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'Fuentepina' and 'Amiga', two new strawberry cultivars: Evaluation of genotype, ripening and seasonal effects on quality characteristics and health-promoting compounds

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Abstract.
BACKGROUND: Strawberries are widely consumed in the world and an important source of health-promoting compounds, such as polyphenols. The nutritional quality as well as the phytochemical composition of strawberry fruits are known to be strongly influenced by genetic, environmental factors, ripeness at harvest, and storage conditions.
OBJECTIVE: The nutritional quality and the phytochemical content of two new strawberry cultivars, namely 'Fuentepina' and 'Amiga', were evaluated. These novel cultivars were compared with 'Camarosa' and 'Candonga', the two most extended cultivars in Spain, and with 'Primoris', an emerging one.
METHODS: The influence of genotype, stage of ripening and season on different properties as colour, firmness, acidity, soluble solids content, antioxidant capacity, and polyphenols profile were evaluated.
RESULTS: Results showed significant effects of genotype, stage of ripening and season on the majority of the measured parameters. Thirty nine phenolic compounds were tentatively identified. Anthocyanins were the most abundant class of polyphenols in 'Amiga', 'Candonga', 'Camarosa' and 'Primoris' cultivars while more flavan-3-ols were recorded in 'Fuentepina'.
CONCLUSIONS: 'Fuentepina' strawberries stand out for their pleasant flavour as a result of a high sugar/acid ratio and 'Amiga' strawberries may offer potential as a new promising cultivar due to its high firmness, good sugar/acid ratio and high content of phytochemicals.

Keywords: *Fragaria* × *ananassa*, healthy compounds, quality, ripening, season, antioxidant capacity

1. Introduction

Strawberries (*Fragaria* × *ananassa,* Duch.) are one of the most popular berries in Europe, with a wide consumer base. More than 4.3 million tons of strawberries were produced worldwide in 2011 [1], Spain being the largest producer in Europe and the third largest in the world. Strawberries represent a rich source of micronutrients,

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such as minerals, vitamin C, folate and phenolic compounds. They contain a complex (poly)phenolic profile consisting mainly of a mixture of flavonoids (anthocyanins and flavonols) and hydrolysable tannins (ellagitannins and gallotannins) along with phenolic acids (hydroxycinnamic and hydroxybenzoic acids) and condensed tannins (proanthocyanidins) [2, 3]. Polyphenols are important antioxidants that exhibit a remarkably high scavenging activity toward chemically generated radicals [4, 5]. Among the phytochemicals occurring in strawberries, it is important to highlight pelargonidin-3-*O*-glucoside which is the major anthocyanin in all strawberry varieties [6]. Another important group of phenolic compounds are ellagitannins, which are present in only a few other berries and nuts [7]. However, in general terms, the nutritional quality and the phytochemical composition of berry fruits are known to be strongly influenced by genetic [8–11] and environmental factors [2, 12–15], including the weather, cultivation method, ripeness at harvest and storage conditions [16].

In addition, it has been reported that environmental changes may affect each cultivar differently [15, 17]. It is interesting to assess the quality of strawberries and their phenolic compound content in different years and thus evaluate how new cultivars are affected by environmental conditions. This assessment could be useful for breeding programs, as it may help select those cultivars that are more susceptible to environmental conditions. In addition to genetic and environmental effects, the stage of ripening at harvest is an important factor to be considered, since it determines postharvest life and final strawberry quality.

The aim of this study was to characterize five strawberry cultivars: 'Fuentepina' and 'Amiga', two new cultivars obtained from the Spanish public breeding program; 'Camarosa' and 'Candonga', the most important commercial cultivars in Spain (with around 65–70% of the total cultivated area in Spain in 2010 and 2011); and 'Primoris', as an example of an emerging cultivar developed in recent years (representing 4% of the total cultivated area in Spain in 2011) [18, 19]. Moreover, in this study, changes in the physico-chemical and nutritional quality (firmness, colour, soluble solid content, acidity, antioxidant activity and phenolic compounds) of those cultivars were considered in two different seasons (2010 and 2011) and in two different stages of ripening (nearly ripe and ripe), both of which were suitable for consumption.

2. Materials and methods

2.1. Strawberry material

Strawberry fruits (*Fragaria* × *ananassa,* Duchesne) from five cultivars, 'Camarosa', 'Candonga', 'Fuentepina', 'Amiga' and 'Primoris', were grown in conventional culture in "El Cebollar", IFAPA's experimental station, located at Moguer (37º16'N, 6º50'W; Huelva, Spain). All the cultivars were grown under the same conditions and in the same field to minimize the effect of environmental and agronomic factors. Details regarding the culture system, irrigation and fertilization are described in Dominguez-Morales [20].

The new strawberry cultivars were obtained from the Spanish public breeding program, 'Amiga' [21] and 'Fuentepina' [22]. The fruits were harvested in two years (2010, 2011) to study the seasonal effect. Selected fruits were those free from physical damage and fungal infection. Strawberries were divided into two groups: ripe fruits (red) and nearly ripe fruits (nearly red) [8] to study the effect of ripening. Both stages of ripening were considered as suitable for consumption.

2.2. Colour

Colour measurements of the fruit surface were carried out with a colorimeter (Konica Minolta CR400). There were two determinations for each strawberry, on two opposite sides of its equatorial diameter. L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness) were recorded. The hue angle [arctan (b^*/a^*)] and chroma $(a^{*2}+b^{*2})^{1/2}$ were calculated. Fruit colour values were an average of 15 strawberry measurements.

2.3. Firmness

Firmness was measured as the maximum penetration force (N) reached during tissue breakage and determined with a 5 mm diameter flat probe. Penetration depth was 5 mm, and the cross-head speed was 5 mm/s using a TA-XT

Plus Texture Analyzer (Stable Micro Systems, Godalming, UK). Strawberries were sliced into halves, and each half was measured in their equatorial zone. Fruit firmness values were an average of 15 strawberry measurements.

2.4. Soluble solid content (SSC) and acidity

The strawberries were cut into small pieces, wrapped in cheesecloth and squeezed by hand; the juice was used to measure acidity and SSC. The acidity of the samples was assessed using a titrator (Mettler Toledo model T70, Mettler Toledo AG, Analytical, Schwerzenbach, Switzerland) and titrated to pH 8.1 using 0.1 M NaOH. The titrable acidity was expressed as a percentage of citric acid. The SSC was determined with an Atago RX-1000 digital refractometer (Atago Co. Ltd., Tokyo, Japan) and was expressed in brix degrees (ºBrix).

2.5. Antioxidant capacity: FRAP and DPPH assays

Freeze-dried strawberry fruits (0.3 g) were extracted with 15 ml acetone:water (60:40 v/v). The mixtures were then centrifuged at 5000 rpm for 5 min at 4ºC. The supernatants were transferred to vials, stored at –80ºC, and later used to analyse antioxidant capacity (DPPH and FRAP).

The FRAP assay was carried out as described by Benzie and Strain [23] with some modifications. To prepare the FRAP reagent, a mixture of 0.1 M acetate buffer (pH = 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM ferric chloride (10:1:1, v/v/v) was made. A volume of 3.9 ml of FRAP reagent was mixed with 0.1 ml of the previously diluted extract. After 5 hours, the signal obtained at 510 nm was measured using a spectrophotometer (Shimadzu UV 2401 PC) and compared with a known concentration range of similarly prepared Trolox standards. The results were expressed as micromoles of Trolox equivalents per gram of dry weight of strawberries (μmol TE/g DW).

DPPH radical scavenging activity was measured according to the technique reported by Brandwilliams et al. [24]. 100 μl of the previously diluted extract was mixed with 1.9 ml of methanolic solution of DPPH (absorbance 1.1). Twenty hours later, absorbance was measured at 515 nm by means of a spectrophotometer (Shimadzu UV 2401 PC), using a methanolic solution of Trolox, at different concentrations, as a control. The results were expressed as micromoles of Trolox equivalents per gram of dry weight of strawberries (μmol TE/g DW).

2.6. HPLC-DAD-FD analysis

Phenolic compounds were extracted from 0.5 g of freeze-dried strawberry powder in 15 ml of acetone:water (60:40 v/v). The mix was homogenized using an Ultraturrax (IKA T25) for 1 min and then the extracts were sonicated for 15 min in an ultrasonic bath. The homogenates were then centrifuged at 5000 rpm for 15 min at 4°C. Each extract was concentrated with a nitrogen flow at low temperature (35°C). The volume of the extract (aqueous residue) was made up to 5 ml with 10% methanol in water. The final extracts were filtered through a 0.45 μm polyethersulfone filter before they were analysed by HPLC.

The phenolic extracts were analysed on a HPLC system (Perkin Elmer) equipped with a photodiode array (Perkin Elmer) and fluorescence (Jasco) detectors. Chromatographic separation was performed on 250 × 4.6 mm, 5 μm particle size reversed phase C18 column and a guard column (3 cm). The solvents were formic acid in water (1% v/v) (A) and methanol (B). The gradient consisted of 5% B for 5 min, 5–45 min 5–80% B, 45–50 min 80–100% B and 50–60 min 100% B at a flow rate of 0.4 ml/min. The injection volume was 20 μl and the column temperature was set at 40 °C.

Spectral data for all peaks were accumulated in the range 190–700 nm and chromatograms were recorded at 260, 320, 360 and 520 nm for simultaneous monitoring of the different groups of phenolic compounds. The excitation and emission wavelengths of the fluorescence detector were set at 290 and 320 nm, respectively. Compounds were identified according to retention time and UV-Vis, fluorescence and mass spectra. Anthocyanins were quantified as pelargonidin-3-*O*-glucoside equivalents (at 520 nm); flavonols as kaempferol-3-*O*-glucoside equivalents (at 360 nm); hydroxycinnamic acids (at 320 nm except cinnamoyl-glucose at 280 nm) as p-coumaric acid equivalents (at 320 nm); ellagic acid derivatives and ellagitannins as ellagic acid equivalents (at 260 nm); and catechin and proanthocyanidins

were quantified in (–)catechin and proanthoycianidin dimer B equivalents, respectively, using fluorescence peak areas.

2.7. HPLC-MS analysis

A Varian 1200 L liquid chromatograph equipped with reversed-phase column (150 mm × 2.1 mm, 3 μm, C18) thermostated at 35°C was used for the HPLC-MS analysis. The mobile phase consisted of two solvents: water-formic acid (99.9/0.1, v/v) (A) and acetonitrile: methanol (75/25, v/v). The gradient condition was 0–20 min, 5–100% B. The flow rate was 0.2 ml/min and the injection volume was 8 μl. Nitrogen was used as the nebulizing gas at a pressure of 50 psi. The MS data were acquired in positive and negative ionization mode. The full scan covered the mass range from m/z 80–1200.

2.8. Statistical analysis

Statistical analyses were performed using Statistix software (version 9.0). Values are given as means. The data were subjected to analysis of variance (ANOVA), followed by a mean comparison using a least significant difference (LSD) test. Differences at $P < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Identification and quantification of phenolic compounds in strawberry cultivars

A total of 39 phenolic compounds were tentatively identified on the basis of their UV-Vis, fluorescence and MS spectra. Figure 1A represents the HPLC-DAD chromatogram at 280 nm and Fig. 1B shows the HPLC-FD profile of 'Camarosa' strawberry extract. The HPLC-DAD analysis of the five strawberry cultivars studied allowed the characterization of 21 compounds and the HPLC-FD analysis the characterization of 18 additional compounds, labelled from 1 to 39 based on the elution order in the chromatogram (Table 1).

Four anthocyanins were identified: cyaniding-3-*O*-glucoside (Cy-3-gluc) (peak 22), pelargonidin-3-*O*-glucoside (Pg-3-gluc) (peak 27), pelargonidin-3-rutinoside (Pg-3-rut) (peak 29) and pelargonidin-3-acetylglucoside (Pg-3–ac) (peak 35). Pg-3-gluc was the main anthocyanin in all the cultivars studied as others have previously reported [8, 25]. All the anthocyanins detected had been previously reported in strawberries [25–27].

In all of the extracts of the strawberry cultivars analysed, 18 flavan-3-ols were detected and quantified. Five peaks were observed for dimers of proanthocyanidins (4, 5, 6, 9 and 26), five for trimers (peaks 1, 7, 13, 20 and 30), seven for tetramers (peaks 11, 12, 14, 17, 24, 25 and 28) and a monomer of catechin (peak 10).

The flavonols detected (peaks 32, 37, 38 and 39) were quercetin-3-glucuronide, kaempferol-3-glucuronide, kaempferol-3-malonylglucoside and kaempferol-3-acetylglucoside.

In all of the extracts of the strawberry cultivars analysed, four hydroxycinnamic acid derivate peaks (15, 18, 23 and 36) were detected and quantified. Peaks 15 and 18 correspond to two p-coumaroylhexoses, peak 23 to ferulic acid hexose and peak 36 to cinnamoyl-glucose. All these compounds have been previously described in strawberries [2, 8, 13].

Two ellagic acid deoxyhexoside (peaks 33 and 34), two bis-HHDP-glucoside (peaks 2 and 3), a HHDP-galloyl-glucose (peak 8), three galloyl-bis-HHDP glucose (peaks 16, 19 and 31) and the ellagitannin sanguiin H6 (peak 21) were tentatively identified.

The concentration of all the phenolic compounds identified in each strawberry cultivar, the stage of ripening and season are presented in the Table 2.

3.2. Effect of genetic factors

All the parameters evaluated were affected by genetic. Colour is probably the most important appearance attribute in strawberries, caused mostly by anthocyanin accumulation. 'Fuentepina' strawberries showed significantly higher

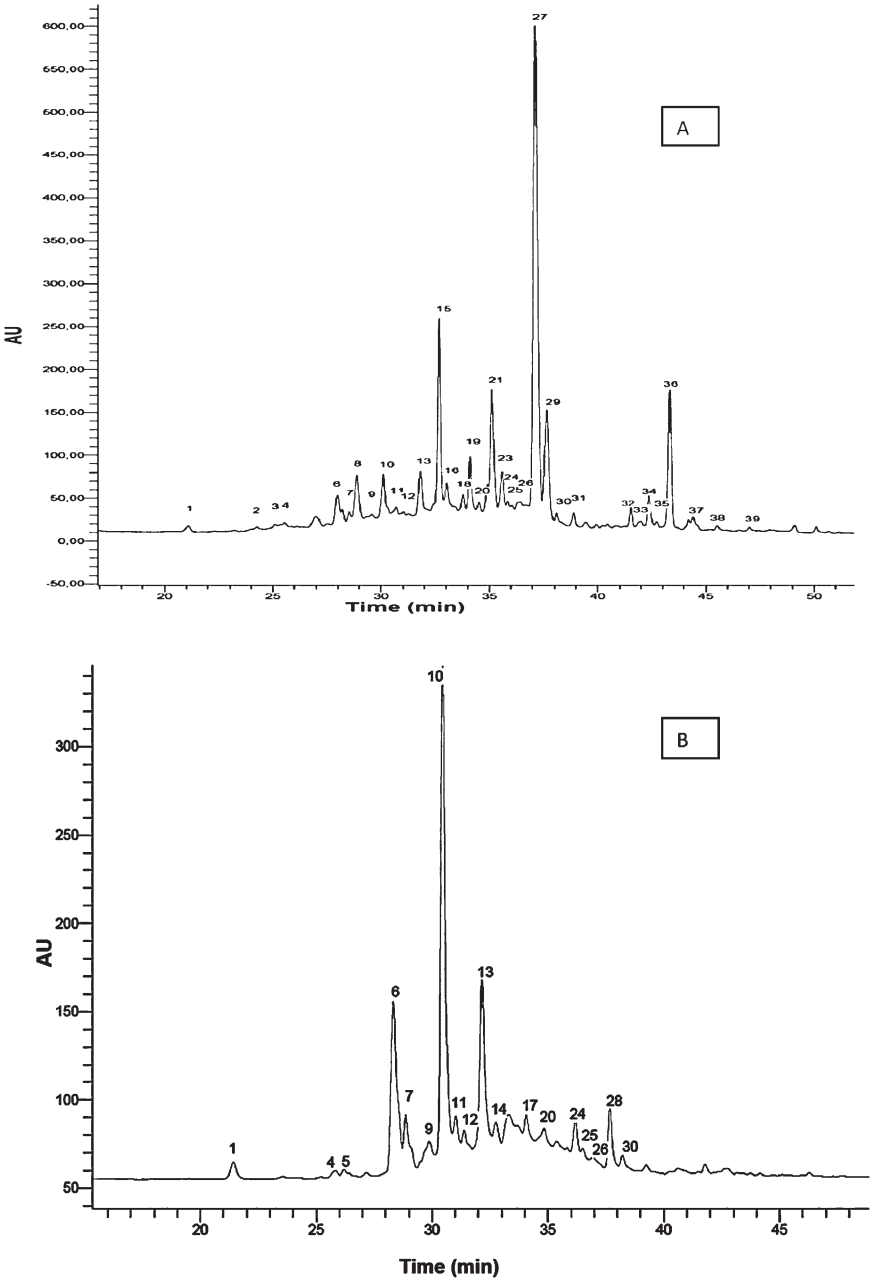

Fig. 1. Chromatograms for strawberry cv. Camarosa. A) HPLC-DAD chromatogram obtained at 280 nm. B) HPLC-FD chromatogram. Peaks numbers refer to Table 1.

lightness ($L^* = 41.5$), hue angle ($h = 36.6$) and chroma ($C^* = 55.4$), thus being less dark and dull red (Table 3). In contrast, 'Camarosa', showed the lowest L^* (33.1), h (29.4) and C^* (42.8) values. 'Camarosa' was the cultivar with the darkest red strawberries and the highest anthocyanin concentration (Table 4). In the case of 'Amiga' there was not a good correlation between its colour values and anthocyanin concentration. 'Amiga' showed intermediate values in the colour parameters studied (Table 3) and registered the highest anthocyanin concentration, together with 'Camarosa' (Table 4). These findings could be explained by the different anthocyanins profile for each cultivar and

Table 1
Maximum wavelength of absorbance and MS of the phenolic compounds detected in the strawberry cultivars studied ('Amiga', 'Camarosa', 'Cangonda', 'Fuentepina' and 'Primoris')

# Peak	Compound	λmax (nm)	m/z $[M]^+$	Cultivars
	Anthocyanins			
22	Cyanidin-3-*O*-glucoside	518, 282	449	AM, CM, CG, FP, PR
27	Pelargonidin-3-*O*-glucoside	504, 430sh, 330sh, 278	433	AM, CM, CG, FP, PR
29	Pelargonidin-3-*O*-rutinoside	504, 428sh, 332sh, 278	579	AM, CM, CG, FP, PR
35	Pelargonidin-3-acetylglucoside	506, 430 h, 332sh, 278	475	AM, CM, CG, PR
			m/z $[M-H]^-$	
	Flavan-3-ols			
1	Proanthocyanidin trimer	278, 234	865	AM, CM, CG, FP, PR
4	Proanthocyanidin dimer	278, 234	577	AM, CM, CG, FP, PR
5	Proanthocyanidin dimer	278, 234	577	AM, CM, CG, FP, PR
6	Proanthocyanidin dimer	278, 234	577	AM, CM, CG, FP, PR
7	Proanthocyanidin trimer	278, 234	865	AM, CM, CG, FP, PR
9	Proanthocyanidin dimer	278, 234	561	AM, CM, CG, FP, PR
10	Catechin	278, 234	289	AM, CM, CG, FP, PR
11	Proanthocyanidin tetramer	278, 234	1153	AM, CM, CG, FP, PR
12	Proanthocyanidin tetramer	278, 234	1153	AM, CM, CG, FP, PR
13	Proanthocyanidin trimer	278, 234	865	AM, CM, CG, FP, PR
14	Proanthocyanidin tetramer	278, 234	1153	AM, CM, CG, FP, PR
17	Proanthocyanidin tetramer	278, 234	1153	AM, CM, CG, FP, PR
20	Proanthocyanidin trimer	278, 234	849	AM, CM, CG, FP, PR
24	Proanthocyanidin tetramer	278, 234	1137	AM, CM, CG, FP, PR
25	Proanthocyanidin tetramer	278, 234	1137	AM, CM, CG, FP, PR
26	Proanthocyanidin dimer	278, 234	561	AM, CM, CG, FP, PR
28	Proanthocyanidin tetramer	278, 234	1137	AM, CM, CG, FP, PR
30	Proanthocyanidin trimer	278, 234	865	AM, CM, CG, FP, PR
	Flavonols			
32	Quercetin-3-glucuronide	356, 300sh, 256	477	AM, CM, CG, FP, PR
37	Kaempferol-3-glucuronide	348, 266	461	AM, CM, CG, FP, PR
38	Kaemperol-3-malonylglucoside	348, 290sh, 266, 234	533	AM, CM, CG, FP, PR
39	Kaempferol-3-acetylglucoside	352, 266	489	AM, PR
	Hydroxycinnamic acid derivates			
15	p-coumaroylhexose	316, 236	325	AM, CM, CG, FP, PR
18	p-coumaroylhexose	316, 236	325	AM, CM, CG, FP, PR
23	Ferulic acid hexose	448, 352sh, 312sh, 248sh	449	AM, CM, CG, FP, PR
36	Cinnamoyl-glucose	283	355 $[M+COO]^-$	AM, CM, CG, FP, PR
	Conjugated forms of ellagic acid			
2	Bis-HHDP-glucoside	234	783	AM, CM, FP
3	Bis-HHDP-glucoside	234	783	AM, CM, CG, FP, PR
8	HHDP-galloyl-glucose	286	633	AM, CM, CG, FP, PR
16	Galloyl-bis-HHDP-glucose	236, 256sh	935	AM, CM, CG, FP, PR
19	Galloyl-bis-HHDP-glucose	236, 256sh	935	AM, CM, CG, FP, PR
21	Sanguiin H6	234, 266sh	934 $[M-2H]^{2-}$	AM, CM, CG, FP, PR
31	Galloyl-bis-HHDP-glucose	236, 256sh	935	CM, CG
33	Ellagic acid deoxyhexoside	372, 250	447	AM, CM, CG, FP, PR
34	Ellagic acid deoxyhexoside	372, 250	447	CM, FP, PR

sh- shoulder AM- amiga; CM- camarosa; CG- candonga; FP- fuentepina; PR- primoris.

Table 2
Concentration of all phenolic compounds identified in nearly ripe and ripe strawberries from the cultivars 'Amiga', 'Camarosa', 'Candonga', 'Fuentepina' and 'Primoris' in 2010 and 2011

	AMIGA				CAMAROSA				CANDONGA				FUENTEPINA				PRIMORIS			
	2010		2011		2010		2011		2010		2011		2010		2011		2010		2011	
	Nearly ripe	Ripe	Nearly ripe	Ripe	Nearly ripe	Ripe	Nearly ripe	Ripe	Nearly ripe	Ripe	Nearly ripe	Ripe	Nearly ripe	Ripe	Nearly ripe	Ripe	Nearly ripe	Ripe	Nearly ripe	Ripe
Anthocyanins	1732	2208	2834	3080	1696	2553	2795	3360	1017	1743	1510	2094	606	639	666	994	1085	1564	1447	1967
Cyanidin-3-*O*-glucoside	25	34	4	18	118	168	161	175	65	115	74	122	5	8	9	16	76	122	85	137
Pelargonidin-3-*O*-glucoside	1524	1938	2571	2783	1320	1976	2209	2647	852	1414	1280	1746	551	576	600	899	970	1373	1298	1739
Pelargonidin-3-O-rutinoside	183	236	255	279	258	401	421	527	100	214	156	216	50	55	57	79	39	69	64	76
Pelargonidin-3-acetylglucoside	–	–	4	–	–	8	4	11	–	–	–	10	–	–	–	–	–	–	–	15
Flavan-3-ols	896	880	904	619	1171	1128	1230	930	1581	1171	1073	808	922	696	855	722	1332	1286	822	844
Catechin	299	298	234	139	395	335	368	231	467	349	323	211	282	203	251	189	501	469	248	249
Proanthocyanidin dimer	262	240	212	149	319	271	315	206	416	310	293	204	248	179	213	178	364	355	200	190
Proanthocyanidin trimer	174	183	273	188	281	322	313	305	353	322	265	254	226	194	237	224	264	264	239	267
Proanthocyanidin tetramer	161	159	185	143	176	200	234	188	345	190	192	139	166	120	154	131	203	198	135	138
Flavonols	221	244	163	163	157	155	195	123	90	144	86	74	71	85	113	128	116	177	89	140
Quercetin-3-glucuronide	134	139	45	48	94	87	113	60	56	96	54	50	35	49	68	81	66	99	44	78
Kaempferol-3-glucuronide	78	94	93	94	45	51	59	48	27	37	24	18	30	31	37	40	35	54	30	40
Kaemperol-3-malonylglucoside	9	8	19	16	18	17	23	15	7	11	8	6	6	5	8	7	15	24	14	17
Kaempferol-3-acetylglucoside	–	3	6	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Hydroxycinamic acid derivates	167	246	353	467	474	578	650	765	670	801	840	997	526	606	513	670	415	558	687	682
p-coumaroylhexose	85	132	183	302	341	433	483	560	472	592	684	878	419	496	407	564	297	420	562	550
Cinnamoyl-glucose	52	77	119	122	101	105	127	153	181	184	131	87	96	99	96	92	100	109	106	107
Ferulic acid hexose	30	37	51	43	32	40	40	52	17	25	25	32	11	11	10	14	18	29	19	25
Conjugated ellagic acids	94	128	142	110	150	177	178	174	127	138	68	56	171	105	170	121	178	188	76	87
Bis HHDP glucoside	1	6	8	23	3	3	5	7	–	2	–	4	–	–	3	2	–	1	3	3
HHDP galloyl glucose	–	5	13	25	–	10	5	14	–	9	–	–	–	2	–	3	–	1	6	4
Galloyl bis HHDP glucose	14	17	19	9	42	42	42	34	43	29	17	10	23	8	23	11	24	25	1	5
Sanguiin H6	58	70	52	22	72	83	88	83	65	70	35	28	128	76	118	81	131	130	47	57
Ellagic acid deoxyhexoside	21	30	49	31	33	39	38	36	19	28	16	14	20	19	26	24	23	31	19	18

Units μg/g DW.

Table 3
Influence of cultivar, ripeness and season on colour parameters (lightness, hue and chroma), firmness, acidity, soluble solid content, ratio and antioxidant capacity (FRAP and DPPH) in strawberries

	L*	h	C	Firmness	Acidity	SSC	Ratio	FRAP	DPPH
Significance[a]									
Cultivar (C)	***	***	***	***	***	***	***	***	*
Ripeness (R)	***	***	***	***	***	***	***	**	ns
Season (S)	***	***	***	**	***	*	***	***	***
C × R	*	*	*	ns	ns	ns	ns	ns	*
C × S	***	***	***	***	***	ns	***	**	ns
R × S	*	ns	***	ns	ns	ns	ns	ns	ns
C × R × S	*	*	*	ns	**	ns	*	ns	ns
Values[b]									
Cultivar									
'Amiga'	36.4 d	33.7 b	48.9 b	8.7 a	0.72 b	8.1 d	11.8 b	249 a	277 b
'Camarosa'	33.1 e	29.4 d	42.8 c	6.1 d	0.93 a	8.4 cd	9.2 c	265 a	310 a
'Candonga'	37.5 c	32.3 c	49.1 b	6.9 c	0.90 a	8.8 b	10.0 c	228 b	265 b
'Fuentepina'	41.5 a	36.6 a	55.4 a	5.7 d	0.64 c	9.5 a	14.8 a	221 b	279 b
'Primoris'	39.4 b	34.0 b	49.4 b	7.6 b	0.73 b	8.7 bc	12.4 b	222 b	280 b
Ripeness									
Nearly ripe	39.2 a	35.7 a	50.7 a	7.7 a	0.83 a	8.4 b	10.7 b	245 a	288
Ripe	36.0 b	30.7 b	47.6 b	6.4 b	0.74 b	9.0 a	12.6 a	229 b	276
Season									
2010	38.1 a	34.7 a	50.8 a	7.3 a	0.84 a	8.6 b	10.7 b	271 a	337 a
2011	37.1 b	31.7 b	47.5 b	6.8 b	0.73 b	8.9 a	12.6 a	203 b	227 b

[a]Level of significance: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, ns- non significant.[b]Average values. Values with different letters are significantly different ($p \leq 0.05$) as determined through LSD test. Firmness (N), acidity (% citric acid), SSC (ºBrix), ratio (SSC/acidity), FRAP and DPPH (μmol TE/g DW).

its individual concentrations. As the Table 2 shows, 'Amiga' cultivar showed a low concentration of cyanidins and a high concentration of pelargonidins.

The pH is another important factor in anthocyanin colour, a low vacuolar pH promoting an orange-red colour in anthocyanins. Thus the pH and the specific anthocyanin profile of 'Amiga' could explain the intermediate colour parameter values in this cultivar.

Strawberry firmness differed strongly among genotypes. 'Amiga' was the firmest cultivar (8.7 N), followed by 'Primoris' (7.6 N) and 'Candonga' (6.9 N), whereas 'Camarosa' (6.1 N) and 'Fuentepina' (5.7 N) cultivars were the softest ones.

'Fuentepina' was the richest genotype in soluble solids (9.5 ºBrix) while 'Camarosa' and 'Amiga' were the cultivars with the lowest soluble solid content (Table 3). In our study the SSC of the 'Camarosa' cultivar (8.4 ºBrix) was slightly higher than the results reported by others [9, 10] (7.2 and 7.1 ºBrix, respectively). These differences could be associated with environmental conditions as well as growing practices.

'Camarosa' and 'Candonga' showed the highest acidity value (0.93 and 0.90% citric acid, respectively) and 'Fuentepina' reported the lowest (1.5 fold difference approx.). The results showed that the most important commercial cultivars in Spain ('Camarosa' and 'Candonga') recorded the highest acidity values. Acidity in these cultivars is above the maximum titratable acidity for acceptable flavour quality of strawberries (0.8%) [28]. It should be noted that the strawberries from the new cultivars ('Fuentepina' and 'Amiga') registered lower acidity, a quality attribute that is being strongly demanded by consumers.

The SSC/acidity ratio also showed significant differences between cultivars. 'Fuentepina' had the highest SSC/acidity ratio (14.8), followed by 'Primoris' (12.4) and 'Amiga' (11.8), whereas the lowest values were measured

Table 4
Influence of cultivar, ripeness and season on anthocyanins, flavan-3-ols, flavonols, hydroxycinnamic acid derivates, conjugated forms of ellagic acid and total phenols in strawberries

	Anthocyanins	Flavan-3-ols	Flavonols	Hydroxycinnamic acid derivates	Conjugated ellagic acids	Total phenols
Significance[a]						
Cultivar (C)	***	***	***	***	***	***
Ripeness (R)	***	***	ns	***	ns	***
Season (S)	***	***	*	***	**	***
C × R	ns	*	*	ns	ns	ns
C × S	*	***	***	*	***	***
R × S	ns	ns	*	ns	ns	ns
C × R × S	ns	ns	ns	ns	ns	ns
Values[b]						
Cultivar						
'Amiga'	2463 a	824.6 b	195.1 a	333.4 c	118.4 bc	3934 b
'Camarosa'	2599 a	1115 a	157.6 b	591.6 b	170.0 a	4634 a
'Candonga'	1590 b	1159 a	98.4 d	844.0 a	95.5 c	3784 bc
'Fuentepina'	724 c	798.7 b	99.3 d	791.7 a	141.4 ab	2555 d
'Primoris'	1513 b	1071 a	129.6 c	639.8 b	131.7 b	3486 c
Ripeness						
Nearly ripe	1538 b	1079 a	129.4	573.2 b	135.3	3455 b
Ripe	2017 a	908 b	142.6	706.9 a	127.4	3903 a
Season						
2010	1483 b	1106 a	145.3 a	535.2 b	144.7 a	3942 a
2011	2072 a	880.7 b	126.6 b	744.9 a	118.0 b	3415 b

[a]Level of significance: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, ns- non significant.[b]Average values. Values with different letters are significantly different ($p \leq 0.05$) as determined through LSD test. Units: μg/g.

for the cultivars 'Camarosa' (9.2) and 'Candonga' (10.0). The low SSC/acidity ratio and consequent poor flavour of strawberries from 'Camarosa' and 'Candonga', the current commercial cultivars, could explain why they are rejected by consumers since sugar to organic acid ratio is a major parameter of strawberry taste [29].

'Camarosa' and 'Amiga' were the cultivars with the highest antioxidant capacity (Table 3); 'Fuentepina' showed no significant differences in its antioxidant capacity (DPPH assay) compared with the remaining cultivars studied (except for 'Camarosa'). Polyphenols and vitamin C are primarily responsible for the antioxidant capacity in strawberries [2, 6]. 'Fuentepina' had the lowest polyphenol concentration, which was not the case, however, of its antioxidant capacity, which suggests that this cultivar could be richer in other antioxidant compounds, such as vitamin C.

The polyphenolic composition of strawberries varies significantly with each genotype [8, 9, 25]. The total phenolic content (TPC), calculated as the sum of individual compounds, in the strawberry cultivars studied varied from 2555 to 4634 μg/g DW (Fig. 2). 'Camarosa' contained significantly the highest concentration of phenolic compounds while 'Fuentepina' had the lowest (Fig. 2).

Anthocyanins were the most abundant class of polyphenols for all the strawberry cultivars studied except 'Fuentepina' (in this cultivar the most abundant class of polyphenols were flavan-3-ols) (Fig. 2). Their contribution to the TPC ranged from 28% ('Fuentepina') to 63% ('Camarosa'). 'Fuentepina' had the lowest total anthocyanin concentration and 'Camarosa' and 'Amiga' the highest, with 'Candonga' and 'Primoris' showing intermediate concentrations of these pigments (Table 4). Pg-3-gluc, was the predominant anthocyanin in all the strawberry cultivars studied (Table 2), as previously reported by other authors [2, 8, 25, 26, 30], followed by Pg-3-rut and Cy-3-gluc. 'Primoris' was the only cultivar not following that rule, showing higher Cy-3-gluc levels than Pg-3-rut. Pg-3-ac was a minor pigment with concentrations below 1% of the total anthocyanin content in all the cultivars. Low levels of

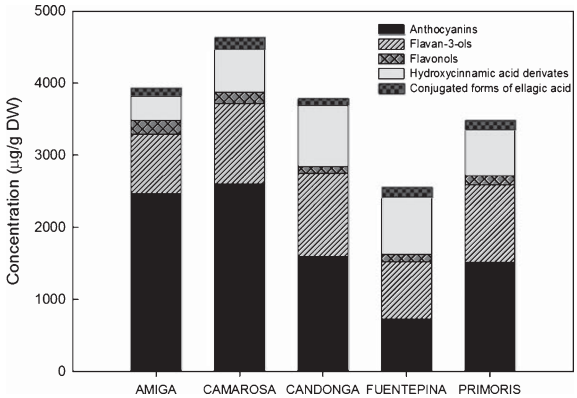

Fig. 2. Comparative of concentrations (µg/g DW) of total phenolic compounds (anthocyanins, flavan-3-ols, flavonols, hydroxycinnamic acid derivates and conjugated forms of ellagic acid) in different strawberry cultivars. Data are an average of years (2010 and 2011) and stages of ripening (nearly ripe and ripe).

acylated anthocyanins were also previously reported in Spanish strawberry cultivars [25, 30]. The percentages found in 'Camarosa' are similar to those described by other authors [25, 30, 31].

The flavan-3-ols group had the highest contribution to the TPC in 'Fuentepina' cultivar (31.3%) and the second highest for the rest of the cultivars (Fig. 2). However, quantitatively, 'Fuentepina' was not the cultivar with the highest concentration of this type of compounds (Table 4). The total proanthocyanidin concentration (as sum of dimers, trimers and tetramers of proanthocyanidins) (567–821 µg/g DW) was in line with the levels found by de Pascual-Teresa et al. [32]. Catechin was the most abundant flavan-3-ol for all the cultivars analysed in this study.

Hydroxycinnamic acid derivatives were the third most abundant group of phenolic compounds in all the strawberry cultivars studied except 'Fuentepina' (second most abundant) (Fig. 2), contributing to the TPC of 8.5% in 'Amiga' up 31% in 'Fuentepina'. p-coumaroil hexose was the predominant hydroxicinnamic acid derivative. The concentrations found for these compounds were in accordance with previous studies in strawberries [8, 33].

The flavonol content varied from 98-99 µg/g DW in 'Candonga' and 'Fuentepina' to 195 µg/g DW in 'Amiga'. This group of compounds comprised between 2.6–5% of the total content of phenolics. Quercetin-3-O-glucuronide was the main flavonol in the samples studied, which was in agreement with the findings of other authors [25, 34].

The total conjugated forms of ellagic acid concentrations in the strawberry cultivars were between 96 µg/g DW ('Candonga') and 170 µg/g DW ('Camarosa'). This group of compounds represented between 2.5% and 5.5% of the TPC in the cultivars studied. Sanguiin H6 was the predominant ellagitanin in all the cultivars. The concentration of other main compounds such as ellagic acid deoxyhexoside was low (19–37 µg/g DW), similar to the values reported by other authors [27].

3.3. Effect of ripening

Loss of firmness increases during strawberry ripening due to the depolymerisation and solubilisation of pectins [35]. In keeping with that, the nearly ripe strawberries from all the cultivars were significantly firmer (Table 3). It should be highlighted that this loss of firmness for the cultivars characterized for the first time in this work ('Fuentepina' and 'Amiga') was less severe (14%) than for the remaining cultivars, in particular 'Camarosa' and 'Primoris' (21% approx.).

Regarding colour, all genotypes showed that ripe fruits were significantly redder (lower h angle values) duller (lower C*) and darker (lower L*) than nearly ripe fruits (Tables 3 and 5), which is consistent with the data reported by other authors [36–38]. The soluble solid concentration increased with ripeness, while the acidity decreased (Table 3), which is consistent with the previously published data [8, 14, 15, 36].

Measured by FRAP assay, the fruit's antioxidant capacity decreased during ripening (Table 3), which is consistent with previous findings [15]. However when this was measured by the DPPH method no significant differences were

Table 5
Colour parameters (Lightness (L*), chroma (C*) and hue(h)), firmness, acidity, soluble solid content (SSC), ratio (SSC/acidity) and antioxidant capacity (FRAP and DPPH) in nearly ripe and ripe strawberries from the cultivars 'Amiga', 'Camarosa', 'Candonga', 'Fuentepina' and 'Primoris' in 2010 and 2011

		L*	h	C*	Firmness	Acidity	SSC	Ratio	FRAP	DPPH
2010										
AMIGA	Nearly ripe	36.3 ± 0.2	35.8 ± 0.1	49.1 ± 1.2	10.5 ± 0.8	0.84 ± 0.01	7.5 ± 0.4	8.9 ± 0.4	292 ± 4	306 ± 33
	Ripe	33.4 ± 0.7	32.8 ± 2.0	46.5 ± 1.8	9.0 ± 0.1	0.81 ± 0.05	8.4 ± 0.7	10.3 ± 1.0	279 ± 26	357 ± 19
CAMAROSA	Nearly ripe	36.9 ± 1.3	35.3 ± 1.1	48.3 ± 0.2	6.8 ± 0.2	0.89 ± 0.01	7.9 ± 0.4	9.2 ± 0.1	289 ± 4	383 ± 28
	Ripe	32.5 ± 1.2	28.1 ± 1.3	43.9 ± 0.6	5.4 ± 0.6	0.93 ± 0.05	8.4 ± 0.2	9.1 ± 0.7	279 ± 28	326 ± 22
CANDONGA	Nearly ripe	39.4 ± 1.8	35.3 ± 0.6	51.6 ± 1.3	7.9 ± 0.5	1.06 ± 0.08	8.5 ± 0.4	8.0 ± 0.9	272 ± 25	289 ± 88
	Ripe	37.3 ± 0.3	32.4 ± 1.6	49.9 ± 2.4	7.0 ± 0.2	0.89 ± 0.08	9.5 ± 0.5	10.7 ± 0.6	258 ± 20	328 ± 35
FUENTEPINA	Nearly ripe	42.4 ± 0.6	37.3 ± 0.8	57.8 ± 0.6	5.3 ± 0.2	0.63 ± 0.04	9.0 ± 0.1	14.2 ± 0.6	258 ± 17	364 ± 12
	Ripe	41.5 ± 1.1	36.3 ± 0.9	57.0 ± 0.8	4.4 ± 0.5	0.61 ± 0.03	9.4 ± 0.4	15.4 ± 0.9	234 ± 12	315 ± 21
PRIMORIS	Nearly ripe	42.1 ± 1.4	39.0 ± 1.6	53.3 ± 0.1	9.6 ± 2.4	0.96 ± 0.15	8.3 ± 0.1	8.7 ± 1.3	281 ± 53	360 ± 18
	Ripe	39.5 ± 1.1	35.0 ± 1.4	50.5 ± 2.0	6.9 ± 0.1	0.73 ± 0.07	8.9 ± 0.3	12.4 ± 1.7	270 ± 57	344 ± 25
2011										
AMIGA	Nearly ripe	41.0 ± 1.0	37.2 ± 0.3	53.8 ± 0.4	8.1 ± 0.9	0.66 ± 0.05	7.8 ± 0.0	11.8 ± 0.8	224 ± 5	229 ± 34
	Ripe	34.9 ± 0.4	28.9 ± 0.3	46.3 ± 0.6	7.0 ± 0.1	0.56 ± 0.08	8.9 ± 0.4	16.2 ± 1.9	201 ± 5	216 ± 24
CAMAROSA	Nearly ripe	33.2 ± 1.6	30.4 ± 1.5	40.7 ± 0.9	6.8 ± 0.1	1.06 ± 0.01	8.1 ± 0.1	7.6 ± 0.1	274 ± 1	287 ± 10
	Ripe	30.0 ± 0.4	23.8 ± 0.9	38.5 ± 0.9	5.4 ± 0.3	0.85 ± 0.02	9.2 ± 0.5	10.8 ± 0.6	220 ± 8	243 ± 9
CANDONGA	Nearly ripe	39.1 ± 0.4	33.6 ± 1.3	50.2 ± 1.3	7.2 ± 0.3	0.88 ± 0.09	8.5 ± 0.5	9.7 ± 0.4	195 ± 14	231 ± 3
	Ripe	34.3 ± 0.9	27.9 ± 1.2	44.8 ± 0.8	5.8 ± 0.4	0.78 ± 0.05	8.9 ± 0.4	11.5 ± 1.3	186 ± 25	212 ± 15
FUENTEPINA	Nearly ripe	43.0 ± 0.8	40.0 ± 1.1	54.4 ± 0.5	7.0 ± 0.8	0.67 ± 0.05	9.9 ± 0.4	14.8 ± 1.7	201 ± 1.5	230 ± 5
	Ripe	39.1 ± 0.3	32.9 ± 1.1	52.2 ± 1.1	6.1 ± 0.4	0.65 ± 0.03	9.7 ± 0.7	15.0 ± 1.9	193 ± 21	205 ± 19
PRIMORIS	Nearly ripe	39.0 ± 0.4	33.0 ± 0.2	47.6 ± 0.2	7.5 ± 0.1	0.63 ± 0.04	8.8 ± 0.1	13.9 ± 1.1	167 ± 11	203 ± 16
	Ripe	37.1 ± 1.1	29.0 ± 1.2	46.1 ± 1.2	6.6 ± 0.4	0.61 ± 0.04	8.9 ± 0.4	14.7 ± 0.9	172 ± 14	212 ± 15

Average values ± standard deviation. Firmness (N), acidity (% citric acid), SSC (ºBrix), ratio (SSC/acidity), FRAP and DPPH (μmol TE/g DW).

found between both ripening stages. These differences could be linked to the different chemical reactions that the methods are based on.

The total anthocyanin concentration significantly increased with increasing ripeness in all the cultivars (Table 4) which is consistent with previous findings in strawberries [8, 14, 15]. The total anthocyanins concentration in ripe strawberries was 1.16–1.52 fold higher than in nearly ripe ones. However, the anthocyanin profile of each cultivar showed no differences during ripening. This indicates that there is a characteristic anthocyanin profile for each cultivar as previously described by Aaby et al. [8]. The concentration of flavan-3-ols decreased with increasing ripeness in all the cultivars except 'Primoris' in 2011 (Table 2). Aaby et al. [8] found the catechin concentration decreased in cv. Blink during ripening but was not altered in other strawberry cultivars. Fait et al. [39] found procyanidins accumulated mainly in the early stages of receptacle development.

In general, no significant differences in flavonols content were found during ripening (Table 4). The changes during ripening in the total flavonol contents were ambiguous, as previously reported [8]. 'Camarosa' showed a higher flavonol concentration in nearly ripe strawberries than in ripe ones in 2011, while 'Amiga', 'Candonga', and 'Primoris' showed the opposite trend in 2010. The other cultivars had a similar flavonol concentration in both stages of ripening (Table 2). Fait et al. [39] found that derivates of the flavonols, kaempferol and quercitin were present at all stages with varying substitutions. They established that the glucuronide derivates of flavonols were only detected in the receptacle from the medium green to the turning stage. In contrast, kaempferol-malonylhexose was detected predominantly in the achenes mainly in late stages of development, while kaempferol-acetylhexose was detected only at the mature red stage of the receptacle. In our study, all the derivates of flavonols were present in both stages and the differences found between cultivars were due to changes in the quercetin-3-*O*-glucuronide concentration.

In general, the concentration of hydroxycinnamic acid derivates was significantly higher in ripe strawberries than in nearly ripe ones, which is in line with previous results [8, 33, 40]. The accumulation of phenolic acids has been associated with an enhanced phenolic compound concentration since these compounds serve as precursors for the various branches of the phenylpropanoid pathway and the metabolism of phenolic compounds [39]. The concentration of conjugated forms of ellagic acid was constant during ripening. In this way, similar concentrations of ellagic acid and ellagitannins during ripening were previously described [8, 40]. However, other authors have described a decrease in the concentration of ellagic acid in fully-ripe strawberries [14, 41].

This study found that total phenol concentration increased during ripening. This can be associated with a large increase in anthocyanins and, to a lesser extent, with an increase in hydroxycinnamic acid derivates. Montero et al. [42] demonstrated a sharp decrease in the total phenol concentration occurred during ripening (first stages of fruit development) after the fruit set and then, at the final stage of ripening, a slight increase in the concentration of these compounds was observed due to an accumulation of anthocyanins [43]. Those results demonstrate a two-phase pattern of expression of the phenylpropanoid pathway genes: they are active in the early green stages, less so in the white phase, and increasingly active again during the turning stage of strawberry fruit development. Fait et al. [39] found the accumulation of different classes of secondary metabolites varied during the development and ripening of strawberries: tannins during early development, and phenolic acids, flavanols and anthocyanins during ripening.

3.4. Seasonal effect

The environment also plays an important role in determining fruit composition and therefore, in its quality. All the evaluated parameters were significantly influenced by the year of harvest (Tables 3 and 4). However, not all the cultivars were affected by environmental conditions in either the same way or to the same extent (Tables 2 and 5).

Generally, the strawberries from 'Amiga', 'Candonga' and 'Primoris' cultivars were firmer in 2010 than in 2011. 'Fuentepina' fruits showed the opposite trend, while 'Camarosa' strawberries showed little difference between years. Regarding acidity, significantly higher concentrations were observed in 2010 for 'Amiga', 'Candonga' and 'Primoris' cultivars, while the fruits from 'Camarosa' and 'Fuentepina' cultivars showed no significant differences. The SSC and ratio showed higher values for strawberries harvested in 2011 than those harvested in 2010.

The antioxidant activity (FRAP and DPPH) of the fruits was notably higher in 2010.

Most of the phenolic compounds were influenced by the environmental conditions (Table 4). A significant interaction effect between cultivar and season was observed in most of the phenolic compounds, indicating the need to evaluate the new cultivars over several years.

The strawberries harvested in 2011 showed higher concentrations of anthocyanins and hydroxycinnamic acid derivates. In contrast, the content of flavonols, flavan-3-ols, ellagic acid conjugates and total phenols was higher in strawberries grown in 2010.

To identify a possible influence of environmental conditions on the phenolic composition of strawberries, data about temperature, solar radiation, humidity and total precipitacion were used from the period of time between 25

Table 6

Temperatures (mean, maximum and minimum), solar radiation, humidity and accumulated rainfall averages for 25 and 5 days previous to harvest for 2010 and 2011. Data are from the closest meteorological station located in Moguer (37º 14' 29" N, 06º 48' 03" W)

	25 DPH		5 DPH	
	2010	2011	2010	2011
T mean (ºC)	17.8	17.3	16.0	20.5
T max (ºC)	23.4	23.0	20.9	29.3
T min (ºC)	12.4	12.2	10.1	12.8
Solar radiation (MJ/m^2 day)	24.4	20.4	23.9	27.7
Humidity mean (%)	70.0	77.2	73.3	62.1
Accumulated Rainfall (mm)	68.4	82.5	2.6	0.2

DPH – days previous to harvest.

and 5 days previous to harvest (DPH) (Table 6). These data are based on the duration of different phenological stages during fruit maturation (25 days) and the final phenological stage of maturation in the region (5 days).

The main differences observed between both years were for accumulated rainfall during 25 DPH and for the temperature and solar radiation registered 5 DPH (Table 6). The accumulated rainfall during 25 DPH in 2011 was 20% higher than in 2010. Additionally, in 2011 the temperatures and solar radiation recorded during the 5 DPH were higher (28% and 16%, respectively). Regarding phenolic composition, the content of flavonols, flavan-3-ols and conjugated forms of ellagic acid were higher in strawberries harvested in 2010, when the accumulated rainfall was lower. Pineli et al. [44] found rain influenced negatively the content of catechins, flavonols and total ellagic acid but this was not the case for anthocyanins. Phenolic compounds, together with vitamin C, are primarily responsible for the antioxidant power of strawberries [2, 6]. Thus, the high antioxidant activity measured in the strawberries harvested in 2010 is consistent with the higher polyphenol concentration found for that season.

In contrast, higher levels of anthocyanins and hydroxycinnamic acid derivates were measured in the strawberries harvested in 2011. Using a PLS regression model, Pineli et al. [44] found a direct relationship between temperature and hours of sunlight in the last 5 days before harvest and the content of anthyocyanins.

4. Conclusions

'Fuentepina' fruits were lighter red and showed the lowest values in health-promoting compounds. However, the most outstanding feature of this cultivar was its excellent flavour as a result of a very high sugar/acid ratio. 'Amiga' had high anthocyanins, polyphenol concentrations and antioxidant capacity. Moreover, this was the firmest variety, a highly valued characteristic for postharvest preservation because softening is a major factor limiting the storage and shelf-life of strawberries. Additionally, 'Amiga' strawberries not only stand out for their good qualities, such as low acidity and high firmness, but also for their high content of health-promoting compounds. These quality attributes make this cultivar a good option for production and commercialisation. This new cultivar had qualities that were superior to those of 'Camarosa' cultivar, which registered high acidity and low firmness. Therefore 'Amiga' could be a good alternative to 'Camarosa' and a strong competitor to 'Candonga'.

Ripeness and season had a significant effect on the quality parameters and on the content of health promoting compounds of strawberry fruits.

Ripe strawberries showed lower acidity and firmness, and higher SSC, anthocyanin and total phenol concentrations than nearly ripe strawberries.

Moreover, this study also demonstrated year-to-year variability in the quality attribute parameters and health-promoting compounds of each cultivar; thus, accurately assessing the nutritional quality of fruit from new genotypes requires longer periods of evaluation.

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