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FREE AND ESTERIFIED CAROTENOIDS IN GREEN AND RED FRUITS OF *CAPSICUM ANNUUM*

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Key Word Index—*Capsicum annuum*; Solanaceae; pepper; carotenoid variation with maturity, free and esterified xanthophylls.

Abstract—Fully green and mature red fruits of the Yolo Wonder A variety of *Capsicum annuum* were analysed for their carotenoid content. The disappearance of chlorophyll was followed by an increased synthesis of carotenoids. Lutein was not detected in the red fruit in which capsanthin was the main carotenoid. It appeared as diester, monoester and free forms, while capsorubin occured as a diester only. Cryptocapsin was not esterified.

INTRODUCTION

Many studies on the metabolism of carotenoids during the ripening of fruits were carried out with tomatoes [1,2]. In this fruit lycopene is the major carotenoid and that makes studies on xanthophyll esterification inopportune. In the case of the pepper which belongs to the same family as tomato, the biosynthetic pattern of the carotenoids is very different [3–7]. The fruit accumulates, closely associated to lipophilic globules and lipoprotein tubules [8–10], dihydroxylated xanthophylls which have been studied in several varieties (*Lycopersiciforme rubrum*, L. *flavum*).

The work reported here was undertaken to investigate the carotenoid content of the Yolo Wonder A variety of *Capsicum annuum* which is very resistant to tobacco mosaïc virus. The fruits were investigated at two stages of maturity (green and red).

RESULTS

The qualitative and the quantitative carotenoid compositions of the Yolo Wonder A variety are given in Table 1 and Table 2 respectively.

With fully green fruits, the absorption spectrum of the

Table 1. Qualitative carotenoid composition of *Capsicum annuum* var. Yolo Wonder A

Carotenoids		λ_{max}(nm)	Hypsochromic shift (nm)	Carbonyl test	Saponification test	Acetylation test
β-Carotene		425–450–479 H*				
β-Cryptoxanthin	F†	425–445–477 H				
	M	424–445–477 H			+	
Cryptocapsin	F	(445)–470–497 H		430–448–480	+	
Lutein	F	422–445–476 E				
Zeaxanthin	M	(427)–451–478 E			+	+
Antheraxanthin	D	(421)–446–476 E	20		+	
Violaxanthin	F	416–441–471 H	40			
		417–442–470 E				
Capsanthin	D	(450)–474–503 H			+	
		485–518 B				
		474 E				
	M	(450)–474–504 H				
		474 E		428–451–479	+	+
	F	(450)–474–504 H				
		472 E				
Capsanthin-5, 6-epoxide	D	473–502 P	~16	425–449–478	+	
		473 E				
Capsorubin	D	452–477–510 H				
		457–488–522 B				
		485 E		419–440–470	+	
Neoxanthin	F	415–437–466 H	16			
		414–437–466 E				

The carotenoids are presented in order of increasing TLC polarity after saponification.

*H: hexane; P: petrol; B: benzene; E: ethanol.

†F = free; M = monoester; D = diester.

Table 2. Individual carotenoid content from *Capsicum annuum* fruit at two stages of maturity

Carotenoid		Fully green µg/g dry wt	Fully green %	Mature red µg/g dry wt	Mature red %
β-Carotene		54.9	24.0	271.8	15.4
β-Cryptoxanthin	F, M*	20.8	9.1	217.0	12.3
Cryptocapsin	F	—	--	90	5.1
Lutein	F	79.9	34.9	—	—
Zeaxanthin	M	—	—	54.7	3.1
Antheraxanthin	D	--	—	162.4	9 2
Violaxanthin	F	33.8	14.8	125 3	7 1
Capsanthin	F,M,D	—	--	587.7	33 3
Capsanthin-5, 6-epoxide	D	--	—	30.0	1.7
Capsorubin	D	—	—	181.7	10.3
Neoxanthin	F	39.1	17.1	35.3	2.0
Total		228.5		1755 9	

*F = free; M = monoester; D = diester

total carotenoid extract (425, 448, 471 nm in petrol) was similar to that of the leaf extract. In the carotene fraction, TLC with system 1 showed one spot corresponding to β-carotene. With TLC system 4, four spots could be detected corresponding to the xanthophylls β-cryptoxanthin, lutein, violaxanthin and neoxanthin, lutein being the most important pigment (Table 2). All dihydroxylated xanthophylls were hypophasic, indicating an absence of esterification (Table 3) at the green stage.

With mature red fruits, the absorption spectrum of the petrol extract showed a bathochromic shift of *ca* 27 nm when compared to the spectrum of the green fruit extract. In the carotene fraction, some colourless compounds giving a flourescence under UV light migrated ahead of β-carotene on Al_2O_3. They were not isolated since they did not contribute to the red colour of the fruit, but generally, compounds having such polarities are mainly the colourless polyenes such as phytoene and phytofluene. The epiphasic esterified xanthophylls could be separated into seven spots with TLC system 2 (β-cryptoxanthin ester, capsanthin diester, capsorubin diester, antheraxanthin diester, capsanthin epoxide diester, zeaxanthin monoester, capsanthin monoester).The hypophasic free xanthophylls gave four spots (cryptocapsin, violaxanthin, capsanthin, neoxanthin).

Table 3. Free and esterified xanthophyll content of *Capsicum annuum* fruit at two stages of maturity

Xanthophyll form	Xanthophyll	Fully green % *	Mature red %
Free	β-Cryptoxanthin	12.0	—
	Cryptocapsin	—	6.1
	Lutein	45.9	- -
	Violaxanthin	19.5	8.5
	Capsanthin	—	2.9
	Neoxanthin	22.4	2.4
	Total	99.8	19.9
Monoester	β-Cryptoxanthin	—	14.6
	Zeaxanthin		3.7
	Capsanthin	--	7.6
	Total		25.9
Diester	Antheraxanthin	---	11 0
	Capsanthin-5, 6-epoxide	—	2.1
	Capsanthin	—	28 8
	Capsorubin	—	12.2
	Total	—	54.1

*Expressed as the percentage of total xanthophylls recovered.

Capsanthin (3*R*,3′*S*,5′*R*)-3,3′-dihydroxy-β, κ carotene-6′ one) was found principally as a diester form, capsorubin (3*S*,5*R*,3′*S*,5′*R*)-3,3′-Dihydroxy-κ,κ-carotene-6,6′-dione) was present only in the form of a diester (Table 2) as was demonstrated by the adsorption polarity before and after saponification. The fine strucutre of the absorption spectrum which appeared after reduction with $NaBH_4$ was typical of the selective reduction of one or both carbonyl groups of the above mentioned structures. The λ_{max}^{EtOH} were 428,451,479 nm for capsanthol and 419,440, 470 nm for capsorubol. Caspsanthin monoester after acetylation showed an increase in R_f. This spot had a slight tail with TLC system 2, indicating the likely presence of two types of monoester owing to the unsymetrical structure of capsanthin.

Capsanthin epoxide was very labile. After reduction, λ_{max}^{EtOH} were 425,449,478 nm. As in the case of antheraxanthin diester, the hypsochromic drift after addition of 2% HCl prior to saponification did not give a valid result; a similar observation was made by Knee [11]. Esterification increased the carotenoid stability.

Cryptocapsin was not esterified and this might explain why this pigment is found in small quantity or destroyed, and also why Simpson *et al.* [7] did not isolate it. After reduction, λ_{max}^{EtOH} 430,448,480 nm were obtained.

Violaxanthin and neoxanthin showed polarities on TLC suggesting that they were not esterified. These results reveal that during ripening, new synthesized chromoplastic xanthophylls are esterified while typical chloroplastic xanthophylls remain unesterified.

Chlorophyll (Chl *a*: 572 µg/g dry wt, Chl *b*: 234 µg/g dry wt, in mature green fruits) disappeared completely in red fruits. This was followed by an increase in the carotenoid content (Table 2). The amount of each carotenoid, expressed as a percentage of the total carotenoids recovered, showed an increase of xanthophylls in red fruits with regard to green ones.

DISCUSSION

Qualitatively, these results seem to confirm those of Cholnoky [3] obtained with the *Lycopersiciforme* variety. In green fruits, we did not observe any zeaxanthin or antheraxanthin which were reported in the study of Davies *et al.* [6] on different peppers [Ornamental Pepper (Capsicum)-Tall Mixed]. This is probably due to the fact that carotenoid content is controlled by factors depending on the environment as well as the genome.

In the green fruit of *Capsicum annuum* var Yolo Wonder A, β-carotene, β-cryptoxanthin and neoxanthin content is higher than in the *Lycopersiciforme* and in ornamental peppers. The capsanthin content in the red fruit is comparable in these different varieties. β-Cryptoxanthin and capsorubin are present at a relatively higher concentration in the red fruit of the Yolo Wonder A variety than in the *Lycopersiciforme* and in ornamental peppers.

The essential phenomenon taking place during ripening is the disappearance of the β,ε-carotene series, similar to situation observed in other pepper varieties [3,6] and in peaches during ripening [12,13].

The chloroplast, when turning into a chromoplast, develops new biosynthetic potentials principally for capsanthin synthesis [14]. Zeaxanthin and antheraxanthin synthesized in the red fruits are presumably involved in the formation of the keto-carotenoids. The mechanisms inducing these changes are still unknown. Moreover, the chloroplast exhibits an enhanced carotenoid synthesis. It is possible that phytol freed by a chlorophyllase becomes available and participates in part for carotenoid biosynthesis [15]. In the red fruit the neutral lipid content was approximately four times greater than in the green fruits [16]. This permits the conclusion that acetate metabolism is intense during maturation, leading to a greater synthesis of geranylgeranyl-pyrophosphate which, in the absence of chlorophyll, could be used mainly in carotenoid biosynthesis. The increase in the plastid permeability to mevalonate could be added to those effects, this fact being consistent with the decrease in membrane polar lipids (phospholipids and glycolipids) which occurs during maturation [16,17].

The xanthophylls present in red fruits were mainly esterified, 25.9% as monoester and 54.1% as diester, while 19.9% were free. The fact that cryptocapsin, violaxanthin and neoxanthin were not esterified was probably due to a selectivity during the esterification reactions.

As far as the presence of capsanthin monoester is concerned this may be an extraction artefact, but our extractions of different plant residues gave the same proportions of capsanthin diester and capsanthin monoester. A similar fact was observed by Booth [18] in *Taraxacum*. This might suggest that esterification is a late step affecting only newly synthesized xanthophylls. Thus, the lipophilic character of capsanthin and capsorubin are enhanced. This considerably increases their accumulation in the lipophilic globules of the red fruit chromoplasts.

EXPERIMENTAL

Materials. The fruits of *Capsicum annuum* (variety Yolo Wonder A) were kindly supplied by BUD Agriculture Plant in Senegal. They were harvested when in a fully green and in a deep red stage, *ca* 60–70 days after blooming. For each analysis, a batch of ten homogeneous fruits was used. After removing the seeds the pericarps were cut into fine pieces, freeze dried, preserved in a N_2 atmosphere and kept in darkness at $-10°$.

Pigment extraction and separation. The material was reduced to a powder using a Dangoumau grinder, all the processing being carried out at 4° [19] and in a dim light. The pigments were extracted with dry Me_2CO and then, for green fruits only, with 20% H_2O in Me_2CO until complete decolouration of the powder. After transfer into petrol (40–60°), carotenes were separated from the chlorophylls and xanthophylls on a cellulose column [20]. Carotenes were eluted with 2% Me_2CO in petrol and the chlorophylls and xanthophylls with dry Me_2CO. Xanthophylls were separated from chlorophylls in a polyethylene powder column according to ref. [21]. A partition between petrol and 5% H_2O in MeOH allowed the separation between epiphasic esterified or one free hydroxyl xanthophylls and hypophasic free xanthophylls. The different fractions were washed, dried with Na_2SO_4 and then submitted to TLC in four different systems according to the polarity of the carotenoids: system 1: MgO and petrol–C_6H_6 (9:10) for carotenes; system 2: neutral Si gel and petrol–Me_2CO–MeOH (88:12:1.5); system 3: neutral Si gel and petrol–Me_2CO–C_6H_6 (7:2:1) for esterified xanthophylls; system 4: neutral Si gel and petrol–Me_2CO (3:2) for free xanthophylls. For red fruit extracts, a preliminary separation was achieved on Al_2O_3 (Woelm neutral-Brockman II-III). Carotenes were eluted from the petrol extract with 2% Me_2CO in petrol and xanthophylls with Me_2CO. The subsequent operations were carried out in the same way as for green fruits.

Identification. Carotenoid spots were scraped from plates, eluted with Me_2CO and rechromatographed in system 2 and 3 for final identification. Absorption spectra in different solvents were compared to those given by Davies [22]. Whenever a co-chromatography was possible, it was performed from extracted and purified carotenoids with methods similar to those used by Costes [20]. 5,6-Epoxides were characterized with 2% HCl and on the plates after saponification [4]. Carbonyl groups were reduced in EtOH [23], and the new maxima noted. The mono and diesters after TLC in system 2 were acetylated by Ac_2O in Py [24] and the new R_f noted. An increase of R_f was found for monoester while the diester R_f showed no change.

Quantitative determinations. Esterification did not change the shape of absorption spectrum; the $E^{1\%}_{1\,cm}$ values given by Davies [22] could therefore be used.

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