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Free Hydroxycinnamic Acids, Lycopene, and Color Parameters in Tomato Cultivars

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Concentrations of antioxidant compounds (total phenolic compounds, free hydroxycinnamic acids, and lycopene) and color parameters (*a**, *b**, and *L**) were determined in 167 tomato samples belonging to five cultivars (Dorothy, Boludo, Dunkan, Dominique, and Thomas) produced on the island of Tenerife. Chlorogenic, caffeic, *p*-coumaric, and ferulic acids were identified and quantified in the tomato samples. Chlorogenic acid had the highest mean concentration, whereas the *p*-coumaric was not detected in almost half of the tomato samples. The cultivar, cultivation method, and production region had little influence on the concentration of analyzed parameters. Considerable seasonal variations in the levels of these parameters were observed. Many correlations were observed between the antioxidant compounds and color parameters. The tomato samples tended to be differentiated according to the sampling period when discriminant analysis was applied.

KEYWORDS: Tomato; cultivation method; season; antioxidant; phenolic compounds; lycopene; color; multivariate analysis

INTRODUCTION

Several epidemiological studies suggest that the consumption of tomatoes reduces the risk of chronic diseases such as cardiovascular disease or cancer (*1*–*3*). In accordance with recent studies, regular intake of small amounts of tomato products can increase cell protection from DNA damage by oxidant species (*4*). This protective effect is commonly attributed to antioxidant components and disease-preventing molecules, including ascorbic acid, vitamin E, phenolic compounds, and carotenoids. Tomatoes are the most highly consumed vegetable in Spain, and as a consequence, they are an important source of these antioxidants in the Spanish diet. In a recent study on dietary sources of vitamin C, vitamin E, and specific carotenoid in Spain (*5*), tomatoes ranked first as a source of lycopene (71.6%); second as a source of vitamin C (12.0%), pro-vitamin A carotenoids (14.6%), and β-carotene (17.2%); and third as a source of vitamin E (6.0%). The Canary Islands is one of the main producer regions of tomatoes in Spain; producing 123.000 Tn in 2006 (*6*).

Environmental factors (light, temperature, air composition, mineral nutrition, growth medium) and cultural practices (cultivar, ripening stage at harvest, training system, irrigation system) are known to affect the chemical composition of tomatoes (*7*–*11*). In practical production, however, these factors are often variable and closely linked to one another. Most reports describe how production factors affect the composition of tomatoes in open field. According to Hart and Scott (*12*), the antioxidant content of the tomato mainly depends on both genetic and environmental factors as well as the ripening stage.

The lycopene is the most abundant carotenoid in the ripened tomato, accounting for approximately 80–90% of the total pigments. The rest are β-carotene and other carotenoids and xanthophylls (*13*). Lycopene mainly accumulates in the final period of ripening and its content is not linearly related to color changes (*14*). Chlorophylls and carotenoids, including lycopene, are the main agents responsible for the color of tomatoes. When the ripening process starts, the chlorophyll is degraded and carotenoids are synthesized (*15*). The development of lycopene with ripening has widely been described (*16*–*19*), and a high correlation has been reported between content of lycopene and the color values (*a**, *b**, *a**/*b**) (*19*). The color of the tomato is a very important marketing factor and a very important attribute for the tomato industry (*20*). Besides which, the lycopene pigment has attracted much interest among researchers because of its biological and physicochemical properties, especially related to its effect as a natural antioxidant and its various benefits for human health (*21*).

Simple hydroxycinnamic acids have recently received much interest, as they constitute a significant proportion of the total phenolics ingested in a normal diet and are readily absorbed from the digestive tract. Hydroxycinnamic acids are found most frequently in plants such as simple esters (*22*). Therefore, many analytical methods include hydrolysis steps in order to separate the phenolic acids from their derivatives and, therefore, to determine the total phenolic acids. But these methods are complex and time consuming (*23*), and some authors preferred the determination of free compounds (*24*). Apart from factors related to the analytical

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Table 1. Distribution of the Tomato Samples Analyzed According to Cultivar, Cultivation Method, Sampling Period, and Region of Production

		cultivation method			sampling period				region of production[a]	
cultivar	total	intensive	organic	hydroponic	Oct '04–Nov '04	Dec '04–Jan '05	Feb '05–March '05	April '05–June '05	West	South
Dorothy	50	25	14	11	14	16	12	8	16	9
Boludo	46	28	14	4	12	12	11	11	15	13
Dominique	19	10	9	0	4	8	5	2	0	0
Thomas	25	16	9	0	8	8	4	5	0	0
Dunkan	27	4	12	11	2	10	9	6	0	0
Overall	**167**	**83**	**58**	**26**	**40**	**54**	**41**	**32**	**31**	**22**

[a] Only in intensive cultivation.

method (with or without hydrolysis of conjugated derivatives), there are many factors, including cultivar, type of cultivation, or seasonal variations, that affect the data regarding to phenolic compounds; which explains the large variation in the concentrations reported in the literature. The content of some antioxidant and antioxidant activity of tomato extracts seem to be greatly affected by the ripening stage (*8, 9, 25, 26*). The variations in the content of the hydroxycinnamic acids are largely due to differences in the degree of maturation of the tomato fruit assayed (*27*).

In this paper, we determined total phenolic compounds, free hydroxycinnamic acids, lycopene, and color parameters in several tomato cultivars produced in the Tenerife island (Spain). The influence of the cultivation method, period of sampling, and region of production has also been evaluated. A correlation study and discriminant analysis were carried out in order to discover the relationships between analyzed parameters and classify the tomatoes into homogeneous groups.

MATERIALS AND METHODS

Tomato Sampling. Five cultivars of tomatoes (Dorothy, Boludo, Dunkan, Dominique, and Thomas) were provided by the main producer of Tenerife (ACETO) and other companies with the purpose of making the analysis. The main characteristics of the tomato samples analyzed are described in **Table 1**. Additional information relative to the tomato samples has been indicated in previous papers (*28, 29*). The tomatoes were vine-ripened and harvested between October 2004 and June 2005 and sampled in four periods: October–November 2004, December 2004–January 2005, February–March 2005 and April–June 2005. Data about temperature, relative humidity, and radiation corresponding to all the months were also taken into account.

Sample Preparation Method. Three tomatoes selected from each sample were hand-rinsed with ultrapure water, shaken to remove any excess water, and gently blotted with a paper towel. The color was measured at several points on the skin of each fresh fruit. Afterwards, the tomatoes were mixed and homogenized to a homogeneous puree (Solac, Spain). The puree was stored in a polyethylene tube at − 80 °C until analysis. Several subsamples were taken in duplicate from this previously defrosted puree to measure lycopene, total phenolic compounds, and hydroxycinnamic acids (chlorogenic, caffeic, *p*-coumaric, and ferulic acids).

Analytical Methods. *Color.* Fruit color (*30*) was measured with a Minolta CR-200 colorimeter (Minolta Camera Co. Ltd., Osaka, Japan) obtaining the CIELAB L^*, a^*, and b^* parameters. In addition, the next indexes were measured using the equations proposed by Hobson et al. (*31*) and Dodds et al. (*32*): a^*/b^*, Hue, chroma (C^*), and tomato color index (TCI).

$$hue = \tan^{-1}\frac{b^*}{a^*} \quad (1)$$

$$C^* = \frac{a^*}{\sqrt{a^{*2} + b^{*2}}} \quad (2)$$

$$\mathrm{TCI} = 2000\frac{a^*}{L^*\sqrt{a^{*2} + b^{*2}}} \quad (3)$$

Total Phenolic Compounds. The phenol content in the tomato samples were determined spectrophotometrically at 750 nm using a Folin–Ciocalteau (Sigma Chemical Co.; St. Louis, MO) colorimetric method described by Kujala et al. (*33*), using gallic acid (Sigma) as a standard. The device used was a spectrophotometer UV–vis (diodo-Array) Hewlett Packard 8453 equipped with a computer Hewlett Packard Vectra XA.

Lycopene. Lycopene concentration was determined spectrophotometrically at 503 nm previous extraction in 20 mL of a 5:5:10 acetone: ethanol:hexane mixture within a flask wrapped with aluminium foil to exclude light, according to the method described by Fish et al. (*34*) The device used was a spectrophotometer UV–vis (diodo-Array) Hewlett Packard 8453 equipped with a computer Hewlett Packard Vectra XA.

Free Hydroxycinnamic Acids. *HPLC Reagents and Standards.* Methanol of HPLC-gradient grade was purchased from Merck (Darmstadt, Germany) and trifluoroacetic acid (TFA) was purchased from Aldrich (Milwaukee, WI, USA). Standards of gallic, *(+)*-catechin, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, *(−)*-epicatechin, *p*-coumaric, ferulic, syringic, and 3,5-dimethoxy-4-hydroxycinnamic acids and kaempferol, quercetin, *(±)*-naringenin, and rutin hydrate were from Sigma (St. Louis, MO) and ellagic acid dihydrate was from Aldrich (Milwaukee, WI). All standards were prepared as stock solutions at 1 g/L, except rutin and quercetin at 0.5 g/L and kaempferol at 0.3 g/L, in methanol (HPLC-gradient grade) 60%, and they were stored in darkness at − 80 °C (*24*). Deionized water was purified with a Milli-Q water system (Millipore Corporation, MA).

HPLC Equipment. The analytical HPLC system is comprised of a Waters 2690 high-performance liquid chromatograph equipped with a Waters 996 photodiode array detector (Water, Milford, MA). The separation was performed using a Nova-Pak C18 steel cartridge (150x3.9 mm i.d.) with a particle diameter of 4 μm, using a Waters C18 guard column to protect the analytical column. The temperature of the column oven was set at 30 °C during all the experiments and the flow rate at 0.7 mL/min. The use of online connected diode-array increases the selectivity and sensitivity for the determination of these compounds. The HPLC pumps, autosampler, column oven, and diode-array system were monitored and controlled using the Millennium[32] system. Several wavelengths were used for the detection of the hydroxycinnamic acids.

HPLC Method. About 1 g of the frozen homogenized tomato puree was weighed directly in polypropylene tubes and mixed with 2 mL of methanol 75% (1.5 mL methanol, and 0.5 mL ultrapure water at pH 2.5 adjusted with TFA). Afterwards, the tubes were put into an ultrasound bath at 40 °C for 30 min. They were then centrifuged for 10 min at 3.500 rpm. The supernatant was carefully recovered to prevent contamination with the homogenized tomato puree pellet and it was passed through a 0.45 filter μm GHP (Waters, Millford, MA) prior to HPLC analysis. Duplicate injections were performed and average peak areas were used for the quantification.

Samples were eluted with a mobile phase similar to that used by Martínez-Valverde et al., (*35*) with a slight modification in the time run of the gradient. It was composed of 0.05% TFA (pH 2) and 100% methanol in the following proportions: 80:20 at time 0 min, changing linearly to 70:30 by 5 min, remaining at this ratio until 14 min. The mobile phase then changed linearly to 55:45 over the course of 15 min, stayed at this ratio for another 3 min, and finally changed back to the initial conditions.

Statistics. All the statistics were performed by means of the SPSS version 14.0 software for Windows. The Kolmogorov–Smirnov test was applied to verify whether the distribution of the variables was

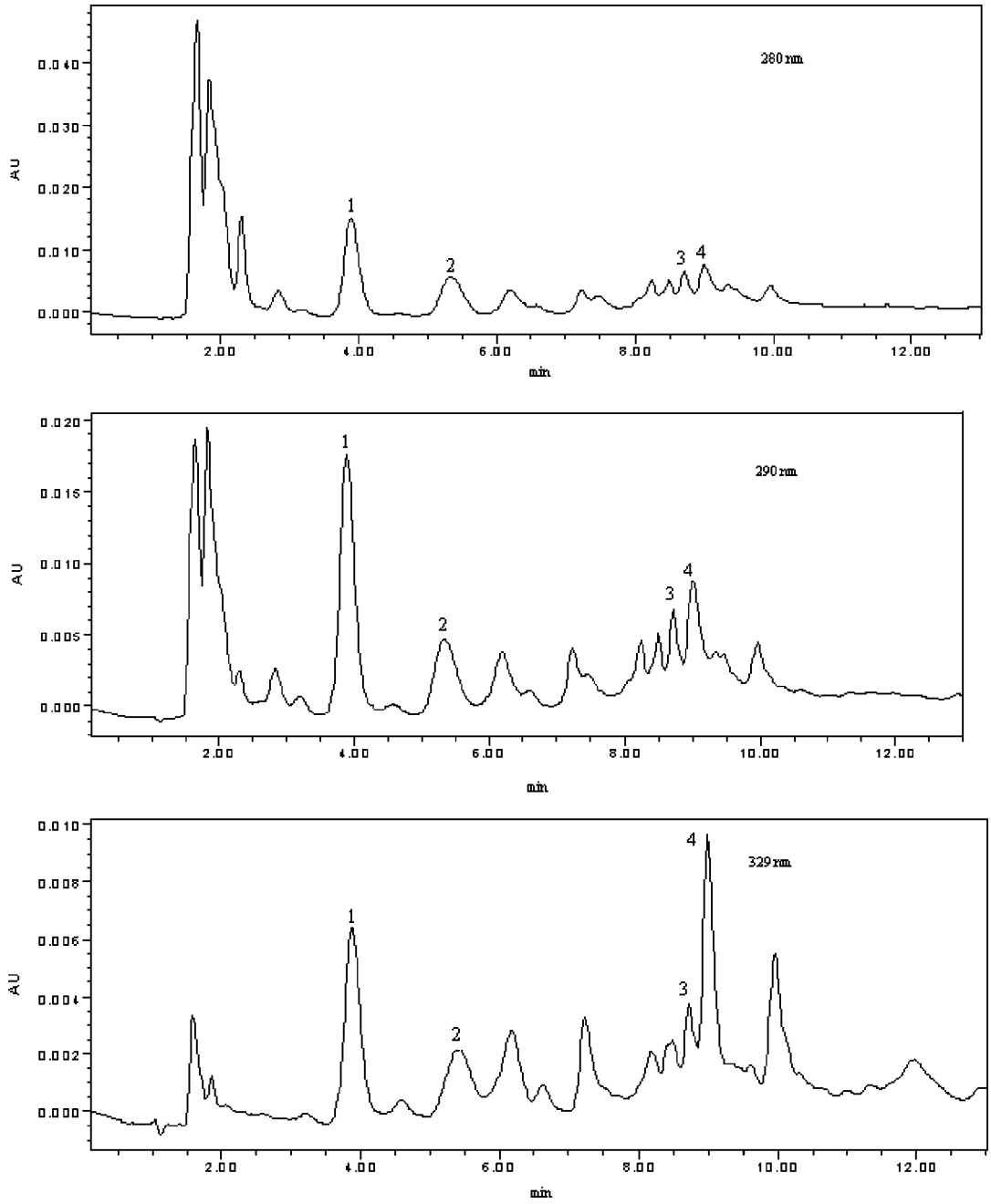

Figure 1. Chromatograms corresponding to a tomato sample at three wavelengths of the UV detector (1, chlorogenic acid; 2, caffeic acid; 3, *p*-coumaric acid; 4, ferulic acid).

normal ($p < 0.05$). When the statistical distribution was not normal, the variables were transformed by applying neperian logarithms to convert them into a normal distribution. The Levene test was applied to verify the homogeneity of the variances. Mean values obtained for the variables studied in the different groups were compared by One-Way ANOVA (Duncan's multiple range) assuming there were significant differences among them when the statistical comparison gave $p < 0.05$. Simple linear and logarithmic correlation analysis was used to indicate a measure of the correlation and the strength of the relationship between two variables. Discriminant analysis (DA) is on the basis of the extraction of linear discriminant functions of the independent variables by means of a qualitative dependent variable and several quantitative independent variables. Two processes wereapplied in DA: (1) stepwise DA that selected the quantitative variables that enhance discrimination of the groups established by the dependent variable; and (2) introduction of all independent variables. The objective of this process is to not lose information, although the system obtained is more complex.

RESULTS AND DISCUSSION

Eleven hydroxycinnamic acids, gallic, (+)-catechin, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, (–)-epicatechin, *p*-coumaric, ferulic, syringic, and 3,5-dimethoxy-4-hydroxycinnamic acids, were used to try to identify the chromatographic peaks in the real samples. The identification of the observed peaks was carried out by checking the retention time and the absorption spectra of the each hydroxycinnamic acid of both real tomato samples and the standards in the range between 190 and 400 nm. In addition, a tomato sample was spiked with the standard and, after HPLC injection, the increase of peak area confirmed the identification. Four hydroxycinnamic acids were separated and identified in the tomato samples: chlorogenic, caffeic, *p*-coumaric, and ferulic acids. **Figure 1** shows three chromatograms corresponding to a tomato sample obtained using the optimized conditions described above, and the following

Table 2. Figures of Merit of Several Hydroxycinnamic Acids

	precision[a]								
	retention time (min)		peak area						
acid	interday	intraday	interday	intraday	recovery (%)	linearity range (mg/L)	R^{2b}	DL[c] (μg/L)	QL[d] (μg/L)
chlorogenic	1.28	0.21	3.22	4.70	95.39	0.3–2.0	0.9995	10.13	10.43
caffeic	2.49	0.08	5.88	2.31	89.10	0.2–0.7	0.9996	2.26	2.28
p-coumaric	1.98	0.11	1.93	0.62	76.05	0.01–0.15	0.9993	6.98	7.03
ferulic	1.58	0.11	1.45	0.26	86.44	0.025–0.25	0.9999	1.03	1.09

[a] Data of precision were expressed as coefficient of variation (%). [b] R^2 = Square of coefficient of correlation of Pearson. [c] DL = Detection limit $D_L=[X_b + 3DS_b$-ordinate]/slope. [d] QL = Quantification limit $Q_L=[X_b + 10DS_b$-ordinate]/slope.

Table 3. Results (mean ± standard deviation; minimum–maximum) of the Free Hydroxycinnamic Acids (mg/100 g FW), Lycopene (mg/100 g FW), and Color Parameters Expressed in Overall Terms and According to Individual Cultivars[a]

param	Dorothy	Boludo	Dominique	Thomas	Dunkan	*P* (sig)
total phenols	21.1 ± 4.4 (11.7 – 29.9)	20.3 ± 4.3 (12.8 – 32.6)	20.5 ± 4.8 (12.2 – 29.6)	19.7 ± 5.0 (9.7 – 28.2)	19.9 ± 3.1 (15.4 – 25.2)	0.849
chlorogenic acid	1.16 ± 0.66 ab (0.38 – 3.00)	1.35 ± 0.66 b (0.61 – 3.27)	1.14 ± 0.50 ab (0.25 – 2.10)	0.90 ± 0.30 a (0.31 – 1.52)	1.00 ± 0.46 a (0.41 – 1.73)	0.035
caffeic acid	0.12 ± 0.04 (0.04 – 0.21)	0.12 ± 0.05 (0.06 – 0.25)	0.11 ± 0.03 (0.07 – 0.18)	0.14 ± 0.06 (0.07 – 0.28)	0.11 ± 0.04 (0.07 – 0.23)	0.514
p-coumaric acid	0.19 ± 0.04 (0.14 – 0.30)	0.17 ± 0.04 (0.10 – 0.28)	0.16 ± 0.04 (0.11 – 0.23)	0.18 ± 0.03 (0.12 – 0.23)	0.16 ± 0.03 (0.14 – 0.21)	0.163
ferulic acid	0.23 ± 0.08 bc (0.10 – 0.45)	0.18 ± 0.06 b (0.08 – 0.36)	0.19 ± 0.05 b (0.11 – 0.29)	0.23 ± 0.06 c (0.13 – 0.36)	0.14 ± 0.07 a (0.04 – 0.28)	0.000
lycopene	2.34 ± 0.68 b (1.0 – 3.6)	2.43 ± 0.77 b (1.2 – 4.5)	2.26 ± 0.78 ab (1.2 – 4.2)	2.56 ± 0.64 b (1.6 – 4.3)	1.89 ± 0.44 a (1.2 – 2.7)	0.004
a^*	22.9 ± 3.9 ab (15.7 – 33.3)	25.0 ± 2.9 c (19.4 – 32.6)	22.9 ± 3.9 ab (16.2 – 29.8)	23.7 ± 4.3 bc (13.5 – 31.6)	21.1 ± 2.9 a (14.0 – 26.7)	0.000
b^*	23.7 ± 3.7 (15.6 – 34.4)	23.3 ± 3.4 (16.0 – 32.1)	22.4 ± 3.1 (17.8 – 28.4)	23.3 ± 4.0 (17.3 – 30.7)	22.4 ± 2.3 (17.6 – 26.9)	0.539
a^*/b^*	0.98 ± 0.19 a (0.6 – 1.6)	1.08 ± 0.15 b (0.8 – 1.4)	1.02 ± 0.13 a (0.8 – 1.3)	1.04 ± 0.23 ab (0.6 – 1.5)	0.95 ± 0.16 a (0.6 – 1.2)	0.009
L^*	44.6 ± 2.1 (40.7) 47.5	44.2 ± 2.3 (40.0 – 47.5)	44.2 ± 2.0 (41.0 – 47.2)	44.2 ± 3.0 (39.7 – 47.7)	43.5 ± 2.2 (39.8 – 46.9)	0.323
C^*	33.1 ± 4.5 ab (23.5 – 45.9)	34.3 ± 3.8 b (27.8 – 43.7)	32.1 ± 4.5 ab (25.0 – 38.3)	33.4 ± 4.6 b (26.4 – 42.3)	30.8 ± 2.5 a (25.6 – 36.2)	0.014
TCI	47.2 ± 7.6 (31.3 – 63.7)	50.3 ± 8.4 (34.1 – 70.4)	49.5 ± 7.7 (36.0 – 61.1)	49.1 ± 11.4 (31.3 – 70.9)	49.1 ± 8.2 (31.9 – 62.4)	0.529
Hue	0.80 ± 0.09 b (0.57 – 1.04)	0.75 ± 0.07 a (0.61 – 0.92)	0.78 ± 0.06 ab (0.65 – 0.90)	0.78 ± 0.11 ab (0.58 – 1.05)	0.82 ± 0.09 b (0.68 – 1.01)	0.009

[a] Results in the same row with the same superscript were not statistically significant ($p < 0.05$) according to the classification obtained by the Duncan Test.

three different wavelengths for the detection of the acids: 280, 290, and 329 nm. One can observe the good resolution and separation of the four identified hydroxycinnamic acids in the chromatogram corresponding to the 329 nm wavelength. Several unidentified peaks corresponding to hydroxycinnamic acids or, probably, to other organic compounds can also be observed on these chromatograms. The analysis time for the determination of these free hydroxycinnamic acids was approximately 12 min, which was lower than the data observed in most of the HPLC methods found in the literature (*24*, *35*). The results corresponding to the validation of the method including the four hydroxycinnamic acids that were detected in the tomato samples are shown in **Table 2**. A relatively high repeatability and reproducibility for the retention times (min) was observed, with variation coefficients for repeatability (intraday precision, between 0.08 and 0.21% for caffeic and chlorogenic acids, respectively) and for reproducibility (interday precision, between 1.28 and 2.49% for chlorogenic and caffeic acids, respectively). With regards to the peak area, the repeatability ranged between 0.26 and 4.70% for ferulic and chlorogenic acids, respectively, and the reproducibility ranged between 1.45% for ferulic acid and 5.88% for caffeic acid. The detection limit of the individual compounds at 329 nm, calculated as the signal multiplied by three times the height of the noise level, varied between and 1.03 and 10.13 μg/L for ferulic and chlorogenic acids, respectively. The detection limits were lower than other values reported by other authors (*24*). The calibration curve for each hydroxycinnamic acid was prepared by injecting 10 μL taken from individual standard solutions in the following ranges of concentrations: 0.3–2 mg/L for the chlorogenic acid, 0.2–0.7 mg/L for the caffeic acid, 0.01–0.15 mg/L for the *p*-coumaric acid, and 0.025–0.25 mg/L for the ferulic acid. The coefficients of correlation for all the phenolic acids were >0.999 and the response of detector was linear in the tested ranges. A recovery study with the four identified hydroxycinnamic acids in tomato samples was performed by spiking the tomato sample extracts with known amounts of these hydroxycinnamic acids. High recovery was obtained for the chlorogenic acid (95.4%); however, the recoveries of caffeic and ferulic acids were moderate (89.1 and 86.4% for both hydroxycinnamic acids, respectively). Mattila and Kumpulainen (*24*) found similar recoveries (87–112%) of several free phenolic acids when developing its method of extraction. A relatively low recovery was observed for *p*-coumaric acid. The analytical methods were applied on the tomato samples described in the Materials and methods section. **Table 3** shows the results obtained in the parameters analyzed and calculated in the all the samples when grouping the tomato samples according to the cultivar. The total phenolic compounds varied between 19.7 and 21.1 mg (expressed as gallic acid)/100 g of fresh weight (FW). No

significant differences in the mean values of total phenolic compounds were found between the cultivars of tomatoes considered, which agrees with Giovanelli et al. (*14*), who did not find significant differences between the two tomato genotypes considered. In contrast, George et al. (*36*) found significant differences in the total phenolic compounds and other antioxidants between cultivars. The concentrations found in this paper were similar to those concentrations reported by Slimestad and Verheul (*37, 38*). Besides, the phenolic compounds determined in five cultivars of tomatoes ranged from 2.25 to 25.84 mg/100 g of FW (*11*). The average phenolic content of tomato pulps belonging to the Tradiro, Excel, and Flavourine cultivars was 15 mg/100 g of FW (*39*). Martínez-Valverde et al. (*35*) reported that the content of total extractable phenolics of nine tomato samples of several cultivars varied between 25.9 and 49.9 mg of ferulic acid/100 g of FW (22.7 and 43.8 mg of gallic acid/100 g) for the Senior and Pera cultivars of tomatoes, respectively, which are comparable to our data.

Four hydroxycinnamic acids, chlorogenic, caffeic, *p*-coumaric, and ferulic, were detected in this study, which agrees with the results by Martínez-Valverde et al. (*35*). However, Mattila et al. (*24*) and Mattila and Hellström (*40*) did not find the caffeic in the tomato samples. Other authors (*26, 37, 38, 41*) also determined, apart from these four hydroxycinnamic acids, flavonoids such as rutin and naringenin. The chlorogenic acid was the major hydroxycinnamic acid found; however, this acid was not detected in 13 tomato samples (7.8% of the total of the tomato samples). The amount of total phenolic compounds was much higher than the sum of hydroxycynnamic acids detected. This is due to the fact that there are other phenolic compounds present in tomatoes, such as flavonoids, which were not quantified in an independent manner. Besides, the Folin–Ciocalteau method used usually overestimates the content of total phenolic compounds, because the presence of other reducing agents, such as ascorbic acid, can interfere (*35, 42*). Our data on chlorogenic acid concentrations (expressed in wet weight) were similar to those reported by other authors who determined free chlorogenic acid (*37, 38, 41*). Martínez-Valverde et al. (*35*) found a mean concentration of this acid in the range of 1.43 and 3.28 mg/100 g of FW for the Pera and Senior cultivars, respectively, which is a slightly higher than our data. Ripe tomatoes have previously been reported to contain around 1–8 mg/100 g of FW of chlorogenic acid (*43*). The highest values of chlorogenic acid found in the literature were reported by Raffo et al. (*26*), who obtained a seasonal variation range of 2.67–5.44 mg/100 g of FW. Many of these data refer to the total chlorogenic acid obtained by applying a previous hydrolysis step, which explains the discrepancies of the data reported in the literature. The caffeic and ferulic acids were detected in all the tomato samples analyzed with concentrations ranging between 0.04 and 0.28 mg/100 g of FW and 0.04–0.45 mg/100 g of FW for the caffeic and ferulic acids, respectively. The concentrations of ferulic acid were similar to those reported by Raffo et al. (*26*) and Martínez-Valverde et al. (*35*) and lower than the data reported by Mattila and Kumpulainen (*24*) for nine cultivars of tomatoes. Our results for caffeic acid were lower than those data reported in the bibliography by other authors (*26, 35*). *p*-Coumaric acid was not detected in 80 tomato samples (47.9% of the total), and the concentration in the detected tomato samples varied between 0.10 and 0.30 mg/100 g of FW. Raffo et al. (*26*) found higher *p*-coumaric acid contents than those contents reported here. Besides, our data were near the lower limit of the interval described by Martínez-Valverde et al. (*35*) for several tomato cultivars.

Some significant differences were observed when the mean concentrations of the hydroxycinnamic acids obtained between the tomato cultivars were compared. The Dunkan and Thomas cultivars showed lower ($p < 0.05$) mean chlorogenic acid concentrations than those found for the Boludo cultivar. In addition, this acid was not detected in 15 and 4% of the tomatoes corresponding to the Dunkan and Boludo cultivars, respectively, and was detected in all the tomato samples belonging to the other cultivars. The Dunkan cultivar presented the lowest mean ferulic acid concentration ($p < 0.05$), and the Thomas cultivar had the highest mean concentration with significant differences in relation to the mean values found for the Dunkan, Dominique, and Boludo cultivars. No significant differences in the mean concentrations of caffeic and *p*-coumaric acids were observed between all the tomato cultivars. The percentage of tomato samples with contents of *p*-coumaric lower than the detection limit was similar in all the cultivars.

The mean concentration of lycopene fell well inside the interval described for other authors (*37, 38, 44*). However, most of the data reported in the literature for ripened tomatoes (*12, 35, 36, 45, 46*) were slightly higher than our data. Slight differences in ripening stage and changes during post-harvest ripening (*37, 38*) could explain these discrepancies. George et al. (*36*) found important differences in the lycopene concentration as a function of the genotype. However, we did not find great differences in the lycopene content between cultivars. Only the Dunkan cultivar had a lower ($p < 0.05$) mean concentration of lycopene than the mean concentration obtained in the other cultivars, except the Dominique cultivar.

The mean value of red color (a^*) of the tomato (*19*) was similar to the values obtained by other investigators for tomatoes in a similar maturity stage, red or slight red color (*19, 47, 48*). As the second chromatic component (b^*) was positive, it measures the yellow color (*30*). The mean value was similar or a little lower than the values described by others (*19, 47, 49*), but higher than the b^* values obtained by Giovanelli and Paradiso (*48*), for tomatoes with a similar maturity stage. The mean value of the a^*/b^* ratio was 1.02, which indicates that the tomatoes analyzed were in the red ripeness stage (*50*). The achromatic component (L^*) measures the darkness ($L^* = 0$) or lightness ($L^*=100$). The mean L^* values found by us were similar to those mean values reported by other authors in tomatoes with a similar maturity stage (*19, 47, 49, 51*). The color indexes, such as ratio a^*/b^*, C^*, and TCI, increase according to the ripening stage. The Hue behavior is the opposite, it is maximum in the green stage (*52*). All the color indexes values were within the range found by Gómez et al. (*47*) and López and Gómez (*52*) for tomatoes with a similar maturity stage. The mean Hue value was similar to that value reported by Arias et al. (*19*). Our results were higher and lower than those results obtained for the elongated and salad tomatoes (green-orange stage), and for cherry and cluster type tomatoes (full-ripening stage), respectively (*51*).

There were significant differences ($p < 0.05$) between cultivars in the mean values of the a^*, a^*/b^*, C^*, and Hue. The Boludo cultivar showed the highest value of a^* with significant differences with respect to the rest of the cultivars, except the Thomas cultivar. The mean values of b^* and L^* found in the cultivars did not present statistically significant differences. However, the Dorothy cultivar had the maximum values of L^* and b^*, whereas the Dunkan cultivar had the lowest values for two these parameters and for a^*.

The mean values of the analyzed parameters differentiating the cultivar and the method of cultivation are shown in **Table**

Table 4. Mean Concentrations of the Free Hydroxycinnamic Acids (mg/100 g FW), Lycopene (mg/100 g FW) and Color Parameters, and Mean Values of the Color Parameters Analyzed in the Tomato Groups According To the Cultivar and the Cultivation Method[a]

		cultivation method			
param	cultivar	intensive	organic	hydroponic	P^b
total phenols	Dorothy	21.16	22.54 bc	19.07 b	0.180
	Boludo	21.65	18.93 a	15.83 a	0.005
	Dominique	19.68	21.36 abc		0.419
	Thomas	17.95	22.80 c		0.016
	Dunkan	19.67	19.67 ab	20.33 b	0.856
	P^c	0.093	0.023	0.043	
chlorogenic acid	Dorothy	1.08	1.34	1.02	0.540
	Boludo	1.39	1.36	0.97	0.548
	Dominique	1.23	1.04		0.212
	Thomas	0.86	0.98		0.238
	Dunkan	1.10	0.91	1.07	0.568
	P^c	0.049	0.247	0.751	
caffeic acid	Dorothy	0.12	0.11 a	0.13 b	0.657
	Boludo	0.12	0.13 a	0.07 a	0.071
	Dominique	0.09	0.13 ab		0.012
	Thomas	0.11	0.18 b		0.003
	Dunkan	0.11	0.11 a	0.11 b	0.936
	P^c	0.579	0.021	0.051	
p-coumaric acid	Dorothy	0.19	0.21	0.16	0.147
	Boludo	0.17	0.17		0.835
	Dominique	0.15	0.17		0.235
	Thomas	0.18	0.19		0.430
	Dunkan	0.15	0.16	0.17	0.717
	P^c	0.255	0.332	0.497	
ferulic acid	Dorothy	0.26 c	0.20 b	0.17	0.001
	Boludo	0.18 ab	0.20 b	0.17	0.647
	Dominique	0.18 ab	0.19 b		0.754
	Thomas	0.21 bc	0.27 b		0.014
	Dunkan	0.14 a	0.12 a	0.17	0.073
	P^c	0.000	0.000	0.982	
lycopene	Dorothy	2.36	2.41	2.20	0.825
	Boludo	2.40	2.44	2.63	0.703
	Dominique	2.16	2.37		0.490
	Thomas	2.45	2.76		0.280
	Dunkan	1.64	2.01	1.87	0.294
	P^c	0.155	0.238	0.080	
a^*	Dorothy	22.78 ab	24.89 b	20.80 a	0.027
	Boludo	24.59 b	25.18 b	26.88 b	0.278
	Dominique	24.23 b	21.47 a		0.125
	Thomas	22.72 ab	25.37 b		0.117
	Dunkan	20.17 a	21.05 a	21.40 a	0.787
	P^c	0.071	0.002	0.017	
b^*	Dorothy	23.89	23.10	23.98 ab	0.820
	Boludo	22.68	23.76	26.56 b	0.102
	Dominique	22.63	22.10		0.619
	Thomas	23.41	22.99		0.810
	Dunkan	23.30	22.48	21.94 a	0.591
	P^c	0.841	0.818	0.026	
L^*	Dorothy	44.9	44.1	44.7 ab	0.578
	Boludo	43.9	44.0	46.7 b	0.071
	Dominique	43.5	45.0		0.114
	Thomas	44.7	43.4		0.308
	Dunkan	44.5	43.4	43.2 a	0.625
	P^c	0.398	0.613	0.023	

[a] Results in the same column with the same superscript were not significantly ($p < 0.05$) different. [b] p value of the comparison by rows. [c] p value of the comparison by column.

4. The mean total phenol concentrations in hydroponic tomato samples belonging to the Boludo cultivar was lower than those found in intensively grown tomatoes, whereas the total content of the phenolic compounds in organic tomatoes tends to be higher. No significant differences in the mean contents of chlorogenic and *p*-coumaric acids were found between the cultivation methods for all the cultivars studied. The organic Dominique and Thomas cultivars had a higher ($p < 0.05$) mean caffeic acid concentration than those found in the intensive cultivations. As with the caffeic acid, the mean ferulic acid concentration in organic tomatoes of the Thomas cultivar was higher than that in intensively grown tomatoes. The Dorothy cultivar hydroponically cultivated had the lowest mean ferulic acid concentration. Organic tomatoes of all the cultivars had higher lycopene concentrations than the corresponding intensive and hydroponic cultivars (except Boludo), although significant differences were not reached. The behavior of the a^* parameter was similar to that of the lycopene (except Dominique), which agrees with the contribution of the lycopene to the red color of the tomatoes. Organic tomatoes belonging to the Dorothy cultivar had a higher a^* value than the intensive and hydroponic tomatoes.

When the cultivar in each cultivation method was considered in an independent manner, differences between the mean values of some parameters were found. The Boludo cultivar of organic and hydroponic cultivations had the lowest mean total phenolic content. Significant differences between the mean concentrations of chlorogenic acid were only observed in intensive tomatoes. The mean caffeic acid concentration of the organic Thomas cultivar was higher ($p < 0.05$) than the mean concentrations found in the rest of the cultivars, except the Dominique cultivar. As regards ferulic acid, there were significant differences between cultivars for the intensive and organic cultivations. The Dunkan cultivars presented the lowest mean ferulic acid content. Significant differences were found with respect to the rest of the cultivars in organic cultivation, and with respect to the Dorothy and Thomas cultivar in intensive cultivation. The behavior of the parameter a* was naturally similar to the lycopene, except for the hydroponic Dorothy cultivar, which had a slightly lower mean value than the Dunkan cultivar. The b*and L* color parameters showed no significant differences in the mean values between intensively and organically cultivated cultivars of tomatoes.

The results regarding the analyzed parameters in all the cultivars according to cultivation method and sampling period are shown in **Table 5**. In addition, **Figure 2** shows the evolution of several climatic factors such as temperature, solar radiation, and relative humidity during these sampling periods. Data from September were included in order to consider the periods of maximum growth and development of the tomatoes in plants before their harvesting. There are many significant differences in the analyzed parameters between the sampling periods considered. No clear tendencies ($p > 0.05$) in the total phenolic compounds were observed between the periods of sampling considered in this paper. The differences found depend on the cultivation method. Organic and hydroponic tomatoes showed a relatively low *p*-value (0.072 and 0.051 respectively), which indicates that the tomatoes tend to differentiate according to the sampling period. So, the tomatoes collected in the December 2004–January 2005 period had the highest mean total phenol concentration. The hydroxycinnamic acids studied behaved in the same way in all the tomatoes cultivated with the three cultivation methods. The tomatoes collected in the October–November 2004 period had the lowest mean concentration of chlorogenic acid, with significant differences with respect to the tomatoes of the February–March 2005 period for intensive and organic tomatoes; this was also true in the case of hydroponic tomatoes from the April–June 2005 period. The mean caffeic acid concentration of the tomatoes collected in the October–November 2004 period was the highest in the three cultivation methods considered, with significant differences in

Table 5. Mean Concentrations of Analyzed Antioxidant Compounds (mg/100 g FW) and Mean Values of Color Parameters in Tomato Groups According to the Cultivation Method and the Sampling Period[a]

period	total phenols	chlorogenic acid	caffeic acid	*p*-coumaric acid	ferulic acid	lycopene	*a**	*b**	*L**
				1) Intensive					
Oct '04–Nov '04	20.5 ± 5.0	0.90 ± 0.31 a	0.17 ± 0.04 b	0.20 ± 0.03	0.26 ± 0.07 b	2.83 ± 0.68 c	26.4 ± 3.16 b	26.8 ± 2.8 c	46.5 ± 0.6 c
Dec '04–Jan '05	20.8 ± 4.9	0.94 ± 0.40 a	0.10 ± 0.03 a	0.15 ± 0.03	0.20 ± 0.07 a	2.23 ± 0.45 b	21.7 ± 3.16 a	23.3 ± 3.1 b	44.5 ± 1.8 b
Feb '05–March '05	20.3 ± 6.1	1.58 ± 0.81 b	0.10 ± 0.02 a	ND[b]	0.18 ± 0.06 a	1.77 ± 0.50 a	23.2 ± 3.8 a	22.1 ± 2.5 b	43.8 ± 2.1 b
Apr '05–June '05	20.0 ± 2.8	1.18 ± 0.44 ab	0.10 ± 0.02 a	ND[b]	0.21 ± 0.08 a	2.72 ± 0.75 c	22.7 ± 3.0 a	19.8 ± 2.0 a	41.8 ± 1.5 a
P (sig)	0.950	**0.003**	**0.000**	**0.000**	**0.003**	**0.000**	**0.000**	**0.000**	**0.000**
				2) Organic					
Oct '04–Nov '04	21.3 ± 3.5	0.82 ± 0.34	0.16 ± 0.05 b	0.20 ± 0.03	0.23 ± 0.07 c	3.18 ± 0.76 c	25.4 ± 3.2 b	26.1 ± 3.0 b	46.5 ± 1.1 b
Dec '04–Jan '05	22.1 ± 2.7	1.16 ± 0.42	0.15 ± 0.06 b	0.16 ± 0.03	0.23 ± 0.07 c	2.23 ± 0.5 b	23.8 ± 3.7 ab	22.5 ± 2.8 a	43.1 ± 2.3 a
Feb '05–March '05	19.2 ± 4.9	1.44 ± 0.67	0.09 ± 0.02 [a]	ND[b]	0.12 ± 0.06 [a]	1.71 ± 0.36 [a]	22.3 ± 4.0 [a]	21.5 ± 3.0 [a]	43.5 ± 2.4 [a]
April '05–June '05	20.2 ± 3.5	1.32 ± 0.80	0.08 ± 0.01 a	ND[b]	0.17 ± 0.06 b	2.31 ± 0.34 b	22.7 ± 3.0 ab	21.1 ± 2.3 a	42.6 ± 1.9 a
P (sig)	0.072	0.061	**0.000**	**0.001**	**0.000**	**0.000**	0.105	**0.000**	**0.000**
				3) Hydroponic					
Oct '04–Nov '04	17.2 ± 2.0 a	0.63 ± 0.2 a	0.18 ± 0.03 c	0.18 ± 0.02	0.22 ± 0.04 c	2.45 ± 0.45 b	24.2 ± 1.1 c	25.5 ± 3.0	45.3 ± 2.4
Dec '04–Jan '05	21.6 ± 2.8 b	0.81 ± 0.27 ab	0.12 ± 0.03 b	0.15 ± 0.01	0.18 ± 0.02 bc	2.21 ± 0.46 b	20.6 ± 1.6 b	22.3 ± 2.4	43.2 ± 1.8
Feb '05–March '05	18.7 ± 2.5 ab	1.36 ± 0.52 c	0.09 ± 0.02 a	ND[b]	0.14 ± 0.03 a	1.37 ± 0.22 a	17.8 ± 3.6 [a]	22.7 ± 2.2	45.0 ± 2.1
April '05–June '05	17.9 ± 3.3 a	1.30 ± 0.53 bc	0.07 ± 0.01 a	ND[b]	0.16 ± 0.02 ab	2.45 ± 0.38 b	25.5 ± 3.2 c	24.2 ± 3.9	44.5 ± 2.7
P (sig)	0.051	**0.010**	**0.000**	**0.007**	**0.001**	**0.000**	**0.000**	0.262	0.375

[a] Significant differences are indicated in bold letters. [b] ND =Not detected.

relation to the rest of the sampling periods considered (except in the case of organic tomatoes collected in the December 2004–January 2005 period). The *p*-coumaric and ferulic acids showed the highest mean values in the October–November 2004 period, with significant differences with respect to many of the other sampling periods considered. The *p*-coumaric acid of tomatoes produced in the February–March 2005 and April–June 2005 periods and cultivated using the three cultivation methods was not detected in all the tomato samples. The behavior of lycopene was similar to that of ferulic acid and inverse to that of chlorogenic acid. Thompson et al. (*46*) found an increase in the lycopene content with the decrease in the chlorogenic acid and increase in ferulic acid, which is in agreement with our results. Chlorogenic acid could be used to synthesize the other hydroxycinnamic acids. It has been observed that chlorogenic acid appeared in abundance in young fruits, but declined rapidly towards the end of growth and during ripening (*27*). Thus, one can deduce that the temperature in the last stages of growth and ripening can play an important role in the content of these antioxidants. Slight changes in the ripening stage of the tomatoes collected in the sampling periods considered can explain the differences in the contents of these hydroxycinnamic acids.

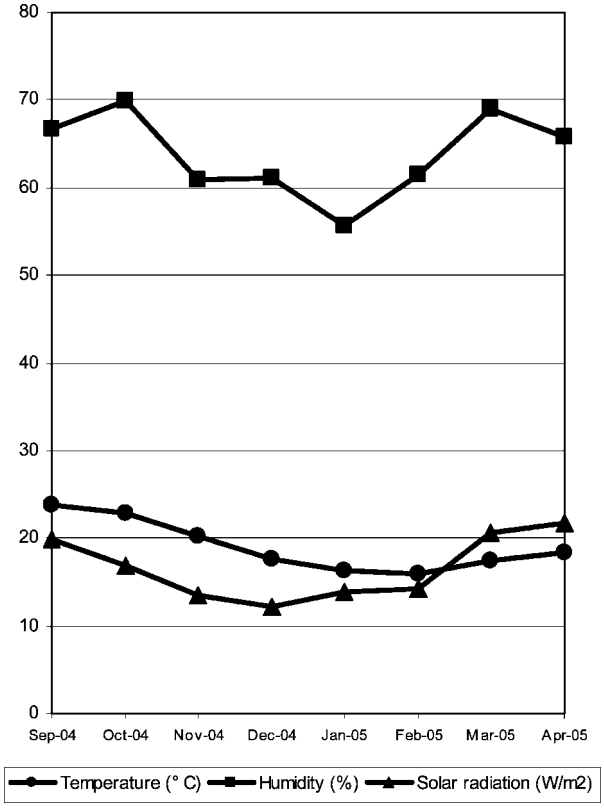

Figure 2. The mean values of several climatic factors during the sampling periods.

The tomato samples collected in the February–March 2005 period and cultivated using the three cultivation methods presented the lowest ($p < 0.05$) mean lycopene concentration. In contrast, the highest mean lycopene concentration was observed in the October–November 2004 period, with significant differences with respect to the rest of the sampling periods in the organic tomatoes; and with respect to the December 2004–January 2005 and February–March 2005 periods in intensive tomatoes. Heinonen et al. (*53*) observed that lycopene concentration was relatively high in summer and low in winter in tomatoes purchased from retail food stores in Finland. This could be due to the fact that tomatoes from the summer period were collected in a more advanced ripening stage. In contrast, Raffo et al. (*26*) did not find clear seasonal trends of carotenoid content or any association with climatic parameters. Moreover, they found lower contents of lycopene in summer. Lycopene synthesis is favoured at temperatures between 16 and 21 °C and inhibited at temperatures above 30 °C (*50, 54*). This explains the lower lycopene content in Liso cultivar compared to other cultivars, as this was the only cultivar harvested in midsummer (*35*). The tomatoes harvested in October–November 2004 period were exposed to a relatively high temperature in the last period of growth and development (September–October 2004), which could explain the high contents of lycopene. Moreover, the sequence of the mean lycopene concentrations was the same as the sequence of temperatures and relative humidity observed in the periods 1 month before the harvesting of the tomatoes. Therefore, Brandt et al. (*50*) reported that the last 10 days of the ripening phase were critical in the lycopene biosynthesis. In general, a similar behavior to the lycopene was observed in the analyzed color parameters. However, significant differences between the mean values were not reached in the a* value for

Table 6. Direct Matrix Correlation for All the Samples[a]

	chlorogenic acid	caffeic acid	*p*-coumaric acid	ferulic acid	lycopene	*a**	*b**	*L**	*a**/*b**	*C**	TCI	Hue
total phenols	0.308[b]			0.287								
chlorogenic acid		−0.169			−0.289			−0.225			0.176	
caffeic acid			0.472	0.542	0.170	0.218	0.295	0.264		0.302		
p-coumaric acid				0.554	0.433	0.357	0.272	0.274		0.369		
ferulic acid					0.287	0.203	0.227	0.165		0.260		
lycopene						0.381	0.242		0.165	0.379		−0.170
*a**							0.374		0.600	0.844	0.419	−0.622
*b**								0.702	−0.501	0.812	−0.626	0.484
*L**									−0.573	0.431	−0.817	0.563
*a**/*b**											0.931	−0.991
*C**												
TCI												−0.935
Hue												

[a] Only the significant ($p < 0.05$) correlations are shown. [b] Pearson's coefficient correlation

organic tomatoes and in *b** and *L** values for hydroponic tomatoes. The mean value of the first chromatic component (*a**) was higher in the October–November 2004 period, with significant differences in relation to other sampling periods. The second chromatic components (*b**) and the achromatic component (*L**) also presented higher levels in intensive, organic, and hydroponic tomatoes collected in the October–November 2004 period than in the rest of the sampling periods considered, with significant differences in intensive and organic tomatoes.

The *a**/*b** ratio represents a simple, significant ripening index. Because of the widely variable metabolism of individual fruits and high dependence of ripening on climatic conditions, both on the growing plant and after harvesting, it would be pointless to report data as a function of time (*14*). These authors investigated the variation in the antioxidant content and color parameters in two tomato genotypes during vine and post-harvest ripening. They observed that ripening conditions affected both the antioxidant accumulation kinetics and the final content, which was higher in post-harvest ripened fruits. Furthermore, when they related variations in the lycopene content as a function of the *a**/*b** value obtained, the carotenoid formation was very slow up to an *a**/*b** value of about 1, and then it became faster, resulting in an exponential relationship between carotenoid synthesis and color variation. Our data about the *a**/*b** value were near to 1, and the lycopene content was about 2.33 mg/100 g of FW. If our data are introduced in the graphic representation shown by these authors (*14*), these should be correctly included in the curve corresponding to vine-ripened tomatoes. These authors indicated that although instrumental determination of red color and evaluation of the *a**/*b** index are the most sensitive and significant indicators for fruit maturity, they did not show a direct and unequivocal correlation with the lycopene content. The same *a**/*b** value can correspond to lycopene contents differing by 100%, which is in agreement with the results of Koskitalo and Ormrod (*55*).

Slimestad and Verheul (*37*, *38*) found that the total phenolic compounds in tomatoes change with the season and their concentrations increase with increasing light intensity. Strong direct radiation on green fruits (~650 W/m^2 or 2990 μmol/m^2/s for 1.5–4 h) inhibited lycopene synthesis (*50*). On the contrary, low light intensity results in uneven fruit color by decreasing the lycopene accumulation (*50*). In our study, no clear influence of the solar radiation on the lycopene and hydroxycinnamic acids compounds content was obtained in the tomato samples analyzed.

The influence of the production region (environmental factors) on the analyzed parameters was considered for the following two tomato cultivars, the intensively cultivated Dorothy and Boludo cultivars. For this purpose, several tomato samples belonging to both cultivars were sampled from the western and southern regions of the island. The mean concentration of total phenols and the hydroxycinnamic acids in the tomatoes of the Dorothy cultivar collected in the west were higher than the tomatoes from the south (22.8 ± 4.6 versus 18.2 ± 4.3 mg/100 g of FW for total phenols; 1.19 ± 0.14 versus 0.75 ± 0.36 mg/100 g of FW for chlorogenic acid; 0.14 ± 0.03 versus 0.09 ± 0.04 mg/100 g of FW for caffeic acid; 0.20 ± 0.04 versus 0.18 ± 0.03 mg/100 g of FW for *p*-coumaric acid; and 0.28 ± 0.10 versus 0.24 ± 0.04 mg/100 g of FW for ferulic acid), with significant differences for total phenols and caffeic acid. The behaviour of lycopene and color parameters was the opposite, although significant differences were not reached for all the parameters. No significant differences were obtained in all the parameters studied for Boludo cultivar. Therefore, little or no influence of the production region on the parameters studied was observed.

Multivariate Analysis. A statistical study of correlation among all the parameters analyzed was carried out beforehand to discover associations between measured pairs of these parameters. There were several significant ($p < 0.05$) correlations (**Table 6**), many of them between chromatic (*a** and *b**) and achromatic (*L**) components and the color indexes, which is due to the fact that the color indexes are calculated from these components.

As regards the antioxidant compounds, there are three positive coefficients of correlations (*r*): caffeic acid with *p*-coumaric ($r = 0.472$) and with ferulic ($r = 0.542$) acids, and *p*-coumaric acid with ferulic acid ($r = 0.554$). These correlations can be explained because these hydroxycinnamic acids have a similar chemical structure, and therefore, they must have a common origin (*24*). **Figure 3** shows the correlation between caffeic and ferulic acids ($r = 0.542$). The total phenols were significantly correlated with all the independently determined hydroxycinnamic acids, except caffeic acid. The coefficient of correlation of the total phenols with the chlorogenic acid was the highest, which is due to the fact that this hydroxycinnamic acid was higher in concentration than the rest of the hydroxycinnamic acids determined, and therefore, its contribution to the total content of hydroxycinnamic acids is higher. Lycopene correlated with some color parameters such as *b**, *a**/*b**, *C** and inversely with Hue. Although there is controversy (*14*), most authors (*19*, *35*, *46*, *50*) have found a correlation between lycopene and the *a**/*b** ratio. This ratio has been proposed as a good indicator of the lycopene content in tomatoes (*19*, *46*) and for establishing the degree of ripeness of tomatoes (*19*). A significant correlation was observed between lycopene and *a**, which could be

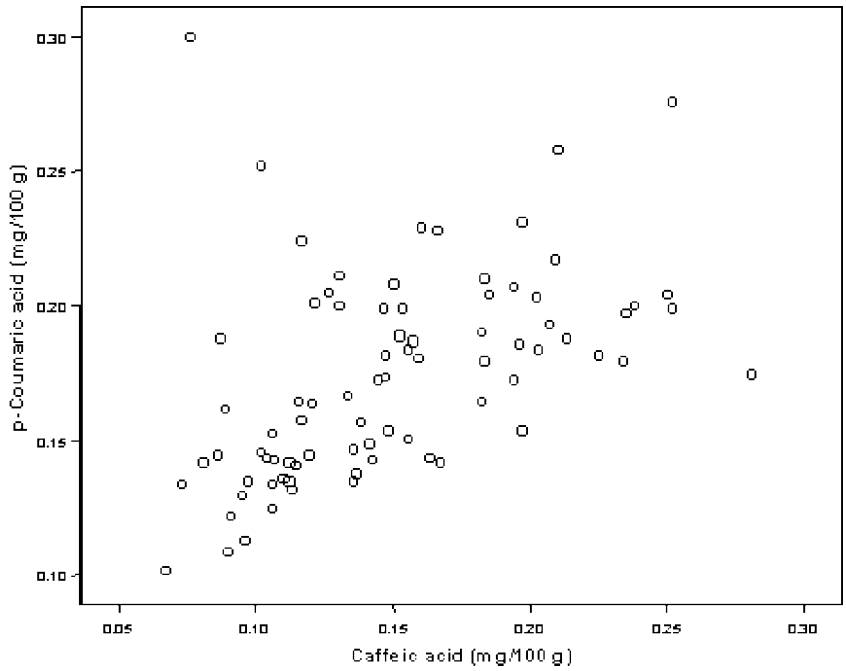

Figure 3. Correlations between *p*-coumaric and caffeic acid.

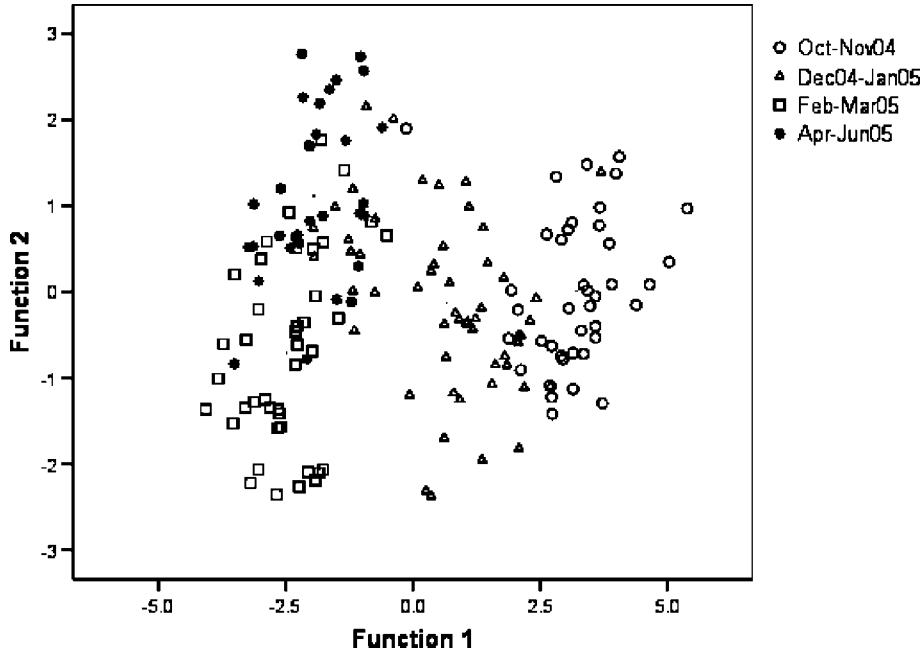

Figure 4. Scatter diagram on the axes representing the first two discriminant functions according to the sampling period.

explained with the fact that this parameter is related to the red color. Some investigators (*19*) have found highly significant and exponential correlations between lycopene and *a**. These investigators analyzed tomato samples at very different maturity stages, and they observed a saturation phenomenon in the latest ripening stages. In the present paper, we collected tomato samples in a relatively narrow interval of ripening, at point 7–8 of the ripening color chart, which could explain the absence of this correlation. Caffeic, *p*-coumaric, and ferulic acids presented significant and positive correlations with lycopene and color parameters such as *b**, *L**, and *C**, and inverse with TCI. Chlorogenic acid showed an inverse correlation with *p*-coumaric, lycopene, and *L** and positive with TCI. Therefore, it can be deduced that the content of hydroxycinnamic acids could be affected by the ripening stage, because these color parameters and lycopene depend on the ripening of the tomatoes.

Discriminant analysis (DA) was performed on the studied quantitative parameters to differentiate the tomato samples according to the cultivation method, cultivar, region of production, and sampling period. After application of stepwise DA to all data, low percentages of correct classification were obtained in the classifications according to the cultivation method (all the variables were eliminated), cultivar (37.7 and 34.1% after cross-validation), and region of production (56.6 and 56.6% after cross-validation). However, when the stepwise DA was applied to differentiate the tomato samples according to sampling period, and 80.2% of the tomato samples were correctly classified (78.4% after cross-validation). **Figure 4** shows the graphic representation of the two first discriminant functions for the tomato samples according to the sampling period. A clear tendency to the differentiation of the tomato samples was observed, particularly with the tomatoes sampled in the October–November 2004 period.

Table 7. Results of the Stepwise Discriminant Analysis for All the Cultivars Considered To Differentiate Cultivation Method and Sampling Period

cultivar	no. of sample and percentage (%) of correct classification		Total of percentage of correct classification (% after cross-validation)	selected variables
	Oct '04–Jan '05	Feb '05–June '05		
		(1) Intensive		
Dorothy	14 (93.3%) 96.0% (96.0%)	10 (100%)	96.0% (96.0%)	TCI, chlorogenic and *p*-coumaric acids
Boludo	15 (93.8%) 96.4% (96.4%)	12 (100%)	96.4% (96.4%)	caffeic and *p*-coumaric acid
Dominique	4 (80%) 90.0% (90.0%)	5 (100%)	90.0% (90.0%)	*p*-coumaric acid
Thomas	7 (87.5%) 93.8% (93.8%)	8 (100%)	93.8% (93.8%)	TCI and *p*-coumaric acid
Dunkan	2 (100%) 100% (50%)	2 (100%)	100% (50.0%)	*L**, *p*-coumric acid
		(2) Organic		
Dorothy	8 (100%) 100% (100%)	6 (100%)	100% (100%)	*L**, chlorogenic, caffeic, and *p*-coumaric acids
Boludo	8 (100%) 10% (92.9%)	6 (100%)	100% (92.9%)	chlorogenic and *p*-coumaric acids
Dominique	7 (100%) 100 % (100%)	2 (100%)	100% (100%)	*b** and *p*-coumaric acid
Thomas	8 (100%) 100% (88.9%)	1 (100%)	100% (88.9%)	*p*-coumaric acid
Dunkan	4 (100%) 100% (91.7%)	8 (100%)	100% (91.79%)	total phenols, lycopene, chlorogenic, and *p*-coumaric acids
		(3) Hydroponic		
Dorothy	9 (85.7%) 90.9% (90.9%)	4 (100%)	90.9% (90.9%)	*p*-coumaric acid
Dunkan	8 (83.3%) 90.9% (90.9%)	5 (100%)	99.9% (99.9%)	*p*-coumaric acid

The stepwise DA was repeated on the samples corresponding to each tomato cultivar in an independent manner to differentiate the tomato samples according to the cultivation method, region of production, or sampling period. Low or moderate percentages of correct classification were obtained when selecting different parameters as a function of the cultivation method and region of production. However, the percentage of correct classification was relatively high when the stepwise DA was applied on the cultivars to differentiate the tomato samples according to the sampling period: Dorothy cultivar, 92.0% (78.0% after cross-validation); Boludo cultivar, 80.4% (78.3% after cross-validation); Dominique cultivar, 84.2% (84.2% after cross-validation); Thomas cultivar, 100.0% (92.0% after cross-validation); and Dunkan cultivar, 70.4% (66.7% after cross-validation).

Table 7 shows the results of the stepwise discriminant analysis in all the cultivars and the cultivation methods in an independent manner to classify the tomato samples according to two sampling periods (October 2004–January 2005 and February–June 2005. High percentages (>90%) of correct classification were observed. The *p*-coumaric acid was selected in all cases. All the hydroponic tomato samples were well-classified into their sampling period. All the tomato samples of the organic cultivars were well-classified according to the sampling period. Therefore, it is confirmed that the sampling period seems to be a more important factor in the differentiation of tomato samples than the cultivar, cultivation method, or production region. This fact could be explained by the small differences observed in the ripening stage between the sampling periods.

In summary, the small differences in the ripening stage of the tomato samples considered are a confounding factor that makes it difficult to interpret the results on differences in the method of cultivation and cultivar. Method of cultivation and cultivar region of cultivation can assert a slight influence on the concentrations of lycopene and phenolic compounds, but the sampling season seems to have a more important influence on these compounds in tomatoes. Climatic conditions during the ripening such as temperature or relative humidity had an influence on the lycopene, chlorogenic, and ferulic acid contents of tomatoes. Correlations between the hydroxycinnamic acids were observed that are due to metabolic relationships between them. Chlorogenic acid has an inverse behaviour to the other hydroxycinnamic acids. Linear discriminant analysis is a useful tool for differentiating the tomato samples according to the sampling period.

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