Color Quality in Paprika Oleoresins

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Color determines the commercial value of paprika oleoresins and is generally associated with the quality of the sample: the greater the coloring capacity, the higher the quality. The present study evaluates different parameters of color measurement, such as ASTA and tint determination. The latter does not give a real value of the ratio between the red and yellow pigments and is not a distinguishing parameter in most cases. However, combination of the information from the ASTA method with that of the carotenoid composition of the red and yellow fractions, acquired by HPLC, enables greater accuracy in judging both the quality of the final sample and the soundness of the process for obtaining it. Finally, an alternative tint determination is proposed, based on measurements at other wavelengths and using the appropriate extinction coefficients, thereby providing the composition of each fraction and the R/Y ratio, values closer to those of the actual sample.

Keywords: *Oleoresin; carotenoid; color; measurement; quality*

INTRODUCTION

The word color (from Latin) means the impression that light rays, reflected by a body, produce in the brain via the retina. Thus, color is a characteristic captured by sight and is often the first perception received of an object, including food. The common association of color with quality makes it a relatively important physical property, and it is given high priority in foodstuffs, so that their aspect is the best possible.

In the pepper, the compounds responsible for color are the carotenoid pigments (Davies, 1976; Fisher and Kocis, 1987). The coloring capacity of these compounds makes them commonly used in the food industry as additives, in the form of paprika or oleoresin (Mínguez-Mosquera et al., 1992, 1993).

As mentioned, color and quality are associated terms, in the sense that the quality of a paprika or oleoresin increases with its coloring capacity (Costa, 1979; Soriano et al., 1990). Thus, color measurement ensures the quality of the product and is the basis of commercial transactions. Currently, various methods are used for such measurement. The most frequently used is the ASTA method (ASTA, 1986), followed by the standard method (Guenter, 1948). Both give a rapid evaluation of color from the measurement of the absorbance, at a determinate wavelength, in a solution of the oleoresin in acetone.

However, previous works (Mínguez-Mosquera et al., 1984, 1992) concluded that these methods were ineffective in evaluating color quality or possible adulterations produced by the addition of other colorants.

Another measurement is that known as tint determination [AFEXPO (Asociación de Fabricantes y Exportadores de Pimentón, Oleorresina y Derivados), private communication, 1993]. This criterion of quality is based on the ratio of absorbances at two determinate wavelengths and is an attempt to obtain the ratio between red and yellow carotenoid pigments in a sample. The parameter could indicate the greater or lesser red coloring capacity of an oleoresin.

These rapid methods do not yield such detailed information as that given by HPLC assay of pigments, as they give only an overall evaluation of the coloring capacity, so closing the door on other data of interest. However, their efficacy could be improved using current knowledge on the characteristics of the carotenoids present in the raw material and with the aid of advanced methodologies.

The present work is a comparative study of tint determination, ASTA degrees, and carotenoid content from HPLC assay, and a swift spectrophotometric method is described that provides information about the carotenoid pigments present in commercial oleoresins.

MATERIALS AND METHODS

Samples. The experimental study was carried out with seven commercial oleoresins from different sources and of different coloring capacity, depending on the producer. The oleoresin is a concentrate of pigments dissolved in an oily matrix, the result of solvent extraction from the fruit, previously dehydrated by drying.

Evaluation of Color According to the ASTA Method. The measurement was carried out by weighing 0.07−0.11 g of oleoresin in a 100 mL measuring flask. Acetone was added to the mark and the flask shaken. With the aid of a 10 mL pipet, 10 mL of the solution was transferred to another 100 mL measuring flask. Acetone was again added and the flask shaken. A portion of this solution was used for the spectrophotometric measurement at 460 nm with an acetone blank. The ASTA units were calculated from the expression

$$\text{ASTA-20.1 units} = A \text{ of acetone solution} \times 164 \times I_f/\text{g of sample}$$

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 oleoresin sample.

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Table 1. ASTA and Tint Parameters of the Commercial Oleoresins (Mean ± SD, $n = 4$)

oleoresin	ASTA	tint value (A_{470}/A_{454})
A	2335 ± 41	0.989 ± (5×10^{-4})
B	3604 ± 26	0.969 ± (13×10^{-4})
C	3592 ± 57	0.991 ± (14×10^{-4})
D	2016 ± 13	0.987 ± (15×10^{-4})
E	1463 ± 30	0.987 ± (4×10^{-4})
F	3551 ± 31	1.011 ± (6×10^{-4})
G	3621 ± 52	0.982 ± (10×10^{-4})

Tint Determination. The parameter tint was determined from the absorption spectrum of the sample in acetone by recording the absorbance at 470 and 454 nm. Approximately 0.025 g of oleoresin was weighed in a 25 mL measuring flask, and acetone was added to the mark. An aliquot of the resulting solution was diluted to known volume, such that the absorbance of the solution was between 0.2 and 0.8 unit. The absorbance was measured in quadruplicate for each sample, using a Hewlett-Packard spectrophotometer, model 8452 (DAD).

Calculation of the Concentrations of Individual Carotenoids and of Isochromatic Fractions of Red and Yellow Pigments. Sample (0.025 g) was placed in a decantation funnel, and 50 mL of ethyl ether was added. The pigments in the ether phase were de-esterified with 25 mL of 10% KOH solution. When de-esterification was completed, 1 mL of a standard solution of β-apo-8′-carotenal was added for later quantification by HPLC according to the methodology described in Mínguez-Mosquera and Hornero-Méndez (1993). The liquid chromatograph used comprised a quaternary pump (Waters, model 600), injection valve (Rheodyne, model 7125), and loop of 20 μL. The detector was a DAD (Waters, model 996). The individual carotenoid pigment composition of each oleoresin was determined in quadruplicate.

RESULTS AND DISCUSSION

The tint value is an attempt to interpret the ratio of red and yellow pigments in the sample. ASTA degrees are related to the total coloring capacity. Table 1 shows the ASTA and tint determination values for each sample of oleoresin. The lack of parallelism between the two measurements is noteworthy, with oleoresins of the same or similar tint values showing very different coloring capacities according to their ASTA degrees. A possible interpretation is that different oleoresins having the same tint ratio differ in total carotenoid concentration. Thus, oleoresins A, D, E, and G, with identical or similar tint values, have different coloring capacities, as shown by the ASTA values. At the same time, oleoresin B has a tint value lower than that of oleoresin F (the maximum) but a higher ASTA value. Thus, there is a lack of equivalence between the two parameters.

The only pigments giving a red color to the fruits of the genus *Capsicum* are the carotenoids. These can be differentiated by color into two groups, the result of their molecular structure, because, depending on their chromophore chain, they take on a red or yellow coloration. The natural ratio between these two pigment fractions in the pepper ranges between 1.2 and 1.5 and is independent of variety. In the products derived from the pepper, depending on processing, this ratio remains constant or is increased by loss of yellow pigmentation (the more labile to thermoxidation) (Mínguez-Mosquera and Hornero-Méndez, 1994). To match this information with that described above, the carotenoid composition of the oleoresins must be examined.

The values for the red fraction (formed by capsanthin and capsorubin) and for the yellow fraction (comprising the rest of the pigments: β-carotene, β-cryptoxanthin, zeaxanthin, capsolutein, and violaxanthin) were deduced from the individual carotenoid composition. The sum of the two fractions gives the total pigments. Table 2 shows the values for each fraction and the R/Y ratio for the seven oleoresins studied. This ratio ranges between 1.1 and 1.7, which includes that of the fruit, but has both lower and higher values. Taking into account that the raw material is subjected to various operations (drying, milling, and extraction) which may result in pigmentation loss, generally caused by the temperatures of these processes, the values found outside the established limits are the result of deterioration, by degradation of one or more pigment fractions, possibly during drying. Thus, paprikas (an intermediate product in the obtaining of oleoresin) can be found with R/Y values higher than those of the fresh fruit, given that generally the yellow carotenoid fraction is the one with higher overall losses (Mínguez-Mosquera et al., 1992). Therefore, the R/Y ratio is a parameter indicating the soundness of the oleoresin-obtaining process, because its constancy indicates a proper control of temperature during processing and that there are no losses in any of the pigment fractions.

As can readily be deduced from Table 2, the oleoresins with highest R/Y ratio are not necessarily those with the highest pigmentation value, as in the case of oleoresin E. Oleoresins F and G, although products of high quality, show loss of constancy in the ratio, demonstrating that losses due to degradation of the yellow fraction have occurred in processing, either at the fruit drying stage or during extraction.

As a result of their different structure, the behavior of the yellow carotenoids in relation to the red ones, in response to thermooxidation reactions that could take place during the oleoresin-obtaining process (time and temperature during drying and extraction), is different, too, so the parameter R/Y is reflects this fact. Joint modification of the parameter R/Y and total carotenoids gives information about the quality of the raw material used or, as already mentioned, the soundness of the processing.

Listing the oleoresins in order of the value of the parameters tested, it is concluded that total carotenoids correspond well with ASTA degrees, whereas the R/Y and tint values show no such correspondence, either one with the other or with total pigments or ASTA. However, it is noteworthy that although the parameter R/Y distinguishes among oleoresins, the tint value does not indicate quality and may even lead to confusion, because it does not distinguish among oleoresins with very different carotenoid contents. It is evident that tint determination does not evaluate the real ratio between red and yellow pigments, nor is it a distinguishing parameter; that is, in most cases it does not allow oleoresins to be distinguished one from the other.

To test the validity of these deductions, an analysis of variance was performed using the procedure of Duncan. This analysis confirmed the conclusions. The ASTA units and total carotenoid content gave parallel results for the coloring capacity of the sample analyzed, the parameter tint had no statistical significance, and the R/Y ratio differentiated among samples. As a consequence of this study, it is concluded that total pigments taken together with the R/Y ratio give an idea not only of the coloring capacity but also of possible pigmentation losses associated with the process.

Table 2. Quantification of Carotenoid Pigments in Seven Commercial Oleoresins by HPLC and Ratio of Pigments between Fractions (Mean ± SD, $n = 4$)

	concentration (g/kg)			
oleoresin	red[a]	yellow[b]	total	R/Y
A	35.21 ± 0.28	24.25 ± 0.22	59.46 ± 0.33	$1.455 \pm (19 \times 10^{-3})$
B	41.32 ± 0.95	36.23 ± 0.79	77.55 ± 1.73	$1.143 \pm (5 \times 10^{-3})$
C	47.30 ± 1.21	35.57 ± 0.80	82.87 ± 1.91	$1.330 \pm (21 \times 10^{-3})$
D	27.28 ± 0.93	17.87 ± 0.50	45.16 ± 1.43	$1.530 \pm (8 \times 10^{-3})$
E	21.93 ± 0.28	12.47 ± 0.25	34.40 ± 0.52	$1.758 \pm (15 \times 10^{-3})$
F	53.68 ± 0.33	28.43 ± 0.73	82.11 ± 0.68	$1.888 \pm (21 \times 10^{-3})$
G	51.37 ± 0.40	31.05 ± 0.56	82.42 ± 0.42	$1.656 \pm (4 \times 10^{-3})$

[a] Red fraction (R) = capsanthin + capsorubin. [b] Yellow fraction (Y) = β-carotene + β-cryptoxanthin + capsolutein + zeaxanthin + violaxanthin.

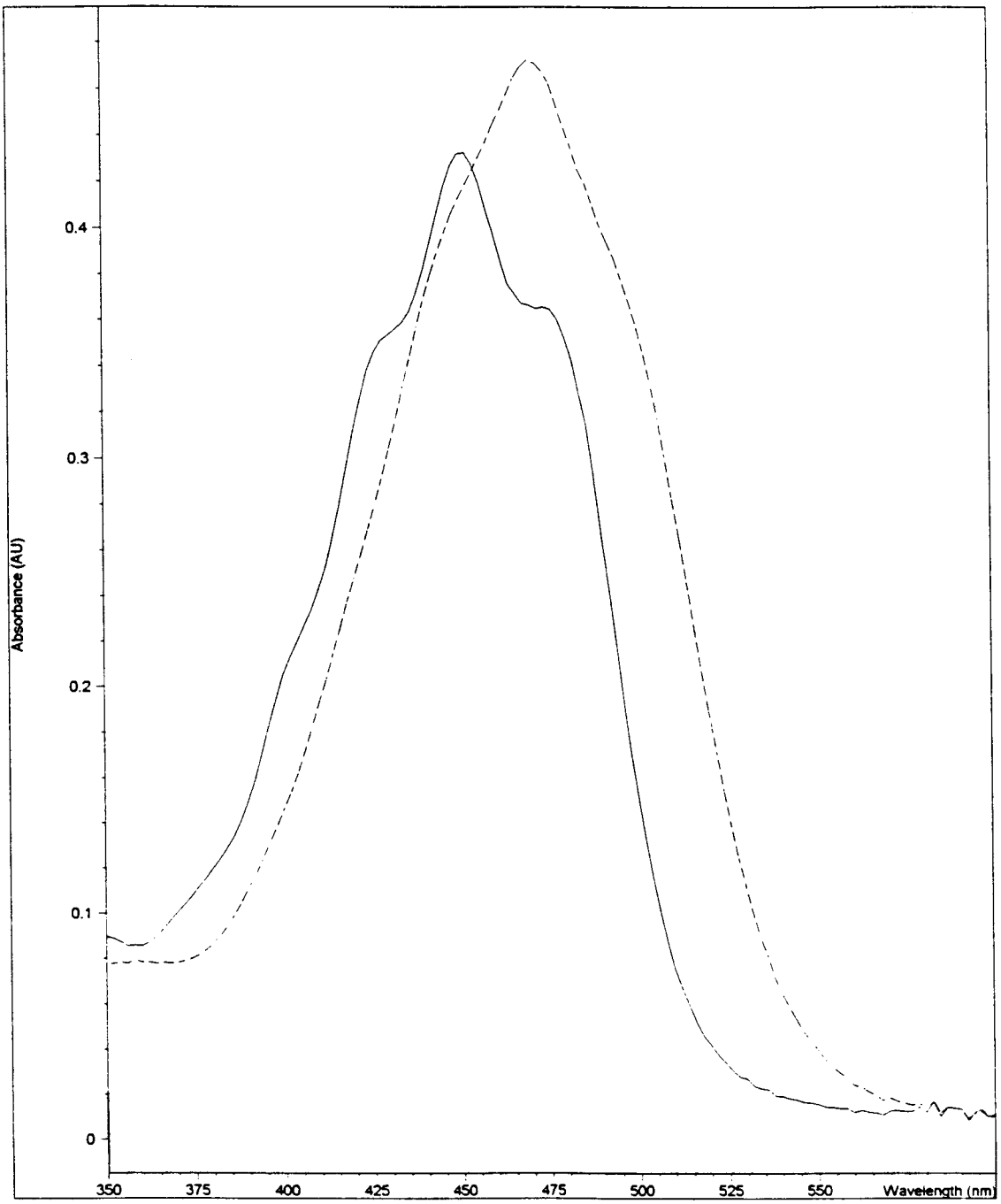

Figure 1. Absorption spectra in acetone of the red carotenoid fraction (- - -) and of the yellow carotenoid fraction (—).

Figure 1 shows the overlapping of the absorption spectra in acetone of the red and yellow carotenoid fractions after their separation by TLC (Mínguez-Mosquera et al., 1984). As can be observed at the wavelengths selected

Table 3. Quantification of Carotenoid Pigments Using the Spectrophotometric Method Proposed and Ratio of Pigments between Fractions (Mean ± SD, n = 4)

	concentration (g/kg)			
oleoresin	red[a]	yellow[b]	total	R/Y
A	34.61 ± 0.57	24.43 ± 0.43	59.04 ± 0.98	1.416 ± (10 × 10^{-3})
B	39.94 ± 0.34	35.14 ± 0.79	75.24 ± 0.63	1.132 ± (8 × 10^{-3})
C	48.24 ± 1.13	34.44 ± 0.21	82.68 ± 1.33	1.401 ± (24 × 10^{-3})
D	27.48 ± 0.19	19.02 ± 0.13	46.51 ± 0.28	1.445 ± (9 × 10^{-3})
E	21.34 ± 0.47	12.38 ± 0.22	33.71 ± 0.69	1.724 ± (9 × 10^{-3})
F	53.84 ± 0.62	27.99 ± 0.46	81.83 ± 0.16	1.924 ± (5 × 10^{-3})
G	53.01 ± 0.76	30.92 ± 0.42	83.93 ± 1.16	1.715 ± (8 × 10^{-3})

[a] Red fraction (R) = capsanthin + capsorubin. [b] Yellow fraction (Y) = β-carotene + β-cryptoxanthin + capsolutein + zeaxanthin + violaxanthin.

for tint determination (λ_{470} and λ_{454}), absorbance is not strictly an evaluation of the different pigment fractions, because their effect is additive at the two wavelengths. Therefore, the ratio of absorbance in the tint determination is not the same as the R/Y ratio, and it is meaningless in the carotenoid composition of the sample analyzed. For technico-commercial reasons the evaluation of oleoresin coloration has to be rapid and quantitative. To meet this need, determination of the pigment fractions directly from the total carotenoid spectrum at the most suitable wavelengths is proposed. The yellow fraction is affected by the red carotenoids over the whole spectrum, whereas the red fraction shows minimal effect of the yellow in the zone between 515 and 535 nm. The following method was therefore used: the total carotenoid concentration was determined at $\lambda = 456$ nm after the coefficient of extinction at this wavelength was calculated, the red fraction was determined at $\lambda = 525$ nm, and the yellow fraction was calculated as their difference. Because, for the oleoresins under study, the concentration of each fraction and the corresponding measurement of absorbance are known, it is easy to calculate the coefficient by applying the following formula

$$E^{1\%}_{1cm} = A_{value}(DF)/CW_s$$

where DF is the dilution factor, W_s is the weight of the sample, and C is the concentration of the fraction in the sample, calculated from HPLC. From this equation, the extinction coefficients of total (E_{0T}) and red (E_{OR}) pigments are calculated by introducing the absorbance at 456 and 525 nm and the concentration of the corresponding fraction.

Thus, four determinations of E_{0T} and E_{OR} were obtained for each oleoresin, generating 28 values of each extinction coefficient. The mean values of each coefficient were $E_{0T} = 2654 \pm 84.868$ and $E_{OR} = 1041 \pm 51.033$. Table 3 shows the values for the different pigment fractions and the theoretical R/Y ratio, calculated using these mean coefficients. The mean percentage relative error of the red and yellow fractions and of the R/Y was 4%. The spectrophotometric method thus gives measurements of the R/Y parameter and of the different pigment fractions that are close to reality, enabling a commercial evaluation of the oleoresins based on their carotenoid composition.

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