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Carotenoid Composition of New Cultivars of Red Pepper for Paprika

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Five cross cultivars of red pepper for paprika and another hot type were compared with a classic sweet variety of pepper from Murcia. Extractable color, moisture, individual carotenoid, red and yellow pigment, and provitamin A content were determined. The results show significant differences between the different cultivars with respect to carotenoid synthesis. There is a good correlation between the moisture content of the cultivars and the provitamin A content and among the carotenoids themselves directly related in the biosynthetic pathway. The results are used as criteria in the selection of cultivars for different industrial processes.

INTRODUCTION

Paprika and its oleoresin are two of the most commonly used natural colorants in the food industry. The pickle industry too makes use of pepper concentrates in the form of paste. The commercial value of these products is based on their richness in carotenoids, which originate in the fruit wall; it is for this reason that the cultivars destined for the production of paprika, oleoresin, or concentrates differ from those intended for direct cosumption. They are fruits with a very strong color and hardly any pulp, so that pigment concentration is higher and dehydration costs are lower. The selection of pepper cultivar with a high level in carotenoids and suitable for industrial processing is another aspect of research on the improvement of paprika quality (Kahn, 1985; Reeves, 1987).

Other studies have been undertaken to identify the different causes of carotenoid deterioration in paprika and oleoresin: chemical–physical factors (Harkay-Vinkler, 1974; Candela et al., 1984; Giménez et al., 1984; Gorospe et al., 1987), industrial treatments (Ramakrishnam et al., 1977; Vámos-Vigyázó et al., 1985), enzymatic actions (Kanner et al., 1977; Barimalaa and Gordon, 1988; Biacs et al., 1989), and storage conditions (Zilberboim et al., 1986; Meseguer et al., 1987; Czinkotai et al., 1989) have all been studied.

These days HPLC is the most widely used technique for pepper carotenoid analysis due to its speed, the absence of alteration in the sample, and the possibility of multiple on-line detection (Gregory et al., 1987; Biacs et al., 1989; Czinkotai et al., 1989; Almela et al., 1990).

In this paper we study the total extractable color by spectrophotometry and the individual carotenoid content by normal-phase high-performance liquid chromatography of several pepper cultivars. A classic sweet variety of pepper from Murcia, Americano, is compared with another five, all crosses, and a sixth very hot type, Belrubi.

EXPERIMENTAL PROCEDURES

The selection of cultivar is based on three criteria: high extractable color, grouped ripening for mechanical harvest, and low moisture for reduced dehydration costs. The fruits were harvested on September 30, 1989, the normal time for this type of crop, and 3-kg samples of uniform maturity were immediately taken to the laboratory. The seeds were removed and the fruits cut into small pieces (about 3 × 3 mm) to obtain a representative sample. Subsamples of 7 g were stored under nitrogen at −28 °C until their analysis.

Table I. Extractable Color and Moisture of Fruits[a]

cultivar	color[b] (ASTA)	moisture, %
Belrubi	563 ± 4.0	82.5 ± 0.6
Negral	513 ± 5.1	58.1 ± 0.7
AmlerB51	474 ± 7.3	85.7 ± 0.5
NAN	456 ± 4.1	39.3 ± 0.6
AmlerB51E81	433 ± 4.5	85.9 ± 0.6
Albar × M.CA	351 ± 6.4	22.1 ± 0.7
Americano	277 ± 5.7	57.5 ± 0.8

[a] Values are means of four determinations ± standard error. [b] 100 ASTA units is the color of a paprika acetone extract (0.164 g brought to 100 mL) and whose absorbance at 460 nm is 1.000.

Moisture Determination. This was realized by drying at 100 °C in an air circulation drying oven until constant weight was reached.

Determination of Extractable Color. Seven-gram samples were extracted with acetone (50 mL) in an Omni-Mixer Sorvall homogenizer whose stainless steel chamber was placed in an ice bath. The extract was filtered through a sintered-glass funnel and the residue re-extracted (three or four times) until total discoloration. The filtrates were mixed together and brought to 250 mL with the same dissolvent. Extractable color was realized by the American Spice Trade Association (ASTA) 20.1 method. For this, the extracts were diluted in the proportion 1:25 and the spectrophotometric absorbance was measured at 460 nm with an acetone blank.

Determination of Individual Carotenoids. Seven-gram samples were extracted as for extractable color determination but by using methanol instead of acetone, with the consequent smaller risk of isomerization. The residue was repeatedly extracted with methanol–diethyl ether (90:10) until the triturate was colorless. The filtrates were brought to 500 mL with the same solvent. A 50-mL aliquot of each sample was saponified with aqueous potassium hydroxide (60%) (Candela et al., 1984), and the free carotenoids were re-extracted with diethyl ether and brought to a final volume of 50 mL.

For HPLC analysis 3-mL aliquots were dried in a nitrogen stream, and the residue was dissolved in 300 μL of acetone. The separation of carotenoids was carried out by normal-phase chromatography on a silica column (25 cm × 4.6 mm i.d.) of Spherisorb (5-μm spherical particles). The solvent mixture was petroleum ether (bp 40–60 °C) and acetone with a stepwise gradient (Almela et al., 1990).

Statistics. Statistical calculations were realized by using the Systat 4.0 package (Systat Inc.).

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Table II. Carotenoid Composition of Different Red Pepper Cultivars[a]

pigment	cultivar						
	Belrubi	Negral	AmlerB51	NAN	AmlerB51E81	Albar × M.CA	Americano
β-carotene	1.22 ± 0.02	1.09 ± 0.02	1.13 ± 0.02	1.05 ± 0.01	1.56 ± 0.01	0.35 ± 0.01	0.43 ± 0.01
cryptocapsin	2.79 ± 0.14	0.10 ± 0.02	1.67 ± 0.08	2.00 ± 0.07	2.42 ± 0.07	0.15 ± 0.01	0.05 ± 0.01
cryptoflavin	0.48 ± 0.03	1.04 ± 0.03		0.41 ± 0.04	0.39 ± 0.02	0.08 ± 0.01	0.34 ± 0.02
β-cryptoxanthin	0.35 ± 0.03	0.42 ± 0.03	0.41 ± 0.04	0.30 ± 0.04	0.51 ± 0.02	0.16 ± 0.02	0.14 ± 0.02
antheraxanthin	0.16 ± 0.02	0.24 ± 0.02	0.13 ± 0.03	0.67 ± 0.03	0.06 ± 0.02	0.31 ± 0.02	0.07 ± 0.01
lutein		0.17 ± 0.03	0.13 ± 0.03	0.35 ± 0.04	0.09 ± 0.03	0.07 ± 0.01	0.03 ± 0.02
capsolutein	0.96 ± 0.01	0.84 ± 0.02	0.84 ± 0.03	0.53 ± 0.03	0.08 ± 0.01	0.07 ± 0.01	0.47 ± 0.03
luteoxanthin	0.38 ± 0.04	0.44 ± 0.03	0.14 ± 0.02	0.20 ± 0.03	0.20 ± 0.03	0.09 ± 0.01	0.18 ± 0.02
zeaxanthin	0.60 ± 0.05	0.43 ± 0.05	0.58 ± 0.04	0.26 ± 0.03	0.53 ± 0.02	0.27 ± 0.02	0.34 ± 0.03
mutatoxanthin	0.73 ± 0.03	0.67 ± 0.04	0.36 ± 0.03	0.62 ± 0.02	0.51 ± 0.02	0.24 ± 0.02	0.27 ± 0.02
capsanthin	4.14 ± 0.20	5.68 ± 0.18	4.69 ± 0.16	2.92 ± 0.15	4.80 ± 0.20	5.36 ± 0.22	3.82 ± 0.16
capsanthin 5,6-epoxide	0.74 ± 0.07	0.37 ± 0.04	0.57 ± 0.06	0.34 ± 0.04	0.10 ± 0.02	0.21 ± 0.03	0.29 ± 0.03
violaxanthin	0.44 ± 0.03	0.58 ± 0.04	0.08 ± 0.05	0.27 ± 0.05	0.06 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
capsorubin	0.61 ± 0.06	0.74 ± 0.07	0.94 ± 0.04	0.25 ± 0.03	0.10 ± 0.02	1.33 ± 0.04	0.53 ± 0.03
capsorubin isomer	0.47 ± 0.03	0.80 ± 0.05	0.68 ± 0.03	0.19 ± 0.02	0.03 ± 0.01	0.69 ± 0.03	0.49 ± 0.03
neoxanthin	0.78 ± 0.02	0.64 ± 0.01	0.33 ± 0.02	0.51 ± 0.02	0.52 ± 0.01	0.13 ± 0.01	0.25 ± 0.01
unknown pigments	0.82 ± 0.27	0.60 ± 0.12	0.34 ± 0.11	1.74 ± 0.31	0.30 ± 0.15	0.45 ± 0.14	0.25 ± 0.10

[a] Presented data (milligrams per gram of dry weight) are means of three analyses ± standard error.

Table III. Pigment Composition (Percentage) of the Red Pepper Cultivars[a]

pigment	cultivar						
	Belrubi	Negral	AmlerB51	NAN	AmlerB51E81	Albar × M.CA	Americano
β-carotene	7.79	7.37	8.48	8.37	12.72	3.53	5.38
cryptocapsin	17.80	0.68	12.54	15.95	19.74	1.51	0.63
cryptoflavin	3.06	7.03		3.27	3.18	0.82	4.26
β-cryptoxanthin	2.23	2.84	3.10	2.30	4.16	0.84	1.75
antheraxanthin	1.02	1.62	0.98	5.34	0.49	3.13	0.88
lutein		1.15	0.98	2.79	0.73	0.70	0.38
capsolutein	6.13	5.68	6.31	4.23	0.65	0.70	5.88
luteoxanthin	2.43	2.57	3.30	1.12	1.63	0.92	2.24
zeaxanthin	3.83	2.91	4.35	2.07	4.32	2.73	4.26
mutatoxanthin	4.66	4.53	2.70	4.94	4.16	2.43	3.38
capsanthin	26.42	38.40	35.21	23.27	39.15	53.98	47.81
capsanthin 5,6-epoxide	4.72	2.50	4.28	2.71	0.82	2.11	3.63
violaxanthin	2.81	3.92	0.60	2.15	0.49	0.41	0.50
capsorubin	3.89	5.00	7.06	1.99	0.82	13.39	6.63
capsorubin isomer	3.00	5.41	5.11	1.52	0.24	6.95	6.13
neoxanthin	4.98	4.33	2.48	4.07	4.24	1.32	3.13
unknown pigments	5.23	4.06	2.52	13.91	2.46	4.53	3.13

[a] Data are calculated from Table II.

RESULTS AND DISCUSSION

Table I shows the moisture content of the different cultivars at harvest and the extractable color expressed in ASTA degrees, referring to 1 g of dry weight of each of the samples.

Although the period of vegetative development was the same for all the cultivars, three of them, AmlerB51E81, AmlerB51, and Belrubi, had a high moisture content which might give the impression that they had not completed their vegetative cycle. However, in control plants studied on successive days, the fruits of these cultivars rotted with no appreciable gain in coloring capacity. The moisture content is very important when the fruit is intended for the production of paprika since in this case it has to be dehydrated to a level which assures the subsequent conservation of the products, the cost of this process being of primary economic importance. From this point of view, the cultivar Albar × Mild California is of great interest since the fruit loses practically all its moisture on the plant without rotting or falling. For the fabrication of concentrates, the degree of moisture at the moment of harvest is not important. In this case too, a simultaneous maturation is preferred for mechanical collection.

The highest value with regard to coloring capacity was obtained with Belrubi, but its tendency to rot and its hot character make it suitable only for the preparation of concentrates or sauces, in which this latter characteristic can be appreciated. Negral has an intense red color but, because of its brown hue, is more suitable for the fabrication of oleoresin. Albar × Mild California has the previously mentioned advantage of being practically converted into rind only but is not interesting because of its pigment content. It would be very interesting to continue the study of new crosses which maintain this characteristic while improving the color. One example of this type of improvement is NAN, which has good coloring capacity but without the brown hue of Negral, which has been extensively cultivated for several years. The NAN and Amler cultivars have recently been introduced for commercial exploitation in some farms.

An analysis of individual carotenoids permits the much more detailed study of the coloring power of the different cultivars of red pepper and the establishment of possible criteria for the selection of species that synthesize red or yellow carotenoids preferentially and have a higher potential for synthesizing the precursors of vitamin A.

Tables II and III show the content and percentages of each one of the pigments obtained by HPLC analysis. The coefficient of variation of triplicate analysis ranged between 5 and 46% depending on the amount of each pigment. Higher values were presented by the minority pigments when eluted near another of higher concentration (i.e., antheraxanthin, capsanthin 5,6-epoxide, or capsorubin isomer). Those pigments which showed a good chromato-

Table IV. Provitamin A Content (IU of Provitamin A/g of Dry Weight) of *Capsicum* Varieties

cultivar	provitamin A,[a] IU	cultivar	provitamin A,[a] IU
Belrubi	5232	AmlerB51E81	5534
Negral	3195	Albar × M.CA	928
AmlerB51	3721	Americano	1185
NAN	4145		

[a] Based on IU of provitamin A = 1667 (mg of β-carotene) + 883 (mg of cryptocapsin + mg of cryptoflavin + mg of β-cryptoxanthin).

Table V. Most Significant Factors of the Correlation Matrix

	moisture	provitamin A	β-carotene	cryptocapsin	β-cryptoxanthin
moisture	1.000	0.714	0.929		0.786
provitamin A		1.000	0.893	0.857	0.643
β-carotene			1.000	0.714	0.821

graphic resolution (i.e., β-carotene, capsanthin, or neoxanthin) presented the smallest coefficients of variation.

The most significant differences between the different cultivars are in the red pigment: capsanthin and capsanthin epoxide, capsorubin and capsorubin isomer, and cryptocapsin. Percentage of total capsanthin varies between 56 and 51% in Albar × Mild California and Americano, respectively, compared to 26 and 31% in NAN and Belrubi. The former are also highest in capsorubin and capsorubin isomer content. The cultivars which synthesize the least quantity of highly hydroxylated xanthophylls are precisely those which show the highest cryptocapsin content: Belrubi, NAN, and AmlerB51E81. It is accepted that capsanthin and capsorubin are formed from zeaxanthin via antheraxanthin and violaxanthin (Cámara, 1980; Rüttiman, 1982). Although cryptocapsin is not described as forming part of this scheme, relative concentrations of cryptocapsin, capsanthin, and capsorubin suggest a close relationship in their biosynthetic pathways.

Table IV shows the provitamin A content of the different cultivars. In obtaining this value, it has been taken into account that carotenoids which contain in their structure at least half of the β-carotene molecule without substitutes in the β-terminal group can be converted more or less effectively into vitamin A (Isler, 1971).

From a comparison of these data with Table I, it can be appreciated that the cultivars which conserve a higher degree of moisture during maturation are those which present the highest provitamin A content. To show this relationship more precisely, a multivarian analysis was carried out with the following variables: color, moisture, provitamin A, and individual carotenoids (except capsanthin and capsanthin epoxide were grouped together and capsorubin and capsorubin isomer were also grouped together). In total, 18 variables applied to seven cultivars of pepper. The most significant correlation indices obtained by using Spearman's correlation matrix are shown in Table V.

A high correlation between moisture and provitamin A content can be appreciated in the seven cultivars, the index reaching 0.929 between moisture and β-carotene. This pigment and cryptocapsin show the highest correlation with regard to the effect which each pigment has on provitamin A content, while β-cryptoxanthin, because of its small proportion, has less influence. Finally, note the close correlation between β-carotene and β-cryptoxanthin, which can be explained by their proximity in the biosynthetic pathway; cryptocapsin, whose position in carotenoid biosynthesis is not well described, shows a lower degree of correlation. High correlations were also obtained between capsanthin and capsorubin (0.678) and violaxanthin and neoxanthin (0.847); these xanthophylls are highly oxygenated, and the latter two are directly related in the biosynthetic pathway.

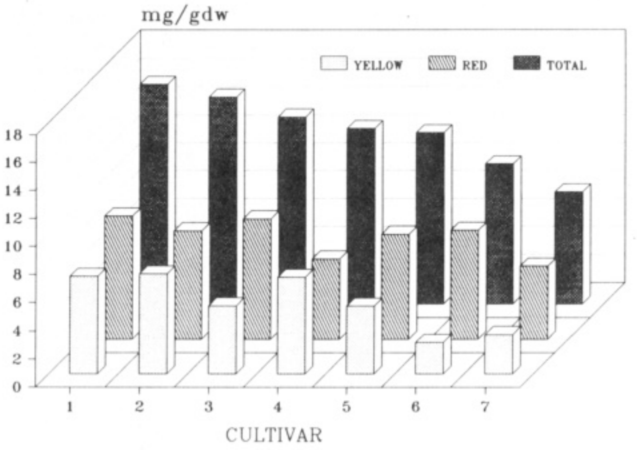

Figure 1. Red and yellow pigments and total carotenoids of the different cultivars. Values are in milligrams per gram of dry weight.

Figure 1 shows the total carotenoid content of each of the cultivars, together with the red and yellow pigment totals. Red pigments were: capsanthin, capsanthin 5,6-epoxide, capsorubin, capsorubin isomer, and cryptocapsin; yellow pigments, the rest.

Belrubi (8.75 mg/g of dry weight) and AmlerB51 (8.55 mg/g of dry weight) are highest in red pigments. Albar × Mild California, whose total carotenoid content is notably smaller, nevertheless showed a high proportion of red pigments (7.74 mg/g of dry weight). The choice of cultivars with a high red pigment content could be of interest for certain applications. For example, red xanthophylls notably increase the pigmentary capacity of the yellow xanthophylls present in poultry feed, whose cost is not insignificant (Saylor, 1986).

In conclusion, this study has revealed the differences which different cultivars of *Capsicum annuum* present in carotenoid pigment synthesis. These differences, together with other factors such as moisture content or their hot character, can be used as criteria in the selection of different cultivars for different purposes.

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Registry No. β-Carot., 7235-40-7; cryptocap., 7044-42-0; β-cryptox., 472-70-8; cryptoflavin, 30311-63-8; antheraxanthin, 68831-78-7; lutein, 127-40-2; luteoxanthin, 1912-50-1; zeaxanthin, 144-68-3; mutatoxanthin, 31661-06-0; capsanthin, 465-42-9; capsanthin 5,6-epoxide, 29486-21-3; violaxathin, 126-29-4; capsorubin, 470-38-2; neoxanthin, 14660-91-4; provit. A, 11103-57-4.