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Carotenoids from *Capsicum annuum* Fruits: Changes during Ripening and Storage

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Abstract. Contents of individual carotenoids in the fruits of Ramillete cultivar of *Capsicum annuum* L. changed during ripening of recently harvested fruits or fruits stored 15 d in a dark and cool room. β-carotene and β-cryptoxanthin contents rose to a maximum and then declined, while the contents of other carotenoids rose to a rather steady level. Capsanthin was the main carotenoid which together with capsorubin were responsible for the ripening colour.

Many studies on the metabolism of carotenoids during the ripening of fruit were carried out in lemon (YOKOYAMA and VANDERCOOK 1967), mango (JOHN *et al.* 1970), rowan berries (VALADON and MUMMERY 1972), peaches and apricots (KATAYAMA *et al.* 1971), bitter lemon (RODRIGUEZ *et al.* 1976), tomato (ZSCHEILE 1966, EDWARDS and REUTER 1967, RAYMUNDO *et al.* 1967, 1970, KHUDAIRI 1972), and pepper (GARCÍA 1951, CHOLNOKY 1954, CURL 1962, DAVIES *et al.* 1970, CAMARA and MONÉGER 1978).

Pepper fruits accumulate dihydroxylated xanthophylls closely associated to lipophilic globules and lipoprotein tubules (KIRK and JUNIPER 1967, LABORDE and SPURR 1973, SIMPSON *et al.* 1974) which have been studied in several varieties and cultivars (*lycopersiciforme rubrum*, *l. flavum*, Yolo Wonder A, and Ornamental Pepper).

The fruit of *Capsicum annuum* constitutes an instructive model for the study of carotenogenesis during ripening not only for the involved route and the capacity to continue the carotenogenesis after harvest, but also because of marked quantitative and structural changes during ripening (CAMARA 1980, CAMARA and BRANGEON 1981, CAMARA *et al.* 1982).

The present studies were undertaken to determine the carotenoid contents of pepper cv. Ramillete (which is used to produce paprika in the Spanish South-East) and to investigate their changes during the ripening and postmaturation of stored fruits.

MATERIAL AND METHODS

Plant Material

The fruits of *Capsicum annuum* L. cv. Ramillete were kindly supplied by Instituto National de Investigationes Agricolas (experimental field in La Alberca, Murcia). At the mature green stage, fruits were daily controlled and

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TABLE 1
Properties and identification of the carotenoids from *Capsicum annuum* fruits separated by thin layer chromatography (TLC) on neutral silica gel with light petrol-acetone (77 : 23) and further purified. — Abbreviations: B = benzene; CH_2Cl_2 = methylene chloride; EtOAc = ethyl acetate; H = hexane; iso-PrOH = isopropanol; Me_2CO = acetone; P = petrol; Si-gel = silica gel (Mn = Macherey-Nagel, P_1 = Merck)

Fraction number and colour	R_f	Purification TLC	R_f	Purification column	λmax [nm]	Hypsochromic shift [nm]	Carotenoids
1 yellow-orange	0.95	Si-gel-MN petrol (80 – 100 °C	0.70	MgO-Hyflo-Super Cell Petrol (40 – 60°)	276, 286, 298 H 425, 449, 477 H		phytoene β-carotene
			0.50 0.40		332, 348, 367 H 380, 400, 425 H		phytofluene ζ-carotene
2 yellow	0.70	Al_2O_3 P : Me_2CO (85 : 15)	0.80 0.60		337, 420, 444, 471 H 425, 447, 476 H		cis β-cryptoxanthin β-cryptoxanthin
2′ yellow-rose	0.65	Si-gel-P1 P : EtOAc : iso-PrOH (95 : 10 : 5)	0.70		445, 469, 498 H		cryptocapsin
3 yellow	0.57	Al_2O_3 P : Me_2CO (85 : 15)	0.60		416, 441, 470 H	40	violaxanthin
4 yellow	0.50	Si-gel-P1 P : EtOAc : iso-PrOH (95 : 10 : 5)	0.75		422, 445, 471 H	20	anteraxanthin
5 orange-yellow	0.45	Si-gel-MN EtOAc : CH_2Cl_2 (20 : 80)	0.50		425, 450, 479 H		zeaxanthin
6 red	0.40	Si-gel-MN P : Me_2CO (60 : 40)	0.80 0.75		483, 516 B 479, 513 B	 15	capsanthin capsanthin-5,6-epoxide
7 rose	0.30	Si-gel-MN P : Me_2CO (60 : 40)	0.40		459, 487, 522 B		capsorubin
8 yellow	0.24	Si-gel-MN P : Me_2CO (75 : 25)	0.30		513, 436, 463 H	16	neoxanthin

harvested with uniform size and maturity: (i) at the beginning the first red colour (± 10 g mass each); (ii) 10 d after this moment (± 6 g); (iii) 21, 29 and 39 d (± 4 g), and (iv) 48 and 58 d from the beginning (± 2 g). For each analysis two batches of 20 homogeneous fruits were used. One of them was immediately analysed and the other one was kept in a cold chamber (15 °C) and dark for 2 weeks, and then analysed in the same way.

Pigment Extraction and Separation

After removing the seeds, the pericarps were cut into fine pieces and ground in a mortar with anhydrous sodium sulphate and sand until a fine powder was formed. The pigments were extracted with methanol, the extract was saponified by adding 60% (m/v) aqueous KOH (1 cm^3 to every 10 cm^3 of methanolic solution) during 12 h in the dark at room temperature. The unsaponified pigments were reextracted with an equal volume of freshly distilled, peroxide-free diethyl ether, washed three times with water to remove methanol and dried over anhydrous sodium sulphate during 2 h in the dark.

The diethyl ether solution was reduced by rotary evaporation until dryness. The unsaponified pigments were resuspended in a mixture of diethyl ether: light petrol (b.p. 40—60 °C) : acetone (2 : 2 : 1) and then submitted to thin layer chromatography (TLC) on neutral silica gel with light petrol (40—60): acetone (77 : 23) (Lopez 1972). The carotenoid spots were rechromatographed by TLC and on columns with different solvents. For details see Table 1.

Carotenoid Identification

Carotenoid spots were scrapped from plates, eluted with diethyl ether or ethanol and rechromatographed in a different system for final identification. Absorption spectra in different solvents were compared to those given by Davies (1976). Carotenoid epoxides were identified by observing the magnitude of the spectral shift on adding a small drop of concentrated HCl to an ethanolic solution of carotenoid in a spectrophotometer cell (Davies 1965). Mono-5,6-epoxides showed a shift of 15—20 nm while 5, 6, 5′, 6′ diepoxides had about 40 nm.

Quantitative Determinations of Carotenoids

Quantitative determinations of carotenoids were carried out spectrophotometrically in 1 cm path length quartz cells in solutions of known volume (Davies 1965). The results (means of three independent samples) were expressed as the amount of individual carotenoid recovered in $\mu g\ g^{-1}$ (dry mass) of tissues.

RESULTS AND DISCUSSION

In the carotene fraction we found some colourless compounds fluorescencing in UV-radiation which migrated ahead of β-carotene on Al_2O_3 (Table 1). They were not isolated since they did not contribute to the red colour of the fruit, but they were identified as the polyenes phytoene, phytofluene and ζ-carotene (Davies 1965).

Cryptocapsin was identified between fractions 2 and 3 in the first TLC. It was not quantified because it appeared only in the last stage of development and in a small quantity, similarly to capsanthin 5,6-epoxide, which

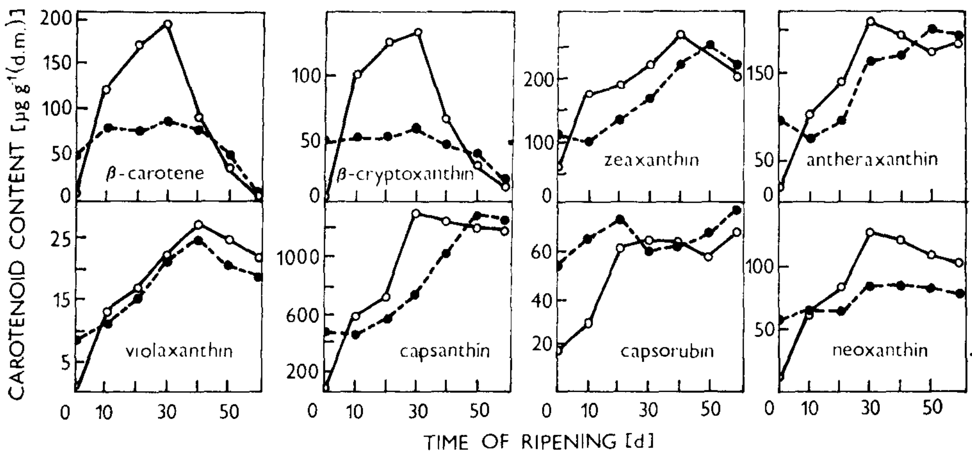

Fig. 1. The occurrence of individual carotenoids in the fruits of *Capsicum annuum* cv. Ramillete during ripening. — Fruits: recently harvested (○—○) and 2 weeks stored (●---●).

appeared between capsanthin and capsorubin and was very labile. After $LiAlH_4$ reduction, the λ_{max}^{EtOH} were 430, 448, 480 nm, 425, 449, 478 nm, 428, 451, 479 nm, and 419, 440, 470 nm for cryptocapsin, capsanthin-5,6-epoxide, capsanthin, and capsorubin, respectively.

During ripening of the fruits, the contents of individual carotenoids of recently harvested and stored fruits were generally significantly different (Fig. 1). Nevertheless, there were similarities in changes of β-carotene and β-cryptoxanthin contents (in recently harvested fruits an increase until about 30 d followed by a quick decrease). In the stored fruits, however, their contents were much lower. It is possible that the fruits, while on the plant, receive some substance like glucose or saccharose, but the harvested fruits need some time to unblock their own reserves.

Contents of zeaxanthin and its transformation products antheraxanthin and violaxanthin followed a similar, but slower development. Their contents in the stored fruits were lower than in recently harvested fruits, but at the end the situation became balanced.

Capsanthin and capsorubin arising from anteraxanthin and violaxanthin, respectively, are responsible for the ripening colour. Their contents did not decrease in the post-maturity stage.

Neoxanthin is formed from violaxanthin, but its formation was inhibited probably by the synthetic pathways of capsanthin and capsorubin.

Hence red pepper is autonomous from the point of view of carotenoid biosynthesis (*cf.* GOODWIN 1980). With the storage conditions employed, the carotenoid contents in most cases increase.

Qualitatively, our results confirm those of CHOLNOKY *et al.* (1956) obtained with the lycopersiciforme variety, of CAMARA and MONÉGER (1978) with the Yolo Wonder cultivar, and of DAVIES *et al.* (1970) with cv. Ornamental Pepper-Tall Mixed, but quantitatively in the first stage of ripening the zeaxanthin content was higher than in the lycopersiciforme and ornamental peppers, while the violaxanthin content was lower. The capsanthin content was the highest among carotenoids. The essential phenomenon during ripening is the increase in the contents of capsanthin and related pigments and the disappearance of the β, ε-carotene series, similarly as in other pepper cultivars (CHOLNOKY *et al.* 1958).

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