DOI:10.1021/jf804058m

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Stability of Paprika without Supplementary Antioxidants during Storage under Industrial Controlled Conditions

Antonio Pérez-Gálvez, Dámaso Hornero-Méndez, and María Isabel Mínguez-Mosquera*

Grupo de Química y Bioquímica de Pigmentos, Departamento de Biotecnología de Alimentos, Instituto de la Grasa (CSIC), Av. Padre García Tejero, 4, 41012-Sevilla, Spain

Different quality parameters of paprika samples stored under controlled conditions (temperature 4 °C and relative humidity 70%) and without reconstitution of the antioxidant levels were analyzed. These included carotenoid composition, ASTA values (as specified by the American Spice Trade Association), fatty acid composition, and peroxide index, in order to determine the progress of autoxidative reactions and directly correlate the loss of carotenoid fraction with the development of prooxidative processes. Evolution of the carotenoid content indicated that autoxidative reactions minimally took place and that coloring capacity was maintained. Peroxide values were very low (1 mequiv/kg) and reached values of 3 mequiv/kg at the end of the storage period. Control of microbial flora during storage also showed how the storage conditions preserved quality of the paprika, as the flora was kept at levels similar to those of the beginning. Therefore, controlled storage conditions were enough to preserve and keep the overall quality of paprika without reconstitution or addition of antioxidants to the product.

KEYWORDS: Paprika; designation of origin; stability; storage; carotenoid content; peroxide value

INTRODUCTION

Paprika is a widely consumed spice condiment, with several applications as a natural colorant in the food industry mainly to correct or even reinforce color to foodstuffs or to provide some flavoring. Two essential aspects of the commercial value of this spice are coloring power and stability. The first is related to the carotenoid concentration of the fruit and depends on pepper variety, ripening stage, and growing conditions (*1*−*4*). The stability is determined by the processing conditions reached during the operation units applied to obtain paprika (*5*, *6*), and once it is processed, suitable storage conditions will ensure commercial life. This is crucial as paprika is not immediately consumed or used for industrial purposes but is stored.

As well as in order foodstuffs, progress of autoxidative reactions, which are responsible of the degradation of carotenoid pigments, could be delayed in paprika by the addition of antioxidants (ascorbic acid, tocopherols, and herbs extracts) and modification of physical parameters (light, temperature, oxygen, and moisture). These methods exogenously avoid the oxidation potential of the sample, which is mainly based on the polyunsaturated fatty acid profile of the paprika (*7*) and promoted by excessive thermal stress during paprika processing (*8*, *9*), inappropriate storage conditions or a combination of both issues. Addition of antioxidants is a common practice to increase the stability of foodstuffs. In the case of paprika, the raw material (the red pepper fruits) contains ascorbic acid, which could be considered as high (3−5 mg/g dry fruit), but during processing, the fruit loses the main part of its ascorbic acid content (*1*, *10*). Thus, addition of ascorbic acid to improve the antioxidant capability of dry material could be considered as a reconstitution technique. This is useful in the sight of results described in some studies (*11*, *12*). Other antioxidants used to improve the coloring stability of paprika are tocopherols and rosemary extract, which showed different coloring protection properties depending on storage conditions. At temperatures higher than 25 °C, the tocopherols display a marked protective effect, while at 5 °C, the rosemary extract has the strongest antioxidant action. The protective effect of added antioxidants seems to depend on conditions used to store paprika such as relative humidity and temperature. High humidity levels provide the proper environment where hydrophilic antioxidants (such as ascorbic acid) could perform their antioxidant activity (*13*). When temperature increases, then the activity of the lipophilic antioxidants becomes key as autoxidative reactions take place at higher rates. To achieve a wide range of antioxidant activity in different storage conditions, more than one antioxidant should be added. In any case, the addition of antioxidants is avoided because of labeling restrictions, when paprika is commercialized under Protected Designation of Origin (PDO) regulations.

Therefore, use of other strategies to improve color stability should be more appropriate instead of reconstitution or adding antioxidants, in order to maintain authenticity of the product. One of those strategies could be the endogenous modification of the oxidation potential. If the lipophilic matrix of the food is prone to oxidation (high content of polyunsaturated fatty acids as

*To whom correspondence should be addressed. Phone: +34954691054. Fax: +34954691262. E-mail: minguez@cica.es.

 Published on Web 05/05/2009

in paprika), changing that composition by a monounsaturated profile could minimize oxidative susceptibility (*14*). This could be achieved by application of a breeding program where cultivars with a less unsaturated fatty acid profile should be selected. But one of the best choices should be application of those technological conditions that allow a correct preservation of natural components and reduced progress of autoxidative reactions, not only in processing but also during storage. In the case of processing of red pepper for paprika production, one of the key operation units that will mark the evolution of carotenoid stability is the dehydration step (*5*, *6*). Conventionally, drying of peppers is performed in convection dryers, with drying temperatures in the range of 50−80 °C. In this case, it has been shown that excessive thermal stress (overdrying) promotes carotenoid oxidation (*9*). The traditional slow drying carried out in La Vera county (Spain) uses heated air by the combustion of oak logs, which is supposed to process with less thermal impact on the fruits, with drying temperatures in the range of 35−40 °C (*10*). This temperature regime allows the fruit to stay metabolically active in the first stage of processing, and biosynthesis of carotenoids is possible, resembling an over-ripening process. Thus, it is common to find after dehydration a similar or even a higher mass balance respect to the carotenoid content (*15*, *16*). This means that a proper technological procedure has been applied. This high quality product presents a smoked characteristic flavor, which is a positive feature within the defined standards of the regulatory council of the PDO *Pimentón de La Vera*. According to the PDO regulation, addition of antioxidants to prevent degradation during storage is out of scope, and preservation techniques should deal with the application of proper storage conditions. Another aspect to consider during storage is that in the traditional dehydration of red pepper for paprika production, carried out in La Vera county, moisture and temperature processing conditions allow the preservation of the natural microbial flora in the final product. It has been shown that molds and yeast are best adapted to the restrictive conditions that are reached during dehydration, but counts are generally low (*17*). Nevertheless, it should be also convenient to control the progress of flora during storage.

The aim of the present work was to monitor the evolution of quality parameters of paprika produced with the traditional slow drying technique (La Vera process) during a storage process with controlled conditions of temperature and humidity, applying those normally used by paprika dealers. Achievement of good results in terms of color retention would imply that a combination of good processing practices with suitable storage conditions is enough to conserve color quality of this product without the addition of antioxidants. The main quality parameter of this product, color, was monitored through the 12 storage months, obtaining the evolution of the ASTA values and of the carotenoid content. This evolution would correlate with other quality indexes that have been scarcely applied in paprika: the fatty acid composition and peroxide index value (PV). Additionally, controls of the microbial flora of the product were performed in order to delimitate the suitability of storage conditions to preservation. The connection between all parameters would give a better approach to the stability and overall quality of this product protected with PDO regulations.

MATERIALS AND METHODS

Raw Material and Sampling Procedure. Four paprika samples (A, B, C, and M, a mixture 1:1:1 of the three former samples) from La Vera county (Cáceres, Spain), produced from red pepper fruits (*Capsicum annuum* L.) of the variety *Jaranda*, and harvested at the same season, were used. Processing conditions at the drying and milling steps correspond to the traditional drying process under La Vera PDO regulations. Samples were kindly provided by Embutidos Palacios S.A. enterprise in 25 kg plastic sacs. For the present study, samples sacs were stored in a dark cool room at 4 °C and 70% of relative humidity for up to 12 months. Sampling was performed every 30 days, taking a 2 kg representative sample from random places within the paprika mass. Subsamples were taken for respective analysis and stored at −30 °C until analysis.

Chemicals and Reagents. HPLC-grade acetone and methanol were supplied by Romil Ltd (Teknokroma, Barcelona, Spain). Diethyl ether containing ca. 7 ppm BHT was purchased from Scharlau (Microdur, Sevilla, Spain). HPLC-grade water was obtained with a Milli-Q water purifying system from Millipore (Milford, MA, USA). All-*trans*-β-apo-8′-carotenal and heptadecanoic acid, used as internal standard for carotenoid determination and fatty acid analysis, respectively, were purchased from Sigma (St. Louis, MO, U.S.). The rest of the reagents were all of analytical grade.

Carotenoid Extraction and Quantification. Two grams of paprika sample were reconstituted with 5 mL of water during 30 min and subsequently extracted with acetone (30 mL), by using a homogenizer Ultraturrax Y25 (Janke Kunkel Ika-LabortechniK, Staufen, Germany). 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. One to five milliliters 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. The organic phase, containing the pigments, was saponified with 40 mL of 20% KOH-methanol during 1 h at room temperature. After the addition of water, the pigments were subsequently extracted with diethyl ether, filtered through a bed of anhydrous sodium sulfate, evaporated in a rotary evaporator, and taken up to 25 mL of acetone. A 1-mL aliquot of the sample was cleaned prior to injection by using a benchtop centrifuge model Mikro 20 (Hettich Zentrifugen, Tuttlingen, Germany) at 16000*g* and stored at −30 °C until analysis. HPLC analyses were performed with a Waters 600E quaternary pump equipped with a Waters PDA 996 diode array detector (Waters, Milford, MA, U.S.) and controlled with a Millennium data acquisition station. The separation and quantification of the carotenoid pigments were carried out using a method previously developed by the authors (*18*). This method uses a C18 reverse-phase column (Spherisorb ODS-2, 5 μm, 0.46 cm × 25 cm supplied by Teknokroma, Barcelona, Spain) and a binary gradient elution system of acetone−H_2O at a flow rate of 1.5 mL/min, a sample injection volume of 5 μL, and detection at 450 nm. Quantification was carried out using all-*trans*-β-apo-8′-carotenal as the internal standard. Data were expressed as retention (%) of total carotenoid content, red (R) and yellow (Y) isochromic fractions. The isochromic R fraction comprises capsanthin, capsorubin, and capsanthin 5,6-epoxide, whereas the Y fraction comprises violaxanthin, antheraxanthin, zeaxanthin, cucurbitaxanthin A, β-cryptoxanthin, and β-carotene.

Microbiological Analysis. Ten grams of paprika was aseptically taken for microbiological analysis every 30 days. Samples were homogenized with 90 mL of 1% peptone water (Pronadisa, Alcobendas, Madrid, Spain). Eight decimal dilutions were prepared and plated in different media. Total mesophilic aerobic bacteria were counted on standard PCA medium, and plates were incubated at 31 °C for 72−96 h; psychrotrophic bacteria were plated on standard PCA medium, and plates were incubated at 4 °C for 6−7 days; enterobacteria were counted on VRBG medium and incubated at 37 °C for 24−48 h; coliforms were counted on VRBA medium and incubated at 31 °C for 24−48 h; lactic acid bacteria were grown on MRS medium at 37 °C for 72−96 h; Micrococcaceae were plated on MSA medium and incubated at 30 °C for 72−96 h; *Clostridium* genus was investigated on SPS medium at 46 °C for 24−48 h; *Enterococcus* was counted on SB medium and incubated at 37 °C for 24−48 h; *Staphylococcus aureus* were counted on BP medium and incubated at 37 °C for 48 h. Results are expressed as logarithms of colony formation units per gram (log cfu/g).

Extraction and Determination of Total Fat Content. Five grams of sample were dehydrated overnight at 35 °C using a vacuum oven and extracted with 50 mL of *n*-hexane for 4 h in a Sohlext apparatus. The fat content was calculated from the difference in weight of the sample before and after extraction and the amount of oil collected in the flask.

Analysis of Fatty Acids. Fatty acids methyl esters (FAMEs) were prepared from the extracted oil by direct interesterification, adding 5 mL

of 0.2 N MeONa solution to a the sample aliquot (0.1 g). The sample was mixed with the reagent in a test tube, adding the appropriate amount of dissolved heptadecanoic acid (C17:0) as internal standard (7−10 mg) for later quantification. The reaction was carried out by heating at 80 °C for 15 min in a water bath. When the reaction was finished, the tube was allowed to cool. Then, 5 mL of 3% (v/v) H_2SO_4−MeOH was added, and the mixture was heated for 15 min. The tube was allowed to cool, and 1 mL of hexane was added, together with 10% (w/v) NaCl solution to help in the transfer of the methyl esters to the organic phase. The methyl esters were separated and quantified using a Hewlett−Packard gas chromatograph (5890 Series II) fitted with FID. The working conditions were the following: oven temperature of 175 °C for 4 min, with a rise of 15 °C/min to 240 °C. The temperature was maintained for 5 min and then returned to the initial 175 °C. Both the detector and the injector were fixed at 250 °C. The solution of methyl esters (1 μL) was injected on to the column (Supelcowax 10, 30 m × 0.32 mm, 0.25 μm film), and the peak areas were measured using an integrator (Hewlett-Packard 3396II). Quantification was performed using the area of the internal standard. Composition data were expressed as percentage values of the total fatty acid content.

Evaluation of Color by the ASTA 20.1 Method. The measurement was carried out according to the Official Analytical Methods of the American Spice Trade Association (*19*). The paprika sample (weigh 0.1 g to the nearest 0.0002 g) was placed in a 100 mL standard flask, and acetone was added to the mark and the flask shaken. The solution was left for 16 h at room temperature in the dark. A portion of this solution was used for the spectrophotometric measurement at 460 nm with an acetone blank. The ASTA units were calculated according to the following equation:

$$\text{ASTA}-20.1 \text{ units} = \text{absorbance} \times 16.4 \times I_f/g \text{ of sample}$$

where I_f is a correction factor for the instrument, calculated from a standard solution of potassium dichromate and ammonium and cobalt sulfate. Four determinations of the ASTA value were made for each paprika sample.

Peroxide Index Value (PV). Determination of the peroxide index was carried out using the method of Hornero-Méndez et al. (*20*).

Statistical Analysis. Analyses were carried out in quadruplicate. Values in the text are the means with their standard deviations. Significance was set at $p < 0.05$. The statistical analysis was performed with a statistical software package (STATISTICA for Windows, 5.5, 1999; Statsoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Evolution of Carotenoid Content. Figure 1 shows the evolution of total carotenoid content during the storage of paprika (**Figure 1a** for paprika A and B; **Figure 1b** for paprika C and M), including a polynomial fit that best represents the trend. The general tendency was similar for all samples, and the amount of carotenoids that were lost was not significantly different on each case (Duncan's test $p > 0.05$). Samples showed a total retention of 83.3%, 85.8%, 81.9%, and 81.3% (A, B, C, and M, respectively), after 1 year of storage, far away from what could be considered as the shelf life of the product (that will be established herein as 50% of loss). It is clear that autoxidation reactions that degrade the carotenoid profile have minimally taken place, as storage conditions diminished the effect of the extrinsic promoting factors of such reactions (light, high temperatures, and oxygen). When kinetic parameters were analyzed (**Table 1**), we obtained a first-order model as the best fit for all samples, but the corresponding correlation parameters (r) were not as good as when autoxidation reactions take place, either naturally or intentionally. Degradation of food components during storage follows a first order model, as in the case of ascorbic acid, chlorophylls, and carotenoids (*21*, *22*, *23*). A fit for the second-order kinetics was also tested but correlation was even lower than that of the first-order model (data not shown). Ladrón-de-Guevara et al. (*24*) describe that degradation of ASTA values through storage of paprika fits different kinetic models (first order or second order) depending on temperature and humidity conditions.

Under the conditions applied in this study, the lag phase of autoxidation involves a substantial part of the storage time analyzed (12 months). This can be observed in **Table 1** where kinetic constants and r-values are presented. A first-order correlation clearly exists, but the contribution of the lag phase disturbs the model. Taking into account these considerations, we will assume this model for subsequent comparisons and to establish the possible stability ranking of analyzed samples, by comparison of the half-life, which is calculated from kinetic constant values of **Table 1**. Half-life values are 1635 days, 2407 days, 1590 days, and 1810 days for paprika A, B, C, and M, respectively. Despite the fact that samples are stored with the same conditions, stability could not be considered equal, with sample B being the most stable and sample C being the first one to lose color qualities and therefore marketability. A different initial composition of fruits, due to harvesting at wide ranges of ripening stages and different processing parameters reached during the production and during the handcrafted drying process (drying time and temperature) of the paprika samples under study, which may vary from one dryer to another, is going to mark the evolution of stability, considering it as the trend of the carotenoid content. A more severe processing commits stability of the products, decreasing the lag phase and promoting the autoxidation reactions. Some previous studies have stated this conclusion, that is, the higher temperature of processing, the lower stability (*8*, *25*). It should be convenient to establish a drying protocol including pretreatments and strategies to reduce oxidation, even more when handcrafted drying methods are applied (*10*). At least, processing conditions of different paprika batches should be traced in order to adjust conditions of storage, which is not done in the traditional dehydration process applied at La Vera.

With regard to the isochromic pigment fractions, carotenoids included in the Y fraction are degraded faster than those of the R one, as can be deduced from kinetic values for the R and Y fractions presented in **Table 1**. Thus, the degradation profile qualitatively follows the tendency stated in previous studies (*15*, *26*, *27*). The higher stability of the R fraction is based on structural features and the kind of fatty acids esterifying those carotenoids. Considering the degradation rate values for the Y fraction, paprika A, C, and M did not show significant differences (Duncan's test, $p > 0.05$), while the Y fraction of sample B followed a lower degradation. When the ratio of the degradation rate of the R fraction to the Y fraction was calculated, paprika A, B, and C showed a close value (ca. 0.62), higher than that of paprika M (0.48). In this sample, which was a mixture of paprika A, B, and C, degradative reactions had a higher incidence on the Y fraction, which will affect the evolution of ASTA values in this sample as follows.

Correlation of the ASTA Method and Carotenoid Content. It is noteworthy to mention the lack of correlation between the evolution of the total carotenoid values and color measurements as ASTA units, mainly when values for the same sample are compared. **Figure 1** allows a direct comparison of the evolution of both measurements as data are expressed as retention percentage. Considering the correlation coefficient values for ASTA, the fit to a first order model is better than the one corresponding to the values of total carotenoid content. In general, the degradation rate is higher, in view of the evolution of ASTA units, and consequently, the half-lives of the samples are lower, with values of 1432 days, 1392 days, 1258 days, and 1092 days for samples A, B, C, and M, respectively. Second, this is a different ranking

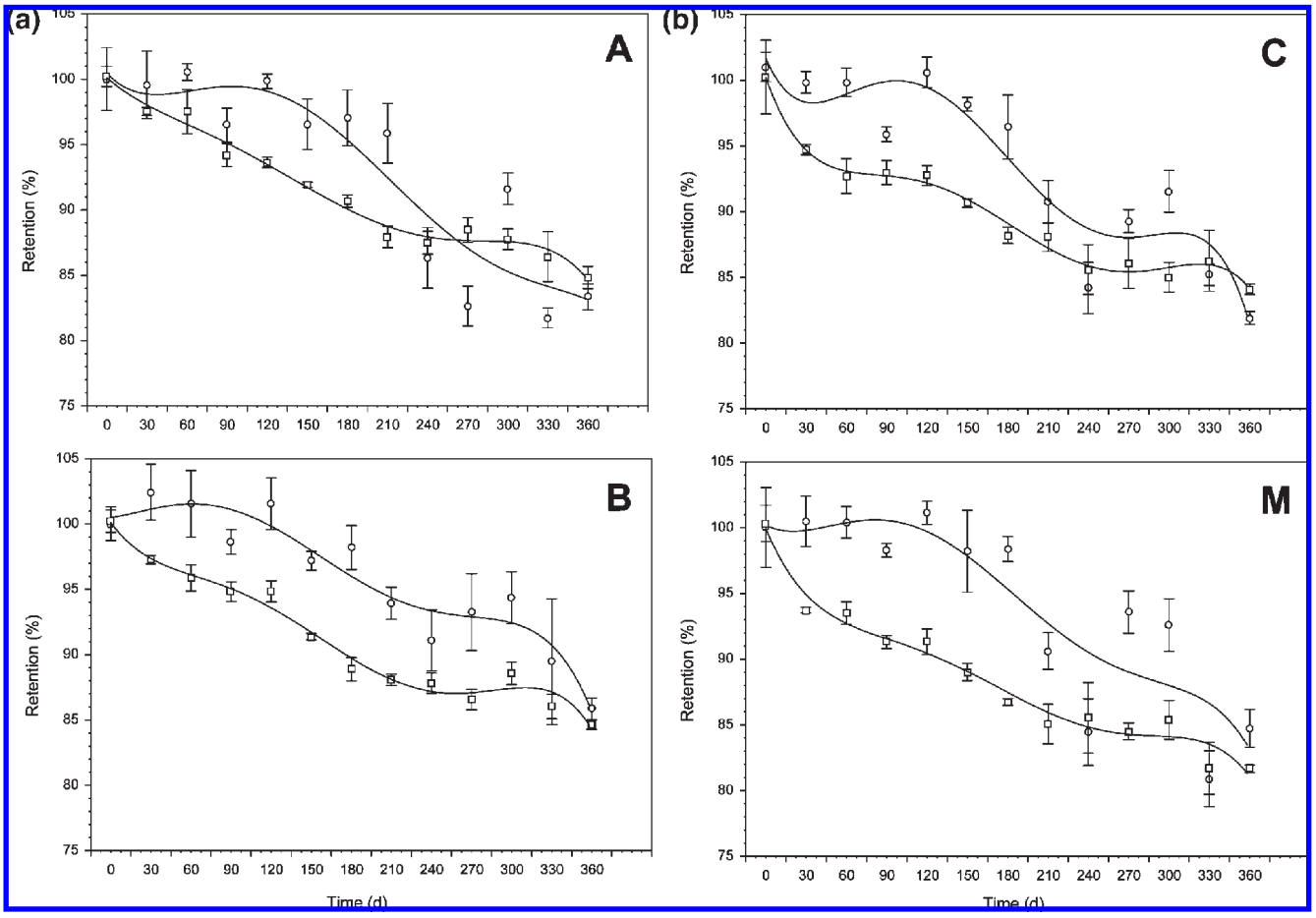

Figure 1. (**a**) Evolution of the total carotenoid content (circles) and ASTA value (squares) (both expressed as retention percentage) during 12 months of storage for paprika A and B. Represented data are mean values including a polynomial fit. (**b**) Evolution of the total carotenoid content (circles) and ASTA value (squares) (both expressed as retention percentage) during 12 months of storage for paprika C and M. Represented data are mean values including a polynomial fit.

Table 1. Degradation Rate Values for Paprika Samples Corresponding to a First Order Model, Considering Red (R) and Yellow (Y) Isochromic Pigment Fractions, Total Carotenoids, and ASTA Units[a]

	Paprika							
	A		B		C		M	
	$(k \pm \text{S.E.}) \times 10^{-4}$	r	$(k \pm \text{S.E.}) \times 10^{-4}$	r	$(k \pm \text{S.E.}) \times 10^{-4}$	r	$(k \pm \text{S.E.}) \times 10^{-4}$	r
R	3.53 ± 0.42	0.6813	2.32 ± 0.17	0.7189	3.47 ± 0.37	0.7747	2.65 ± 0.20	0.6326
Y	5.52 ± 0.25	0.7986	3.78 ± 0.23	0.8414	5.80 ± 0.25	0.8920	5.57 ± 0.14	0.7381
total	4.24 ± 0.17	0.7353	2.88 ± 0.24	0.7849	4.36 ± 0.16	0.8405	3.83 ± 0.25	0.7248
ASTA	4.84 ± 0.51	0.9446	4.98 ± 0.43	0.9440	5.51 ± 0.31	0.8500	6.35 ± 0.61	0.8407

[a] k is expressed in d^{-1}. S.E. is standard error. Fit corresponds to the model $\ln(\%\text{ret}) = 4.605 - k \times t$. R fraction comprises capsanthin, capsorubin, and capsanthin 5,6-epoxide. Y fraction comprises violaxanthin, antheraxanthin, zeaxanthin, cucurbitaxanthin A, β-cryptoxanthin, and β-carotene.

from that obtained from the total carotenoid trend (B > M > A > C). Rate values for ASTA units are closer to those of the Y fraction as can be denoted in **Table 1**. Spectrophotometric determination of ASTA units is performed at 460 nm, where the maximum of the absorbance spectra is mainly due to the Y pigment, while the maximum of the R pigment fraction lies on 480 nm so that evolution of the ASTA values closely represents that of the Y pigments (*28*).

The higher significant differences between the rank obtained from the carotenoid content and the one obtained from the ASTA method were those of paprika M. As mentioned before, the Y fraction of paprika M was especially affected by autoxidative reactions but not the R pigments, the fact that decreased the ratio between degradation rate values. This could explain why paprika M showed the lower half-life value considering ASTA evolution. The ASTA method is commonly applied in industry for evaluation of the coloring capacity of paprika and paprika oleoresins. When this method is applied, previous considerations should be taken into account, as this measurement does not correlate with the components responsible for the color of this product, the carotenoids, and should include in the measurement other extractable unknown compounds, whose evolution may be difficult to control.

Evolution of Fatty Acid Content and PV. In relation to the evolution of fatty acids and PV, the evolution of both quality parameters also reflects the lack of autoxidation during most of

the storage period. **Table 2** includes the composition of unsaturated fatty acids (expressed as percentage values of the total fatty acid content) for two paprika samples (A and M) stored through 12 months. For each unsaturated fatty acid, no significant changes were found between data at different storage times within a sample and even when data from different samples were compared (Duncan's test $p > 0.05$). The same was observed for samples B and C (data not shown). The PV for the paprika samples tested in this study are depicted in **Figure 2**. In the present study, the applied spectrophotometric method allows the correct measurement of the PV in foodstuffs with high carotenoid content that has been shown to correlate with the AOAC official method (*20*, *29*). All samples started with the same levels (1.12 ± 0.17 expressed as mequiv/kg sample; mean value of samples A, B, C, and M), a value that was maintained during 90 days of storage. The low initial PV is indicative of the applied processing techniques having minimally developed autoxidative reactions, and the quality of samples could be considered as very high, taking into account this quality parameter. After that time period (90 days), the PV started to rise, with the same increase for all paprika samples. This increase corresponds with the decline in carotenoid and ASTA values, which decreased from the initial time and in the following time controls, while the PV continued increasing, reaching a mean value of 3.34 ± 0.23 at 180 days (expressed as mequiv/kg sample; see **Figures 1** and **2**). This may indicate that the lag phase probably had finished and that autoxidative reactions had started to take place, although the PV kept on the same levels at 180 to 330 days. At 360 days, the values increased significantly for all samples, and we could assume the trend to rise.

The evolution of the PV observed in the present study could be considered as low in comparison with the one observed in other stored foodstuffs. Thus, pistachio nuts slightly increase the PV after 15 moths of storage at 10 °C (starting value of ca. 1 mequiv/kg) (*30*). Storage of mayonnaise at 4 °C did not produce a significant increase of the PV after 165 days (*31*). In our case, paprika samples stored at 4 °C could be considered as high quality samples as neither coloring capacity nor other organoleptic

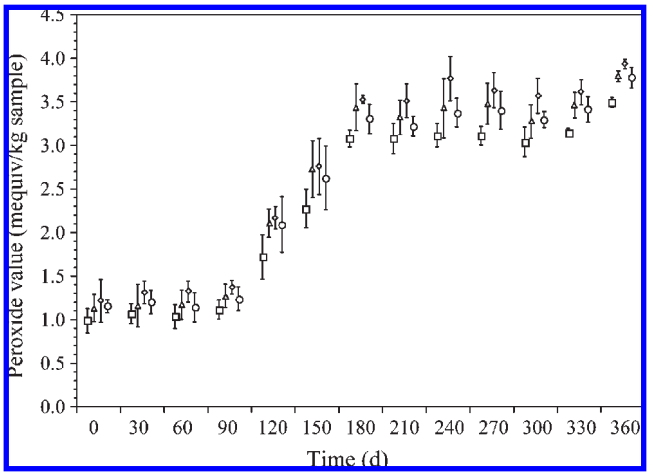

Figure 2. Progress of the peroxide index value during 12 months of storage for paprika A (□), B (△), C (◇), and M (○). Data are represented as the mean ± standard deviation and expressed as mequiv/kg sample.

Table 2. Composition in Unsaturated Fatty Acids (Expressed As Percentage Values of the Total Fatty Acid Content) of Paprika A and M Stored during Twelve Months at 4 °C and 70% Humidity

	Paprika					
	A			M		
time (d)	oleic	linoleic	linolenic	oleic	linoleic	linolenic
0	9.85 ± 0.03	67.5 ± 0.2	3.55 ± 0.01	9.90 ± 0.04	67.9 ± 0.1	3.49 ± 0.13
30	9.90 ± 0.04	67.0 ± 0.2	3.54 ± 0.10	10.1 ± 0.1	67.7 ± 0.2	3.36 ± 0.03
60	10.0 ± 0.23	67.1 ± 0.5	3.46 ± 0.02	9.93 ± 0.06	67.4 ± 0.2	3.38 ± 0.04
90	10.0 ± 0.03	67.1 ± 0.5	3.59 ± 0.04	10.1 ± 0.1	67.5 ± 0.6	3.46 ± 0.03
120	9.41 ± 0.15	68.4 ± 1.1	3.25 ± 0.14	9.30 ± 0.06	68.5 ± 0.3	3.40 ± 0.24
150	9.36 ± 0.27	69.1 ± 1.2	3.38 ± 0.25	9.36 ± 0.13	68.2 ± 0.1	3.29 ± 0.14
180	9.44 ± 0.15	67.5 ± 0.1	3.44 ± 0.01	9.63 ± 0.16	67.8 ± 0.1	3.22 ± 0.10
210	9.68 ± 0.03	66.9 ± 0.8	4.60 ± 0.92	9.32 ± 0.94	70.5 ± 3.9	3.90 ± 0.24
240	9.76 ± 1.11	67.8 ± 1.0	3.34 ± 0.04	8.97 ± 0.02	68.9 ± 0.1	3.56 ± 0.07
270	9.03 ± 0.03	68.1 ± 0.1	3.63 ± 0.11	9.03 ± 0.03	69.1 ± 0.2	3.57 ± 0.09
300	9.00 ± 0.02	68.5 ± 0.1	3.66 ± 0.01	9.02 ± 0.06	69.1 ± 0.2	3.41 ± 0.13
330	8.98 ± 0.04	68.4 ± 0.2	3.54 ± 0.18	9.08 ± 0.12	69.0 ± 0.1	3.44 ± 0.08
360	9.04 ± 0.05	68.8 ± 0.1	3.51 ± 0.13	8.93 ± 0.09	69.2 ± 0.6	3.39 ± 0.24

Table 3. Microbial Flora during Storage of Paprika A, B, and C[a]

	A			B			C		
microbiota	$t = 0$	$t = 180$	$t = 360$	$t = 0$	$t = 180$	$t = 360$	$t = 0$	$t = 180$	$t = 360$
total aerobic	7.60	8.18	7.78	6.90	9.63	8.48	7.00	10.2	8.53
enterobacteria	5.48	5.43	5.00	5.18	5.36	4.78	4.95	5.89	4.85
coliforms	6.18	5.30	5.00	4.60	5.00	5.30	5.60	5.70	6.00
lactic acid bacteria	4.46	5.26	4.60	3.85	5.00	4.30	3.18	6.30	4.70
Micrococcaceae (*Staphylococcus* sp.)	3.60	n. d.	2.60	2.90	n. d.	3.49	4.30	n. d.	3.30
Clostridium (sulfit-reducers)	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
S. aureus	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Enterococcus	5.08	5.30	5.32	4.30	5.04	4.30	5.04	5.36	4.30

[a] Data expressed as log cfu/g. n. d., not detected.

properties were significantly altered. Imposed storage conditions also contributed to preserve samples as the initial microbial flora was not altered and did not significantly develop during the 360 storage days. **Table 3** shows the control of the microbial flora for paprika samples A, B, and C at 0, 6, and 12 storage months. The initial counts are considered low and within the limits of the International Commission on Microbiological Specifications for Foods (ICMSF).

In light of the present results, it can be concluded that the combination of the traditional slow drying and application of commercial controlled conditions of storage is enough to keep coloring capacity, in terms of carotenoid content, and preserve the overall quality of paprika with the designation of origin (PDO *Pimentón de La Vera*). The initial PV determined in the samples could be used in the future as a reference to categorize paprika. Thus, another parameter could be added to color controls and a global approach to quality could be achieved. Indeed, this parameter could be indicative of the processing technique employed and the potential stability. Use of preservation techniques, as reconstitution with added antioxidants, is considered as unnecessary as the prediction of stability of the carotenoid content covers the commercial cycle.

ACKNOWLEDGMENT

We express our sincere gratitude to Dr. E. Aranda for his assistance with the microbiological analysis. Thanks are also due to Sergio Alcañiz-García for technical work.

LITERATURE CITED

(1) Daood, H. G.; Vinkler, M.; Markus, F.; Hebshi, E. A.; Biacs, P. A. Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors. *Food Chem*. **1996**, *55*, 365–372.

(2) Hornero-Méndez, D.; Costa-García, J.; Mínguez-Mosquera, M. I. Characterization of carotenoid high-producing *Capsicum annuum* cultivars selected for paprika production. *J. Agric. Food Chem*. **2002**, *50*, 5711–5716.

(3) Howard, L. R.; Smith, R. T.; Wagner, A. B.; Villalon, B.; Burns, E. E. Provitamin A and ascorbic acid content of fresh peppers cultivars (*Capsicum annuum*) and processed jalapeños. *J. Food Sci*. **1994**, *59*, 362–365.

(4) Howard, L. R.; Talcott, S. T.; Brenes, C. H.; Villalon, B. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum species*) as influenced by maturity. *J. Agric. Food Chem*. **2000**, *48*, 1713–1720.

(5) Mínguez-Mosquera, M. I.; Jarén-Galán, M.; Garrido-Fernández, J. Influence of the industrial drying processes of pepper fruits (*Capsicun annuum* cv. Bola) for paprika on the carotenoid content. *J. Agric. Food Chem*. **1994**, *42*, 1190–1193.

(6) Pérez-Gálvez, A.; Hornero-Méndez, D.; Mínguez-Mosquera, M. I. Dependence of carotenoid content and temperature-time regimes during the traditional slow drying of red pepper for paprika production at La Vera county. *Eur. Food Res. Technol*. **2005**, *221*, 645–652.

(7) Pérez-Gálvez, A.; Garrido-Fernández, J.; Mínguez-Mosquera, M. I.; Lozano-Ruiz, M.; Montero-de-Espinosa, V. Composition of two new pepper varieties (*Capsicun annuum* L.) Jaranda and Jariza. Effect of drying process and nutritional aspects. *J. Am. Oil Chem. Soc*. **1999**, *76*, 205–208.

(8) Malchev, E.; Ioncheva, N.; Tanchev, S.; Kalpakchieva, K. Quantitative changes in carotenoids during the storage of dried red pepper and red pepper powder. *Nahrung* **1982**, *26*, 415–420.

(9) 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.

(10) Pérez-Gálvez, A.; Hornero-Méndez, D.; Mínguez-Mosquera, M. I. Changes in the carotenoid metabolism of capsicum fruits during application of modelized slow drying process for paprika production. *J. Agric. Food Chem*. **2004**, *52*, 518–522.

(11) Biacs, P.; Czinkotai, B.; Hoschke, A. Factors affecting stability of coloured substances in paprika powders. *J. Agric. Food Chem*. **1992**, *40*, 363–367.

(12) Carvajal, M.; Martínez, M. R.; Martínez-Sánchez, F.; Alcaraz, C. F. Effect of ascorbic acid addition to peppers on paprika quality. *J. Sci. Food Agric*. **1997**, *75*, 442–446.

(13) Kanner, J.; Mendel, H.; Budowski, P. Carotene oxidizing in red pepper fruits (*Capsicum annuum*, L.), effect of ascorbic acid and copper in a beta-carotene-linoleic acid solid model. *J. Food. Sci*. **1978**, *43*, 709–712.

(14) Pérez-Gálvez, A.; Garrido-Fernández, J.; Mínguez-Mosquera, M. I. Effect of high-oleic sunflower seed on the carotenoid stability of ground pepper. *J. Am. Oil Chem. Soc*. **2000**, *77*, 79–83.

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

(16) Mínguez-Mosquera, M. I.; Pérez-Gálvez, A.; Garrido-Fernández, J. Carotenoid content of the varieties Jaranda and Jariza (*Capsicun annuum* L.) and response during the industrial slow drying and grinding steps in paprika processing. *J. Agric. Food Chem*. **2000**, *48*, 2972–2976.

(17) Martín, A.; Aranda, E.; Benito, M. J.; Pérez-Nevado, F.; Córdoba, M. G. Identification of fungal contamination and determination of mycotoxigenic molds by micellar electrokinetic capillary chromatography in smoked paprika. *J. Food Prot*. **2005**, *68*, 815–822.

(18) Mínguez-Mosquera, M. I.; Hornero-Méndez, D. Separation and quantification of the carotenoid pigments in red peppers (*Capsicun annuum* L.), paprika and oleoresin by reversed-phase HPLC. *J. Agric. Food Chem*. **1993**, *43*, 1613–1620.

(19) ASTA. *Official Analytical Methods of the American Spice Trade Association*, 2nd ed.; American Spice Trade Association: Washington, DC, 1986.

(20) Hornero-Méndez, D.; Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. A rapid spectrophotometric method for the determination of peroxide value in food lipids with high carotenoid content. *J. Am. Oil Chem. Soc*. **2001**, *78*, 1151–1155.

(21) Labuza, T. P. Enthalphy/Entropy compensation in food reactions. *Food Technol*. **1980**, *15*, 67–77.

(22) Lee, M. T.; Chen, B. H. Stability of lycopene during heating and illumination in a model system. *Food Chem*. **2002**, *78*, 425–432.

(23) Tang, Y. C.; Chen, B. C. Pigment change of freeze-dried carotenoid powder during storage. *Food Chem*. **2000**, *69*, 11–17.

(24) Ladrón-de-Guevara, R. G.; González, M.; García-Meseguer, M. J.; Nieto, J. M.; Amo, M.; Varón, R. Effect of adding natural antioxidants on colour stability of paprika. *J. Sci. Food Agric*. **2002**, *82*, 1061–1069.

(25) Ramesh, M. N.; Wolf, W.; Tevini, D.; Jung, G. Influence of processing parameters on the drying of spice paprika. *J. Food Eng*. **2001**, *49*, 63–72.

(26) Pérez-Gálvez, A.; Garrido-Fernández, J.; Mínguez-Mosquera, M. I. Participation of pepper seed in the stability of paprika carotenoids. *J. Am. Oil Chem. Soc*. **1999**, *76*, 1449–1454.

(27) Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. Structure-reactivity relationship in the oxidation of carotenoid pigments of the pepper (*Capsicum annuum* L.). *J. Agric. Food Chem*. **2001**, *49*, 4864–4869.

(28) Hornero-Méndez, D.; Mínguez-Mosquera, M. I. Rapid spectrophotometric determination of red and yellow isochromic carotenoid fractions in paprika and red pepper oleoresins. *J. Agric. Food Chem*. **2001**, *49*, 3584–3588.

(29) *Official Methods of Analysis*, 15th ed.; Helrach, K., Ed.; AOAC: Arlington, VA, 1990; Sec 965.33.

(30) Maskan, M.; Karatas, S. Storage stability of whole-split pistachio nuts (*Pistachia vera* L.) at various conditions. *Food Chem*. **1999**, *66*, 227–233.

(31) Morales-Aizpurúa, I. C.; Tenuta-Filho, A. Oxidation of cholesterol in mayonnaise during storage. *Food Chem*. **2005**, *89*, 611–615.

Received for review December 31, 2008. Revised manuscript received March 29, 2009. Accepted April 8, 2009. This study was financed by CICYT (Spanish Government; projects AGL2000-0699 and AGL2007-60092/ALI).