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Effect of reduced glutathione on the stability of pigments in paprika powders studied by multiwavelength spectrometry and high-performance liquid chromatography

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Abstract

Multiwavelength spectrometry and reversed-phase high-performance liquid chromatography have been employed for the study of the effect of reduced glutathione and storage time on the pigments of paprika powders. The evaluation of the data by principal component analysis and cluster analysis proved that the storage time exerts the highest effect of the decomposition rate of pigments. The stability of some pigment fractions was modified in the presence of reduced glutathione. However, the effect was of secondary importance. Multiwavelength spectrometry combined with HPLC can be successfully used for the study of the stability of pigments of paprika powder.

Keywords: Food analysis; Principal component analysis; Pigments; Glutathione

1. Introduction

Pigments in foods and food products exert a considerable impact on the marketability, on the consumer acceptance and show marked biological activity too [1–3]. Spectrophotometric methods are excellent tools for the measurement of the total amount of pigments [4,5]; however, the composition of pigments cannot be established [6]. Liquid chromatographic methods such as thin-layer and high-performance liquid chromatography (HPLC) have been extensively applied for the separation and quantitative determination of pigments [7]. HPLC methods have been used for for the determination of anthocyanins [8], catechins and flavonol glucosides [9], carotenoids [10], etc. The majority of HPLC techniques use reversed-phase separation mode [11]; however, size-exclusion chromatography [12,13] have also been found application in the HPLC analysis of pigments.

Carotenoids have been determined by HPLC in vegetables [14,15], green leafy vegetables [16], vegetable juices [17], thermally processed tomato-based food products [18], carrots [19], plant tissues [20] and paprika [21,22]. Because of the high number of fractions the identification of the individual carotenoids is difficult in HPLC. Authentic standards are available only for a limited number of unsaponified pigments, and these standards are liable to oxidative decomposition. As the spectra of pig-

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ments are similar the indentification of pigments according to their spectra is cumbersome and inaccurate.

The objectives of the study were the determination of the effect of reduced glutathione (GLT) on the stability of pigments in paprika (*Capsicum annuum*) powders using multiwavelength spectrometry and reversed-phase HPLC and the evaluation of the data by various multivariate mathematical–statistical methods. The use of GLT was motivated by the assumption that GLT being a strong and natural antioxidant may protect the pigments against bleaching caused by oxidative processes.

2. Materials and methods

Samples of paprika powder containing 0, 5, 7.5 and 10% (w/w) reduced glutathione (GLT) were prepared and stored at ambient temperature (22±1°C) in diffuse light in Petri dishes covered by glass plates. Paprika powder without GLT was stored at 1°C in dark and served as control. Aliquots of samples (1 g) were taken after 14 and 28 days, the pigments were exhaustively extracted by acetone, the combined acetone extracts were concentrated to 3 ml and used for further analyses after appropriate dilutions. The absorption of the pigment extracts was measured at 395, 410, 425, 440, 455, 470, 485 and 500 nm wavelengths. HPLC measurements were carried out with a Waters LC Module I high-performance liquid chromatograph system with 20 μl sample loop and a Waters 746 Data Module integrator (Waters-Millipore, Milford, MA, USA). A Spherisorb ODS (150×4 mm I.D., 5 μm particle size) column was used for the separation. The mobile phase consisted of a mixture of methanol–acetonitrile (80:20) (solvent A) and bidistilled water (solvent B). Gradient elution started with 80% A changing to 90% A in 10 min, to 95% A in 5 min, to 97% A in 2 min, final hold. The flow-rate was 2 ml/min, pigments were detected at 400 nm. Authentic pure capsanthin, zeaxanthin and β-carotene served as standards.

The results of multiwavelength spectrometry were evaluated with principal component analysis (PCA) [23]. The dimensionality of the matrices of PC loadings and variables was reduced to two by the nonlinear mapping technique [24].

HPLC data were assessed by cluster analysis. The absolute peak area of eight pigment fractions (including the peaks of capsanthin, zeaxanthin and β-carotene) formed the original data matrix. In order to find the differences between the treatments and storage times cluster analysis has also been carried out on the transverse of the original data matrix.

3. Results and discussion

Multiwavelength spectrometry established that the concentration of pigments decreased during storage even in samples containing the highest concentration of GLT; however, it modified the decomposition rate of individual pigment fractions. The results of PCA clearly show that three background (theoretical) components explain the overwhelming majority of variance represented by the eight original variables only with 2.01% loss of information (Table 1). Except for 500 nm, the other wavelengths have high loadings in the first PC indicating their similarity. Wavelengths 395–485 form a distinct cluster on the two-dimensional nonlinear map of PC loadings while the point of 500 nm is far from the others (Fig. 1). This results suggests that the determination of absorbance at 500 nm and one other wavelength between 395 and 485 practically contains the same information as the measurement of all eight wavelengths. The differences among the spectral characteristics of samples were higher after 28 than after 14 days of storage (Fig. 2). The separation of clusters of 14 and 28 days of storage indicates the decisive role of storage time in the decomposition rate of pigments.

Typical chromatograms are shown in Fig. 3. Peaks numbered have been used for further calculations. Pigments are well separated in the HPLC system indicating that this method is suitable for the separation of saponified and unsaponified carotenoids extracted from paprika powder. Similarly to the results of multi-wavelength spectrometry the chromatograms also prove the decomposition of pigments during storage and the differences in the decomposition rates. The retention time of eight characteristic peaks and the inter-day reproducibility (RSD) are

Table 1
Similarities and dissimilarities of the effect of various treatments and storage time on the amount of pigments in a paprika powders

No. of principal components	Eigenvalues	Variance explained (%)	Total variance explained (%)
1	5.95	74.34	74.34
2	1.40	17.51	91.84
3	0.49	6.14	97.99
Principal component	Principal components		
loadings	1	2	3
395 nm	*0.87*	−0.40	0.26
410 nm	*0.90*	−0.36	0.24
425 nm	*0.92*	−0.15	0.17
440 nm	*0.99*	0.01	0.01
455 nm	*0.89*	0.38	−0.21
470 nm	*0.93*	0.29	−0.19
485 nm	*0.93*	0.29	−0.20
500 nm	−0.10	*0.88*	0.47

Results of principal component analysis.

compiled in Table 2. The data prove the good stability of HPLC system even after 28 days and indicate that the reproducibility is lower at higher retention times. The RSD values of peak area showed higher deviations (3.4–7.2%) probably due to the fact that baseline separation of peaks has not been achieved for each pigments fraction increasing the error of integration.

Cluster analysis of HPLC results entirely supported the conclusions drawn from the spectrometric data concerning the decisive role of time in the decomposition rate of pigments. Taking into consid-

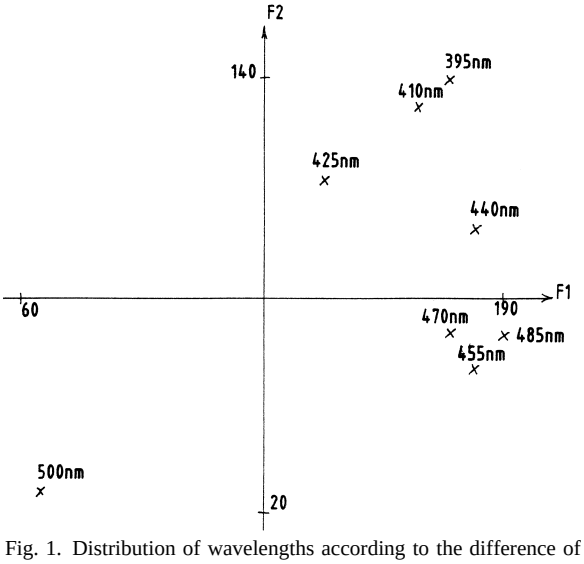

Fig. 1. Distribution of wavelengths according to the difference of their information content. Two-dimensional nonlinear map of principal component loadings. Number of iterations, 70; maximum error, $4.32 \cdot 10^{-3}$.

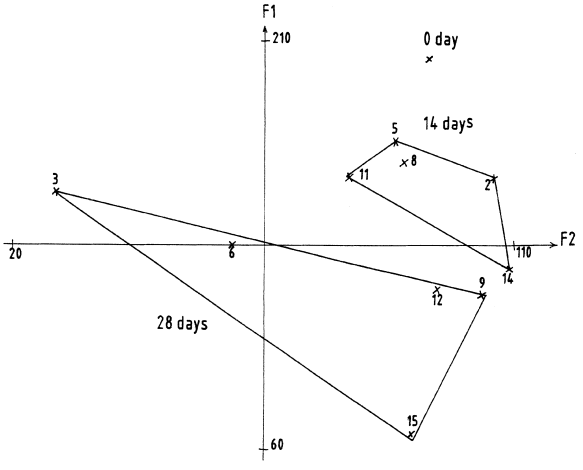

Fig. 2. Similarities and dissimilarities among the treatments and storage times. Two-dimensional nonlinear map of principal component variables. Number of iterations, 426; maximum error, $4.52 \cdot 10^{-3}$. (2) 0% GLT, 14 days; (3) 0% GLT, 28 days; (5) 5% GLT, 14 days; (6) 5% GLT, 28 days; (8) 7.5% GLT, 14 days; (11) 10% GLT, 14 days; (12) 10% GLT, 28 days; (14) 0% GLT, 14 days; (15) 0% GLT, 28 days. GLT, reduced glutathione.

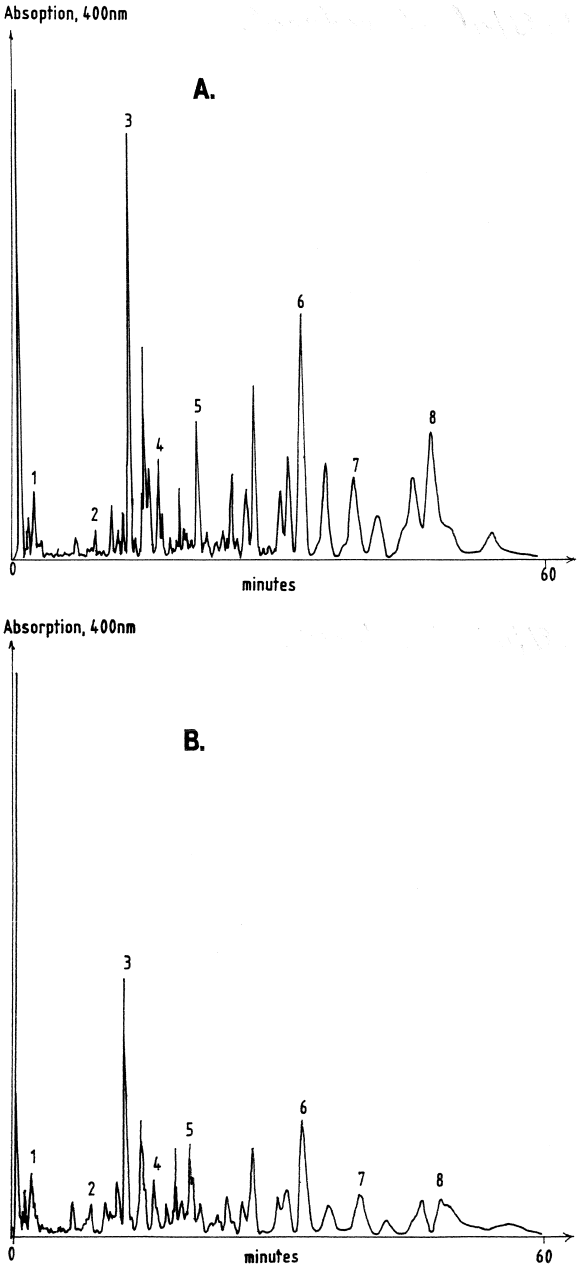

Fig. 3. Reversed-phase HPLC chromatograms of color pigments of paprika powder. (A) Control 0% GLT, 0 day; (B) 10% GLT, 28 days. Peak identification: (3) capsanthin; (4) zeaxanthin; (8) β-carotene; other peaks are unidentified.

Table 2
Retention time and relative standard deviations of inter-day reproducibility (RSD) of eight characteristic pigment fractions (28 days)

No. of pigment fraction	Retention time	
	Mean (min)	RSD (%)
1	2.48	1.00
2	8.67	1.03
3 (capsanthin)	13.18	1.76
4 (zeaxanthin)	16.50	1.39
5	20.68	0.90
6	33.70	2.90
7	40.05	3.30
8 (β-carotene)	49.52	3.19

eration simultaneously the peak areas of eight selected pigment fractions, the points are clustered according to the storage time and not according to the type of treatment (Fig. 4).

The differences among pigment fractions on their cluster dendrogram proves again that GLT modifies the decomposition rate of the individual pigment fraction differently (Fig. 5).

It can be concluded from the data that multi-wavelength spectrometry and the reversed-phase HPLC technique combined with gradient elution can be successfully used for the study of the stability of pigments of paprika powder. The decomposition rate mainly depends on the time of storage, the presence of reduced glutathione is of secondary importance.

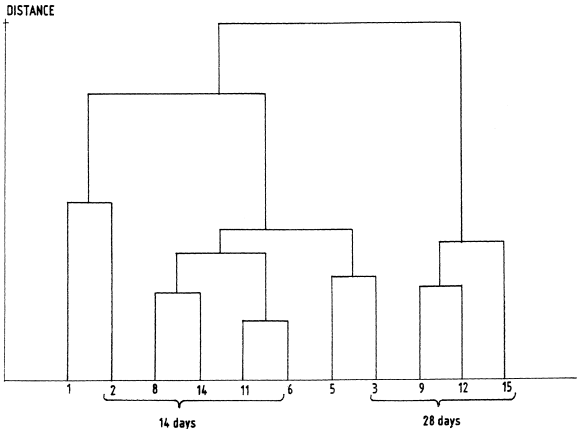

Fig. 4. Cluster dendrogram of samples taking into consideration simultaneously the peak areas of eight selected pigment fractions. Numbers refer to treatments in Fig. 2.

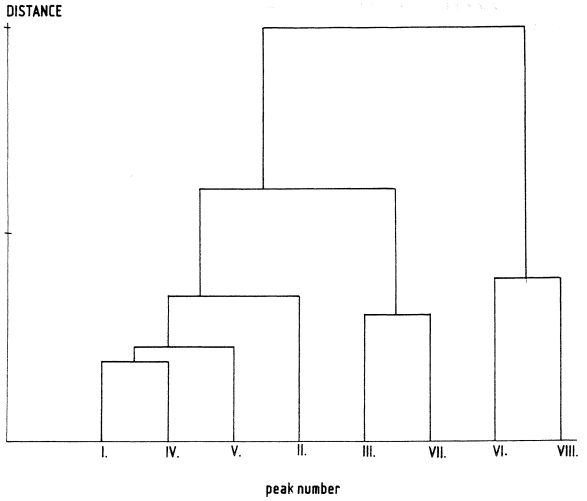

Fig. 5. Cluster dendrogram of pigments fractions taking into consideration simultaneously each sample. Numbers refer to pigment fractions in Fig. 3.

GLT only modifies the decomposition of some pigment fractions resulting in different spectral characteristics and chromatographic profiles.

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