

# Change in the Carotenoid and Antioxidant Content of Spice Red Pepper (Paprika) as a Function of Ripening and Some Technological Factors

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A study was conducted to investigate the change in quality attributes of red pepper (paprika) (*Capsicum annuum* L. var. Km-622) as a function of ripening and some technological factors. Of quality attributes, carotenoids and bioantioxidants (ascorbic acid and tocopherols) have been studied. It was found that the dynamics of fruit ripening with regard to carotenoids and bioantioxidants was influenced to a considerable extent by weather conditions of the production season. A rainy and cool season yielded fruits with more  $\beta$ -carotene but less diesters of red xanthophylls as compared to those produced in a relatively dry and warm season. The ripening stage at harvest was found to affect the quality of paprika. Harvest at unripe stages (color break or faint red) resulted in a high accumulation of dehydroascorbic acid in the overripe fruits, whereas de novo biosynthesis of carotenoids and tocopherols was partially retarded. Application of pre-drying centrifugation resulted in a marked loss of ascorbic acid, and as a consequence, carotenoid stability was impaired during the storage of ground paprika. Sugar caramelization caused dry pods and ground paprika to retain more pigments and tocopherol as compared to those from control or centrifuged red pepper samples. During the storage of ground paprika, color stability was improved by grinding the seeds with the pericarp.

**Keywords:** Red pepper; carotenoids; antioxidant; technology

## INTRODUCTION

An increasing interest is being paid to the spice red pepper (*Capsicum annuum* L.) (spice paprika) not only because of its economical importance but also because of its interesting composition. It is an excellent source of natural colors and vital micronutrients such as vitamins C and E (Somos, 1984), which have been confirmed by many epidemiological studies to reduce the risk of cancer and cardiovascular disease when taken daily in adequate amounts (Gey and Puska, 1989; Sies, 1991; Gerster, 1991).

Spice paprika has been subjected to quality control in Hungary for over 78 years. From the very beginning, this developed from visual inspection of color (color test), but once Benedek (1958) developed a method for the determination of pigment content, visual inspection of the color became the second parameter of grading. The Hungarian Standard (1983) for the first time specified spice paprika according to the pigment content. Several authors applied colorimetric and spectrophotometric methods in the testing of paprika (Drdák et al., 1980; Fekete et al., 1976; Horváth and Kafka, 1973; Haspel-Horatovic and Horickova, 1976; Malchev et al., 1982). However, colorimetry could not give detailed information on the compositional changes in red pepper as a function of ripening, processing, and storage.

The adventure and development of liquid chromatography opened up the possibility for more informative

data and better realization on the compositional change in the pigment of spice paprika. Using open column (Curl, 1962), thin-layer (Vinkler and Richter, 1972; Minguez-Mosquera et al., 1984), overpressure thin-layer (Acél, 1988), and recently high-performance liquid chromatography (Baranyai et al., 1982; Fischer and Kocsis, 1987; Gregory et al., 1987; Biacs et al., 1989; Almela et al., 1990; Minguez-Mosquera and Hornero-Méndez, 1993), the chemical nature, formation, and stability of paprika pigment have been examined in depth.

The effect of some varietal and technological factors on content and composition of pigment and other vital bioactive materials in spice paprika has been the subject of many recent investigations. In a study by Minguez-Mosquera et al. (1992), different Spanish varieties have been found to differ substantially in the red xanthophyll content and the ratio of red/yellow. The differences between varieties for the capsanthin/capsorubin ratio and the esterified carotenoid content have been reported to be significant (Biacs et al., 1993). Recently, varietal factors have been found to affect not only carotenoid but also bioactive compounds such as vitamins E and C that play an important role in the stability of the colored substances during ripeness, processing, and storage of red pepper (Biacs et al., 1992; Daood et al., 1996).

Technological factors most likely to affect the quality of paprika may include overripening, drying (time and temperature), milling (type, temperature, and powder fineness), and storage conditions. As overripening is indispensable for the accomplishment of carotenogenesis and the stabilization of pigment via esterification with fatty acids (Biacs et al., 1989; Minguez-Mosquera and Hornero-Méndez, 1994a; Daood et al., 1996), red pepper

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should be overripened for a few weeks either on the plant or in the stores after harvest to reach technological ripeness.

It is well-known that industrial operations cause a marked loss of quality components, particularly colored substances (Lease and Lease, 1956, 1962; Minguez-Mosquera and Hornero-Méndez, 1993, 1994b). Stability of the main carotenoids has been found to depend on drying conditions (Malachev et al., 1982). Storage of ground paprika would be affecting the rate of color degradation depending on temperature (Carbonell et al., 1986), residual moisture content of the product (Salwin, 1959; Lease and Lease, 1956), and increased surface area particularly in the presence of unsaturated lipids (Kannar et al., 1976, 1977).

In the present work, the change in the content of carotenoids, carotenoid esters, and bioantioxidants in red pepper as impacted by ripening conditions and some technological factors was investigated.

## MATERIALS AND METHODS

The seeds of spice red paprika (*Capsicum annum* L. var. Km-622) were obtained from the Red Pepper Developing Ltd., Kalocsa, Hungary. This variety is early, suitable for direct sowing in the field, of short growing time, and has high disease tolerance. The plants are semi-determinate in growth, yielding long fruits of high position (ascending) that makes it convenient for mechanical harvest. The Km-622 cultivar is characterized by concentrated ripening and a high content of red carotenoids, especially capsanthin. Analytical-grade organic solvents were from Reanal (Budapest) while all HPLC-grade solvents were from Merck. Standard  $\beta$ -carotene, capsanthin, tocopherols, ascorbic acid, and 1,2-phenylenediamine hydrochloride (OPDA-HCl) were purchased from Sigma (St. Louis, MO).

**Agricultural Conditions.** The soil of the experimental station (Kalocsa, Hungary) is a plowed layer that contains humus and  $\text{CaCO}_3$  in about 36% and 18.5%, respectively. To ensure a sufficient micronutrient supply in the whole experiment, 11 g/m<sup>2</sup> of N, P, and K were given every March. The seeds were sown (25 g/m<sup>2</sup>) between April 2 and April 10.

After overlaying the area, it was sprayed with Rideon 80 WP (0.7 g/m<sup>2</sup>) and then with 0.2% Zineb, Dithane, and Funduzol (1l/m<sup>2</sup>) as antimicrobial disease treatments followed by irrigation with water. In mid-May, the plants were twice given kemira Ferticare-1 nutrient solution (2 g/L).

Pesticides were applied when needed. Planting was carried out mechanically at the end of May with a plant density of 200–220 thousand plant/ha. The plants were irrigated 2 times more with 30 mm of water. The fruits were harvested between September 15 and September 20, 1995 and 1996.

**Technological Conditions.** After hand-harvesting, the pods were packaged in wood crates and stored at ambient conditions in an open area to undergo overripening (technological ripening) for 2–3 weeks.

The pods were then transferred to the drying unit of a paprika processing pilot plant. The drying unit consists of washing equipment, chopper, holed washing cylinder, centrifuge, reservoir, and drying equipment (cabinet) with conveying belt moving in six levels inside the drying cabinet. The temperature of the drying air was maintained between 80 and 90 °C for the upper four lines and between 30 and 40 °C for the lower two levels. Depending on the temperature used, the time required for getting the semi-final product (11–12% moisture) ranged between 5 and 7 h. Hot dry air in a counter current was used to dehydrate the paprika pods. The semi-final product was packaged in murky polyethylene sacs stored in dark stores until milling.

The milling process included coarse grinding with two hammer mills followed by fine milling using a stone mill at around 45 °C. Fine particles of 200–300  $\mu\text{m}$  were separated on mechanically shaken sieves while the coarse particles were

sent back to the stone mill. The final product (ground paprika) was packaged in polyethylene sacs and stored in the dark at 15–20 °C.

**Extraction of Carotenoids, Tocopherols, and Ascorbic Acid.** Lipids and fat-soluble pigments and tocopherols from raw spice paprika were extracted according to a previously described procedure (Daood et al., 1987) with slight modification. Five-gram samples from the pericarp of paprika pod were taken in triplicate (at least) and disintegrated in a crucible mortar in the presence of quartz sand. The water was removed by adding 25 mL of methanol with further disintegration of the aggregating bulk. The mixture was then transferred quantitatively to a 100 mL conical flask, and 70 mL of a 6:1 dichloroethane–methanol solution was added. The mixture was shaken for 15 min by a mechanical shaker. A few drops of distilled water should be added if the dichloroethane phase is not clearly separated from the polar phase (water + methanol). The two phases were separated by a separatory funnel, and the lower layer containing lipids in dichloroethane was dried over anhydrous sodium sulfate. Finally, the organic solvent was evaporated under vacuum by rotary evaporator at not higher than 40 °C. The residues were either redissolved in the HPLC eluent for analysis of carotenoids or saponified for analysis of tocopherols.

Ascorbic acid (AA) and its oxidized form, dehydroascorbic acid (DHAA), were extracted from 5 g of fresh pericarp or 0.5 g of powder of spice paprika by 100 mL of 4% metaphosphoric acid solution after disintegration in a mortar with quartz sand (in case of raw paprika). To stop enzymatic conversion of AA to DHAA, a small aliquot of metaphosphoric acid was added at the beginning of disintegration. The macerate was transferred to a 200 mL conical flask, shaken for 15 min, and filtered through filter paper (MN-640). The first turbid aliquot was discarded, and the clear extract was applied to the HPLC analysis.

**HPLC Equipments and Conditions.** To analyze carotenoid type compounds, a Beckman liquid chromatograph consisting of a model Gold System 116 M pump, a model 165 variable wavelength UV–visible detector, and a model 340 organizer equipped with a 20  $\mu\text{L}$  loop injector was used. The separation was performed on Chromsil-C18 (240  $\times$  4.6 mm i.d.) 6  $\mu\text{m}$  column using 2:1:1 acetonitrile–2-propanol–methanol as the mobile phase at a flow rate of 0.9–1.2 mL/min (Biacs and Daood, 1994). The column effluents were detected at 480 nm, and the signals were recorded by Shimadzu C-R3A integrator.

For tocopherol analysis, a combination of a Beckman 114M isocratic pump, a model RF-535 Shimadzu fluorometric detector, and a Waters-740 Data Module integrator was used. The separation was performed on Lichosorb 10  $\mu\text{m}$  (250  $\times$  4.6 mm i.d.) with a mobile phase consisting of 99.5:0.5 *n*-hexanes–ethanol. The fluorometric detector was set at 295 and 320 nm as the excitation and emission wavelength, respectively (Speek et al., 1985).

AA and DHAA were simultaneously determined by a procedure including ion pair chromatography separation (Daood et al., 1994) and postcolumn derivatization of DHAA to fluorescent compound by reacting it with 1,2 phenylene diamine hydrochloride (OPDA-HCl) according to Kacem et al. (1986). The analytical system that was used consisted of two isocratic pumps (Beckman model 114M), a UV–visible detector (Beckman model 165), a fluorometric detector (Shimadzu RF-535), and two integrators. The separation was performed on Spherisorb C-18, 10  $\mu\text{m}$  (250  $\times$  4.6 mm i.d.) column using 97:3:0.05 v/v/v 0.01 M potassium dihydrogen phosphate–methanol–tetrabutylammonium hydroxide (pH 2.85) at a flow rate of 1.0 mL/min. AA was detected at 244 nm. The stream from the UV detector was mixed in PTFE-Tee (Upchurch Scientific, Inc., Catalog No. (800) 426-0191) with that of the fluorogenic reagent (0.35% OPDA-HCl in water) at a flow rate of 0.6:0.3. The mixture passes a 3-m Teflon tubing of 0.4 mm heated to 70 °C in a column heater. The derivatized DHAA was monitored by fluorometric detector set at 350 and 430 nm as the excitation and emission wavelength, respectively (Kacem et al., 1986).

The peaks of individual carotenoids were identified by comparing their spectral features as measured by photodiode-array detector (Waters 996) with the spectral characteristics and retention times of pure (70% lutein and 95%  $\beta$ -carotene from Sigma, St. Louis, MO) and authentic standards (for fatty acid esters) prepared by thin-layer chromatography (Vinkler and Richter, 1972) before and after saponification of spice paprika extract. Some pigments were tentatively identified according to their spectral characteristics as compared with the literature data (Baurenfeind, 1981). We referred to a previous work (Biacs, 1989; Biacs and Daood, 1994) to characterize fatty acid esters of the major carotenoids. The yellow-colored carotenoids were quantified as  $\beta$ -carotene equivalent, while the red ones were quantified as capsanthin equivalent. To identify tocopherols, retention times were compared with those of standard materials ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherol, Sigma, St. Louis, MO), which were used also for quantitation.

Identification of AA in the samples was based on comparison of spectral characteristics and retention time with that of the standard. To prepare standard DHA, 100 mL of AA solution (50  $\mu$ g/mL in 4% metaphosphoric acid containing 8% acetic acid) was freshly oxidized by shaking with 1 g of activated charcoal for 10 s followed by filtration through filter paper. This solution is stable for more than 24 h.

## RESULTS AND DISCUSSION

**Dynamics of Ripening.** As stated by Biacs et al. (1989) and Minguez-Mosquera and Hornero-Méndez (1994a), there are five stages of biological ripeness of spice paprika. The climacteric ripeness starts in green fruits and proceeds through two color break stages toward the biological ripeness, which can be characterized by two stages: faint red and deep red. However, the last stage does not represent the technological ripeness at which red pepper can be processed. Usually, spice paprika needs to overripen 2–3 weeks before processing.

Table 1 shows the change in pigment composition of red pepper as a function of ripening of the Km-622 variety. The green pod distributes polar unesterified xanthophylls and  $\beta$ -carotene that are synthesized via a light-dependent chlorogenesis pathway together with the chlorophylls on the biomembranes of the chloroplasts. With the onset of ripeness there was an accumulation of brownish-red and later red pigment as a result of light-independent (de novo) synthesis of carotenoids. During the course of ripening, the content of the major xanthophylls, violaxanthin and lutein, in the chloroplasts tended to decrease; meanwhile, new xanthophylls were being formed and immediately esterified with fatty acids to form mono- and/or diesters. The same trend has been observed with another Hungarian variety, SZ-20 (Biacs et al., 1989) and two of Spanish varieties, Bola and Agridulce (Minguez-Mosquera and Hornero-Méndez, 1994a,b). An overall 24-fold increase in the carotenoid content was recorded in spice paprika as a result of the ripening process. Although xanthophylls intensively esterify with fatty acids during ripening, their unesterified forms increased from 161 to 1452  $\mu$ g/g dry matter. The dramatic change took place on the content of mono- and diesters, which changed from 0 in green fruit to 4.41 and 2.85 mg/g dry matter in ripe red fruits, respectively. Despite the fact that red and yellow xanthophylls in red pepper are synthesized via hydroxylation and epoxidation of  $\beta$ -carotene (Baurenfeind, 1981), the concentration of the latter increased 31 times during ripening. One should expect differences between different cultivars in the rate of esterification of caro-

**Table 1. Change in the Carotenoid Content ( $\mu$ g/g Dry Matter) of Red Pepper as a Function of Progressive Ripening during 1996<sup>a</sup>**

carotenoids	pigment stages				
	green	color break-1	color break-2	faint red	deep red
free xanthophylls					
capsorubin	-	-	tr	tr	tr
violaxanthin	72	113	197	198	220
capsanthin		273	571	473	621
lutein	89	178	295	179	381
cryptocapsin				89	230
monoesters					
capsorubin			116	189	655
violaxanthin					426
capsanthin			117	243	1106
auroxanthin		11	81	289	831
antheroxanthin					
cryptocapsin		7	33	187	543
cryptoxanthin			38	127	166
$\beta$ -carotene (all trans)	19	112	525	731	1120
diesters					
capsorubin-1		17	26	138	313
capsorubin-2			22	65	161
capsanthin-1			59	108	298
capsanthin-2		6	103	181	768
capsanthin-3		33	116	209	611
capsanthin-4					
violaxanthin-1		44	53	144	210
violaxanthin-2		35	36	134	152
lutein + zeaxanthin		38	73	174	328
unidentified	231	375	395	445	640
total carotenoids	410	1130	2930	4430	10160

<sup>a</sup> The values are the means of three replicate samples.

**Table 2. Main Meteorological Characteristics of Production Season of 1995 and 1996, Kalocsa, Hungary**

weather elements	month						av or total
	V	VI	VII	VIII	IX	X	V-X
temp ( $^{\circ}$ C) at 12:00							
50 yr av	16.70	20.00	22.20	21.10	17.10	11.30	18.06
1995	15.12	18.35	23.18	20.12	14.70	11.40	17.14
1996	17.75	20.29	19.22	20.02	12.32	11.10	16.78
sunshine (h)							
50 yr av	254.0	270.0	299.0	281.0	212.0	149.0	1465.0
1995	206.7	251.9	327.2	236.5	152.3	152.4	1327.0
1996	237.5	274.3	284.0	204.9	99.0	130.8	1230.6
rainfall (mm) monthly							
50 yr av	62.0	64.0	51.0	52.0	50.0	52.0	331.0
1995	61.9	104.2	23.1	59.5	115.0	4.8	368.5
1996	27.5	50.6	46.4	100.1	151.2	20.0	395.8

tenoids with fatty acid during biological or technological ripeness. Unfortunately, due to the unavailability of published analytical data on the individual mono- or diesters in red pepper during ripening, it is not possible to make a comparison between the used cultivar Km-622 and cultivars from other sources. The objective of our next work is to collect data on the rate of esterification of red xanthophylls in different red pepper cultivars from different countries including Hungary, Spain, and Bulgaria.

It seemed that conversion of  $\beta$ -carotene to esterified or non-esterified xanthophyll is influenced by environmental and varietal factors. As shown in Table 2, in the past few years there was a great alteration in the meteorological factors as compared to the average of 50 years. In August and September, the period during which red pepper ripens on plants, the sunshine and temperature were well below average, whereas rainfall

**Table 3. Carotenoid Content (mg/g Dry Matter) in a Fully Ripened Red Pepper Cultivated in Different Production Season under the Same Agricultural Conditions<sup>a</sup>**

carotenoids	production season	
	1995	1996
free xanthophylls	1.36 (16)	1.40 (14)
monoesters	2.76 (33)	4.41 (43)
diesters	3.34 (40)	2.85 (28)
$\beta$ -carotene	0.78 (9)	1.12 (11)
total carotenoids	8.45 (100)	10.16 (100)

<sup>a</sup> Values in parentheses represent % each fraction counters out in the carotenoid extract.

**Table 4. Bioantioxidant Content of Red Pepper Harvested at Different Stage of Ripeness in Different Seasons under the Same Agricultural Conditions<sup>a</sup>**

bioanti oxidants	ripening stages at harvest				
	green	color break-1	color break-2	faint red	deep red
ascorbic acid (mg/g dry matter)					
1995	12.4 $\pm$ 0.4	14.8 $\pm$ 0.2	10.5 $\pm$ 0.3	9.4 $\pm$ 0.2	7.5 $\pm$ 0.1
1996	13.9 $\pm$ 0.1	14.9 $\pm$ 0.4	12.9 $\pm$ 0.5	12.3 $\pm$ 0.3	10.2 $\pm$ 0.4
dehydroascorbic (mg/g dry matter)					
1995	nd	nd	nd	nd	nd
1996	3.5 $\pm$ 0.2	4.1 $\pm$ 0.3	2.8 $\pm$ 0.1	2.2 $\pm$ 0.1	1.4 $\pm$ 0.1
$\alpha$ -tocopherol ( $\mu$ g/g dry matter)					
1995	169 $\pm$ 13	480 $\pm$ 10	854 $\pm$ 17	920 $\pm$ 55	1085 $\pm$ 68
1996	340 $\pm$ 20	350 $\pm$ 24	430 $\pm$ 34	480 $\pm$ 43	1150 $\pm$ 92

<sup>a</sup> Values are means of three replicate samples  $\pm$  standard deviation. nd, not determined.

was well above the average of the last 50 years. Such a change in the weather elements has, of course, an effect on the dynamics of fruit ripeness and the quality of paprika. Data in Table 3 implied that although the fully ripe fruits harvested in 1995 (long sunshine period, low rainfall level, and high temperature) had less carotenoid, they distributed more diesters and less monoesters and  $\beta$ -carotene than those harvested in 1996 (short sunshine period, high rainfall level, and low temperature). These results probably reflect the fact that a cool, rainy season can disturb to a considerable extent the transformation of  $\beta$ -carotene to the subsequent xanthophylls and their further esterification with fatty acids to form diesters. Because diesters are more stable and have better lipophilic properties than carotenes, monoesters, and unesterified forms, it is appreciable and desirable that red pepper contains a high quantity of diesters even though the total carotenoid content is relatively low. Therefore, the 1995 ground paprika was characterized by high color stability during storage (data not shown) as compared to that of the 1996 paprika.

Of the quality attributes, bioantioxidant content is of high interest from the technological and nutritional points of view. As stated by Biacs et al. (1992), bioantioxidant content is strongly correlating with the color stability and retention in ground spice paprika. The naturally occurring antioxidants such as vitamins C and E play different roles during the ripeness processing and storage (Daood et al., 1996). Table 4 summarizes the result obtained from the HPLC analysis of vitamins C (AA and DHAA) and E ( $\alpha$ -tocopherol) during the ripening of spice paprika. Vitamin C tended to increase at color break-1 stage but then decreased gradually with the advanced ripening most probably due to its antioxidant role, which increases with the increasing respiration rate in the climacteric fruit. These results

**Table 5. Carotenoid and Bioantioxidant Content of Ground Paprika Produced from Red Pepper Fruits Harvested at Different Stages of Ripening and Overripened at Ambient Conditions (1996)<sup>a</sup>**

components	ripening stage at harvest		
	color break	faint red	deep red
carotenoids ( $\mu$ g/g dry matter)			
unesterified	498 $\pm$ 40	736 $\pm$ 37	1448 $\pm$ 58
monoesters	2789 $\pm$ 153	3593 $\pm$ 103	5147 $\pm$ 232
diesters	2248 $\pm$ 183	2372 $\pm$ 131	3517 $\pm$ 124
$\beta$ -carotene	1259 $\pm$ 113	2018 $\pm$ 101	1963 $\pm$ 108
total	6770 $\pm$ 474	8820 $\pm$ 450	12108 $\pm$ 775
antioxidant (mg/g dry matter)			
ascorbic acid	4.03 $\pm$ 0.21	4.08 $\pm$ 0.28	5.40 $\pm$ 0.26
dehydroascorbic acid	5.25 $\pm$ 0.30	5.30 $\pm$ 0.15	2.55 $\pm$ 0.05
$\alpha$ -tocopherol	1.01 $\pm$ 0.03	0.86 $\pm$ 0.02	0.75 $\pm$ 0.04

<sup>a</sup> The values are means of three replicate samples  $\pm$  standard deviation.

supported those reported by Biacs et al. (1992). In 1995, during the last stage of ripening (color break-2 and deep red), there was a loss of 7.3 mg/g dry matter for ascorbic acid. This magnitude is well above the 2.7 mg/g dry matter loss recorded in 1996 for the same cultivar cultivated in the same field and under the same agricultural conditions. The weather conditions were the only changing factors to which this variation may be attributed. Dehydroascorbic acid tended to change parallel to ascorbic acid, indicating that it is further broken down as a result of the ripening process.

The tendency of change in  $\alpha$ -tocopherol content was also found to be influenced by the weather conditions. In 1995, when the weather was favorable for growing and early ripening of red pepper,  $\alpha$ -tocopherol increased immediately after the onset of ripening to reach its maximum level at color break-2 or faint red stages. The state was different in 1996 when the weather was unfavorable (cool and rainy). The tocopherol content was slowly increasing during the first stages of ripening but dramatically increased at the last stage when the fruits became deep red. However, the concentration of  $\alpha$ -tocopherol in the dry matter of red pepper fruits harvested at the two different seasons was almost the same. In both seasons, no decrease was observed on the  $\alpha$ -tocopherol content of the ripening fruits where the water content was high, supporting the results reported by Biacs et al. (1992) and Daood et al. (1996). The low antioxidant activity of  $\alpha$ -tocopherols in aqueous media may be due to its low mobility in aggregated media and hydrogen bonding by water at phenolic or chroman ether group as stated by Iwatsuki et al. (1994) or to regeneration, by ascorbic acid, of oxidized tocopherol molecules as a function of synergism between them (Lambelet et al., 1985).

**Effect of Overripening.** Overripening is an indispensable step of spice paprika processing. Carotenoid biosynthesis continues to produce more red xanthophylls in a form of stable fatty acid mono- and diesters. The highest color capacity and stability should be approached that makes the raw material ready for industrial operations. In Hungary, the harvest of spice paprika should be ended before October every year to avoid early frost. To approach technological ripeness, the crop should be overripened in wood crates for 2–3 weeks at ambient conditions. The mechanically harvested spice paprika batches contain fruits at different stages of ripeness. All have to be overripened. Table 5 shows the antioxidant and carotenoid content of spice paprikas harvested at different ripeness stages and

**Table 6. Change in the Carotenoid Content ( $\mu\text{g/g}$  Dry Matter) of Ground Paprika as a Function of Ambient Storage and Seed Addition (1996)<sup>a</sup>**

carotenoids	treatments			
	before storage		after 3-month storage	
	pure pericarp	pericarp + 20% seeds	pure pericarp	pericarp + 20% seeds
free xanthophylls				
violaxanthin	56	53	79 (141)	80 (151)
capsanthin	104	114	74 (71.3)	85 (74.6)
lutein	231	170	74 (32.2)	140 (82.3)
cryptocapsin	98	65	75 (76.5)	79 (121)
cryptoxanthin	32	32	27 (84.3)	33 (103)
monoester				
capsorubin	354	256	81 (22.9)	121 (47.3)
violaxanthin	267	259	68 (25.5)	116 (44.7)
capsanthin	698	648	158 (22.6)	285 (44.0)
auroxanthin	227	205	42 (18.5)	80 (39.0)
antheroxanthin	531	456	130 (24.5)	242 (53.1)
cryptocapsin	423	377	44 (10.4)	96 (25.5)
$\beta$ -cryptoxanthin	281	175	43 (15.3)	103 (58.8)
$\beta$ -carotene (all trans)	1114	803	162 (14.5)	306 (38.1)
$\beta$ -carotene (cis)	211	135	38 (18.0)	97 (71.8)
diesters				
capsorubin-1	261	175	45 (17.2)	92 (52.6)
capsorubin-2	191	79	24 (12.6)	49 (62.0)
capsanthin-1	227	110	29 (12.8)	79 (71.8)
capsanthin-2	524	407	72 (13.7)	176 (43.2)
capsanthin-3	640	470	76 (11.8)	188 (40.0)
capsanthin-4	303	283	35 (11.6)	88 (31.1)
violaxanthin-1	177	158	22 (12.4)	72 (45.5)
violaxanthin-2	85	56	6 (7.1)	25 (44.6)
lutein + zeaxanthin	299	232	22 (7.4)	92 (39.7)
total carotenoids	7966	6275	1701 (21.4)	3110 (49.6)

<sup>a</sup> Values in parentheses are % retention of pigment by paprika powder after a 3-month storage. Number of replicate samples is 3–4. Percentage coefficient of variation (% CV) between replicate samples was 5.2–9.4%.

overripened in a well-aerated storage for 3 weeks. The overripe fruits harvested at color break and faint red stages contained a low level of carotenoids and AA and the highest level of DHAA and  $\alpha$ -tocopherol. It is of interest that harvesting at early ripeness stages promoted the formation of DHAA. When harvested at the full ripeness stage and subsequently overripened, the fruits distributed 1–2 times more free xanthophylls, monoesters, and diesters than those harvested at earlier ripeness stages (faint red fruits). On the other hand, no substantial difference was found in  $\beta$ -carotene content between paprikas harvested at the last stages of ripeness. This confirms that the early harvest leads to a high accumulation of free xanthophylls and  $\beta$ -carotene, while on-plant full ripeness caused any excess of  $\beta$ -carotene to be converted completely to mono- or diesters of red xanthophylls during overripening. The latter trend is essentially required to reach high color capacity and color stability during processing and storage.

**Effect of Seed Addition.** A part of this work was designed to clarify whether grinding the seeds together with the pericarp improves storage stability of paprika pigments. Dried pericarp alone and with 20% seeds was milled using the stone mill system of the Research Station for spice red pepper as described in Materials and Methods. The two samples, in triplicate, were packaged in nylon sacs and stored at ambient conditions in the laboratory for 3 months. Table 6 shows the data concerning the change in the carotenoid content of ground paprika as a function of seed addition and storage. Except for unesterified violaxanthin, capsan-

thin, and  $\beta$ -cryptoxanthin, all the separated carotenoids showed a marked decrease after seed addition as a result of dilution. The presence of carotenoids in the seeds (Minguez-Mosquera and Hornero-Méndez, 1993) as well as a preventive of seed antioxidants may explain why they were unchanged after seed addition.

From comparison of freshly prepared and 3-month-old stored samples, it was observed that with all pigments (except unesterified violaxanthin and auroxanthin) there was a loss of 15–90% and 0–74% in non-seed-containing and seed-containing samples, respectively, indicating the preventive effect provided by the seeds. The unexpected increase in the violaxanthin content (51%) of non-seed-containing samples and violaxanthin (41%) and cryptocapsin (21%) in seed-containing samples as a function of storage may be ascribed to the interference of degraded products of other carotenoids with these constituents in the HPLC separation.

Among monoesters, auroxanthin and cryptocapsin showed high sensitivity toward oxidative degradation. The ground paprika without seeds retained 10.4% and 18.5% while paprika with seeds retained 25.5% and 39.0% of cryptocapsin and auroxanthin, respectively. Other monoesters lost about 41–56% and 74–85% of their original content as a function of ambient storage of paprika with seeds and without seeds, respectively.

The highest magnitude of degradation was recorded with the diester fraction with violaxanthin and zeaxanthin + lutein diesters in non-seed-containing paprika and capsanthin diester 3 and 4 in seed-containing paprika being the most susceptible constituents, most probably due to the unsaturated fatty acid moiety of these esters (Biacs et al., 1989; Minguez-Mosquera and Hornero-Méndez, 1994a), which can easily be cooxidized in the course of lipid oxidation. The most stable diesters were those of capsorubin and capsanthin-1 followed by capsanthin diester-2 that contains saturated fatty acids. Concerning  $\beta$ -carotene, it was among the sensitive and labile components in both paprika samples. These results agree with those of Minguez-Mosquera and Hornero-Méndez (1994b), who investigated two different Spanish varieties.

From the above-mentioned results, it could be concluded that grinding the seeds can improve to a considerable extent the stability of carotenoid-type pigments in spice paprika during storage with unfavorable conditions. This is not in agreement with the conclusion of Czinkotai et al. (1989), who established that seed addition has no effect on storage stability of carotenoids in ground paprika stored for 6 months. This variation can be attributed to the fact that Czinkotai and co-workers used a coffee mill to prepare the samples, so the milling temperature was not rising to the degree at which seed oil can diffuse out and cover the colored particles in the form of a protective layer as usually happens in the stone and roller mills.

To investigate whether the protective effect of seeds on carotenoids is attributable to the  $\gamma$ -tocopherol content of the seed oil, a detailed study of the tocopherol composition of paprika seed was conducted (Table 7). As expected,  $\alpha$ - and  $\beta$ -tocopherol content decreased in seed-containing paprika as a result of dilution, whereas the high  $\gamma$ -tocopherol content of the seeds caused the level of this analogue to be 3 times higher. As a function of storage of paprika without seeds there was a loss of 59%, 23%, and 25% for  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherol, respec-

**Table 7. Content ( $\mu\text{g/g}$  Dry Base) and Storage Stability of Tocopherols in Ground Paprika as Affected by Seed Addition and Ambient Storage of 3 Months<sup>a</sup>**

tocopherols analogues	without seeds			with 20% seeds		
	before storage	after storage	change %	before storage	after storage	change %
$\alpha$ -tocopherol	697 $\pm$ 28	2889.5 $\pm$ 28	-59	534 $\pm$ 25	408 $\pm$ 34	-24
$\beta$ -tocopherol	13.7 $\pm$ 0.3	10.5 $\pm$ 0.6	-23	11.3 $\pm$ 0.5	10.6 $\pm$ 0.6	-6
$\gamma$ -tocopherol	11.6 $\pm$ 0.4	8.2 $\pm$ 0.3	-29	37.4 $\pm$ 1.7	33.2 $\pm$ 2.2	-11

<sup>a</sup> The values are means of three–four replicate samples  $\pm$  standard deviation.

**Table 8. Change in the Carotenoid Content ( $\mu\text{g/g}$  Dry Matter) of Ground Paprika as a Function of Different Industrial Operations and Refrigerated Storage of 6 Months<sup>a</sup>**

carotenoids	control		centrifuged		caramelized	
	0 time	6 months	0 time	6 months	0 time	6 months
free xanthophylls monoesters	704 $\pm$ 56	597 $\pm$ 24	467 $\pm$ 42	478 $\pm$ 38	595 $\pm$ 36	544 $\pm$ 38
$\beta$ -carotene	1293 $\pm$ 86	1112 $\pm$ 71	1190 $\pm$ 105	1101 $\pm$ 83	1445 $\pm$ 116	1139 $\pm$ 108
<i>cis</i> - $\beta$ -carotene diesters	638 $\pm$ 29	630 $\pm$ 32	608 $\pm$ 36	599 $\pm$ 53	664 $\pm$ 37	649 $\pm$ 40
	156 $\pm$ 11	139 $\pm$ 8	165 $\pm$ 11	94 $\pm$ 6	236 $\pm$ 23	182 $\pm$ 20
total	741 $\pm$ 62	779 $\pm$ 70	959 $\pm$ 77	823 $\pm$ 74	804 $\pm$ 56	775 $\pm$ 62
	3532 $\pm$ 233	3256 $\pm$ 260	3611 $\pm$ 325	3096 $\pm$ 341	3743 $\pm$ 301	3288 $\pm$ 293

<sup>a</sup> The values are means of three replicate samples  $\pm$  standard deviation.

**Table 9. Content and Storage Stability of Antioxidants in Ground Paprika as Affected by Predrying Centrifugation and during Drying Caramelization<sup>a</sup>**

antioxidant	samples and storage time (month)								
	control			centrifuged			caramelized		
	0	6	loss %	0	6	loss %	0	6	loss %
ascorbic acid (mg/g dry matter)	3.7 $\pm$ 0.2	2.6 $\pm$ 0.1	30	2.3 $\pm$ 0.2	2.3 $\pm$ 0.2	0	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	10
dehydroascorbic acid (mg/g dry matter)	1.6 $\pm$ 0.1	0.2 $\pm$ 0.02	87	0.9 $\pm$ 0.06	0.2 $\pm$ 0.01	78	0.6 $\pm$ 0.05	0.2 $\pm$ 0.04	67
tocopherols ( $\mu\text{g/g}$ dry matter)									
$\alpha$ -tocopherol	181 $\pm$ 10	144 $\pm$ 6	21	163 $\pm$ 2	164 $\pm$ 5	0	196 $\pm$ 9	153 $\pm$ 8	22
$\beta$ -tocopherol	10 $\pm$ 0.5	6.2 $\pm$ 0.2	38	12 $\pm$ 0.6	6.2 $\pm$ 0.2	48	10 $\pm$ 0.4	6.3 $\pm$ 0.3	37
$\gamma$ -tocopherol	30 $\pm$ 1.0	20 $\pm$ 1.5	33	43 $\pm$ 2.0	36 $\pm$ 4.0	16	44 $\pm$ 4.0	30 $\pm$ 1.0	32

<sup>a</sup> Values are means of three replicate samples  $\pm$  standard deviation.

tively, revealing the antioxidative activity of such fat-soluble antioxidants. Addition of seeds caused all tocopherol analogues to slightly change. This may be due to either the regeneration, by some reducing agents, of oxidized tocopherol or the presence of an unidentified antioxidant that can compete for lipid peroxy radicals much faster than can tocopherols.

**Effect of Centrifugation and Sugar Caramelization.** In Hungary, the industrial process of spice paprika may include centrifugation of sliced, washed, paprika pods as a normal practice to reduce water content and eventually to shorten drying time. This step was studied for its effect on pigment composition and bioantioxidant content of spice paprika. The effect of application of high drying temperature, that leads to sugar caramelization, on carotenoids and bioantioxidants was also investigated. Caramelization in spice paprika is usually occurring when technologically unripe (non-overripe) pods having a high sugar quantity are processed, particularly when the slicing and washing steps are not severe enough to remove excessive sugars. For drying freshly harvested paprikas (technologically unripe), high-temperature, long-time drying is indispensable, which in no way yields caramelized paprika products having deep brownish red color.

Table 8 shows the results obtained from HPLC analysis of carotenoids in paprikas prepared by different ways and refrigerated for 6 months. It is to be mentioned that all the ground paprika samples tested contained seeds up to 20%. As a consequence of water removal by centrifugation, the dry matter of centrifuged

paprika contained less yellow unesterified, monoesterified xanthophylls, and  $\beta$ -carotene than that of control sample. Since diesters are highly lipophilic, they were strongly retained by the tissues, and thus, their concentration in the dry matter increased because of the water-soluble materials that were removed by centrifugation. However, the total carotenoid content stayed unchanged.

In the case of caramelized powder, although the processing conditions were drastic enough to enhance great destruction of color, carotenoid content was not lower than that of the other samples. As a result of high-drying temperature, more *cis*- $\beta$ -carotene was found in the caramelized powder. Since  $\beta$ -carotene concentrations were very similar in the three samples, the high concentration of *cis*- $\beta$ -carotene in caramelized paprika is probably due to the stabilizing effect of the caramelization product that might retard further degradation of the *cis* form. The protective effect against thermal degradation can also be noticed on the major carotenoids such as mono- and diesters.

After a 6-month refrigerated storage, a gradual decrease in carotenoid content was recorded in the powders prepared from the three samples investigated in this work. The low rate of carotenoid decomposition will most likely be attributed to the preventive effect of seeds and to the low storage temperature ( $>15$  °C) at which color degradation takes place with reduced rate (Carbonell et al., 1986). The loss in yellow pigments was greater than that recorded for red ones, indicating their low stability during storage. Similar trend has been

observed by Minguez-Mosquera and Hornero-Méndez (1994b). The overall loss of carotenoid in the centrifuged, caramelized, and control paprika was 14%, 12%, and 8%, respectively, so one can conclude that in association with the reduced antioxidation potency of the semi-final product and high technology costs, the centrifugation of paprika pods after slicing and washing is not recommended to be used as a step in a short-time, relatively low-temperature practice in paprika drying. In addition, although caramelization in paprika was accompanied by good color retention during processing, it should be avoided because of the low antioxidation capacity of the caramelized paprika that makes it no longer tolerant during storage, particularly at ambient conditions. Shown in Table 9 is the change in the most effective antioxidants as a function of a 6-month refrigerated storage. The highest concentration of ascorbic acid was found in the control paprika followed by the centrifuged and caramelized samples. As being water-soluble, ascorbic acid lost almost 40% of its content as a result of centrifugation while caramelized paprika retained only 27% of the antioxidant. Concerning tocopherols, the fat-soluble antioxidant, the highest level was found in caramelized paprika, revealing that caramelization of sugar can protect fat-soluble materials including pigments and tocopherols against thermal destruction. The initial concentrations of natural antioxidants in the samples used in this experiment seemed to be well above their effective range to perform antioxidant function (Biacs et al., 1991) during storage. However, ascorbic acid performed effectively its antioxidant function only in the control paprika (30% loss). The 0% and 10% loss estimated for ascorbic acid in centrifuged and caramelized samples, respectively, after 6 months storage pointed to a very weak antioxidation potency. From the overall change of dehydroascorbic acid, it seems that this derivative is sensitive to the storage conditions; its interaction in the system of ground paprika reaches an equilibrium at concentration beyond 0.2 mg/g dry matter.

It was also of interest that  $\alpha$ -tocopherol in centrifuged paprika exhibited no antioxidant effect since a slight change in its concentration was recorded as a function of storage. The effective analogue of tocopherol in the powder of centrifuged paprika was  $\beta$ - and  $\gamma$ -, which showed 50% and 15% loss, respectively, during storage.

The different analogues of tocopherol showed a similar trend in their antioxidant function in the control and caramelized paprika. Therefore, it can be said that the high stability of carotenoid pigments during storage of the control paprika was associated with the high-antioxidant activity of both ascorbic acid and tocopherols. The hindrance of ascorbic acid activity as an antioxidant (by unknown factors) in the centrifuged and caramelized paprikas weakened their natural defense against oxidative damage even at refrigerated storage. Finally, it could be concluded that natural coordination between ascorbic acid and tocopherols is an important factor in keeping the quality of paprika at a high level, and in order to have paprika products with high color stability, the concentration of both antioxidants should be kept well above the critical limit below which the antioxidants are scarcely effective as first oxidation barriers against oxidation of carotenoids in ground paprika products as demonstrated previously (Biacs et al., 1991; Daood et al., 1996).

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