Participation of Pepper Seed in the Stability of Paprika Carotenoids

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ABSTRACT: The degradation of carotenoid pigments present in dried, milled pepper fruit in the absence and presence of different proportions of seed at 70ºC in an oven was studied. The thermodegradation reaction rate was inversely proportional to the percentage of seed in the mixture in a first stage. Beyond that stage, the mixtures with lower proportions of seed lost pigmentation more slowly with increasing reaction time. In taking the experiment as a whole, the value of the reaction rate was inverted, and was higher the greater the amount of seed. The presence of higher seed levels involve the addition of unsaturated fatty acids (mainly linoleic) that are prone to oxidation. Finally, in a mixture representing a commercial paprika, the effect of the presence of some 40% of seed on the degradation of the red and yellow pigment fractions was determined.

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KEY WORDS: Carotenoid, degradation, fatty acid, paprika, seed, stability.

Paprika is a widely used natural colorant. Its good pigmentary qualities, together with its high content of provitamin A carotenoids, make it particularly appropriate for use as a food ingredient. Two essential aspects of its commercial value are coloring power and color stability. The former refers to the capacity to afford color, a direct consequence of its carotenoid concentration. This depends mainly on the pepper variety used, with very notable differences in carotenoid content between varieties (1–3). The latter factor—the maintenance of coloring power with time—is determined by the processing conditions in the different stages through which the pepper fruit passes before becoming paprika. These stages are dehydration of the fruit, milling, and homogenization (4,5).

The milling process in the production of paprika includes the seed from the fruit, which improves the organoleptic characteristics of the paprika and gives it greater stability. These are due on one hand to the contribution of oil contained in the seed, giving a more attractive color, and on the other to the inclusion of liposoluble antioxidants, such as tocopherols, which can considerably increase color permanence during storage. The proportion of seed in the fruit usually is around 40% and, as the milled seed is colorless, its presence means a dilution of color intensity in spite of the apparently improved appearance.

Different works on paprika pigments have focused on aspects such as degradation by air and light (6), type of drying and length of storage (5–9), and the relationship with sugar content (10), but there is only one reference specifically about seed and its effect on the carotenoid fraction of the fruit. This was a study of color change in paprikas with differing percentages of seed (11), and the conclusion was that the presence of a high content of seed had beneficial effects on color conservation. However, this positive effect has not been measured or monitored very methodically or widely. As the addition of seed contributes unsaturated fatty acids (FA), mainly linoleic acid (12), the opposite effect would be expected, given the readiness with which polyunsaturated FA are oxidized, generating hydroperoxides (13).

Thus, this work aims to study in detail the possible effect of the addition of seed on the stability of paprika carotenoids. The range tested was from 0 (absence of seed) to 60%. These values were considered limits by lack of (seed-free) or by excess (abnormally high) seed content for assessing the behavior of the carotenoid fraction present in intermediate mixtures of paprika. A previous work (14) demonstrated that in paprikas, oven-heated from 30 to 100ºC, there was a notable decrease in carotenoid concentration above 70ºC. Thus, 70ºC was chosen as the best storage temperature at which to test rapidly the positive and/or negative effects coming from the presence of seed.

EXPERIMENTAL PROCEDURES

Starting material. The fruits used for the experiment were of the variety Jaranda, grown in the area of La Vera (Cáceres, Spain), dehydrated at mild temperature, with oak-log burning as heat source.

Mixtures of dehydrated fruit and pepper seed were prepared in the laboratory as follows. A definite weight of dried, sliced fruit was mixed with different proportions of seed: 0, 20, 40, and 60% (w/w). Each mixture was milled, using a hammer mill, several times—each step producing a finer grain, until the final particle diameter was 0.5 mm, similar to that obtained in industry.

Sampling. From each homogenate, an amount of around 40 g was taken and placed on a petri dish in an oven at 70°C in the absence of light. Sampling was carried out each 24 h

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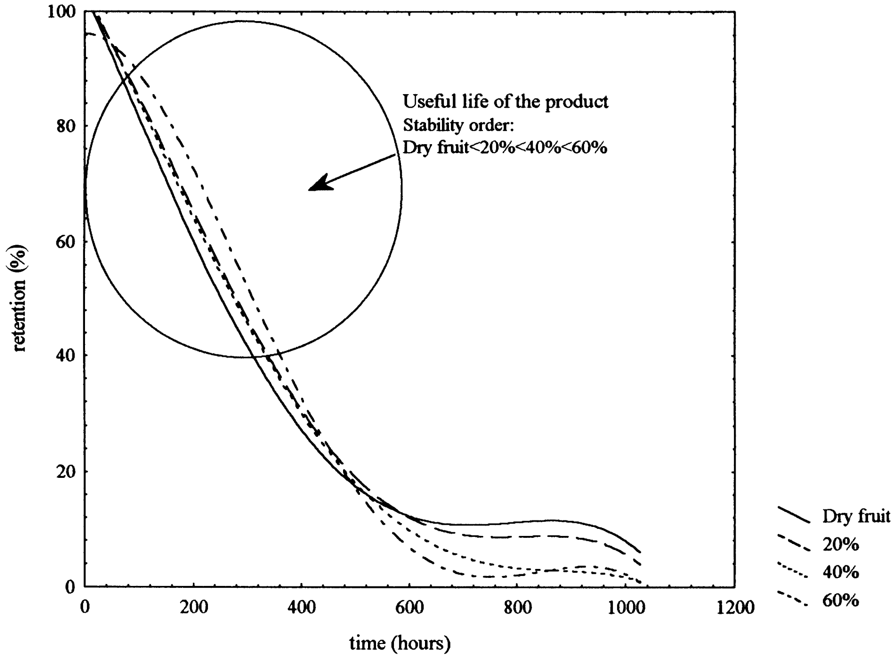

FIG. 1. Percentage total retention of pigments vs. time for the tested seed and dry fruit mixtures, fitted to a fourth-order polynomial.

during the first 3 d, then each 2–4 d, and, toward the end of the experiment, after 7 and 9 d. At first, the amounts of mixture sampled were around 0.8 g. With increasing pigment degradation, it was necessary to increase the sample weight to 1.5 g at the end of the experiment.

Extraction, saponification, and quantification of pigments. The methodology used was that described in previous works (15,16). After extraction with acetone, the organic phase (containing the pigments) was hydrolyzed by adding 10% (wt/vol) methanolic potassium hydroxide and an appropriate amount of internal standard (β-apo-8′-carotenal) for later quantification. Following hydrolysis, neutralization, filtration, and evaporation, the residue containing the pigments was collected in acetone [high-performance liquid chromatography (HPLC)] and kept in the freezer at –20°C until quantification by HPLC analysis.

RESULTS AND DISCUSSION

Changes in carotenoids in the tested mixtures. Figure 1 shows the change in percentage retention of total pigment with time fitted to a fourth-order polynomial. In this figure, it can be observed that, up to a carotenoid retention of 50%, the most stable mixture is that with the highest percentage of seed, whereas the mixture without added seed shows the greatest degradation. However, when the percentage of pigment retention falls below 30%, the order of stability is inverted, and the mixtures with highest percentage of seed show the highest rate of degradation, whereas the milled dehydrated fruit (without seed) presents in this stage a slower degradation.

One may deduce that, during the first stage of dehydration, the addition of a higher percentage of seed has protective effects on the carotenoid fraction. As already mentioned, seed is included during the commercial production of paprika for its beneficial effect, as the seed oil gives a better apparent color, and the tocopherols, being natural antioxidants, provide the first barrier to possible carotenoid oxidation, thus protecting the coloring power (17,18). However, the presence of FA in both dry fruit and seed provides raw material for the formation of hydroperoxides (13) which initiate degradation of carotenoids to colorless compounds.

Table 1 shows the mean values of FA and tocopherol composition in both dry fruit and seed, of the Jaranda variety. Previous studies (12) established that the seed contains some 20% of oil, which is directly integrated into the composition of the paprika, and the dry fruit contains about 3% of oil. Thus, with the

TABLE 1
Fatty Acid Composition and Tocopherol Level in the Oil from Seed and Dry Pepper Fruit, (*Capsicum annuum* L. cv. Jaranda)[a]

	Seed		Dry fruit	
Fatty acid	mg/g	(%)	mg/g	(%)
Lauric (12:0)	ND	0	0.75	3.0
Dodecenoic (12:1)	ND	0	0.24	0.96
Myristic (14:0)	1.1	0.12	2.27	9.1
Palmitic (16:0)	96.1	10.9	5.03	20.1
Palmitoleic (16:1)	2.0	0.22	0.27	1.1
Stearic (18:0)	25.7	2.9	1.05	4.2
Oleic (18:1)	70.7	8.0	3.21	12.8
Linoleic (18:2)	685.2	77.4	5.91	23.6
Linolenic (18:3)	2.8	0.31	6.10	24.4
Arachidic (20:0)	2.1	0.24	0.20	0.80
α-Tocopherol (mg/kg)	755		356	

[a]ND, not detected.

range of seed considered (0–60%), the paprika has an oil concentration ranging from 3 to 13%. Linoleic (23%) and linolenic (24%) acids are the major FA in the dry fruit. In the seed, the proportion of linoleic acid is threefold higher (77%), whereas the linolenic acid content is low (0.33%). This difference in the content of FA with two and three double bonds leads to the different extents of formation of hydroperoxides under the prevailing environmental conditions. The presence of α-tocopherol decreases the formation of free radicals in the medium. The dehydrated fruit has an α-tocopherol concentration of 356 mg/kg, but in the seed it is 755 mg/kg. The other isomers of tocopherol can be effective as antioxidants (17), but their concentrations in both dehydrated fruit and seed are very small.

In the mixtures of dehydrated fruit and seeds there are thus two types of constituents, i.e., FA, which are hydroperoxide generators, and tocopherols, which are natural antioxidants that are responsible for the changes shown in Figure 1. If a good balance is maintained between these two constituents, the product will keep its initial characteristics. When the balance is changed toward a high proportion of unsaturated FA, pathways are opened for irreversible reactions that may lead to accelerated changes in components, in particular, pigments.

In milled dehydrated fruit, the small particles have a large exposed surface area, and the constituent carotenoids are subjected to air, light, and heat. When seed is added, its oil covers the particles with a thin film, filling the hollow spaces and greatly decreasing the surface exposed, most importantly to oxygen. This may explain, in part, the retarding of pigment degradation in mixtures having a greater oil film on the dry fruit particles. The other factor is the presence of tocopherols in the oil, which curb the action of any hydroperoxides formed, preventing their direct action on the pigments.

Thus, in the milled dehydrated fruit without addition of seed, oxygen is more readily available, and the small amount of hydroperoxides formed (since the amount of oil is small) can more easily attack the carotenoids. Factors favoring oxidation are the presence of linoleic and linolenic acids and low content of tocopherols. All this helps to explain the higher pigment degradation rate. However, as observed in Figure 1, the values of carotenoid retention in mixtures with seed, from a determined time range, decrease more quickly until there is a change in the order of stability. A reasonable explanation might be that initially the greater oil layer covering the particles is a better physical barrier, preventing direct contact between hydroperoxides and pigments. Moreover, tocopherols from the added seed contribute a antioxidant effect. The presence of larger amounts of oil, with its oleic- and linoleic-type FA constituents, can in principle increase the degradative effects on the pigments. However, as seen in Table 1, the major FA of the seed, unlike the dry fruit, is linoleic rather than linolenic, which is less susceptible to oxidation. The mixtures with more seed will have a higher concentration of unsaturated FA so that once oxidative reactions are set off, a large amount of raw material is available in which these reactions can continue, thus overcoming the antioxidant effectiveness of tocopherols. The possible enzymatic involvement of lipoxygenase in these reactions has not been considered; previous studies in dry pepper did not detect hydroperoxide formation by enzyme action, and in the dry seed lipoxygenases seemed to be in very low concentration (19,20).

Carotenoid changes depending on percentage of seed used in model systems. A first kinetic study was performed up to a color retention of 50%, the stage considered the useful life of paprika, and later, a complete kinetic study was made consid-

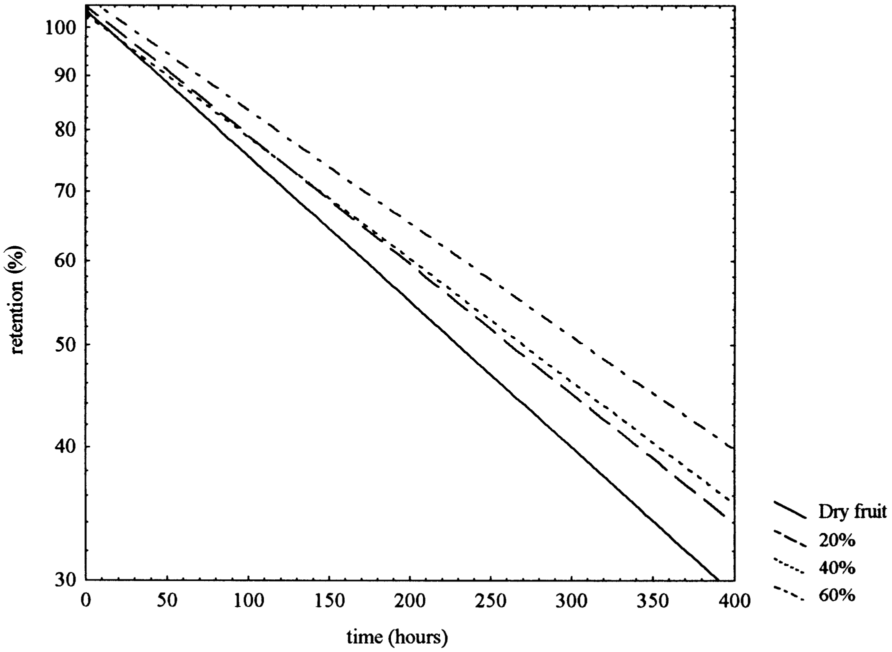

FIG. 2. Percentage total retention vs. time fitted to a first-order kinetic model (logarithmic ordinate).

TABLE 2
Kinetic Parameters of the Thermodegradation Reaction of the Red, Yellow, and Total Pigment Fractions, up to 50% Color Retention

	Percentage of seed in the mixture			
Pigment fraction[a]	0	20	40	60
	Kinetic constant[b] (*k* ± SD)$*10^{-4}$			
Red	27.84 ± 0.7	25.01 ± 0.6	24.22 ± 1.5	21.51 ± 2.4
Yellow	38.42 ± 1.2	32.82 ± 0.8	30.70 ± 1.3	28.90 ± 1.8
Total	31.80 ± 0.9	28.23 ± 0.6	26.69 ± 1.4	24.69 ± 2.1
	Ordinate at the origin (logarithmic coordinate)			
Red	4.654	4.644	4.654	4.672
Yellow	4.635	4.654	4.605	4.654
Total	4.644	4.654	4.635	4.672
	Correlation coefficient *R*			
Red	0.966	0.952	0.976	0.901
Yellow	0.973	0.953	0.970	0.948
Total	0.970	0.956	0.977	0.927

[a] Red fraction = capsorubin + capsanthin. Yellow fraction = violaxanthin + cucurbitaxanthin A + zeaxanthin + β-cryptoxanthin + β-carotene.
[b] First-order reaction. Kinetic model: ln (%retention) = Ordinate – $k \times t$.

ering the entire carotenoid degradation. The kinetic parameters of the reaction were deduced using the integral method. This uses a procedure of trial and error to find the reaction order: the order is assumed, and the kinetic equation is integrated. If the assumed order is correct, the graphical representation (determined from integration) of the concentration–time data should be linear (21). By using this procedure and including data up to 50% retention, it is deduced that the thermodegradation reaction of carotenoids is first order and is independent of the percentage of seed used in the production of the paprika (Fig. 2). A greater presence of seed means a lower rate of degradation. Data derived from Figure 2 are summarized numerically in Table 2, which shows the kinetic parameters deduced (rate constant *k*, ordinate at the origin, and correlation coefficient *R*) according to red, yellow, and total pigment fractions as function of percentage of seed in the paprika. Given that the reaction kinetics are first order, the value of the constant *k* is determined by the higher or lower rate at which the reaction takes place. In general terms, the red fraction (which is independent of the percentage of seed incorporated) presents lower values of *k*, and the yellow fraction higher values. Thus, to the conclusion that a higher percentage of seed results in a lower rate of degradation can be added the fact that the yellow pigment fraction is always degraded at a higher rate than the red.

From the experiment on total color loss, a kinetic study was performed with the same method. Table 3 displays the results. Carotenoid degradation, as in the first study, was first order, but the pigment degradation rate as a function of increasing percentage of seed was inverted. The values of the kinetic constant from all the data obtained showed that a higher percentage of seed gave a higher degradation rate. In Figure 1 there is a final stage in which degradation is greater in the presence of a higher percentage of seed. Consequently, this kinetic study confirms that an excessive seed addition leads to the low carotenoid concentration in the final stages of the reaction and a rejectable product owing to its low colorant capacity. The commercially significant result deduced

TABLE 3
Kinetic Parameters of the Thermodegradation Reaction of the Red, Yellow, and Total Pigment Fractions, Considering All Data Collected[a]

	Percentage of seed in the mixture			
Pigment fraction[a]	0	20	40	60
	Kinetic constant[b] (*k* ± SD)$*10^{-4}$			
Red	26.91 ± 2.5	29.09 ± 1.3	38.33 ± 1.3	43.71 ± 2.6
Yellow	34.37 ± 3.0	36.96 ± 1.7	45.99 ± 1.6	46.08 ± 1.7
Total	29.63 ± 2.7	31.56 ± 1.4	41.31 ± 1.4	45.11 ± 2.2
	Ordinate at the origin (logarithmic coordinate)			
Red	4.605	4.625	4.625	4.605
Yellow	4.605	4.605	4.654	4.605
Total	4.605	4.605	4.654	4.644
	Correlation coefficient *R*			
Red	0.990	0.992	0.972	0.950
Yellow	0.985	0.991	0.984	0.971
Total	0.989	0.991	0.979	0.963

[a] See footnotes in Table 2.

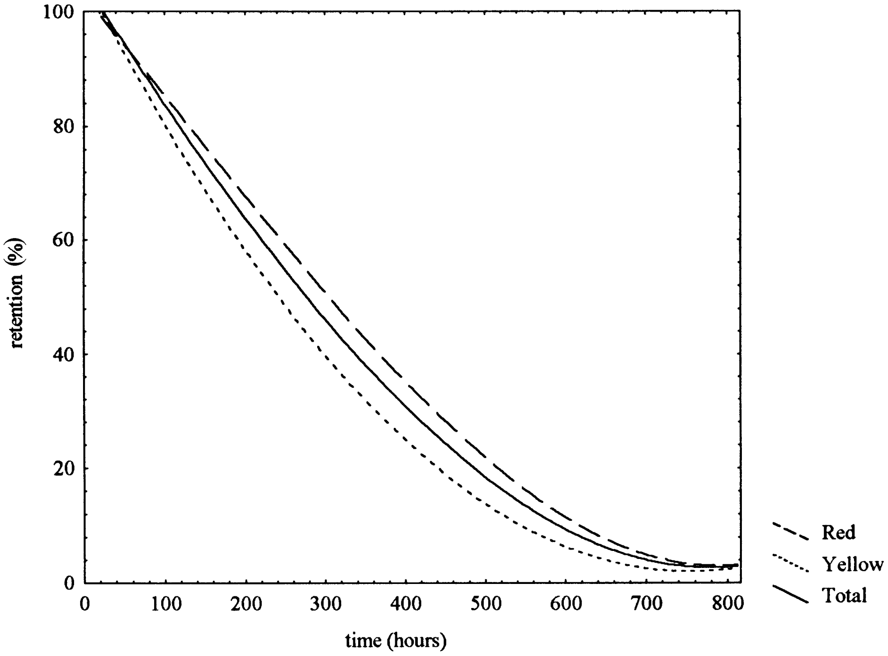

FIG. 3. Percentage retention of the red, yellow, and total pigment fractions vs. time in the mixture resembling a commercial paprika (40% seed).

in this study is that a high percentage of added seed has a protective effect during the useful life of the product.

Changes in pigments in the mixture reproducing a commercial paprika. Isomerization reactions. The mixture representing commercial paprika, which contained 40% seed, was studied in detail to determine pigment changes. Figure 3 shows the changes in total pigments and in the red and yellow pigment fractions, fitted to a fourth order polynomial. The red pigments were more stable than the yellow fraction throughout the experimental period. The curve of total pigments, as the sum of the two partial fractions, is intermediate between the two. Based on the initial 120 h of the experiment the total coloring power of the paprika did not decrease during the first 50 h. Figure 3 complements the conclusions of

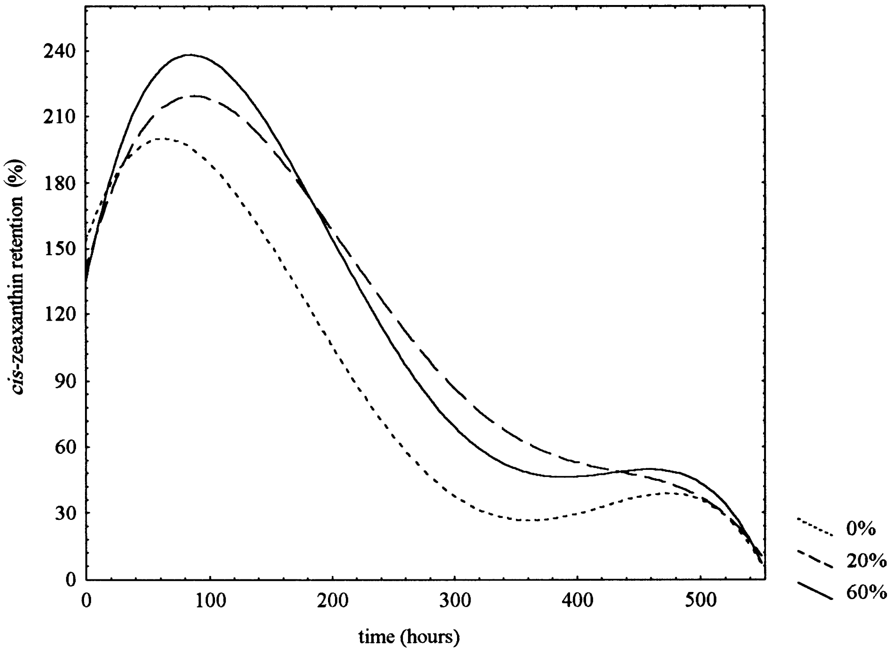

FIG. 4. Effect of seed addition on the percentage retention of *cis*-zeaxanthin vs. time.

the kinetic study, which establishes higher values of k for the yellow fraction than for the red, with intermediate values of k for total pigments.

Marked isomerization that was a function of the percentage of seed used took place in the first reaction period. In the first 24 h, only the pigments capsanthin, zeaxanthin, and β-carotene were transformed to their corresponding *cis* isomers (whose concentration increases notably), leading to colorless products (14). After 48 h, this transformation was still taking place. For example, Figure 4 shows the considerable initial increase in concentration of the *cis* isomer of zeaxanthin. The formation of isomer increased with greater presence of seed, and retention values exceeded 200%. This effect is seen only in the first hours, after which the behavior follows the general lines of the rest of the pigments. The other two *cis* isomers (of capsanthin and β-carotene) presented time-related changes similar to that shown by *cis*-zeaxanthin. These isomer-forming reactions probably take place in the milling process in the industrial production of paprika, when the heat given off during the process creates conditions appropriate for isomerization. This relatively greater presence of *cis* isomers has been observed when studying changes in carotenoid content during the paprika production (22).

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