Color Quality in Paprika

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The carotenoid pigmentation of five varieties of pepper plant and their respective paprikas was quantified in three fractions: (1) β-carotene, esterified cryptoxanthin, and diesterified zeaxanthin (Y); (2) diesterified capsanthin and capsorubin (R); (3) remaining pigments (B). Although the varieties analyzed vary in pigment richness, the ratio between fractions can be considered constant within the normal limits for natural products. These same ratios were confirmed in paprikas obtained in the laboratory from the fruits analyzed. Thus, this parameter can be used for quality control. Fluctuations outside the set limits may indicate either losses in pigmentation by oxidative processes or enhancement of color with natural or artificial pigments. The suitability of this methodology for quality evaluation and detection of alterations was also tested in four commercial paprikas. The results were compared with those obtained from the most commonly used methods of evaluation in this field (ASTA and STANDARD).

INTRODUCTION

The main parameter used for commercial evaluation of paprika quality is color. This is usually measured by pigment extraction with an organic solvent, followed by spectrophotometric quantification using a coefficient. Although numerous methods of evaluation are described in the bibliography, all have the same basis (Guenter, 1948; Moster and Prater, 1952; Nagle et al., 1979; FAO/WHO, 1984). The wavelength of measurement is usually 460 nm, but other wavelengths between 458 and 462 nm have been used; the extraction solvent in most cases is acetone (Moster and Prater, 1952). The only differences between methods are in sample weight, time and form of extraction, and final volume of the solution. The two most used methods in industry are ASTA-20 and STANDARD, and although the value given by each is different for the same sample, the correlation between measurements is good (Salmerón Salmerón, 1973). The step from one system to the other can be made by means of a conversion factor.

Although the coloration in paprika is due almost exclusively to the carotenoid fraction, there is also a small amount of water-soluble coloration, presumably polyphenolic (Salmerón Salmerón, 1973). This latter coloration, not having any affinity for organic solvents, is excluded from the overall evaluation in the usual methods. These methods do not discriminate between paprikas having similar colorant power but different pigment composition, so that both oxidative alteration of natural pepper pigments and the presence of added pigments are undetectable.

The present work evaluates by fractions the carotenoid content in pepper plants of different varieties and sources on the basis of the previous work of Mínguez Mosquera and Garrido Fernandez (1984). The relationships between them have been studied in detail, and the same study has been repeated in paprika obtained from the pepper plants.

This technique for evaluating pigment content in commercial paprika, characterized in parallel by the ASTA and STANDARD methods, affords a quality index as valid as that of the two official methods. It gives additional information on the degree of deterioration of the product, since pigment lability is different depending on the oxidations taking place during storage.

To evaluate capacity to discriminate the presence of colorants foreign to the raw material, increasing amounts of colorants were added to the paprikas. Evaluation by the three methods was repeated, with special reference to capacity to detect any addition.

MATERIALS AND METHODS

Apparatus. A Hewlett-Packard UV–vis spectrophotometer photodiode array, Model 8450, provided with a Hewlett-Packard recorder, Model 7225 A, was used.

Procedure. *Raw Material.* Pigments were characterized in five varieties of the pepper plant (*Capsicum annuum*) used to obtain paprika: *Bola carmelina*, *Bola americano*, *Bola negral*, *Decano corto*, and *Decano largo*. The corresponding paprikas were prepared from these samples by drying for 12 days in a recycled-air stove at 30 °C. Some 35% seed was added with respect to the husk used, to resemble the commercial product.

Four commercial paprikas from different sources were also analyzed. Canthaxanthin and β-apo-8′-carotenal (Roche and Sigma, respectively) were used as added colorants.

Color Measurement by ASTA and STANDARD. In the ASTA-20 method (FAO/WHO, 1984), 0.07–0.11 g of paprika was put into a tared 100-mL flask. Acetone was added to the mark, the mixture was stirred, and after 4 h, an aliquot of the transparent decanted extract was taken. The absorbance of the solution at 460 nm was measured. Units of color were calculated from

$$\text{ASTA-20 units} = \frac{\text{absorption of extract} \times 16.4 \times I_f}{\text{g of sample}}$$

in which I_f is a correction factor for the apparatus, calculated from the absorbance of a standard solution of potassium dichromate and ammonium and cobalt sulfate.

In the STANDARD method (Guenter, 1948), 2 g of paprika was put into a tared 100-mL flask and made up with acetone. After 24 h with frequent stirring, 1 mL of the transparent decanted solution was taken and made up to a final volume of 100 mL with acetone. The absorbance of this solution at 462 nm was determined and the result multiplied by 328 000.

Evaluation of Soluble Color. The same procedure as in the ASTA and STANDARD determinations was followed, but the organic solvent was substituted by water. The coefficients and wavelengths used were the same.

Extraction, Separation, and Identification of Pigments. A sample of 10 g of fresh pepper fruit or 2 g of paprika was extracted with acetone until colorless. The extracts were combined and transferred to diethyl ether for evaporation in a rotavapor. The dry residue was collected with 10 mL of acetone (Mínguez Mosquera and Fernández Diez, 1981).

Pigments were separated by TLC on silica gel 60 GF_{254}, with the mixture hexane/ethyl acetate/ethanol/acetone (95:3:2:2) (Mínguez Mosquera et al., 1982). Each pigment was identified

0021-8561/92/1440-2384$03.00/0

Table I. Chromatographic and Spectral Characteristics of the Carotenoid Pigments of the Pepper Plant: Identification Tests

fraction	band	identified pigment	R_f	color	absorption maxima, nm: ethanol	absorption maxima, nm: benzene	IR: C—OH	IR: C=O	epoxide test: TLC[a]	epoxide test: HS,[b] nm
Y	1	β-carotene	1	yellow	(426), 446, 470	(436), 458, 486	−	−	−	0
		ζ-carotene	1	yellow	(379), 400, 422	387, 406, 432	−	−	−	0
		phytoene	1	uncolored	285	296	−	−	−	0
		phytofluene	1	uncolored	331, 348, 368	337, 355, 375	−	−	−	0
	2	cryptoxanthin	0.85	yellow	(428), 446, 470	(438), 458, 484	+	−	−	0
	3	zeaxanthin	0.69	yellow	(424), 444, 470	(436), 458, 486	+	−	−	0
R	4	capsanthin	0.40	brick red	472	484, (504)	+	+	−	0
	5	capsorubin	0.25	red-brown	(454), 478, (502)	460, 486, 522	+	+	−	0
B[c]	6	xanthophylls[d] mono- and de-esterified								

[a] Color variation of pigment on the chromatographic plate on spraying with hydrochloric acid. [b] Hypsochromic displacement of pigment spectrum in ethanol on adding hydrochloric acid. [c] Identification tests of the pigments of this fraction not included as they are the same as those of the R and Y fractions, differing only in the degree of esterification. [d] Derivates of zeaxanthin, cryptoxanthin, capsanthin, and capsorubin.

Table II. Quantification of Carotenoid Pigments in Five Varieties of Pepper Plant: Ratios between Fractions

variety	carotenoid content by fraction,[a] mg/kg: Y	carotenoid content by fraction,[a] mg/kg: R	carotenoid content by fraction,[a] mg/kg: B	ratio[b]: R/Y	ratio[b]: R/B
B. carmelina	93.67 ± 4.6	285.04 ± 18.2	273.23 ± 14.0	3.04 ± 0.10	1.03 ± 0.05
B. americano	113.67 ± 4.8	364.20 ± 13.7	375.38 ± 11.9	3.20 ± 0.05	0.97 ± 0.04
B. negral	142.40 ± 5.7	420.40 ± 17.2	439.12 ± 27.3	2.95 ± 0.20	0.96 ± 0.05
D. corto	144.33 ± 11.0	480.83 ± 28.4	412.56 ± 13.2	3.35 ± 0.13	1.14 ± 0.06
D. largo	156.00 ± 5.5	531.50 ± 15.9	476.60 ± 10.8	3.42 ± 0.07	1.10 ± 0.04

[a] Mean of six replicates. Limit of confidence ($p < 0.05$). Y, β-carotene, zeaxanthin, and cryptoxanthin; R, diesterified capsanthin and capsorubin; B, rest of mono- and de-esterified xanthophylls. [b] Mean of the six individual ratios. Limit of confidence ($p < 0.05$).

using R_f values, spectrum, shape, localization of absorption maxima in the spectra in different solvents and peak ratios, functional group tests (epoxide, hydroxyl, and carbonyl), infrared spectrum, and finally cochromatography with standards. Details of the methodology are described in a previous work (Mínguez Mosquera and Hornero-Méndez, 1992).

Quantification. Using the system described, TLC gives a clear separation of three fractions. For quantification (Mínguez Mosquera and Garrido Fernandez, 1984), the pigments making up each fraction were scraped from the plate together and eluted with ethanol to a volume of 25 mL. Spectrophotometric measurement was made at 450 nm for the first fraction and 470 nm for the second and third, using the coefficient of the major pigment in each case. The results were obtained in milligrams of pigment for each kilogram of fresh sample, using the expression

$$\text{mg/kg of pigment} = AV_iV_f \times 10.000/E_0V_{cr}P_m$$

in which V_i is the volume of the total pigment extract, V_f is the volume to which the pigment fraction (once scraped and eluted) is taken, V_{cr} is the volume of chromatographed extract, P_m is the weight of sample used to obtain the extract, E_0 is the extinction coefficient in ethanol at λ_{max} of the major pigment of the fraction at 1%, for a light path of 1 cm, and A is the absorbance at λ_{max}.

Addition of Colorants. Canthaxanthin and β-apo-8′-carotenal were added to a commercial paprika in three known concentrations in the extraction flask. The units of color of the new sample were determined by the two traditional methods. The pigment content of each fraction and the ratios between them were also determined.

RESULTS AND DISCUSSION

Quantification of the Principal Pigment Fractions in the Pepper Plant Fruit and Corresponding Paprikas. Table I shows the characteristics of the chromatographic development in TLC of the pigment extracts and the tests carried out to identify them. For the quantification, three clearly differentiated zones were considered. The first was formed by the three yellow bands between R_f 1 and 0.6. As can be seen, band 1 was formed exclusively by carotenes, including phytoene, phytofluene, ζ-carotene and β-carotene. In the elution system used, these advance together at the front of the chromatogram. They were individually separated by TLC on silica gel using light petroleum ether as developer. Bands 2 and 3 comprise the principal esterified yellow xanthophylls, cryptoxanthin and zeaxanthin, respectively. These three bands form the fraction we have denoted yellow (Y). They were quantified by scraping off the plate together and eluting with ethanol. As β-carotene was the major pigment, the evaluation was performed with the $E^{1\%}_{1\,cm,450\,nm}$ of β-carotene in ethanol, 2500.

The second zone, between R_f 0.6 and 0.35, was formed by two major red bands, bands 4 and 5, with upper halos of the same color as the band. They were the principal diesterified red xanthophylls, capsanthin and capsorubin, which for the present study formed the fraction denoted red (R). The diesterified form of both compounds represents more than 50% of the individual concentration of each in the fruit. This fraction was quantified jointly on the basis of E_o of diesterified capsanthin, 1760, as this contributes more than 60% of the pigment concentration.

The third zone, between R_f 0.35 and 0, is formed by numerous bands of little individual entity—the rest of the mono- and de-esterified xanthophylls. As these remain in the lower zone of the chromatogram during development, they have been denoted base (B). Evaluation, as in the two previous fractions, was performed jointly, using the coefficient corresponding to diesterified capsanthin. As the pigments are a heterogeneous set in both composition and degree of esterification, any coefficient is appropriate for their quantification. In fact, it is not essential that a very exact coefficient be used, since quantification will systematically have the same error.

Table II shows the pigment concentration found in fractions Y, R, and B for the different varieties of pepper plant. The distinct varieties show great differences in pigment content. To determine if these differences are statistically significant, a variance analysis was performed

Table III. Quantification of Carotenoid Pigments in Five Paprikas: Ratios of Pigments between Fractions

paprika from variety	carotenoid content by fraction,[a] mg/kg			ratio[b]	
	Y	R	B	R/Y	R/B
B. carmelina	189.54 ± 9.1	562.68 ± 34.3	549.46 ± 29.6	2.96 ± 0.11	1.02 ± 0.05
B. americano	227.34 ± 9.3	735.40 ± 27.6	738.76 ± 19.5	3.23 ± 0.06	0.99 ± 0.04
B. negral	286.80 ± 10.6	846.80 ± 30.2	875.24 ± 53.2	2.97 ± 0.21	0.96 ± 0.04
D. corto	278.66 ± 19.0	968.36 ± 42.8	859.02 ± 33.6	3.47 ± 0.12	1.12 ± 0.06
D. largo	319.00 ± 11.2	1085.30 ± 37.7	965.20 ± 26.5	3.40 ± 0.06	1.12 ± 0.06

[a] Mean of six replicates. Limit of confidence ($p < 0.05$). Y, β-carotene, zeaxanthin, and cryptoxanthin; R, diesterified capsanthin and capsorubin; B, rest of mono- and de-esterified xanthophylls. [b] Mean of the six individual ratios. Limit of confidence ($p < 0.05$).

Table IV. Estimation of Color Quality in Four Commercial Paprikas: Comparison between Methods

paprika	color evaluation method[a]			carotenoid content by fraction,[a,b] mg/kg			ratio	
	U.S.	ASTA	total pigments, mg/kg	Y	R	B	R/Y	R/B
a	33995 ± 492	84.98 ± 1.22	1152 ± 48	60 ± 2	556 ± 14	536 ± 36	9.2 ± 0.3	1.03 ± 0.50
b	26446 ± 263	66.11 ± 0.66	924 ± 8	83 ± 8	439 ± 3	411 ± 9	5.2 ± 0.5	1.05 ± 0.02
c	26179 ± 164	65.44 ± 0.41	912 ± 23	120 ± 4	399 ± 22	392 ± 16	3.3 ± 0.1	1.01 ± 0.09
d	10928 ± 297	27.31 ± 0.75	328 ± 10	20 ± 2	161 ± 5	147 ± 4	7.9 ± 0.5	1.09 ± 0.01

[a] Means of four analyses. Limit of confidence ($p < 0.05$). [b] Y, yellow fraction (β-carotene, zeaxanthin, and cryptoxanthin); R, red fraction (Diesterified capsanthin and capsorubin). B, base fraction (rest of mono- and de-esterified xanthophylls).

using Duncan's procedure. The results indicate that the differences are statistically significant, considering either pigment sets or total content ($p < 0.05$). Thus, we can arrange the varieties by their pigment richness as follows: *D. largo*, *D. corto*, *B. negral*, *B. americano*, *B. camelina*.

The natural relationships between the pigment fractions in the pepper plant are seen from the results shown in Table II. It can be seen that, leaving aside the question of variety, the value of the ratio R/Y tends to 3 while R/B tends to 1. A statistical analysis similar to that described above indicated that the differences between varieties for a single ratio are also significant. Nevertheless, it must be pointed out that the values of the ratios are very close to each other, so that the differences are not as marked as in the case of pigment content. As with the chlorophyll a/b ratio in higher plants (Lichtenthaler, 1983) or the β-carotene/carotenoids ratio in the orange (Bernath and Swisher, 1969), it can be concluded that in the fruits of the pepper plant, leaving aside variety and carotenoid content, the ratios between pigments are within the normal variability of natural products.

These differences, although statistically significant, fall within a relatively narrow range. Statistically, the minimum and maximum values of the range—confident limits—can be calculated from the values of the corresponding ratios. For R/Y, considering the population of *B. negral* to have the lowest value and *D. largo* the highest, a minimum limit can be established $[(\bar{x}_{BN} - ts)/n^{1/2}]$ above which the means of n experimental values can be found with 97.5% probability. Similarly, a maximum limit can be established $[(\bar{x}_{DL} + ts)/n^{1/2}]$, below which should be the means of n measurements made ($p < 0.025$). The same reasoning can be used for the ratio R/B.

From the standard deviation and for the worst case of duplicate analyses, these limits are 2.7–3.5 for the ratio R/Y and 0.9–1.2 for R/B. Thus, the normal range ($p < 0.05$) of this ratio is fixed in the varieties studied.

Establishment of the range opens the way to color quality control in paprika. An index that remains constant within set limits, independent of variety, can be converted into a useful tool for differentiation of quality, based on an intrinsic characteristic of the pepper plant. Histological and biochemical studies reveal the composition of a product and detect whether it corresponds to specification, but in practice, a simple parameter is needed that with a single analysis can differentiate quality and guarantee product reliability.

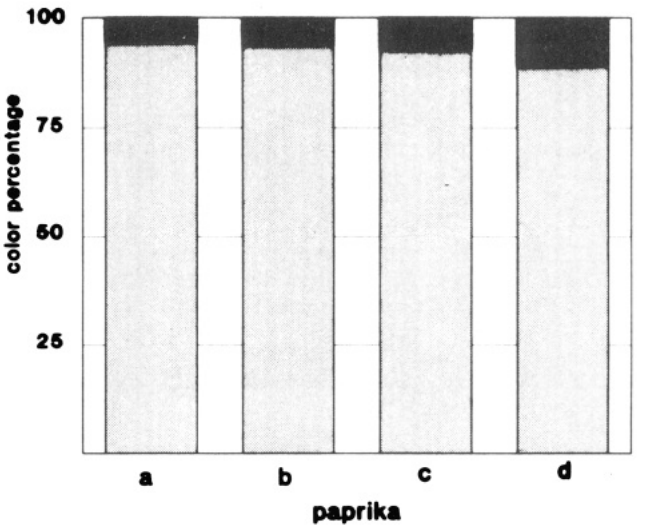

Figure 1. Percentage of water-soluble color (dark shading) and acetone-soluble color (light shading) in four commercial paprikas (a–d).

The carotenoid pigments of paprika should be in the same ratios as in the pepper plant within the set limits, even when obtained from the varieties used to establish the limits.

Chromatographic analysis of the paprikas obtained from the varieties of pepper plant studied reveal similar pigment composition, and they were quantified in the same way as in the case of fresh pepper plant.

The results obtained are shown in Table III. It can be seen that the total pigment content is again a parameter for differentiating the varieties, while the ratios between fractions remain within the limits fixed for the fresh fruits. All of the results are within the limits. The processing did not affect the relationships between pigments, and under these conditions none of the fractions underwent oxidative processes.

From the results, it can be ensured that any enhancement of color (addition of natural or artificial red colorants) would displace the value of either R/Y or R/B, depending on the polarity of the product added. Any such adulteration would be detected by the resulting values falling outside the set limits.

Table V. Estimation of the Quality of Paprika with Added Colorants: Comparison of Methods and Detection of Fraud[a]

	color evaluation method			carotenoid content by fraction,[b] mg/kg			ratio	
mg/kg colorant added	U.S.	ASTA	total pigments, mg/kg	Y	R	B	R/Y	R/B
canthaxanthin								
81	12 385	30.96	418	21	167	230	7.76	0.72
162	13 692	34.23	486	19	159	308	8.62	0.53
324	16 735	41.84	656	19	168	468	8.65	0.35
β-apo-8′-carotenal								
81	11 903	29.75	417	20	244	153	11.91	1.59
162	13 407	33.40	499	20	333	145	16.45	2.30
324	16 256	40.63	648	19	486	143	25.70	3.40

[a] Means of two analyses. [b] Y, yellow, fraction (β-carotene, zeaxanthin, and cryptoxanthin); R, red fraction (diesterified capsanthin and capsorubin); B, base fraction (rest of mono- and de-esterified xanthophylls).

Upward displacement of the R/Y value could be produced by addition of red colorants of medium polarity or by losses from oxdative processes of the Y fraction. Either case would be detected ($p < 0.05$) whenever the R/Y value fell outside the set limits. Downward displacement of this ratio could result from color enhancement in the yellow fraction with apolar colorants or the use (although not provable) of unripe fruits to obtain the paprika. This latter case, infrequent because of the interests of the industry itself, would decrease the ratio, although not below 2.

The R/B value is more constant under normal conditions, since it is unaffected by natural oxidative processes or the degree of ripeness of the raw material. Thus, any displacement outside the set limits is due exclusively to addition of natural or artificial colorants.

In practical terms, an addition of colorant that produces a 10% increase of color would be detectable.

Measurement of Color in Paprika. Color evaluations of four commercial paprikas according to the ASTA and STANDARD methods gave identical quality rankings (Table IV). When the method of quantification by fractions is applied to these same paprikas, it is observed that the ranking for total pigment content is similar to that given by the two previously mentioned methods. Table IV shows the relationships between the different pigment fractions. It can be seen that only paprika c remained with the limits for ripe pepper fruit and recently and correctly-processed paprika. The high R/Y value in paprika a can be explained by the practical absence of the Y fraction. Chromatographic analysis showed the almost total absence of β-carotene, cryptoxanthin, and zeaxanthin in the sample. The loss of these pigments must have taken place during processing or storage, which seems to indicate that the lability to oxidative processes is higher in these pigments. The ratio between the R and B fractions in all of the paprikas studied remained within the set limits, as they are formed mainly by pigments that are apparently more resistant and have similar stabilities.

Consequently, the quality evaluation of a paprika by the quantification by fractions procedure gives an idea of coloring power similar to that of the two official methods based on total pigment content. It also reveals the degree of deterioration of the product from the rise in the ratio between red and yellow pigments, given the greater lability of the Y fraction components.

Nonquantified Coloration. The water-soluble color is shown in Figure 1. All of the paprikas have between 5 and 15% water-soluble color, so that, in some cases, this parameter should not be ignored. On extraction with acetone, in both the official and proposed methods, this coloration is excluded.

Although the official methods do not evaluate this additional coloration, it is in fact present. Its presence may or may not be desirable but should be taken into account in quality classification. While its incidence may be minimal in the final color, it is perhaps a good parameter of the degree of deterioration of the product, as it probably comes from browning reactions.

Detection of Added Colorants. Canthaxanthin and β-apo-8′-carotenal—pure commercial pigments foreign to pepper—were added to the paprikas.

The addition of these colorants increased the color units considerably (Table V). Plotting the quantity of colorant added against units of color reached gave similar straight line slopes. This indicates that in the range of concentration used for the addition, both compounds have a similar coloring power. The added-to paprikas were evaluated as being of better quality than the initial ones by the official methods, without having shown that this improvement was due to an addition.

Table V also shows how quantification by fractions again coincides with the official methods for paprika quality based on total pigment content. The study of the relationships between fractions, however, shows inappropriate values for this product. In the case of β-apo-8′-carotenal, the concentration of pigments in the R fraction increases, thus increasing the ratios R/Y and R/B. When canthaxanthin is added, the concentration of pigments in the B fraction increases, resulting in a decrease in the ratio R/B.

It can therefore be concluded that the proposed method easily and quickly detects the addition of colorants that are structurally closely related with the natural pigments of the pepper plant.

ACKNOWLEDGMENT

We express our sincere gratitude to CICYT (Spanish Goverment, ALI-91-1166-C03-02) for financial support of this research.

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Received for review June 26, 1992. Accepted September 15, 1992.

Registry No. Canthaxanthin, 514-78-3; β-apo-8′-carotenal, 1107-26-2; β-carotene, 7235-40-7; ζ-carotene, 72746-33-9; phytoene, 13920-14-4; phytofluene, 27664-65-9; cryptoxanthin, 472-70-8; zeaxanthin, 144-68-3; capsanthin, 465-42-9; capsorubin, 470-38-2.