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Effects of fluorescent light and vacuum packaging on the rate of decomposition of pigments in paprika (*Capsicum annuum*) powder determined by reversed-phase high-performance liquid chromatography

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Abstract

The effect of storage time, the presence of light and oxygen on the decomposition rate of carotenoid pigments in paprika (*Capsicum annuum*) powders was determined by reversed-phase high-performance liquid chromatography (RP-HPLC). The similarities and dissimilarities of pigment composition of samples under various storage conditions was elucidated by principal component analysis (PCA) and stepwise regression analysis (SRA). Calculations proved that the overall decomposition rate of pigment sections equally depended on the storage time and on the presence of light and oxygen, the effect of storage time being the most decisive factor while the impact of oxygen was the lowest. The selectivity of decomposition also depended on the storage time and on the presence of oxygen the influence of storage time being the most important. RP-HPLC followed by PCA and SRA can be successfully used for the study of the impact of environmental conditions on the decomposition of carotenoid pigments of paprika powders.

Keywords: *Capsicum annuum*; Food analysis; Carotenoids; Pigments

1. Introduction

The quantity and quality of pigments exert a considerable influence on the commercial value and consumer acceptance of various foodstuff and food products. The quantity of colour pigments can be exactly determined by various spectrophotometric methods either without extraction in the instance of liquid products or after extraction of pigments from solid matrices. Although these spectrophotometric methods expose high repeatability and reproducibility and can be successfully used for routine analytical purposes, they are not suitable for the differentiation of the individual pigment fractions [1,2].

Because its high selectivity and sensitivity, high-performance liquid chromatography (HPLC) has been extensively employed for the separation and quantitative determination of pigments in plants, foods and food products. Earlier results have been previously reviewed [3]. Because of the considerable commercial importance much effort has been devoted to the application of various chromatographic techniques for the separation of carotenoid pigments of paprika (*Capsicum annuum*). These studies have

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been motivated by the finding that the chemical structures of the pigments (degree of esterification) is related to their higher stability [4,5]. The chromatographic determination of carotenoids in foods has been recently reviewed [6].

Principal component analysis (PCA) has been developed for the evaluation of large data matrices [7]. PCA calculates the similarities and dissimilarities between the columns and rows of a data matrix of any dimension without one column or one row being the dependent variable. Although PCA reduces the dimensionality of the original data matrix the resulting matrices of principal component (PC) loadings and PC variables are sometimes even multidimensional making the visual evaluation of the results difficult. In order to facilitate the evaluation the dimensions of the matrices of PC loadings and variables can be reduced either to two by a nonlinear mapping technique (NLMAP) [8] or to one by cluster analysis [9]. Because of its simplicity PCA has been frequently used in food science and technology for the classification of foodstuffs according to their chemical composition [10–13]. The disadvantage of PCA is that it does not define the theoretical (background) variables as concrete physicochemical entities. This difficulty can be overcome by calculating linear relationships between the coordinates of the two-dimensional nonlinear maps of PC loadings and variables and the concrete physicochemical parameters included in the experiments. However, in the traditional multilinear regression analysis the presence of independent variables that exert no significant influence on the dependent variable lessens the significant level of the independent variables that significantly influence the dependent variable. Stepwise regression analysis (SRA) overcomes this difficulty [14]. SRA automatically eliminates from the selected equation the insignificant independent variables increasing in this manner the information power of the calculation.

The objectives of our study were the separation of the carotenoid pigments of paprika (*Capsicum annuum*) powder by reversed-phase (RP) HPLC and the elucidation of the effect of fluorescent light and vacuum packaging on the selectivity and overall rate of decomposition of pigments by the simultaneous use of SRA and PCA.

2. Experimental

All solvents and reagents were analytical or HPLC grade and were purchased from Merck (Darmstadt, Germany).

2.1. Storage experiments

Hot red pepper powder (*Capsicum annuum*) was obtained from a pepper plant (Macao, Portugal). The vacuum-packed and normal atmosphere packed samples were stored in plastic bags and half of them were kept in a dry dark place and other half were kept in a metal box with an 18 W daylight tube (Osram, Germany). The fluorescent tube was suspended approximately 20 cm above the bags. Every day the tube was placed in a different position. Each experiment was carried out at 22 ± 1°C. Two bags from each treatment were randomly selected after 4, 8, 12, 16, 20, 24 and 28 days of storage time for RP-HPLC analysis.

2.2. Extraction procedure

Pigments were extracted from the 2.0-g samples using 15.0 ml of acetone in an Ultra Turrax homogeniser for 3 min and then they were centrifuged. This process was repeated until the extracts were colourless (normally three times was enough). All the extracts were pooled in a separator and dried over anhydrous Na_2SO_4 and then dried in a rotary evaporator at 35°C. The residue was dissolved in 10.0 ml of acetone, and filtered through a 0.20-μm membrane filter before HPLC injection. Aliquots were frozen at −20°C until RP-HPLC separation.

2.3. RP-HPLC separation

Separation of carotenoid pigments was carried out on a HPLC system consisting of an L-7450 diode array detector, an L-6200 Intelligent pump (Hitachi, Tokyo, Japan) and a Valco injector (Houston, TX, USA) with 20-μl injector loop. Columns were LiChrocart endcapped RP-18 (250×4 mm I.D.; particle size, 10 μm) with a LiChrocart RP-18 (4×4 mm I.D.; particle size, 5 μm) precolumn (Merck). The detection wavelength was 450 nm. The eluents

were A: acetone–water (75:25, v/v), B: acetone–methanol (75:25, v/v). The binary gradient was: from 0 to 65% B in 10 min, to 100% B in 60 min at a flow-rate of 1 ml/min [15]. To avoid carotenoid decomposition each step of the analysis was carried out under subdued light.

2.4. Statistical analysis

SRA was used for the elucidation of the effect of light and oxygen on the overall decomposition rate of pigments. The total peak areas measured by HPLC were the dependent variables. The independent variables were the storage time (days) and the presence (marked by 1) or absence (marked by 0) of light and oxygen. The number of accepted variables was not limited. The acceptance level of the individual independent variables was set to a 95% significance level. PCA was employed for the elucidation of the similarities and dissimilarities of the pigment composition of the various samples. The exact identification of the individual pigment fractions is very difficult. Reliable standards can be purchased only for some basic pigment fractions, and they decompose rapidly. The identification according to the UV and visible spectra is not reliable because the spectra of the pigments are similar. As the retention times (RSD 0.8–2.1%) and peak areas (RSD 3.5–5.2%) of pigment fractions showed marked variations among the samples and the identification of the fractions cannot be performed accurately the individual pigment fractions cannot be used as observations for PCA. Therefore, the relative peak areas were summarized separately for the two parallel determinations between the retention time intervals of 0–10, 10–20, 20–30, 30–40, 40–50, 50–60 min and these segments were used as variables for PCA (altogether 12 variables). We are well aware that this interval division probably does not reflect the distribution of carotenoids of the chromatograms (free pigments probably 0–20 min; monoesterified xanthophylls probably 20–30 min; carotenes probably 30–34 min, and diesterified carotenoids probably 34–60 min). However, the exact mathematical evaluation requires the equidistant distribution of intervals without taking into consideration the distribution of pigments according to their chemical structure. The different treatments (light+oxygen, light+vacuum, dark+oxygen, dark+vacuum) and sampling times (4, 8, 12, 16, 20, 24 and 28 days) were taken as observations (altogether 28 observations). The limit of the variance explained was set to 99%. In order to facilitate the evaluation of the multidimensional matrices of PC loadings and variables, their dimensionality was reduced to two by the NLMAP technique. The iteration of the nonlinear maps was carried out to the point when the difference between the two last iterations was lower than 10^{-8}. It was assumed that the maps reflect the selectivity of the treatments. In order to find the relationship between the selectivity of treatments and the storage conditions SRA was applied. The first and second coordinates of the two-dimensional nonlinear map of PC variables were separately the dependent variables. Other conditions were as described above.

Softwares for PCA and nonlinear mapping was developed by Dr. Barna Bordás (Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary). Software for SRA was purchased from Compudrug (Budapest, Hungary).

3. Results and discussion

More than 35 pigments could be separated on the LiChrocart RP-18 column using water–acetone–methanol gradient elution, indicating that many different pigments contribute to the colour of paprika powders (Fig. 1). SRA found significant linear relationship between the overall rate of decomposition of pigments and the storage conditions. The parameters of the correlation are compiled in Table 1 (Eq. (1)). The normalized slope values (b') indicate that the storage time exerts the highest impact on the decomposition rate while the effect of oxygen is relatively low. The relationship was highly significant, the significance level being over 99.9% (see calculated F value).

The results of PCA are compiled in Table 2. Six background variables explain the overwhelming majority of information present in the original variables with only 11.47% loss of information. This result indicates that the treatments influence differently the

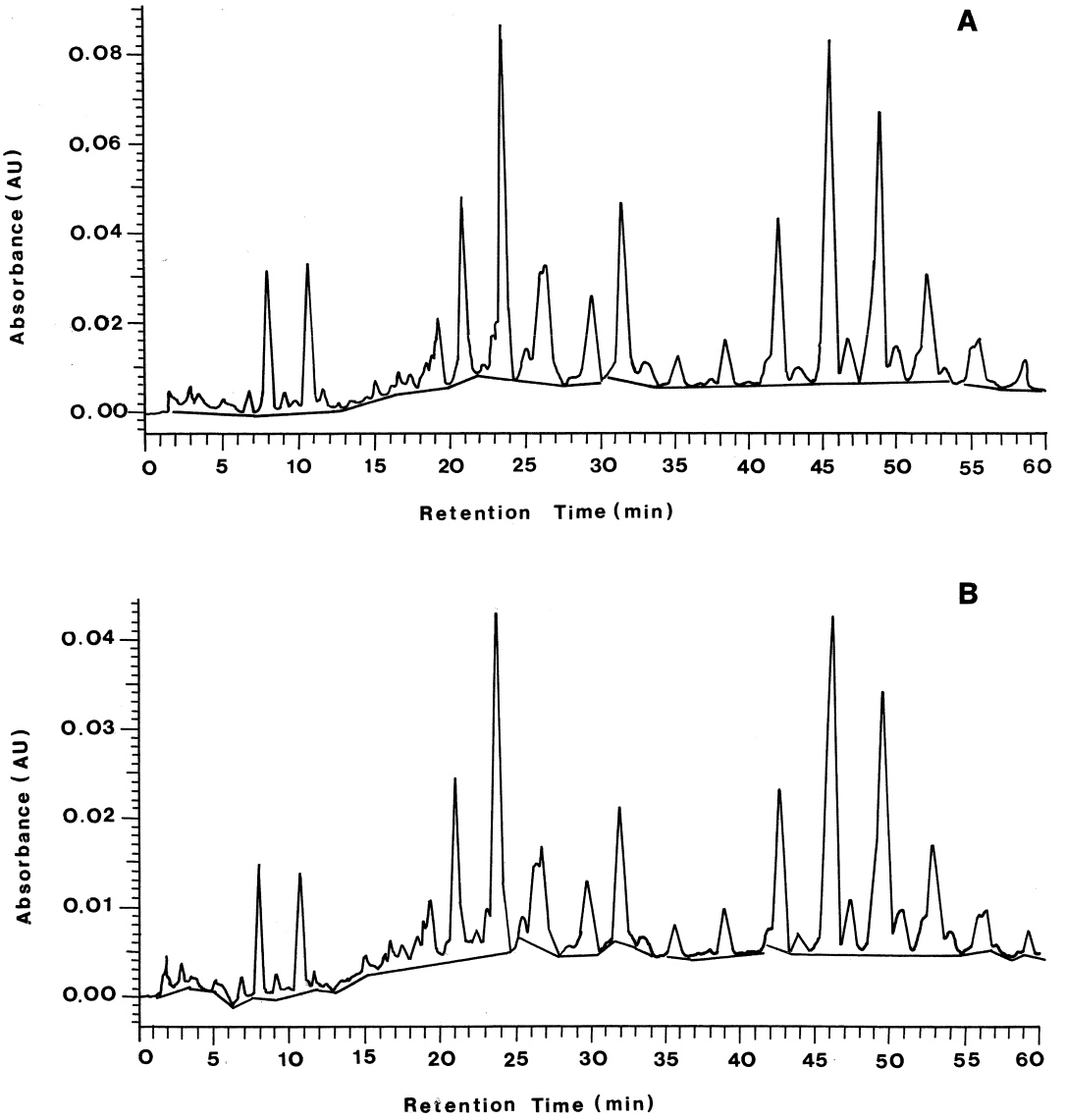

Fig. 1. Separation of pigments of (*Capsicum annuum*) on a LiChrocart RP-18 column after 28 days of storage, using water–acetone–methanol gradient elution, detection wavelength 450 nm, flow-rate: 1 ml/min. Storage conditions: (A: darkness, absence of oxygen, B: light, absence of oxygen).

various segments of chromatogram that is the inclusion of each segment in the calculation is justified. The average values of chromatogram segments are shown on the two-dimensional nonlinear map of PC loadings (Fig. 2). Interestingly, the segments form two well-defined clusters. The first parts of the chromatograms (0–10, 10–20, 20–30 and 30–40 min) behave similarly, while the segments 40–50 and 50–60 form a separated cluster.

Treatments are randomly distributed on the two-dimensional nonlinear map of principal component variables (Fig. 3). This finding suggests that not only the overall rate of decomposition but also the selectivity of decomposition depends on more than

Table 1
Relationship between the total peak areas of carotenoid pigments of paprika (*Capsicum annuum*) powder and the storage conditions (related to the overall rate of decomposition), and the first coordinate of the two-dimensional nonlinear map of PC variables and the storage conditions (related to the selectivity of decomposition) – results of stepwise regression analysisI Total peak area = $a + b_1x_1 + b_2x_2 + b_3x_3$ (1)II First coordinate = $a + b_1x_1 + b_3x_3$ (2)

Parameter	I	II
n	56	28
a	$4.92 \cdot 10^4$	73.6
b_1	$-3.94 \cdot 10^2$	4.00
s_{b1}	$8.53 \cdot 10^1$	0.71
b_2	$-5.75 \cdot 10^3$	–
s_{b2}	$1.37 \cdot 10^3$	–
b_3	$-3.90 \cdot 10^3$	42.1
s_{b3}	$1.37 \cdot 10^3$	11.3
b_1'%	39.50	60.27
b_2'%	36.03	–
b_3'%	24.47	39.73
$F_{calc.}$	15.72	22.96
r^2	0.4756	0.6475

x_1 = Storage time (days); x_2 = presence of light; x_3 = presence of oxygen; s_{bi} = standard deviations of the regression coefficients b_1, b_2 and b_3; b_i' = normalized slope values indicating the relative impact of independent variables.

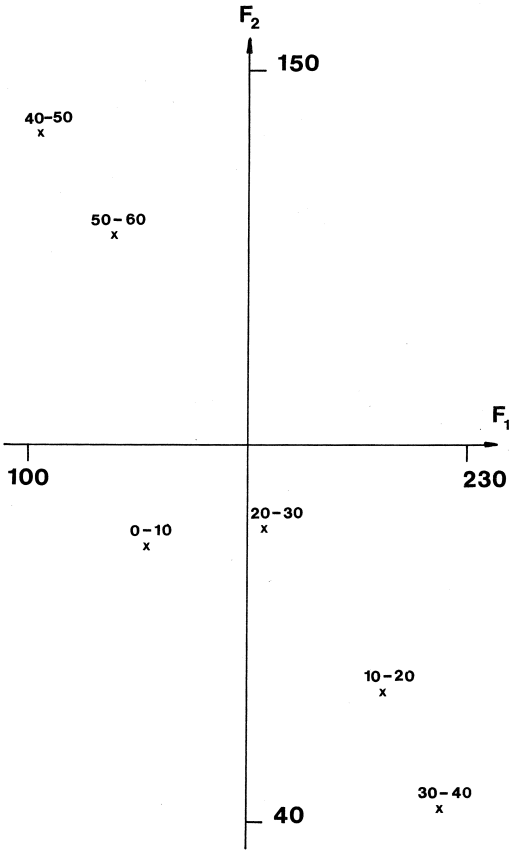

Fig. 2. Similarities and dissimilarities between the pigment fractions. Two-dimensional nonlinear map of principal component loadings. No. of iterations: 165; maximal error: $8.09 \cdot 10^{-2}$. Numbers refer to time intervals of chromatograms (min).

one storage parameters such as storage time, presence of light and oxygen. SRA entirely supported our previous qualitative conclusions (Table 1, Eq. (2)). The first coordinate of the two-dimensional nonlinear map was significantly correlated with the storage time and the presence of oxygen the significance level being over 99.9%. It was found that the second coordinate did not depend on the parameters included into the calculations.

4. Conclusions

It can be concluded from the data that the overall rate and selectivity of the decomposition of carotenoid pigments of paprika powder can be successful-

Table 2
Similarities and dissimilarities between the pigment fractions – results of principal component analysis

No. of PCs	Eigenvalue	Variance explained (%)	Total variance explained (%)
1	3.23	26.93	26.93
2	2.18	18.13	45.06
3	2.01	16.73	61.79
4	1.45	12.10	73.89
5	1.07	8.88	82.76
6	0.69	5.77	88.53

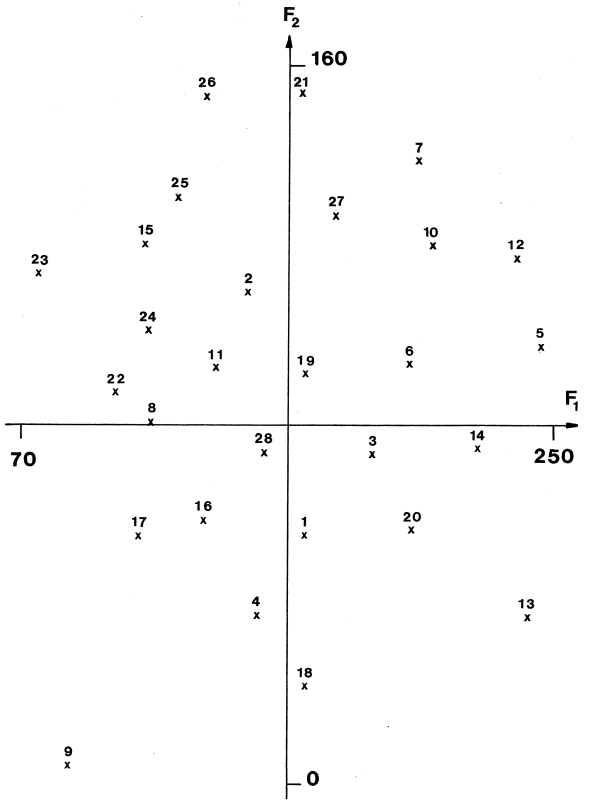

Fig. 3. Similarities and dissimilarities among the treatments according to the composition of carotenoid pigments. Two-dimensional nonlinear map of principal component variables. No. of iterations: 128; maximal error: $8.13 \cdot 10^{-2}$. Treatments: 1=light, oxygen, 4 days; 2=light, oxygen, 8 days; 3=light, oxygen, 12 days; 4=light, oxygen, 16 days; 5=light, oxygen, 20 days; 6=light, oxygen, 24 days; 7=light, oxygen, 28 days; 8=light, vacuum, 4 days; 9=light, vacuum, 8 days; 10=light, vacuum, 12 days; 11=light, vacuum, 16 days; 12=light, vacuum, 20 days; 13=light, vacuum, 24 days; 14=light, vacuum, 28 days; 15=dark, oxygen, 4 days; 16=dark, oxygen, 8 days; 17=dark, oxygen, 12 days; 18=dark, oxygen, 16 days; 19=dark, oxygen, 20 days; 20=dark, oxygen, 24 days; 21=dark, oxygen, 28 days; 22=dark, vacuum, 4 days; 23=dark, vacuum, 8 days; 24=drak, vacuum, 12 days; 25=dark, vacuum, 24 days; 26=dark, vacuum, 20 days; 27=dark, vacuum, 24 days; 28=light, vacuum, 28 days.

ly followed by RP-HPLC. PCA and SRA proved to be useful tools to determine the effect of storage conditions on the pigment composition of paprika powder.

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