Effect of High-Oleic Sunflower Seed on the Carotenoid Stability of Ground Pepper

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ABSTRACT: Mixtures of dehydrated pepper fruit (*Capsicum annuum* L.) with pepper seed (PS) and with high-oleic sunflower seed (SFS) in proportions of 0, 20, 30, and 40% (w/w) were subjected to heat degradation to compare the effect of a polyunsaturated medium (PS mixtures) and a monounsaturated medium (SFS mixtures) on the carotenoid fraction. The kinetic study carried out indicates that the carotenoid degradation, in both types of mixture and for all the proportions, fits a first-order mathematical model. The values of the kinetic constant *k* in each case and for the stage of commercial usefulness of the paprika, which ends when 50% of its initial coloring power has been lost, indicate that the presence of a higher percentage of seed, either pepper or high-oleic sunflower, in the mixture means a greater stability of the carotenoid fraction. However the presence of a lipid medium less sensitive to oxidation (SFS mixtures) strengthens this effect, prolonging the stage of practical usefulness of the product, so manufacture of paprika by grinding dehydrated pepper fruit with high-oleic SFS will provide a product with a longer shelf life. Also the improvement of the natural lipid substrate of the pepper fruit, to delay carotenoid degradation in the end product, is a possible line of investigation.

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KEY WORDS: Carotenoid, degradation, linoleic, oleic, paprika, seed, stability.

In the preparation and commercialization of foodstuffs or prepared foods, the aim is always to obtain a product of maximal quality that is as well-conserved as possible before reaching the consumer. From the period when a product is prepared until it is used can vary from hours to days to months to years, depending on the shelf life of the product and on the chain of distribution. To improve foodstuffs, a normal practice is to add substances that either prevent degradation (stabilizers and antioxidants), improve flavor (sweeteners), increase firmness (thickeners, agglutinants), or increase vitamin content. Historically, pepper was used as a food additive in the form of the dried fruit, and later as paprika, a product obtained by grinding the dry fruit and its seed. Such mixing dilutes coloring power, as the seed supplies no color, but dilution improves the organoleptic properties of the product and increases consumer acceptability. In addition, grinding of the intact dried fruit increases the presence of antioxidant substances, such as the tocopherols, giving greater stability to the carotenoids present (1).

Previous studies showed that the lipid composition of paprika, pericarp, and seed (2) is relatively high in polyunsaturated fatty acids, in particular linoleic (77% in the seed and 25% in the dehydrated fruit) and linolenic (24% in the dehydrated fruit) acids. These acids are sensitive to oxidation, which gives rise to hydroperoxides (3). The latter in turn can oxidize pigments, thereby decreasing the coloring power of paprika. The oxidation of fatty acids consists basically of three reactions: initiation, propagation, and termination, with propagation being the limiting step. The oxidation of polyunsaturated fatty acids is significant at ambient temperature whereas monounsaturated fatty acids are oxidized at high temperature (4). The antioxidant action of the tocopherols present in the medium would be more effective if degradative processes caused by fatty acid oxidation were minimized. To achieve this, the fatty acid profile of paprika could be altered from a composition that is mainly unsaturated to saturated. However, such a composition may not be appropriate for foodstuffs, as the current prototype of an ideal fat is virgin olive oil, which is rich in oleic acid and low in polyunsaturated and saturated fatty acids (5). For this reason, the present study considers the use of the dried pericarp of pepper fruit (*Capsicum annuum* L.) as a colorant for a seed, other than pepper, with a lipid composition less sensitive to oxidation. The seed chosen for the experiment was a high-oleic acid variety of sunflower seed. This variant of sunflower was originally obtained by chemical mutagenesis (6).

The aim of the work was to study the effect of pepper carotenoid pigments in a lipid medium with a low polyunsaturated acid (9%) content as compared to the lipids of pepper seed (7), which are higher in polyunsaturated fatty acids. The proposed experiment complements a previous one carried out with pepper seed and provides an opportunity to compare pigment stability in the two products: a mixture of dehydrated fruit with pepper seed, and a mixture of dehydrated fruit with high-oleic sunflower seed.

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MATERIAL AND METHODS

Preparation of paprika. The pepper fruits and seed used in the experiment were of the variety Jaranda, grown in the zone of La Vera (Cáceres, Spain), dehydrated at mild temperature, with heat from the burning of oak logs. The high-oleic sunflower seeds (SFS) were supplied by the company Cortillo S.A. (Écija, Spain). The mixtures of dehydrated fruit with pepper seed (PS) and dehydrated fruit with high-oleic SFS were prepared in the laboratory as follows. A determined weight of dried, sliced fruit was mixed with different amounts of seed: 0, 20, 30, and 40 % (w/w). Each mixture was milled in a hammer mill several times, each step producing a finer particle, until the final particle diameter was 0.5 mm, similar to that obtained in the industry.

Sampling. From each homogenate, around 40 g was taken and placed in a Petri dish in the oven at 70°C in the absence of light. Sampling was carried out each 24 h during the first 3 d, then each 2–4 d, and, toward the end of the experiment, 7 and 9 d from the previous one. Sampling was done in a matter of a few minutes, and the mixtures were returned to the oven. At the beginning, sample weight was 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. Each sample point is average of quadruplicate samples.

Extraction, saponification, and quantification of pigments. The processes have already been described (8,9). Each sample was subjected to extraction in a homogenizer (Ultra-Turrax T25; Janke & Kunkel, Staufen, Germany) with 50 mL of acetone. This treatment was repeated several times until no more color was extracted. All extracts from the same sample were pooled in a separatory funnel; 200 mL of 10% (wt/vol) NaCl solution was then added, and the resultant mixture was treated with 100 mL of ethyl ether to transfer pigment to the ether phase. Once the aqueous phase was removed, the ether phase (containing the pigments) was deesterified by adding 10% (wt/vol) methanolic potassium hydroxide and an appropriate amount of internal standard (β-apo-8′-carotenal) for subsequent quantification of pigments. After deesterification and the operations of neutralization, filtration, and evaporation, the residue was collected in acetone [high-performance liquid chromatography (HPLC) grade] and kept in the refrigerator at –20°C until its analysis by HPLC.

RESULTS AND DISCUSSION

In Table 1 is listed the carotenoid composition of the dry pericarp of the pepper (without seed) used as starting material and of samples prepared with dehusked sunflower seed (kernel only). The presence of pigments in sunflower seed was reduced to that of lutein, at a concentration of 1 mg/kg of oil. Because the prepared mixtures had a minimal concentration of lutein, its presence was not considered in this study.

In Table 2 is listed the fatty acid composition of the SFS used. The oleic acid content was 86%, with the rest (saturated and unsaturated) similar to that of olive oil. For comparison, the fatty acid profile of the PS (2) is included in Table 2. The most striking difference is the polyunsaturated fatty acid content of the PS compared to the monounsaturated content of the high-oleic SFS. Table 2 includes the α-tocopherol concentration in the two types of seed.

Figure 1 shows the change in percent retention of total pigments with time for the mixtures of dehydrated fruit with 20, 30, and 40% of added high-oleic SFS and for the control. There was an obvious difference between the sequence followed by the carotenoid concentration of the dehydrated fruit ground without seed and that of the different mixtures including seed. The latter behaved similarly, although a trend toward higher stability with increasing percentage of seed was

TABLE 1
Initial Carotenoid Composition (mean values; mg/kg) of the Different Tested Mixtures of Pepper Seed Mixed with High-Oleic Sunflower Seed[a]

	Percentage of seed in the lipid mixture			
Pigment	0	20	30	40
Capsorubin	250.4	215.7	194.2	180.8
Violaxanthin	280.8	230.5	188.1	142.8
Capsanthin epoxide	212.5	151.3	118.3	110.0
Capsanthin	1475.2	1173.5	972.8	873.0
cis-Capsanthin	301.5	275.9	308.3	212.5
Cucurbitaxanthin A	300.6	211.2	206.8	169.5
Zeaxanthin	315.0	259.6	232.1	179.1
cis-Zeaxanthin	46.6	43.1	38.2	31.0
β-Criptoxanthin	329.4	263.6	245.9	192.8
β-Carotene	291.5	244.7	228.3	242.0
cis-β-Carotene	83.0	63.5	63.7	27.4
Red[b]	2239.5	1816.3	1593.6	1376.3
Yellow[c]	1646.9	1326.6	1203.0	984.6
Total[d]	3886.4	3142.9	2796.6	2360.9

[a]Individual pigments, grouped by chromatic fraction (red and yellow), and total pigments are shown.
[b]Red = capsorubin + capsanthin + capsanthin epoxide + *cis*-capsanthin.
[c]Yellow = violaxanthin + cucurbitaxanthin A + zeaxanthin + *cis*-zeaxanthin + β-criptoxanthin + β-carotene + *cis*-β-carotene.
[d]Total= red + yellow.

TABLE 2
Initial Fatty Acid Composition of the Oil of Pepper Seed and High-Oleic Sunflower Seed

	Pepper seed		Sunflower seed	
Fatty acid	mg/g	%	mg/g	%
Lauric (12:0)	ND[a]	0	ND	0
Dodecenoic (12:1)	ND	0	ND	0
Myristic (14:0)	1.08	0.12	0.32	0.05
Palmitic (16:0)	96.12	10.85	29.13	4.15
Palmitoleic (16:1)	1.99	0.22	1.09	0.16
Stearic (18:0)	25.68	2.90	ND	0
Oleic (18:1)	70.73	7.99	607.68	86.48
Linoleic (18:2)	685.17	77.36	62.68	8.92
Linolenic (18:3)	2.75	0.31	0.25	0.04
Arachidic (20:0)	2.14	0.24	1.50	0.21
α-Tocopherol (mg/kg oil)	755		264	

[a]ND = not detected.

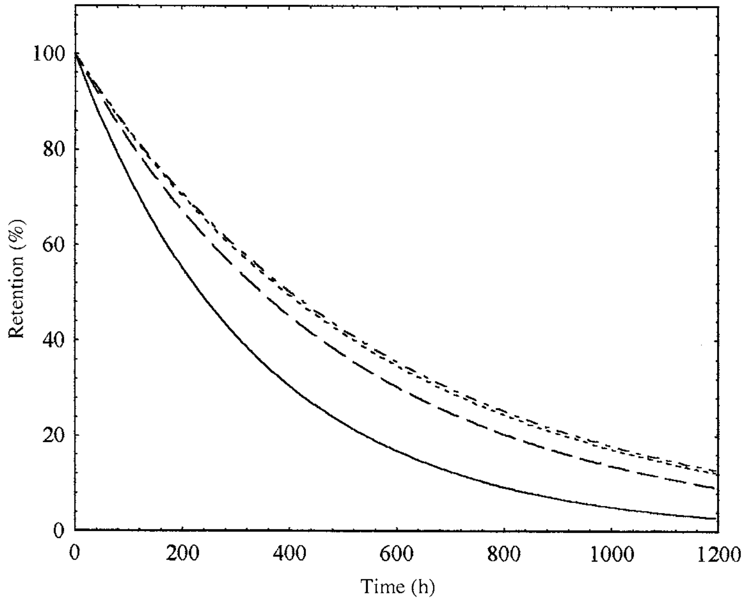

FIG. 1. Change in the percentage of retention of total pigments with time for (—) dehydrated fruit, (– – –) fruit + 20% high-oleic sunflower seed (SFS), (---) fruit + 30% SFS, and (-·-·) fruit + 40% SFS.

seen. In the containers in which the experiment was conducted, the samples containing only dehydrated fruit were decolored earlier and more rapidly than the others, which retained their striking red color until the end of the experiment.

Previous work (7) studied the effect of different proportions of PS on the stability of carotenoid pigments. It showed that in a first stage (covering the useful life of the product), stability increased with increasing amounts of seed. The kinetic model describing the degradation of total pigments was first-order, with kinetic constant k varying from 24.69×10^{-4} to 31.80×10^{-4} as the percentage of seed decreased. From this it was concluded that a greater presence of PS had a beneficial effect. However, as the experiment progressed, the degradation rates inverted, and the paprikas with more PS showed the lowest stability. The kinetic model for the overall experiment had values for the kinetic constant k from 29.63×10^{-4} to 45.11×10^{-4} with increasing percentage of seed in the mixture. This effect at the beginning and end of the experiment was not observed in the present case, using high-oleic SFS, where stability increased with higher percentage of seed from beginning to end.

To quantify the observed effect, a kinetic study was carried out on the data obtained. The kinetic parameters of the carotenoid degradation reaction were deduced using the integral method. This method uses a procedure of trial-and-error to find the order of the reaction. The order is assumed, and the kinetic equation is integrated. If the order assumed is correct, the graphical representation (determined from integration) of the concentration–time data should be linear (10). Table 3 lists the kinetic parameters obtained with this methodology for the tested mixtures, i.e., those with high-oleic SFS and those of dehydrated fruit ground without seed. In all cases, the best kinetic model describing carotenoid pigment degradation was first order. In addition, from the rate constant k, carotenoid degradation takes place more slowly the greater the presence of seed in the mixture.

Although the addition of PS is beneficial (the values of the kinetic constant k in the SFS mixtures are lower in all cases), the change of lipid substrate from polyunsaturated to monounsaturated means improved carotenoid stability in the test mixtures and a prolonged shelf life for such mixtures. Table 4 shows the change in retention percentage of total pigments for the different PS and SFS mixtures, the approximate time when the mixture is no longer commercially useful (retention percentage ≤50%), and a comparison of the increase in hours of useful life between the SFS mixture and the corresponding PS mixture. It was also observed that the use of a 20% SFS mixture provided a slightly higher stability with time than a 30% PS mixture, and that the stabilities of 30 and 40% SFS mixtures were similar.

The mixtures of greatest interest are those containing between 30 and 40% of seed by weight, the normal dilutions in the manufacture of paprika. Table 4 shows that these two mixtures are the most stable, as they are the last to exceed 50% of carotenoid retention; but the SFS mixtures do so approximately 100 and 150 h later than the PS mixture. This is the quantification, in hours, of the increase in stability of the carotenoid content when using a lipid substrate less prone to oxidation, so manufacture of paprikas using high-oleic SFS will provide a product with a longer shelf life. Mixtures with high-oleic SFS proportions greater than 40% will yield a product lacking the normal appearance of paprika because of the high oil content in the sunflower kernel.

Although the percentages of seed were equal in both kinds of mixtures (PS and SFS), with the same color dilution and the same tocopherol concentration, the presence of oil was

TABLE 3
Kinetic Parameters Obtained from the Fit of the Concentration of the Red and Yellow Fractions and Total Pigments to First-Order Kinetics for Paprikas Prepared with High-Oleic Sunflower Seed

	Percentage of seed in the mixture			
Pigment fraction[a]	0	20	30	40
	Kinetic constant[b] ($k \pm$ SD) $\times 10^{-4}$			
Red	26.91 ± 2.5	18.37 ± 0.7	15.93 ± 0.5	15.69 ± 0.6
Yellow	34.37 ± 3.0	22.17 ± 0.7	20.14 ± 0.5	18.96 ± 0.5
Total	29.63 ± 2.7	19.85 ± 0.7	17.57 ± 0.5	17.18 ± 0.6
	Ordinate at the origin (logarithmic coordinate)			
Red	4.605	4.605	4.605	4.605
Yellow	4.605	4.605	4.605	4.605
Total	4.605	4.605	4.605	4.605
	Correlation coefficient *R*			
Red	0.990	0.977	0.985	0.981
Yellow	0.985	0.983	0.990	0.992
Total	0.989	0.980	0.987	0.987

[a]Red = capsorubin + capsanthin + capsanthin epoxide + *cis*-capsanthin.
[b]Yellow = violaxanthin + cucurbitaxanthin A + zeaxanthin + *cis*-zeaxanthin + β-criptoxanthin + β-carotene + *cis*-β-carotene.
[c]Total = red + yellow.

not the same, due to the different fat content of the two types of seed, i.e., 18% in PS and 50% in sunflower kernel. The differences in behavior of the two systems are the result of the qualitative difference between the two types of oil, particularly regarding the presence of polyunsaturated fatty acids. The fat of the starting material—the dehydrated fruit—has 24% linolenic acid (18:3), which is very prone to oxidation. With the addition of one type of seed oil or another, this acid is diluted in the final paprika. At the same time, depending on the type of seed oil added, the proportion of fatty acids is altered, in the sense that a lower percentage of polyunsaturateds are added with SFS. The lipid medium contributed by the seed, which surrounds and impregnates the pigmentation of the fruit, is different from case to case, and it affects the degradation. PS impregnates the pigments in a highly polyunsaturated medium, whose main component is linoleic acid (18:2), whereas SFS contributes oleic acid (18:1) (Fig. 2). It is this difference in composition of the lipid medium that causes such differences in oxidation.

TABLE 4
Change in the Percentage of Retention of Total Pigments in the Mixtures of Dehydrated Fruit with Pepper Seed and Dehydrated Pepper Fruit with High-Oleic Sunflower Seed

	Total pigment retention (%)					
	20[a]		30		40	
Time (h)	PS[b]	SFS	PS	SFS	PS	SFS
50	87	89	88	91	91	92
100	75	79	77	83	79	85
150	66	70	67	76	69	78
200	57	62	59	69	60	71
250	**49**	55	51	63	53	66
300	43	**49**	**45**	57	**46**	60
350	37	44	39	52	40	56
400	32	39	34	**48**	35	51
450	28	34	30	43	31	**47**
500	24	31	26	39	27	43

[a]Percentage of added seed in the mixture.
[b]Type of seed used: PS, pepper seed; SFS, high-oleic sunflower seed. Boldface type indicates product is no longer commercially useful.

The fatty acid content which is responsible for the generation of peroxides and hydroperoxides by autoxidation plays a primary role in color stability of the tested mixtures (4). Thus, the type of fatty acids composing the lipid substrate determines the rate at which oxidation takes place. Degradation of pigments still occurs, but addition of a protecting lipid medium generates oxidizing products more slowly.

It can be concluded that the profile of the lipid substrate plays an important role in the oxidative process, and, in the case of paprika, the presence of linoleic acid favors such processes, in contrast to other mixtures with oleic acid as the major fatty acid component. The lipid substrate supplies oxidizing agents that affect the carotenoid fraction, and the normal method to delay degradation is to add antioxidants, such as ascorbic acid or tocopherols. This experiment provides an alternative method to delay the negative effect of polyunsaturated substrates.

A change in the type of seed used in the grinding (high-oleic SFS instead of the seed of the fruit itself) could be a drastic alteration in the manufacture of paprika and thus may not be the most appropriate solution. Genetic engineering has become a common and very well known tool and could be used in the development of new varieties of pepper whose lipid profile is different from current varieties. In this way, fruits could be obtained with a lower polyunsaturated fatty

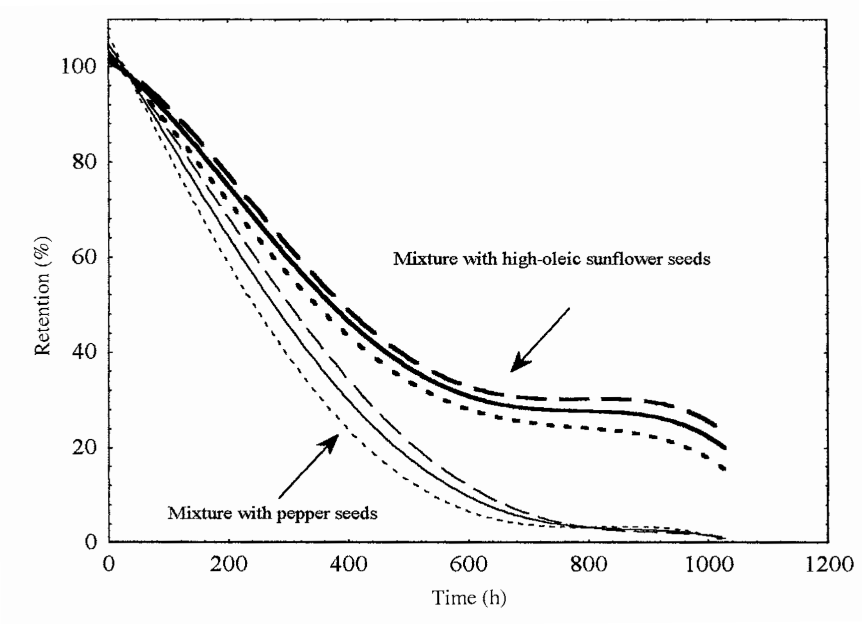

FIG. 2. Change in the percentage of retention of the fractions of red (– – –), yellow (---), and total (—) pigments with time for the mixture equivalent to a commercial paprika, and that of a mixture with high-oleic sunflower seed of the same proportion (40%).

acid content, possibly by controlling the desaturase enzymes that produce these fatty acids (11).

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REFERENCES

1. Mínguez-Mosquera, M.I., M. Jarén-Galán, J. Garrido-Fernández, and D. Hornero-Méndez, *Carotenoides en el pimentón. Factores responsables de su degradación*, edited by M.I. Mínguez-Mosquera, CSIC, Madrid, 1996, pp. 3–11.
2. Pérez-Galvez, A., J. Garrido-Fernández, M.I. Mínguez-Mosquera, M. Lozano-Ruiz, and V. Montero-de-Espinosa, Fatty Acids Composition of Two New Pepper Varieties (*Capsicum annuum* L. cv. Jaranda and Jariza), *J. Am. Oil Chem. Soc. 76*:205–208 (1999).
3. Frankel, E.N. Chemistry of Free Radical and Singlet Oxidation of Lipids. *Prog. Lipid Res. 23*:197–221 (1985).
4. Porter, N.A., S.E. Caldwell, and K.A. Mills, Mechanisms of Free Radical Oxidation of Unsaturated Lipids, *Lipids 30*: 277–290 (1995).
5. Alexander-North, L.S., J.A. North, K.P. Kiminyo, G.A. Buettner, and A.A. Spector, Polyunsaturated Fatty Acids Increase Lipid Radical Formation Induced by Oxidant Stress in Endothelial Cells, *J. Lipid Res. 35*:1773–1785 (1994).
6. Garcés, R., J.M. García, and M. Mancha, Lipid Characterization in Seeds of a High Oleic Acid Sunflower Mutant, *Phytochemistry 28*:2597–2600 (1989).
7. Pérez-Galvez, A., J. Garrido-Fernández, and M.I. Mínguez-Mosquera, Participation of Pepper Seed in the Stability of Paprika Carotenoids, *J. Am. Oil Chem. Soc. 76*:1449–1454 (1999).
8. Mínguez-Mosquera, M.I., and D. Hornero-Méndez, Separation and Quantification of the Carotenoid Pigments in Red Peppers (*Capsicum annuum* L.), Paprika, and Oleoresin by Reversed-Phase HPLC, *J. Agric. Food Chem. 41*:1616–1620 (1993).
9. Mínguez-Mosquera, M.I., and A. Pérez-Gálvez, Study of Lability and Kinetics of the Main Carotenoid Pigments of Red Pepper in the De-esterification Reaction, *J. Agric. Food Chem. 46*: 566–569 (1998).
10. Fogler, S., *Elements of Chemical Reaction Engineering*, edited by N.R. Amundson, Prentice-Hall: Englewood Cliffs, 1992, pp. 190–240.
11. Harwood, J.L., Recent Advances in the Biosynthesis of Plant Fatty Acids, *Biochim. Biophys. Acta. 1301*:7–56 *(*1996).

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