Structure−Reactivity Relationship in the Oxidation of Carotenoid Pigments of the Pepper (*Capsicum annuum* L.)

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The relationship between the degradation rate and structure of each pigment of the pepper carotenoid profile was studied in mixtures of dehydrated fruit with lipid substrates of differing degrees of unsaturation and in different proportions (20 and 40%). The differences in structural nature of the carotenoids present in the pepper fruit produce a variable rate of oxidation, resulting in nonuniform degradation. The yellow xanthophylls and β-carotene have the highest rates of oxidation, with the ketocarotenoids and violaxanthin degrading at lower rates. Autoxidation is greater or lesser depending on the functional groups, which stabilize the radical intermediaries of the reaction. The behavior of capsanthin and capsorubin is that expected of carotenoids having structures that include keto groups: a markedly greater stability to autoxidation processes. This increases their antioxidant capacity, adding to their beneficial impact by reducing the proliferation of radical processes, which are detrimental to health.

Keywords: *Carotenoid; lipid substrate; oxidation; paprika; reactivity; structure*

INTRODUCTION

The main organoleptic characteristic of the pepper (*Capsicum annuum* L.) is color, an attribute of the carotenoid profile of the fruit. Its commercial derivatives—paprika and oleoresin—are widely used, apart from their various culinary uses, to correct or supply color in other foodstuffs (*1*). This property is important not only in the commercial evaluation of paprika and oleoresin but also during storage, when the initial color of these products must be either retained or acceptably stable (*2*). The need to maintain coloring capacity—and thus the carotenoid profile—and the types of reaction, intimately related with the structural nature of the carotenoids, that cause it to disappear have been the subject of research (*3*). It is in fact their chain of conjugated double bonds that allows carotenoids to be degraded via oxidation processes originated by reactive species (singlet oxygen, free radicals often generated during lipid peroxidation, etc.) that are added to the polyenoic chain: a structure identical in and common to all of the carotenoids present in the pepper (*4*, *5*).

The effect—antioxidation or autoxidation—of the mechanism depends on the conditions of oxygen tension under which the pigment degradation takes place and on other structural properties of the pigment (*6*, *7*). Thus, the presence of functional groups (hydroxyl, epoxide, or keto) and the type of ring located at the ends of the polyenoic chain (β or κ in the case of pepper carotenoids) can vary the rate of pigment oxidation. Terao (*8*) attributes the greater antioxidant effect (and thus the lower rate of oxidation) of canthaxanthin and astaxanthin, compared with β-carotene, to the presence of the keto groups in the xanthophyll structure. The rate of other, secondary, mechanisms of oxidation also depends on structural features involving the cyclic end-groups (allyl hydrogen abstraction and addition to the ring) (*9*).

Earlier works have determined that modifying the nature (from poly-unsaturated to mono-unsaturated) of the lipid fraction surrounding and impregnating the carotenoid series reduces their oxidation rates (*10*, *11*). Those experiments, and the concept that the pepper carotenoid profile is divided into two isochromic fractions (red and yellow), have established that the structural nature determines a different rate of oxidation, with the red fraction showing a greater stability. The structural feature distinguishing the fractions is the existence, or not, of keto functional groups. However, the carotenoid profile of the pepper fruit presents more complex structural differences. Figure 1 shows the structure of major carotenoids present in red pepper fruits. The yellow fraction includes β-carotene (a hydrocarbon), β-cryptoxanthin and zeaxanthin (mono- and dihydroxylated carotenoids), and cucurbitaxanthin A and violaxanthin (epoxidized carotenoids). The yellow isochromic fraction thus includes carotenoids having different degrees of oxidation. In the red fraction, the structural difference is the substitution of one or both 3-hydroxy-β rings (present in the yellow xanthophylls) by one, or two, 3-hydroxyacylcyclopentane rings (*12*, *13*). Such diverse and attractive structural features of the carotenoid profile can be used to broaden the knowledge of the relationship between structure and reactivity, by monitoring individual changes during an oxidation process. Nonuniformity in pigment oxidation will probably be found not only between one fraction and the other but also among components of the same isochromic group.

The aim of the present work is to analyze the degradation of the pepper carotenoid profile, using changes in individual carotenoids to reveal relationships between their oxidation and structural nature. Because the degradative reaction, in which pigment−fatty acid

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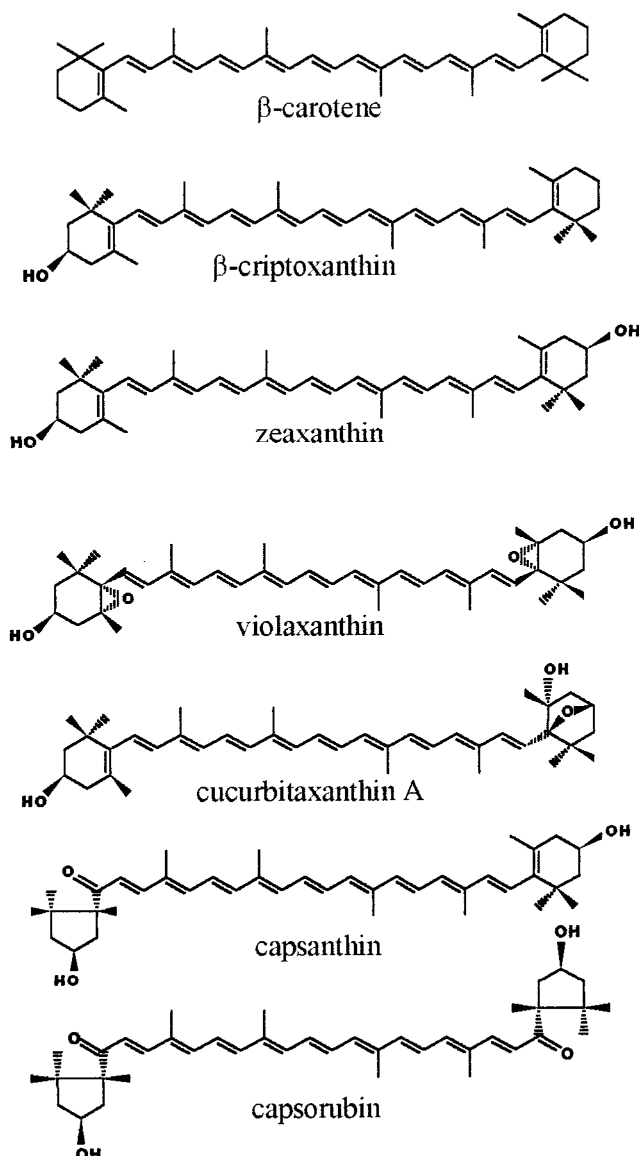

Figure 1. Chemical structures of major carotenoids present in red pepper fruits (*C. annuum* L.).

interactions are responsible for the lipid peroxidation processes, takes place in a lipid environment, two lipid substrates are considered, with differing degrees of unsaturation (poly-unsaturated and mono-unsaturated).

MATERIALS AND METHODS

Samples. The pigment concentrate employed comes from a mixture of dehydrated pepper fruit and seeds in different proportions to supply the poly-unsaturated (pepper seeds) or mono-unsaturated (high-oleic sunflower seeds) lipid substrate. The dehydrated fruits are sliced, separating pericarp, stalk, and seeds. The appropriate amounts of dried pepper pericarp and lipid (mono-unsaturated or poly-unsaturated) substrate are weighed to obtain mixtures containing 20 and 40% (by weight) of each lipid substrate. Approximately 100 g of each mixture is obtained by milling in a hammer mill to a particle size of <500 μm.

Monitoring the Oxidation Reaction. The prepared mixtures are subjected to heat as physical agent to initiate oxidation reactions of the lipid substrate and consequently of the carotenoid content. Earlier assays (*14*) established 70 °C as the most appropriate temperature. Each mixture of dehydrated fruit and lipid substrate (in the different proportions employed) is placed on a Petri dish and stored in an oven at a constant temperature of 70 °C throughout the experiment. Initial samplings are done each 24 h, then at increasingly longer periods (48, 72, and 96 h), with the last two at 168 and 216 h. In this way, a minimum of 80% of the course of the degradation reaction is monitored. The total number of time data for the oxidation reaction is 14, each involving a sampling in quadruplicate—a total of 56 analyses for each mixture assayed. The weight of sample analyzed varies because the pigment concentration decreases during the course of the reaction. The initial weight is ~1 g of sample, increasing gradually to 2 g at the end of the experiment. The sample is placed in a quartz flask, 50 mL of acetone/water (75:25, analytical grade) is added, and the flask is kept in a refrigerator at −20 °C and in darkness until analysis.

Extraction and De-esterification of Carotenoid Pigments. The analysis of the carotenoid composition in each sample requires an extraction with organic solvent followed by de-esterification of the carotenoid fraction (*15*). The sample is homogenized with 50 mL of acetone (analytical grade) and the pigmentation extracted by adding repeated identical volumes of acetone until the filtrates are colorless. The colored filtrate is placed in a decanting flask, and 150 mL of ethyl ether (analytical grade) and 200 mL of 10% (w/v) sodium chloride solution are added. Any pigmentation is transferred to this phase. The process is repeated. All of the organic phases containing carotenoids are combined in the same flask and washed several times with 200 mL of 2% (w/v) anhydrous sodium sulfate solution.

At the start of saponification, 1 mL of a standard solution of *β*-apo-8′-carotenal in 40−60 °C light petroleum ether (concentration between 150 and 200 μg/mL) is added for the subsequent quantification, 50 mL of 10% potassium hydroxide in methanol is added, and the mixture is stirred vigorously. After 1 h of reaction at room temperature, 200 mL of 10% sodium chloride in water is added, and the aqueous and organic phases are left to separate. The aqueous phase is discarded, and the organic phase is washed several times with 200 mL of distilled water until the washings are neutral. The organic phase, containing the pigmentation, is filtered through a solid bed of anhydrous sodium sulfate, and the filtrate is evaporated to dryness under vacuum. The residue is dissolved in 10 mL of acetone (HPLC-grade) and stored at −20 °C until its analysis by HPLC.

Pigment Separation and Quantification by HPLC. The carotenoid profile of the extract is quantified using *β*-apo-8′-carotenal as internal standard (*13*). Chromatographic separation is verified on a reversed phase column (Spherisorb C_{18} ODS2; particle size = 5 μm; and 250 mm × 4 mm i.d.). The eluent comprises a binary gradient (acetone/water at an initial proportion of 75:25) at a constant flow rate of 1.5 mL/min. The gradient is initially with that mixture for 5 min and then linearly to a composition of 95:5 in 5 min, keeping this proportion for 7 min. At the end of the analysis, the column is washed with acetone for 3 min and returned to the initial conditions. The volume of sample injected is 20 μL. Detection was performed at 450 nm with a photodiode array detector.

Kinetic Study of the Data. The oxidation reaction transforms the carotenoid pigments into one or more degradation products that are colorless or of lower coloring capacity than the initial reactant. The kinetic parameters (order of reaction n and kinetic constant κ) are determined according to the integral method (*16*). This method uses a procedure of trial and error, in which the reaction order of the rate equation (E-1) is initially assumed.

$$-(dC_p/dt) = \kappa[C_p]^n \tag{E-1}$$

With the assumed order, eq E-1 is integrated, giving a linear expression relating C_p (carotenoid concentration expressed as percentage of retention) with time t. From the expression best representing the changes in the experimental data with reaction time, the order (assumed ab initio) is verified and the kinetic constant κ obtained.

Table 1. Initial Carotenoid Composition (Milligrams per Kilogram) of Mixtures Prepared with Dehydrated Pepper Fruit and Lipid Substrate of Different Degrees of Unsaturation

	poly-unsaturated		mono-unsaturated	
pigment	20%	40%	20%	40%
capsorubin	202.9[a]	154.2	215.7	180.8
violaxanthin	241.8	169.5	230.5	142.8
capsanthin 5,6-epoxide	205.1	104.0	151.3	110.0
capsanthin	1161.9	893.0	1173.5	873.0
cis-capsanthin	298.1	217.1	275.9	212.5
cucurbitaxanthin A	194.6	170.0	211.2	169.5
zeaxanthin	237.8	181.1	259.6	179.1
cis-zeaxanthin	33.7	29.4	43.1	31.0
β-cryptoxanthin	257.3	184.1	263.6	192.8
β-carotene	236.8	163.0	244.7	174.0
cis-β-carotene	67.9	48.2	63.5	27.4
red fraction[b]	1868.0	1368.3	1816.4	1376.2
yellow fraction[b]	1269.9	945.3	1316.2	916.6
total[c]	3137.9	2313.6	3132.6	2292.8

[a] Mean values, $n = 4$. [b] Red fraction = capsorubin + capsanthin and isomers + epoxide of capsanthin. Yellow fraction = violaxanthin + cucurbitaxanthin A + zeaxanthin and isomer + β-cryptoxanthin + β-carotene and isomer. [c] Total = red + yellow.

RESULTS AND DISCUSSION

Table 1 shows the individual carotenoid composition of each mixture prepared with the lipid substrates of different degrees of unsaturation. The carotenoid composition has capsanthin as major pigment, with the concentration levels of the rest of the yellow xanthophylls and β-carotene being lower but similar to each other. Tables 2 and 3 show the data for the changes in each pigment in the prepared mixtures (20 and 40%, respectively) with lipid substrates of different unsaturation levels: poly-unsaturated and mono-unsaturated. Expression of the results as percentage of retention enables direct comparison of the alteration in each carotenoid. In general, no pigment shows either a marked decrease in concentration or a specific maintenance against degradation; all follow a degradative path of the same order, although different individual trends are apparent. During the first 72 h, there are no decreases exceeding 20%, except in the pigments capsanthin, β-carotene, and (particularly) zeaxanthin, which in some mixtures exceed a 30% loss of carotenoid retention. These pigments not only are degraded to colorless products but also undergo a parallel isomerization to their corresponding *cis* forms. Figures 2 and 3 show the variation of the *cis* isomers of capsanthin and β-carotene, respectively, in the mixtures prepared with 40% of poly-unsaturated (A) and mono-unsaturated (B) lipid substrate. It can be observed that in a first stage, the presence of isomers increases—to levels >180% in the case of β-carotene in the mono-unsaturated lipid environment. It can be deduced that in this stage, isomerization is the preferential reaction, relegating degradation reactions to second place. This situation continues through to later periods of the experiment, although, from the changes in the *cis* isomers, the rate of isomerization falls below that of oxidation and, finally, only relatively sharp decreases are seen in the isomer fraction.

From 168 h of reaction, there is a second stage in which degradation reactions are preferential. Capsorubin has the lowest loss of retention in all cases, at ~50–55% loss after 360 h, followed by violaxanthin, with slightly higher losses. Of the rest, β-carotene stands out with a general 70% loss in its initial retention. The lack of uniformity in the degradation of the carotenoid profile to colorless products continues to the end of the experiment and is associated with the different features in the structure of each pigment.

Table 2. Changes in the Carotenoid Profile during the Oxidation of the Mixtures with 20% of Lipid Substrate Added[a]

time (h)	capsorubin	capsanthin	violaxanthin	cucurbitaxanthin A	zeaxanthin	β-cryptoxanthin	β-carotene
			Poly-unsaturated Substrate				
0	100.0 ± 2.1[a]	100.0 ± 2.3	100.0 ± 2.1	100.0 ± 0.9	100.0 ± 3.4	100.0 ± 3.0	100.0 ± 1.6
24	100.0 ± 3.1	87.2 ± 0.9	99.2 ± 3.4	100.0 ± 2.5	87.7 ± 1.6	100.0 ± 1.9	93.3 ± 2.8
48	94.5 ± 0.5	75.6 ± 1.0	100.0 ± 1.4	92.5 ± 3.9	81.7 ± 1.1	93.8 ± 1.4	82.4 ± 2.7
72	89.3 ± 1.6	68.6 ± 3.4	92.1 ± 2.6	93.7 ± 2.1	80.9 ± 2.2	92.7 ± 2.6	78.6 ± 2.1
120	88.3 ± 2.1	66.3 ± 1.1	87.2 ± 3.4	79.5 ± 1.5	66.9 ± 3.0	76.8 ± 1.6	63.0 ± 2.6
168	72.1 ± 2.1	60.8 ± 1.4	71.0 ± 2.3	59.9 ± 2.5	55.0 ± 1.2	62.1 ± 0.3	53.6 ± 2.3
216	74.7 ± 3.3	53.6 ± 2.6	57.5 ± 3.1	54.8 ± 3.6	53.6 ± 3.3	61.2 ± 4.5	50.9 ± 3.7
288	68.2 ± 2.4	43.2 ± 2.9	51.0 ± 2.9	41.1 ± 4.7	44.2 ± 1.1	40.9 ± 1.8	36.1 ± 1.3
360	44.7 ± 2.5	26.2 ± 0.5	30.9 ± 1.8	24.7 ± 2.5	22.8 ± 2.3	24.4 ± 1.6	23.6 ± 1.1
456	39.1 ± 1.1	21.1 ± 1.0	28.6 ± 0.4	17.5 ± 1.2	16.4 ± 0.7	17.1 ± 0.8	17.9 ± 0.8
552	36.6 ± 3.5	14.0 ± 0.3	21.0 ± 1.5	12.6 ± 1.1	10.9 ± 0.6	10.5 ± 0.9	10.6 ± 0.6
648	26.6 ± 0.5	11.7 ± 0.2	16.3 ± 0.7	7.9 ± 1.4	7.3 ± 1.1	7.4 ± 0.4	7.2 ± 0.3
816	16.1 ± 0.1	5.9 ± 0.3	8.1 ± 1.3	4.3 ± 0.1	3.8 ± 0.4	3.2 ± 0.1	3.2 ± 0.1
1032	13.6 ± 1.9	3.7 ± 0.1	4.9 ± 0.3	1.5 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.0 ± 0.1
			Mono-unsaturated Substrate				
0	100.0 ± 2.3	100.0 ± 2.4	100.0 ± 1.0	100.0 ± 0.7	100.0 ± 2.4	100.0 ± 4.3	100.0 ± 3.7
24	98.4 ± 2.6	84.3 ± 2.6	96.7 ± 3.7	100.0 ± 0.5	81.0 ± 2.9	97.9 ± 3.5	82.0 ± 2.6
48	88.1 ± 1.9	75.0 ± 2.4	86.4 ± 4.4	90.0 ± 1.0	73.4 ± 0.7	86.2 ± 0.4	73.3 ± 0.1
72	86.5 ± 1.2	71.0 ± 1.7	82.6 ± 2.1	93.5 ± 2.2	73.7 ± 2.6	86.7 ± 2.7	71.9 ± 0.3
120	78.8 ± 2.7	65.8 ± 2.4	73.7 ± 2.2	68.2 ± 3.4	54.0 ± 2.6	68.8 ± 2.8	59.7 ± 3.0
168	71.1 ± 2.3	60.9 ± 4.2	68.4 ± 3.6	63.1 ± 0.8	54.3 ± 1.6	65.0 ± 0.7	53.8 ± 0.4
216	71.9 ± 1.9	53.0 ± 1.2	64.9 ± 3.2	66.0 ± 0.7	49.1 ± 1.2	61.3 ± 0.8	49.3 ± 0.4
288	64.1 ± 3.3	41.3 ± 3.8	46.0 ± 1.9	54.3 ± 3.5	45.5 ± 3.9	49.0 ± 3.1	39.7 ± 1.7
360	47.0 ± 0.9	34.2 ± 0.8	38.3 ± 1.6	37.1 ± 0.9	31.0 ± 0.7	34.2 ± 0.9	34.3 ± 0.5
456	41.5 ± 1.4	26.3 ± 0.6	31.4 ± 1.5	33.4 ± 1.6	26.0 ± 1.2	28.1 ± 1.4	27.4 ± 1.1
552	39.9 ± 0.9	25.6 ± 2.4	30.9 ± 1.8	29.7 ± 1.8	25.5 ± 1.4	27.5 ± 1.1	24.3 ± 1.1
648	37.4 ± 1.3	22.9 ± 1.9	27.3 ± 0.2	24.3 ± 0.9	20.4 ± 0.4	21.0 ± 1.0	22.8 ± 1.3
816	31.5 ± 0.3	18.1 ± 1.9	18.8 ± 0.4	17.4 ± 0.8	14.6 ± 0.8	15.0 ± 0.9	11.3 ± 0.7
1032	27.2 ± 1.6	16.1 ± 1.0	16.9 ± 1.2	14.4 ± 1.1	12.2 ± 0.9	11.8 ± 0.9	9.9 ± 0.1

[a] Data expressed as percentage of retention. Mean value ± standard error.

Table 3. Changes in the Carotenoid Profile during the Oxidation of the Mixtures with 40% of Lipid Substrate Added[a]

time (h)	capsorubin	capsanthin	violaxanthin	cucurbitaxanthin A	zeaxanthin	β-cryptoxanthin	β-carotene
			Poly-unsaturated Substrate				
0	100.0 ± 1.8[a]	100.0 ± 1.9	100.0 ± 0.6	100.0 ± 2.5	100.0 ± 2.2	100.0 ± 2.0	100.0 ± 0.3
24	96.6 ± 3.1	89.3 ± 0.3	100.0 ± 0.5	100.0 ± 1.7	92.7 ± 4.2	100.0 ± 1.6	90.9 ± 3.7
48	91.3 ± 1.4	87.3 ± 1.5	95.4 ± 1.3	92.9 ± 3.1	79.7 ± 1.1	95.5 ± 1.5	81.5 ± 1.6
72	85.9 ± 2.9	76.1 ± 1.3	90.7 ± 2.6	89.7 ± 1.9	85.4 ± 3.0	92.6 ± 3.4	78.0 ± 2.9
120	79.6 ± 2.4	67.5 ± 3.1	81.8 ± 2.3	80.9 ± 5.8	68.2 ± 4.2	81.1 ± 4.2	66.9 ± 1.2
168	72.3 ± 6.0	61.4 ± 2.6	53.1 ± 2.5	58.0 ± 1.3	56.3 ± 3.9	68.4 ± 0.5	58.5 ± 0.5
216	64.5 ± 3.7	50.9 ± 0.5	46.0 ± 0.4	49.5 ± 1.4	49.1 ± 2.3	57.2 ± 2.5	46.6 ± 2.4
288	63.4 ± 3.2	44.8 ± 1.9	40.1 ± 1.9	42.8 ± 3.4	40.9 ± 3.3	45.2 ± 0.5	37.1 ± 2.2
360	56.4 ± 2.5	29.2 ± 0.2	37.5 ± 1.4	29.5 ± 0.6	26.0 ± 0.6	27.5 ± 0.5	24.2 ± 2.0
456	37.3 ± 1.4	19.7 ± 0.3	25.8 ± 2.9	17.6 ± 0.8	16.4 ± 1.7	16.7 ± 2.2	14.3 ± 0.3
552	25.9 ± 1.9	13.2 ± 0.9	20.4 ± 1.1	9.7 ± 0.6	9.4 ± 0.5	7.4 ± 0.4	7.5 ± 0.5
648	14.0 ± 0.7	6.7 ± 0.5	16.3 ± 1.0	5.3 ± 0.3	3.6 ± 0.5	2.8 ± 0.1	2.8 ± 0.1
816	8.1 ± 0.7	2.1 ± 0.1	6.4 ± 1.5	1.5 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.3 ± 0.1
1032	5.4 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	–[b]	–	–	–
			Mono-unsaturated Substrate				
0	100.0 ± 2.9	100.0 ± 1.7	100.0 ± 1.0	100.0 ± 0.7	100.0 ± 2.4	100.0 ± 0.9	100.0 ± 0.3
24	97.2 ± 0.5	88.9 ± 1.6	97.9 ± 1.6	97.0 ± 1.3	92.5 ± 0.5	100.0 ± 2.4	79.6 ± 1.4
48	95.9 ± 3.7	82.3 ± 0.7	98.3 ± 2.8	94.9 ± 0.6	86.6 ± 0.8	98.3 ± 4.6	71.9 ± 1.1
72	89.0 ± 3.7	78.1 ± 0.7	95.0 ± 1.9	96.5 ± 0.9	83.4 ± 2.3	96.4 ± 2.6	66.4 ± 0.3
120	83.3 ± 1.3	75.1 ± 3.7	80.4 ± 4.7	81.9 ± 4.3	76.3 ± 4.5	87.9 ± 0.4	57.6 ± 0.6
168	76.6 ± 1.4	73.8 ± 2.9	74.6 ± 2.2	73.8 ± 2.8	70.3 ± 1.0	79.3 ± 1.0	50.7 ± 1.2
216	70.6 ± 0.9	66.0 ± 1.2	73.5 ± 2.1	71.4 ± 3.2	67.6 ± 3.3	82.0 ± 1.2	49.1 ± 0.6
288	63.1 ± 2.1	51.3 ± 4.2	60.6 ± 2.1	54.9 ± 2.9	56.1 ± 2.9	62.9 ± 5.7	40.1 ± 3.5
360	56.9 ± 2.5	42.9 ± 0.1	56.6 ± 1.2	44.2 ± 1.0	41.6 ± 0.1	45.4 ± 1.3	34.5 ± 1.3
456	45.7 ± 0.8	35.3 ± 2.6	47.8 ± 0.6	36.9 ± 1.1	34.1 ± 0.1	36.4 ± 0.2	28.6 ± 2.9
552	40.9 ± 1.7	28.6 ± 2.2	38.2 ± 2.4	29.4 ± 4.7	29.5 ± 2.3	29.0 ± 4.1	24.8 ± 0.9
648	37.5 ± 1.0	25.9 ± 0.8	36.0 ± 0.9	28.6 ± 0.3	26.7 ± 0.5	27.6 ± 0.6	18.8 ± 2.4
816	33.9 ± 0.3	23.3 ± 1.6	30.9 ± 0.2	23.4 ± 1.6	22.3 ± 0.3	22.3 ± 0.1	13.1 ± 0.1
1032	28.1 ± 2.2	21.2 ± 0.5	21.7 ± 1.2	16.1 ± 0.6	15.4 ± 0.4	14.9 ± 0.3	8.5 ± 0.1

[a] Data expressed as percentage of retention. Mean value ± standard error. [b] Not detected.

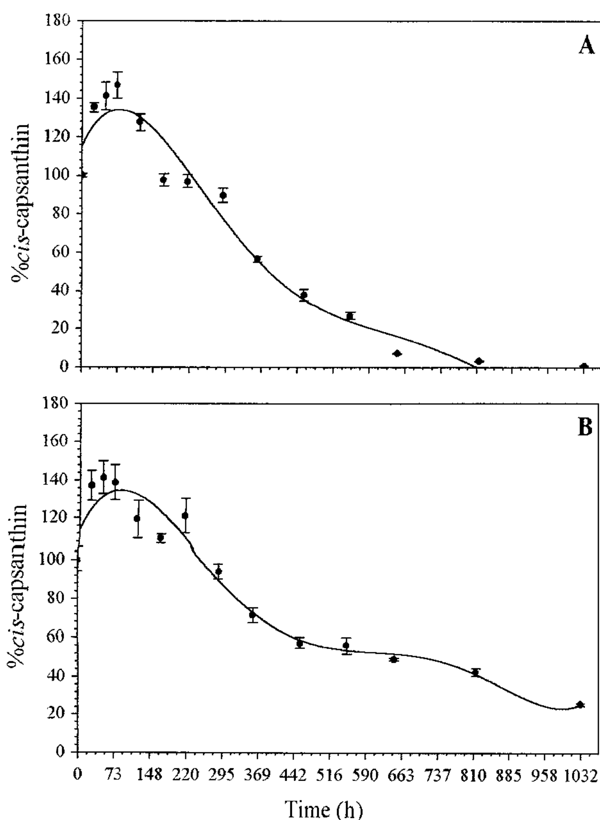

Figure 2. Changes in the percentage of retention of the *cis* isomers of capsanthin during the degradation of carotenoids in the 40% mixture with poly-unsaturated (A) and mono-unsaturated (B) lipid substrate.

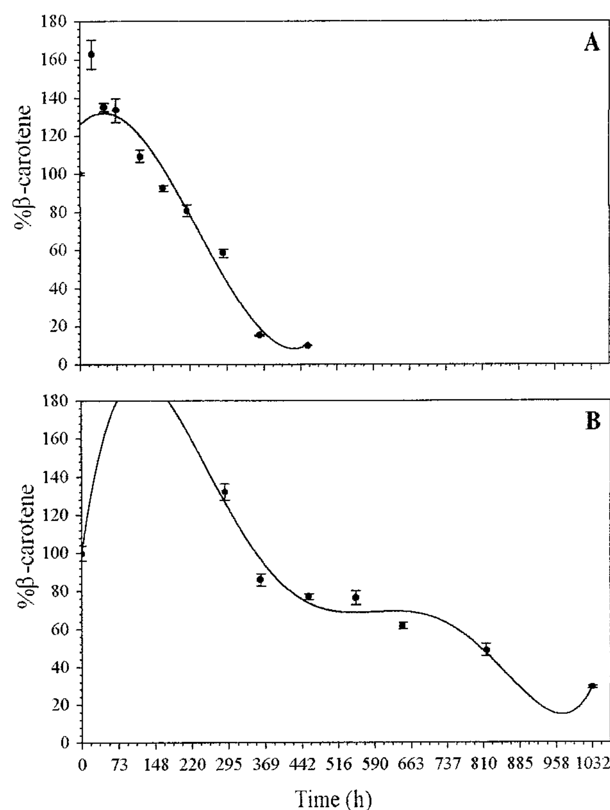

Figure 3. Changes in the percentage of retention of the *cis* isomers of β-carotene during the degradation of carotenoids in the 40% mixture with poly-unsaturated (A) and mono-unsaturated (B) lipid substrate.

These differences in the oxidation of the pepper carotenoid fraction are established by calculating the kinetic parameters (order of reaction and kinetic constant κ) using the integral method. The parameters best

Table 4. Kinetic Parameters of the Oxidation Reaction of Pepper Carotenoid Pigments in Lipid Substrates with Different Degrees of Unsaturation

	20% lipid substrate				40% lipid substrate			
	poly-unsaturated		mono-unsaturated		poly-unsaturated		mono-unsaturated	
pigment	kinetic constant ($\kappa \pm$ SE) $\times 10^{-4}$ [a]	R	kinetic constant ($\kappa \pm$ SE) $\times 10^{-4}$ [a]	R	kinetic constant ($\kappa \pm$ SE) $\times 10^{-4}$ [a]	R	kinetic constant ($\kappa \pm$ SE) $\times 10^{-4}$ [a]	R
β-carotene	39.40 ± 0.91	0.995	25.27 ± 0.73	0.987	48.38 ± 1.74	0.983	25.76 ± 0.51	0.993
β-cryptoxanthin	39.34 ± 0.82	0.991	23.18 ± 0.62	0.985	49.42 ± 0.56	0.972	19.13 ± 0.94	0.983
zeaxanthin	38.95 ± 0.75	0.995	24.09 ± 0.75	0.979	49.77 ± 1.80	0.980	19.70 ± 0.64	0.989
cucurbitaxanthin A	38.74 ± 1.27	0.992	21.11 ± 0.55	0.982	47.65 ± 0.68	0.976	18.89 ± 0.81	0.985
violaxanthin	28.89 ± 0.78	0.991	20.19 ± 0.84	0.971	36.52 ± 0.78	0.954	15.35 ± 0.62	0.990
capsanthin	33.43 ± 0.60	0.995	22.05 ± 1.04	0.965	44.26 ± 1.67	0.977	18.63 ± 0.87	0.966
capsorubin	20.02 ± 0.66	0.985	14.85 ± 0.66	0.968	27.18 ± 1.14	0.976	14.03 ± 0.51	0.982

[a] First-order reaction, kinetic model: $\ln(\%\ \text{retention}) = 4.605 - \kappa t$. SE, standard error.

representing the data are those for a first-order kinetics model for all of the pigments and in all of the mixtures assayed. Table 4 displays the results of the kinetic study by percentage and type of lipid substrate (poly-unsaturated or mono-unsaturated).

In general, the kinetic constant κ for the pigment group comprising β-carotene and the yellow xanthophylls (except violaxanthin) remains at a similar level, with cucurbitaxanthin A always showing the lowest values. The rest of the pigments—violaxanthin and the ketocarotenoids capsanthin and capsorubin—are degraded at rates that are similar but, as a whole, always lower than the first group. Thus, two pigment groups of similar autoxidation levels can be established, although, in the two groups, each carotenoid presents a particular kinetic constant, corroborating the lack of uniformity. For both groups, the kinetic constant increases with fat content if the lipid environment supplied is poly-unsaturated and decreases if it is mono-unsaturated. This different change in oxidation rate, depending on the degree of unsaturation of the lipid environment and which affects the total carotenoid fraction, has already been demonstrated in earlier works (*10*, *11*).

From the results set out in Table 4, a correlation can be established between the carotenoid's structure and its reactivity. The experimental conditions produce carotenoid autoxidation; that is, radicals are propagated without producing an antioxidant effect (*4*, *17*). The rate at which each pigment is degraded depends on the stability of the intermediate peroxycarotenoid radical, formed during the autoxidation process, and this stability is based essentially on structural features (*3*). In all of the mixtures assayed, capsorubin presents the lowest autoxidation rate. This can be attributed to its structural properties: the keto groups situated at the ends of the polyenoic chain probably increase the stability of the derived radical by favoring electron delocalization along the chain of conjugated double bonds. Consequently, there is a lower tendency for the oxidation process to continue, at least immediately, in accord with the results of various authors (*8*, *9*, *18*). Stabilization of the intermediate radical originated by the keto groups present in pigments such as astaxanthin, canthaxanthin, rhodoxanthin, and actinoerythrol decreases the autoxidation rate of these carotenoids compared with that of others not having this structural feature.

In the case of capsanthin, its single keto group probably does not contribute to stabilizing the degradation-derived radical, as it concentrates the electron charge at the end of the chain near the functional group, increasing the reactivity and consequently the oxidation rate, which exceeds that of capsorubin. The kinetic constant of violaxanthin includes it within the ketocarotenoid group in all cases, although the pigment does not possess such a structural property. Its lower autoxidation rate can be attributed to the epoxide groups at positions 5,6 and 5′,6′, the presence of which restricts the reactive points of the molecule to the polyenoic chain, as they impede the abstraction of allyl hydrogens (there being none on the violaxanthin rings), and the addition of radicals to those positions, occupied by the epoxide groups, and to the adjacent carbons (*9*). The rest of the pigments, in which all of the degradation routes mentioned are accessible without any possibility of structure-based stabilization during the autoxidation mechanism, present a higher degradation rate, in accord with the results of other authors (*8*, *18*, *19*).

The lower autoxidation of the ketocarotenoids is an interesting quality, both commercially—the slower loss of the chromatic properties (especially the red color) of paprika—and nutritionally. The incorporation of these pigments (and other carotenoids) via the diet makes them available as antioxidants against various radical reactions (*20*, *21*). Because these pigments have a low rate of autoxidation, the effectiveness of their protective action is greater than that of other carotenoids more liable to autoxidation processes, such as β-carotene (*17*).

Finally, the modulating role played by the lipid environment during the degradation reaction of the carotenoid fraction should be noted. As demonstrated previously, the carotenoid degradation rate increases or decreases depending on the degree of unsaturation of the supplied lipid substrate, although the ranking of stability in the mixture is unchanged. However, the differences between the kinetic constants of oxidation are smaller with increasing presence of fat, independent of the degree of unsaturation. Thus, capsanthin is degraded at rates closer to those of β-carotene and zeaxanthin when the percentage of lipid substrate in the mixture is higher. This indicates that the common lipid environment makes the oxidation reactions more uniform, despite the different structural nature of each pigment. Thus, the latter is not the only factor determining the behavior of the carotenoid molecule when the reaction takes place in highly lipid media. In such cases, the degradation reactions taking place on the polyenoic chain (a structure that is identical in all of the pigments studied here) by identical mechanisms are not as affected by other structural properties of the molecule, so that the degradation rate of the carotenoid profile tends to be more uniform.

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