41 e	8.68 b	5.24 e	6.35 cd	5.40 bc
	5	–	9.30 b	7.41 de	7.95 cd	8.44 b	5.67 de	5.19 e	6.98 ab
Far red	20	7.90 b	8.34 cd	5.98 f	7.60 e	9.16 a	6.12 cd	6.80 bc	4.73 bc
	10	–	7.77 e	7.96 c	7.68 de	7.70 c	5.69 cde	5.36 e	5.54 bc
	5	–	8.41 cd	8.62 b	9.12 a	7.61 c	6.90 b	4.94 ef	8.26 a
Blue	20	5.87 c	8.96 b	8.55 b	6.30 g	8.76 ab	6.85 b	7.29 a	5.24 bc
	10	–	8.07 de	7.20 e	6.90 f	7.87 c	6.93 b	4.67 f	4.24 c
	5	–	8.16 d	7.78 cd	8.31 b	6.60 d	8.23 a	6.21 d	6.87 ab
Control	0	6.25 c	8.56 c	6.10 f	8.05 bc	6.25 d	5.93 cd	3.89 g	4.43 bc

Table 4

Total polyphenols in strawberry (mg of catechin equivalents per g of fresh weight) grown under three different LED light treatments. Different letters indicate significant differences at $p \leq 0.05$.

LED light treatment	Lights per lineal m	First trial		Second trial					
		1st sampling	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling
Red	20	1.37 a	0.70 bc	0.49 a	0.48 ab	0.46 abc	0.65 ab	0.69 abc	0.70 a
	10	–	0.63 bcd	0.46 ab	0.24 c	0.36 bc	0.56 b	0.48 d	0.62 a
	5	–	0.71 ab	0.43 ab	0.38 abc	0.68 a	0.63 ab	0.74 a	0.59 a
Far red	20	1.51 a	0.53 bcde	0.28 bcd	0.25 c	0.49 abc	0.55 b	0.71 ab	0.64 a
	10	–	0.91 a	0.19 d	0.27 c	0.39 bc	0.57 b	0.55 cd	0.58 ab
	5	–	0.36 e	0.29 bcd	0.38 abc	0.46 abc	0.66 ab	0.70 ab	0.59 ab
Blue	20	0.68 b	0.65 bcd	0.38 abc	0.24 c	0.54 ab	0.55 b	0.69 abc	0.62 a
	10	–	0.49 cde	0.18 d	0.49 a	0.44 abc	0.55 b	0.60 bcd	0.65 a
	5	–	0.45 de	0.28 bcd	0.47 ab	0.46 abc	0.80 a	0.67 abc	0.60 a
Control	0	0.69 b	0.50 cde	0.22 cd	0.34 bc	0.26 c	0.57 b	0.59 bcd	0.41 b

treatments, although differences were not statistically significant for all samplings, the three LED light densities did not show differences in total polyphenols content, that means that total polyphenols can increase using a low light density. Finally the total polyphenols showed a trend towards increase in the content during the season, since it was higher for those samples collected at the end of the season than those that were collected at the beginning of the season.

3.5. Yield

Yield is one of the most important parameters in agriculture. First trial was focused on quality parameters, the second trial due to was performed in a commercial farm, in addition to study quality parameters, was included the quantification of yield. (Table 5). The results showed that it was higher for all LED light treatments and densities, with the exception of strawberry plants grown under blue light with a density of 5 lights per meter.

3.6. Soluble sugars

The effect of LED light on soluble sugars was examined in the first and second trials of this study, the results are presented in Tables 6 and 7. Overall no significant differences were found in the first trial, however glucose and fructose were slightly higher and sucrose slightly lower for strawberry fruits that were cultivated under red and far red lights. In the second trial three samplings collected during the season were analysed, no trend was detected for any light treatment studied, however glucose, fructose and sucrose were higher at the beginning and at the end of the season for three treatments compared with control, which could indicate that the use of light could be useful for increasing sugar at the beginning and at the end of season and hence result in higher product commercial value.

3.7. Total soluble solids, acidity and Brix/acidity ratio

Total Soluble Solids (°Brix) and acidity are important parameters that affect fruit flavor, and the Brix/acidity ratio correlates with consumer acceptance (Basson et al., 2010). These parameters were analysed in the second trial, the results are included in Table 8. Regarding Total Soluble Solids, the results showed a tendency towards increasing during the season, overall samples from the fourth, fifth and sixth samplings showed higher content, the same tend could be observed for the acidity, therefore samples from the last two samplings (fifth and sixth samplings) showed higher acidity. In consequence of these results the Brix/acidity ratio was higher for strawberry fruit collected at the end of the season. In terms of comparison of LED light treatments on each sampling, no clear differences could be observed, but it some significant differences for some samplings at lower light density are noticeable (5 lights per lineal meter), thus Total Soluble Solids was higher for red LED light in the first, third and fifth samplings, for far red LED lights in the third sampling and finally for blue LED light in the fifth sampling, acidity was higher for red LED light in the first, third, fifth and sixth samplings, for far red LED light in the third, fourth and sixth samplings and finally for blue LED light in the third, fourth and fifth samplings.

Table 5
Yield (g strawberry plant⁻¹) on second LED light trial.

LED light treatment	Lights per meter			
	20	10	5	0
Red	766	773	678	–
Far red	679	874	915	–
Blue	771	1023	505	–
Control	–	–	–	613

3.8. Ascorbic acid

Ascorbic acid content (AsA) was evaluated in the first trial and no significant differences were found (Table 9), however AsA was higher for strawberry that was grown under red LED light and lower for strawberry that was grown under blue and far red LED lights compared with control.

3.9. α -amylase and lipase in vitro inhibition

Extracts from all strawberry fruit treatments exhibited the capacity to inhibit the digestive enzymes pancreatic lipase and α -amylase in vitro and extracts from fruit grown in the presence of supplemental lighting had a greater inhibitory capacity than fruit grown under control conditions. The higher lipase in vitro inhibition was obtained at far red light treatment with 20 lights per lineal meter, inhibitory capacity for the rest of light and densities was higher than control except for blue light treatment at 5 lights per lineal meter. The higher α -amylase inhibition in vitro was obtained at red light treatment with 10 lights per lineal meter and blue light treatment with 5 lights per lineal meter, the rest of the combinations tested did not show statistically significant differences compared with control (Fig. 1).

4. Discussion

LED light influence on functional compounds and yield have been proved in different crops (Dlugosz-Grochowska et al. 2016; Kondo et al. 2016; Tewolde et al. 2016). Previous studies in strawberry, obtained different results in phytochemical contents depending on wavelength used, the results obtained by Nadalini et al. (2017) showed lower anthocyanins content for blue and red LED lights whereas Choi et al. (2013) showed higher anthocyanin content for red plus blue LED lights, Kadomura-Ishikawa et al. (2013) results showed higher anthocyanins content for blue LED light and Zhang et al. (2018) results showed higher anthocyanins content for blue and red LED lights, regarding TPC, the previous studies performed in strawberry exhibited higher content for fruit grown under red light (Choi et al. 2015; Paparozzi et al., 2018) or not differences between red and blue light compared to control (Piovene et al., 2015).

Overall our results showed that spectral manipulation, especially for red and far red LED light treatments, enhance all the functional compounds analysed in the strawberry fruits for both trials, nevertheless the results of the second trial varied during the season in the seven samplings performed, and provided a wide data range. This data indicated that in general functional compounds and antioxidant activity did not show big differences between treatments and control in sampling performed at the middle of the cycle. It has been described on previous studies that accumulation of these compounds is correlated with color of fruit, dark red fruits have higher content and the color is related with illumination. At the middle of the cycle plants are the higher number of leaves, and these can produce shade on some fruit, decreasing the amount of light that fruit receive, and therefore decreasing the red fruit color and functional compounds. As well it is known that light is not the only factor that modifies functional compounds, other parameters such as temperature and radiation have an important impact on fruit quality (Zhang et al., 1997), autumn temperatures and radiation in northern countries are constantly changing, which also could explain the differences in our study for the second trial depending on sampling date. A detailed discussion of each of functional compounds studied in this work is presented below.

Biosynthesis of anthocyanins is affected by environment (Jaakola, 2013), highlighting the important role of light in anthocyanins accumulation (Zoratti, 2014). The results obtained on previous studies performed with different wavelength of light present contradictory results on this issue (Choi et al., 2013; Kadomura-Ishikawa et al., 2013; Nadalini et al., 2017). Our results shows that the anthocyanins content

Table 6

Soluble sugars, Glucose (Gl), Fructose (Fr) and Sucrose (Su) expressed as mg/g of fresh weight, in strawberry grown under three different LED light treatments in the first and second trial. Different letters indicate significant differences at $p \leq 0.05$.

LED light treatment	Lights per lineal m	First trial			Second trial								
		First sampling			First sampling			Second sampling			Third sampling		
		Gl	Fr	Su	Gl	Fr	Su	Gl	Fr	Su	Gl	Fr	Su
Red	20	33.76 a	36.59 a	15.91 a	26.79 abc	30.22 abc	18.41 de	23.84 ab	24.93 ab	23.45 a	19.01 cd	21.47 cd	5.34 f
	10	–	–	–	31.06 a	34.94 a	21.72 cd	19.64 bc	20.98 bcd	11.80 bc	20.26 bcd	22.72 bcd	13.17 a
	5	–	–	–	22.44 c	24.87 c	16.06 e	17.80 c	19.23 cd	7.84 cd	18.17 d	20.26 d	7.03 e
Far red	20	35.84 a	39.74 a	12.83 a	22.79 c	24.98 c	26.08 bc	17.90 c	18.65 d	14.61 b	20.24 bcd	22.69 bcd	8.78 c
	10	–	–	–	23.77 c	26.42 bc	22.06 cd	23.27 ab	24.18 abc	23.98 a	20.85 bc	23.65 abc	11.83 b
	5	–	–	–	29.06 ab	32.07 ab	32.30 a	20.56 bc	21.18 bcd	15.42 b	22.44 ab	24.88 ab	12.19 ab
Blue	20	29.97 a	32.24 a	14.52 a	24.59 bc	26.36 bc	30.50 ab	24.20 ab	24.92 ab	27.74 a	23.49 a	26.03 a	12.25 ab
	10	–	–	–	26.06 bc	28.87 bc	33.27 a	16.45 c	17.69 d	8.01 cd	19.05 cd	21.38 cd	8.10 cd
	5	–	–	–	24.77 bc	27.09 bc	24.81 c	15.68 c	16.73 d	6.45 d	19.33 cd	21.74 cd	7.32 de
Control	0	30.63 a	33.88 a	17.23 a	15.96 d	17.99 d	6.73 f	26.62 a	27.42 a	23.71 a	21.14 abc	23.47 bc	7.62 de

Table 7

Soluble sugars, Glucose (Gl), Fructose (Fr) and Sucrose (Su) (mg/g) FW, in strawberry grown under three different LED light treatments in the second trial. Different letters indicate significant differences at $p \leq 0.05$.

LED light treatment	Lights per lineal m	Second trial											
		Fourth sampling			Fifth sampling			Sixth sampling			Seventh sampling		
		Gl	Fr.	Su.	Gl	Fr.	Su.	Gl	Fr.	Su.	Gl	Fr.	Su.
Red	20	24.32 ab	26.40 abc	26.96 a	27.68 a	30.64 a	20.52 b	26.19 bcd	29.46 bcd	23.13 bcd	41.31 a	45.72 a	50.27 a
	10	27.05 a	29.68 a	27.46 a	25.31 ab	28.09 ab	18.62 bc	26.45 bcd	29.74 bcd	24.00 bc	28.39 bc	32.30 bc	30.70 bc
	5	18.74 bc	20.57 cd	15.92 de	25.14 ab	27.01 abc	28.96 a	28.15 bc	31.81 bc	35.92 a	23.82 defg	26.63 efg	25.69 d
Far red	20	21.67 ab	23.62 bc	20.78 bc	21.44 bc	23.42 bcd	15.37 cd	23.02 def	25.77 def	20.15 cde	26.08 cde	28.88 cde	27.86 cd
	10	15.58 c	17.75 d	13.84 e	28.09 a	31.14 a	20.94 b	19.08 f	22.08 f	12.16 f	22.97 efg	26.68 efg	15.95 f
	5	24.49 a	26.55 ab	27.17 a	20.01 c	21.84 d	16.44 cd	36.36 a	41.57 a	37.57 a	20.45 g	22.59 g	17.80 ef
Blue	20	24.82 a	27.00 ab	24.35 ab	20.94 bc	22.46 cd	22.08 b	26.24 bcd	29.47 bcd	25.85 b	27.07 bcd	30.81 bcd	24.84 d
	10	21.72 ab	24.00 abc	15.32 de	22.49 bc	24.56 bcd	14.56 d	24.85 cde	27.86 cde	19.66 de	30.73 b	34.43 b	33.55 b
	5	22.34 ab	24.18 abc	18.86 cd	18.37 c	20.02 d	14.40 d	30.04 b	34.07 b	35.39 a	24.54 def	28.05 def	19.79 e
Control	0	27.19 a	29.46 ab	21.79 bc	25.08 ab	27.04 abc	26.31 a	20.69 ef	23.63 ef	18.05 e	22.19 fg	24.56 fg	20.00 e

in strawberry fruit was significantly improved for plants that was grown under supplemental lights. Far red and red LED light showed the better results, no differences between densities was found which suggests that density LED light can be decreased which represents economic saving without decrease beneficial impact of lights.

TPC in fruit is primarily influenced by ripening, growing environment and genetic differences in plants (Shahidi and Nacz, 2004). Our results present clear evidences of the positive effect of supplemental LED lights in TPC in fruit. Both red, far red and blue LED lights improved content, whereas previous studies only described the positive effect of red LED light (Choi et al. 2015; Paparozzi et al., 2018). No differences between densities were found for most of sampling, even low densities showed better performance for sampling in the middle of cycle, therefore, similar to the results obtained for anthocyanins, we can reduce the density without compromising TPC.

Numerous studies have indicated that certain interactions within

antioxidant compounds, including anthocyanins, flavonoids, and phenolics, might exhibit synergistic effects. Consequently, conducting a comprehensive analysis of total antioxidant activity has the potential to provide a more accurate assessment of the collective contribution of antioxidant components (Meyers et al., 2003). Strawberry fruit is characterized for have high antioxidant activity (Cordenunsi et al., 2005). The results obtained in this work provides evidences that LED lights can be used to enhance the antioxidant activity of strawberry fruit. The red light proved to be more effective in enhancing antioxidant activity. Red light is involved in the production of anthocyanins (Zhang et al., 2018), a natural antioxidant compound found in strawberry fruit. As observed in the results of this study, the anthocyanin content in most samples was higher for fruit grown under red light, which would result in a greater antioxidant activity. Regarding to light density it is observed for red light that higher intensity exhibits higher antioxidant activity, while for blue light the effect of intensity over antioxidant activity varies

Table 8 Brix, acidity (Ac) and brix divided by acidity (Brix/Ac.), in strawberry grown under three different LED light treatments in the first and second trial. Different letters indicate significant differences at $p \leq 0.05$.

LED light treatment	Lights per lineal m	First sampling		Second sampling		Third sampling		Fourth sampling		Fifth sampling		Sixth sampling							
		Brix	Ac.	Brix/Ac.	Brix	Ac.	Brix/Ac.	Brix	Ac.	Brix/Ac.	Brix	Ac.	Brix/Ac.	Brix/Ac.					
Red	20	5.60 ab	0.48 ab	11.90 a	6.53 a	0.50 abc	13.13 a	5.73 ab	0.50 bc	11.53 a	5.03 b	0.45 bc	11.27 a	8.90 a	0.61 ab	14.61 a	7.27 bcd	0.53 ab	13.69 a
	10	5.97 ab	0.48 ab	12.43 a	4.87 b	0.47 abc	10.33 b	6.50 ab	0.51 abc	12.77 a	5.57 ab	0.40 c	13.75 a	6.17 cd	0.50 cd	12.81 ab	8.10 abc	0.57 a	14.17 a
	5	6.83 a	0.54 a	12.82 a	5.87 ab	0.45 bc	12.97 ab	7.30 a	0.54 ab	13.67 a	6.37 ab	0.48 ab	13.35 a	8.77 a	0.59 abc	15.07 a	7.87 abc	0.57 a	13.89 a
Far red	20	6.00 ab	0.50 ab	12.10 a	5.60 ab	0.47 abc	11.83 ab	6.27 ab	0.46 c	13.57 a	6.77 a	0.49 ab	13.85 a	8.17 ab	0.61 a	13.36 ab	9.03 a	0.57 a	15.72 a
	10	5.33 ab	0.45 b	11.67 a	6.63 a	0.53 a	12.48 ab	5.43 b	0.45 c	12.10 a	6.20 ab	0.49 ab	12.55 a	6.83 bcd	0.50 cd	13.78 ab	6.17 d	0.49 b	12.81 a
	5	5.27 ab	0.46 b	11.53 a	6.53 a	0.51 abc	12.89 ab	7.27 a	0.57 a	12.76 a	6.43 ab	0.52 a	12.41 a	7.10 bcd	0.55 abcd	12.94 ab	8.33 ab	0.57 a	14.50 a
Blue	20	5.97 ab	0.48 ab	12.52 a	5.03 ab	0.46 bc	10.97 ab	5.10 b	0.48 bc	10.69 a	6.33 ab	0.48 ab	13.16 a	5.10 d	0.51 bcd	9.94 b	8.43 ab	0.55 ab	15.79 a
	10	6.07 ab	0.50 ab	12.26 a	5.67 ab	0.52 ab	10.80 ab	5.23 b	0.48 bc	10.79 a	5.40 ab	0.42 c	12.95 a	7.17 abc	0.55 abcd	13.01 ab	6.53 cd	0.54 ab	12.08 a
	5	5.17 b	0.53 ab	9.77 a	5.53 ab	0.45 c	12.35 ab	5.60 ab	0.53 ab	10.60 a	6.80 a	0.53 a	12.75 a	8.10 ab	0.61 ab	13.30 ab	7.10 bcd	0.52 ab	13.60 a
Control	0	6.10 ab	0.51 ab	12.08 a	5.50 ab	0.47 abc	11.64 ab	5.07 b	0.45 c	11.02 a	6.40 ab	0.46 bc	13.92 a	6.07 cd	0.47 d	12.82 ab	7.00 bcd	0.49 b	14.43 a

Table 9

AsA in strawberry grown under three different LED light treatments in the first trial. Different letters indicate significant differences at $p \leq 0.05$.

	mg AsA/g FW
Control	0.80 ab
Red	0.91 a
Blue	0.76 b
Far Red	0.72 b

along the cycle and for far red the best results was obtained in the most of sampling for lower density (5 light per lineal meter). These results remark the importance of testing different intensities to determine the most suitable intensity for improve each fruit quality parameter.

To the best of our knowledge, this is the first study that analyze the total polyphenols in fruit grown under different LED lights, since the previous studies performed on this issue was focused on the study of different classes of polyphenols such as flavonoids (Choi et al., 2013; Paparozzi et al., 2018) and anthocyanin (Naldiani et al., 2017). The results of this work show that LED lights treatment improved total polyphenols in the fruit compared with control. In general red light achieved higher content compares to other lights treatments. Regarding densities, this work indicates that fruit grown under red light tended to exhibit higher content of total polyphenols than lower densities, while no differences between densities were found for far red and blue lights. Based on these results, we can conclude that light treatments had a beneficial impact on total polyphenol content, and choice of the most suitable density depends on wavelength.

The influence of LED light on organoleptic quality have been studied previously in strawberry, obtaining different results depending on wavelength used. Regarding soluble sugars, the effect of using supplemental light on this fruit quality parameter in strawberries is not clear (Nadalini et al., 2017), although Choi et al. (2013, 2015) demonstrated higher fructose for strawberry fruit grown under blue plus red LED light, and additionally Choi et al. (2015) demonstrated higher glucose for strawberry fruit grown under blue plus red and blue LED lights. Our results did not show significant differences between LED light treatments in seven samplings collected during the growing season; however, some differences were observed depending on sampling date. Thus, LED light produced increases in individual sugars at the beginning and the end of the growing season. This suggests an important application of the using LED lights at this time of year to increase sugar content in fruit, improve consumer acceptability and consequently enhance commercial value. Total Soluble Solids and acidity are key factors that affect strawberry flavor (Kallio et al., 2000). Our results found some difference as we described in the results, but did not demonstrate a clear effect of LED light treatments on these strawberry quality parameters. Similar results were shown in others studies conducted on strawberry (Nadalini et al., 2017), whereas in other studies, spectral manipulation achieved alterations in these parameters in strawberry fruit (Choi et al., 2013). The plant response to light is very specific, varying between crops and even cultivars. Different studies used different cultivars, it can explain the variability of the results. Ascorbic acid was not influenced by LED light treatment, we performed the assay only in the first trial, we think these results are not conclusive, and it is necessary to perform additional ascorbic acid assays.

Diabetes and obesity have become an important health problem in developing countries (Anon, 2006; Shaw et al., 2010), and many fruits have been demonstrated to have an important effect on prevention of these diseases (Limberaki et al., 2012; Lobo et al., 2010), therefore the study of agronomic techniques that enhance healthy fruit and vegetable quality is an interesting issue. Many berry fruits, included strawberry fruit, have been demonstrated to be an inhibitor of pancreatic lipase (Zhu et al., 2015) and α -amylase (Pinto et al., 2010) in vitro. The results obtained in this study support this finding, however the most important

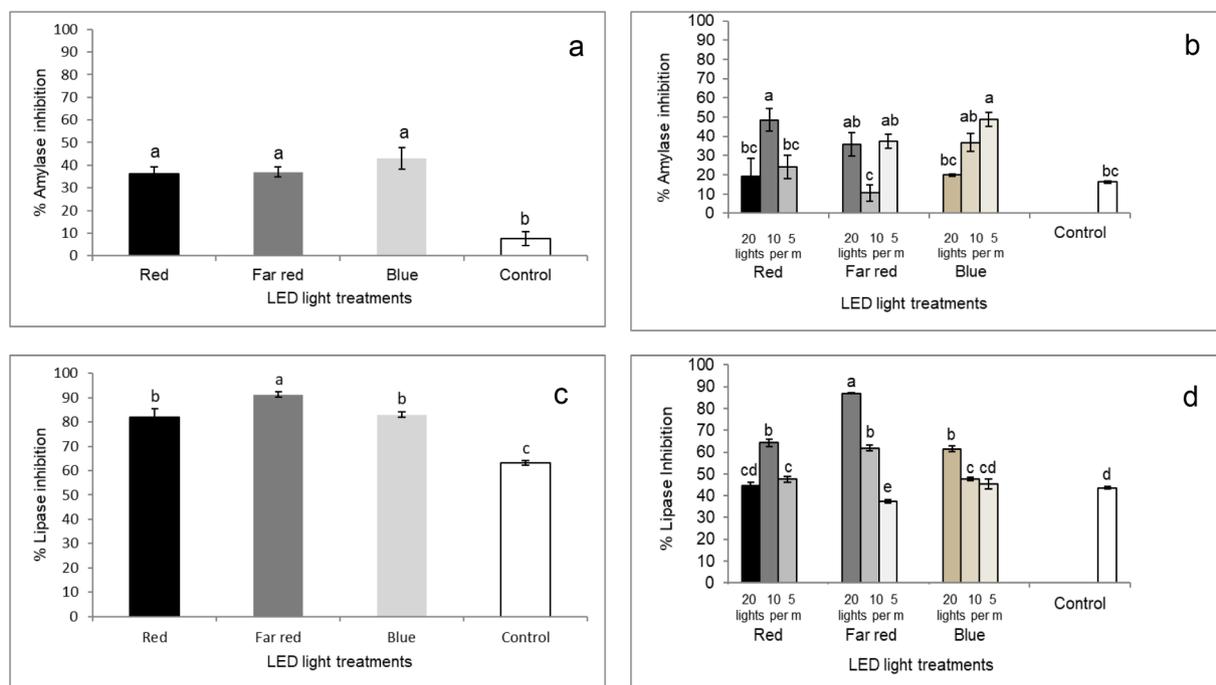


Fig. 1. Effect of strawberry extracts grown under three different LED light treatments on α -amylase in the first trial (a) and the second trial (b), and lipase activity in the first trial (c) and the second trial (d). Values are expressed as % inhibition. Different letters indicate significant differences at $p \leq 0.05$.

findings of our results are the positive effect of the use of LED lights to increase inhibition of both digestive enzymes.

Environment is a key factor in agriculture, that play an important role in yield. Adverse environmental conditions can reduce yield (Gauci et al., 2009). Berry fruits grown in the north of Europe are affected by limitations in photosynthesis efficiency, mainly due to cloud cover that produces fluctuations in light irradiance (Petridis et al., 2018), this adverse weather condition occurs more frequently in autumn in the area where the study was performed. Yield data recorded in the second trial showed a significant increase for plants grown under LED lights, with the exception of blue light at 5 light per meter where yield was lower than control. Thus, it can be indicate that LED light treatments was useful to increase yield.

5. Conclusion

The comprehensive study of the impact of red, far red, and blue LED lights on strawberry fruit provided substantial insights across various parameters. It is detailed in this epigraph. Red, far red, and blue LED light was effective in enhancing anthocyanin, total phenolic content, antioxidant activity and total polyphenol contents. Although the three treatments improved the content of functional compounds, red and far red LED light were the most effective wavelengths for enhancing these compounds. Low light densities can be used to achieve significant benefits from LED light which represents economic saving. No significant differences were found for Total Soluble Solid, however soluble sugars (glucose, fructose and sucrose) showed higher content in light treatments at the beginning and the end of the cycle compared to control. Acidity was lower in the middle and at the end of cycle. This is a relevant issue concerning the achievement of increase fruit sugars and decrease acidity that achieve better flavor at the time of the year when sugar content is usually low and acidity high. The modification of these quality parameter, as revealed in the results of this work, could contribute to reevaluation of the product, consequently, enhance farmer profitability. Yield is another important issue in agriculture activity, the use of LED lights in this study increase production, this will allow enhanced grower benefits. The inhibitory activity of extracts from all treatments exhibited

the capacity to inhibit the digestive enzymes pancreatic lipase and α -amylase activity in vitro, extract from fruit grown under supplemental lighting had a greater inhibitory capacity than control. The inhibitory activity vary depending on density. Overall this study supplies interesting data that demonstrate that strawberry fruit grown in the presence of supplemental light may impart health benefits via enhanced antioxidant capacity, functional compounds and by limiting caloric assimilation due to the increase in the capacity to inhibit the digestive enzymes pancreatic lipase and α -amylase.

To the best of our knowledge, this is the first study in which an LED lighting system was tested in a commercial glasshouse, sampling several times throughout the cycle, exploring different LED light densities and demonstrating an increase in the inhibition of digestive enzymes through spectral manipulation. These results will contribute to the development of commercial-scale LED systems.

CRediT authorship contribution statement

L.F. Pérez-Romero: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **P.J. Stirling:** Funding acquisition, Supervision. **R.D. Hancock:** Conceptualization, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113192.

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