ELSEVIER

Journal of Chromatography A, 915 (2001) 149–154

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Profiling of colour pigments of chili powders of different origin by high-performance liquid chromatography ☆

Agnes Kósa[a], Tibor Cserháti[a,*], Esther Forgács[a], Helena Morais[b], Teresa Mota[b], A.C. Ramos[b]

[a]*Chemical Research Centre, Institute of Chemistry, Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary*
[b]*Instituto Nacional de Investigacao Agraria, Oeiras, Portugal*

Received 11 October 2000; received in revised form 20 January 2001; accepted 5 February 2001

Abstract

The colour pigments of five chili powders of different origins were separated and quantified by reversed-phase high-performance liquid chromatography (RP-HPLC). The similarities and dissimilarities of pigment composition of chili powders were elucidated by principal component analysis (PCA). RP-HPLC separated 50–100 pigment fractions depending on the detection wavelength and on the origin of chili powder. It was found that the pigment composition of chili powders from Malaysia and China and from India and Pakistan show marked similarities while the composition of colour pigments of chili powder from Thailand was different. It was further established that the chromatograms are similar in the first 5–35 min of development, they are highly different between 35 and 75 min and moderately different at the end of the chromatograms. It was concluded that RP-HPLC followed by PCA can be successfully used for the identification of chili powders according to the composition of their colour pigments.

Keywords: Food analysis; Principal component analysis; *Capsicum frutescens*; Pigments

1. Introduction

Various high-performance liquid chromatographic methods (HPLC) have been frequently used for the separation and quantitative determination of natural and synthetic colour pigments in foods and food products. Earlier results have been previously reviewed [1]. Thus, procyanidins from apples, fruits and fruit juices were fractionated by size-exclusion chromatography and the solutes were identified by time-of-flight mass spectrometry [2], flavonol aglycones and glucosides were determined in berries by reversed-phase HPLC (RP-HPLC) using electrospray ionization mass spectrometry and diode array ultraviolet detection [3], anthocyanic pigments in red fruit juices [4], etc. The pigment composition of onion and celery [5], tomato juice [6], red peppers (*Capsicum annuum*) [7], grape skin extracts [8], red wines [9], and hops and beer [10] have also been investigated.

Besides the traditional HPLC techniques, the application of ion-interaction chromatography [11], high-performance ion chromatography [12], gel-per-

☆Presented at the 6th International Symposium on New Achievements in Chromatography, Plitvice Lakes, 11–13 October 2000

*Corresponding author. Tel.: +36-1-325-7900; fax: +36-1-325-7554.

E-mail address: forgacs@cric.chemres.hu (T. Cserháti).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00640-9

meation chromatography combined with RP-HPLC [13] and capillary zone electrophoresis [14,15] has been reported. The considerable interest in the analysis of pigments was motivated by the fact that the exact knowledge of pigment composition may help the identification (the authenticity) of the products [16].

The evaluation of large data matrices containing the retention parameters of various pigment fractions determined in different foods and food products is not possible with the traditional linear regression analysis. The application of various multivariate mathematical statistical methods overcomes this difficulty [17]. Principal component analysis (PCA), a multivariate statistical method has been frequently employed for the evaluation of retention data matrices [18,19]. PCA finds the relationships among all parameters of the original data matrix without defining one of two being the dependent variable. PCA calculates the correlations among the columns and rows of the matrix elucidating the similarities and dissimilarities among them. PCA has been successfully used for the differentiation of Spanish white wines [20], for the study of the interaction of some steroid drugs with a β-cyclodextrin polymer [21], for the elucidation of the relationship between retention and molecular characteristics of environmental pollutants [22], etc. As the matrices of PC loadings and variables are generally multidimensional too, the reduction of the dimensionality of the matrices by nonlinear mapping technique highly facilitate the evaluation [23].

The objectives of our study were the separation and quantitative determination of the colour pigments of chili (*Capsicum frutescens*) powders of different origin by RP-HPLC and the use of PCA for the differentiation of the chili powders according to the chromatographic profile of their pigments.

2. Experimental

The pigments of five chili powders of different origin (sample I, Thailand; sample II, Malaysia; sample III, China; sample IV, India; sample V, Pakistan) were extracted by shaking 3 g of chili powder with 10 ml of acetone for 30 min. The suspension was centrifuged and the supernatant decanted. This procedure was repeated as the solid rest was nearly white. The collected supernatants were evaporated and redissolved in the mobile phase. Each solvent for extraction and HPLC development was purchased from Merck (Darmstadt, Germany) and was of HPLC quality. HPLC separation of pigments was performed with a Waters LC Module I HPLC instrument with an injection device of variable volume and a Waters 746 Data Module integrator (Waters-Millipore, Milford, MA, USA). Separations were carried out on a narrow-bore Novapak octadecylsilica column (150×2 mm I.D., carbon loading, 9.5%, Waters Division of Millipore, Millipore, Milford, MA, USA). The column was not thermostated; each analysis was performed at room temperature (21±1°C). In order to determined the ratio of yellow and red pigments, each measurement was carried out at 340 (yellow pigments) and 440 nm (red pigments). The flow-rate was 0.17 ml/min. The gradient steps used for the separation of pigments are compiled in Table 1. Each gradient step was linear. Determinations were performed in triplicate and the relative standard deviation (RSD%) of the retention times and peak areas were calculated (intra-day reproducibility). The inter-day reproducibility was calculated from the three parallel determinations carried out for four consecutive working days. In order to find the differences between the intra- and inter-day reproducibilities the corresponding RSD values were compared with the 'F' probe.

PCA has been employed for the elucidation of the similarities and dissimilarities of the pigment composition of chili powders. The five different chili

Table 1
Gradient elution used for the separation of the colour pigments of chili (*Capsicum frutescens*) powders[a]

Time (min)	Solvent A (%)	Solvent B (%)
0	15	85
25	80	20
35	80	20
45	90	10
55	90	10
58	97	3
120	97	3

[a] Solvent A, methanol–acetonitrile (80:20, v/v); solvent B, bidistilled water.

powders were taken as variables. As the retention time of pigment fractions showed high variations among the chili powders the retention times and relative peak areas of the individual peaks cannot be used as observations for PCA. Therefore, the peak areas were summarized between the retention time intervals of 5–15, 15–25, 25–35, 35–45, 45–55, 55–65, 65–75, 75–85, 85–95, 95–105 and 105–115 min at both 340 and 440 nm, and were used as observation for PCA (altogether 22 variables). The limit of the variance explained was set to 99%. In order to concentrate the information in a plane the varimax rotation of the PC loadings around two axis was carried out. The two-dimensional nonlinear map of the PC loadings and variables were also calculated. The iteration of the nonlinear map was carried out to the point when the difference between the two last iterations was lower than 10^{-8}.

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

3. Results and discussion

Colour pigments of each chili powder were well separated under the RP-HPLC conditions applied (Figs 1–4). The chromatographic profiles of pigment fractions are different at 340 and 440 nm demonstrating that chili powders contains both red and

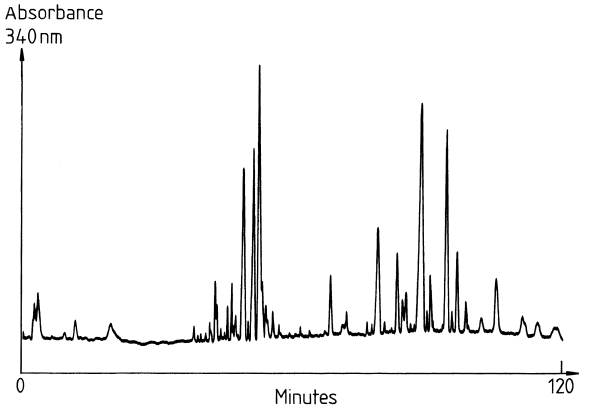

Fig. 1. Separation of color pigments of chili powder (origin, Malaysia) at 340 nm. For conditions see Section 2.

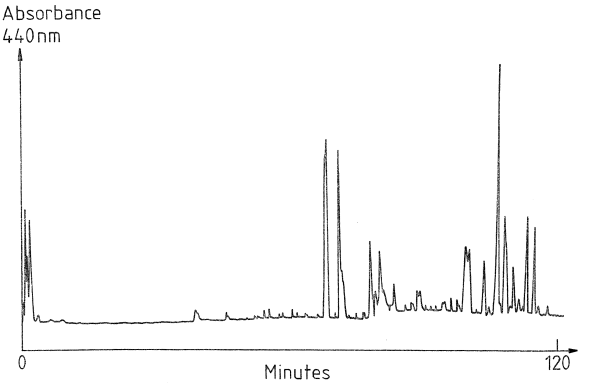

Fig. 2. Separation of color pigments of chili powder (origin, Malaysia) at 440 nm. For conditions see Section 2.

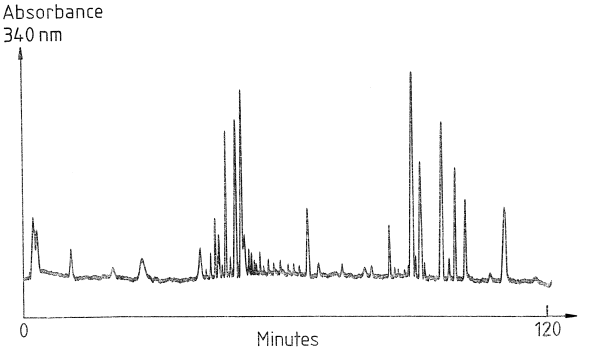

Fig. 3. Separation of color pigments of chili powder (origin, Thailand) at 340 nm. For conditions see Section 2.

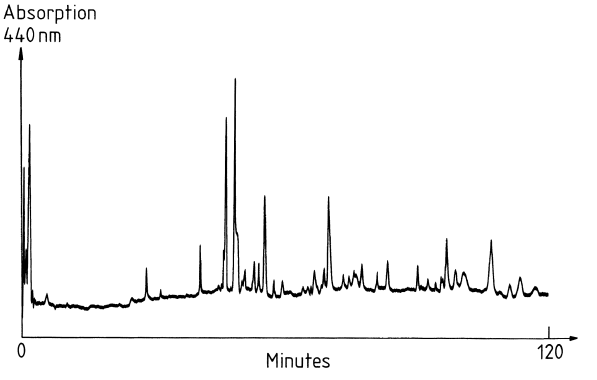

Fig. 4. Separation of color pigments of chili powder (origin, Thailand) at 440 nm. For conditions see Section 2.

yellow pigments (compare Figs. 1 & 2 and 3 & 4). This result suggests that the measurement of the pigment composition at minimally two different wavelengths is necessary for the correct evaluation of the pigment composition. The chromatograms of pigments also differ according to the origin of the samples (compare Figs. 1 & 3 and 2 & 4). This fact indicates that the separation and quantitative determination of pigment fractions may help the identification of the origin of chili powders. To the best of our knowledge the pigment composition of chili powder have never been studied in detail, and the pigments have never been reliably identified. As paprika and chili are closely related it can be assumed that the pigments of chili are also carotenoid derivatives but they are not necessarily identical with the pigments of paprika.

The relative peak areas of pigment fractions measured at 340 and 440 nm are compiled in Table 2. Similarly to the conclusions drawn from Figs 1–4 it can be established again that the relative concentration of pigments depends on both the origin of the sample and the wavelength of the detection, that means, that the chromatographic profiles of pigments are characteristic for the chili powder.

The results of PCA using are compiled in Table 3.

Table 3
Similarities and dissimilarities between the pigment composition of chili powders: results of principal component analysis

No. of PC	Eigen-value	Variance explained (%)	Total variance explained (%)
1	3.21	64.17	64.17
2	1.39	27.72	91.88
3	0.25	4.98	96.86
4	0.10	2.10	98.97

Principal component loadings

Chili powder	No of principal component		
	1	2	3
Thailand	*0.90*	−0.14	−0.40
Malaysia	*0.87*	−0.45	0.15
China	*0.86*	−0.45	0.21
India	*0.56*	*0.79*	0.15
Pakistan	*0.78*	*0.58*	−0.03

Table 2
Relative peak areas (%) of pigment fractions detected at 340 (A) and 440 nm (B)

No.	Time of development (min)	Wave-length	Origin of chili powder				
			Thailand	Malaysia	China	India	Pakistan
1	5–15	A	0.444	1.932	1.897	0.579	0.094
2		B	0.698	0.507	1.101	0.725	1.397
3	15–25	A	0.355	1.130	0.144	0.137	0.517
4		B	0.036	0.188	0.460	0.555	0.314
5	25–35	A	0.643	0.396	0.325	0.142	0.437
6		B	1.523	0.157	0.180	1.389	1.960
7	35–45	A	5.286	3.339	6.422	4.043	18.927
8		B	7.477	0.296	0.059	8.903	8.296
9	45–55	A	13.956	13.140	13.486	80.108	41.271
10		B	16.049	2.081	0.832	21.627	21.498
11	55–65	A	7.431	5.302	3.368	3.221	0.418
12		B	12.672	7.273	5.110	19.817	12.925
13	65–75	A	7.806	6.183	5.603	2.750	1.610
14		B	12.387	18.580	11.804	11.245	13.213
15	75–85	A	12.500	12.057	10.433	2.804	3.844
16		B	13.520	15.670	19.600	13.818	11.318
17	85–95	A	21.801	28.770	26.882	4.261	21.500
18		B	13.877	15.710	24.336	10.383	13.766
19	95–105	A	17.867	17.195	18.945	1.853	7.116
20		B	13.340	18.234	15.768	7.915	9.454
21	105–115	A	11.912	10.557	12.494	0.102	4.260
22		B	8.423	21.304	20.750	3.624	5.860

Two principal components explain the majority of variance indicating that the five original variables can be substituted by three background (abstract) variables with only 3.14% loss of information. Unfortunately, PCA does not prove the existence of such background variables as concrete physicochemical entities, but only indicates their mathematical possibility. The fact that each chili powder has a high loading in the first PC indicates the basic similarity of pigment composition of chili powders.

The rotated factor loadings after varimax rotation around two axis are compiled in Table 4. The data suggest that the highest similarity is between the pigments of chili powders from China and Malaysia and India and Pakistan. Chili powder of Thailand is related to the chili powders of Malaysia and China. The two-dimensional nonlinear map of PC loadings is shown in Fig. 5. The distribution of the chili powders entirely supports the conclusions drawn from the result of varimax rotation: the points representing the chili powders originating from Malaysia and China are the nearest to each other.

The two-dimensional nonlinear map of PC variables (relative peak areas determined at different time intervals and detection wavelengths) is shown in Fig. 6. The points from three well-defined clusters. The retention time intervals, 5–35 min (cluster A), are very near to each other suggesting that the first part of chromatogram is similar for each sample, therefore, it cannot be used for the identification of the samples. The points of 35–75 min intervals (cluster B) show high variety that means that this part of the chromatograms is characteristic for the samples and can be used for identification. The points representing the end of the chromatogram (75–115 min, cluster C) show less variety than the central part of the chromatograms. Interestingly the relative peak areas measured at 340 and 440 nm are not separated suggesting that both of them can be used for the identification of chili powders.

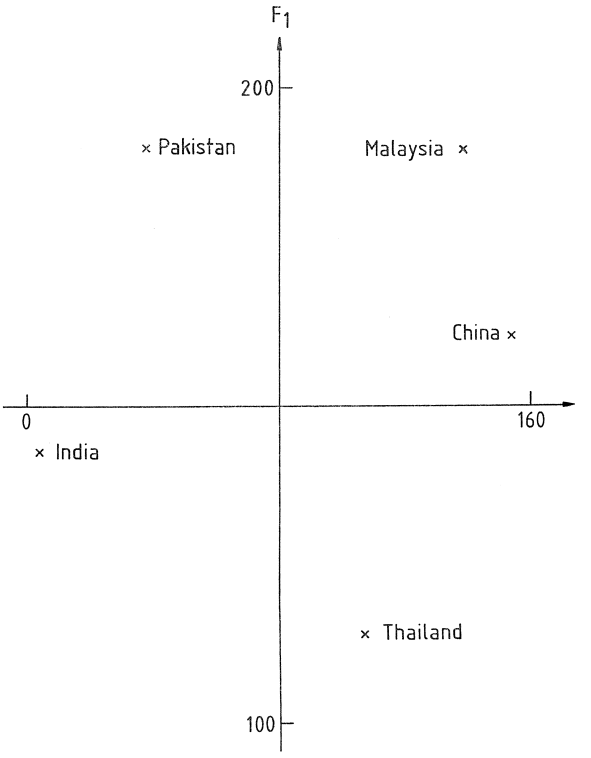

Fig. 5. Similarities and dissimilarities of chili powders according to the composition of colour pigments. Two-dimensional nonlinear map of principal component loadings. Number of iterations, 43; maximal error. 8.81×10^{-3}.

Table 4
Varimax rotation of principal component loadings around two axes

Chili powder	Rotated factor loadings	
	1	2
Thailand	*0.82*	0.38
Malaysia	*0.97*	0.11
China	*0.97*	0.10
India	0.03	*0.97*
Pakistan	0.32	*0.91*

No significant differences were found between the intra- and inter-day repoducibilities of retention times and peak areas proving the similar stability and reproducibility of column. The RSD values for both intra-day and inter-day reproducibilities were 0.6–1.7% for retention times and 3.2–4.9% for peak areas. The relatively high RSD values for peak areas reflects the inadequate separation of some pigment fractions.

It can be concluded from the data that the colour pigments of chili powders can be separated by RP-HPLC. The ratio of pigments measured at 440 and 340 nm is characteristic for the individual chili powders. Principal component analysis is a useful tool to detect the similarities and dissimilarities between the pigment composition of chili powders

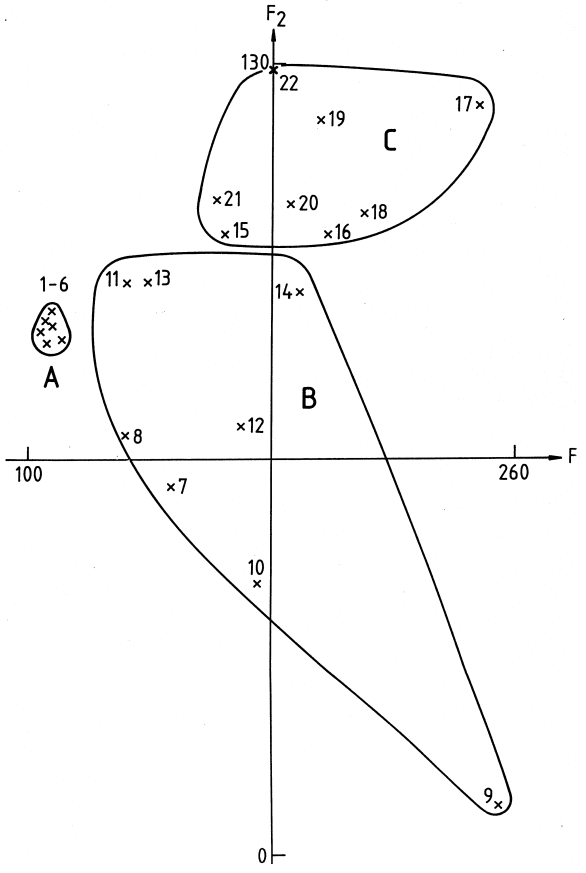

Fig. 6. Similarities and dissimilarities between the pigment fractions. Two-dimensional nonlinear map of principal component variables. Number of iterations, 85; maximal error, 5.50×10^{-3}. Numbers refer to pigment fractions in Table 2.

and to classify the powders according to the pigment composition.

Acknowledgements

This work was supported by the Portuguese–Hungarian cooperation project 'Development of new methods and their application for the assessment of the effect of environmental conditions on the stability of color pigments in foods'.

References

[1] K. Robards, M. Antolovich, Analyst 122 (1997) 11R.
[2] A. Yanagida, T. Kanda, T. Shoji, M. Ohnishi-Kameyama, T. Nagata, J. Chromatogr. A 855 (1999) 181.
[3] S. Häkkinen, S. Auriola, J. Chromatogr. A 829 (1998) 91.
[4] J.-P. Goiffon, P.P. Mouly, E.M. Gaydou, Anal. Chim. Acta 382 (1999) 39.
[5] A. Crozier, E. Jensen, M.E.J. Lean, M.S. McDonald, J. Chromatogr. A 761 (1997) 315.
[6] V. Böhm, Chromatographia 50 (1999) 282.
[7] M. Weissenberg, I. Schaeffler, E. Menagem, M. Barzilai, A. Levy, J. Chromatogr. A 757 (1997) 89.
[8] I. Revilla, S. Pérez-Magarino, M.L. González-SanJosé, S. Beltrán, J. Chromatogr. A 847 (1999) 83.
[9] S. Pérez-Magarino, I. Revilla, M.L. González-SanJosé, S. Beltrán, J. Chromatogr. A 847 (1999) 75.
[10] J.F. Stevens, A.W. Taylor, M.L. Deinzer, J. Chromatogr. A 832 (1999) 97.
[11] M.C. Gennaro, E. Gioannini, S. Angelino, R. Aigotti, D. Giacosa, J. Chromatogr. A 767 (1997) 87.
[12] Q.-C. Chen, S.-F. Mou, X.-P. Hou, J.M. Riviello, Z.-M. Ni, J. Chromatogr. A 827 (1998) 73.
[13] C. Froytog, R. Slimestad, O.M. Andersen, J. Chromatogr. A 825 (1998) 89.
[14] J.J. Berzas Nevado, C. Guiberteau Cabanillas, A.M. Contento Salcedo, Anal. Chim. Acta 378 (1999) 63.
[15] L. Arce, A. Rios, M. Valcárcel, J. Chromatogr. A 827 (1998) 113.
[16] P.P. Mouly, E.M. Gaydou, C.R. Arzouyan, J.M. Estienne, Analusis 24 (1996) 230.
[17] T. Cserháti, E. Forgács, Adv. Chromatogr. 36 (1996) 1.
[18] M. Persson, K. Sjödin, A.-K. Borg-Karlson, T. Norin, I. Ekberg, Phytochemistry 42 (1996) 1289.
[19] K. Sjödin, M. Persson, A.-K. Borg-Karlson, T. Norin, Phytochemistry 41 (1996) 439.
[20] C.M. Garcia-Jares, M.S. Garcia-Martin, N. Carro-Marino, R. Cela-Torrijos, J. Sci. Food Agric. 69 (1995) 175.
[21] E. Forgács, T. Cserháti, J. Chromatogr. A 845 (1999) 447.
[22] T. Cserháti, E. Forgács, J. Chromatogr. A 869 (2000) 41.
[23] J.W. Sammon Jr., IEEE Trans. Comput. C18 (1969) 401.