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Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors

Hussein G. Daood,[a]* M. Vinkler,[b] F. Márkus,[c] E. A. Hebshi[a] & P. A. Biacs[a]

[a]*Central Food Research Institute, H-1022, Herman Ottó u. 15, Budapest, Hungary*
[b]*Universitry of Horticulture and Food Industry, H-1115 Villányi út. 39, Budapest, Hungary*
[c]*Research Station of Paprika Development, Kalocsa, Hungary*

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A study was conducted to investigate [by high-performance liquid chromatography (HPLC)] the antioxidant vitamin content of paprika during ripening, processing and storage. The most biologically effective antioxidant vitamins, such as ascorbic acid, tocopherols and carotenoids, were separated, identified and quantitated in different samples.

The rate of *in vivo* synthesis of the three antioxidants increased after onset of the ripening process was induced in the climacteric fruits. As ripeness advanced, antioxidant vitamins tended to increase proportionally except that ascorbic acid reached a maximum level at the colour break-II stage and then declined. During drying and storage there was a dramatic decrease in the concentration of tocopherol and ascorbic acid as a result of active antioxidation performance, while carotenoid content decreased at a lower rate.

Application of a forced-air drying technique resulted in a significantly high retention of antioxidant vitamins by dried or ground paprika. The different cultivars showed significant differences in their antioxidant vitamin contents.
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INTRODUCTION

Recent epidemiological studies have associated the use of antioxidant vitamin supplements with substantial reductions in the risk of cancer and coronary heart diseases (Sies, 1991; Gey & Puska, 1989; Gerster, 1991). These compounds perform their function by counteracting the oxidizing effects on lipids by scavenging highly reactive oxygen free radicals, the major oxidizing factors for the oxidative modification of low density lipoprotein and nucleic acids.

Among the protective mechanisms against free radicals, antioxidant vitamins (vitamin E, vitamin C and β-carotene) are of special interest. As a result of the increased level of environmental pollution there is now an increasing risk of disturbance of the prooxidant–antioxidant balance in favour of the former in the human body (Sies, 1991). This has caused some organizations such as the US National Cancer Institute to recommend higher values of dietary intake and recommended dietary allowance (RDA) of antioxidant vitamins. The newly recommended increase of dietary intake of such vitamins corresponds to five or more servings of combinations of vegetables and fruits per day, which is much more than the previously estimated amounts for a healthy man (Lanchance, 1988; National Research Council, 1989).

It has been demonstrated that fresh or well-processed plant-derived foods (mainly fruits, vegetables and cereals) are the best sources of antioxidant vitamins. Numerous papers on the individual vitamins in plant products have been published. However, little information is available on the antioxidant vitamin content of spice red pepper (paprika) products which are very familiar ingredients of human diets in Hungary and many other countries.

The objective of this work was to study the change in the antioxidant vitamin content of paprika as a function of ripeness, drying and storage, and to evaluate the nutritive value of paprika products using recently developed HPLC methods.

MATERIALS AND METHODS

The fruits of different cultivars of spice paprika (*Capsicum annuum* L.) were obtained from the Research

*To whom correspondence should be addressed.

Station for Paprika Development (Kalocsa and Paprika and Food Processing Enterprise, Szeged, Hungary). Paprika fruits were harvested in the last week of September 1992 and 1993. To characterize dynamics of ripeness, fruits at various ripening stages (green, colour break-1, colour break-2, red and deep red) were taken and immediatly analyzed.

To study the effect of drying on the antioxidant vitamin content of paprika, the fruits were naturally dried under ambient conditions. At certain dates (Fig. 1) a batch of paprika was taken and the pericarp was cut into small pieces and dried by forced-air technique using an experimental cabinet dryer (Labor MIM Hungary) with variable conditions (air temperature and moisture). The air flow was at a superficial velocity of 2 m s^{-1}. Five kilogrammes of fresh or over-ripe paprika was dried by this method. For chemical analysis and the storage experiment, dried paprika was ground by a coffee mill to pass a sieve of 0.5 mm mesh.

Extraction methods

Lipid fractions including carotenoids and tocopherols from fresh and ground paprikas were extracted according to a previously described method (Biacs *et al.*, 1989, 1992) using methanol to remove the water after disintegration of fresh paprika. The pigments and tocopherol was then extracted by a mixture of 1:1 CCl_4–chloroform containing methanol up to 20%. A mixture of 2:1:1 chloroform–acetone–isopropanol was applied to extract lipid fractions from ground paprika samples.

For tocopherol analyses, the extracted lipid fraction was saponified by refluxing with 4 ml of 30% methanolic KOH for 40 min at the boiling point of methanol in the presence of 0.5 g ascorbic acid. Following cooling of the flask and addition of 20 ml salted water, the tocopherol homologues were extracted twice by shaking with 40 ml petroleum ether in a separating funnel. The ether fractions were collected, washed 3 times with 40 ml distilled water and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and the residues were redissolved in 5–10 ml of HPLC-grade hexane.

For ascorbic acid, aqueous extraction using 4% metaphosphoric acid solution was carried out and followed by centrifugation and filtration of the extract. In the case of fresh paprika, disintegration of the samples with quartz sand in a mortar before extraction is necessary (Daood *et al.*, 1994).

HPLC determination

A Beckman Liquid chromatograph was used, consisting of a Model 114 M isocratic pump, a Model 165 variable wavelength UV–visible detector and a Model 340 organizer equipped with a 20 μl loop injector. For tocopherol analysis, a Shimadzu fluorometric detector was used at 295 and 320 nm as the excitation and emission wavelengths, respectively. The signal was electronically

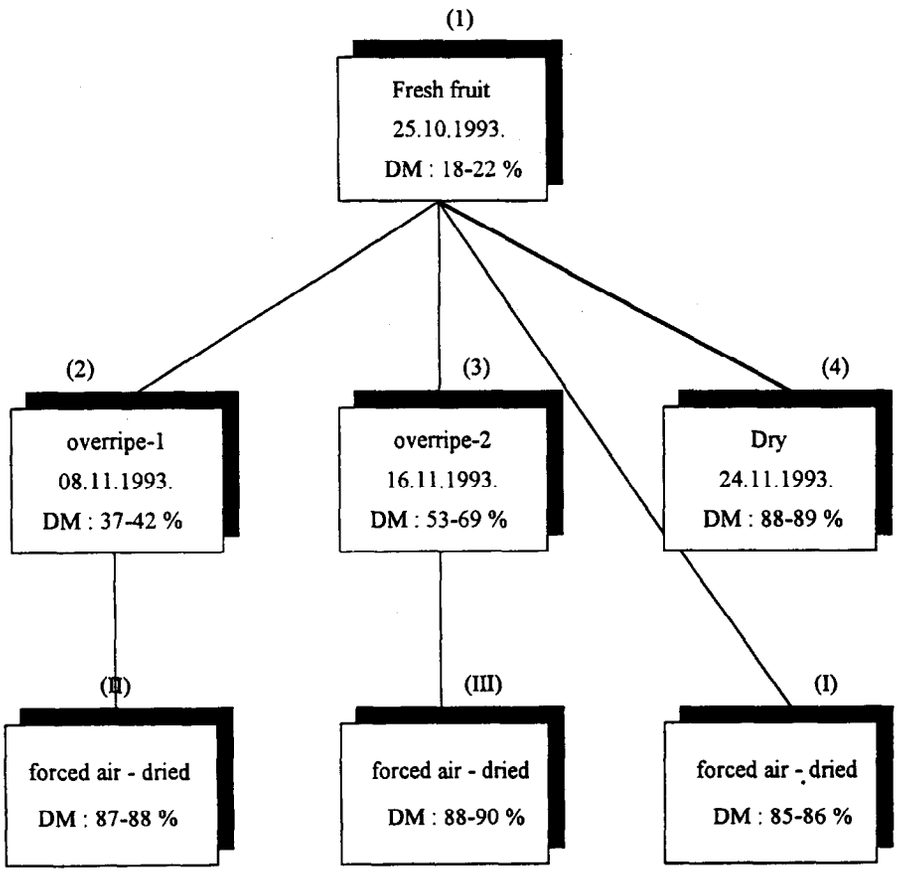

Fig. 1. Different stages of natural dryness (ambient) at which paprika was taken and dried by forced-air method.

Table 1. Parameters and conditions used for the separation of ascorbic, acid, tocopherol and carotenoids of paprika by HPLC techniques

Parameters	Conditions of separation		
	Ascorbic acid	Tocopherol	Carotinoid
Stationary phase	Lichosorb C_{18} 5 μm, 250×4.6 mm ID	Lichosorb 10 μm, 250×4.6 mm ID	Chromsil C_{18} 6 μm, 250×4.6 mm ID
Mobile phase	0.01 M KH_2PO_4–MeOH 97:3 containing 0.75 mM tetrabutylammonium hydroxide	*n*-hexane–ethanol 99:5:0.5	acetonitrile–2–propanol–methanol–water 39:52:5:4
Flow rate	1 ml/min	1.5 ml/min	1 ml/min
Detection	225 nm	ex = 290 nm em = 320 nm	440 nm
Reference	Daood *et al.* (1994)	Speek *et al.* (1985)	Biacs & Daood (1994)

integrated by a Shimadzu C-R3A or Waters-740 Data Module integrator. Conditions for HPLC separations are described in Table 1.

For peak identification the retention times and maximum absorption spectra of tocopherol, ascorbic acid and β-carotene were compared with those of standard materials (Sigma, USA) which were used also for quantification. To study the spectral characteristics of the antioxidant vitamins and to check peak purity, a Model Waters-990 photodiode-array detection system under the control of chromatographic software was used.

RESULTS AND DISCUSSION

HPLC analysis

Figure 2 shows the HPLC separation of tocopherol isomers extracted from spice paprika. The normal phase system consisting of silica gel, as the stationary phase and 99.5:0.5 hexane–ethanol as the mobile phase, provided a good separation of α-, β- and γ-tocopherols with their triene derivatives. Fluorescence detection allowed the monitoring of not only tocopherols but also ubiquinone, which are regarded as effective fat-soluble antioxidants. This could not be achieved by UV detection, which is well known to be substantially less sensitive than the fluorescence detection.

Table 2 shows the data of five measurements of antioxidant vitamins using the same fresh fruits or ground paprika. Values of 5.5, 5.1 and 2.8 of C.V. % for β-carotene, α-tocopherol and ascorbic acid, respectively, were obtained when fresh fruits were analyzed, whereas lower values were recorded for the same vitamins when determined in ground paprikas.

The second effective antioxidant vitamin in paprika is vitamin C (ascorbic acid). By ion-pair chromatography (Fig. 3) it could be separated from its oxidized form (dehydroascorbic acid) and other interfering materials. The peak purity display of the chromatographic software showed a high purity level of the estimated vitamin. Furthermore, interfering materials have maximum absorption wavelengths far from that used to monitor ascorbic acid (245 nm) and therefore, it can be said that the detection, by this method, of vitamin C is highly accurate. This is supported by the low C.V. % value obtained for ascorbic acid when several measurements were performed for the same raw or processed paprika samples (Table 2).

Because of the complexity of the carotenoid extract of paprika, it was difficult task to precisely estimate β-carotene by a simple, one-step analytical procedure. The Lipid Group of the Central Food Research Institute has developed a liquid chromatographic method that provides excellent isocratic separation of carotenoid and carotenoid esters from fruits and vegetables (Biacs &

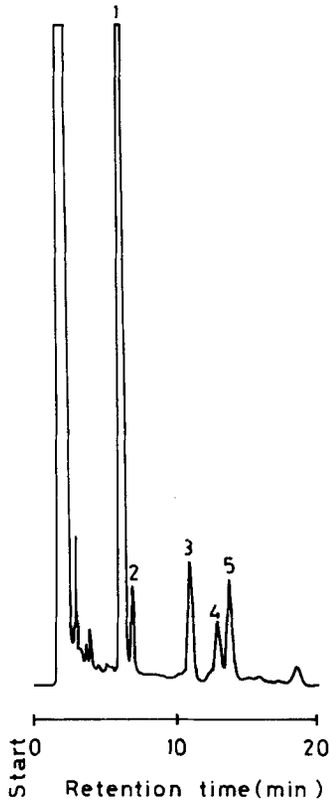

Fig. 2. HPLC separation of fat-soluble antioxidant from paprika: 1, α-tocopherol; 2, α-tocotriene; 3, ubiquinone; 4, β-tocopherol; 5, γ-tocopherol.

Table 2. HPLC determination of antioxidant vitamins in fresh and dried paprika samples (Mihályteleki cultivars, 1994, Soroksár, Budapest)

Samples	Concentration of antioxidant vitamins	
	Fresh paprika	Dried paprika
	β-carotene μg/g DM	
1	419	610
2	407	624
3	434	631
4	447	650
5	468	652
$\bar{X}$	435	633
SD	24	18
C.V.%	5.5	2.8
	α-tocopherol μg/g DM	
1	430	598
2	393	654
3	422	622
4	380	642
5	402	611
Mean $\bar{x}$	405	625
SD	20.6	23
C.V.%	5.1	3.7
	Ascorbic acid mg/g DM	
1	10.5	9.2
2	10.1	9.4
3	10.7	9.8
4	10.4	9.5
5	10.9	9.1
Mean $\bar{x}$	10.5	9.4
SD	0.3	0.27
C.V.%	2.8	2.9

C.V.% = per cent coefficient of variation.
$LSD_{5\%}$ = least significant difference at 5% probablity level.

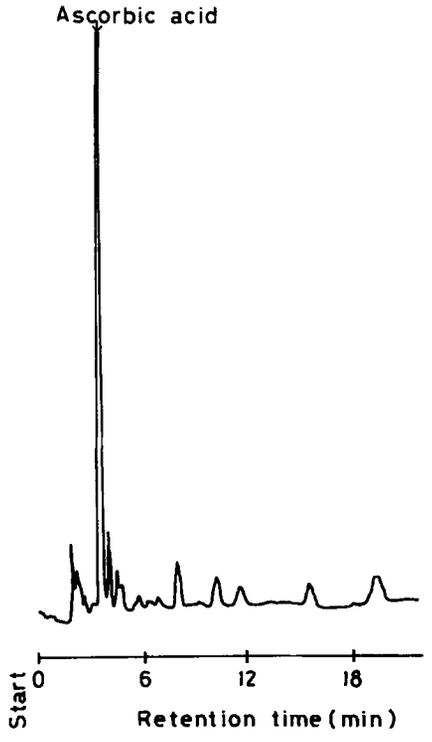

Fig. 3. HPLC profile of vitamin C and other organic acid extracted from paprika.

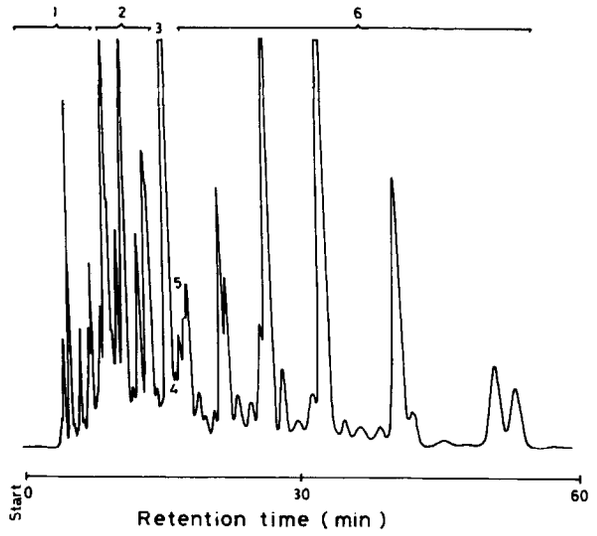

Fig. 4. HPLC separation of carotenoid and carotenoid esters under isocratic elution conditions: 1, free xanthophylls; 2, monoesters; 3, β-carotene; 4, monocis β-carotene; 5, polycis β-carotene; 6, diesters.

Daood, 1994). Shown in Fig. 4 is the HPLC separation of carotenoids from fresh, ripe paprika fruit. The method allows the qualitative and quantitative analysis of about 42 carotenoid compounds without saponification and that has been used by some authors (Matus *et al.*, 1981; Chandler & Schwartz, 1987; Almela *et al.*, 1991) to simplify the sample. It is especially important to avoid alkaline hydrolysis of fatty acid esters of carotenoids because this step is often carried out with artifact formation from the major and minor constituents of the pigment giving rise to a greater experimental error. Another advantage of the developed method is the separation of the *cis*-structure of β-carotene from its all-*trans* form, the biologically most important form of provitamin A. Similar separation has been achieved by other authors, only with gradient elutions (Matus *et al.*, 1991; Deli *et al.*, 1992).

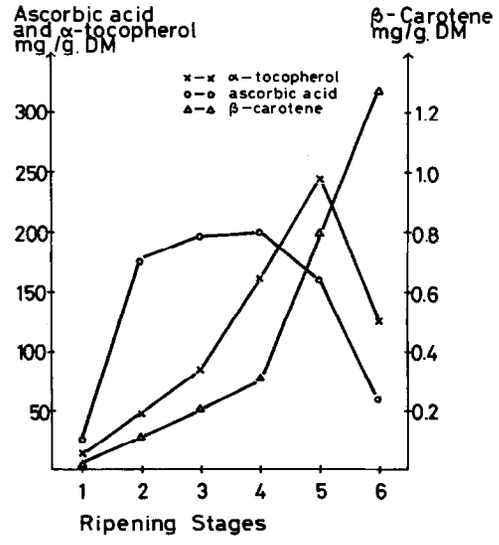

Fig. 5. Change in the antioxidant vitamin content of paprika during ripening stages.

Dynamics of ripening

Colour ripening of paprika fruit can be characterized by five stages. At the first stage (green fruits) small amounts of ascorbic acid, tocopherol and β-carotene could be estimated, with ascorbic acid being the main antioxidant vitamin. The concentration of β-carotene that is formed through light-dependent carotenoid biosynthesis in green tissues depends to a great extenf on the nature and photosynthetic activity of the chloroplasts. Higher levels of β-carotene have been found in leafy vegetables such as spinach and celery (Daood *et al.*, 1989).

Because of climacteric ripening of paprika fruit, ascorbic acid tended to increase markedly (Fig. 5) while slower increases were seen for tocopherol and β-carotene. During ripening of ascorbic acid there was a steady-state between stages 2 and 4 followed by a decline in its concentration at the post-ripeness (stage 6). When the fruit lost more than 50% of its water content and both lipophilic and hydrophilic systems were exposed to high oxidation stress, both ascorbic acid and tocopherol performed antioxidant functions. One of the most important ripening processes in climacteric fruits and vegetables is the induction of light-independent carotenoid biosynthesis which often occurs simultaneously with the destruction of the chloroplast system (chlorophyll destruction). The concentration of β-carotene, a major component of paprika pigment, increased gradually at the first stages and to a high level at the last stages of the progressive ripening. During the ripening and post-ripening stages no decrease was recorded in β-carotene content, revealing that it is well protected from oxidative degradation.

Effect of drying

A study by Carbonell *et al.* (1986) demonstrated that drying conditions can substantialy influence the quality attributes of paprika. It has been shown that the higher the drying temperature the greater the quality impairment in fresh or stored ground paprika (Malchev *et al.*, 1982). As reported by Carbonell *et al.* (1986), dehydration of paprika with ambient air (25°C) is advantageous for colour retention and hygroscopic properties of dried products, but no attention has been paid to the antioxidant vitamin content as affected by different drying conditions.

In the present work we compare natural drying with forced-air dehydration in an experimental cabinet dryer using ambient air with special respect to the antioxidant vitamin contents (vitamin C, vitamin E and β-carotene). Table 3 summarizes the data obtained for ascorbic acid, α-tocopherol and carotenoids in two cultivars dried differently. Retention of ascorbic acid and tocopherol by paprika was significantly lower in naturally dried (sample 4) than in forced air-dried paprikas (samples I, II and III). The fruit lost about 63% of its ascorbic acid content when naturally dried while losses of about 54 and 4.4% were recorded when freshly harvested and over-ripe fruits were dried by the forced-air method (samples I and II), respectively. The cultivar V-2 showed a similar ascorbic acid retention when the fruits were dried by the two different methods.

α-Tocopherol *in vivo* synthesis continued during natural drying, reaching its maximum concentration when the dry matter content of the fruit was between 53 and 68% (sample 3). A significant decrease was observed

Table 3. Bioantioxidant content of naturally and forced air-dried paprika samples, 1993

Samples*	Concentration mg/g DM			
	Ascorbic acid	α-tocopherol	β-carotene	Total carotenoids
		Km-622		
1	12.1	1.01	0.88	7.98
2	10.1	0.98	1.42	12.6
3	4.55	1.14	0.73	7.92
4	4.45	0.96	0.59	6.10
I	5.63	0.56	0.81	6.78
II	9.64	0.73	0.91	8.07
III	2.96	1.00	0.49	4.85
Mean $\bar{x}$	7.06	0.91	0.83	7.76
$LSD_{5\%}$	0.50	0.08	0.07	0.86
		K-V2		
1	10.2	1.25	0.45	6.02
2	9.62	1.18	0.66	7.46
3	8.04	1.07	0.52	6.53
4	3.18	0.75	0.34	4.05
I	5.14	0.49	0.47	4.30
II	9.22	0.70	0.74	6.67
III	6.85	0.68	0.36	4.14
Mean** $\bar{x}$	7.46	0.87	0.51	5.60
$LSD_{5\%}$	0.65	0.07	0.04	0.50

*Samples description is illustrated in Fig. 1.
**Three replications were made in this experiment.
$LSD_{5\%}$ = least significant difference at 5% probablity level.

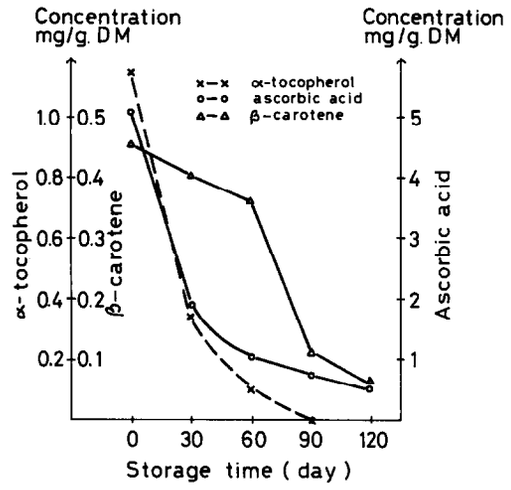

Fig. 6. Change in the antioxidant content of ground paprika during ambient storage.

only with fully dry fruits having a dry matter content of 89% (sample 4). With forced-air drying a marked deterioration of α-tocopherol was noticed, as a result of rapid oxidation, particulaly when fresh fruits were used as starting materials. The best retention of α-tocopherol was found in dried paprika obtained by drying over-ripe fruit having 53–68% dry matter (sample III). Values of per cent loss of 12.4 and 41.2 were estimated for Km-622 and V-2 cultivars, respectively, when their over-ripe fruits were dried by forced-air method. From these results it became evident that different cultivars show different responses to the drying process and accordingly it is very necessary to test the response to the forced-air drying conditions of most, if not all, of the economically important paprika.

The behaviour of β-carotene was somewhat different from that of ascorbic acid and tocopherol. In a previous work (Biacs *et al.*, 1989) carotenogenesis has been shown to continue at over-ripeness of paprika fruits giving rise to an intensive red colour that determines the technological ripeness of such vegetables. In accordance with the increased carotenoid accumulation, β-carotene increased 1.6 times as a result of the over-ripeness process. The oxidative damage was evident only at the subsequent stages towards full dryness.

It is of interest that dehydration of fresh fruits by the forced-air technique resulted in only 8% loss of β-carotene, while over-ripe fruits, when fully dried by the same technique, lost 53–56% of their β-carotene content. However, the β-carotene content of forced air-dried samples (I and II) was significantly higher than that of naturally dried paprika (sample 4). This agrees with the results of Carbonell *et al.* (1986) who found that rapid dehydration of paprika at 25°C is advantageous for colour retention and hygroscopic properties.

Another interesting observation is that with V-2 cultivar, forced-air drying of fresh and over-ripe (37% DM) paprika brought about an increase in the β-carotene content of the final products. To explain such behaviour further specific biochemical studies are required.

Storage of ground paprika

Spice paprika is usually ground to a powder of about 0.5 μm particulates. This increases to a great extent the surface area and thus enhances lipid oxidation several fold. The detrimental effect of lipid oxidation on the whole biological system can be eliminated if the natural antioxidants are available and able to perform their function. Figure 6 shows the change in α-tocopherol, ascorbic acid and β-carotene as a function of grinding and storage of ground paprika. Dramatic decrease was noticed in the concentration of both α-tocopherol and ascorbic acid, indicating that they act as the first oxidation barrier. Ground paprika lost 70, 90 and 100% of its α-tocopherol content after 30, 60 and 90 days of ambient storage, respectively. Ascorbic acid content of the same sample decreased to 35, 20 and 10% of the original level after a storage of 30, 60 and 120 days, respectively. β-Carotene, slightly decreased during the first storage period (2 months) but dramatically shifted to low levels during the second half of the storage when the concentrations of α-tocopherol and ascorbic acid were too low to be effective in the anti-oxidation process. The behaviour of antioxidant vitamins and β-carotene during storage of finely ground paprika was similar to that observed in blood samples by Esterbauer (1991) who stated that vitamin E provides a first oxidation barrier, while ascorbic acid is needed for its regeneration and the carotenoids function as a second oxidation barrier against lipid oxidation. The role of β-carotene as a single oxygen quencher in food systems (Yung & Min, 1991) and free radical trapper in biological systems of low oxygen pressure (Burton & Ingold, 1992) has been well demonstrated.

Effect of varietal factor

Table 4 shows the ascorbic acid, tocopherol and β-carotene contents of the different cultivars at over-ripeness-1 stage (37–41% DM). The highest value with regard to β-carotene was obtained with SZ-80, semi-determinate 7/92, K-801 and Km-622. The lowest values were recorded for the pungent K-V2 and K-90 and most of the Szegedi cultivars. The level of β-carotene found in the Spanish cultivar Negral (0.9 mg/g DM) is lower than that reported by Almela *et al.* (1991). This variation (about 0.2 mg/g DM) is probably owing to environmental and agricultural factors.

Based on tocopherol content the examined cultivars can be divided into three groups: (1) of high tocopherol content (Semi-determinate 7/92, K-90, K-V2, Km-622, K-50); (2) of medium tocopherol content (K-801, SZ-20, Strain-100, SZ-80, Mihályteleki); (3) of low tocopherol content (Bibor, Napfény, Negral). Concentration of tocopherol varied between 2.97 and 0.89 in Semi-determinate 7/92 and Negral, respectively.

Significant differences were also found between the different cultivars in ascorbic acid content. The highest

Table 4. Bioantioxidant content of paprika fruit (over-ripe-1) from different cultivars

Cultivars	Concentration mg/g DM		
	β-Carotene	α-Tocopherol	Ascorbic acid
	Cultivated in Kalocsa, 1992		
K-50	1.36	1.80	3.19
Km-622	1.41	1.92	4.79
K-801	1.58	1.61	4.37
Semi-determinate 7/92	1.64	2.97	3.57
SZ-80	1.96	1.39	3.37
K-V2	0.73	2.05	5.17
K-90	0.76	2.65	1.25
Strain-100	0.81	1.41	4.18
Mean $\bar{x}$	1.28	1.97	3.74
$LSD_{5\%}$	0.10	0.15	0.62
	Cultivated in Szeged, 1993		
Mihályteleki	0.62	1.33	3.61
SZ-20	0.71	1.52	3.52
Bibor	0.73	0.95	7.20
Napfény	0.56	0.90	7.18
Negral	0.93	0.87	5.46
Mean $\bar{x}$	0.71	1.11	5.39
$LSD_{5\%}$	0.07	0.12	0.84

Number of replicate samples used for each cultivar was four. $LSD_{5\%}$ = least significant difference at 5% probablity level.

concentrations were 7.18 and 2.20 mg/g DM in Napfény and Bibor, respectively, while the lowest value was obtained with the pungent K-90 (1.25 mg/g DM). Ascorbic acid is often regarded as a limiting factor along with red pigments in the evaluation of paprika quality. Accordingly, Bibor and Napfény have already been recognized as good new cultivars, and are to be introduced for commercial production of paprika products in the Szegedi Paprika and Food Processing Enterprise. In the Kalocsa factories, there is more interest in K-V2 than K-90 as a good pungent cultivar owing to the high ascorbic acid content of the former (5.17 mg/g DM) even though it contains less α-tocopherol.

It is appreciated that the Kalocsa Paprika Enterprise produces paprika products (powder, paste, oleoresin) from a mixture of cultivars having outstanding characteristics (e.g. Km-622, K-801, Semi-determinate 7/92,SZ-20). These cultivars have not only the best colouring capacity but also the best bioantioxidant potency.

In conclusion, it is worthy mentioning that 1 g of freshly ground paprika can provide 1180–2130 IU of vitamin A (1 IU = 0.6 μg β-carotene according to NAS–NRC, 1980), 1.0–1.7 mg of vitamin E and 3.2–4.5 mg of vitamin C. These values are high enough to make spice paprika of particular importance as a vital food ingredient that can easily and practically compensate dietary bioantioxidant vitamins, the essentially required micronutrients for reducing the incidence of cancer and cardiovascular diseases (Moon & Micozzi, 1988; Simon, 1992).

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