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Characterization of Carotenoid High-Producing *Capsicum annuum* Cultivars Selected for Paprika Production

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Twelve selected pepper (*Capsicum annuum* L.) cultivars, bred for mechanical harvesting (grouped ripeness) and adaptation to different cultivation cycles (short to long), have been characterized by their carotenoid pigment content and composition with the aim of producing high-quality paprika. A detailed analysis of the carotenogenesis was performed throughout the ripening process, but with special emphasis on the ripe stage, with the aim of selecting the best cultivar for paprika production. The MA1 cultivar (with grouped ripeness and very short cultivation cycle) showed the highest carotenoid content (12697.58 mg/kg dwt), followed by DN5 and RN2 cultivars with 11086.88 and 10393.29 mg/kg dwt, respectively. Most of the cultivars (MA3, RN1, LR2, LR7, DN3, DR6, Datler, and Mulato) showed a total carotenoid content in the range of 7000−9700 mg/kg dwt. In general, chlorophyll-retaining character was related to high carotenoid content (cultivars DN3, DN5, MA3, Mulato, RN1, and RN2). The general trend of the cultivation cycle was that the shorter the cycle, the higher the total carotenoid content (as exemplified by the cultivar MA1). The lowest total carotenoid content was found for the RR1 cultivar (4856.77 mg/kg dwt), which showed the longest cultivation cycle. Carotenogenic capacity of the cultivars has been discussed relative to total carotenoid content and the R/Y and Caps/Zeax ratios, the main quality traits for breeding cultivars for production of high-quality paprika. The cultivar MA1, with the highest total carotenoid content, high R/Y (2.11) ratio, and highest Caps/Zeax (9.85) ratio, was found to be the most suitable cultivar for paprika production in terms of carotenoid pigment biosynthesis capacity. Moreover, this cultivar has a short cultivation cycle and grouped ripeness, which are both important characteristics for a proper application of mechanical harvesting. The potential improvement of other varieties is also discussed.

KEYWORDS: ***Capsicum annuum*; carotenoid; breeding cultivars; paprika; mechanical harvesting**

INTRODUCTION

The mature fruits of the red pepper (*Capsicum annuum* L.) are considered one of the richest sources of natural pigments. This is the main reason pepper fruits have been traditionally used as food colorant in the form of paprika (ground powder) and, more recently, as oleoresins. The intense and characteristic red color of *Capsicum* fruits is due to carotenoid pigments that are synthesized massively during fruit ripening. Some of them (capsanthin, capsorubin, and capsanthin 5,6-epoxide) are considered to belong almost exclusively to the genus *Capsicum* (*1*). All the carotenoid pigments present in the pepper are C_{40} isoprenoids containing 9 conjugated double bonds in the central polyenic chain, with different end groups (β, ϵ, κ, 3-hydroxy-5,6-epoxide) which change the chromophore properties of each pigment, allowing them to be classified in two isochromic families: red (R) and yellow (Y). The red fraction contains mainly capsanthin, capsanthin-5,6-epoxide, and capsorubin (together with other minor carotenoids), while the yellow fraction comprises the rest of the pigments (mainly zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene, and cucurbitaxanthin A), which are biosynthetic precursors of the red fraction (*2*).

Due to the health-beneficial properties related to carotenoid-rich diets (antioxidant, free-radical scavenging, cancer-risk reducing, immune-response enhancing, and provitamin A providing) (*3*−*5*), there is an increasing interest in large-scale production of carotenoids from alternative or improved natural sources (for instance Tagetes, dunaliella, haematococcus, etc.). Metabolic engineering of carotenoid biosynthesis has been achieved recently in various plants (rice, canola seeds, tomato fruits, etc.) [reviewed by Giuliano et al. (*6*)], thanks to the cloning of genes that codify for carotenogenic enzymes (*7*−*11*). This has led to a better understanding of carotenoid

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Table 1. Characterization of the Selected Cultivars with Respect to Cultivation Cycle, Degree of Fruit Grouping during Ripening, Fruit Shape, and Chlorophyll Retention in the Ripe Fruit

cultivar	cultivation cycle	ripeness	fruit shape	chlorophyll-retaining character
MA1	very short	grouped	elongated	−
MA3	very short	grouped	round	+
LR2	medium	half-grouped	elongated	−
LR7	medium	half-grouped	elongated	−
DN3	medium	half-grouped	elongated	+
DN5	medium	half-grouped	elongated	+
DR6	medium	half-grouped	elongated	−
Datler	medium	half-grouped	elongated	−
Mulato	medium	half-grouped	elongated	+
RR1	long	echeloned	round	−
RN1	long	echeloned	round	+
RN2	long	echeloned	round	+

biosynthesis and regulation, enabling traditional plant-breeding to be carried out on a more solid basis.

Ever since the pepper was introduced into Europe and spread to the rest of the world, growers have been selecting and breeding different pepper cultivars for the properties and characteristics that were most interesting for different purposes. The result is a great number of very different cultivars showing a wide range of morphological and organoleptic characteristics, including color and pungency, which determine their use. In the case of *Capsicum* there are about 59 000 accessions located in more than 140 germplasm banks (*12*), representing an invaluable source of potential genetic variation that can be used in plant-breeding. The most highly valued characteristic is a high content in carotenoids, as ultimately the commercial value of paprika is based on its coloring capacity, which depends directly on relative pigment richness. New trends in the paprika-producing industry have aroused interest in new, more-competitive cultivars. In previous works (*2*), we selected red-to-yellow isochromic fraction ratios (R/Y) and the capsanthin-to-zeaxanthin ratio (Caps/Zeax) as the most-useful and most-appropriate indexes, together with the total carotenoid content (TCC), for breeding and selecting carotenoid high-producing varieties for paprika. In the present study, 12 selected pepper cultivars, bred for paprika production and adaptation to different cultivation cycles and mechanical harvesting (*13*), have been characterized by their carotenoid pigment content and composition. Cultivars with grouped fruit ripening are suitable for mechanical harvesting, whereas cultivars with short and medium cultivation cycles are suitable for cultivation in cooler areas, avoiding the undesirable and harmful action of the TSWV (tomato spotted wilt virus).

MATERIALS AND METHODS

Plant Material. Fruits of the pepper (*Capsicum annuum* L.) of selected cultivars (MA1, MA3, RN1, RN2, LR2, LR7, DN3, DN5, RR1, DR6, Datler, and Mulato) were used for the present study. Plants were grown in open fields at the CIDA (La Alberca, Murcia, Spain), with a plantation density of 50 000 plants/ha and under drip irrigation. Cultivars presented genetic variability in terms of fruit shape, cultivation cycle length, degree of fruit grouping, and chlorophyll retention in the ripe fruit, as summarized in **Table 1**. Five to ten fruits at different ripening stages were harvested every 15 days during the growing period of 75 days. Fruits were devoid of peduncles and seeds, cut into small pieces, lyophilized, and kept at −30 °C until analysis.

Pigment Extraction. A known weight of lyophilized sample equivalent to 2−10 g of fresh fruit (depending on the degree of ripeness) was reconstituted with water during 30 min, and subsequently extracted with acetone, by using a homogenizer Ultraturrax Y25 (Janke Kunkel Ika-LabortechniK). Extraction was repeated until the complete exhaustion of color (usually 4−5 extractions were enough). All extracts were pooled in a separator and shaken with diethyl ether. A sufficient quantity of 10% NaCl was added at the end to aid in the separation of the phases. The organic phase was saponified with 40 mL of 20% KOH−methanol during 1 h at room temperature. After addition of water, the pigments were subsequently extracted with diethyl ether, evaporated in a rotary evaporator, and taken up to 25 mL of acetone. A 1-mL aliquot of this solution was centrifuged at 12 000 rpm and stored at −30 °C until analyzed. Losses occurring during the process were monitored with use of *all-trans*-β-apo-8′-carotenal as internal standard; 1−5 mL (depending on the expected total carotenoid content) of 100 μg/mL internal standard stock solution was added to the sample at the start of the extraction process. All analyses were carried out in quadruplicate.

High-Performance Liquid Chromatography. HPLC analyses were performed with a Waters 600E quaternary pump equipped with a diode array detector (PDA 996, Waters) and controlled with a Millennium data acquisition station. The HPLC system was equipped with a reverse-phase Spherisorb ODS-2 (5 μm, 0.46 cm × 25 cm) column (Teknokroma, Barcelona, Spain). A precolumn (1 cm × 4 mm i.d.) of the same material was fitted to protect the main column. Samples were cleaned previous to injection by using a benchtop centrifuge model Micro-Centaur (MSE Scientific Instruments, Sussex, England).

HPLC Separation and Quantification of Carotenoids. Separation and quantification of the carotenoid pigments was carried out following a method previously developed by the authors (*14*). The method uses a C-18 reverse-Phase column and a binary gradient elution system of acetone−H_2O as follows: Initially 75% acetone is maintained for 5 min, changing linearly to 95% in 5 min and kept during 7 min. The flow rate was 1.5 mL/min, the sample injection volume was 5 μL, and spectrophotometric detection was performed at 450 nm. *all-trans*-β-Apo-8′-carotenal was used as internal standard for calibration and quantification. Response factors relative to the internal standard were calculated for each individual pigment by performing calibration plots (peak area ratio versus concentration ratio) in the presence of a known amount of internal standard. In the case of minor carotenoids (epoxides and *Z*-isomers), for which enough standard was available, quantification was performed by using the response factor of the carotenoid more closely related in structure and chromophore, e.g. capsanthin 5,6-epoxide was quantified as capsanthin. A stock solution of known concentration for each individual carotenoid was prepared by using the absorption coefficients avilable in the literature (*15*). Separation and quantification of zeaxanthin and lutein was achieved by using the method of Juhler and Cox (*16*).

Pigment Identification. These have been described in detail in previous publications (*14*), and consist of the following: separation and isolation of the pigments by TLC and co-chromatography with purified pigments; acquisition of UV−visible spectra in different solvents and comparison with the values reported in the literature; as well as chemical derivatization and microscale tests for the examination of 5,6-epoxide, hydroxyl, and carbonyl groups. Carbonyl and hydroxyl groups were also investigated by FT-IR spectroscopy.

RESULTS AND DISCUSSION

Changes in the Carotenoid Profile during Fruit Development and Ripening. Table 2 shows changes in the individual carotenoid content during fruit ripening for the 12 studied cultivars. According to the results, carotenoid profile changes during fruit development and ripening follow the carotenogenic pathway characteristic of the *Capsicum* genus, with no detection of either unusual or exclusive pigments in any cultivar. Unripe green fruits presented the typical carotenoid pattern for chloroplast-containing tissues, with lutein as the major pigment (45−55%), followed by violaxanthin (15−20%), β-carotene (15−20%), neoxanthin (12−15%), and antheraxanthin (4−6%). This was the general trend for the carotenoid composition up to day 30, although in some cultivars (Datler, Mulato, DR6, LR7, LR2,

Table 2. Changes in the Individual Carotenoid Composition (mg/kg dwt) during Fruit Ripening of Selected Cultivars for Paprika Production (MA1, MA3, LR2, LR7, RN1, RN2, RR1, DN3, DN5, DR6, Mulato, and Datler)[a]

	ripening stage (days)					ripening stage (days)				
pigment	15	30	45	60	75	15	30	45	60	75
			cv MA1					cv MA3		
neoxanthin	31.38	8.72	0.00	0.00	0.00	32.76	47.31	0.00	0.00	0.00
capsorubin	0.00	13.99	66.66	277.81	322.54	0.00	7.20	196.28	255.19	288.77
violaxanthin	49.55	13.51	97.27	194.35	169.71	37.07	53.48	87.41	272.82	244.02
caps 5,6-epoxide	0.00	24.95	109.18	268.48	341.40	0.00	17.12	191.28	231.05	296.72
capsanthin	0.00	281.24	3711.36	7994.43	7949.68	0.00	187.56	2587.02	3628.77	4141.03
antheraxanthin	5.31	0.00	0.00	785.25	860.60	3.94	20.71	267.18	412.30	459.96
cucurbitaxanthin A	0.00	43.94	426.37	916.83	919.75	0.00	22.64	341.59	693.50	665.13
zeaxanthin	0.00	30.96	635.89	953.79	806.78	0.00	23.29	320.67	667.28	454.31
lutein	96.83	24.96	12.63	0.00	0.00	110.83	33.05	14.92	0.00	0.00
β-cryptoxanthin	0.00	20.21	470.08	472.73	607.61	0.00	21.34	475.71	959.77	475.59
β-carotene	25.56	31.93	454.03	510.99	719.51	36.58	31.27	423.08	736.69	539.85
			cv LR2					cv LR7		
neoxanthin	44.85	34.71	4.83	0.00	0.00	21.13	27.36	7.91	0.00	0.00
capsorubin	0.00	37.33	66.04	153.50	232.91	0.00	3.26	90.22	162.80	197.32
violaxanthin	68.39	43.62	39.54	60.89	217.85	28.29	23.95	71.15	176.40	209.01
caps 5,6-epoxide	0.00	24.49	74.51	141.23	215.96	0.00	8.45	107.00	160.85	178.83
capsanthin	0.00	213.91	1594.86	2715.06	4435.35	0.00	76.83	2455.14	3569.31	4142.19
antheraxanthin	5.66	25.12	179.67	328.59	474.77	3.79	8.54	302.13	371.18	452.95
cucurbitaxanthin A	0.00	26.89	215.56	429.47	606.45	0.00	8.16	352.73	515.31	591.30
zeaxanthin	0.00	87.11	300.36	358.45	635.90	0.00	36.41	476.61	559.09	562.93
lutein	140.47	40.05	32.77	0.00	0.00	93.09	21.94	21.29	0.00	0.00
β-cryptoxanthin	0.00	14.04	147.99	285.20	392.63	0.00	3.51	226.48	315.50	304.44
β-carotene	49.78	41.50	112.17	199.72	245.20	25.12	14.96	192.89	327.51	265.28
			cv RN1					cv RN2		
neoxanthin	17.68	23.30	13.56	0.00	0.00	24.45	21.06	11.02	0.00	0.00
capsorubin	0.00	0.00	159.19	190.93	408.87	0.00	0.00	139.33	220.88	354.42
violaxanthin	22.86	33.69	83.21	163.36	332.39	37.02	35.69	80.26	187.44	298.57
caps 5,6-epoxide	0.00	0.00	149.66	181.36	366.05	0.00	0.00	140.25	188.13	330.11
capsanthin	0.00	0.00	2743.98	3286.44	5605.70	0.00	0.00	2534.45	3811.59	6363.08
antheraxanthin	5.64	4.65	303.50	296.89	535.63	5.53	4.99	267.44	351.28	494.06
cucurbitaxanthin A	0.00	0.00	414.94	490.00	975.64	0.00	0.00	370.13	568.76	899.20
zeaxanthin	0.00	41.73	563.75	577.15	678.27	0.00	0.00	492.69	531.42	716.92
lutein	58.75	34.97	32.00	0.00	0.00	75.96	61.38	49.06	0.00	0.00
β-cryptoxanthin	0.00	0.00	417.52	319.34	364.62	0.00	0.00	343.87	308.16	450.00
β-carotene	41.24	23.66	400.33	317.50	419.72	36.84	21.50	324.12	347.59	486.92
			cv RR1					cv DN3		
neoxanthin	21.13	19.70	7.35	0.00	0.00	19.73	23.13	0.00	0.00	0.00
capsorubin	0.00	0.00	64.40	105.78	125.76	0.00	0.00	90.08	156.05	181.19
violaxanthin	23.42	27.79	42.76	102.47	109.54	15.80	32.10	0.00	155.06	219.43
caps 5,6-epoxide	0.00	0.00	58.34	91.08	102.78	0.00	0.00	74.60	126.24	155.58
capsanthin	0.00	0.00	1467.06	2579.22	3012.57	0.00	0.00	2169.62	4103.92	5714.35
antheraxanthin	3.64	3.58	158.57	226.98	262.75	8.76	4.91	193.67	366.19	449.70
cucurbitaxanthin A	0.00	0.00	206.46	345.81	389.55	0.00	0.00	304.34	608.23	809.03
zeaxanthin	0.00	0.00	355.56	361.86	405.70	0.00	0.00	650.97	948.39	1351.78
lutein	72.78	54.99	22.59	0.00	0.00	71.13	65.48	44.95	0.00	0.00
β-cryptoxanthin	0.00	0.00	179.61	234.56	195.33	0.00	0.00	412.91	376.26	344.33
β-carotene	21.76	17.01	153.69	304.14	252.79	23.02	23.25	342.12	651.55	447.54
			cv DN5					cv DR6		
neoxanthin	42.17	50.55	0.00	0.00	0.00	14.18	29.77	25.58	0.00	0.00
capsorubin	0.00	0.00	84.88	153.45	231.50	0.00	0.00	230.57	257.38	290.79
violaxanthin	63.12	77.14	77.42	103.05	255.35	17.26	44.21	239.94	230.25	223.03
caps 5,6-epoxide	0.00	0.00	61.05	167.18	197.76	0.00	0.00	213.96	208.52	236.35
capsanthin	0.00	0.00	2452.19	3904.88	5985.10	0.00	3.07	4697.15	5405.12	6051.35
antheraxanthin	6.57	6.57	202.57	278.90	536.74	1.64	5.45	552.58	613.26	548.32
cucurbitaxanthin A	0.00	0.00	369.53	454.77	865.58	0.00	0.00	766.11	787.77	795.41
zeaxanthin	0.00	0.00	1231.13	835.00	1650.10	0.00	5.19	1032.75	1059.90	740.89
lutein	118.26	148.37	0.00	0.00	0.00	30.61	61.16	0.00	0.00	0.00
β-cryptoxanthin	0.00	0.00	500.69	572.07	681.73	0.00	0.00	633.51	552.88	335.30
β-carotene	45.72	51.95	399.87	489.12	683.01	13.80	23.01	623.52	493.73	435.01
			cv Mulato					cv Datler		
neoxanthin	74.80	120.05	0.00	0.00	0.00	18.27	20.41	0.00	0.00	0.00
capsorubin	0.00	35.16	145.01	152.03	181.83	0.00	2.77	178.33	194.30	284.33
violaxanthin	81.25	92.27	95.71	185.45	238.80	16.08	20.55	122.21	237.67	255.11
caps 5,6-epoxide	0.00	26.96	113.01	140.03	177.78	0.00	3.67	150.54	177.93	270.00
capsanthin	0.00	325.00	2220.02	3645.00	5011.88	0.00	18.29	4447.85	4669.91	5432.16
antheraxanthin	5.33	36.25	159.75	223.60	550.02	6.74	2.30	481.34	504.83	636.33
cucurbitaxanthin A	0.00	0.00	292.76	365.91	653.78	0.00	1.75	575.74	707.13	820.42
zeaxanthin	0.00	45.10	466.56	600.44	729.50	0.00	30.78	974.48	820.84	809.20
lutein	246.43	320.20	0.00	0.00	0.00	59.40	11.99	9.12	0.00	0.00

[a] Data represent means for four replicates.

MA3, and MA1) the exclusive ketocarotenoids (capsanthin, capsorubin, etc.) of the ripe fruit started to appear, which is in accordance with the shorter cultivation cycle shown by these cultivars, and therefore of agronomic interest. In all cultivars,

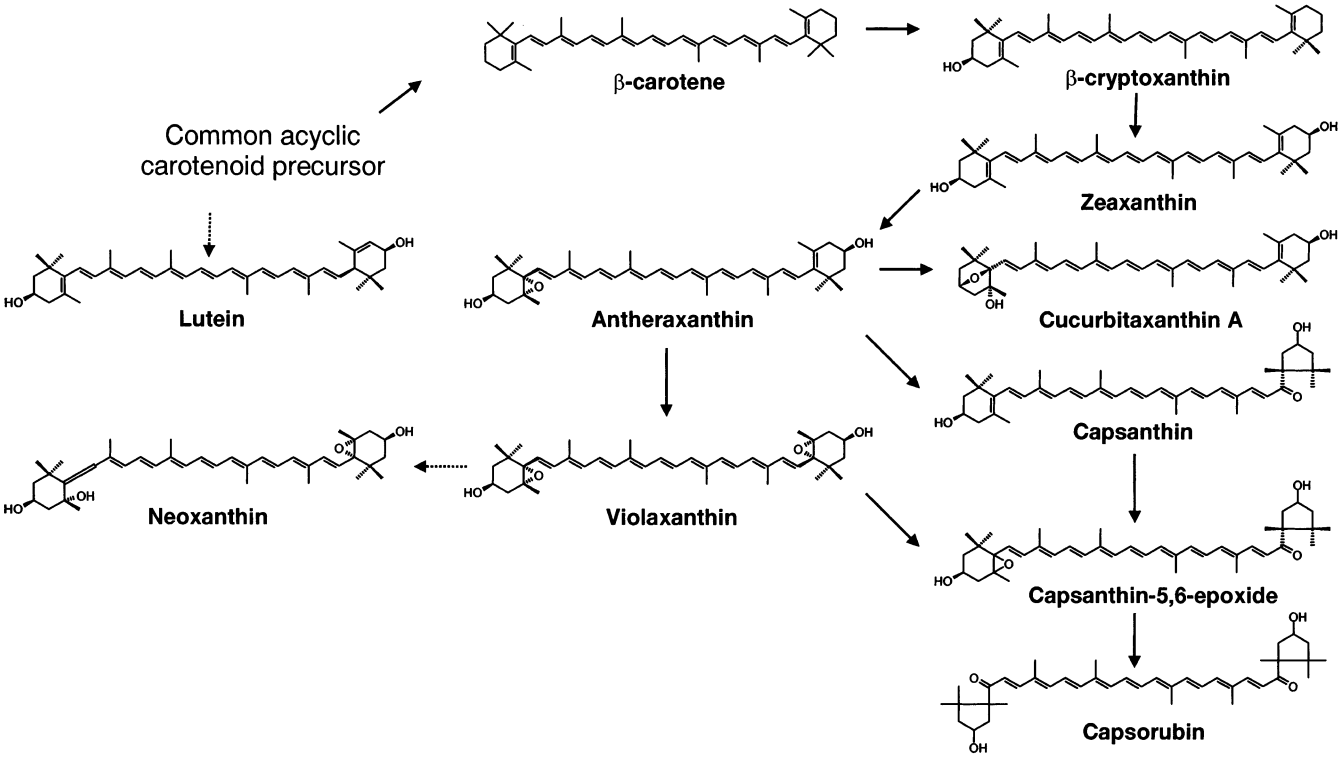

Figure 1. Carotenoid biosynthetic pathway in *Capsicum annuum* fruits.

as the ripening process progressed, a de novo carotenoid biosynthesis associated with chloroplast-to-chromoplast transformation was observed. This carotenogenesis involved the exclusive biosynthesis of the β,β-series carotenoids, with the formation of the characteristic ketocarotenoids (capsanthin, capsorubin, and capsanthin 5,6-epoxide) as well as other xanthophylls, namely β-cryptoxanthin, zeaxanthin, and cucurbitaxanthin A. In contrast, and in accordance with previous observations, lutein and neoxanthin (xanthophylls related to green vegetables, and whose synthesis is blocked once the ripening process is triggered) disappeared. **Figure 1** shows a scheme of the carotenogenic pathway for the *Capsicum* fruits.

In the present study, capsanthin stands out as the major pigment in all cultivars from the beginning of the ripening process. Although capsanthin is detectable in some cultivars at day 30, its presence is generalized from day 45, outstanding in DR6 and Datler cultivars, with the highest levels (4697.15 and 4447.85 mg/kg dwt) for this ripening stage. Fruits from these two cultivars do not experience notable increases during subsequent ripening stages (1.29- and 1.22-fold, respectively), in contrast with those from the other cultivars, with the highest increase in fruits of cultivar MA1 (2.14-fold). In the ripe fruit (day 75, and over-ripe stage for short- and medium-cycle cultivars), the relative content for capsanthin reached 60% in all cultivars, cultivar MA1 again showing the highest value (7949.68 mg/kg dwt). Capsorubin and capsanthin-5,6-epoxide underwent changes in parallel to capsanthin due to their biosynthetic relationship (see **Figure 1**), but at lower concentration levels—no more than 2.5−3.0% of the total carotenoid content in the ripe fruit. The individual contents of the carotenes and xanthophylls belonging to the yellow isochromic fraction (β-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin, and violaxanthin)—biosynthetic precursors of the red pigments—increased sharply as a result of ripening. MA1 and DN5 cultivars showed the highest levels for β-carotene and β-cryptoxanthin at day 75, and therefore these are the cultivars with highest provitamin A content. During the course of this study, the MA-3 cultivar showed the highest levels for β-carotene and β-cryptoxanthin at day 60 (736.69 and 959.77 mg/kg dwt, respectively) although over-ripening of fruits resulted in a decrease of 25 and 50% of the two pigments respectively by interconversion into others of the red isochromic fraction. This phenomenon has been found in previous studies (*17*) with red pepper cultivars used for paprika production, so that over-ripening of fruits is a convenient way to exploit to a maximum the carotenogenic potential of the fruit. In the case of zeaxanthin, concentration levels rose gradually until the late-ripening stages, and then sharply. Zeaxanthin content was highest for cultivars DN3 and DN5 (1351.78 and 1650.10 mg/kg dwt, respectively). Previous studies (*2*) suggested that the zeaxanthin pool plays an important role in the control of carotenoid biosynthesis in red pepper, and therefore high zeaxanthin content denotes an overexpressed carotenogenic capacity that can be exploited for breeding and selecting carotenoid high-producing cultivars for paprika production.

Total Carotenoid Content and Pigment Ratios. Table 3 shows changes in the total carotenoid content, red-to-yellow isochromic fraction ratio (R/Y) and capsanthin-to-zeaxanthin ratio (Caps/Zeax). The highest total carotenoid content was found in fruits of the MA1 cultivar (12 697.58 mg/kg dwt), followed by DN5 and RN2 cultivars (11 086.88 and 10 393.29 mg/kg dwt, respectively). Of the other cultivars, DN3, RN1, Datler, and DR6 showed high total carotenoid content (about 9600 mg/kg dwt), and the rest contents under 8200 mg/kg dwt. The lowest carotenoid content was found for RR1 cultivar (4856.77 mg/kg dwt). In terms of the net increase of the total carotenoid content during ripening (from day 30 to day 75), cultivars Datler, RN2, and DN3 had the highest values (74.5-, 71.8-, and 64.9-fold, respectively), denoting their high carotenogenic capacity. In the rest of the cultivars, the extent of the increase in total carotenoid content was less, although this is not an indication of lower carotenogenic capacity, because in most cultivars the carotenoid content at day 30 was quite high. At day 45, cultivars DR6 and Datler stand out from the rest

Table 3. Evolution of the Total Carotenoid Content (mg/kg dwt), Yellow (Y)-to-Red (R) and Isochromic Fractions Ratio (R/Y), and Capsanthin-to-Zeaxanthin Ratio (Caps/Zeax) during Fruit Ripening[a]

cultivar	pigment fraction or ratio	ripening stage (days) 15	30	45	60	75
MA1	total car. content	208.63 ± 2.52	494.41 ± 29.66	5983.46 ± 359.01	12374.67 ± 742.48	12697.58 ± 711.06
	R/Y ratio		1.84 ± 0.15	1.85 ± 0.11	2.23 ± 0.18	2.11 ± 0.08
	Caps/Zeax ratio		9.08 ± 0.82	5.84 ± 0.33	8.38 ± 0.75	9.85 ± 0.75
MA3	total car. content	221.19 ± 13.27	464.97 ± 27.90	4905.14 ± 294.31	7857.38 ± 471.44	7565.38 ± 453.92
	R/Y ratio		0.84 ± 0.05	1.54 ± 0.12	1.10 ± 0.07	1.66 ± 0.13
	Caps/Zeax ratio		8.05 ± 0.45	8.07 ± 0.73	5.44 ± 0.30	9.12 ± 0.82
LR2	total car. content	309.15 ± 18.55	558.78 ± 33.53	2768.29 ± 138.41	4672.10 ± 261.64	7457.04 ± 447.42
	R/Y ratio		0.88 ± 0.05	1.68 ± 0.12	1.79 ± 0.07	1.88 ± 0.11
	Caps/Zeax ratio		2.46 ±0.14	5.31 ± 0.48	7.57 ± 0.58	6.97 ± 0.39
LR7	total car. content	171.42 ± 10.29	233.35 ± 14.00	4303.54 ± 258.21	6157.95 ± 344.85	6904.25 ± 345.21
	R/Y ratio		0.61 ± 0.05	1.61 ± 0.13	1.72 ± 0.07	1.89 ± 0.13
	Caps/Zeax ratio		2.11 ± 0.19	5.15 ± 0.46	6.38 ± 0.48	7.36 ± 0.66
RN1	total car. content	146.17 ± 11.26	162.00 ± 9.07	5281.65 ± 295.77	5822.97 ± 349.38	9686.89 ± 581.21
	R/Y ratio			1.37 ± 0.05	1.69 ± 0.10	1.93 ± 0.15
	Caps/Zeax ratio			4.87 ± 0.37	5.69 ± 0.32	8.26 ± 0.74
RN2	total car. content	179.80 ± 8.99	144.62 ± 7.23	4752.61 ± 285.16	6515.26 ± 325.76	10393.29 ± 582.02
	R/Y ratio			1.45 ± 0.12	1.84 ± 0.13	2.11 ± 0.08
	Caps/Zeax ratio			5.14 ± 0.46	7.17 ± 0.65	8.88 ± 0.67
RR1	total car. content	121.59 ± 9.36	103.37 ± 5.79	2709.02 ± 135.45	4351.89 ± 261.11	4856.77 ± 242.84
	R/Y ratio			1.41 ± 0.10	1.76 ± 0.14	2.01 ± 0.14
	Caps/Zeax ratio			4.13 ± 0.37	7.13 ± 0.64	7.43 ± 0.67
DN3	total car. content	138.45 ± 10.66	148.86 ± 11.46	4283.26 ± 239.86	7491.90 ± 576.88	9672.94 ± 580.38
	R/Y ratio			1.20 ± 0.05	1.41 ± 0.10	1.67 ± 0.13
	Caps/Zeax ratio			3.33 ± 0.25	4.33 ± 0.39	4.23 ± 0.38
DN5	total car. content	275.84 ± 13.79	334.58 ± 25.76	5379.33 ± 301.24	6958.42 ± 417.51	11086.88 ± 831.52
	R/Y ratio			0.93 ± 0.04	1.55 ± 0.12	1.37 ± 0.06
	Caps/Zeax ratio			1.99 ± 0.15	4.67 ± 0.42	3.63 ± 0.33
DR6	total car. content	77.50 ± 4.65	171.86 ± 10.31	9015.67 ± 694.21	9608.80 ± 480.44	9656.47 ±743.55
	R/Y ratio		0.02 ± 0.00	1.33 ± 0.09	1.57 ± 0.11	2.14 ± 0.15
	Caps/Zeax ratio		0.59 ± 0.03	4.55 ± ±0.41	5.10 ± 0.46	8.17 ± 0.74
Mulato	total car. content	462.13 ± 27.73	1204.07 ± 60.20	3908.26 ± 234.50	5808.37 ± 447.24	8103.55 ± 486.21
	R/Y ratio		0.47 ±0.03	1.73 ± 0.14	2.10 ±0.15	1.97 ± 0.16
	Caps/Zeax ratio		7.21 ± 0.65	4.76 ± 0.43	6.07 ±0.55	6.87 ± 0.62
Datler	total car. content	120.66 ± 6.03	129.02 ± 7.74	7933.75 ± 396.69	8325.52 ± 641.07	9615.86 ± 740.42
	R/Y ratio		0.24 ± 0.01	1.51 ± 0.11	1.54 ± 0.11	1.65 ± 0.12
	Caps/Zeax ratio		0.59 ± 0.03	4.56 ± 0.41	5.69 ± 0.51	6.71 ± 0.60

[a] Data represent means ± SD for four replicates.

with regard to total carotenoid content (9015.67 and 7933.75 mg/kg dwt, respectively), which is in accordance with their medium cultivation cycle (see **Table 1**), with no substantial increases up to day 75. In most cultivars, the rise in total carotenoid content from day 60 to day 75 was small, but in all cases there were significant increases in the R/Y and Caps/Zeax ratios (**Table 3**), showing that during the late ripening stages there was an interconversion of yellow fraction pigments into red ones. Therefore, in the case of red pepper cultivation for paprika production, it is important that the fruits be left to over-ripen to promote the biosynthesis of red pigments, thereby increasing the quality of the raw and processed products.

There was a general increase in the R/Y ratio from day 30, due to the biosynthesis of red pigments (capsanthin, capsorubin, etc.). In previous studies (*2*), an inverse correlation was found between total carotenoid content and R/Y ratio, which seems to be related to a limitation of the carotenogenic ability of the cultivar. This has led to proposing both total carotenoid content and R/Y ratio as reference traits when breeding and selecting new red pepper cultivars for paprika production. Cultivars characterized during the present work showed in general high values for both traits, demonstrating that it is possible to select and breed for both characters. Ripe fruits of the MA1 cultivar showed the highest total carotenoid content (12697.58 mg/kg dwt) and R/Y ratio (2.11). In most of the studied cultivars, the R/Y ratio was in the range 1.9−2.0. The exception was the DN5 cultivar which, while showing a high total carotenoid content, had the lowest R/Y ratio; as a consequence, this cultivar should be improved for this trait. Perhaps extending an over-ripening period beyond day 75 would result in a rise of the red isochromic fraction at the expense of the yellow fraction. Cultivars DN3, MA3, and Datler, with high carotenoid content, showed low R/Y ratios (1.67, 1.66, and 1.65, respectively), which correlates with the highest contents for β-carotene and β-cryptoxanthin (about 6%). Cultivars MA1, DN5, and Datler showed the greatest content for the two pigments and, as a result, the highest provitamin A value. According to the American National Research Council (*18*), between 4.8 and 6.0 mg of β-carotene are needed to supply 100% of the RDA for vitamin A in adult female and male humans, respectively (*19*). Therefore, taking into account the β-carotene and β-cryptoxanthin contents, and the fact that an average moisture value of about 90% was found for the studied cultivars, a daily intake of 100 g of fresh ripe fruit would be enough to cover the RDA for vitamin A (assuming total absorption and conversion of β-carotene to vitamin A in the body).

Most of the studied cultivars presented a good correlation between total carotenoid content and Caps/Zeax ratio. An exception was cultivar RR1 which, while presenting the lowest total carotenoid content, had a high Caps/Zeax ratio (7.43). DN5 and DN3 cultivars, with high carotenoid content, showed the lowest Caps/Zeax ratios (as well as low R/Y ratios), suggesting that these cultivars have reached their maximum carotenogenic capacity.

Selection of Cultivars. This work includes the characterization of red pepper cultivars bred not only for their high carotenoid-producing capacity (needed for paprika production) but also with the aim of developing cultivars suitable for

mechanical harvesting (cultivars with grouped fruit ripening), as well as cultivars with shorter cultivation cycles, allowing them to be grown in cooler producing areas not affected by the TSWV (*13*). The studied cultivars, and especially MA1, DN5, and RN2, stand out for their high total carotenoid contents (above 10 000 mg/kg dwt), pigment ratios, and carotenogenic potential over other cultivars previously characterized in our laboratory. For instance, cultivars Agridulce and Bola, traditionally used in Spain for paprika production, showed carotenoid contents about 7500 and 5000 mg/kg dwt, and R/Y ratios of 1.3 and 1.7, respectively (*20*). Cultivars Jaranda and Jariza, introduced in the past decade in the producing area of La Vera (Cáceres, Spain), showed carotenoid contents of about 7250 mg/kg dwt, with R/Y ratios of 1.2 in both cases (*21*). More recently, five paprika-producing cultivars (Negral, NuMex, Belrubi, Delfín, and Mana) have been characterized for use in breeding programs, having carotenoid contents in the range 7000−8500 mg/kg dwt, with the exception of the Mana cultivar, with 13 000 mg/kg dwt (*2*).

As shown in **Table 1**, there is a good correlation between length of cultivation cycle and degree of fruit grouping, so that the shorter the cultivation cycle, the more grouped the ripe fruits. Cultivars with round fruits (such as RN1 and RN2) usually yield lower carotenoid contents, so that they need a longer cultivation cycle to reach high carotenoid content. These two cultivars also show an echeloned fruit ripening, and therefore are not suitable for mechanical harvesting. Cultivars with shorter cultivation cycles and grouped fruit ripening (MA1 and MA3) are more appropriate for mechanical harvesting. Moreover, the short cultivation cycle for these two cultivars makes them the most suitable for cultivation in cooler areas to avoid the action of TSWV. In addition, the high carotenoid contents and pigment ratios of these two cultivars make them good candidates for paprika production. The rest of the studied cultivars (LR2, LR7, DN3, DN5, DR6, Datler, and Mulato) showed medium cultivation cycles and half-grouped fruits, and all have elongated fruits. Total carotenoid content in these cultivars is from medium to high. These characteristics make the cultivars promising.

Finally, it has been found that chlorophyll-retaining character does not affect carotenoid accumulation, which is in accordance with previous works (*2*). In fact, apart from cultivar MA1, the following 4 cultivars in descending order of total carotenoid content (DN5, RN2, RN1, DN3) retained chlorophyll in the ripe fruit. As this is an undesirable color character for paprika production (it makes the product brownish), further research is needed to understand the biochemistry of the altered chlorophyll catabolism in these cultivars.

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