Optimisation of the microwave-assisted extraction of pigments from paprika (*Capsicum annuum* L.) powders

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Abstract

The efficiency of microwave-assisted extraction (MAE) for the extraction of colour pigments from paprika (*Capsicum annuum*) powders was evaluated using 30 extracting solvent mixtures. The separation efficacy and selectivity of MAE was carried out using a spectral mapping technique and the relationship between the efficacy and selectivity of extraction and the physicochemical parameters of solvent mixtures was calculated by stepwise regression analysis. The calculation results were verified experimentally by the separation of pigment fractions by high-performance liquid chromatography. It was established that both the efficacy and selectivity of MAE depend significantly on the dielectric constant of the extraction solvent mixture. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Carotenoids are responsible for the desirable colour of many foods and food products. They form one of the most important groups of natural pigments found in many families of the flora and fauna. Carotenoids show marked biological activity [1], such as anticarcinogenic [2], antioxidant [3] and cancer chemopreventive [4] activity. Furthermore, the fact that natural pigments are more acceptable to consumers as they have always been present in foods and are readily metabolised gives them special importance. The commercial value of paprika (*Capsicum annuum* L.) powders depends considerably on the colour of the product.

Various spectroscopic methods [5,6], triestimulus colorimetry [7] and adsorption [8] and reversed-phase [9] thin-layer chromatography have been employed for the determination of the overall quantity of pigments or for their separation. Because of its high separation power and repoducibility, high-performance liquid chromatography (HPLC) has became the method of choice for the analysis of paprika pigments [10] using mainly silica [11,12] and octadecylsilica [13,14] stationary phases. Pigments have generally been extracted from paprika powders by the traditional shaken flask method, which is time-consuming, and the reproducibility and repeatability are relatively low. The majority of methods used for the extraction of pigments from paprika powder employ acetone as solvent [15,16],

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however the use of methanol has also been reported [11].

Microwave-assisted extraction (MAE) has been widely used for sample preparation to replace other extraction methods such as Soxhlet, sonication, supercritical fluid extraction, etc. MAE considerably reduces extraction time [17] and/or enhances the efficiency of the extraction [18]. MAE is frequently applied for sample preparation in the trace analysis of solid [19] (sediment, warp) and liquid [20] (rain, waste water) samples contaminated by organic compounds, and in the analysis of samples of biological origin (e.g., extraction of alkaloids [21] and selected drugs from serum [22], flavonoids from foodstuffs [23], and lipids [24]). The technique has a number of advantages over the traditional extraction methods: since it is not tedious and not restricted in solvent selectivity, no concentration/evaporation step is required, and the possibility of contaminating the sample with solvent impurities is much lower. These problems can also be successfully avoided by using supercritical fluid extraction, but its application requires complicated instrumentation.

Spectral mapping analysis (SPM) [25] and stepwise multiple linear regression (SLR) have frequently been employed in chromatography. The SPM technique has previously been used for the characterisation of solid phases in liquid chromatography [26], for the elucidation of the relationship between chromatographic retention data and biological activity of nonionic surfactants [27] and for the detection of adulteration of different origin paprika and chilli powders [28]. As visual evaluation of the multidimensional selectivity map is difficult, the nonlinear mapping technique can be used for the reduction of the dimensionality of the selectivity map [29]. Not only SPM but also SLR has found employment in various fields of chromatography such as thin-layer [30,31], gas [32], and high-performance liquid chromatography [33]. In addition to these well-established techniques, the partial least-square method has also found application in the evaluation of chromatographic retention data matrices; however, its information power is lower than that of the other multivariate techniques [34].

The objectives of this work were to elucidate the conditions for MAE of colour pigments from paprika powders and to find the relationship between the strength and selectivity of the extracting solvent mixtures and their physicochemical parameters using multivariate mathematical statistical methods.

2. Experimental

The optimal extraction time was determined by mixing 40 mg of red paprika powder (Sol das Beiras, Manuel Rosa Eusébio & Filhos, Mação, Portugal) with 2 ml of acetone–water (1:1, v/v) and extracting the suspension by an Ethos Sel microwave extractor (Milestone, Sorisole, Italy) for 30, 60, 90 and 120 s at various electric power. The employment of acetone as organic component was motivated by the fact that acetone has frequently been used for the extraction of pigments from paprika powders by the traditional shaken flask method.

After extraction the samples were centrifuged at 6000 rpm for 10 min. The absorption of the clear supernatants was determined by a Jasco V-570 UV–Vis–near IR spectrophotometer (Jasco, Edo, Japan) at 395, 410, 425, 455, 470 and 485 nm, the zero point being set to 500 nm. Extraction was finished when the sample reached 60°C.

Solvents used for extraction were water mixed with acetone, dioxane, ethanol, methanol and tetrahydrofuran (THF) in volume ratios of 15, 30, 45, 60, 75 and 90 (percent of organic modifier). The number and exact composition used for the optimisation of the MAE process are compiled in Table 1. The samples after extraction were centrifuged as described above, decanted and the absorbances were measured with a spectrophotometer at six different wavelengths, also listed above.

SPM was employed to separate the strength and selectivity of the effect of solvent mixtures on the absorbance of paprika extracts. The data matrix consisted of the absorbance values of the samples determined at six different wavelengths as variables and the solvent mixtures as observations. Potency values calculated by SPM are related to the overall effect of solvents on the extracted amount of pigments, and the selectivity data show the differences in the selectivity of the effect of solvent mixtures independent of the amount of extracted pigments. The nonlinear mapping technique was applied for two-dimensional visualisation of the multidimension-

Table 1
Number and composition of solvent mixtures used for the MAE of colour pigments of paprika

Solvent No.	Organic modifier		Solvent No.	Organic modifier	
	Type	% (v/v)		Type	% (v/v)
1	Acetone	15	16	Ethanol	60
2		30	17		75
3		45	18		90
4		60	19	Methanol	15
5		75	20		30
6		90	21		45
7	Dioxane	15	22		60
8		30	23		75
9		45	24		90
10		60	25	Tetrahy-drofuran	15
11		75	26		30
12		90	27		45
13	Ethanol	15	28		60
14		30	29		75
15		45	30		90

al spectral map. The iteration was carried out to the point where the difference between the last two iterations was less than 10^{-8}.

Stepwise regression analysis was used to elucidate the relationships between the results of spectral mapping and the physicochemical parameters of the solvent mixtures [35]. The physicochemical parameters included in the calculations were the dielectric constant (ϵ), dipole moment (μ) and refractive index (n) of the solvent mixtures. The physicochemical parameters of the mixtures were calculated from the parameters of the pure components by the additivity rule. These are compiled in Table 2. We are well aware that the relationship between the composition and additive physicochemical parameters of solvent mixtures is not strictly linear. As data on the physicochemical parameters of the solvent mixtures

used in this study are practically nonexistent we are forced to use this inaccurate approximation for the calculation. SLR was carried out three times, the potency values and the coordinates of the two-dimensional nonlinear map for solvents being the dependent variables. Independent variables in each instance were the physicochemical parameters of the solvent mixtures mentioned above. To test the quality of the generated regression equations, the regression coefficient (R), the standard error of the estimate (s_{XY}), and a test of the null hypothesis (F-test) were used.

Reversed-phase HPLC was employed for experimental verification of the difference in the selectivity of MAE procedures. The HPLC system consisted of a 125×3 mm I.D. Purospher RP-18 column (Merck, Darmstadt, Germany, particle size 5 μm). A Waters LC Module I (Millipore, Milford, MA, USA), a Waters variable-wavelength UV detector (Millipore), a Waters injector (Millipore) with a 2 μL sample loop and a Waters 746 integrator (Millipore) completed the equipment. The pigments of paprika powder extracted with the two different solvent mixtures at opposite ends of the selectivity map were separated. Because of the large differences between the retention characteristics of paprika pigments, gradient elution was employed for their separation. Methanol–acetonitrile (80:20, v/v) was used as eluent A, and bidistilled water was used as eluent B. The gradient program is shown in Table 3. The flow-rate was 0.5 mL/min and the detection wavelength was set to 440 nm. Separations were carried out at room temperature (21±1°C). The mean values

Table 2
Dielectric constant (ϵ), dipole moment (μ) and refractive index (n) of the pure organic solvents used in the experiments

Organic solvent	Dielectric constant (ϵ)	Dipole moment (μ)	Refractive index (n)
Acetone	20.7	2.88	1.359
Dioxane	22.1	2.06	1.422
Ethanol	24.3	1.69	1.361
Methanol	32.6	1.70	1.328
Tetrahydrofuran	7.6	1.75	1.407

Table 3
Gradient elution for the reversed-phase high-performance liquid chromatographic separation of colour pigments in the extracts of paprika (*Capsicum annum*). Eluent A, methanol–acetonitrile (80:20, v/v); eluent B, bidistilled water

Time (min)	A (%)	B (%)
0	15	85
10	40	60
25	80	20
35	80	20
45	90	10
55	90	10
58	97	3
120	97	3

and relative standard deviation of retention times and peak areas were calculated from three consecutive determinations. In order to compare the selectivities of these two extracting solvent mixtures, the linear correlation was calculated between the mean values of the peak areas of 13 characteristic peaks. Peaks well separated from the others and showing large peak areas were selected because these criteria minimise the absolute and relative error, enhancing the reliability of the calculation. Peak areas measured in solvent mixture 6 were the dependent variables and peak areas measured in solvent mixture 26 were the independent variables.

Spectral mapping and nonlinear mapping calculations were carried out with software prepared by Dr. Barna Bordás (Plant Protection Institute of the Hungarian Academy of Sciences, Budapest, Hungary), while software for stepwise regression analysis was prepared by Compudrug (Budapest, Hungary). Software for spectral mapping and nonlinear mapping was prepared according to the algorithms described in the original publications [25,29].

3. Results and discussion

The optimal conditions for MAE of pigments of paprika powder were 120 s extraction time and 50 W. The energy level of 50 W was chosen because, at higher power, the temperature increase in the samples was very rapid. Under these MAE conditions the temperature of the sample remained under 60°C. The 60°C temperature limit was chosen because heating carotenoids at higher temperature may cause rearrangement of the molecules and a decrease in the content of total carotenes [36]. Thermooxidisation may also occur: the hydrophobic carotenoids may oxidise to more hydrophilic compounds [37]. The relationship between the extraction time and sample temperature is shown in Fig. 1. The diagrams clearly show that the temperature of the samples approximates the limit temperature of 60°C at about 120 s. The rise in temperature verified for acetone, dioxane and THF was lower than for ethanol and methanol. It was established that each sample contained nonextracted pigments after MAE (solid rests showed a rose colour). The same phenomenon was observed using a one-step traditional shaken flask extraction

with acetone. The results demonstrated that more than one extraction step is needed for the exhaustive extraction of pigments from paprika powders even using MAE. The potency values (overall effect of solvents on the amount of extracted pigments) are listed in Table 4. The data clearly indicate that solvent mixtures showed marked differences in their effect in dissolving pigments, the extraction capacity varying between 0.15 and 0.06 (arbitrary units). According to the potency values the extraction strength increases with increasing concentration of organic component in the extraction solvent mixture and also depends on the character of the organic modifier. This result can be explained by the fact that the highly lipophilic pigments are more soluble in the more hydrophobic solvent mixtures.

The two-dimensional nonlinear selectivity map of extraction solvents is shown in Fig. 2. The markedly low value of the maximum error ($5.51\cdot10^{-4}$) guarantees the reliability of the mapping. Interestingly, the solvent mixtures form clusters according to the concentration of the organic modifier in the higher concentration range (cluster A, concentration of organic modifiers 90%, v/v; cluster B, concentration or organic modifiers 75%, v/v) but not in the lower concentration ranges. The data also suggest that the character of the organic modifier has a smaller impact on the selectivity of MAE than the concentration. However, it can be established that dioxane + THF, methanol + ethanol and acetone form different selectivity groups in cluster A. The distribution of solvents on the map indicates that the selectivity of the extraction of colour pigments of paprika powders can be influenced rather by changing the concentration of the organic component than by changing the organic modifier.

The results of stepwise linear regression analysis are compiled in Table 5. Stepwise regression analysis found significant linear relationships between the strengths and selectivity of extraction and the physicochemical parameters of the solvents. The calculated dielectric constant of the solvent mixtures exerted a significant influence both on the strength and selectivity of extraction, while dipole moment and refractive index have no significant effect, suggesting that the characteristics of MAE extraction can be modified by changing the dielectric constant of the extracting agent. We should emphasise that the
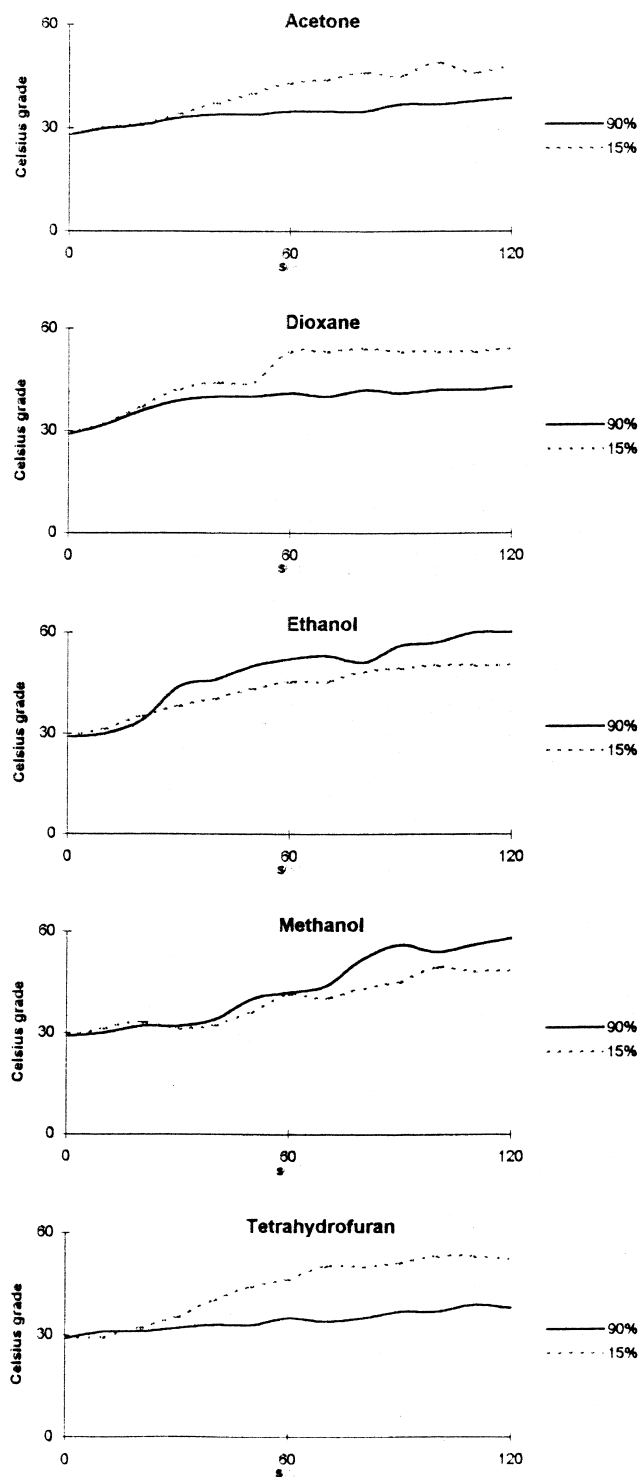
Fig. 1. Dependence of the sample temperature on the time of extraction using various solvent mixtures. Dotted lines refer to the mixtures water–organic modifier (85:15, v/v), continuous lines refer to the mixtures containing 90% (v/v) organic modifier.

Table 4
Overall effect of extraction solvents on the quantity of extracted pigments. Potency values (arbitrary units) calculated by spectral mapping techniques. Numbers refer to solvent mixtures in Table 1

Solvent	Potency	Solvent	Potency	Solvent	Potency
1	0.08	11	0.11	21	0.07
2	0.08	12	0.13	22	0.09
3	0.09	13	0.09	23	0.10
4	0.11	14	0.07	24	0.14
5	0.12	15	0.09	25	0.07
6	0.11	16	0.10	26	0.09
7	0.06	17	0.11	27	0.09
8	0.06	18	0.12	28	0.15
9	0.08	19	0.08	29	0.13
10	0.11	20	0.08	30	0.14

Table 5
Relationships between the strength and selectivity of extraction and the physicochemical parameters of the solvent mixtures. Results of stepwise linear regression analysis. F_1 and F_2 are the corresponding coordinates of the two-dimensional selectivity map, ϵ is the calculated dielectric constant, R is the regression coefficient, $F_{5\%}$ is the test of the null hypothesis, and s_{XY} is the standard error of the estimate ($n = 30$)

$Potency = 0.153 - 1.42 \cdot 10^{-3} \, (\pm 2.18 \cdot 10^{-4}) \cdot \epsilon_r$
$R = 0.8075, \, F_{5\%} = 52.47, \, s_{XY} = 0.015, \, P < 0.0001$

$F_1 = 96.03 + 1.48 \, (\pm 0.26) \cdot \epsilon$
$R = 0.7351, \, F_{5\%} = 32.92, \, s_{XY} = 21.65, \, P < 0.0001$

$F_2 = 147.69 - 1.37 \, (\pm 0.22) \cdot \epsilon$
$R = 0.7646, \, F_{5\%} = 39.41, \, s_{XY} = 18.31, \, P < 0.0001$
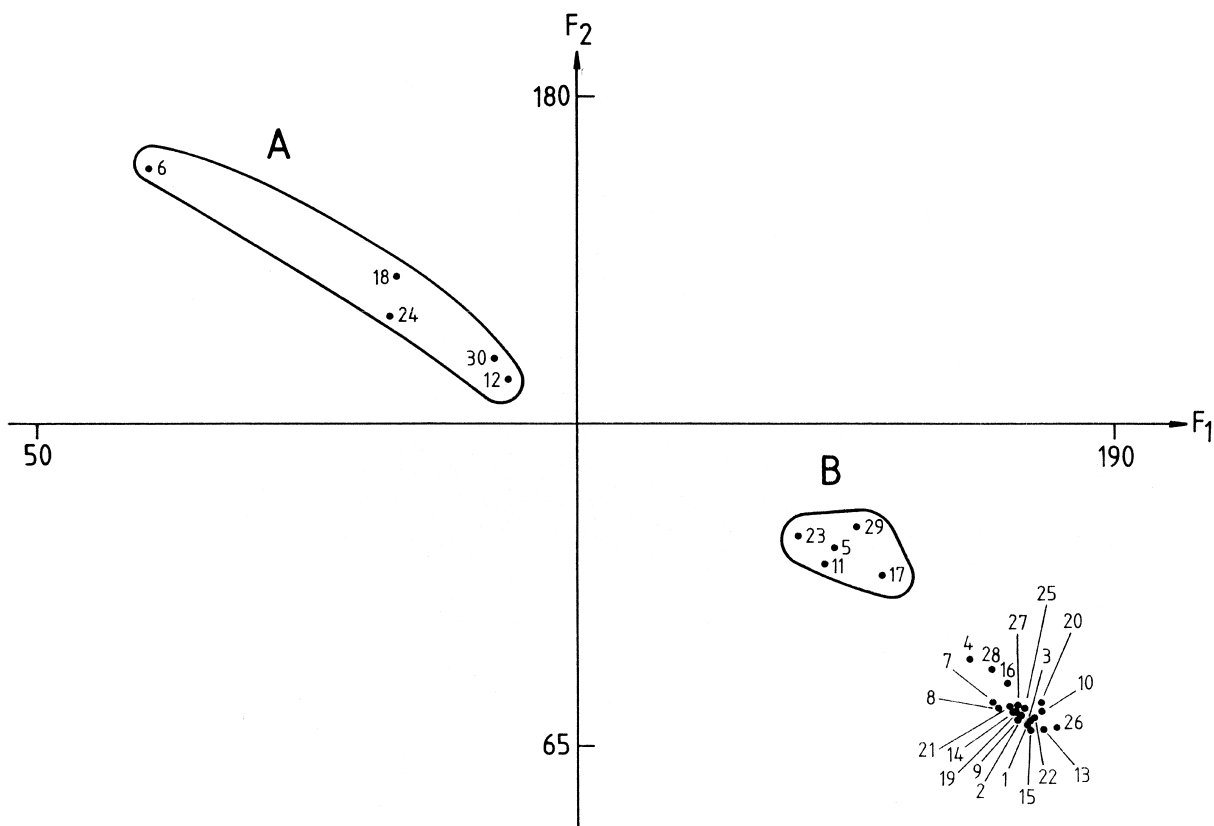

Fig. 2. Similarities and dissimilarities between the selectivity of extraction solvent mixtures. Two-dimensional nonlinear selectivity map of solvent mixtures. Number of iterations, 698; maximum error, $5.51 \cdot 10^{-4}$. Numbers refer to solvent mixtures in Table 1.
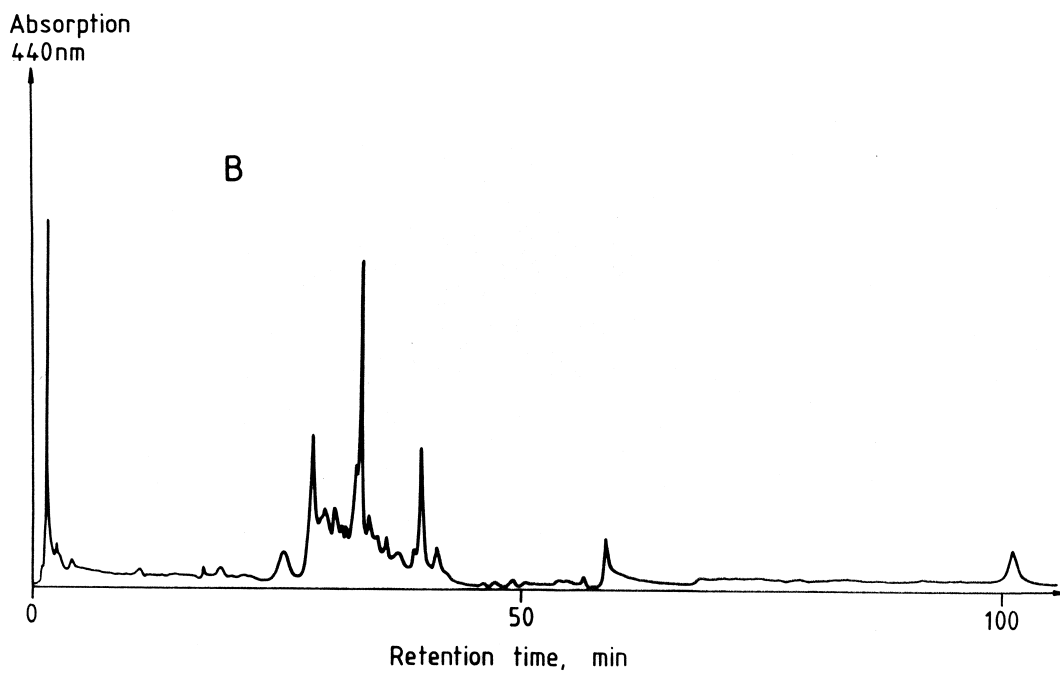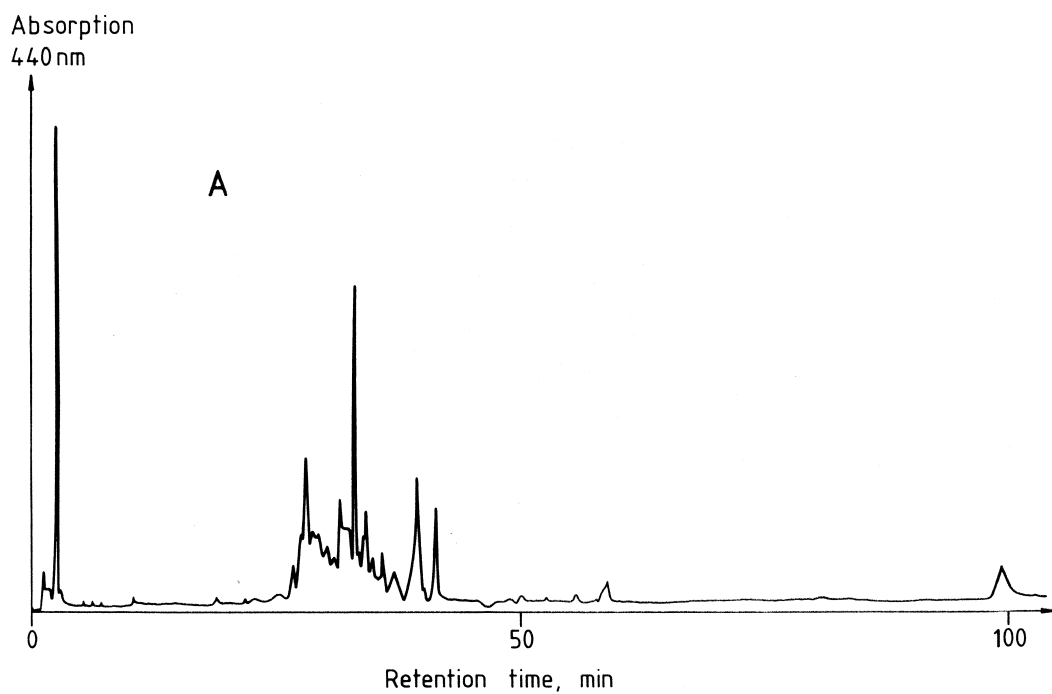
Fig. 3. Separation of the colour pigments of paprika powders extracted with water–acetone (10:90, v/v; chromatogram A) and with water–THF (72:25, v/v; chromatogram B). For chromatographic conditions, see Experimental.

conclusions discussed above are not based on theoretical considerations, therefore their extrapolation to other matrices may lead to serious misinterpretation. The regression coefficients indicate that the change of dielectric constant explains about 54–65% of the change of the characteristics of extraction. This result can be explained by the assumption that other physicochemical parameters of the solvent mixtures not included in the calculation may exert a considerable impact on MAE.

The HPLC chromatograms of pigments extracted with water–acetone (10:90, v/v; mixture 6 in Table 1) and with water–THF (75:35, v/v; mixture 29 in Table 1) are shown in Fig. 3A and B, respectively. The differences in the chromatograms indicate the diverse selectivities of the solvent mixtures. The relative standard deviation of the retention times was lower than 1.5%, showing the good stability of the HPLC system. The relative standard deviation of peak areas was between 3.2 and 4.8%. The relatively low precision of the determination of peak areas can be explained by the presence of overlapping peaks on the chromatogram which markedly increase the error of integration.

The average retention times and peak areas of the selected 13 pigment fractions are listed in Table 6. The data indicate that not only the absolute values of peak areas but also their ratio are different, demonstrating the different selectivity of the extracting solvent mixtures. A significant linear correlation was found between the peak areas:

$$\text{peak area}_{\text{solvent 6}} = 70\,844 + (1.36 \pm 0.36)$$
$$\cdot \text{peak area}_{\text{solvent 26}},$$
$$n = 13, r_{\text{calc.}} = 0.7498, r_{99.0\%} = 0.6614$$

Although the correlation is significant, the correlation coefficient ($r_{\text{calc.}}$) indicates that the distribution of pigment fractions extracted by solvent mixtures 6 and 26 is not identical. The data support the results of SPM calculations concerning the different selectivities of the extracting solvent mixtures.

It can be concluded that the spectral mapping technique combined with two-dimensional non-linear mapping and with stepwise linear regression analysis is a valuable tool for the evaluation of the MAE process and are able to separate the strength and selectivity of various extracting solvent mixtures. SPM indicated that both the overall efficacy and selectivity of the extraction depend considerably on the dielectric constant of the extraction solvent mixture.

Acknowledgements

Table 6
Mean values of retention times (t_R) and peak areas of the selected pigment fractions used for the comparison of solvent mixtures 6 and 26 showing different selectivity

Peak No.	t_R (min)	Area	
		Solvent mixture 6	Solvent mixture 26
1	10.87	4894	6084
2	25.74	462 568	288 455
3	28.20	778 138	466 373
4	29.60	672 933	164 637
5	30.57	272 171	276 718
6	33.14	522 956	389 851
7	34.04	307 870	263 210
8	35.54	221 954	130 151
9	36.04	190 014	194 637
10	39.47	604 011	328 291
11	41.20	467 096	160 948
12	58.87	283 952	137 316
13	100.17	95 366	100 570

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