Evolution of Color during the Ripening of Selected Varieties of Paprika Pepper (*Capsicum annuum* L.)

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For the first time the evolution of the color of 13 paprika pepper varieties, some of them with chlorophyll-retaining genes, is studied, from the moment the fruit is considered completely formed until harvesting, i.e., during ripening. The final quality of each variety was evaluated by determining the extractable color by the American Spice Trade Association (ASTA) and the tint. The use of the chromatic attributes L^*, a^*, and b^* suggested by the Commission Internationale de l'Eclairage (CIE), i.e., of the CIELAB space, made it possible to follow the evolution of color which takes place during fruit ripening and to identify the chlorophyll-retaining varieties. Saturation (S^*) was the most suitable attribute for distinguishing the different phases of ripening.

Keywords: *Paprika pepper; chromatic coordinates; evolution of color*

INTRODUCTION

The external appearance of fruits, particularly their color, is of prime importance when considering the different attributes which define quality, and in the case of fruits destined for fresh consumption, a visual impression which does not coincide with the established standard easily leads to refusal.

In the case of pepper varieties destined for the processing of paprikas and oleoresins, two of the most widely used natural colorants in the food industry (Fisher and Kocis, 1987), the obtention of lines rich in carotenoids, basically red ones, is of great importance, since their quality is closely related to their coloring power (Costa, 1979, 1980; Soriano et al., 1990). Thus, the ripening of pepper fruit has been the object of interest of many researchers, not only because of the spectacular change they undergo but also because of the complexity of the mechanisms which take part in the process of biosynthesis of capsanthin, a typical colorant of this fruit (López-Sanchez, 1972; Alemán and Navarro, 1973; De la Torre and Farre, 1975; Candela et al., 1984; Fisher and Kocis, 1987; Alcaraz, 1990; Ortiz et al., 1990; Almela et al., 1990, 1991; Minguez and Hornero, 1993, 1994; Biacs and Daood, 1994).

The completely ripe fruit behaves as a balanced biological unity. This stage is reached through a common biosynthetic route, in which it is the regulation of the individual carotenoid production that decides the characteristic differences of each variety (Almela et al., 1991; Minguez and Hornero, 1994).

Normally, the change in color of the pepper surface takes places as a result of chlorophyll degradation and a considerable increase in its carotenoid content, which is influenced by the temperature and illumination to which the fruit is exposed. In the varieties containing chlorophyll-retaining genes the total chlorophyll content suddenly increases at the onset of ripening, principally due to the sharp increase of chlorophyll *b*. From this moment onward, the content of chlorophill (particularly chlorophyll *a*) decreases, but not to the extent that it completely disappears in the ripe fruit (Ferrer and Costa, 1991).

This paper studies the evolution of color during fruit ripening in 13 selected paprika pepper varieties (three of them with chlorophyll-retaining genes), from the moment we consider that the green fruit is completely developed and has reached its maximum size until harvesting. The study and comparison of the chromatic attributes Y, x, and y as well as the L^*, a^*, and b^* ones, suggested by the Commission Internationale de l'Eclairage (CIE) (i.e., the CIE Yxy and CIELAB systems (CIE, 1978; CIE, 1986), respectively), obtained from direct reflection measurements on the fruits, as well as the plot of the chromatic coordinates in the CIELAB color space, makes it possible to identify the different maturation phases, which are important for the quality and classification of the varieties, and to establish the most appropriate moment for harvesting. In addition, the final quality of each of the varieties is determined.

MATERIALS AND METHODS

Plant Materials. Thirteen selected pepper varieties used for producing paprika were studied. Seven (Alargado rojo, Belrubí, Bunegro, Cokaner, Danero, Datler, and Negral) were obtained by the Centro de Investigación y Desarollo Agroalimentario in La Alberca (Murcia, Spain); the variety Americano is a traditional cultivar in the province of Murcia (Spain) and Numex Conquistador was obtained in California (U.S.A.); the remaining varieties (Larguillo, Morro negro, and Morro rojo) were obtained in the Escuela Técnica Superior de Ingenieros Agrónomos (E.T.S.I.A.) of Albacete (Spain). Of these varieties, Negral, Bunegro, and Morro rojo contain chlorophyll-retaining

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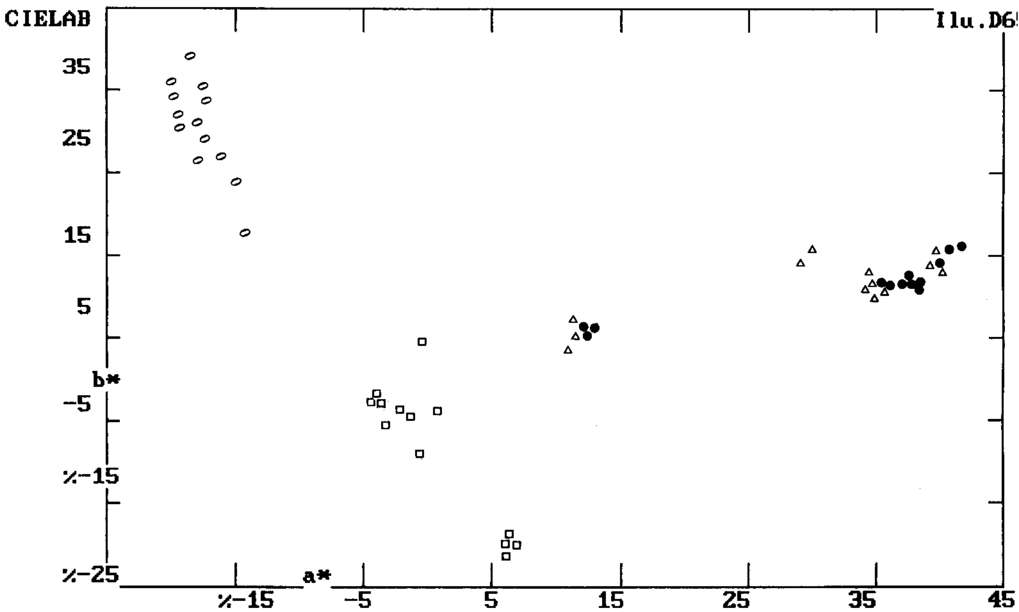

Figure 1. Evolution of color of the fruit in the CIELAB diagram for the different samples of each variety: (○) green, (□) reddish-green, (△) reddish, and (●) red peppers.

Table 1. CIE Yxy Chromatic Attributes, Dominant Wavelength (d.w.), and Excitation Purity (Purity) of the Green, Reddish-Green, Reddish, and Red Fruits of the Different Varieties

	green		reddish-green		reddish		red	
variety	d.w.	purity	d.w.	purity	d.w.	purity	d.w.	purity
Alargado rojo	564.04	53.36	575.50	7.61	617.77	46.95	617.42	51.98
Belrubí	564.82	53.71	463.79	11.43	614.88	44.27	621.76	45.94
Bunegro	565.19	49.78	486.03	1.89	598.93	21.13	597.71	26.47
Cokaner	565.25	44.67	465.93	13.01	626.91	40.01	629.65	43.14
Danero	564.79	58.17	501.11	1.56	623.90	47.19	617.42	50.72
Datler	564.65	54.16	512.17	1.10	614.71	51.17	619.94	48.96
Larguillo	566.70	59.68	554.16	0.67	624.96	41.36	626.62	44.26
Morro negro	562.83	37.58	467.84	14.84	596.68	23.57	596.20	26.09
Morro rojo	563.56	47.75	498.67	1.42	622.29	43.73	625.76	42.92
Negral	566.03	57.28	491.00	1.65	592.70	28.58	596.76	26.45
Numex c.	564.92	51.02	479.03	5.61	605.03	45.52	619.82	43.18
Ocal	564.31	53.86	487.68	3.82	603.53	47.57	627.66	44.93
Americano	565.96	56.58	462.96	11.87	621.46	44.07	621.35	43.60

genes in the ripe fruits (Navarro and Costa, 1993; Pardo et al., 1995; Costa et al., 1995).

Experimental Design. The varieties were sown in random blocks with four repetitions on the grounds of the E.T.S.I.A. of Albacete. Transplanting was made on April 15, 1994, completely developed green peppers being obtained for varieties on August 9 of the same year. At that moment, 40 fruits in each of the four blocks of each variety were marked using tags. The fruit were harvested at different times throughout ripening—August 9, August 16, August 31, and September 19, 1994—thus providing four samples in terms of their external color: green, reddish-green, reddish, and red. Each sample consisted of 40 fruits, 10 per repetition, selected at random, for each variety.

Chromatic Coordinates. The color of the fruits of each of the samples was measured by reflectance using a Minolta CR-200 colorimeter through direct reading on three different areas of the surface of randomly selected fruit, considering as the definitive measurement the mean of the three made. The values obtained were used to calculate the chromatic attributes of the CIE Yxy systems shown in Table 1: dominant wavelength (d.w.) and excitation purity (purity). The color CIELAB coordinates L^* (brightness), a^* (red component), and b^* (yellow component), and chromatic attributes C^* (chrome), H^* (metric angle), and S^* (saturation) are given in Table 2. The graphic representation of this latter color space (Figures 1 and 2), with Illuminant D65, was made from the corresponding a^* and b^* values, which are given in the mentioned table by means of a specific program (not published) developed by Prof. Dr. Antonio Serna Serna of the Departamento de Química-Física of the University of Murcia (Spain).

Evaluation of the Extractable Color by the American Spice Trade Association (ASTA). In the ASTA-20.1 method (ASTA, 1968), 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

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

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. The total pigment content was determined using a Shimadzu UV-160 spectrophotometer. Table 3 shows the mean value, in ASTA units, of the four repetitions of each variety, as well as their standard deviation.

Tint Determination. To evaluate the tint, or quotient of the absorbances between the red and yellow carotenoid pigments of each variety, the absorption spectra of the extracts of each variety in acetone were registered in the visible zone (380−600 nm). This quality criterion is specifically quantified by the following quotient: T = absorbance at 470 nm/absorbance at 455 nm (Afexpo, 1993). The tint value (Table 3) was calculated from only one sample per variety, obtained by mixing the same quantity of paprika for each of the repetitions.

Chlorophyll Determination. The determination of the total chlorophyll (Table 3), from the samples obtained for the tint evaluation, was carried out according to the procedure of

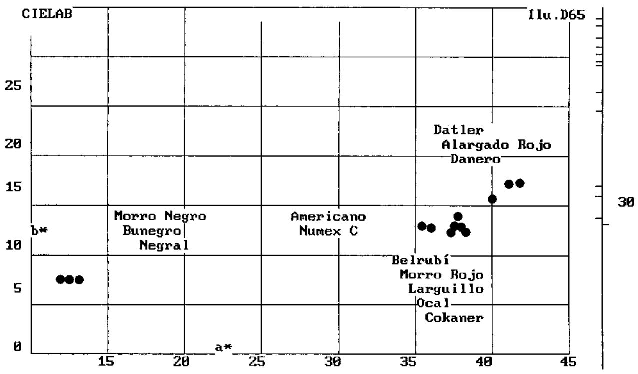

Figure 2. Distribution in the CIELAB diagram of the pepper varieties in the last ripening phase (red fruits).

Vernon (1960) through extraction with 80% acetone, adsorption chromatography, and spectrophotometry.

RESULTS AND DISCUSSION

Variation of the Chromatic Attributes in the CIE Yxy System. Table 1 shows how in green fruits the dominant wavelength varies only slightly (between 562 and 566 nm) among the different varieties, since chlorophyll is the most abundant pigment. In the second phase of ripening, corresponding to the reddish-green fruits, the value of this chromatic characteristic decreases sharply, due to the disappearance of part of the chlorophylls and the progressive increase of the chromoplasts (Ferrer and Costa, 1991). As ripening progresses, the dominant wavelength increases again until it reaches final values of 596−597 and 617−629 nm for the varieties with and without chlorophyll-retaining genes, respectively. This difference between both types of variety arises from the incomplete disappearance of chlorophyll from the retaining varieties. Similar behavior is observed for the chromatic characteristic of excitation purity, the final values varying between 42 and 52% for the nonretaining varieties and between 36 and 37% for the chlorophyll-retaining ones.

Evolution of Ripening and Behavior of the Chromatic Attributes in the CIELAB System. The color diagram CIELAB for all the samples (Figure 1) clearly illustrates the different ripening phases of the varieties. Depending on the moment of harvest, the points which represent the varieties move from the negative *a** (green component) to the positive *a** values (red component). In the two last harvests, the chlorophyll-retaining varieties stop their moving since this pigment does not disappear in the ripe fruits. Parameter *b** (yellow-blue component) shows the characteristic inflection corresponding to the beginning of ripening.

Figure 2 shows the position of the varieties during the CIELAB diagram in the last maturation phase, corresponding to the harvesting of the red fruits (September 19, 1994). The chlorophyll-retaining varieties (Morro negro, Bunegro, and Negral) with very close *a** values (between 12 and 13) are on the left hand side. The other varieties, with values of between 35 and 42, are on the right hand side. At this stage of ripening, the most attractive colors are those with high attribute *C** values (Table 2), placed high on the right hand side of the diagram (Datler, Alargado rojo, and Danero).

From Table 2 we deduce that the maximum *L** value is reached during the reddish-green stage of the fruit, and that it falls during the final stages of ripening. The *C** values stabilize at the end of the mentioned process, at 15−16, for the fruits with chlorophyll-retaining genes and at 38−46 for the fruits of the remaining varieties.

Table 2. Color Coordinates and CIELAB Chromatic Attributes, Brightness (*L), Red Component (*a**), Yellow Component (*b**), Chrome (*C**), Metric Angle (*H**), and Saturation (*S**) of the Samples of the Different Varieties (Green (1), Reddish-Green (2), Reddish (3), and Red (4) Peppers)**

variety	sample	*L**	*a**	*b**	*C**	*H**	*S**
Alargado rojo	1	48.45	−19.50	34.54	39.66	119.44	32.47
	2	96.01	−0.34	7.65	7.66	92.54	0.61
	3	38.22	39.52	17.20	43.10	23.53	48.60
	4	35.71	41.04	18.75	45.12	24.56	57.01
Belrubí	1	48.35	−18.31	34.58	39.13	117.91	31.66
	2	82.72	5.84	−14.56	15.69	291.87	2.97
	3	36.00	34.61	15.56	37.94	24.21	39.99
	4	34.52	37.78	15.32	40.77	22.07	48.14
Bunegro	1	45.95	−16.10	30.65	34.62	117.71	26.08
	2	87.39	−1.30	−1.52	2.00	229.52	0.04
	3	29.61	10.73	6.70	12.65	32.00	5.40
	4	30.78	12.92	8.87	15.67	34.46	7.98
Cokaner	1	46.90	−14.96	27.81	31.58	118.27	21.26
	2	86.21	6.34	−17.04	18.18	290.41	3.83
	3	34.44	34.86	12.61	37.07	19.89	39.40
	4	34.72	38.29	13.81	40.70	19.83	47.72
Danero	1	51.89	−20.56	39.77	44.77	117.34	38.62
	2	94.97	−4.23	0.50	4.26	173.26	0.19
	3	35.36	40.15	15.98	43.22	21.70	52.81
	4	37.86	41.83	18.91	45.91	24.33	55.66
Datler	1	53.22	−20.11	37.56	42.61	118.16	34.10
	2	94.41	−4.01	1.26	4.20	162.61	0.18
	3	36.93	39.89	19.05	44.21	25.53	52.91
	4	35.52	39.99	17.08	43.48	23.13	53.23
Larguillo	1	56.55	−18.71	43.33	47.20	113.35	39.39
	2	97.99	0.85	−0.72	1.11	319.41	0.01
	3	35.16	35.87	13.42	38.30	20.51	41.71
	4	33.65	37.50	14.09	40.06	20.60	47.69
Morro negro	1	40.31	−14.23	21.26	25.58	123.79	16.23
	2	82.09	6.27	−18.48	19.51	288.73	4.63
	3	32.28	11.52	8.16	14.12	35.30	6.17
	4	31.04	12.07	8.91	15.00	36.43	7.25
Morro rojo	1	46.59	−18.07	29.96	34.99	121.10	24.77
	2	96.79	−3.46	0.15	3.46	177.55	0.12
	3	31.92	34.48	13.62	37.08	21.56	43.05
	4	35.18	37.35	14.02	39.89	20.58	45.24
Negral	1	51.62	−18.06	38.69	42.70	115.02	33.96
	2	90.20	−2.11	−0.85	2.28	201.93	0.05
	3	32.03	11.29	10.40	15.35	42.65	7.35
	4	30.18	12.27	8.82	15.12	35.72	7.56
Numex c.	1	48.35	−17.47	32.72	37.09	118.09	28.45
	2	87.09	−0.58	−6.05	6.08	264.53	0.42
	3	35.89	29.16	17.10	33.80	30.39	31.84
	4	34.90	35.29	14.40	38.12	22.19	41.62
Ocal	1	49.74	−19.58	35.53	40.56	118.86	33.08
	2	87.52	−3.32	−2.70	4.28	219.20	0.21
	3	37.37	29.89	18.77	35.30	32.12	33.33
	4	33.14	37.91	14.16	40.47	20.49	49.41
Americano	1	49.68	−17.50	37.09	41.01	115.26	33.85
	2	87.78	6.60	−15.97	17.28	292.46	3.40
	3	32.59	34.91	14.00	37.61	21.85	43.41
	4	34.40	35.82	14.33	38.58	21.80	43.27

The *H** value also makes it possible to distinguish the different maturation phases, since it stabilizes at the end of the process at 34−37 for the normal and 19−25 for the retaining varieties. The *S** values at the last harvest vary between 41−57 for the normal and 7−8 for the retaining varieties. In the Datler, Morro rojo, Danero, and Americano varieties, as well as in the varieties carrying chlorophyll-retaining genes, there are minimum differences in the values between the last two harvests (August 31 and September 19, 1994), since fruit maturation has already been reached at an earlier stage.

Chemical Color Determination. The ASTA units and the tint of the different varieties (Table 3), as determined from the paprika obtained from the fruit of the last harvest (dried and milled fruits), are indicative of the quality reached in each variety at the end of the ripening process, since they show the total pigment concentration synthesized in the fruit and the relation between the red and yellow carotenoids. In this sense,

Table 3. Mean and Standard Deviation of the Extractable Color (ASTA), Tint, and Chlorophyll Content (g/kg of Paprika) of the Varieties

variety	ASTA means ± d*t*	tint	content in chlorophyll
Alargado rojo	215 ± 3	0.983	
Belrubí	274 ± 4	0.983	
Bunegro	280 ± 4	0.990	0.48
Cokaner	245 ± 3	0.990	
Danero	250 ± 4	0.977	
Datler	269 ± 3	0.997	
Larguillo	370 ± 5	0.986	
Morro negro	215 ± 3	1.000	0.40
Morro rojo	263 ± 3	0.990	
Negral	265 ± 5	0.989	0.50
Numez c.	206 ± 3	0.966	
Ocal	294 ± 4	0.988	
Americano	162 ± 3	0.988	

the variety Larguillo is particularly interesting. In the same table we also show the chlorophyll content of the varieties studied. In spite of the fact that the variety Bunegro presents the maximum extractable color value of the chlorophyll-retaining group, its use for paprika production is not widespread in certain markets due to the peculiar visual color that the presence of this pigment gives it.

CONCLUSIONS

It is possible to distinguish the normal varieties from those which have chlorophyll-retaining genes. This is shown by the final values reached by the chromatic attributes of the CIE Yxy system: dominant wavelength and purity.

The color diagram CIELAB clearly illustrates the chromatic evolution which takes place during fruit ripening. The samples represented in this diagram show, in terms of their ripening stage, increasing a^* values. Moreover, it distinguishes the chlorophyll-retaining varieties: the higher the quantity of this pigment retained in the ripe fruit, the more to the left they appear.

Among the CIELAB color attributes, saturation (S^*) is the most appropriate for distinguishing the different fruit ripening phases. The constancy of this parameter in the final ripening phases points to the varieties which ripen earlier.

The ASTA method and the tint values reveal precisely the quality each variety reaches at the end of the process, since they show the total pigment concentration synthesized in the fruit and the relation between red and yellow carotenoids.

ACKNOWLEDGMENT

We thank Prof. Dr. Antonio Serna Serna for the use of the specific program developed for calculating the color in the different spaces with a Hitachi-3200 spectrophotometer (program of the Departamento de Química-Física de la Universidad de Murcia (Spain)).

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Received for review July 20, 1995. Revised manuscript received May 14, 1996. Accepted May 30, 1996.[⊗]

JF950465M

⊗ Abstract published in *Advance ACS Abstracts*, July 15, 1996.