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Multivariate Statistical Analysis of the Color−Anthocyanin Relationships in Different Soilless-Grown Strawberry Genotypes

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Apart from the need to assess the color of foods due to its preponderant role in their acceptability, there is currently a new trend consisting in the study of the relationships between the color and the pigments accounting for it. The color of five strawberry varieties cultivated in two different soilless systems has been studied, and an array of multivariate statistical methods have been performed to single out the color parameters that best discriminate among the different samples surveyed and to correlate them with the pigment content. It is concluded that there is not a direct relationship between the external and flesh colorations of the berries. Additionally, after discriminant methods were applied, it was noticed that, taking into account the strawberry varieties, >90% of the cases could be correctly classified, a noticeably lower percentage of correct classification (around 60%) being obtained when the type of cultivation system was selected as the criterion for discrimination. The best correlations of pigment−color coordinates were found between pelargonidin-3-rutinoside and the external a^* ($r = -0.87$) followed by pelargonidin-3-glucoside and the internal L^* ($r = -0.72$).

KEYWORDS: Analysis of variance (ANOVA); anthocyanins; color; General Linear Models (GLM); hydroponics; linear discriminant function analysis (LDA); pigments; principal component analysis (PCA); soilless systems; strawberry (*Fragaria* × *ananassa* Duch.)

INTRODUCTION

The color of ripe strawberries (*Fragaria* × *ananassa* Duch.) is furnished by anthocyanins, which account for the reddish, purplish, and bluish colors of many flowers, fruits, and vegetables and are attributed diverse health benefits stemming from their likely antioxidant, anticarcinogenic, anti-inflammatory, and antiangiogenic properties (*1*–*3*). Fruits and berries (apples, red grapes, cranberries, blueberries, raspberry, blackcurrant, etc.) are the largest source of anthocyanins in nature, where these pigments are mainly located in the peel, but also sometimes in the pulp. Previous investigations have revealed that the color of strawberries is conditioned by genetic, climatic, and agronomic factors and that postharvest color changes associated with biosynthetic changes of anthocyanins can occur (*4, 5*). Equally, their coloration is affected by all of the factors that affect the stability of the pigments such as pH, storage temperature, presence of enzymes, light, and oxygen, but especially by the temperature (*6*–*8*). In any case, the need to properly evaluate the color of strawberries and foodstuffs in general on the part of industry and scientists is unquestionable, because such an attribute is one of the most important quality indices affecting consumers' perception of their quality and hence their acceptability (*9*–*11*). To do so, objective methods based on Tristimulus Colorimetry are gaining relevance as the subjectivity underlying sensory analysis is overcome. Thus, such techniques have been widely applied in foods with different purposes, such as the assessment of color changes during food processing (*12, 13*), ripening (*14*–*16*), and degradation (*17*). More importantly, and as a response to the renewed interest in pigments due to the health benefits conferred by some of them, the usefulness of the objective assessment of color for their quality control is drawing much attention, due to the inherent advantages of this kind of measurement (nondestructiveness, ultrarapidity, portability, adaptability, etc.). Thus, the coordinates of universally accepted color spaces have been correlated with the pigment content of diverse foods such as tomato (*18*), orange juices (*19*), or wine (*20*), among others. In connection with this trend, the color of strawberries has also aroused much interest, some studies having shown good correlations between the a^* color coordinate and the anthocyanin levels (*21*) and inverse

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Table 1. Summary of the CIELAB Color Coordinates Defining the External and Internal Colors of the Samples

	strawberry cultivars and cultivation systems[a]									
	cv. Aromas		cv. Camarosa		cv. Diamante		cv. Medina		cv. Ventana	
color[b]	OS	CS	OS	CS	OS	CS	OS	CS	OS	CS
$L^*_{(e)}$	38.34 ± 4.86	37.26 ± 3.23	41.44 ± 6.82	40.47 ± 4.30	49.33 ± 5.98	50.29 ± 5.52	43.77 ± 3.65	39.93 ± 2.98	40.36 ± 5.40	41.71 ± 4.33
$a^*_{(e)}$	40.53 ± 3.77	40.70 ± 2.82	34.74 ± 4.80	37.34 ± 3.37	42.30 ± 4.71	43.83 ± 3.55	40.63 ± 1.58	41.47 ± 3.18	39.26 ± 3.03	40.28 ± 2.69
$b^*_{(e)}$	112.80 ± 16.35	121.76 ± 13.86	84.75 ± 16.26	111.00 ± 11.02	38.19 ± 8.34	53.92 ± 13.06	59.86 ± 15.22	90.82 ± 10.56	97.07 ± 14.50	88.55 ± 9.42
$C^*_{ab(e)}$	120.02 ± 15.77	128.41 ± 13.83	92.30 ± 24.55	117.26 ± 10.31	57.11 ± 8.74	69.76 ± 11.92	72.85 ± 12.99	99.88 ± 10.67	104.76 ± 12.88	97.67 ± 9.14
$h_{ab(e)}$	69.50 ± 3.80	71.41 ± 1.39	64.87 ± 8.97	70.98 ± 3.25	41.61 ± 4.10	50.01 ± 5.67	54.49 ± 6.65	65.35 ± 1.58	67.87 ± 6.99	64.17 ± 1.97
$L^*_{(i)}$	76.01 ± 5.90	73.78 ± 5.30	65.39 ± 5.35	66.69 ± 5.14	80.61 ± 4.87	81.37 ± 4.94	70.31 ± 5.01	63.89 ± 4.09	67.66 ± 3.35	76.49 ± 3.29
$a^*_{(i)}$	17.90 ± 5.81	19.67 ± 7.54	32.58 ± 6.74	29.66 ± 6.94	17.06 ± 8.33	17.31 ± 6.41	39.25 ± 8.30	39.56 ± 3.30	31.13 ± 4.20	20.81 ± 2.42
$b^*_{(i)}$	31.71 ± 5.64	35.59 ± 10.05	42.83 ± 7.77	43.17 ± 8.26	29.44 ± 3.74	29.32 ± 3.15	42.66 ± 6.36	44.09 ± 5.42	36.95 ± 3.34	26.87 ± 2.99
$C^*_{ab(i)}$	36.47 ± 7.77	40.84 ± 11.86	53.82 ± 10.21	52.40 ± 10.65	34.36 ± 7.36	34.24 ± 5.91	57.97 ± 8.45	59.25 ± 6.20	48.33 ± 5.14	34.01 ± 3.57
$h_{ab(i)}$	61.16 ± 3.60	61.73 ± 6.28	52.87 ± 1.46	55.64 ± 2.07	61.36 ± 8.90	60.36 ± 6.91	47.38 ± 5.34	47.99 ± 1.26	49.88 ± 2.50	52.52 ± 1.66

[a] Open system (OS), without recirculation of nutrient solution; closed system (CS), with recirculation of nutrient solution. [b] (e) External color measured on the fruit surface; (i) internal color measured on the flesh.

correlations between a^* and pelargonidin-3-glucoside and total pelargonidin contents (*5*). Likewise, changes in the color and anthocyanin composition of strawberries during fruit development (*9*), storage (*8, 22*), and processing (*23*) have been previously studied.

Considering the new tendencies described above, we have carried out a thorough study of the color and anthocyanin content of a series of strawberry varieties grown in soilless systems (*24*) (also known as hydroponic systems). This type of strawberry had been scarcely studied despite the system's growing relevance in the Mediterranean basin as a replacement for methyl bromide to control pests and as a means to use water and nutrients in a more sustainable manner; soilless systems with recirculation of nutrients have been developed, and their inherent risk of transmission of pathogens can be efficiently controlled by different disinfection methods. To draw the most meaningful information, we have applied an array of statistical techniques not only to correlate CIELAB color coordinates (*25*) with the pigment content but also to single out the parameters that best discriminate among the different sets of samples surveyed. Specifically, one-way analysis of variance (ANOVA) and pattern recognition techniques, such as principal component analysis (PCA) and linear discriminant analysis (LDA), were carried out, and the correlations between anthocyanin content and color parameters were studied by both simple and multiple regressions computed by General Linear Models (GLM).

MATERIALS AND METHODS

Plant Material. The plants were cultivated in a multitunnel polycarbonate experimental greenhouse managed by the University of Huelva (southwestern Spain). Five varieties of strawberries (Aromas, Camarosa, Diamante, Medina, and Ventana) and two different soilless cultivation systems, namely, open (OS) and closed (CS) systems, were evaluated. In the OS there was not recirculation of the nutrient solution, whereas in the CS, where the nutrient was recirculated, the lixiviates were disinfected by slow sand filtration. Twenty plants of each variety were grown in five hanging trays filled with perlite substrate with two lines of plants per tray. The berries were picked at commercial ripeness, specifically when 75% of the surface showed a red color, which corresponds to stage 5 in terms of the commercial criterion. The fruits ranged from 19 to 30 mm in diameter and from 15 to 27 g in weight. For each variety, 500 g of fruits was considered and, after the color measurements, they were homogeneized to a paste, which was stored at −21 °C for no longer than 2 months until the analyses. Further information regarding the strawberry samples can be found in a previous study (*26*).

Color Measurement. The color of the samples was measured within 24 h of the harvest by spectroradiometry, a technique that mimics the operation of the human eye and which has already proved to be successful in the objective assessment of the color of diverse foodstuffs (*27–29*). The reflectance spectra were obtained using a CAS140-B compact array spectroradiometer (Instrument Systems, Munich, Germany), equipped with a Top 100 telescope optical probe (Instrument Systems) and a Tamron zoom model SP 23A (Tamron USA, Inc., Commack, NY) and fitted with an external incandescent lamp (150 W) as source of illumination. A white reference $BaSO_4$ pressed plate (USRS-99-010, Labsphere Inc., North Sutton, NH) was used to blank the instrument.

The entire visible reflectance spectrum (380–770 nm) was recorded ($\Delta\lambda = 1$ nm), and the CIE Standard Illuminant D65 and the CIE 1964 Standard Observer (10° Observer) were considered in the calculations. The color parameters corresponding to the uniform color space CIELAB were computed directly by the apparatus through the Specwin v. 1.8.1.6 software provided by the manufacturer. The apparatus was set to take three sequential measurements (5 s of exposure each) such that the color coordinates obtained were means of them.

Within the CIELAB uniform space a psychometric index of lightness, L^*, and two color coordinates, a^* and b^*, are defined. The coordinate a^* takes positive values for reddish colors and negative values for greenish ones, whereas b^* is positive for yellowish colors and negative for the bluish ones. L^* is an approximate measure of lightness, allowing each color to be considered as equivalent to a member of the gray scale, taking consequently values ranging from 0 (black) to 100 (white). From these coordinates, other color parameters, namely, chroma and hue, are defined within the space. The hue angle (h_{ab}) is the qualitative attribute of color according to which each one has been traditionally regarded as bluish, yellowish, reddish, etc. Chroma (C^*_{ab}) is the attribute that allows the assessment of the degree of difference of any given hue relative to a gray color with the same lightness, being considered the quantitative attribute of colorfulness.

For the color measurements 10 fruits of each variety were analyzed. The color of the surface (external color) was measured at five different points of the berry, such that 50 measurements were taken for each variety. After measurement of the external color, the berries were sliced lengthwise, and the internal color (flesh color) was measured at three different points of the two halves obtained, giving a total of 60 measurements per variety. The data shown in the tables correspond to the mean of these 50 or 60 measurements.

After their color analysis, the sliced strawberries were gently homogenized with the aid of a kitchen mixer, and the pastes obtained were eventually stored at –21 °C until their analysis.

Anthocyanidins. Anthocyanidins were extracted as previously described by Seeram et al. (*30*) and analyzed by HPLC-PDA according to the methodology described by Gómez-Míguez and Heredia (*31*). Their identification was performed at 525 nm by comparing their retention times, the spectrum of a pelargonidin 3-glucoside standard (Extrasynthese, Genay, France), and the spectroscopic features with those given in the literature (*32*). The anthocyanidin levels in the samples were estimated from a dose–response curve made with the pelargonidin-3-glucoside standard. Three replicates from each sample were extracted and analyzed, and each replicate was injected three times to obtain the corresponding mean.

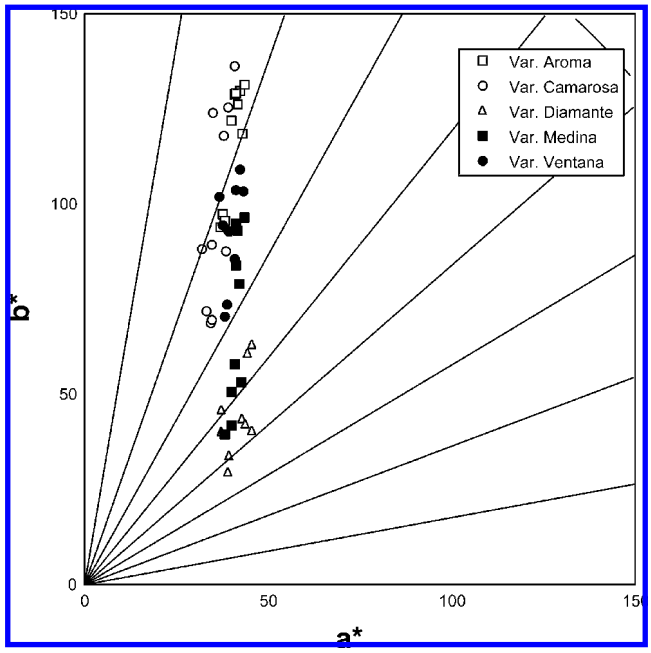

Figure 1. External color: location of the strawberry samples in the (a^*b^*) plane by cultivar.

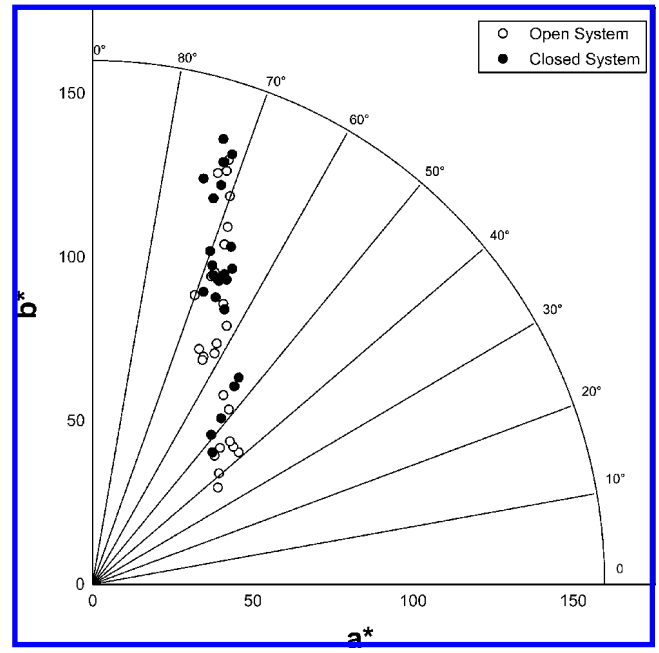

Figure 2. External color: location of the strawberry samples in the (a^*b^*) plane by cultivation system.

Statistical Analysis. Appropriate statistical techniques were applied to evaluate the existence of significant differences among strawberry varieties and/or cultivation systems and to discriminate between different samples. Specifically, one-way analysis of variance (ANOVA) and pattern recognition techniques, such as principal component analysis (PCA) and linear discriminant analysis (LDA), were carried out for those purposes. Significant differences were determined at $p < 0.05$.

Correlations between anthocyanin content and color parameters were studied by both simple and multiple regressions computed by GLM. Statistica v. 6.0 (*33*) software was used for all statistical treatments.

RESULTS AND DISCUSSION

Color Analysis. Table 1 shows the mean values of the CIELAB colorimetric parameters obtained for the five varieties of strawberry studied. Both internal and external colors are included, the internal variables being denoted with the subindex (i) and the external ones with the subindex (e).

External Color. Significant differences ($p < 0.001$) were found regarding the external color when all of the varieties were considered altogether. The berries from cv. Diamante showed the highest lightness and $a^*_{(e)}$ values and the lowest $b^*_{(e)}$, chroma, and hue angle values. On the other hand, cv. Aromas berries exhibited the smallest $L^*_{(e)}$ and the highest $b^*_{(e)}$, $C^*_{ab(e)}$, and $h_{ab(e)}$ values. Thus, as can be observed graphically in **Figure 1**, the strawberries from the variety Aromas had a red-orange hue and showed the darkest and most intense color, whereas those from the cv. Diamante, which clustered nearer the coordinate origin but in a redder zone, were the least colorful. In previous studies on the color of strawberries grown by conventional practices (*9, 21*), similar values for $L^*_{(e)}$ and $a^*_{(e)}$ but lower values for $C^*_{ab(e)}$ and $h_{ab(e)}$ were reported. The great variability in the b^* values among the strawberry samples is noteworthy. Thus, in **Figure 1** it can be readily seen that the samples are rather clustered in a narrow interval of positive a^* values (red component of their color), but much scattered along the positive part of the b^* axis (yellow component of their color).

To evaluate the influence of the soilless cultivation systems on the external color of the strawberries, comparisons between samples corresponding to the two cultivation systems (OS and CS) were established. The distribution of the samples in the (a^*b^*) plane grouped according to the cultivation system is shown in **Figure 2**, where it can be observed that there is not a clear separation, although a certain trend toward redder hues could be seen in OS samples. As for the CS samples, a trend toward more intense colors could also be appreciated. Furthermore, after application of one-way ANOVA, it was concluded that significant differences ($p < 0.05$) between OS and CS samples regarding b^*, C^*_{ab}, and h_{ab} values existed.

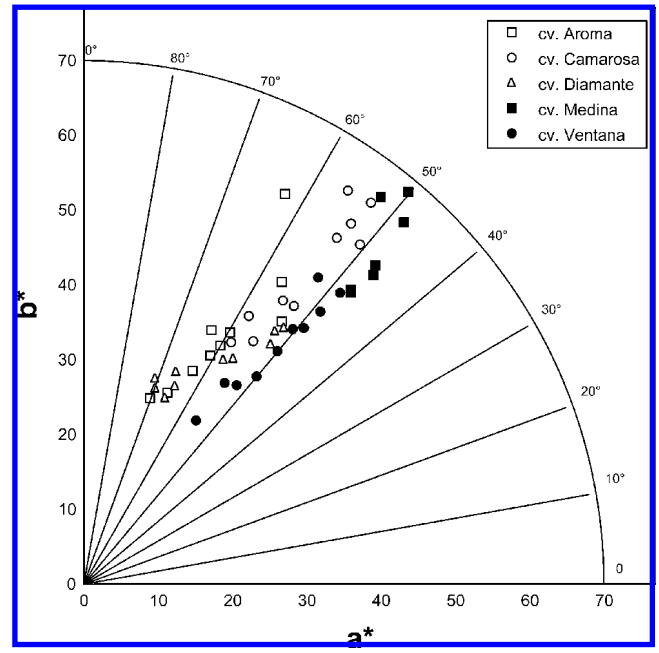

Figure 3. Internal color: location of the strawberry samples in the (a^*b^*) plane by cultivar.

Internal Color. The internal or flesh color of the fruits also showed statistically significant differences at $p < 0.001$ when all of the cultivars were considered altogether. The distribution of the strawberries grouped according to variety in the (a^*b^*) plane is plotted in **Figure 3**. In this instance, the cv. Diamante berries had the highest values of $L^*_{(i)}$ as for the external color, but showed the lowest $a^*_{(i)}$ values. The cv. Medina samples

Table 2. Anthocyanin Contents (Micrograms per Gram of Fresh Fruit) in the Different Strawberry Cultivars Studied as a Function of Cultivation System

	strawberry cultivars and cultivation systems[a]									
	cv. Aromas		cv. Camarosa		cv. Diamante		cv. Medina		cv. Ventana	
compound	OS	CS	OS	CS	OS	CS	OS	CS	OS	CS
plg-derivative 1	0.54 ± 0.04	1.00 ± 0.04	0.76 ± 0.01	0.62 ± 0.03	0.37 ± 0.01	0.54 ± 0.02	0.47 ± 0.01	0.42 ± 0.02	0.18 ± 0.00	0.72 ± 0.02
cy-3-glucoside	5.30 ± 0.01	4.81 ± 0.06	4.87 ± 0.05	3.15 ± 0.06	1.66 ± 0.01	2.40 ± 0.11	3.71 ± 0.10	3.97 ± 0.27	0.64 ± 0.01	1.29 ± 0.08
plg-3-glucoside	107.03 ± 2.54	141.59 ± 4.07	165.66 ± 0.94	103.43 ± 3.61	76.46 ± 1.12	73.69 ± 1.68	113.36 ± 0.63	130.82 ± 3.93	74.01 ± 1.92	113.31 ± 0.15
plg-3-rutinoside	7.23 ± 0.10	5.46 ± 0.62	23.92 ± 0.88	12.87 ± 1.44	3.73 ± 0.13	5.20 ± 0.46	6.07 ± 0.01	6.22 ± 0.30	3.24 ± 0.37	9.03 ± 0.61
plg-derivative 2	0.43 ± 0.07	0.63 ± 0.05	0.38 ± 0.00	0.42 ± 0.05	0.44 ± 0.06	0.43 ± 0.08	0.60 ± 0.03	0.65 ± 0.02	0.18 ± 0.00	1.30 ± 0.01
plg-acetylglucoside	2.71 ± 0.01	3.62 ± 0.00	3.39 ± 0.09	2.36 ± 0.07	1.13 ± 0.03	1.68 ± 0.06	2.73 ± 0.03	2.90 ± 0.05	0.55 ± 0.02	1.18 ± 0.00
anthocyanins	123.25	157.11	198.88	122.85	83.79	83.94	126.94	144.97	78.81	126.84

[a] Mean values ± standard deviation. Open system (OS), without recirculation of nutrient solution; closed system (CS), with recirculation of nutrient solution.

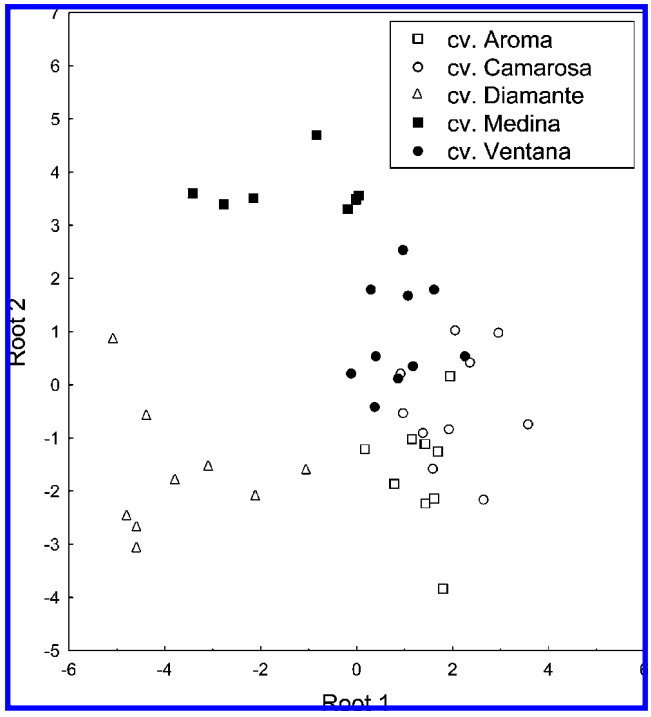

Figure 4. LDA: scatterplot of canonical functions by cultivar.

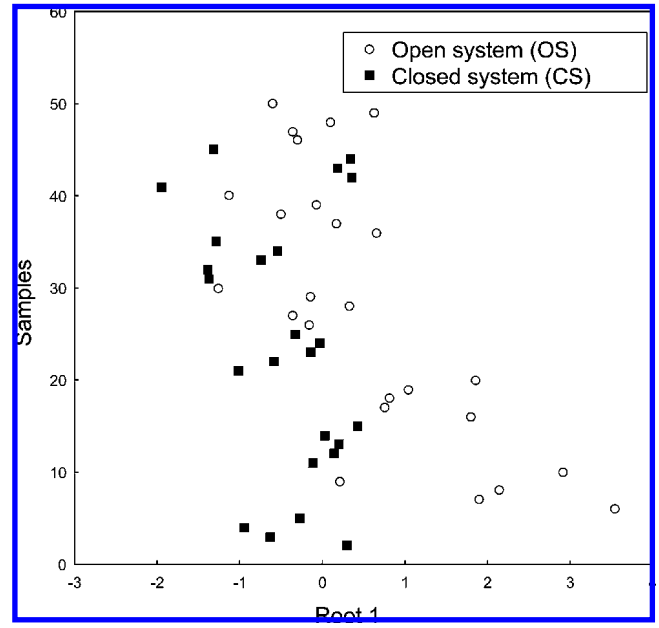

Figure 5. LDA: scatterplot of canonical functions by cultivation system.

showed the smallest $L^*_{(i)}$ and $h_{ab(i)}$ values and the highest $a^*_{(i)}$, $b^*_{(i)}$, and $C^*_{3(i)}$ values. The cv. Aromas berries had also the highest $h_{ab(i)}$, whereas the lowest $C^*_{ab(i)}$ value corresponded to the Ventana cultivar. These results indicate that, internally, the cv. Medina berries showed the darkest and reddest color, whereas those from the Ventana variety exhibited the least vivid coloration. When the samples from the two cultivation systems (OS vs CS) were compared in terms of color, no statistically significant differences were found, indicating that the systems did not have an influence on the internal color of the strawberries.

Considering the external and the internal color together, it can be concluded that there is not a direct relationship between the coloration of the exterior and the flesh of the berries, in the sense that the samples showing the most or the least vivid external color do not coincide with the ones showing the most or the least vivid flesh coloration.

Anthocyanin Composition. Table 2 shows the mean values of the anthocyanin contents as a function of the strawberry variety and the type of cultivation as well as the total content, considering the sum of all the individual anthocyanins. Up to six different anthocyanins (pelargonidin-3-glucoside, pelargonidin-3-rutinoside, cyanidin-3-glucoside, pelargonidin acetylglucoside, and two trace pelargonidin derivatives) were detected, pelargonidin-3-glucoside being by far the main pigment regardless of the strawberry variety, followed by pelargonidin-3-rutinoside. The differences in the anthocyanins and other strawberry phenolics as a function of the cultivar or the soilless cultivation systems have been studied in depth in a previous work, in which it was concluded that the contents of cyanidin-3-glucoside, pelargonidin-3-rutinoside, *p*-coumaric acid, and pelargonidin-3-glucoside were the most appropriate variables to discriminate among varieties, as were those of *p*-hydroxybenzoic acid and pelargonidin derivative 1 to discriminate between cultivation systems (*26*).

Statistical Analysis. To assess the existence or not of statistically significant differences among the strawberry samples regarding their color, ANOVAs were applied. For this purpose all of the CIELAB color parameters (L^*, a^*, b^*, C^*_{ab}, and h_{ab}) were taken into account, and the cultivars and the cultivation systems were considered separately. To evaluate the influence of the cultivars on the color, the data from the two cultivation systems (OS, CS) for each strawberry variety were taken into account and significant differences ($p < 0.05$) among all of the color variables were found. Analogously, the data from the five cultivars for each cultivation system were considered to evaluate the influence of the soilless cultivation system, as a consequence of which it was found that solely the external color variables $b^*_{(e)}$, $C^*_{ab(e)}$, and $h_{ab(e)}$ and the internal $L^*_{(i)}$ proved to be statistically different between the groups. When two-way ANOVA analyses were applied, significant interactions between cultivar and cultivation system were observed only for $L^*_{(i)}$.

Table 3. Correlation Matrices between Chromatic Parameters and Anthocyanin Levels[a]

	external color					internal color				
	$L^*_{(e)}$	$a^*_{(e)}$	$b^*_{(e)}$	$C^*_{ab(e)}$	$h_{ab(e)}$	$L^*_{(i)}$	$a^*_{(i)}$	$b^*_{(i)}$	C^*_{ab}(i)	$h_{ab(i)}$
plg-derivative 1	*−0.45*	−0.38	*0.51*	*0.50*	*0.50*	−0.28	−0.00	0.23	0.15	0.25
cy-3-glucoside	−0.41	−0.25	0.39	0.38	0.36	−0.29	0.18	0.42	0.33	0.14
plg-3-glucoside	*−0.56*	*−0.53*	0.42	0.40	*0.47*	*−0.72*	*0.54*	*0.65*	*0.64*	−0.30
plg-3-rutinoside	−0.29	*−0.87*	0.24	0.21	0.36	*−0.59*	0.36	*0.47*	*0.46*	−0.20
plg-derivative 2	−0.18	0.07	0.12	0.12	0.16	−0.37	0.36	0.28	0.34	−0.34
plg-acetylglucoside	−0.41	−0.31	0.37	0.36	0.35	−0.41	0.32	*0.56*	*0.47*	0.01
anthocyanins	*−0.54*	*−0.60*	0.41	0.39	*0.48*	*−0.71*	*0.52*	*0.65*	*0.63*	−0.27

[a] Significant effects ($p < 0.05$) are denoted by italics.

Table 4. R^2 Values Obtained from the Multiple Regression Models Used for the Study of the Relationships Anthocyanin Levels versus Sets of Scalar and Anthocyanin Levels versus Angular Coordinates[a]

	external color		internal color	
	$L^*_{(e)}$, $a^*_{(e)}$, $b^*_{(e)}$	$L^*_{(e)}$, $h_{ab(e)}$, $C^*_{ab(e)}$	$L^*_{(i)}$, $a^*_{(i)}$, $b^*_{(i)}$	$L^*_{(i)}$, $h_{ab(i)}$, $C^*_{ab(i)}$
plg-derivative 1	0.334641	0.257343	*0.521158*	*0.471573*
cy-3-glucoside	0.184907	0.178954	*0.461385*	*0.527569*
plg-3-glucoside	*0.461826*	*0.405234*	*0.657889*	*0.638269*
plg-3-rutinoside	*0.755848*	0.338249	*0.489297*	*0.431798*
plg-derivative 2	0.066669	0.048048	0.164254	0.154720
plg-acetylglucoside	0.198665	0.168311	*0.565423*	*0.616473*
anthocyanins	*0.493043*	*0.391936*	*0.683034*	*0.656196*

[a] Significant effects ($p < 0.05$) are denoted by italics.

To tackle the second overall objective of the study, that is, to examine the ability of the variables to differentiate among strawberry cultivars and cultivation systems, both PCA and LDA were performed considering standardized experimental data.

After the application of the PCA to the data set, it was seen that the two principal components explained 81.89% of the total variance. The first factor, which explained 48.18% of the variance, was mainly linked to the internal color variables $L^*_{(i)}$, $a^*_{(i)}$, $b^*_{(i)}$, and $C^*_{ab(i)}$, whereas the second principal component (PC), which explained 33.71% of the total variance, was related to external color variables, specifically $b^*_{(e)}$, $C^*_{ab(e)}$, and $h_{ab(e)}$.

To determine which variables were the most appropriate for discriminating between samples, LDA was performed, because stepwise LDA selects the variables that enhance the discrimination of the groups established by the dependent variable. The criterion for the selection is the Wilks lambda, which maximizes the ratio of variance between groups to that within groups. Thus, two LDAs were carried out, considering on the one hand the strawberry cultivars and, on the other hand, the soilless cultivation systems. Taking into account the strawberry varieties, a mathematical model that selected five variables, namely, external hue ($h_{ab(e)}$), internal hue ($h_{ab(i)}$), external $a^*_{(e)}$, internal $a^*_{(i)}$, and internal chroma ($C^*_{ab(i)}$), was obtained. It classified correctly 91% of the cases with high significance levels ($p < 0.001$). Additionally, the prediction percentages were 100% for cv. Diamante and cv. Medina, 90% for cv. Camarosa and cv. Ventana, and 80% for cv. Aromas. Canonical function 1 is mainly related to the external variables $a^*_{(e)}$ (negative sign) and hue ($h_{ab(e)}$) (positive sign), whereas canonical function 2 is mainly linked to internal color variables $a^*_{(i)}$ (positive sign), chroma ($C^*_{ab(i)}$), and hue ($h_{ab(i)}$) (both with negative sign). The location of the strawberry samples surveyed within the plane defined by the two corresponding canonical functions is depicted in **Figure 4**. The scatterplot shows a quite good separation among the samples as a function of the cultivar. Thus, it can be observed that the first function allowed the samples to be classified into two groups, one of them including the samples from cv. Diamante and cv. Medina (with higher values of $a^*_{(e)}$, that is, the reddest ones, which is what it is sought by the consumers) and the other including those from the remaining cultivars. As for the second canonical function, it is mainly linked to internal variables $a^*_{(i)}$ and $C^*_{ab(i)}$. Because $C^*_{ab(i)}$ had a negative sign and $a^*_{(i)}$ a positive sign, the strawberry samples with higher internal chroma and lower internal a^* values were located at the bottom of the scatterplot.

Considering the type of cultivation system as criterion for the stepwise LDA analysis, 63% of the samples were correctly classified through the external color variables hue ($h_{ab(e)}$) and lightness ($L^*_{(e)}$). In this case, the prediction percentages obtained were 71% for the closed system and 56% for the open one. Due to the fact that solely two sets were taken into consideration, only one classification function, related to $h_{ab(e)}$ and $L^*_{(e)}$, both with negative sign, was obtained, which yielded a rather good separation trend among the samples as can be observed in **Figure 5**.

Statistical Relationships between the CIELAB Color Coordinates and the Anthocyanin Content. First, the relationships between the color parameters and the anthocyanin pigments were explored by means of simple correlations (**Table 3**). Significant correlations ($p < 0.05$) were found between internal $L^*_{(i)}$, $b^*_{(i)}$, $C^*_{ab(i)}$ and the pelargonidin-3-glucoside levels ($r = -0.72$, 0.65, and 0.64, respectively) and the total anthocyanin content ($r = -0.71$, 0.65, and 0.63, respectively). A significant negative correlation was also found between external a^* and pelargonidin-3-rutinoside ($r = -0.87$), which indicated that the lower the levels of this pigment, the higher the external redness of the strawberries. In any case, the correlations observed are lower than what could be presumed considering that the reddish color of strawberries is due to anthocyanic pigments, which seems to indicate that there are factors other than the pigments that contribute to the overall appearance of the berries.

Due to its three-dimensional nature, the complete definition of the color of any object by means of the CIELAB coordinates requires the joint consideration of the scalar (L^*, a^*, b^*) or the angular ones (L^*, h_{ab}, and C^*_{ab}). Hence, to achieve a more meaningful evaluation of the correlations existing between the color of the strawberries and their anthocyanin pigments, multiple regression studies by means of GLM were applied. For this purpose, the content of each anthocyanin was considered as a dependent variable and the sets L^*, a^*, b^* and L^*, h_{ab}, C^*_{ab} as predictor or independent variables, the R^2 values being shown in **Table 4**. When the set $L^*_{(e)}$, $a^*_{(e)}$, $b^*_{(e)}$ was considered,

the highest R^2 values were obtained for the contents of pelargonidin-3-rutinoside, total anthocyanin content, and plg-3-glucoside, in this order. However, when the set $L^*_{(e)}$, $h_{ab(e)}$, $C^*_{ab(e)}$ was taken into account, pelargonidin-3-glucoside was the anthocyanin showing the highest correlation. In relation to the contribution of each predictor variable to the regression models mentioned above, in the case of external color, when the scalar parameters ($L^*_{(e)}$, $a^*_{(e)}$, $b^*_{(e)}$) were considered, redness ($a^*_{(e)}$) was the variable with the highest weight, followed by $L^*_{(e)}$ and $b^*_{(e)}$. When the psychometric parameters ($L^*_{(e)}$, $h_{ab(e)}$, $C^*_{ab(e)}$) were considered, $C^*_{ab(e)}$ was the color parameter with the highest weight, followed by $h_{ab(e)}$ and $L^*_{(e)}$. On the other hand, when the internal color was studied, $a^*_{(i)}$ was also the scalar parameter with the highest weight in the models and $h_{ab(i)}$ was the variable with the highest weight for the psychometric parameters. In this case, the highest R^2 values were obtained for the contents of both total anthocyanin content and pelargonidin-3-glucoside, regardless of the set of color parameters considered.

The analyses performed showed that, averaging the values obtained for OS and CS, the Camarosa variety berries had the highest anthocyanin contents and lowest a^* values, whereas the opposite was noted in the samples from the cv. Diamante, which is in agreement with the results reported for strawberries grown according to different practices in Poland and Florida (*5, 21*). In any case, some of the differences found among samples were not statistically different, as already pointed out earlier.

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