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Assessment of the Differences in the Phenolic Composition of Five Strawberry Cultivars (*Fragaria* × *ananassa* Duch.) Grown in Two Different Soilless Systems

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The phenolics from different strawberry cultivars (Aromas, Camarosa, Diamante, Medina, and Ventana) cultivated in two different soilless systems (with and without recycling nutrient solution) were quantified to assess differences in their profiles as a function of both the variety and the cultivation system. Considering groups of phenols, it was found that either anthocyanins (including pelargonidin-3-glucoside, cyanidin-3-glucoside, pelargonidin-3-rutinoside, pelargonidin-3-acetylglucoside, and two unidentified pelargonidin derivatives) or phenolic acids (including caffeic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, and ellagic acid) were quantitatively more important than those of flavonols (quercetin and kaempferol); the ranges of values were 78.81−198.88, 49.77−128.37, and 12.85−43.04 μg/g, respectively. Considering individual compounds and after applying relevant pattern recognition techniques, 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, whereas those of *p*-hydroxybenzoic acid and pelargonidin-derivative 1 were the most appropriate to discriminate between cultivation systems. The first factor of PCA was mainly linked to anthocyanins and quercetin, whereas the second principal component (PC) was related to kaempferol and *p*-coumaric acid.

KEYWORDS: Strawberry (*Fragaria* × *ananassa* Duch.); hydroponics; phenolics; flavonoids; analysis of variance (ANOVA); linear discriminant function analysis (LDA); principal component analysis (PCA)

INTRODUCTION

A certain correlation between diets rich in fruits and vegetables and a lower incidence of some major human chronic diseases, including several forms of cancer and cardiovascular disease, among others, has been observed in a wide variety of epidemiological studies (*1*). These beneficial effects have been attributed to a wide variety of naturally occurring compounds (often referred to as "phytochemicals") that have not been traditionally regarded as nutrients, phenolics being one of the most important groups (*2*). High levels of these compounds can be found in deep-colored berries such as strawberries (*Fragaria* × *ananassa* Duch.), whose phenolics content is known to be higher relative to that of other fruits (*3*).

The phenolic profile of strawberries from diverse origins has been studied by several authors, so the occurrence of ellagic acid, *p*-coumaric acid, caffeic acid, ferulic acid, and *p*-hydroxybenzoic acid and the flavonols kaempferol, quercetin, and myricetin (*4*) is well-known. Among these compounds, the levels of ellagic acid, a widespread compound in the plant kingdom, are worthy of note as it has been reported to make up 51% of the total phenolic content in some strawberries (*5*). Additionally, it has been found that the levels of ellagic acid in strawberry are some 3-fold higher relative to other commonly consumed fruits, such as apple, banana, and citrus (*6*).

As for anthocyanin pigments, flavonoids that account for the color of many fruits, vegetables, and derived products, several studies have revealed the occurrence of cyanidin 3-glucoside, pelargonidin 3-rutinoside, and pelargonidin 3-glucoside in strawberries, the latter being the major one (*7*). Aside from these anthocyanins, others, such as pelargonidin-3-arabinoside and cyanidin-3-rutinoside, have also been reported to occur in some cultivars, as well as some acylated ones (*8*). The quantitative analysis of anthocyanins in strawberries is important not only to assess their degree of maturity (*9*), but also due to the fact that they are responsible for their color, and this attribute does influence a great deal the consumers' preferences (*10*, *11*).

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Over the last years, the impact of a wide variety of factors such as climate, temperature, processing, type of cultivar, etc., on the levels of bioactive species in strawberries has been the aim of several surveys (*12*, *13*). Additionally, the changes in the phenolic composition of this product over ripening, postharvest, and storage have also been evaluated in many recent studies (*14*, *15*). Nevertheless, there is still very scarce information on the phenolic composition of strawberries grown in soilless systems (also known as hydroponic systems), which are being increasingly used in Spain and other Mediterranean countries as an alternative to methyl bromide (MB) for the control of insect and soil-borne pests before planting. This kind of system allows a more sustainable use of both water and nutrients, since hydroponic systems with recirculation of nutrients have been successfully developed, as well as a reduction of the contamination of the soil. One of the main downsides of these systems is the risk of transmission of pathogen microorganisms due to the recirculation system, although lixiviates can be readily disinfected by means of heat treatment, ozone and hydrogen peroxide treatment, ultraviolet radiation, membrane and/or slow sand filtration, etc. (*16*). Because of all the means commented above, strawberries grown in soilless systems have drawn the attention of a number of researchers in the past decade, who have focused mainly on the establishment of differences between strawberries grown in that way and those cultivated in a traditional fashion (*17*, *18*). Thus, it has been recently concluded that there are not statistical significant differences between organic acids, soluble sugars, anions, and mineral contents corresponding to strawberries grown in soilless systems relative to those cultivated in soil, although the levels of those parameters in the former were usually slightly lower (*19*, *20*). In this sense, this study was conducted to evaluate the differences in the phenolics contents of five strawberry varieties (Aromas, Camarosa, Diamante, Medina, and Ventana) cultivated in two different soilless growing systems (with and without recycling nutrient solution systems, that is, closed and open soilless growing systems, respectively). Additionally, this survey was also aimed at assessing the ability of the phenolics found in the samples to discriminate among strawberries cultivar and cultivation systems, for which appropriate pattern recognition (PR) techniques (specifically principal component analysis and linear discriminant function analysis) were applied.

MATERIALS AND METHODS

Plant Material. The study was conducted in multi-tunnel polycarbonate experimental greenhouses managed by the University of Huelva, in southwestern Spain (latitude 37°14′N, longitude 6°53′W, and altitude 23 m) where strawberry plants (*Fragaria* × *ananassa* Duch.) were grown in soilless systems. Five varieties (Camarosa, Ventana, Aromas, Diamante, and Medina) and two different soilless cultivation systems (open (OS) and closed (CS) systems) were evaluated. A total of 20 plants for each variety were cultivated in five hanging trays filled with perlite substrate with two lines of plants per tray. The varieties were randomly disposed along the trays to minimize possible differences due to the effect of illumination, nutrients addition, etc. The trays were aligned along a support placed at roughly 150 cm from the ground.

The naked-root seedlings were initially grown in Avila, where the climate is more appropriate for their initial development, and then shipped to our greenhouses in Huelva. They were then planted in October (density: 11 plants/m^2), and strawberries were harvested between the middle of December and the end of January. For this study, all of the samples were collected at the same maturation stage, which occurred at the first of January.

In the closed soilless systems, lixiviates were disinfected by slow sand filtration. The radiation source in the greenhouse consisted of natural daylight. The temperature ranged from 25 to 8 °C (day/night) with relative humidity held at 75 ± 5%, manually controlled by opening/closing the ceiling. Additionally, the perlite moisture was monitored by a probe.

The nitritional solution was composed of KNO_3, $CaNO_3$, $Mg(NO_3)_2$, KH_2PO_4, P_2O_5, K_2O, H_3PO_4, and other microelements at standard concentration for hydroponic cultures (*21*). pH and electrical conductivity of the nutrient solution were measured before each addition as well as in the drainage solution. Their values ranged from 5.6 to 6.5 for pH and around 1 dS/m for conductivity. Additionally, pH and conductivity of the nutrient solution were daily controlled.

The strawberries were harvested at commercial ripeness, specifically when 75% of the surface showed a red color, which corresponds to stage 5 in terms of commercial criterion. The fruit size ranged from 19 to 30 mm diameter and from 15 to 27 g weight.

500 g of fruit (which included a variable number of strawberries depending on the individual weight) was selected for each variety. The berries were gently homogenized by means of a kitchen mixer, and the pastes obtained were subsequently stored for 2 months at −21 °C until their analysis.

Extraction and Hydrolysis of Phenols. Two different extraction and hydrolysis procedures were performed. Flavonols and phenolic acids were extracted and hydrolyzed according to the methodology described by Häkkinen et al. (*22*). In brief, the extraction involved hydrolyzation with 50% aqueous methanol (v/v) containing hydrochloric acid (1.2 M) and ascorbic acid (80 mg) as antioxidant. The extracts thus obtained were sonicated for 2 min, and the remaining air was replaced with an atmosphere of nitrogen to prevent oxidative reactions, after which they were shaken in a water bath (35 °C) in the dark for 16 h. 15-mL aliquots were concentrated in a rotary evaporator at 35 °C, and the residues were redissolved in 1.5 mL of methanol.

On the other hand, anthocyanidins were extracted as previously described by Seeram et al. (*23*). Briefly, the samples were homogenized in 25 mL of methanol containing hydrochloric acid (0.1%) and then centrifuged (10 min at 3000 rpm). The supernatants were concentrated by means of a rotary evaporator at 37 °C and redissolved in 4 mL of the mixture water:acidic methanol (1:1) by sonication during 4 min.

In both cases, the concentrated extracts were filtered through 0.45 μm filters (Hydrophilic PVDF, Millipore Millex-HV, Bedford, MA) prior to their injection in the HPLC system.

High-Performance Liquid Chromatography. HPLC analyses were carried out by means of an Agilent 1100 series HPLC system (Palo Alto, CA) equipped with a diode-array detector, which was set to scan from 200 to 770 nm. A C_{18} Nova-pack column (5 μm, 30 cm × 3.9 mm i.d.) was used as stationary phase, and the injection volume was set at 20 μL.

For the analysis of flavonols and phenolic acids, the mixtures of solvents water−methanol−acetic acid (93:5:2, v/v/v, solvent A) and methanol−acetic acid (98:2, v/v, solvent B) were combined according to the following gradient: 0−60 min, 60% B linear; 60−70 min, 100% B linear; 70−100 min, washing and re-equilibration of the column. The flow was 1.0 mL/min, and the temperature of the column was set at 20 °C.

As for anthocyanins, the HPLC analyses were carried out using acetonitrile/formic acid/water as follows: eluent (A) 3:10:87; eluent (B) 50:10:40, according to the methodology described by Gómez-Míguez and Heredia (*24*).

Identification and Quantitative Analysis of Phenolics. The identification of phenolic acids and flavonols was achieved by comparison of their retention times and spectra with those of appropriate standards, their levels being determined by external calibration considering the following wavelengths: 280 nm for benzoic acids, 320 nm for cinnamic acids, and 360 nm for flavonols. The standards were acquired from Merck (Darmstadt, Germany) (*p*-hydroxy benzoic acid, ferulic acid), Fluka Chemie AG (Buchs, Switzerland) (caffeic acid and *p*-coumaric acid), and Sigma Chemical Co. (St. Louis, MO) (quercetin, kaempferol, ellagic acid).

Anthocyanins were identified at 525 nm by comparison of their retention times, spectrum of pelargonidin 3-glucoside standard, and spectroscopic features with those given in the literature (*25*). Their levels

in the samples were estimated from a calibration curve made with pelargonidin-3-glucoside standard (Extrasynthese, Genay, France).

Analytical Quality Control. The within-laboratory repeatability (within-day precision) was developed according to UNE 82009-1:1998. It was ascertained by analyzing the phenolic content of an extract six times within the same day. Within-laboratory reproducibility (day-to-day precision) was assessed by analyzing in duplicate an extract over a period of 1 month, the control sample being kept at −20 °C between the analyses.

The stock solutions of phenolics standards were made up in methanol to a concentration of 1000 μg/mL. The corresponding calibration curves were constructed with five dilutions of the stock solutions. Recoveries were determined in two varieties (Camarosa and Aromas) by appropriately spiking the extraction solutions with pure standards prior to the analysis of the samples. The standards were spiked at the following concentrations, according to their estimated concentration in strawberry: 1 mg/L for caffeic, ferulic, and *p*-hydroxybenzoic acid, and for quercetin; 15 mg/L for *p*-coumaric acid; 50 mg/L for ellagic acid and kaempferol; and 150 mg/L for pelargonidin-3-glucoside.Three replicates from each sample were analyzed, and all of the samples and standards were injected three times to obtain the averages.

Statistical Analysis. The assessment of the existence of significant differences among strawberry varieties and/or cultivation systems, and the discrimination between samples, was performed by appropriate statistical methods. Specifically, one-way analysis of variance (ANOVA) and pattern recognition (PR) techniques, including principal component analysis (PCA) and linear discriminant function analysis (LDA), were carried out for those purposes. The Statistica v.6.0 (*26*) software was used for all of the statistical treatments.

RESULTS AND DISCUSSION

Thirteen different compounds were identified and quantified, which are classified into three different groups of phenolics: (a) phenolic acids (caffeic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, and ellagic acid); (b) flavonols (quercetin, kaempferol); and (c) anthocyanins (pelargonidin-3-glucoside, cyanidin-3-glucoside, pelargonidin-3-rutinoside, pelargonidin-3-acetylglucoside, and two pelargonidin derivatives).

Method Validation. The coefficients of variation (CV) obtained for the within-laboratory repeatability and reproducibility assessment of the HPLC method were <9.9% and <18%, respectively. The highest CV for the repeatability study corresponded to ferulic acid and pelargonidin-derivative 1, whereas those for the reproducibility study corresponded to *p*-coumaric acid and pelargonidin-derivative 2.

The calibration curves were linear in the concentration range 0.1−250 μg/mL ($r^2 > 0.999$). The limits of detection, worked out from them as 3 times the ratio of the standard error of the intercept to the slope, ranged from 6 μg/L (for ellagic acid) to 89 μg/L (for quercetin). The recoveries, which were taken into account in the calculations for the analysis of samples, ranged from 69% to 89% for phenolic acids, from 79% to 95% for flavonols, and from 75% to 95% for anthocyanins, the lowest corresponding to ellagic acid.

Influence of the Cultivar on the Phenolic Composition. The average phenolics contents as a function of the strawberry variety and the type of cultivation are displayed in **Table 1**. The total phenolic content (considering the sum of all of the individual phenolics) ranged from 179.19 μg/g fresh fruit (cv. Diamante-CS) to 299.07 μg/g fresh fruit (cv. Camarosa-OS), showing statistically significant differences ($p < 0.001$) among cultivars.

The highest levels of flavonoids (flavonols + anthocyanins) were found in cv. Camarosa-OS (237.26 μg/g fresh fruit) and the lowest in cv. Diamante-OS (101.66 μg/g fresh fruit). The total anthocyanin content (considering the sum of all of the individual anthocyanins) varied from 78.81 μg/g fresh fruit (cv.

Table 1. Phenolics Contents (μg/g Fresh Fruit) in the Different Strawberry Cultivars Studied[a]

	strawberry cultivar/cultivation system[b]											
	Aromas		Camarosa		Diamante		Medina		Ventana	
compound	OS	CS	OS	CS	OS	CS	OS	CS	OS	CS
p-OH-benzoic acid	0.43 ± 0.07 acgh	0.2 ± 0.05 a	2.29 ± 0.12 h	n.d.	2.40 ± 0.05 bef	n.d.	0.13 ± 0.04 bcegh	0.12 ± 0.03 egh	0.21 ± 0.06 f	n.d.
caffeic acid	0.30 ± 0.02 ac	0.55 ± 0.27 ace	0.78 ± 0.05 e	0.64 ± 0.02 abce	0.11 ± 0.01 ac	0.05 ± 0.01 a	0.32 ± 0.01 ace	0.53 ± 0.05 bce	1.21 ± 0.13 bde	0.18 ± 0.01 bde
p-coumaric acid	8.15 ± 0.26 acd	10.14 ± 1.28 acd	11.12 ± 1.22 ad	5.77 ± 0.31 bce	6.20 ± 0.20 c	1.43 ± 0.04 be	1.58 ± 0.67 e	8.88 ± 0.42 d	16.35 ± 0.09 g	25.47 ± 1.82 f
ferulic acid	0.13 ± 0.03 ac	n.d.[c]	0.17 ± 0.03 c	0.28 ± 0.03 ac	0.72 ± 0.04 bc	n.d.	0.08 ± 0.02 ac	0.13 ± 0.04 ac	0.37 ± 0.01 bc	0.25 ± 0.01 bc
ellagic acid	49.36 ± 2.68 a	45.87 ± 0.94 a	47.35 ± 2.21 ab	43.08 ± 2.89 a	83.47 ± 2.18 ab	66.46 ± 1.16 ab	59.79 ± 1.82 ab	72.28 ± 2.98 ab	110.23 ± 1.24 b	80.38 ± 2.63 ab
quercetin	1.34 ± 0.08 ace	1.24 ± 0.02 ae	1.50 ± 0.02 e	1.76 ± 0.12 e	n.d.	1.40 ± 0.00 acde	1.33 ± 0.00 cde	n.d.	n.d.	n.d.
kaempferol	25.88 ± 0.48 ad	32.23 ± 2.91 acde	36.88 ± 1.74 be	41.28 ± 1.73 bce	17.87 ± 1.44 a	25.91 ± 0.30 acd	32.54 ± 0.35 bcde	12.85 ± 2.51 a	27.50 ± 1.62 acde	41.13 ± 1.97 b
Plg-derivative 1	0.54 ± 0.04 bcd	1.00 ± 0.04 a	0.76 ± 0.01 bcd	0.62 ± 0.03 bcd	0.37 ± 0.01 b	0.54 ± 0.02 bcd	0.47 ± 0.01 bcd	0.42 ± 0.02 bcd	0.18 ± 0.00 d	0.72 ± 0.02 c
Cy-3-glucoside	5.30 ± 0.01 a	4.81 ± 0.06 a	4.87 ± 0.05 b	3.15 ± 0.06 b	1.66 ± 0.01 bd	2.40 ± 0.11 b	3.71 ± 0.10 b	3.97 ± 0.27 b	0.64 ± 0.01 cd	1.29 ± 0.08 c
Plg-3-glucoside	107.03 ± 2.54 bd	141.59 ± 4.07 a	165.66 ± 0.94 a	103.43 ± 3.61 bd	76.46 ± 1.12 c	73.69 ± 1.68 c	113.36 ± 0.63 cde	130.82 ± 3.93 ab	74.01 ± 1.92 ce	113.31 ± 0.15 bd
Plg-3-rutinoside	7.23 ± 0.10 cdef	5.46 ± 0.62 c	23.92 ± 0.88 b	12.87 ± 1.44 a	3.73 ± 0.13 d	5.20 ± 0.46 cdef	6.07 ± 0.01 cdef	6.22 ± 0.30 cdef	3.24 ± 0.37 e	9.03 ± 0.61 f
Plg-derivative 2	0.43 ± 0.07 ac	0.63 ± 0.05 ab	0.38 ± 0.00 ac	0.42 ± 0.05 ac	0.44 ± 0.06 ac	0.43 ± 0.08 ac	0.60 ± 0.03 ac	0.65 ± 0.02 ac	0.18 ± 0.00 c	1.30 ± 0.01 b
Plg-acetylglucoside	2.71 ± 0.01 a	3.62 ± 0.00 ab	3.39 ± 0.09 h	2.36 ± 0.07 gh	1.13 ± 0.03 bef	1.68 ± 0.06 bceg	2.73 ± 0.03 bcegh	2.90 ± 0.05 cgh	0.55 ± 0.02 f	1.18 ± 0.00 ef
phenolic acids	58.36 ± 2.69 a	56.77 ± 1.61 a	61.72 ± 2.53 abd	49.77 ± 2.91 ad	92.89 ± 2.19 abc	67.94 ± 1.16 abc	61.90 ± 1.94 ab	81.94 ± 3.01 abc	128.37 ± 1.25 c	106.29 ± 3.20 bc
flavonols	27.23 ± 0.49 abef	33.47 ± 2.91 adef	38.38 ± 1.74 ce	43.04 ± 1.73 cd	17.87 ± 1.44 ab	27.30 ± 0.30 abdef	33.87 ± 0.35 cf	12.85 ± 2.51 b	27.50 ± 1.62 abdef	41.13 ± 1.97 c
anthocyanins	123.25 ± 2.54 b	157.11 ± 4.12 a	198.88 ± 1.29 a	122.85 ± 3.89 bd	83.79 ± 1.13 c	83.94 ± 1.75 c	126.94 ± 0.64 cde	144.97 ± 3.95 ab	78.81 ± 1.96 ce	126.84 ± 0.63 bd
flavonoids[d]	150.48 ± 2.59 b	190.58 ± 5.04 af	237.26 ± 2.17 f	165.89 ± 4.26 abf	101.66 ± 1.83 c	111.24 ± 1.77 c	160.81 ± 0.73 abde	157.82 ± 4.68 abd	106.31 ± 2.54 ce	167.97 ± 2.07 abf
total phenolics	208.84 ± 2.74 abcde	247.35 ± 3.38 ade	299.07 ± 3.19 d	215.66 ± 3.68 abcde	194.55 ± 2.63 b	179.19 ± 1.29 b	222.71 ± 1.97 c	239.76 ± 3.94 abcde	234.68 ± 2.08 abcde	274.26 ± 3.81 de

[a] Means ± SD in the same row followed by different letters are significantly different by LSD test ($p < 0.05$). [b] Open system (OS), without recirculation of nutrient solution; closed system (CS), with recirculation of nutrient solution. [c] Not detected. [d] Flavonols + anthocyanins.

Ventana-OS) to 198.88 μg/g fresh fruit (cv. Camarosa-OS), showing statistically significant differences ($p < 0.001$) among cultivars. Overall, the anthocyanin content was lower relative to other samples from strawberry varieties (Camarosa, Carisma, Eris, Oso Grande, and Tudnew) recently surveyed in Spain (*27*). Considering individual anthocyanins, six of them were identified and quantified in the samples. Pelargonidin-3-glucoside was the predominant anthocyanin in the five strawberry cultivars surveyed, accounting for 83−94% of the total anthocyanin content. In absolute terms, its levels varied from 73.69 μg/g fresh fruit (cv. Diamante-CS) to 165.66 μg/g fresh fruit (cv. Camarosa-OS). The second most important anthocyanin in quantitative terms was pelargondin-3-rutinoside, whose content ranged from 3.24 (cv. Ventana-OS) to 23.92 μg/g fresh fruit (cv. Camarosa-OS), which agreed well with the findings of other authors for strawberries grown in temperature climate areas (*28*). Regarding cyanidin-3-glucoside, the largest amounts were found in cv. Aromas-OS (5.30 μg/g), which was in agreement with the levels of pelargonidin-3-glucoside and cyanidine-3-glucoside reported by Kosar et al. (*9*) in cv. Camarosa grown in walk-in plastic tunnels. As for the minor anthocyanins pelargonidin-acetylglucoside and the two pelargonidin derivatives, it was observed that they accounted for <3% of the total contents. As far as flavonols were concerned, it was checked that their total levels were also statistically different ($p < 0.001$) among the cultivars studied. Kaempferol was the most important quantitatively, such that its levels ranged from 12.85 μg/g fresh fruit in cv. Medina-CS cultivar to 41.28 μg/g fresh fruit in cv. Camarosa-CS cultivar. Quercetin was not detected in some of the strawberry cultivars surveyed, the highest amounts being found in cv. Camarosa-CS cultivar (1.76 μg/g fresh fruit), whereas myricetin was not found at detectable levels in the samples analyzed. In this respect, it is important to keep in mind that the flavonoids contents in strawberries, as in any source, depend on a series of factors, such as the stage of maturity, cultivar, storage conditions, and analytical methods, among some others, which can be readily inferred when comparing different studies on this topic (*29*−*31*). Thus, Gil et al. (*29*) reported relatively high contents of quercetin (40 μg/g) and kaempferol (14 μg/g) in a commercial plantation of cv. Selva, whereas Häkkinen and Törrönen (*30*) found much lower contents of these same flavonols in nine strawberry cultivars grown under conventional farming practice in Finland (from 4 to 7 μg/g for quercetin and from 7 to 9 μg/g for kaempferol), and Lugasi and Hovari (*31*) reported the presence of quercetin (10−53 mg/kg) but no kaempferol in other commercial strawberry samples. With regard to the effect of storage, Häkkinen and Törrönen (*30*) observed a rise in the levels of quercetin accompanied by a concomitant decrease of kaempferol and myricetin over 9 months of storage at −20 °C.

Taking into consideration only phenolic acids, it was seen that the total contents varied from 49.77 (cv. Camarosa-CS) to 128.37 μg/g fresh fruit (cv. Ventana-OS). This group of compounds comprised 33% of the total content of phenolics, their levels being statistically different ($p < 0.001$) among the different types of strawberry studied, as it was previously observed in the case of anthocyanins and flavonols. Ellagic and *p*-coumaric acids were the major phenolic acids in the samples studied, which was in consonance with the findings of other authors (*4*). The levels of ellagic acid, which accounted for approximately 86% of the total phenolic acids, ranged from 43.08 μg/g (cv. Camarosa-CS) to 110.23 μg/g (cv. Ventana-OS), amounts that were in agreement with those reported by Määttä-Riihinen et al. (*28*) and Cordenunsi et al. in a commercial plantation located in Brazil (*32*). In the case of *p*-coumaric acid, its levels varied from 1.43 μg/g (cv. Diamante-CS) to 25.47 μg/g (cv. Ventana-CS). In relation to the occurrence of this compound in strawberries, it is known that its levels can also vary considerably within the same cultivar depending on the geographical origin. Thus, for instance, the amounts reported in cultivars of the Senga sengana variety grown in Finland happened to be clearly higher than those found in cultivars from Poland (18 and 7 μg/g, respectively) (*30*).

Influence of the Cultivation System on the Phenolics Content. The levels of total phenolics in the varieties Aromas, Diamante, Medina, and Ventana were apparently quite similar regardless of the soilless cultivation technique used (**Table 1**). Conversely, the levels found in Camarosa strawberries from the open system (299.07 μg/g fresh fruit) were clearly higher than those corresponding to samples from the closed system (215.66 μg/g fresh fruit), above all because of the differences in the contents of pelargonidin-3-glucoside (165.66 and 103.43 μg/g, respectively) and, to a lesser extent, of the differences in the contents of pelargonidin-3-rutinoside (23.92 and 12.87 μg/g fresh fruit, respectively) and *p*-coumaric acid (11.12 and 5.77 μg/g fresh fruit, respectively). Taking into consideration strictly the information extracted from the statistical analyses performed, it was concluded that there were no significant differences in the levels of phenolic acids and flavonols as a function of the soilless cultivation system used, which was not true in the case of anthocyanins ($p < 0.05$). The conclusions drawn from different studies in which the effect of different cultivation techniques on the content of phenolics in strawberries was assessed are varied. Thus, Häkkinen and Törrönen (*30*) concluded that organic cultivation had no significant effects on the levels of phenolic acids and flavonols as compared to traditional cultivation, although higher levels of total phenolics were reported by other authors in organically and sustainably grown strawberries relative to those grown according to conventional agricultural practices (*33*). Likewise, differences in the levels of phenolic acids, flavonols, and anthocyanins between two different cultural systems, hill plasticulture and matted row, have also been reported elsewhere (*34*).

Statistical Analysis. The existence of statistical significant differences in the levels of individual phenolics among the strawberry samples surveyed was evaluated by means of one-way analysis of variance (ANOVA), taken separately into account the cultivar and the cultivation system. The significance levels of the factors as well as their interaction on the concentration of phenolic compounds are shown in **Table 2**.When the data from the two cultivation systems for each strawberry variety were taken into account to evaluate the influence of the cultivar, it was concluded that there were significant differences ($p < 0.05$) among the levels of all of the individual phenolics studied except for *p*-hydroxybenzoic acid, ellagic acid, and pelargonidin derivative 2.

In the same way, to evaluate the influence of the soilless cultivation system, data from the five cultivars for each cultivation system were taken into account. Solely, the levels of *p*-hydroxybenzoic acid, pelargonidin-3-glucoside, pelargonidin-derivative 1, and pelargonidin-derivative 2 proved to be statistically different, the amounts of *p*-hydroxybenzoic acid being always lower in samples from the closed system, irrespective of the variety. When two-way ANOVA was applied, significant interactions appear between cultivar and cultivation/culture system for pelargonidin-3-rutinoside, pelargonidin derivative 2, *p*-hydroxybenzoic acid, ferulic acid, kaempferol,

Table 2. Summary of the ANOVA Results Regarding the Cultivar and the Cultivation System[a]

	p-value[b]		
	cultivar	cultivation system	interaction cultivar × cultivation system
p-OH-benzoic acid	0.067790	*0.000051*	*0.000038*
caffeic acid	*0.000035*	0.755561	0.531849
p-coumaric acid	*0.000000*	0.301457	*0.000000*
ferulic acid	*0.005233*	0.067971	*0.028136*
ellagic acid	0.090537	0.640572	0.901113
quercetin	*0.013259*	0.793661	0.083465
kaempferol	*0.022865*	0.369431	*0.021542*
Plg-derivative 1	*0.023221*	*0.001101*	0.360618
Cy-3-glucoside	*0.000000*	0.303868	0.617463
Plg-3-glucoside	*0.001080*	*0.049890*	*0.001583*
Plg-rutinoside	*0.000000*	0.724960	*0.006768*
Plg-derivative 2	0.743358	*0.011436*	*0.030132*
Plg-acetylglucoside	*0.000000*	0.147768	0.302054

[a] Significant effects ($p < 0.05$) are denoted by italics. [b] *p*-Values obtained by Fisher's test.

Table 3. Variance Explained by the Principal Components

PC	eigenvalue	percentage variance (%)	cumulative variance (%)
1	4.19	32.27	32.27
2	2.35	18.06	50.33
3	1.93	14.88	65.22
4	1.55	11.94	77.16
5	1.17	9.05	86.21

total flavonols, and total anthocyanins. These interactions have been included in **Table 2**.

To meet the second overall objective of the study, that is, to examine the ability of the variables obtained to differentiate among strawberry cultivars and cultivation systems, principal component analysis (PCA) and linear discriminant function analysis (LDA) were carried out, for which standardized experimental data were considered.

After the PCA analysis was applied to the data set, it was seen that the five principal components explained 86.21% of the total variance (**Table 3**). The first factor, which explained 32.27% of the variance, was mainly linked to anthocyanins (cyanidin-3-glucoside, pelargonidin-3-acetylglucoside, and pelargonidin derivative 1) and quercetin, whereas the second principal component (PC), which explained 18.06% of the total variance, was related to kaempferol and *p*-coumaric acid. Data of strawberry samples were graphically expressed as a projection of linear PC scores along the first two eigenvector axes, each a function of all variables under study. Both scatterplots constructed with respect to cultivar and soilless cultivation system display overlap of all samples cases (data not shown).

To determine which variables were the most appropriate for discriminating between samples, linear discriminant function analysis (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 Wilks's λ, which maximizes the ratio of variance between groups to that within groups. Thus, two LDA analyses were carried out in this study, considering, on 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 the variables cyanidin-3-glucoside, pelargonidin-3-rutinoside, *p*-coumaric acid, and pelargonidin-3-glucoside and classified correctly 90% of the cases with high levels of significance ($p < 0.001$) was obtained (*F*-values and *p*-levels in **Table 4**). Additionally, it was seen that the prediction percentages were 100% for cv. Diamante, cv. Ventana, and cv. Camarosa, 80% for cv. Aromas, and 75% for cv. Medina (data not presented).

Table 4. LDA Analysis as a Function of the Cultivars: *p*-Levels and *F*-Values of the Selected Variables in the Model

variable	*F*	*p*-level
Cy-3-glucoside	32.43	0.000000
Plg-rutinoside	30.19	0.000000
p-coumaric acid	28.28	0.000000
Plg-3-glucoside	6.69	0.000440

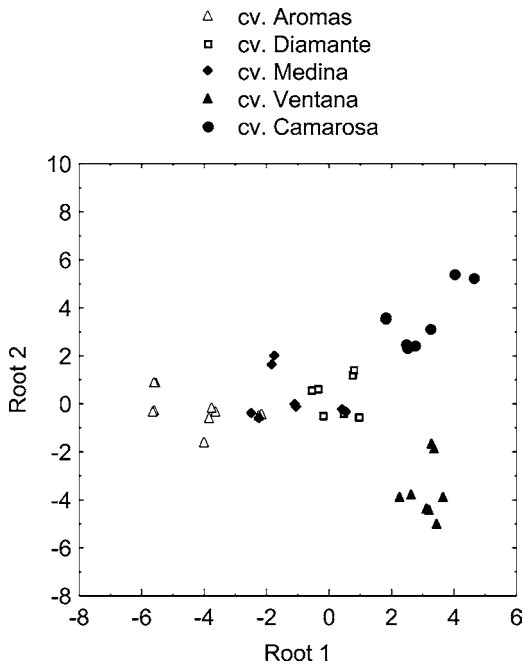

Figure 1. Scatterplot of the samples in the plane defined by the canonical functions when the strawberry cultivars are considered for discrimination.

The location of the strawberry samples surveyed within the plane defined by the two corresponding canonical functions is depicted in **Figure 1**. The discriminant function 1 is mainly related to anthocyanins, specifically cyanidin-3-glucoside and pelargonidin-3-glucoside (negative sign) and pelargonidin-3-rutinoside (positive sign), whereas the discriminant function 2 is mainly linked to *p*-coumaric acid (negative sign). 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 the varieties Camarosa and Ventana (with larger contents of pelargonidin-3-rutinoside and lower amounts of cyanidin-3-glucoside and pelargonidin-3-glucoside) and the other including those from the remaining cultivars. As for the second canonical function, it is mainly linked to the levels of *p*-coumaric acid. Because this variable had a negative sign, the strawberry samples with the highest levels of this compound were located at the bottom of the scatterplot.

Considering the type of cultivation system as criterion for the stepwise LDA analysis, 86% of the samples were correctly classified through the variables *p*-hydroxybenzoic acid and pelargonidin-derivate 1 (*F*-values and *p*-levels in **Table 5**). In this case, the prediction percentages obtained were 90% for the

Table 5. LDA Analysis as a Function of the Soilless Cultivation Systems: *p*-Levels and *F*-Values of the Selected Variables in the Model

variable	*F*	*p*-level
p-hydroxybenzoic acid	20.58	0.000026
pelargonidin-derivate 1	14.38	0.000506

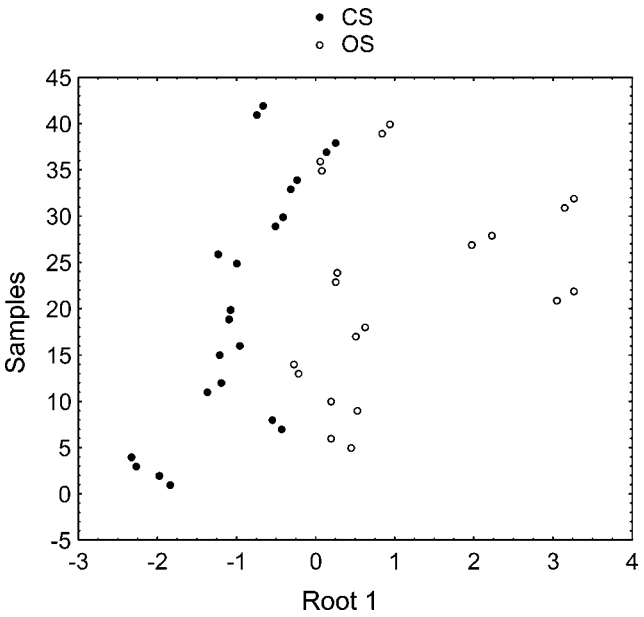

Figure 2. Scatterplot of the samples in the plane defined by the canonical functions as a function of the cultivation systems (open (OS) and closed (CS) soilless cultivation systems).

closed system and 80% for the open system (data not presented). Because of the fact that solely two sets were taken into consideration, only one classification function, related to *p*-hydroxybenzoic acid (positive sign) and pelargonidin derivative 1 (negative sign), was obtained, which yielded a quite good separation among the samples, as it can be observed in **Figure 2**. Regarding the soilless cultivation system (OS vs CS) and considering that the closed cultivation system avoids water and nutrient waste, in this study it has been stated that, in general, strawberries obtained by hydroponics with recycling nutrient solution have higher total anthocyanins and flavonols contents, and hence total phenolic content, than those for fruits obtained in open systems. This could yield a higher quality product, both sensorial and nutritional, because these compounds are responsible for the color and for the antioxidant capacity of strawberries.

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