Competition between the Processes of Biosynthesis and Degradation of Carotenoids during the Drying of Peppers

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The changes in the levels of carotenoid pigments during the drying of peppers of the var. *Bola* for paprika demonstrate that in these fruits there is a period of carotenogenesis after harvesting. The pathway followed seems to be the same as that which occurs during the ripening of peppers. This biosynthetic period is strongly favored by light, while in darkness, this process is not as fast. At the final stages of drying, the biosynthetic process is interrupted and until the complete dehydration of the fruit, depending on the external factors, degradation can be enhanced or minimized. Combining, during the drying process, a first step of illumination with a second step of darkness, it is possible to get dry peppers for paprika with some 20–40% increase in pigment concentration.

INTRODUCTION

For more than 100 years, certain varieties of peppers have been progressively selected with the sole aim of producing paprika. This is obtained by drying the fruits and subsequently milling them, thereby achieving a fine powder with a high coloring capacity, traditionally used both domestically and industrially to modify the color of foodstuffs, improve their appearance, and, in some cases, confer particular organoleptic characteristics.

Traditionally, in Murcia (Spain), the product for industrial use is obtained by drying peppers in the sun. This form of drying is both excessively slow and costly, requiring 1 or 2 weeks of exposure and an extensive surface area for drying. The sunlight probably promotes oxidative degradation during drying (Carnevale et al., 1980). It is necessary to use varieties with high physical resistance that can endure such a prolonged process without suffering breakage, and in order to guarantee the hygienic quality of the final product, strict analytical checks are required.

In the "Vera" zone (Cáceres, Spain), a system of drying has been developed, which not only achieves a perfect dehydration of the fruit but also confers particular organoleptic characteristics to the final product. Via log burners, the area in which the fruits are piled receives a gentle and continuous supply of heat, that in 10 or 15 days completely dehydrates the peppers. The wood smoke produced contributes to the development of particular, and highly valued, odors and flavors, which are subsequently recognizable in the paprika.

Both of these traditional forms of drying are slow. Nowadays there is a tendency toward the use of industrial drying processes which are far more rapid, more uniform, and more hygienic. The fresh fruits are deposited on trays and circulate on continuous belts through a drying tunnel. The temperature of the drying depends on the flow of air, on the amount of sample to be dried, and on the time spent in the tunnel. As a general rule, the temperatures do not exceed 50–60 °C and the drying period is less than 20 h (Zapata et al., 1992).

Previous studies have shown that partial synthesis of pigments possibly can occur during drying of peppers from var. *Bola*, since during the dehydration the activity of some enzymes will increase (Mínguez-Mosquera et al., 1993). However, the final effect of these biosynthetic processes has been difficult to clarify due to them occurring at the same time as degradative processes, and thus, it is accepted in the literature that the postharvesting period and the drying step always induce the degradative loss of carotenoids (Ramakrishnan and Francis, 1973; Malchev et al., 1989). For this reason, it has been deemed necessary to carry out a study in which the factors inducing degradation could be limited.

The objective of the present work is to test the hypothesis that synthesis of pigments occurs in the var. *Bola* of pepper during the drying process, while elucidating the intensity of the possible coupled degradative processes. To perform this study, paprika peppers of the var. *Bola* were dried both in the light and in the dark. The illumination used introduced conditions to the environment, in which the drying was performed, that possibly promote oxidative degradation. The temperature used permitted a slow dehydration, thus allowing the fruits to develop naturally for a certain period of time.

MATERIALS AND METHODS

Raw Material Used. *Plant Material.* Peppers of the var. *Bola*, harvested in the red mature stage from the Vera zone (Cáceres, Spain), were used. Fruits were only used if their degree of firmness indicated that they had not become overripe.

Drying Chamber of the Fruits. A thermostatically controlled chamber was subdivided into two so that one section could be used for photothermooxidation studies and the other for purely thermooxidation studies. In both zones, the temperature was maintained at 35 °C and an air extractor was fitted to favor drying and to approximate conditions, as far as possible, to those prevailing in the natural drying process. The compartment destined for the thermooxidation studies was lined in black, and all joints were sealed to prevent any light entering. The degree of illumination thus achieved was less than 5 lx. The chamber dedicated to photooxidation studies was coated in white, and the fluorescent tubes providing the illumination were distributed homogeneously, thereby obtaining a constant and uniform lightness fixed at 1000 lx. The fruits were hung up in the chambers to avoid any contact between them and any surface, which could have a detrimental effect on the uniformity of the drying as well as on the amount of light received.

Sampling. Samples were taken from both compartments of the chamber every 24 h during the first 4 days of drying. Once drying had slowed down, sampling was carried out at longer time intervals until the end of the process. Random subsamples, from 200 g of fresh fruit to 20 g of dry fruit, were homogenized, and from these homogenates, for the analysis, samples were weighed in triplicate, from 10 g for fresh fruit to 1.5 g for dried fruit. The

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Table 1. Evolution of the Individual Concentration of Carotenoids during the Drying under Light of Var. *Bola* Peppers

drying time (h)	pigment concentrations[a] (mg/kg of dry matter)							dry-matter content (%)
	β-carotene	cryptoxanthin	zeaxanthin	capsanthin	violaxanthin	capsorubin	totals	
0	314.8	134.1	170.3	1750.2	446.06	381.6	3197.0	12.0
25	522.6	269.0	276.4	2052.5	326.3	359.1	3815.9	18.4
65	529.3	358.7	227.9	2217.9	242.2	426.9	4002.9	28.4
90	502.8	414.8	309.0	2770.2	274.8	428.9	4700.5	34.2
161	552.2	324.8	178.0	1750.6	241.28	326.9	3273.8	91.9
233	508.2	345.6	275.6	1867.6	259.0	345.3	3601.3	94.2
329	529.7	267.6	231.1	1939.6	234.9	312.1	3515.0	95.7

[a] Average of three determinations.

weight of the samples used is progressively lower due to the loss of moisture but is adjusted to get samples of similar dry-matter content during the whole process. The weighed samples were frozen at −30 °C until extraction of pigments.

Pigment Analysis. *Pigment Extraction.* In all samples, the dry-matter content was determined using a vacuum heater to establish the progress of the drying process. To homogenize the extraction conditions, a certain amount of water was added in each case to make the moisture content of all samples similar to that of the fresh fruit. The extraction was performed after rehydration, and the procedure followed was that described in previous publications (Mínguez-Mosquera et al., 1992). Samples were extracted with acetone until the color was exhausted. The combined extracts were transferred into ethyl ether for saponification with an equal volume of 20% KOH–methanol (w/v). After 2 h, the carotenoids were washed and transferred into diethyl ether with water. The ether was then evaporated to dryness and the pigment recovered in 25 mL of acetone. An aliquot of this was filtered through a 0.45-μm millipore membrane for separation and quantification of the pigments by liquid chromatography.

Identification, Separation, and Quantification of Pigments. The carotenoid pigment analysis was carried out by high-performance liquid chromatography, using a Perkin-Elmer system with a Series 4 quaternary pump, fitted with an injection valve (Rheodyne Model 7125) with a 5-μL sample loop. Separation was realized on a reverse-phase C_{18} column (Spherisorb ODS2, 5 μm, 250 × 4 mm, Hewlett-Packard) protected by a precolumn of the same material (10 × 4 mm), eluting the sample using a binary gradient (acetone/water). Detection was performed at 450 nm with a UV–vis detector, Perkin-Elmer Model LC-85B, and a Hewlett-Packard Model A-3396 integrator. Quantification was realized using β-apo-8′-carotenal as internal standard. The different moisture contents in the samples during the process make it necessary to refer the pigment concentration to the dry matter to make the results comparable.

More details about the identification process and the system of separation and quantification of pepper pigments by HPLC are included in a previous publication (Mínguez-Mosquera and Hornero-Méndez, 1993).

RESULTS AND DISCUSSION

Pigments identified in the present work are the same as those reported in a previous paper (Mínguez-Mosquera and Hornero-Méndez, 1993). The main carotenoid components are β-carotene, cryptoxanthin, zeaxanthin, capsanthin, violaxanthin, and capsorubin. Although other pigments such as capsanthin 5,6-epoxide, antheraxanthin, mutatoxanthin, capsolutein, and cryptocapsin have been detected, they have not been taken into account in this study because of their minimal contribution to the total pigment concentration.

Changes in Pigment Concentration during Drying. In Tables 1 and 2 are shown the changes in pigment concentration during drying in the light and in the dark, respectively. In the same tables are shown the changes in dry-matter percentage throughout the drying process. The pattern of change in pigment concentration until the final composition of the fruit dried is reached is qualitatively similar in both drying systems and can be divided into two steps: a first biosynthetic step with an increase in the pigment concentration and, later, a degradative step with gradual pigment loss. The first step is detectable until the fruit reaches a dry-matter content between 35–40%, and at this moment, the second step begins. In both drying systems used, the change from one step to another occurs approximately 60–90 h after the beginning of the process.

Comparing the dried fruit obtained from both drying systems with the fresh fruit, it can be seen that there exist a net increase in the concentrations of β-carotene, cryptoxanthin, zeaxanthin, and capsanthin and a decrease in violaxanthin and capsorubin. For violaxanthin, the whole drying process had a degradative effect, while for capsorubin, the degradative effect was stronger than the biosynthetic step and the final result is a lower concentration than in the fresh fruit. In both drying systems, the final dry fruit obtained has a higher overall pigment content than the fresh one.

Effect of Illumination. Illumination promotes some specific differences with respect to the pattern described. As can be seen in Table 1, during the biosynthetic step the maximum in concentration of each pigment in the fruit is not reached simultaneously. β-Carotene reaches its maximum concentration in the first 25 h and, from this moment, remains constant. Cryptoxanthin and capsanthin increase their concentration gradually, reaching a maximum after 90 h of drying. Zeaxanthin shows an erratic tendency, an initial increase in concentration followed by a decrease and finally a new increase. Capsorubin has a small initial loss in concentration and then a subsequent increase, reaching its maximum after 90 h of drying. The increases in pigment concentrations compared to those of the fresh fruit during this first step could be estimated at around 12% for capsorubin, 58% for capsanthin, 66% for β-carotene, 81% for zeaxanthin, and more than 200% for cryptoxanthin. Losses only occur in the concentration of violaxanthin, and these are around 45%.

The proof that this first phase represents a biosynthetic stage was achieved by performing a balance on the pigment material, which demonstrated that there was a net increase of 2.96×10^{-3} mol/kg, eliminating the possibility that the changes seen in the concentrations arose as a result of interconvertion of the pigments found in the fresh fruit.

The second step in the pattern of change of pigments begins when the fruit reaches a dry-matter percentage of around 35%, approximately after 90 h of drying. During this second period, only β-carotene and violaxanthin maintain their concentrations, with no degradation occurring at this step, and then show a high stability. With respect to the rest of the carotenoid pigments, generalized losses in the concentration occurs. The high concentrations reached by every pigment during the biosynthetic step begin to diminish due to the harsh conditions. Capsanthin, capsorubin, zeaxanthin, and cryptoxanthin only retain some 70% of maxima reached previously. Although the extent of the degradative reaction is very great in the present conditions, the intensity of the prior

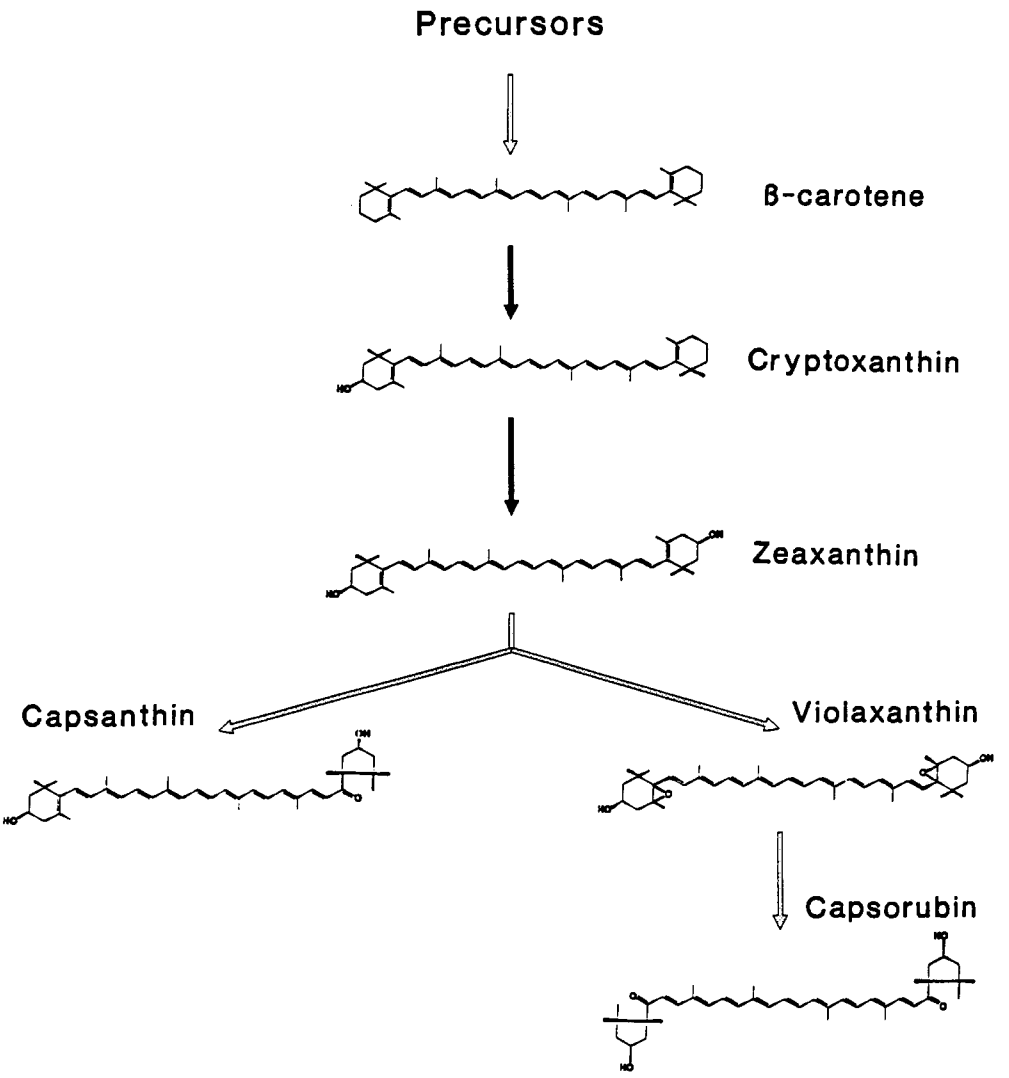

Figure 1. Carotenogenic pathways in red peppers. White arrows are steps with other minority pigments not included in the figure.

Table 2. Evolution of the Individual Concentration of Carotenoids during the Drying in Darkness of Var. *Bola* Peppers

	pigment concentrations[a] (mg/kg of dry matter)							
drying time (h)	β-carotene	cryptoxanthin	zeaxanthin	capsanthin	violaxanthin	capsorubin	totals	dry-matter content (%)
0	314.8	134.1	170.3	1750.2	446.06	381.6	3197.0	12.0
25	277.4	186.9	248.0	1646.3	184.0	346.8	2889.4	18.5
65	408.7	250.1	264.1	2002.4	217.0	313.3	3455.6	44.7
90	373.1	206.1	253.2	2221.3	254.2	362.0	3669.9	76.2
161	380.4	209.0	268.3	2185.5	258.5	418.6	3720.3	94.5
233	379.3	174.2	239.2	2099.6	260.9	385.8	3539.0	95.0
329	474.95	225.8	260.6	1999.0	244.1	333.6	3538.1	96.1

[a] Average of three determinations.

biosynthetic step leads to a positive final balance between losses and gains.

Only four determinations have been performed for this stage of the process. They are clearly insufficient to determine the kinetic parameters of degradation, but it can be conjectured from the appearance of the degradation lines that they probably obey zeroth-order kinetics.

Effect of Darkness. During drying in darkness, there are also some specific differences with respect to the general pattern described previously for the biosynthetic step. In Table 2, the results show that in the first 25 h, β-carotene, capsanthin, and capsorubin lose between 6% and 12% of their initial concentration. Violaxanthin experiences a drastic fall in its concentration of more than 55%, while cryptoxanthin and zeaxanthin increase their concentrations by some 38%. After these first hours, all pigments increase their concentration, not only overcoming the initial loss but reaching concentrations higher than those in the fresh fruit, except for violaxanthin which never recovers its initial concentration. Again, there is a time difference in reaching the maximum concentration for each pigment.

The second step practically does not exist in darkness, and only slight oscillations around the maximun are detected, probably due to an incomplete biosynthetic process occurring in the first step.

Comparison between Drying Processes. The differences between the intensity of carotenoid synthesis during each drying system are notable. In the light, the biosynthetic step increases the total pigment concentration some 47%, while in darkness, this increase is close to 15%. The intensity of the degradative step is also different: while in the light it is strong, in the dark it is almost imperceptible. Comparing the pigment concentrations

of fresh fruit with those of the dried fruits from both drying processes, it is possible to see that in both cases the final dried fruit has similar pigmentary characteristics, both qualitatively and quantitatively. From this final result, it can be concluded that neither of the drying systems used can be considered better than the other. In one of them, a strong degradative process occurs, which provokes significant losses with respect to the maximum reached, and in the other, the rate of biosynthesis is reduced and the fruits do not develop their full pigmentary capacity. Probably by combining a first step in the light with a second one in darkness, it could be possible to develop the highest pigment concentration in the fruit without any subsequent degradation.

Having established the existence of a biosynthetic step during drying which is clearly favored by light, it could be interesting to study the implications of the possible pathways followed in both processes. Some changes in the pigment concentration (Tables 1 and 2) seem randomly distributed, but they probably have a physiological interpretation. The usual theory of carotenogenesis in peppers is summarized in Figure 1. The pathway of carotenogenesis begins with phytoene and phytofluene, colorless precursors of β-carotene. From this point, by progressive oxygenation, those components with a maximum degree of oxidation are formed, capsanthin and capsorubin. Both of these are formed in parallel by excision of the pathway at the antheraxanthin-formation step (Davies et al., 1970; Isler, 1971; Davies, 1976; Gross, 1991).

During drying in the light, the fact that β-carotene reaches its maximum concentration in the first 24 h could be interpreted as a fast and massive synthesis so that the increases in the other pigments can take place at its expense, reaching their maximum concentrations subsequently. At the same time, it can be observed that synthesis of β-carotene does not stop until there is a generalized halt in the biosynthetic process, suggesting that there is an equilibrium between its catabolism and anabolism. The pathway preferentially followed is that which leads to the formation of capsanthin, and the parallel route to violaxanthin synthesis seem interrupted. Nevertheless, an increase in capsorubin concentration occurs. The balance of material in this branch of the pathway shows a loss of 2.06×10^{-4} mol/kg, which would seem to indicate that the synthesis of capsorubin occurs exclusively from preexisting violaxanthin and, furthermore, that a portion of the violaxanthin is lost.

During drying in the dark, it is observed that capsanthin initially decreases in concentration, as does capsorubin and violaxanthin. It would appear that the pathways of formation of these pigments run in the opposite direction to their synthesis, since there is a simultaneous increase in the concentration of cryptoxanthin and zeaxanthin, which in turn coincides with a decrease in the concentration of β-carotene. The decrease in the concentration of pigments in the first few hours is 72.2×10^{-5} mol/kg, while the increase only represents about a third of this amount. The process leads to a net loss of 51.8×10^{-5} mol/kg. Given that, subsequently, there is not only a compensation for this loss but an overall increase in the concentration of the pigments, the initial loss can only be explained by there being intermediary compounds which were not measured and which were used eventually for the synthesis of fundamental pigments in the biosynthetic pathway.

In general terms, during the drying of fruits of var. *Bola*, there is a synthesis of carotenoids, increasing the initial concentration of the fresh fruit. The magnitude of the increase in concentrations of these pigments and the speed with which they are synthesized depend on the presence or absence of light. It seems that β-carotene formation is a photoinduced reaction and that in these conditions, far from being destructive, induces a great increase in all pigments during a restricted period. Once the biosynthetic step is finished, light has a strong degradative effect, and its presence is undesirable. The experiments performed offer the possibility of modifying the carotenogenic pathways simply by altering external factors.

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LITERATURE CITED

Carnevale, J.; Cole, E. R.; Crank, G. Photocatalyzed oxidation of paprika pigments. *J. Agric. Food Chem.* **1980**, *28*, 953–956.

Davies, B. H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: London, 1976; Vol. 2.

Davies, B. H.; Matthews, S.; Kirk, J. T. O. The nature and biosynthesis of the carotenoids of different colour varieties of *Capsicum annuum*. *Phytochemistry* **1970**, *9*, 797–800.

Gross, J. Carotenoids in vegetables. In *Pigments in Vegetables*; Van Nostrand Reinhold: New York, 1991; pp 75–278.

Isler, O., Ed. *Carotenoids*; Birkhäuser Verlag: Basel, 1971.

Malchev, E.; Tanchev, S.; Ioncheva, N.; Kalpakchieva, K. Changes in carotenoids during the storage of red pepper powder obtained by drying of red pepper paste. *Nahrung* 1989, *33*, 799–803.

Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika and oleoresin by reversed-phase HPLC. *J. Agric. Food Chem.* **1993**, *41*, 1616–20.

Mínguez-Mosquera, M. I.; Jarén-Galán, M.; Garrido-Fernández, J. Color quality in paprika. *J. Agric. Food Chem.* **1992**, *40*, 2384–8.

Mínguez-Mosquera, M. I.; Jarén-Galán, M.; Garrido-Fernández, J. Effect of processing of paprika on the main carotenes and esterified xanthophylls in fresh fruit. *J. Agric. Food Chem.* **1993**, *41*, 2120-2124.

Ramakrishnan, T. V.; Francis, F. J. Color and carotenoid changes in heated paprika. *J. Food Sci.* **1973**, *38*, 25–28.

Zapata, M.; Bañón, S.; Cabrera, P. In *El pimiento para pimentón*; Mundi Prensa: Madrid, 1992.

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