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Changes in the Carotenoid Metabolism of Capsicum Fruits during Application of Modelized Slow Drying Process for Paprika Production

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A temperature profile simulating the traditional slow drying process of red pepper fruits, which is conducted in La Vera region (Spain) for paprika production, was developed. Carotenoid and ascorbic acid content, as well as moisture of fruits, were monitored during the slow drying process designed. Data obtained suggested that the evolution of carotenoid concentration, the main quality trait for paprika, directly depend on the physical conditions imposed. During the drying process, three different stages could be observed in relation to the carotenoids. The first stage corresponds to a physiological adaptation to the new imposed conditions that implied a decrease (ca. 20%) in the carotenoid content during the first 24 h. After that short period and during 5 days, a second stage was noticed, recovering the biosynthetic (carotenogenic) capability of the fruits, which denotes an accommodation of the fruits to the new environmental conditions. During the following 48 h (third stage) a sharp increase in the carotenoid content was observed. This last phenomenon seems to be related with an oxidative-thermal stress, which took place during the first stage, inducing a carotenogenesis similar to that occurring in over-ripening fruits. Results demonstrate that a fine control of the temperature and moisture content would help to positively modulate carotenogenesis and minimize catabolism, making it possible to adjust the drying process to the ripeness stage of fruits with the aim of improving carotenoid retention and therefore quality of the resulting product. In the case of ascorbic acid, data demonstrated that this compound is very sensitive to the drying process, with a decrease of about 76% during the first 24 h and remaining only at trace levels during the rest of the process. Therefore, no antioxidant role should be expected from ascorbic acid during the whole process and in the corresponding final product (paprika), despite that red pepper fruit is well-known to be rich on this compound.

KEYWORDS: ***Capsicum annuum*; carotenoids; ascorbic acid; drying process; paprika processing**

INTRODUCTION

Color quality and quantity are factors especially applied in commercial trade of pepper fruits (*Capsicum annuum* L.) and their processed products, paprika and oleoresins, to assess their economic value. As carotenoids are responsible for pepper color (*1*), numerous studies in this area are centered on ensuring the aforementioned factors from growing of fruits until storage of final product, but especially in control of processes applied for obtaining paprika (*2*−*4*). Pepper fruits contain not only carotenoids as natural antioxidants but also others like ascorbic acid, present in relative high amounts in ripened fruits, and they could play a positive role to ensure stability of the final product (*5*) and therefore the color.

Production of paprika consists of two basic operations: drying and milling. A traditional way of drying is commonly applied in La Vera County, one of the main paprika productive areas of Spain. Dehydration of fruits is carried out in drying houses where the heat source is the burning of oak logs (*3*, *6*). In this traditional and handcrafted process, dehydration conditions (temperature and time) are controlled by man without technical equipments, using only his own experience. The initial moisture content and its modification during processing of fruits indicates to the operator how to modify the temperature of the process, which has two significant features: mild and lengthy conditions. Although in some cases decreases on the carotenoid content after drying have been detected, generally, the mild conditions of temperature and initial moisture content of fruits preserve the original carotenoid content of the fruits and even allow an increase on it due to a biological activity of fruits that produces, to some extent, de novo biosynthesis of carotenoids (*2*).

This kind of anabolic metabolism is promoted when initial values of moisture are high, and thermal conditions are very gentle. That is certainly the drawback of this traditional drying process: the lack of information of processing parameters applied because the farmers do not use technical equipments to

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control temperature applied and evolution of fruits moisture. In addition, previous studies have shown that not only is a control of processing parameters necessary but also they should be adapted to ripening stage of fruits (*7*). Some ripeness indexes such as water content or xanthophyll esterification that have been considered recently (*8*) should be used. Standardization of traditional process comprising both temperature control and adaptation to the ripening degree of fruits would provide a way to increase quality of the final product and homogeneity on production. In addition, prediction of color upholding during storing could be done on the basis of processing parameters applied. To reach this objective, two items should be accomplished.

First, it is necessary to have a uniform raw material; that is, a batch of fruits with similar ripeness stage (that also implies a similar initial moisture content). This problem has already been solved with the introduction of new cultivars, like Jaranda and Jariza, that ripen at the same time, also allowing mechanical harvesting in a single operation. The second step is to establish optimal processing conditions in order to prevent or minimize decreases on the carotenoid content of fruits, the main quality trait, always keeping in mind the characteristics of the traditional process carried out in La Vera County.

The aim of this work was to follow the carotenoid metabolism of pepper fruits under the controlled conditions of a modelized slow drying process (time and temperature), reproducing a traditional one, to establish the relationship between the evolution of imposed conditions and its effect on both carotenoid and ascorbic acid content of fruits.

MATERIALS AND METHODS

Plant Material. Fruit of peppers (*Capsicum annuum* L.) of Jaranda cultivar were used for the present study. Plants were grown in an open field at La Vera county (Cáceres, Spain) and harvested at the fully ripe stage.

Chemicals and Reagents. HPLC-grade acetone and methanol were supplied by Teknokroma (Barcelona, Spain). Diethyl ether containing ca. 7 ppm BHT was purchased from Microdur, S. L. (Sevilla, Spain). HPLC-grade water was obtained with a MilliQ water purifying system from Millipore (Milford, MA). All-trans-β-apo-8′-carotenal, used as internal standard for carotenoid determination, and ascorbic acid were purchased from Sigma (Barcelona, Spain). Other reagents were all of analytical grade.

Slow Drying Model Applied. Drying of fruits was developed in a heater with digital control of temperature (Memmert, model IPC 500, Germany) and controlled by software-PC (Celsius for Windows, Memmert, Germany). The temperature−time profile was a reproduction of that of real dryers from La Vera County (**Figure 1**). The model was developed using the average values for time−temperature profiles of eight industrial drying chambers from La Vera County (*9*). The model diagram includes the characteristic falls and rises in temperature associated with the traditional fire drying. Ripe fruits (5 kg) were placed in the drying chamber, where each fruit was suspended by its pedicele. Samples were taken every 24 h for a period of 8 days, to analyze the carotenoid and ascorbic acid content that allows a time-response curve to the conditions used to be obtained. Samples were lyophilised and frozen at −30 °C until analysis.

Pigment Extraction. A known weight of lyophilised sample equivalent to 2−10 g of fresh fruit (depending on the degree of ripeness) was reconstituted with water during 30 min and subsequently extracted with acetone by using a homogenizer Ultraturrax Y25 (Janke Kunkel Ika-LabortechniK). Extraction was repeated until the complete exhaustion of color (usually 4−5 extractions were enough). All extracts were pooled in a separator flask and shaken with diethyl ether. A 1−5-mL sample of a 100 μg/mL all-trans-β-apo-8′-carotenal stock solution was added to the extract as internal standard. A sufficient quantity of 10% NaCl was added at the end to aid in the separation of the phases.

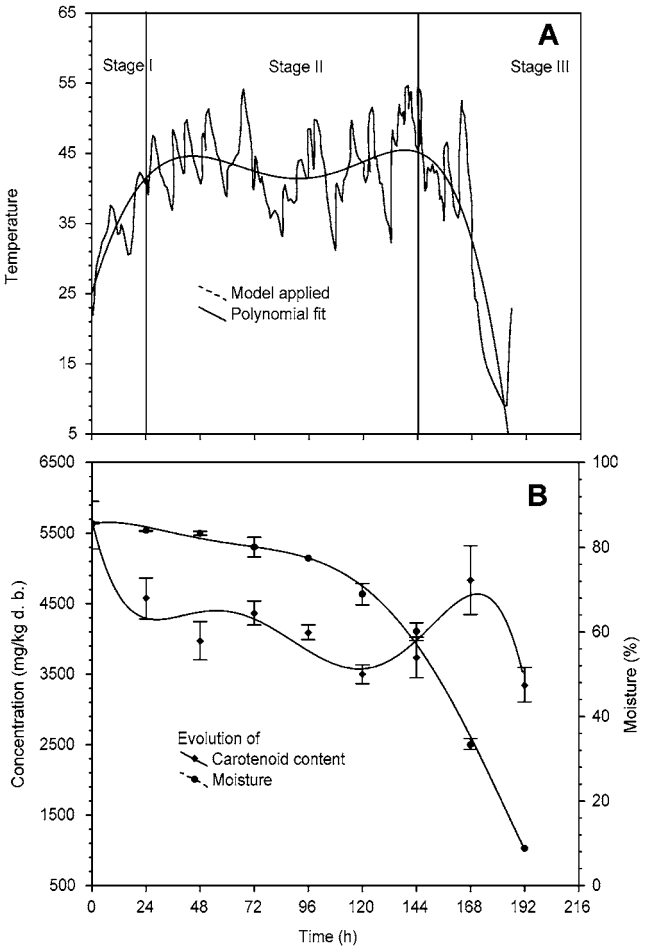

Figure 1. Temperature–time profile applied in the slow drying of pepper fruits (A) and response of carotenoid and moisture content to conditions imposed (B).

Organic phase, containing the pigments, was saponified with 40 mL of 20% KOH−methanol during 1 h at room temperature. After addition of water, the pigments were subsequently extracted with diethyl ether, evaporated in a rotary evaporator, and taken up to 25 mL of acetone. A 1-mL aliquot of the sample was cleaned previous to injection by using a benchtop centrifuge model Micro-Centaur (MSE Scientific Instruments, Sussex, England) at 12 000 rpm and stored at −30 °C until analysis by HPLC.

HPLC Separation and Quantification of Carotenoids. HPLC analyses were performed with a Waters 600E quaternary pump equipped with a Waters PDA 996 diode array detector (Waters, Barcelona, Spain) and controlled with a Millennium data acquisition station. The HPLC system was equipped with a reverse phase Spherisorb ODS-2 (25- × 0.46-cm, 5-μm) column (Teknokroma, Barcelona, Spain). A precolumn (1- × 0.4-cm) of the same material was fitted to protect the main column. Separation and quantification of the carotenoid pigments was carried out using a method previously developed by the authors (*10*). The method uses a binary gradient elution system of acetone−H_2O as follows: initially, 75% acetone is maintained for 5 min, changing linearly to 95% in 5 min ,and kept for 7 min. Flow rate was 1.5 mL/min, sample injection volume was 5 μL, and spectrophotometric detection was performed at 450 nm. All-trans-β-apo-8′-carotenal was used as internal standard for calibration and quantification. Pigment identification has been described in detail in previous publications (*10*).

Extraction and analysis of ascorbic acid. Vitamin C (L-ascorbic acid) was determined by HPLC, following the method described by Howard et al. (*11*) with some modifications. Pepper samples (10 g), free of stems and seeds, were cut in small pieces, homogenized with 40 mL of 3% (w/v) citric acid, and centrifuged at 20 000 rpm. The resulting pellet was reextracted with 40 mL of citric acid solution and centrifuged.

Table 1. Concentration (mg/kg d.b.) of Main Carotenoid Pigments Present in Red Pepper Fruits Measured during Their Modelized Dehydration Process

time (h)	β-carotene	β-cryptoxanthin	zeaxanthin	antheraxanthin
0	655.97 ± 41.31[a]	575.50 ± 30.54	640.50 ± 40.40	184.82 ± 7.51
24	495.95 ± 33.41	450.81 ± 18.03	455.60 ± 34.41	183.51 ± 12.72
48	395.55 ± 7.28	385.48 ± 21.34	381.61 ± 34.62	146.05 ± 10.20
72	402.23 ± 25.65	425.17 ± 12.06	447.64 ± 23.49	123.06 ± 9.46
96	381.38 ± 11.01	392.30 ± 7.26	382.00 ± 14.02	88.56 ± 10.32
120	308.58 ± 9.01	344.36 ± 7.48	356.59 ± 15.86	66.09 ± 5.20
144	282.04 ± 18.64	349.55 ± 10.59	350.58 ± 8.11	46.53 ± 5.48
168	355.90 ± 20.70	491.70 ± 10.71	462.25 ± 46.87	58.74 ± 6.13
192	244.09 ± 9.54	303.95 ± 16.52	313.11 ± 17.50	38.12 ± 4.45

	violaxanthin	capsanthin	5,6-epoxide capsanthin	capsorubin
0	234.04 ± 8.79	2210.6 ± 114.65	69.42 ± 8.60	218.32 ± 11.32
24	177.30 ± 12.40	1739.7 ± 111.81	91.04 ± 5.19	209.99 ± 13.11
48	158.72 ± 10.66	1477.1 ± 123.37	72.15 ± 1.64	186.22 ± 3.86
72	171.28 ± 6.54	1669.0 ± 128.90	62.54 ± 8.87	212.95 ± 3.53
96	160.65 ± 5.22	1538.2 ± 34.13	45.56 ± 0.88	215.69 ± 7.64
120	140.17 ± 5.63	1350.1 ± 36.22	35.43 ± 2.44	194.59 ± 8.34
144	259.32 ± 11.16	1371.9 ± 92.74	34.44 ± 3.24	202.54 ± 12.07
168	318.45 ± 7.11	1826.2 ± 71.16	208.54 ± 10.87	257.46 ± 14.91
192	173.87 ± 61.31	1152.1 ± 30.67	155.26 ± 8.28	190.70 ± 5.61

[a] Mean ± standard deviation for four replicates.

Both supernatants were collected and taken to a final volume of 100 mL. An aliquot of 1 mL was stored in the freezer (−30 °C) until analysis.

Samples were analyzed by HPLC using an NH_2−Spherisorb (12- × 0.46-cm, 5-μm) column (Teknokroma, Barcelona, Spain) and 100 mM NaH_2PO_4, pH = 6.5 buffer solution as mobile phase at a flow rate of 1 mL/min. Detection was performed spectrophotometrically at 260 nm. Quantitation was achieved by constructing a calibration curve with ascorbic acid standard solutions. HPLC analyses were carried out in an HP 1100 chromatographic system (Hewlett-Packard, Germany) equipped with a quaternary pump, a diode array detector, and controlled with an HP ChemStation A.05.02 data acquisition station (Hewlett-Packard, Germany).

Statistical Analysis. Analyses were carried out in quadruplicate. Values in the text are means with their standard deviations. Data were analyzed parametrically, and mean values at different time points of red or yellow fractions were compared to test for significant differences (Duncan test). Significance was set at $p < 0.05$. A polynomial fit was used to follow the changes of data during the experiment as well as to reproduce and modelize the temperature−time profile. The statistical analysis was performed with a statistical software package (STATISTICA for Windows, 5.5, 1999; Statsoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Reproduction of traditional drying process means to apply an irregular temperature profile with continuous periods (intervals of ca. 24 h) of rises and falls with initial and end phases of fire up and down. Evolution of carotenoid content is the response of pepper fruits to the aforementioned physical imposed conditions, and a direct correlation can be established between the temperature pattern, reproduced in **Figure 1A**, and evolution of carotenoid content, represented in **Figure 1B**. From the polynomial fit to the temperature−time profile, the modelized dehydration process applied may be divided in three periods (stage I, II, and III). **Table 1** shows individual evolution of main carotenoids present in pepper fruits during their complete dehydration process, so that particular changes, including increases and decreases, may be followed. Some of these carotenoids, such as antheraxanthin and 5,6-epoxide-capsanthin, do not mainly contribute to carotenoid profile in quantitative terms, but they are key pigments in the biosynthetic pathway of red pepper carotenoids (*12*). Individual carotenoids are grouped in two isochromic fractions, red and yellow, and their evolution is illustrated in **Figure 2**.

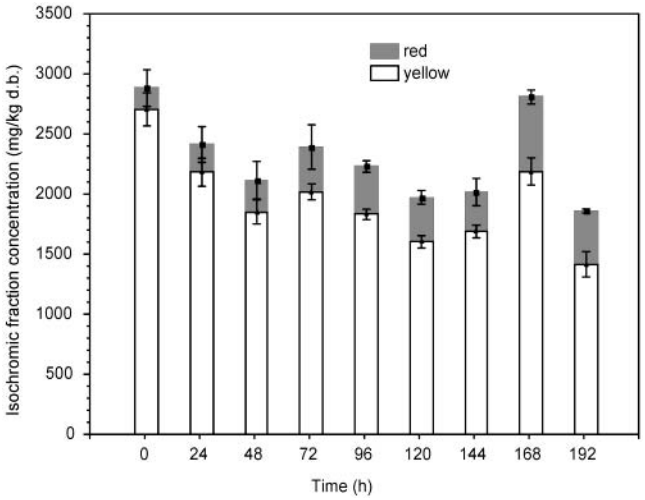

Figure 2. Evolution of red and yellow isochromic fractions present in pepper fruits during the modelized dehydration process applied.

In stage I, an initial period of slow increase in temperature (fire up simulation during 24 h) produced a sharp decrease (ca. 20%) of the carotenoid content, likewise affecting the red and yellow fractions. During this stage, top temperatures of 42 °C were imposed, with an increase on mean temperature from 22 to ca. 34 °C, conditions that, as can be observed in **Figure 1A**, slightly affected the moisture content. This fact together with the temperature profile could enhance activity of oxidative enzymes such as lipoxygenase and peroxidase that, as for all enzymes in general, increase their action with increasing temperatures. This group of enzymes produces an amplified level of oxidative stress promoting degradative reactions. Lipoxygenase has been described in fresh pepper fruits, and during dehydration process its activity remains almost unaltered, at least during the first 2 days of drying (*13*). This enzyme is involved in carotenoid degradation via hydroperoxides formation from lipid oxidation. Peroxidases alter organoleptic properties such as color during fruit processing by generation of activated oxygen species that subsequently react with fruit components (*14*). These enzymes are known to increase their activity during fruit processing as in the case of dehydration of cocoa beans that showed a 10-fold peroxidase activity during drying with respect to fresh fruit (*15*). Although temperatures applied in this stage may produce inactivation of this enzyme, it is known that heat-denatured peroxidases enhance lipid peroxidation reactions (*16*). Therefore, enzymatic processes produce a chain oxidation reaction, promoted by remaining water content, and kinetically favored by the increase on mean temperature, which affects not only fruit carotenoid concentration but also ascorbic acid content (**Figure 3** shows the evolution) decreasing a 66% during stage I, which is in agreement with other works on thermal processing of fruits and vegetables where enzymatic oxidation of ascorbic acid is favored by temperature (*17*, *18*).

Stage II covers the next 5 days with a mean temperature of 44 °C, reaching 55 °C as top value, and always above 31 °C. The carotenoid content initially fluctuated down and up to finally reach a final constant value without significant differences with respect to that at the beginning of stage II. The first degradative processes that took place at the fire up phase are still active and initially reduced even more carotenoid concentration. However, it is noteworthy that the increase in both isochromic

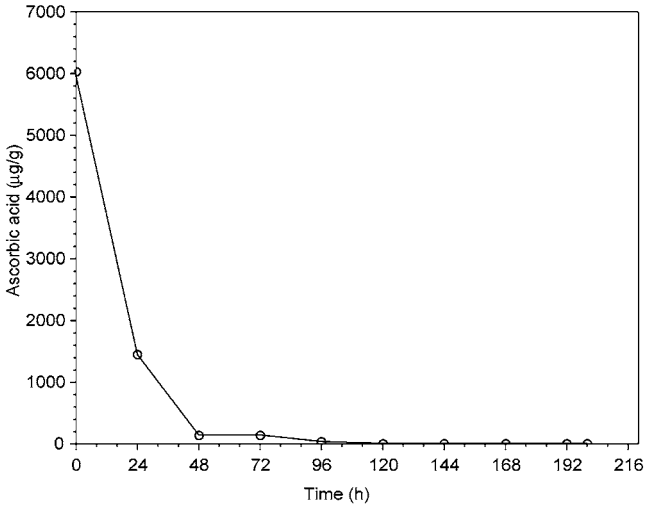

Figure 3. Evolution of the ascorbic acid content during the slow drying of pepper fruits.

fractions ocurred at 72 h, so fruits showed an improvement of the anabolic ability. This could be interpreted as a defense mechanism against the continuous oxidative stress initiated at the fire up phase. Ascorbic acid content, that may act as antioxidant, is reduced to trace levels just at the beginning of this stage, so no antioxidant role should be expected from ascorbic acid during the whole drying process. Therefore, biosynthesis of carotenoids, antioxidants, is employed to counteract the oxidation progress. However, this recovering phase is immediately followed in the next 24 h by a decline in total carotenoid content. During this prolonged mild temperature treatment joined with significant losses in water content (moisture content fell from 80 to 60%), it seems that fruits are trying to acclimate to imposed conditions, reaching an equilibrium between biosynthetic and degradative processes that it is finally reached and maintained at $t = 120$ and $t = 144$ h, respectively.

Anabolic capability is fully denoted, that is, biosynthesis of both pigment fractions took place, but as can be seen in **Figure 2**, only values of red fraction are upheld at later time points without significant changes, while the yellow pigment concentration fell continuously. Changes on the isochromic pigment fractions include degradative reactions and biotransformation of pigment following the carotenoid biosynthesis pathway operating in *Capsicum* fruits (*2*, *3*, *12*). The imbalance between those processes produced a decline in yellow fraction content, while losses in red concentration are compensated with the biotransformation turn-over (**Figure 4A**).

Stage III (144−192 h) is characterized by sharp increase on total carotenoid concentration (ca. 35% with respect to content at 144 h) at 168 h, immediately followed by a sharp decrease in the next 24 h that represents the fire down. Both accumulation and degradation affected all carotenoids, as may be observed in **Figure 2** and **Table 1**, and they are joined with a definitive fall of moisture, 35% at 168 h and 9% at 192 h. Like at the beginning of stage II, the oxidative-thermal stress induced anabolic processes, simulating an over-ripening process, as could be achieved in plant. The dehydration process ended with general losses on carotenoid concentration. Perhaps a prolonged decrease of processing temperature at the end phase would have favored a higher retention of carotenoid content.

No more than promotion of degradation and inhibition of anabolic processes could be expected from the mean temperatures and warm holding applied to fruits, but in the present

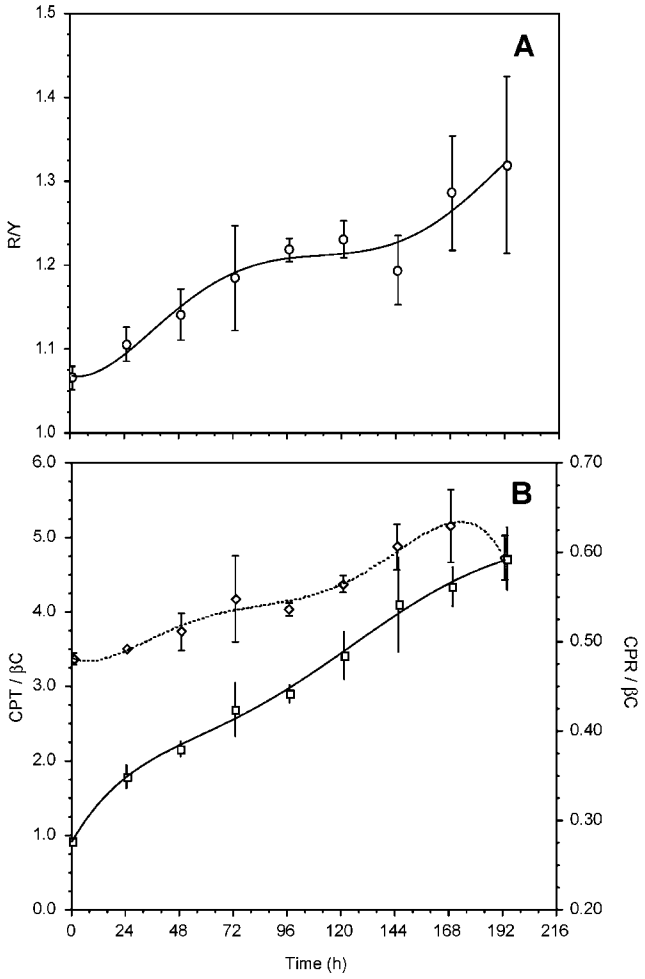

Figure 4. (A) Evolution of red to yellow concentration ratio (R/Y). (B) Capsanthin to β-carotene (CPT/βC, dotted line) and capsorubin to β-carotene (CPR/βC, full line) concentration ratio during the slow drying of pepper fruits.

study this is not the case. The opposite effect is completely denoted at some time intervals. That is the response of fruits against oxidative stress caused by temperature, decreases of water content and ascorbic acid, and accumulation of oxidant species. To promote carotenoid biosynthesis is a protection mechanism because carotenoids produced a moderate effect on temperature and counteracted the pro-oxidant environment (*19*, *20*). Reactivation of biosynthesis of antioxidants under environmental or processing stress has been demonstrated in cucumber cotyledons and rice seedlings (*21*, *22*). Pigment biosynthesis has been associated with the response of some carotenogenic fruits to stress conditions, as in the case of moderate and high-temperature processing of bittermelon, *Momordica charantia* L., and tomatoes, *Lycopersicon esculentum* (*23*, *24*). Barry et al. (*25*) demonstrated in drought-stressed barley seedlings that not only carotenoid biosynthesis but also their esterification may be induced under stressing conditions.

It is noteworthy that the preferential accumulation of red carotenoids (i.e., capsanthin and capsorubin) are continuously formed from their precursors in the yellow fraction. This fact is clearly confirmed by the evolution of red to yellow pigments ratio (R/Y), shown in **Figure 4A**. The tendency of this ratio at any of the three dehydration stages is to rise. **Figure 4B**, in particular, depicts the evolution of the concentration ratio between capsanthin to β-carotene and capsorubin to β-carotene, an evolution that follows the same increasing line. The same

pattern is observed for the evolution of the concentration ratio between capsanthin to β-carotene and capsorubin to β-carotene, which are good indicators for the biosynthesis recovery by linking end-products (capsanthin and capsorubin) and a precursor (β-carotene) of the carotenogenic pathway (*12*).

These biotransformation processes make the imbalance between the anabolic and catabolic process less unfavorable. Therefore, control of temperature and moisture are main factors to be optimized and controlled to modulate carotenogenesis and minimize catabolic reactions that are mainly present at initial and end periods of processing. Performing this kind of model processing will make it possible to adjust the drying process conditions to the ripeness stage of fruits, with the aim of promoting carotenoid retention and therefore quality of the resulting product.

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