Changes in Carotenoid Esterification during the Fruit Ripening of *Capsicum annuum* Cv. *Bola*

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During fruit ripening of pepper (*Capsicum annuum*), *de novo* biosynthesis and esterification of carotenoids occur simultaneously. From the very first stages of ripening the totally esterified fraction of xanthophylls is the majority (around 50%). During the ripening process, free xanthophylls decrease in proportion to the rest and at the same time the partial esterified xanthophylls increase in proportion at the expense of the free fraction and simultaneously a portion of the partial esterified xanthophylls are transformed in totally esterified xanthophylls. In the fully ripe fruit the percentages of the free carotenoid pigments and the partially and totally esterified forms of these are 21.3%, 35.6%, and 43.1%, respectively. The fatty acids esterifying yellow xanthophylls are chiefly linoleic ($18{:}2^{\Delta 9,12}$), myristic (14:0), and palmitic (16:0), whereas in red xanthophylls they are lauric (12:0), myristic (14:0), and palmitic (16:0). This fact helps to explain the greater stability of the red xanthophylls compared to the yellow ones, because of the number of double bonds in the fatty acid chains.

INTRODUCTION

The carotenoid pigments are widely found throughout the plant kingdom. In fruits, however, their presence is particularly obvious, since these, during ripening, change color from greens, due to chlorophylls, to yellows or reds, arising, in the majority of cases, from the presence of carotenoids (Weedon, 1971). Generally, as fruits ripen and as the chlorophylls disappear, not only is the color of the typical chloroplastic carotenoids [β-carotene (β,β-carotene), lutein (β,ϵ-carotene-3,3′-diol), neoxanthin (5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′-triol), and violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol)] revealed, but also new carotenoids, not present in the unripe fruits, are synthesized. Examples of fruits in which such synthesis occurs are peppers (*Capsicum annuum*), tomatoes (*Lycopersicon esculentum*), and oranges (*Citrus aurantium*) (Goodwin, 1976). It has been shown that these changes during the course of ripening are closely related to the degeneration of chloroplasts and the appearance of chromoplasts (Sitte et al., 1980; Gross, 1991), which contain the carotenogenic enzymes. There are fruits, such as the olive (*Olea europaea*), in which the carotenoids, both in the unripe and ripe states, are present in their natural free (nonesterified) form (Mínguez-Mosquera and Garrido-Fernández, 1989). In other fruits, in contrast, as ripening progresses, the carotenoids are progressively esterified with fatty acids, this process giving rise to a whole family of esterified forms (nonesterified, partially esterified, and totally esterified). Pumpkins (*Cucurbita pepo*) and peppers are examples of the latter type. According to the results of some studies (Biacs et al., 1989), the esterified forms are more stable than the nonesterified forms and, furthermore, those that are esterified with saturated fatty acids are more stable than those that are esterified with unsaturated fatty acids. At the same time esterification makes the carotenoids more liposoluble and, therefore, more easily incorporated into the structure of membranes (Karrer and Jucker, 1948; Goodwin, 1952).

Fruits of the pepper (*C. annuum* L.) undergo a profound change during the course of ripening (Davies et al., 1970; Camara and Monéger, 1978). As far as the carotenoid pigments are concerned, two linked processes almost certainly occur: the conversion of the existing pigments and the *de novo* synthesis of these same pigments. The occurrence of the latter process is indicated by the fact that the overall carotenoid content increases by 1 or 2 orders of magnitude. A previous study (Mínguez-Mosquera and Hornero-Méndez, 1994) on ripening in this variety found that the total carotenoid content increased from 34.76 mg/kg in the unripe fruit to 962.50 mg/kg in the ripe fruit. The green color of the fruit is principally due to the presence of chlorophylls and to the carotenoids typical of the chloroplasts, such as the xanthophylls violaxanthin, neoxanthin, and lutein as well as β-carotene, the main carotene. As ripening begins, the fruit adopts tones of orange and at the end of the process it has an intense red color. This coloration is chiefly due to newly formed oxygenated carotenoids (with keto groups), mainly capsanthin (3,3′-dihydroxy-β,κ-caroten-6′-one) and capsorubin (3,3′-dihydroxy-κ,κ-carotene-6,6′-dione), both of these being exclusive to peppers.

In the present work, the variations in the degrees of esterification of the principal pigments are studied during ripening of the pepper. In addition, the nature of the fatty acids that esterify these pigments is determined in peppers of the *Bola* variety (*C. annuum grossum*), which is normally used in Spain for the production of paprika.

EXPERIMENTAL PROCEDURES

Samples. Fruits of *C. annuum* cv. *Bola*, grown in the La Vera region (Cáceres, Spain), were used. Fruits were harvested at five successive stages of ripeness on the basis of their color: green, green with orange zones (color break I), predominantly orange but with green zones (color break II), dark orange (red I), and dark red (red II). The period of time from the fecundation of flowers to the optimun harvest point of the fruits is some 35–40 days. One kilogram of fruit at each stage of ripeness was available. Once the peduncles and seeds had been removed from the samples, they were triturated and 200-g aliquots taken. These aliquots were homogenized, and four 10-gram samples were taken for the extraction and subsequent quantification of pigments.

Pigment Extraction. The process has already been described in previous publications (Mínguez-Mosquera and Hornero-Méndez, 1993). Pigments were extracted from a sample weighing 10 g using Me_2CO, until the complete exhaustion of all color. All extracts were pooled in a separator and shaken with Et_2O. A sufficient quantity of 10% NaCl was added at the end to aid in the separation of the phases. Subsequently, the organic phase

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Table 1. Changes in Concentration (Milligrams per Kilogram of Fresh Weight)[a] of Carotenoid Pigments in the *Bola* Variety with Stage of Ripening

pigment	stage of ripening				
	green	color break I	color break II	red I	red II
neoxanthin	7.23 ± 0.87	0.17 ± 0.10	tr[b]		
capsorubin		4.18 ± 0.82	12.02 ± 1.04	27.75 ± 0.51	62.03 ± 1.72
violaxanthin	9.72 ± 0.83	11.02 ± 1.20	15.83 ± 1.61	24.17 ± 1.50	58.36 ± 2.06
capsanthin 5,6-epoxide		4.05 ± 0.73	7.88 ± 0.95	19.30 ± 1.12	39.97 ± 1.93
capsanthin		22.72 ± 2.01	53.45 ± 2.13	202.78 ± 10.81	522.49 ± 14.05
antheraxanthin	0.28 ± 0.03	1.56 ± 0.08	5.12 ± 0.11	16.13 ± 0.28	32.53 ± 0.51
cis-capsanthin		tr	4.86 ± 0.20	18.71 ± 1.60	59.42 ± 1.83
capsolutein		3.02 ± 0.11	7.33 ± 0.69	30.19 ± 1.29	70.05 ± 1.22
zeaxanthin		6.09 ± 0.47	13.86 ± 0.93	39.80 ± 1.78	46.03 ± 1.36
cis-zeaxanthin		1.32 ± 0.05	2.16 ± 0.04	3.98 ± 0.75	5.15 ± 0.43
lutein	8.89 ± 0.91	2.05 ± 0.07	0.97 ± 0.02		
cis-lutein	1.02 ± 0.01	tr	tr		
β-cryptoxanthin		3.34 ± 0.20	8.02 ± 0.62	30.93 ± 0.83	41.22 ± 2.39
β-carotene	6.43 ± 0.18	7.52 ± 0.24	8.81 ± 0.57	38.10 ± 1.12	53.80 ± 1.96
total carotenoids	33.57 ± 2.85	67.04 ± 6.10	140.31 ± 8.91	451.84 ± 21.60	991.05 ± 29.46
% yellow pigments	100.00	53.84	44.26	40.57	30.99
% red pigments	0.00	46.16	55.74	59.43	69.01

[a] Mean ± standard deviation of four determinations. [b] tr, traces (less than 0.05 mg/kg).

was washed several times with 2% anhydrous Na_2SO_4. This phase contains the pigments in various stages of esterification with fatty acids and can be used for chromatographic purposes once the volume has been reduced by drying under vacuum in a rotary evaporator at 30 °C. An aliquot of the extract is saponified for HPLC quantification of the carotenoid composition in each stage of ripeness.

Identification of Pigments. The pigment identification has been described in detail in a previous publication (Mínguez-Mosquera and Hornero-Méndez, 1993), and consists of the following: separation of pigment by TLC and cochromatography with purified pigments; observation of the pigment color on TLC plates under white, UV_{254nm}, and UV_{360nm} lights with a Desaga UV–vis lamp; recording of UV–visible spectra in different solvents with a Hewlett-Packard UV–vis diode array spectrophotometer Model 8452A and comparison with the values reported in the literature (Foppen, 1971; Davies, 1976; Davies and Köst, 1988); examination of 5,6-epoxide groups investigated by addition of 2% HCl in EtOH; carbonyl and hydroxyl groups investigated by FT-IR spectroscopy using a Bio-Rad FTS-7 IR spectrophotometer and also by acetylation with Ac_2O/Py to test for hydroxyl groups and by reduction with $NaBH_4$ in EtOH to test for carbonyl groups (Davies, 1976; Liaaen-Jensen, 1971).

Thin-Layer Chromatography. The method developed by Mínguez-Mosquera et al. (1984) was used. In this method plates of silica gel 60 GF_{254} (20 × 20 cm plates, thickness 0.7 mm) (Merck, Darmstadt, Germany) were employed with a mixture of hexane–ethyl acetate–ethanol–acetone (95:3:2:2) as developing fluid.

High-Performance Liquid Chromatography. For HPLC analysis a Waters Model 600E quaternary pump was used with a Model 994 diode UV–vis detector (DAD) to obtain the spectra of each peak in the mobile phase as well as its purity. The chromatograph was equipped with a Rheodyne Model 7125 injection valve. A reversed-phase C_{18} column packed with Spherisorb ODS 2 (5 μm, 25 cm × 4 mm i.d.), supplied by Hewlett-Packard, was used. A precolumn (1 cm × 4 mm i.d.) of the same material protected the main column.

Gas Chromatography. For analysis of the fatty acids by gas chromatography a Perkin-Elmer Model 3920B gas chromatograph was used, this being equipped with a FID detector and a HP-3394A integrator. The column used was a Supelcowax 10 fused silica capillary column (30 m, 0.53 mm i.d., 1.0-μm film thickness).

HPLC Separation and Quantification of Carotenoids. Monitoring and quantification of the carotenoid pigments was carried out using a method previously developed by the authors (Mínguez-Mosquera and Hornero-Méndez, 1993). This method uses a C_{18} reversed-phase column and a binary gradient elution system of H_2O–Me_2CO at a flow rate of 1.5 mL/min, a sample injection volume of 5 μL (loop), and detection at 450 nm. Quantification was carried out using β-apo-8′-carotenal (Sigma Chemical Co., St. Louis, MO) as internal standard for calibration. This pigment has the advantages of being absent from the pepper and of being easily separated from the rest of the carotenoids, and, furthermore, its recovery is greater than 98%. Calculation of the response factors from the calibration curves and their values have been described in the previous publication (Mínguez-Mosquera and Hornero-Méndez, 1993). For the separation and quantification of zeaxanthin and lutein the method of Juhler and Cox (1990) was used. This method employs the same reversed-phase column previously described with an isocratic elution system of THF and H_2O (52:48 v/v) at a flow rate of 1 mL/min and detection at 450 nm.

Fatty Acid Analysis. Prior to this stage, each pigment was separated by TLC using the method of Mínguez-Mosquera et al. (1984), which has been previously described. Using these conditions the totally esterified forms of capsanthin, capsorubin, zeaxanthin (β,β-carotene-3,3′-diol), and β-cryptoxanthin (β,β-caroten-3-ol) were clearly separated. Each pigment was scraped from the plate and eluted with acetone. The process was repeated until a sufficient quantity of each pigment was obtained (ca. 100 μg). Subsequently, the solvent was evaporated and the methyl ester forms were derivatized. For derivatization, the esterified pigment was subjected to methanolysis by the addition of 10 mL of a NaMeO solution to a flask containing the pigment dissolved in 1 mL of MeOH. This was refluxed for 10 min. At the end of this time, esterification was carried out by the addition of one drop of phenolphthalein and 10 mL of a HCl–MeOH solution. Again this was refluxed until the indicator changed color. Once the solution had cooled, 1 mL of *n*-hexane and a sufficient amount of a concentrated NaCl solution were added to make the level of the hexane up to the level of the neck of the flask. The chromatographic conditions used were as follows: injection volume, 1.5–2 μL; injector temperature, 250 °C; detector temperature, 250 °C; oven temperature, maintained for 4 min at 195 °C and then raised in 4 min to 250 °C and kept this temperature for 5 min. The carrier gas (helium) flow rate was 1 mL/min.

Reagents. All of the reagents used in the development and application of the HPLC method were of HPLC grade (ROMIL Chemicals, Teknokroma, Barcelona, Spain). For all other purposes analytical grade (ACS) reagents were employed. The solvents used for HPLC were Me_2CO and deionized water, both of which were filtered through a 0.45-μm membrane and degassed prior to being used.

RESULTS AND DISCUSSION

Changes in the Carotenoid Composition during Fruit Ripening. Table 1 shows how the individual and total carotenoid contents of fruits of the *Bola* variety change during the course of ripening. These results agree with those from a previous study (Mínguez-Mosquera and

Hornero-Méndez, 1994) in which the changes occurring in the *Bola* and *Agridulce* varieties of pepper durig rippening were studied in detail. In general, the same conclusion can be drawn from these results as from those obtained from the cited publication. There was *de novo* synthesis of carotenoids, giving rise to an overall 30-fold increase in the carotenoid content. On the one hand, neoxanthin and lutein (the only representative of the β,ϵ series) disappear, while on the other hand, new pigments, principally zeaxanthin, β-cryptoxanthin, capsanthin, and capsorubin, appear. Capsanthin and capsorubin are exclusive to the genus *Capsicum* and are responsible for the red color of the ripe fruit.

Degree of Esterification of the Pigments from Red Pepper. Carotenoid pigments except β-carotene in ripe peppers are esterified with the fatty acids present in this fruit (Curl, 1962; Baranyai et al., 1982). As some of the pigments have one, two, or more OH groups susceptible to esterification, total, partial, or no esterification is possible. This fact gives rise to complex chromatograms, particularly with HPLC, the resolution of which is greater than that of TLC (Gregory et al., 1987; Khachik and Beecher, 1988; Philip and Chen, 1988).

To determine the degree of esterification of the main pigments of peppers, we made use of TLC development from a direct extract [previously described by Mínguez-Mosquera et al. (1984)], from which we had already separated and identified the main bands: β-carotene (band 1, R_f = 0.11); β-cryptoxanthin (band 2, R_f = 0.60); zeaxanthin (band 3, R_f = 0.29); capsanthin (band 4, R_f = 0.37); and capsorubin (band 5, R_f = 0.21). The other pigments remained in a region of low R_f (less than or equal to 0.18), which we called the "base", and will be discussed below. Each separated xanthophyll (capsanthin, capsorubin, zeaxanthin, β-cryptoxanthin) was individually scraped off and eluted with acetone. Three aliquots were taken of each elution. The first was immediately subjected to the acetylation test to determine its grade of esterification. All forms were found to be totally esterified. The second was saponified with KOH–MeOH (10%). Acetylation tests on the resulting pigments showed that they had been totally transformed to nonesterified forms, which remained in the base on TLC development. The third aliquot was partially saponified by an almost instantaneous contact with KOH-MeOH (10%). When developed on the plate, the original pigment, the nonesterified pigment, and an intermediate band were obtained. The latter possibly corresponded to partial esterification states, as confirmed by the acetylation test. Both the nonesterified and the partially esterified forms remained in the base (with R_f less than 0.18).

These results suggest that the base of the TLC chromatogram could be a mixture of partially esterified and nonesterified forms of the same pigments. To test this hypothesis, the following HPLC experiment was performed. Using comparable concentrations, the direct extract of pigments (Figure 1A), the solution of pigments comprising the base (Figure 1B), and the respective saponified extracts (Figures 1C,D) were injected into the HPLC. In addition, the pigments comprising each band of the TLC chromatogram were injected into the HPLC before and after saponification (partial and total) to identify each component. Table 2 shows the correspondence between the pigments developed by TLC and their location in the HPLC chromatogram.

From the results we can deduce the following: (1) With HPLC, the totally esterified forms, i.e., the diesterified forms of capsanthin, capsorubin, and zeaxanthin and the monoesterified forms of β-cryptoxanthin, have longer retention times than β-carotene. (2) The partially esterified forms, i.e., the monoesterified forms of capsanthin, capsorubin, and zeaxanthin, have a narrow range of retention times, between those of nonesterified β-cryptoxanthin (12.63 min) and β-carotene (15.08 min). At shorter retention times (2.04–12.63 min), the pigments appear in their nonesterified form. (3) The base of the development on the plate contains the monoesterified forms of capsanthin, capsorubin, and zeaxanthin and small amounts of the respective nonesterified forms, as shown by the study of the degree of esterification. It also contains a considerable amount of the nonesterified form of β-cryptoxanthin. This is therefore the only major pigment that does not vary its t_R on being saponified (see peak 10 in chromatograms B and D of Figure 1). These points are

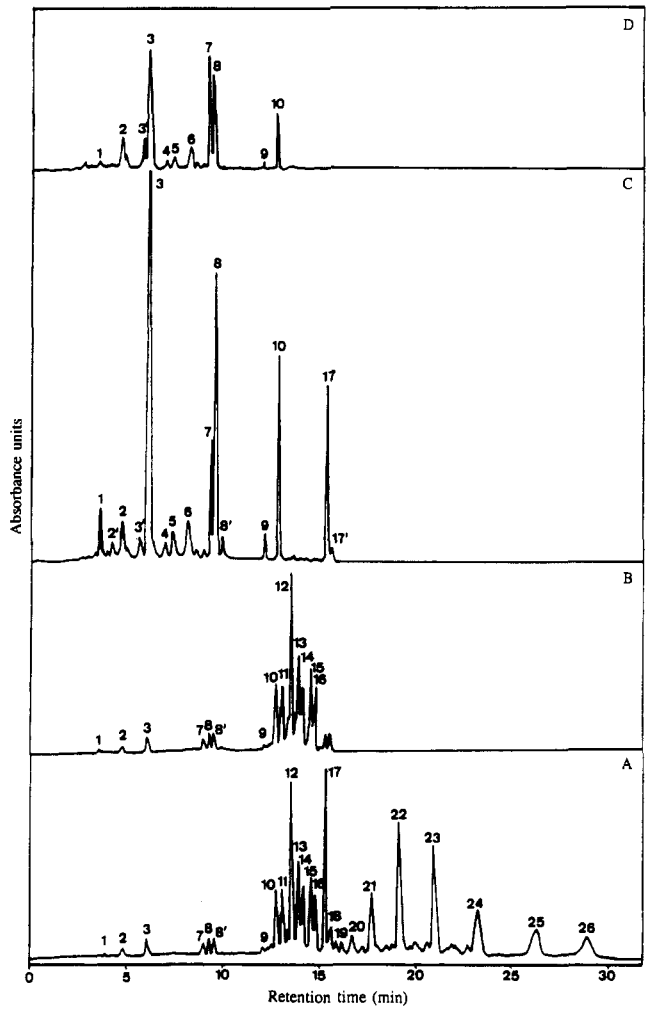

Figure 1. HPLC chromatograms: (A) direct extract; (B) base from a developed TLC plate of a direct extract using as developer 95:3:2:2 hexane–ethyl acetate–ethanol–acetone; (C) saponified direct extract; (D) saponified Base. Peak identity: 1, capsorubin; 2, violaxanthin; 2′, violaxanthin isomer; 3, capsanthin; 3′, capsanthin 5,6-epoxide; 4, antheraxanthin; 5, *cis*-capsanthin; 6, mutatoxanthin; 7, capsolutein; 8, zeaxanthin; 8′, *cis*-zeaxanthin, 9, cryptocapsin; 10, β-cryptoxanthin; 11, capsanthin monoester (capsorubin monoester); 12, capsanthin monoester (capsorubin monoester and zeaxanthin monoester); 13, capsanthin monoester; 14, zeaxanthin monoester; 15, capsanthin monoester; 16, zeaxanthin monoester; 17, β-carotene; 17′, *cis*-β-carotene (β-cryptoxanthin monoester); 18, β-cryptoxanthin monoester; 19, capsorubin diester (β-cryptoxanthin monoester); 20, capsorubin diester; 21, capsanthin diester (capsorubin diester); 22, capsanthin diester (capsorubin diester); 23, capsanthin diester; 24, capsanthin diester (zeaxanthin diester); 25, zeaxanthin diester; 26, zeaxanthin diester.

Table 2. TLC and Reversed-Phase HPLC Localization of the Esterification Forms of the Main Pigments from Red Pepper[a]

pigment	degree of esterification	pigment localization in TLC[b] (band)	pigment localization in HPLC[c] (peaks)
β-carotene	none	1	17, 17′
β-cryptoxanthin	monoesterified	2	18
	nonesterified	base	10
zeaxanthin	diesterified	3	25, 26
	monoesterified	base	14, 16
	nonesterified	base	8, 8′
capsanthin	diesterified	4	21, 22, 23, 24
	monoesterified	base	11, 12, 13, 15
	nonesterified	base	3
capsorubin	diesterified	5	19, 20
	monoesterified	base	11, 12
	nonesterified	base	1

[a] Support in TLC: silica gel 60 GF254. Solvent system of TLC: hexane–ethyl acetate–ethanol–acetone (95:3:2:2). [b] R_f values of bands are given under Results and Discussion. [c] These peaks are the most prominent in the chromatogram.

Table 3. Percent Composition of Fatty Acids Esterifying the Main Xanthophylls

fatty acid	xanthophyll: β-cryptoxanthin	zeaxanthin	capsanthin	capsorubin
lauric	9.7	8.6	26.5	26.0
myristic	18.2	19.1	48.3	47.4
palmitic	23.6	21.7	17.6	18.0
palmitoleic	tr[a]	tr	tr	tr
stearic	tr	tr	tr	tr
oleic	2.4	1.8	tr	tr
linoleic	48.4	50.6	7.5	8.6
linolenic	tr	tr	tr	tr

[a] tr, trace (≤0.5%).

in accordance with the previous results of Fisher and Kocis (1987) and of other authors.

In addition, by a similar analytical process, it was shown that violaxanthin and neoxanthin are not esterified during ripening, although the latter disappears together with lutein during the process (Davies et al., 1970; Mínguez-Mosquera and Hornero-Méndez, 1994). This fact, which agrees with the observations of other authors, supports the theory that esterification only occurs at the level of the carotenoids which are newly formed in the chromoplasts and does not occur in those in the chloroplasts (Camara and Monéger, 1978). This suggests that as the chromoplasts form at the expense of the chloroplasts, the carotenoids pass into the plastoglobules which also contain fatty acids and which take part in the esterification. In the pepper, distinct types of chromoplasts have been observed, principally globular, membraneous, and fibrillar. The first type is the most important, this being the spherical or ovoid type which contains an abundance of plastoglobules with fatty acids and carotenoids (Gross, 1991).

Fatty Acids Esterifying the Main Xanthophylls. The results obtained from fruits of the *Bola* variety in the ripe red stage are shown in Table 3. In this table, the fatty acids chiefly responsible for the esterification of each of the principal carotenoid pigments of the fruit (capsanthin, capsorubin, zeaxanthin, and β-cryptoxanthin) are shown. These fatty acids are lauric (12:0), myristic (14:0), palmitic (16:0), and linoleic ($18:2^{\Delta 9,12}$) acids, in agreement with the observations of other authors (Philip et al., 1971; Camara and Monéger, 1978; Tsatsaronis and Kehayoglou, 1971).

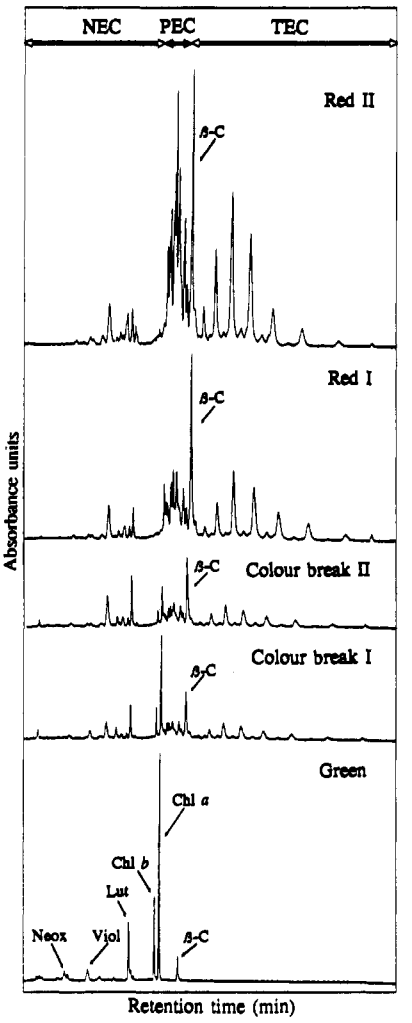

Figure 2. Changes in pigment composition and xanthophyll esterification during ripening of pepper fruits of the *Bola* variety. NEC, nonesterified carotenoids; PEC, partially esterified carotenoids; TEC, totally esterified carotenoids. Peak identity is as in Figure 1A, in the green stage: Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Lut, lutein; Viol, violaxanthin; Neox, neoxanthin; β-C, β-carotene.

Furthermore, small amounts of other fatty acids, such as palmitoleic ($16:1^{\Delta 9}$), oleic ($18:1^{\Delta 9}$), stearic (18:0), and linolenic ($18:3^{\Delta 9,12,15}$) acids, are found, although these do not represent more than 2% of the total fatty acids and so will not be taken into account.

In Table 3, it can be seen that the red pigments capsorubin and capsanthin are esterified with short-chain saturated fatty acids (chiefly lauric, myristic, and palmitic), while in the yellow carotenoids the main fatty acid (approximately 50%) has two double bonds (linoleic acid). This supports the findings of other authors (Daood and Biacs, 1986; Gross, 1991) with respect to stability and makes it easy to understand the greater stability of the red pigments. At the same time, the greater stability of the esterified pigments seem to be related to their more lipophilic nature and, hence, to their better integration into membrane structures in which they are less susceptible to adverse conditions in their environment.

Changes in Esterification during Fruit Ripening. As mentioned previously, in the ripe red fruit the carotenoids are esterified, to a greater or lesser extent, with fatty acids, nonesterified, partially esterified, and totally esterified forms being found. Experiments on different esterified and nonesterified forms of capsanthin (Biacs et al., 1989) have shown that the esterified forms are more

Table 4. Percentage of Esterification of Xanthophylls during the Fruit Ripening of *C. annuum* Cv. *Bola*

	stage of ripening				
carotenoid fraction	green	color break I	color break II	red I	red II
nonesterified	100.0	49.6	46.2	32.6	21.3
partially esterified	0.0	9.8	16.6	24.6	35.6
totally esterified	0.0	40.6	37.2	42.7	43.1
total carotenoid content (mg/kg of fr wt)	33.6	67.1	140.3	451.8	991.1

stable than the nonesterified forms. The greater stability is found in the totally esterified pigments. As these are present in the highest amounts, it is evident that nature has not esterified these compounds without good reason. Furthermore, it has also been reported (Biacs et al., 1989) that those pigments which are esterified with saturated fatty acids are more stable, being less easily attacked by photo- and thermo-oxidative processes and by other processes in which the enzyme lipoxygenase is involved.

The changes in the degree of esterification have been studied in five successive stages of ripening (green or unripe stage, color breaks I and II, red I and II) of peppers of the *Bola* variety. The changes occurring in the free fraction and in the partially and totally esterified fractions of the carotenoid pigments during ripening are clearly illustrated in Figure 2. The chromatograms in this figure correspond to the five successive stages of ripening studied and have been obtained using directly comparable conditions. Table 4 shows the results obtained from these chromatograms. The results have been calculated from the area under each peak, each fraction being expressed as a percentage of the total carotenoids.

It can be deduced from these results that from the very first stages of ripening, approximately half of the carotenoid pigments present are esterified to the maximum degree possible and that as ripening proceeds this tendency is maintained: the proportion of monoesterified forms increases while that of the free forms decreases. At the same time there is a net synthesis of pigments, which progressively become esterified. The percentages of the free carotenoid pigments and the partially and totally esterified forms of these in the ripe fruit are 21.3%, 35.6%, and 43.1%, respectively. These results could be of use in future studies as indices of the physiological maturity of the fruit.

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