Carotenoid Metabolism during the Slow Drying of Pepper Fruits of the Agridulce Variety

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During the industrial drying of peppers of the Agridulce variety, the changes that occur in the concentration of the pigments cannot be explained using one single kinetic pattern that considers only degradation, since both decreases and increases in the pigment concentration occur simultaneously, suggesting a metabolic modulation. Monitoring the effects of the process is carried out using a model system, introducing, as an additional variable, the presence or absence of light. The patterns of change in the concentration of the pigments during drying of fruits in the light and in darkness are similar and can be divided into three phases. In the first phase, there is a metabolic decrease in the pigment content of the fruits. In the second phase, there is an increase in pigment concentration, although this phase does not compensate for the losses in the first phase. This lack of compensation means that there are changes in the concentrations of the pigments found in the fresh fruit. After these metabolically induced changes, there is a third, degradative, phase promoted by external factors. This phase occurs from the very beginning of drying, although its effect, even when photodegradative reactions are induced, is only evident when metabolic activity has ceased in the fruit.

Keywords: *Carotenoids; pigment metabolism; pepper drying; photo- and thermodegradation*

INTRODUCTION

The colorant capacity of paprika is the main parameter that determines its commercial quality. This colorant capacity is due mainly to the initial carotenoid content of the fresh fruits from which it is derived and also to the degree of pigment degradation induced by the industrial process. According to Lease and Lease (1956), the initial carotenoid content is a varietal characteristic, while pigment retention during the process depends on the environmental conditions employed during the steps of drying of peppers and storage of paprika. Several authors have centered their studies on elucidating the effects of both steps (Ramkrishnan and Francis, 1973; Carnevale et al., 1980; Malchev et al., 1982).

During drying, the temperature used provokes oxidative alterations in the pigment content (Zapata, 1992), while during storage of paprika, particular prooxidant conditions exist, such as the presence of polyunsaturated acid plus a high surface area of exposed pigment, and those can promote a faster carotenoid degradation (Carnevale et al., 1980). In parallel with these degradative reactions, both enzymatic and nonenzymatic browning reactions take place. These reactions promote the development of another type of pigmentation which initially is insignificant but which, in some cases, reaches such an extreme that it has to be taken into account (Mínguez-Mosquera et al., 1992).

There is a trend in industry toward dehydrating the fruit as quickly as possible. The most frequently used system is oven-drying, employing temperatures around 60 °C. An alternative method developed in recent years is dry-spraying. With this system, peppers are triturated until a paste is obtained. This is subsequently dried by nebulizing it in hot air. Malchev et al., in 1989, performed a study comparing the pigment content and stability of paprikas obtained using oven-drying and dry-spraying.

It is commonly accepted that processing of peppers has an intrinsic degradative effect on pigments, and the final effect depends exclusively on the harshness of the conditions used. In studies performed previously (Mínguez-Mosquera et al., 1993), it was found that fruits from pepper varieties Agridulce and Bola, with similar initial pigment contents and submitted to the same process, gave rise to two paprikas with very different qualities. Paprikas obtained from peppers of the Agridulce variety had a higher pigment content than those obtained from the Bola variety. Thus, the effects of the process were modified by varietal characteristics.

After this previous study, it was considered necessary to study in detail the specific changes in the carotenoid content during the drying of the fruits of both varieties. In the Bola variety, Mínguez-Mosquera et al. (1994) have shown that some changes in the pigment content during drying do not follow a degradative pattern, as would be expected from earlier studies in the literature (Lease and Lease, 1956). When drying of fruits from this variety is carried out slowly, the synthesis of carotenoids takes place together with degradations induced by external factors. Nevertheless, with fast drying, the biosynthetic reactions do not exist and only the degradative losses are apparent. In the Bola variety drying is not necessarily synonymous with pigment degradation.

Peppers of the Agridulce variety have morphological characteristics easily distinguishable from those of the Bola fruit, such as a thinner pericarp or higher surface area/weight ratio. In addition, the industrial drying of this variety is usually slow and at low temperature. These facts probably lead to a pattern of change in pigment concentration during drying of Agridulce fruits different from that observed in the Bola variety. A knowledge of the pattern of pigment changes during the drying of the Agridulce variety would permit optimization of the drying conditions. To determine the effects of the industrial drying of fruits of the Agridulce variety

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on the pigment content, the carotenoid concentration was measured in industrially supplied fresh and dry fruits. Subsequently, a model drying system was developed in the laboratory to study in detail the individual changes throughout slow drying.

MATERIALS AND METHODS

Raw Material Used. *Industrial Drying System.* Ripe peppers of the Agridulce variety and their corresponding dried fruits were supplied by Netasa (Plasencia, Spain). The industrial drying of the fruits is according to the traditional method used in this area for this variety and consists of a slow dehydration at low temperature (30–35 °C) produced by wood combustion. Simultaneous smoking of the fruits occurs, and this contributes to a modification of the final organoleptic characteristics. This process is performed in the dark and lasts approximately 10–15 days.

Model Drying System. Peppers of the Agridulce variety, harvested in the red mature stage from the Vera zone (Cáceres, Spain), were used. To study the effect of the slow dehydration, the laboratory drying was performed in a thermostatically controlled chamber at a temperature of 35 °C, with recirculation of air. The chamber was divided into one zone with light and another in darkness. The samples placed under illumination are submitted to an illuminance of 1000 lx. Fruits were dried in one or the other of the chambers for a period of some 5–10 days.

Sampling. Samples of fresh and dry fruit were taken randomly from industry lots. In the model drying system, samples were taken from both compartments 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. The weights of samples used were 200 g of pepper pericarp for fresh fruit and 20 g for dry fruit. During the model drying system, the weight of sample used throughout the process changed between both extremes according to the moisture content.

The samples were cut into pieces the thickness of the pericarp and having a surface area of 2 mm^2 by means of a system of blades assembled in the laboratory. The pieces of pulp were mixed by hand to ensure that the samples taken were representative of the whole. Four samples were randomly weighed for analysis: 10 g for fresh fruit, 1.5 g for dried fruit. In all cases the weight used is adjusted to obtain samples of similar dry-matter content during the whole process. The weighed samples were frozen at −30 °C until extraction of pigments. In all samples the dry-matter content was determined using a vacuum heater to establish the progress of the drying process and to calculate the pigment concentrations in dry matter.

Pigment Analysis. All techniques described above were carried out under low green illumination to prevent pigment degradation and/or transformation.

Pigment Extraction. To make the extraction conditions uniform, a certain amount of water was added to each sample to make their moisture contents similar to that of the fresh fruit. A sample with a dry weight of around 1–1.5 g was extracted with acetone until the color was exhausted. The combined extracts were transferred into ethyl ether for saponification with 20% KOH methanol (w/v). An aliquot of saponified extract 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. Each pigment from the saponified extract was isolated and purified by preparative TLC and submitted to the usual identification test for carotenoids (acetylation, reduction with borohydride, acid conversion of 5,6-epoxide groups into 5,8-furanoid, UV–vis spectrum in differents solvents, IR spectrum, etc.). Pigment separation was performed by HPLC with a reversed phase C_{18} column and a water/acetone gradient elution. Quantification was achieved using β-apo-8′-carotenal (Sigma, A 5303) as internal standard with detection at 450 nm. More details about the procedure followed for identification, separation, and quantification of the carotenoid pigments present in this variety were given in a previous publication (Mínguez-Mosquera and Hornero-Méndez, 1993).

Table 1. Changes in the Pigment Content of Peppers of the Agridulce Variety during Industrial Drying and Smoking

pigment	concentration (mg/kg of dry matter)	
	fresh fruit[a]	dry fruit[b]
β-carotene	698.78 ± 98.94	796.85 ± 134.05
cryptoxanthin	502.65 ± 83.99	565.46 ± 102.69
zeaxanthin	894.96 ± 156.80	661.96 ± 134.77
capsanthin	2538.23 ± 235.33	2454.82 ± 353.01
violaxanthin	420.86 ± 19.03	237.82 ± 25.20
capsorubin	274.07 ± 42.89	312.67 ± 23.10

[a] Percent moisture, 88.36. [b] Percent moisture, 7.12.

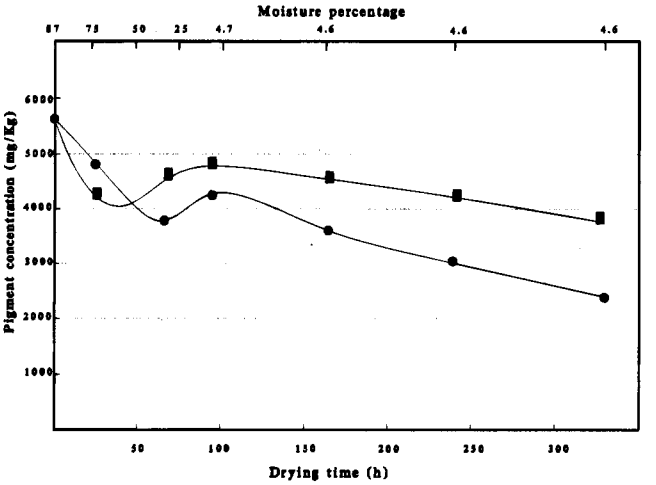

Figure 1. Evolution of the overall carotenoid concentration (milligrams per kilogram of dry matter) during the drying of pepper at 35 °C in darkness (■) and under illumination (●): changes in the moisture content during the processes.

RESULTS AND DISCUSSION

Industrial Drying. During processing of the fruits, fluctuations are provoked in the concentrations of the carotenoid pigments, some of which decrease in concentration while others increase (Table 1). This fact suggests that reorganizations and interconversions take place in the carotenoid metabolic pathway. The balance of the pigment material during the drying phase shows a 6% overall loss. Monitoring the individual pigment concentration shows that the overall loss is due to an increase in the concentration of capsorubin and decreases in the concentrations of zeaxanthin and violaxanthin, while the concentrations of β-carotene, cryptoxanthin, and capsanthin are not modified. These differences are statistically significant ($p < 0.05$). The losses could appear to be quantitatively insignificant, but it is not clear if they are really so small or if they are the result of a coupled synthesis and degradation process as suggested by the increase in concentration of some pigments. To study the effect of slow drying on the pigment content, an experiment was performed using a model drying system at low temperature in which the presence and absence of light were included, to enhance any degradative processes that may have been masked by biosynthetic processes.

Model Drying System. The changes in total concentration of carotenoid pigments during the drying of pepper fruits under illumination and in darkness are shown in Figure 1. In the same figure are detailed the

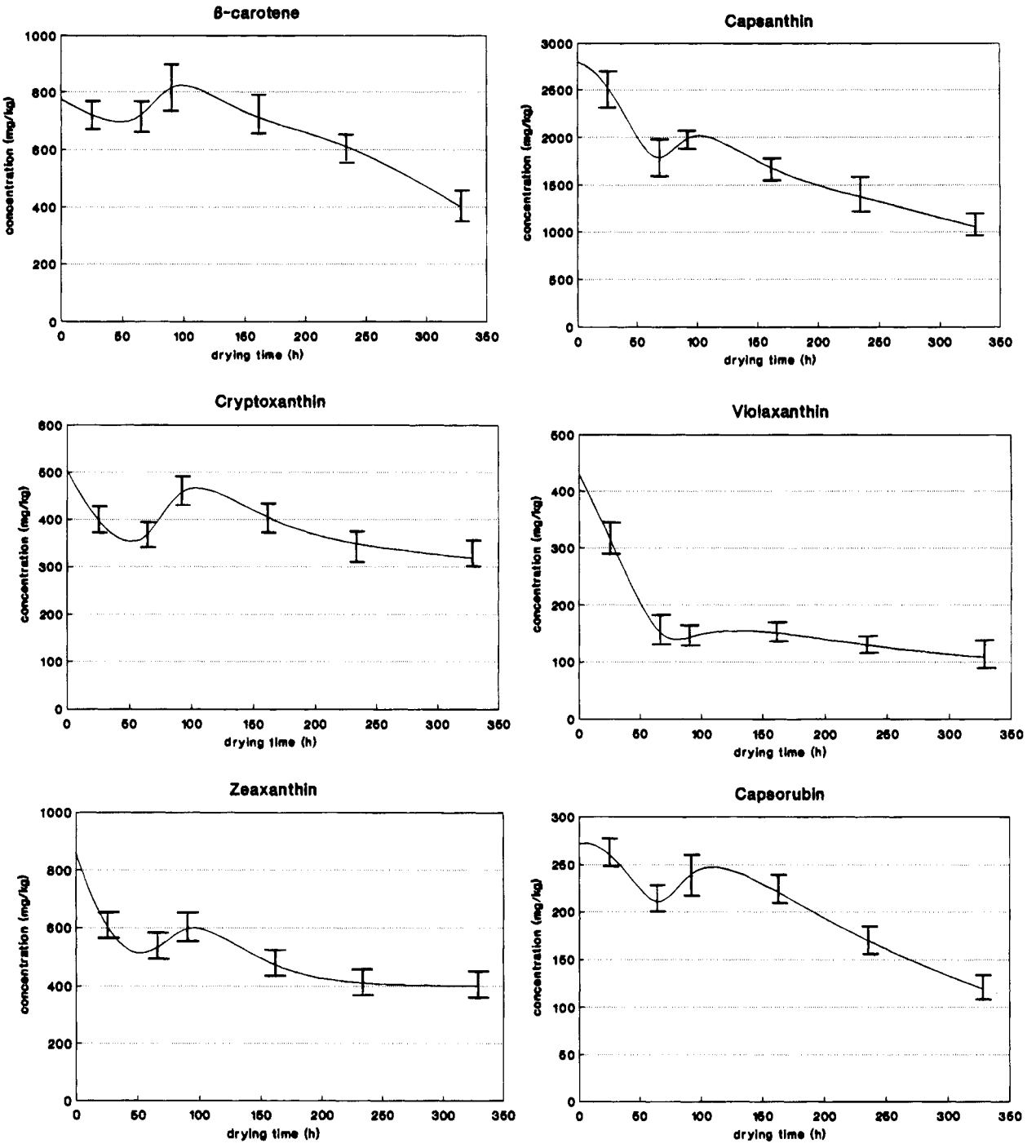

Figure 2. Evolution of the individual carotenoid concentration (milligrams per kilogram of dry matter) during the drying of pepper at 35 °C under illumination. Average of four determinations and limits of confidence as shown.

percentages of moisture throughout the drying process. As drying progresses, there is an increased degradative effect on the carotenoid content. This effect is greater when drying is performed in the light. Nevertheless, the existence of similar fluctuations in the pattern of changes in both light and dark at the beginning of the process suggests a possible metabolic effect.

In Figures 2 and 3 are shown the changes in the concentration of each carotenoid during drying of fruits under illumination and in darkness, respectively. In all pigments, three clearly differentiated phases in the pattern of changes can be observed.

First Phase. Under illumination this phase begins at harvesting and continues until the fruits have a moisture content lower than 30%. With the drying system employed, this phase lasts for the first 50–70 h of drying. The effect found in this period is degradative without formation of new carotenoids. This decrease is very notable for some pigments such as violaxanthin, which retains only some 33% of its initial concentration. Capsanthin and zeaxanthin also decrease in concentration, retaining around 62% of the pigment concentration of the fresh fruit. In the other pigments such as β-carotene, cryptoxanthin, and capsorubin, the decrease is less acute, and retention can be estimated at around 70–85%. The overall pigment retention in this phase with respect to the fresh fruit is around 66%.

In darkness, this phase is shorter than under illumination, occurring only during the first 30–50 h of drying, when the fruits pass from a moisture content of 87% to one of 60%. In quantitative terms the changes in pigment concentration are less acute than in the

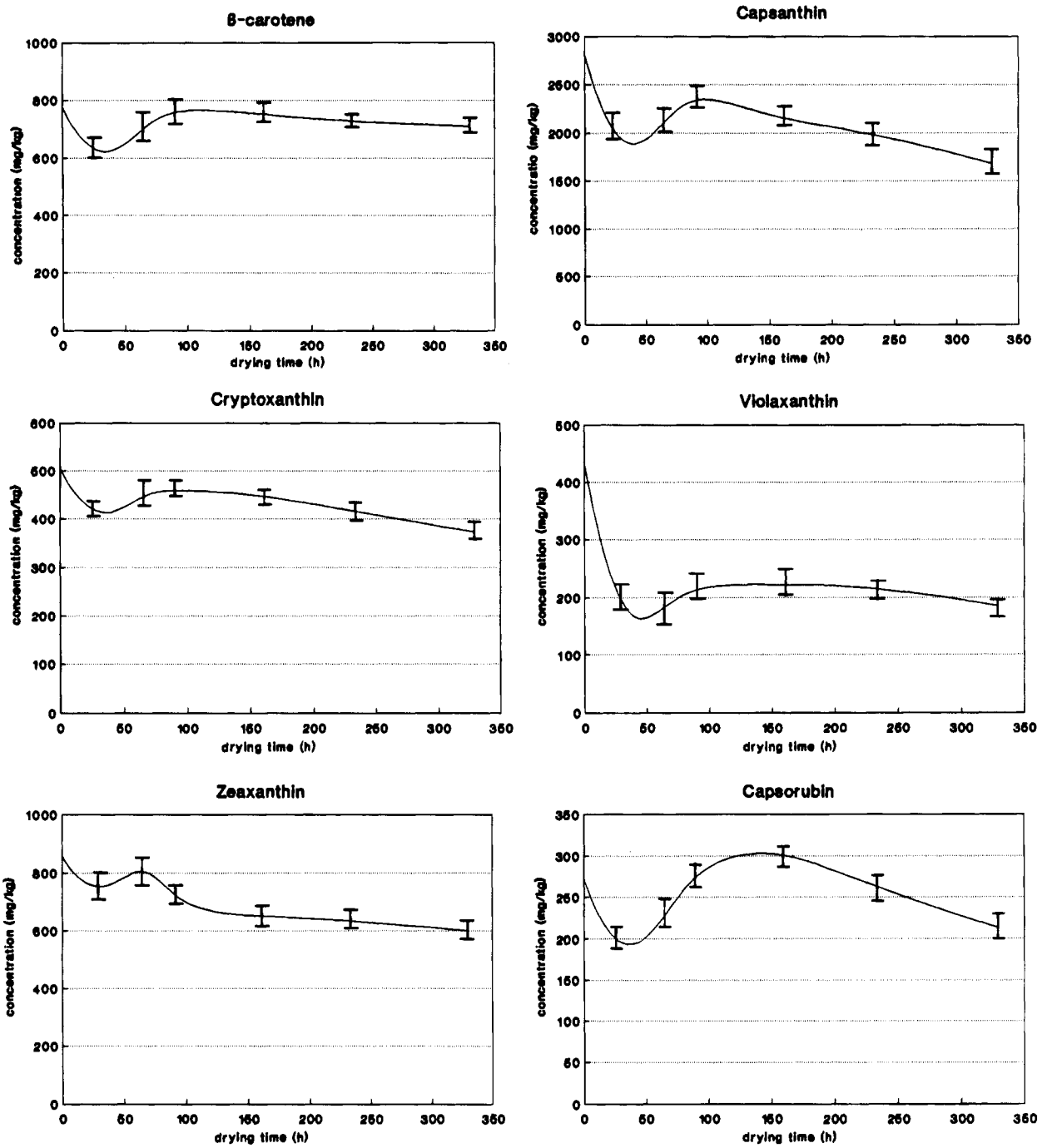

Figure 3. Evolution of the individual carotenoid concentration (milligrams per kilogram of dry matter) during the drying of pepper at 35 °C in darkness. Average of four determinations and limits of confidence are shown.

light. The pigments most affected are violaxanthin, capsanthin, and capsorubin, and the overall pigment retention in this phase is close to 73%.

Second Phase. In both model drying systems this phase continues until the moisture content of the fruit falls to 5%, after approximately 100 h of drying time, and the overall effect is an increase in the pigment concentration of the fruits in comparison to the minimum values resulting from the previous phase.

In the light, β-carotene reaches an even higher concentration than in the fresh fruit, while capsorubin and cryptoxanthin only reach some 90% of the initial pigment content. The effect on the concentration of zeaxanthin, capsanthin, and violaxanthin is less acute, there being only a slight increase in their concentrations. The overall increase in the pigment concentration is not sufficient to compensate for the losses in the previous phase, the retention at the end of the second phase being around 75% compared with the levels in the fresh fruit. The initial decrease followed by a later increase suggests that both phases have a metabolic origin.

In the dark this phase compensates for the initial losses in β-carotene while the concentration of capsorubin reaches levels higher than the initial values. Cryptoxanthin and zeaxanthin almost reach concentrations similar to those of the fresh fruit, while the increase in the concentration of capsanthin is only sufficient to return its level to 85% of the initial content. Violaxanthin is the only pigment that experiences an insignificant increase, which places its retention around 50%. The overall retention levels after this phase are 83%.

Third Phase. This phase occurs when the drying process is finished and the fruit has physical characteristics similar to those of industrially dried fruit. During this phase, the fruits have a moisture content stabilized at 5%; this phase can be considered as storage stage under the conditions of the drying process. It is a phase of continuous degradation. Under illumination, all of the pigments degrade with time and there is an approximately linear relationship between the percentage retention of pigments and time, with a retention at the end of this phase close to 45%. The losses can be attributed to the additive effects of thermo- and photodegradative reactions. In darkness, the absence of photodegradative reactions means that this phase has less effect. Although there are small fluctuations with time, all of the pigments show a slight decrease in concentration and the pigment retention after this "storage" at 35 °C is around 70%.

Comparison between Processes. During both model drying systems, violaxanthin is the pigment that behaves somewhat differently from the general pattern described. There is a strong initial degradation of violaxanthin, under both illumination and darkness (first phase), followed by an almost nonexistent biosynthetic phase (second phase); from this moment on, the concentration of this pigment remains almost constant (third phase). In this last phase under illumination there is a degradation due to the light, but this can be considered insignificant compared with that which has a metabolic origin.

As was expected, illumination is the greatest inducer of pigment degradation under the conditions used. Light has also a metabolic effect, which is masked by its degradative effect. In Figure 1, it can be observed that the overall pigment retention at the end of the first phase under illumination is 7% lower than in darkness, and this can be explained as the degradative contribution of light since the only difference between the drying systems is the presence or absence of light. During the second phase under illumination, this degradative contribution should also occur, and theoretically to a similar extent, since this phase lasts the same time as the previous one. Thus, if the increase in concentration during the second phase under illumination is around 9%, the real synthesis should be 16% to compensate for the simultaneous degradations. In darkness this synthesis is around 10% and coincides with the increase in concentration. These results suggest that the biosynthetic response of the fruit under illumination is stronger than in darkness and this fact allows the fruit to compensate for the photodegradative reactions. Thus, during the second phase, light plays two opposite roles, as an accelerator of pigment catabolism and as an inducer of degradation.

The fluctuations in the pigment concentration detected during industrial drying can be attributed to a metabolic origin, as has been shown by the model experiment. Coupled to the metabolic reactions, a pigment thermodegradation exists, and this is similar in all of the experiments as they were performed at identical temperatures. Nevertheless, at the beginning of drying, pigment thermodegradation is masked by the metabolic reactions and becomes apparent only when drying is finished and the metabolism of the fruit is stopped. Thus, during slow drying, pigment metabolism is the factor that is chiefly responsible for the final quality of the dried fruit. Under illumination, the addition of thermo- and photodegradative reactions enhance the losses during the whole drying procces. In these aggressive conditions the metabolic phases are no longer responsible for the quality.

The existence of metabolic reactions which take place during drying could permit to optimization of the industrial drying conditions to minimize thermodegradative losses. Optimization would allow a final product with a higher colorant capacity and with the best quality possible for the variety to be achieved.

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