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Separation and quantitation of colour pigments of chili powder (*Capsicum frutescens*) by high-performance liquid chromatography–diode array detection

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Abstract

The performance of reversed-phase thin-layer (RP-TLC) and reversed-phase high-performance liquid chromatography (RP-HPLC) was compared for the separation and determination of the colour pigments of chili (*Capsicum frutescens*) powder using a wide variety of eluent systems. No separation of pigments was achieved in RP-TLC, however, it was established that tetrahydrofuran shows an unusually high solvent strength. RP-HPLC using water–methanol–acetonitrile gradient elution separated the chili pigments in many fractions. Diode array detection (DAD) indicated that yellow pigments are eluted earlier than the red ones and chili powder contains more yellow pigments than common paprika powders. It was established that the very different absorption spectra of pigments make the use of DAD necessary.

Keywords: *Capsicum frutescens*; Food analysis; Pigments

1. Introduction

The quantity and composition of colour pigments in foods and food products exert a marked influence on consumer acceptance, and consequently, on the commercial value of the products. It has been established many times that one of the main properties employed for the evaluation of the product quality is the colour, that is an adequate colour considerably enhances marketability. The selection of paprika cultivar with a high level of colour pigments is a significant aspect of research on the improvement of paprika quality. The total amount of pigments is generally determined by various spectroscopic methods [1,2]. The pigments are extracted from the accompanying matrix with an organic solvent (generally acetone) and the absorption of the solution is measured at one or more wavelengths (usually 460 nm). Unfortunately, these spectrometric methods do not discriminate between paprika and chili powders having similar colour but different pigment composition, so that both oxidative alteration of natural pigments and the presence of added pigments are undetectable.

Due to the considerable commercial importance of pigment composition much effort has been devoted to the development of liquid chromatographic methods suitable for the separation of colour pigments of paprika and chili powders. Various techniques such as adsorption [3] and reversed-phase thin-layer chromatography (TLC and RP-TLC) [4], adsorption and reversed-phase high-performance liquid chromatog-

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raphy (HPLC and RP-HPLC) [5] using silica [6,7] and octadecylsilica [8,9] supports have been successfully used for the separation of the pigment fractions of paprika. However, the number of separated pigment fractions markedly depends on the chromatographic conditions (quality of support, composition of the mobile phase, etc.). As the quantity and composition of colour pigments considerably depends on the variety of *Capsicum* species [10] the separation of pigments provides information not only about the industrial and commercial value of the product but may also indicate the type of the cultivar used for the production. As both red and yellow pigments are equal indicators of the commercial value, storage stability and provenance (cultivar type) of paprika and chili powders it is very important to determine the concentration and composition of both pigment types. Although many chromatographic methods have been developed for the separation and determination of colour pigments of paprika powders, according to our knowledge the pigments of chili powders have not been studied in detail.

The objectives of our work were the development of new RP-TLC and RP-HPLC methods for the separation and determination of the colour pigments of chili powder, the evaluation of RP-TLC as a possible pilot method for RP-HPLC [11,12] and the assessment of the influence of diode array detection (DAD) on the performance of RP-HPLC analysis. Moreover, the inclusion of RP-TLC in the investigations was motivated by the fact that it is suitable for the detection of pigment fractions that are not eluted in RP-HPLC. These non-moving or slow moving fractions can cause considerable problems in the following analyses. The non-moving fractions can slowly contaminate the RP-HPLC column, and the slow-moving pigment fractions can cause a drifting baseline [13].

2. Materials and methods

A 1 g amount of commercial chili powder (Cholula, Mexico) was shaken for 30 min with 3.0 ml acetone. The suspension was centrifuged and the supernatant was separated. This procedure was repeated as the solid rest was nearly white. The collected supernatants were evaporated to 1.0 ml. Pigment solution (3 μl) was spotted onto the RP-TLC plates. A tenfold dilution of this solution was used for RP-HPLC. Solvents were purchased from Merck (Darmstadt, Germany) and were HPLC quality.

2.1. RP-TLC

Polgram UV_{254} (Macherey–Nagel, Düren, Germany) plates were impregnated by overnight predevelopment in *n*-hexane–paraffin oil (95:5, v/v). Bidistilled water, methanol, acetonitrile, acetone, tetrahydrofuran and dioxane mixed in different ratios were used as mobile phases. To evaluate the effect of pH and sodium chloride for the separation bidistilled water was replaced with diluted acetic acid, sodium acetate and sodium chloride in some instances. Developments were carried out in sandwich chambers at ambient temperature, the running distance being about 16 cm. Due to the light sensitivity of pigments, developments were carried out in the dark. After development the plates were dried at room temperature and were evaluated visually.

2.2. RP-HPLC

The HPLC system consisted of a ISCO Pump Model 2360 (ISCO, Lincoln, NE, USA), a Waters 991 photodiode array detection system (Waters Chromatography Division, Milford, MA, USA), an NEC PowerMate SX/16 computer with PDA program and Valco injector (Valco, Houston, TX, USA) with 20-μl sample loop. The column was 150×4 mm I.D. filled with octadecylsilica support (particle size 5 μm, Merck) in our laboratory with a Shandon analytical HPLC packing pump (Pittsburg, PA, USA). The optimal mobile phase composition for gradient elution of colour pigments consisted of water and methanol–acetonitrile (8:2, v/v) as organic phase. The gradient elution was from 15% to 80% organic modifier in 50 min, isocratic hold for 30 min, to 97% organic phase in 10 min, and isocratic hold for 10 min. Flow-rate was 1 ml/min, the detection wavelength ranged from 300 to 460 nm, including the absorption maximum of yellow and red pigments (340 and 440 nm, respectively). Developments were carried out at room temperature. Each determination was run in quadruplicate and the

relative standard deviations (RSDs) of the retention time, peak height and peak area were calculated. To assess the impact of detection wavelength on the quantitation of pigment fractions absorption spectra of the peaks was compared. The efficiency of separation was checked by the peak purity test carried out at the maximum absorbance.

3. Results and discussion

3.1. RP-TLC

Pigments were not well separated on impregnated silica layers in any of the eluent systems. These results indicate that RP-TLC carried out on impregnated silica support is not suitable for the analysis of pigments of chili powders. Even at higher methanol and acetonitrile concentrations some pigments showed very low mobility while other fractions were eluted together with the solvent front proving that the hydrophobicity of pigments governing RP-TLC retention is highly different. This retention behaviour suggests that the pigments of chili powder can be separated only with gradient elution techniques in the reversed-phase separation mode. Interestingly, tetrahydrofuran showed the highest elution (solvent) strength, although – according to the traditional solvent strength system – tetrahydrofuran is a moderately strong solvent. The good separation capacity of tetrahydrofuran has been previously reported [14]. We have to admit that we do not have any valid hypothesis for the explanation of this discrepancy. In our opinion the high elution strength of tetrahydrofuran can be exploited in the future for the separation of slowly-moving pigments in any reversed-phase liquid chromatographic system.

3.2. RP-HPLC

Colour pigments of chili powder were separated into many fractions under the optimal RP-HPLC conditions as demonstrated in Figs. 1 and 2. The number of pigment fractions is higher at 340 nm than at 440 nm, indicating that this chili powder contains more yellow than red pigments. This observation is in contrast with that observed for paprika powders

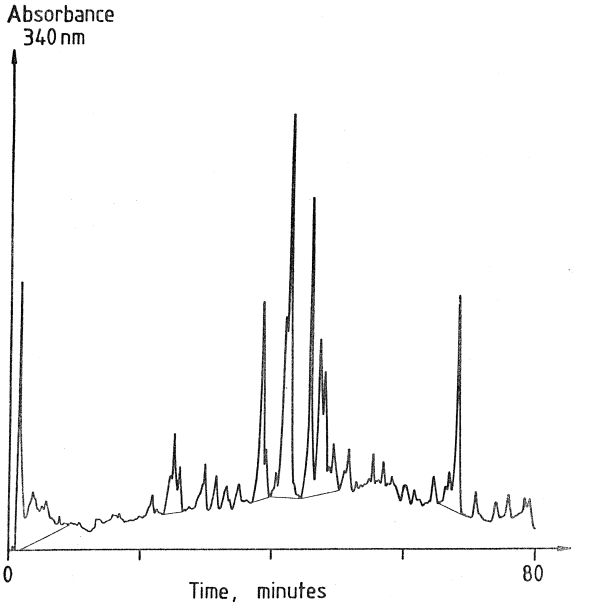

Fig. 1. Separation of the colour pigments of a chili powder on an octadecylsilica HPLC column. Detection wavelength 340 nm. For chromatographic conditions see Materials and methods.

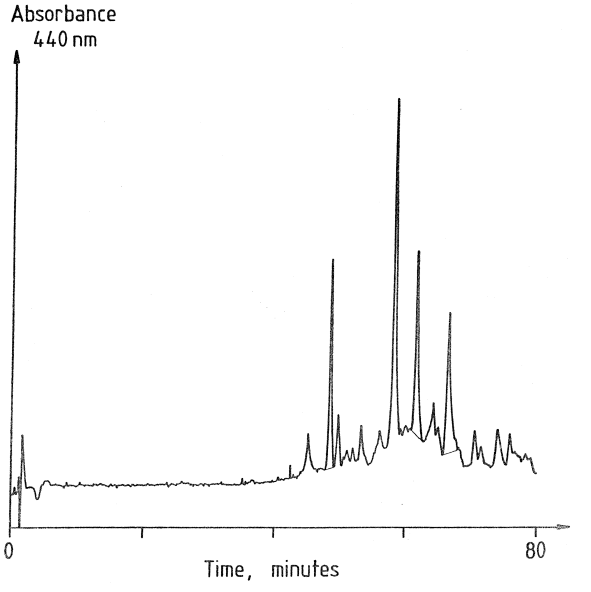

Fig. 2. Separation of the colour pigments of a chili powder on an octadecylsilica HPLC column. Detection wavelength 440 nm. For chromatographic conditions see Materials and methods.

where more pigment fractions can be detected at 440 than at 340 nm [15]. The chromatograms further show that the yellow and red pigment fractions are more and less separately eluted from the column; the yellow ones are earlier eluted suggesting that they are less hydrophobic than the red ones. When authentic standard compounds are not available, the relative concentrations of solutes are generally calculated from their absorption (peak area or peak height) at the wavelength of detection. In the instance this approximation leads to considerable error.

Some fractions show very low adsorption at 340 or 440 nm (Fig. 3) indicating that the calculation of pigment composition according to the absorbance either at 340 or at 440 nm is basically wrong.

More or less correct quantitative evaluation can be carried out at the maximum absorbance of each fraction which is feasible with DAD. The retention time, relative concentration of colour pigments according to the detection at 340 and 440 nm are summarised in Tables 1 and 2, respectively. The low values of the RSDs indicate the good reproducibility of the method and the stability and reliability of the

Table 1
Retention times and relative concentrations of pigment fractions of Chili seco[a]

Peak No.	Retention time (min)	Relative concentration (%)
1	1.41	9.64
2	3.52	5.71
3	4.92	1.25
4	5.58	0.43
5	24.67	2.09
6	25.17	2.73
7	26.02	1.48
8	29.83	2.60
9	31.54	1.38
10	38.39	7.78
11	39.01	1.48
12	41.88	8.42
13	42.49	14.92
14	45.70	11.37
15	47.09	7.02
16	47.79	5.59
17	49.24	1.94
18	51.57	2.31
19	66.83	2.17
20	72.23	9.03
21	75.82	1.11

[a] Detection wavelength 340 nm.

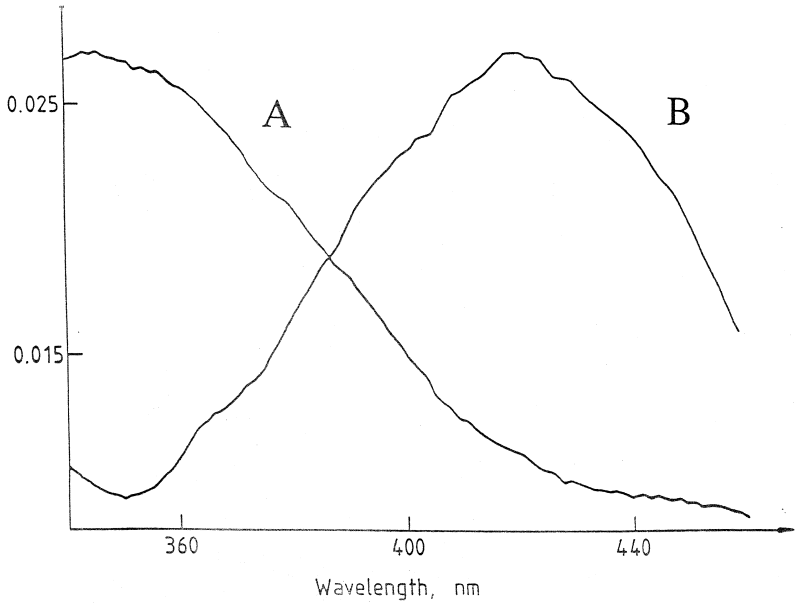

Fig. 3. Absorption spectra of pigment fractions eluted at 42.49 (A) and 48.61 min (B).

Table 2
Retention times and the relative concentrations of pigment fractions of Chili seco[a]

Peak No.	Retention time (min)	Relative concentration (%)
1	45.12	7.92
2	48.61	19.25
3	49.11	5.11
4.	59.12	24.34
5	61.55	22.17
6	66.34	14.51
7	66.83	1.92
8	72.23	2.02
9	75.82	2.76

[a] Detection wavelength 440 nm.

HPLC–DAD system. The matching values of the peak purity test varied between 80.67 and 97.21% indicating that the colour pigments of chili powder are well separated and the quantity of co-eluted pigment impurities is fairly low. However, it has to be emphasized that the peak homogeneity check does not necessarily measure the peak homogeneity when the fractions are structurally similar and have the same chromophores and retention characteristics.

4. Conclusion

It can be concluded from our data that RP-TLC is not suitable for the analysis of the colour pigments of chili powder, therefore it cannot be used as a pilot method for RP-HPLC. Conversely, HPLC–DAD using a water–methanol–acetonitrile eluent system and gradient elution has been proved to be a valuable tool for the separation and determination of the pigment fractions of chili powder. The pigment composition deviates considerably from that of common paprika powder. As chili powder contains both yellow and red pigment components, DAD is necessary for the exact evaluation of the chromatograms.

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