Carotenoids and Carotenoid Esters from New Cross-Cultivars of Paprika

Péter A. Biacs,*,† Hussein G. Daood,† Tibor T. Huszka,‡ and Péter K. Biacs†

Central Food Research Institute, Herman Ottó u. 15, H-1022 Budapest, Hungary, and Szegedi Paprika Rt, Szövetkezeti ut 1, Szeged, Hungary

Carotenoids and carotenoid esters present in spice paprika powders (*Capsicum annuum*) were separated and analyzed by high-performance liquid chromatography with a rotating monochromator detector controlled by user-friendly chromatographic software. The carotenoid composition of paprika powders produced from new cross-cultivars was compared with that of powders from the original (Hungarian and Spanish) parents. The results showed significant differences between the new and old cultivars with respect to esterification of carotenoid with fatty acid and the capsanthin/capsorubin ratio. It was also found that the first generation (F-1) from Hungarian red longum and Spanish lilac round cultivars had a carotenoid composition similar to that of its Spanish parent, but the (F-5) subsequent generation gave a yield of improved characteristics such as high color intensity and high capsanthin/capsorubin ratio.

INTRODUCTION

Spice paprika (red pepper) is commercially cultivated in Hungary and Spain. Its various products such as powder, paste, and oleoresin are excellent food colorants. However, it is still a serious problem for paprika producers and consumers that ground products lose their color during a relatively short storage period. It has been demonstrated that a radical shift in the color of the product, even though accompanied by no change in flavor, can make it completely unacceptable.

Several analytical studies focused on the carotenoid ester composition of paprika fruits (Vinkler and Richter, 1972; Fischer and Kocsis, 1987; Gregory et al., 1987; Biacs et al., 1989). The results showed that fatty acid esters of capsanthin and capsorubin are the major constituents of paprika pigment. Although acylation with fatty acids is advantageous for pigment stability, capsanthin and capsorubin lose their color when exposed to oxidizing agents such as light, metals, molecular oxygen, and oxidizing enzymes. In a previous study (Biacs et al., 1987) capsanthin has been found to be more stable than capsorubin, and diesters of these xanthophylls are more resistant than monoesters to oxidative degradation. These results should be taken into consideration when the quality of paprika is to be modified by cross-breeding or gene technology experiments which seem to be the best alternative to solve the problem of color loss during processing and storage. During recent years, in scientific cooperation, Hungarian and Spanish plant breeders have begun a series of experiments to produce new cultivars having high color intensity and stability. Cross-breeding of Hungarian red longum cultivar with the Spanish lilac round cultivar was part of the scheduled program.

The objective of the present work was to study the carotenoid and carotenoid ester content of new cross-cultivars using modified reversed-phase high-performance liquid chromatography. Traditional sweet varieties of paprika from Szeged (Sz-20) and Mihályteleki (MT) were compared with the cultivars produced by cross-breeding of MT and the Spanish cultivar (Negral), which has an intense brownish red color.

MATERIALS AND METHODS

Ground paprika samples were obtained from the research station of Szegedi Paprika RT (Hungary), where breeding experiments are carried out. The powders were produced by milling dry fruits (without seeds) using a cylinder mill which gave powders of 0.5-μm particles. Triplicate samples were taken and stored in sealed nylon bags at −20 °C until their analysis.

Organic solvents used for extraction and chromatograpic analysis were of HPLC grade and obtained from Reanal (Budapest).

Pigment Extraction. Half-gram samples were extracted by mechanical shaking of each with 50 mL of 2:1:1 (v/v/v) chloroform–2-propanol–acetone for 20 min at room temperature as reported by Pavisa et al. (1987). The extract was filtered through a Rundfilter MN640m filter paper and the filter residue re-extracted with an extra 50 mL of solvent mixture until residues of faint brown color were obtained. The filtrates were collected and brought to 100 mL with the same solvent. A 25-mL aliquot was transferred to a round-bottom flask. Then the solvent was evaporated under vacuum by rotatory evaporator. The residues were redissolved in 2 mL of chloroform, and the volume was then brought to 10 mL with the HPLC eluent.

Liquid Chromatography. A Nucleosil ODS 5-μm column (Labor MIM, 250 × 4.6 mm, stainless steel) was used for separation of paprika carotenoids. The mobile phase consisted of 39:57:4 (v/v/v) acetonitrile–2-propanol–water (Czinkotai et al., 1989). Other chromatographic conditions were as follows: flow rate, 1.2 mL/min; detection wavelength, 438 nm, range of detection, 0.1 AUFS.

The HPLC system comprised a Beckman series liquid chromatograph equipped with a Model 114 solvent delivery pump, a Model 340 organizer supplied with a 20-μL loop injector, and a Model 165 variable-wavelength UV–visible detector. Spectral analysis was carried out using a Model Chrom-A-Scope (Barspec) rotating monochromator detector. The detector signals were transferred to an IBM compatible computer under the control of chromatographic (user-friendly) software.

Peak Identification and Quantification. The individual components of isocratic separation were identified according to their spectral characteristics and chromatographic behavior compared with those of authentic standards prepared from saponified and unsaponified extracts of fresh ripe paprika fruit by thin-layer chromatography (Vinkler and Ritcher, 1972; Biacs et al., 1987).

* Author to whom correspondence should be addressed.
† Central Food Research Institute.
‡ Szegedi Paprika.

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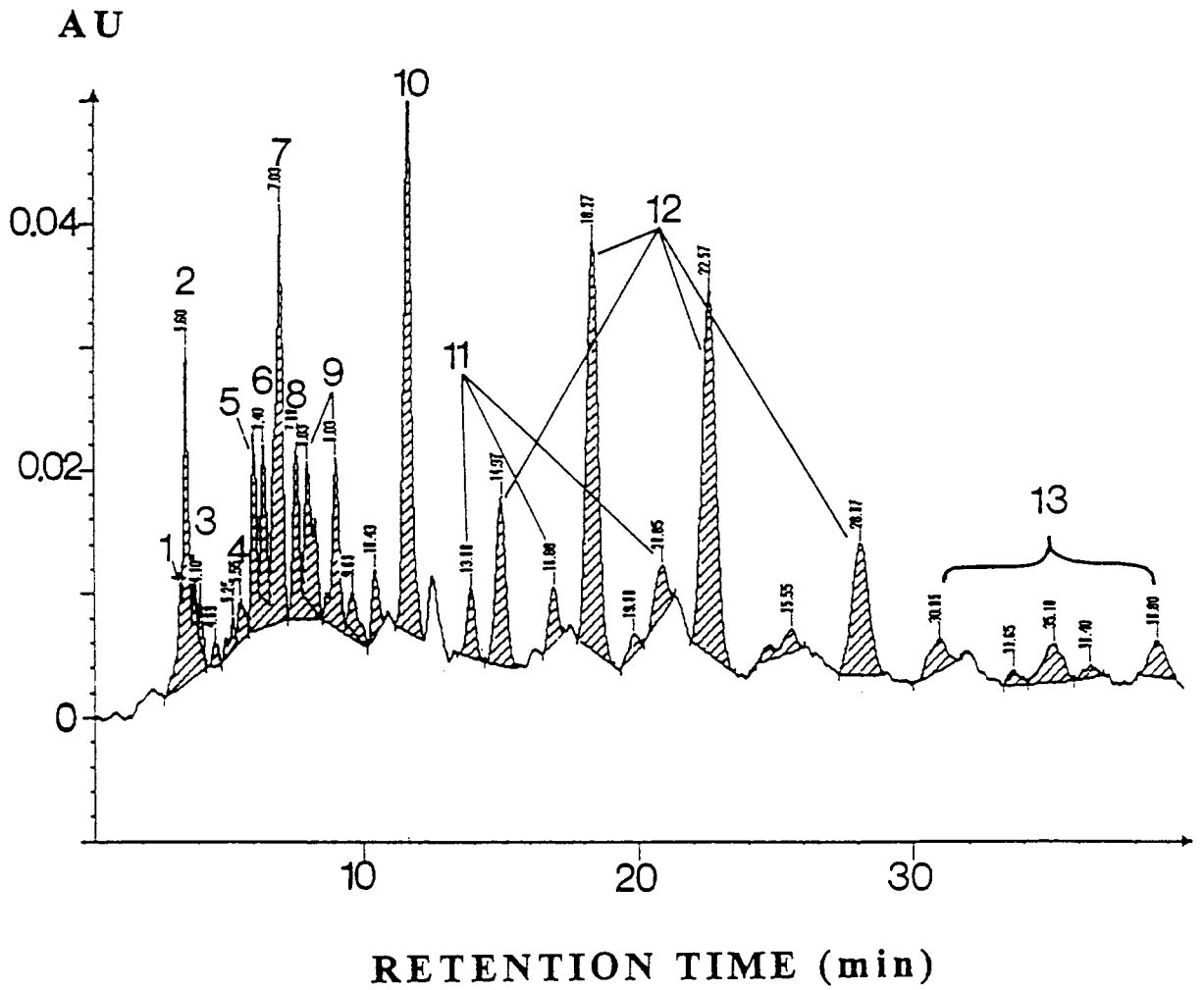

Figure 1. HPLC profile of paprika extract separated on a Nucleosil ODS column and detected at 438 nm. 1, Capsorubin; 2, capsanthin; 3, lutein; 4, β-cryptoxanthin; 5, 6, capsorubin monoester; 7–9, capsanthin monoesters; 10, β-carotene; 11, capsorubin diesters; 12, capsanthin diesters; 13, lutein and zeaxanthin diesters.

As for quantification, the chromatograms were evaluated by relating the integrated area of the individual components to that of authentic standard. Pigment concentration of the authentic solutions was determined spectrophotometrically using extinction coefficient ($E^{1\%}$) values of 2072 and 2200 for capsanthin and capsorubin, respectively (Bauernfeind, 1981).

RESULTS AND DISCUSSION

In previous works (Biacs et al., 1989; Czinkotai et al., 1989) HPLC separation of paprika carotenoids has been carried out on a reversed-phase (C_{18}) column containing 10-μm particles. By such a procedure carotenoid extracts could be fractionated into only 15 components. Therefore, the separation compared weakly with those achieved by a stepwise or linear gradient elution on similar columns (Fisher and Kocsis; 1987; Gregory et al., 1989). Additionally, although the method was not harmful to the samples, some geometrical isomers and epoxides were not well resolved. This disadvantage limited the method's applicability in the biochemical investigation of carotenoids. As an increase of the surface area of column particles can improve partitioning between carotenoids (lipophilic materials) and C_{18} phase, better separation was expected on a C_{18} column of 5-μm particles (Figure 1). A total of 32 peaks were resolved in less than 35 min by using an isocratic solution system which provided a clear separation of carotenoids, carotenoid esters, and their geometrical isomers.

Rapid and advanced spectrum analysis provided by a rotating monochromator detector made easier the identification of carotenoid pigments. During analysis, chromatographic software acquired data covering the whole spectra range between 360 and 700 nm and provided a contour map, three-dimensional plot, and peak purity display. The contour plot is a pseudo-tridimensional representation of the absorbance, wavelength, and time data, for a complete separation profile. The chromatographic information summarized in the isogram assisted in obtaining a rapid preliminary conception of the major and minor constituents of the sample and aided in the selection of the optimal detection wavelength (438 nm), which is suitable for monitoring both the yellow and red xanthophylls simultaneously. A better spectrum analysis was found in the perspective display of three-dimesional data: absorption as a function of wavelength and time (Figure 2). The major carotenoids such as capsorubin, capsanthin, and β-carotene were easily distinguishable on the plot. However, geometrical isomers and epoxides of these pigments could not be differentiated from their origins according to such a spectrum analysis. The exact wavelengths of maximum absorption of these derivatives were recorded when a separate spectrum analysis of each peak was processed by the chromatographic software. The latter analysis was also performed on three different points of each peak to obtain the so-called peak purity display.

The result of qualitative analysis implied that ground paprika distributes capsorubin, capsanthin, antheroxanthin, mutatoxanthin, zeaxanthin, lutein, β-cryptoxanthin, capsorubin monoesters, capsanthin monoesters, β-carotene

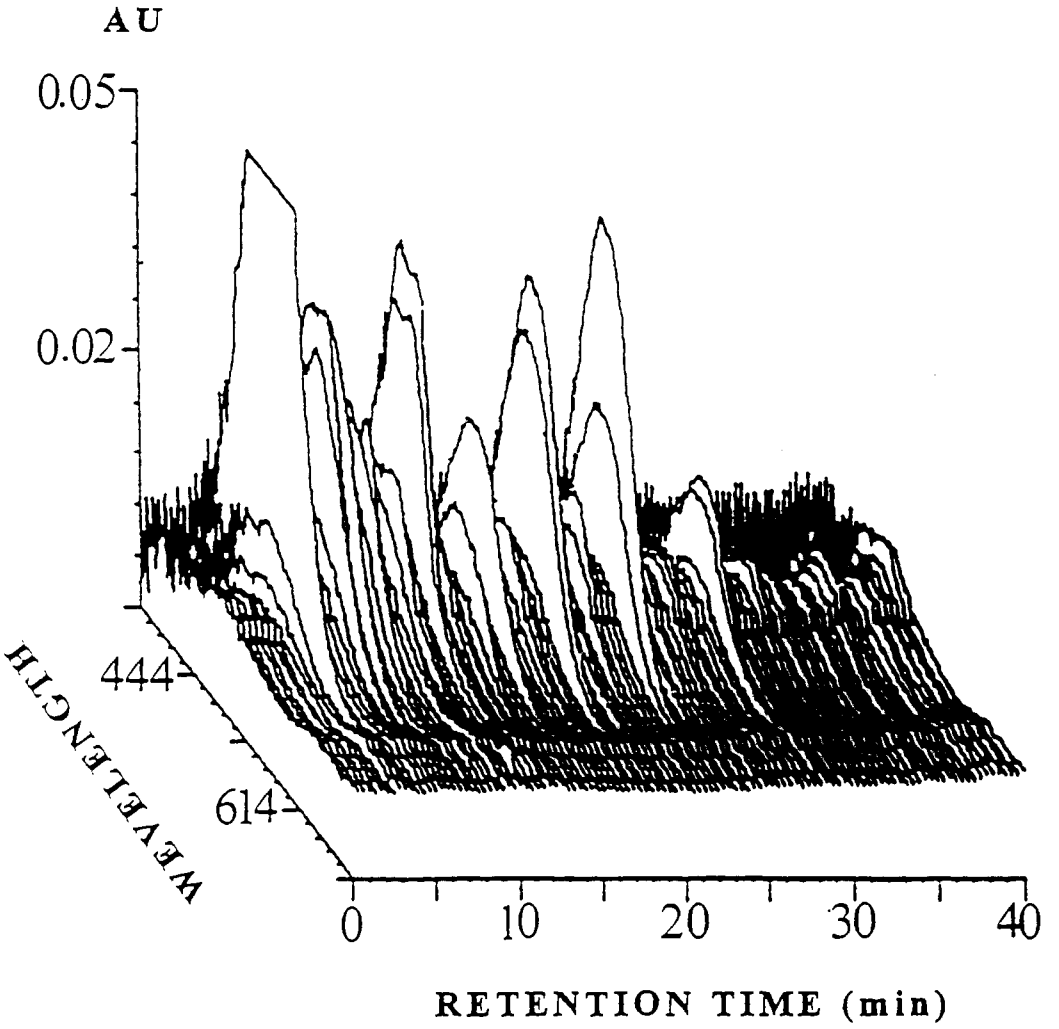

Figure 2. Three-dimensional plot of paprika carotenoids separated by HPLC method.

Table I. Concentration (Micrograms per Gram of Powder) of Red Xanthophylls in Different Paprika Cultivars and Cross-Cultivars

carotenoid[a]	Sz-20	Mihályteleki	Negral	F-1	F-5
free CA	8.1 ± 0.8	7.6 ± 0.5	6.5 ± 0.5	4.9 ± 0.2	6.4 ± 0.4
CA-ME	34.5 ± 4.3	26.9 ± 1.9	14.9 ± 0.6	18.1 ± 0.4	15.5 ± 2.6
CA-DE	32.7 ± 1.2	37.3 ± 1.7	6.7 ± 0.1	10.4 ± 1.5	21.4 ± 2.4
total CA	67.2 ± 5.7	64.2 ± 3.6	21.6 ± 0.7	28.5 ± 1.9	36.9 ± 5.0
free CR	0.8 ± 0.1	0.6 ± 0.1	1.0 ± 0.05	1.0 ± 1.0	0.8 ● 0.06
CR-ME	11.4 ± 1.1	6.4 ± 0.8	10.4 ± 0.4	12.9 ± 1.0	9.8 ± 1.7
CR-DE	8.4 ± 0.4	10.2 ± 1.6	1.7 ± 0.1	2.4 ± 0.1	6.6 ± 1.0
total CR	19.8 ± 1.5	16.6 ± 2.4	12.1 ± 0.5	15.3 ± 1.1	16.4 ± 2.7
total red	95.9	89.0	41.2	49.7	60.5
CA/CR					
free	10.1	12.7	6.5	4.9	8.0
total	3.39	3.86	1.78	1.86	2.25

[a] ME, monoester; DE, diesters; CA, capsanthin; CR, capsorubin. Total red includes epoxides and geometrical isomers of capsanthin and capsorubin.

epoxide β-carotene, neo-β-carotenes, capsorubin diesters, capsanthin diesters, zeaxanthin diesters, lutein diesters, and other unidentified components.

Carotenoids of New Cross-Cultivars. Table I shows the concentration of unesterified mono- and diesters of capsorubin and capsanthin from different cultivars. On the basis of red pigment content, two groups could be distinguished. The cultivars of the first group (Sz-20, MT) were characterized by their high red xanthophyll content, as well as by high capsanthin/capsorubin ratios. The second group, which involves Negral (Spanish round lilac) and its hybrids F-1 and F-5, had a lower red pigment content but a higher capsorubin level than did the first group. As the different cultivars contained very low quantities of free capsanthin and capsorubin, the principal differences between them seemed to be the fatty acid esters (mono- or diesters) of the major red xanthophylls. The result of the HPLC analysis revealed that cross-breeding of the Spanish cultivar (Negral) with the Hungarian red longum produced a hybrid of relatively higher red pigment content than that of the Spanish origin.

As the main component of red pigment in paprika, capsanthin attracts more attention, and its determination is, therefore, important. In general, its concentration in Hungarian cultivars was considerably higher than that in the Spanish one and its hybrids. A similar trend was observed for the total capsorubin.

With regard to esterification of the main red xantho-

Table II. Percentage of Capsanthin and Capsorubin in the Pigment Extract of Different Paprika Cultivars

cultivar	proportion,[a] % of total carotenoids	
	capsanthin	capsorubin
Szegedi-20 (Sz-20)	51.6 ± 2.0	15.8 ● 0.4
Mihályteleki (MT)	54.3 ± 2.2	17.3 ● 0.4
Negral	41.7 ± 0.9	22.1 ± 0.5
F-1 semideterminate	44.3 ± 3.0	19.4 ● 2.1
F-5 semideterminate	47.9 ± 1.2	15.8 ● 1.0

[a] Calculated from the integrated peak areas. The values represent means of three to four replicates ± standard deviation.

phylls with fatty acids, it was found that, unlike the Spanish cultivar, Hungarian cultivars contained low amounts of free (unesterified) and high amounts of fatty acid di- and monoesters of capsanthin and capsorubin. From the point of view of paprika quality, cultivars having high amounts of esterified xanthophylls exhibit better storeability (low color loss during storage) since esterification with fatty acids is advantageous for pigment stability (Biacs et al., 1987). It was of interest that the first generation (F-1) of the hybrids from the Hungarian and Spanish cultivars showed a carotenoid composition similar to that of the Spanish parent. It was very interesting that the fifth generation (F-5) of the same hybrid possessed a pigment composition similar to that of the Hungarian parent and higher color intesity (total red carotenoids) in comparison with the F-1 generation.

Another parameter of paprika quality is the capsanthin/capsorubin ratio. Since capsanthin contains fewer polar groups on its structure than capsorubin, it is more stable toward oxidative degradation (Biacs et al., 1987; Czinkotai et al., 1989). So the cultivars of high color stability are characterized by a high capsanthin/capsorubin ratio such as that found in the Hungarian red longum. An improvement of this ratio was achieved by the cross-breeding of Hungarian red cultivars from which the semideterminate cross was produced. It is very interesting that no significant difference was obtained in capsanthin/capsorubin total between Negral and F-1, but in the fifth generation this ratio increased. The same held true for both free and esterified forms of capsanthin and capsorubin.

Table II shows the percentage of capsanthin and capsorubin in the pigments extracted from different paprika powders. According to the percentage of capsanthin and capsorubin in the carotenoid extracts, the different cultivars showed significant variation. The red longum cultivars contained the highest level of capsanthin, whereas the highest level of capsorubin was found in the Spanish cultivar Negral and its F-1 hybrid, which exhibited the lowest percentage of capsanthin. The inverse correlation between capsanthin and capsorubin indicated that the differences between the cultivars examined are due to the varietal factors which may alter the pathway of carotenoid biosynthesis. Comparing the estimated values to those in the literature, the Hungarian red longum cultivars MT and Sz-20, which showed capsanthin percentages of 54.3 and 51.6, respectively, were similar to the Spanish Albarx M.CA and Americano, respectively (Almela et al., 1991). As for the lowest level, a 41% level recorded for the Negral cultivar was very close to the value reported for Negral, Amler/B51, and Amler-B51-E81 produced in Murcia, Spain (Almela et al., 1991).

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