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Extraction and determination of capsaicinoids in fruit of hot pepper *Capsicum annuum* L. by spectrophotometry and high-performance liquid chromatography

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Abstract

The simple spectrophotometric method of determination of capsaicinoids in fresh fruit of hot pepper and the HPLC method were compared. Capsaicinoids were extracted from hot pepper fruit with the petroleum ether:acetone mixture, and then separated using thin layer chromatography on silica gel and evaluated quantitatively using the HPLC method and spectrophotometrically. The high correlation factor obtained for these methods (0.93) proved that either method can be used for determining the total of capsaicinoids in fresh and powdered hot pepper fruits isolated by the TLC method and that both can be successfully used in laboratories that are not so well equipped. © 2000 Published by Elsevier Science Ltd.

Keywords: *Capsicum annuum*; TLC; Spectrophotometry; HPLC; Capsaicinoid evaluation

1. Introduction

For a long time hot pepper fruit has been known all over the world as a delicious spice with a characteristic smell and taste. It is used for preparing spicy sauces and also in Mexican and Asian cuisines. Popularity of the latter has been growing recently (Henderson & Henderson, 1992). The value of hot pepper consists in its sensorial attributes — color, spiciness and flavor. The group of alkaloids called capsaicinoids that are typical of this plant are responsible for spiciness of pepper fruit. Twelve compounds have been identified (Kobata, Todo, Yazawa, Iwai & Watanabe, 1998) but capsaicin and dihydrocapsaicin are responsible for about 90% of the spiciness. The remaining analogues differ in the length of the hydrocarbonaceous chain and the degree of unsaturation (Iwai, Suzuki & Fujiwake, 1979; Kawada et al., 1985; Lopez-Hernandez, Oruna-Coucha, Simal-Lozano, Gonzalez-Castro & Vasquez-Blanco, 1996).

Capsaicinoids are derivatives of phenylopropanoid compounds (Govindarajan & Sathyanarayana, 1991; Sudhakar, Ravishankar & Venkataraman, 1992; Perucka, 1996a). They are characterized by a high biological activity and their pharmacological, neurological and dietetic effectiveness is well known. They influence homologous pain receptors, peripheral and central heat detectors and pulmonary aortas (Saria, Lembeck & Skofitsch, 1981). Capsaicinoids, even when their contents is lower, show a positive effect on aliphatic and carbohydrate metabolism (Govindarajan & Sathyanarayana, 1991). The concentration of capsaicinoids in hot pepper varieties ranges from 0.003 to 0.01%; varieties of mild chillies contain from 0.5 to 0.3%, and strong chillies are characterized by a content higher than 0.3%, reaching about 1%. Both hot pepper and chillies are produced in increasing amounts in Europe and India. International demand for strongly colored hot pepper fruit arouses hope for an increase in the preference for spiciness in food products.

For quality control fast and simple methods that do not require very modern equipment are needed (Govindarajan & Sathyanarayana, 1991). Immediate tests of the fruit proved unsatisfactory (quoted after Anan, Ito, Matsunaga & Monma, 1996). On the other hand the application of instrumental methods of gas–liquid chromatography (Krajewska & Powers, 1987; Todd, 1977) and of high performance liquid chromatography

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Contreras-Padilla & Yahia, 1998; Hall, Holden & Yeoman, 1987; Lopez-Hernandez, Oruna-Coucha, Simal-Lozano, Gonzalez-Castro & Vasquez-Blanco, 1996; Saria et al., 1981; Sudhakar et al., 1992 Weaver & Awde, 1986; Yao, Nair & Chandra, 1994) calls for complicated preparations and costly instruments.

The method meeting all the requirements, i.e. one that is reliable, not too expensive, portable and easy to use is the thin layer chromatography method (TLC). It can be used not only for preliminary purification of the extract before the HPLC analysis (Iwai et al., 1979) or for quantitative separation on HPTLC (Aczel, 1989; Fujiwake, Suzuki, Oka & Iwai, 1980), but also for quantitative determination of total capsaicinoids (Jentzsch, Kulbeka & Pock, 1969; Iwai, Suzuki, Lee, Kobashi & Oka, 1977). The aim of the present method was to compare the simple spectrophotometric method of determination of capsaicinoids in fresh fruit of hot pepper with the HPLC method, after preliminary purification of capsaicinoids by TLC.

2. Materials and methods

Hot pepper *Capsicum annum* L. var. Bronowiecka was grown in a field of the Agricultural Experimental Institute in Felin near Lublin. Fruits were picked at the stage of full ripeness and divided into pericarps, placenta and seeds. The pericarps were used for analysis. All chemicals used were of HPLC grade. Capsaicin, 2,6-dichlorochinonechloroimide and TLC ready to use plates (Silica gel 60 GF$_{254}$) were from Merck.

2.1. Extraction

Samples of hot pepper pericarps (10 g of fresh material) were homogenised with acetone and then with a mixture of acetone and petroleum ether (1:1) until the tissue was decolorized. The extract was filtered under reduced pressure and was put into a separatory funnel where acetone was washed out with small portions (0.01 ml) of water. The obtained ether layer was dried with anhydrous Na_2SO_4 and concentrated with vacuum evaporator at 30°C. The extraction procedure is presented in Fig. 1.

2.2. Isolation and identification of capsaicinoids

The ether extract (1 ml) was put on the TLC plate and developed in petroleum ether:acetate:methanol (75:20:5), according to the procedure recommended for separation of carotenoids (Davies, Matthews & Kirk, 1970). Three plates with purified extracts and one with capsaicin standard (0.4 ml, 0.2 mg/ml) were developed in parallel. In order to locate the capsaicinoids on the plate, one of them was sprayed with the solution of 2,6-dichlorochinonechloroimide (Jentzsch et al., 1969). The R_f values were determined for capsaicinoids and carotenoids, respectively (Table 1). The bands corresponding to capsaicinoids were scraped off the plates not being sprayed and the compounds were washed out of silica gel with methanol. They were used for colorimetric and HPLC determination.

2.3. Determination of capsaicinoids

The TLC purified capsaicinoids were dissolved in methanol (5 ml) and the solution was treated with 2,6-dichlorochinonechloroimide (5 ml) and the absorption was measured at 590 nm with a spectrophotometer. For

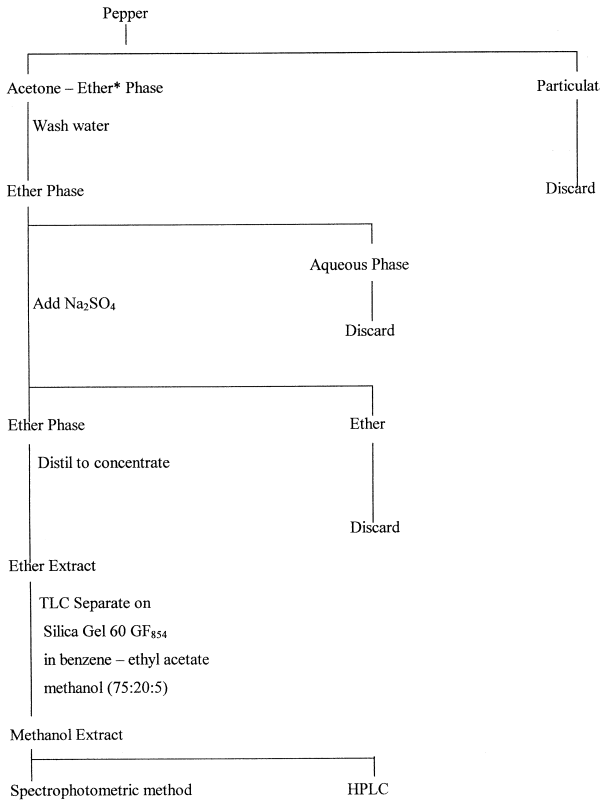

Fig. 1. Flow diagram for extraction of capsaicinoids from fresh pepper fruits.

Table 1
The R_f values of carotenoids and capsaicinoids from *Capsicum annuum* L. fruits

Carotenoids	R_f
β-Carotene	0.98
Cryptoxanthin	0.64
Zeaxanthin	0.58
Antheraxanthin	0.42
Violaxanthin	0.35
Capsanthin	0.29
Capsorubin	0.23
Neoxanthin	0.18
Capsaicinoids	0.31

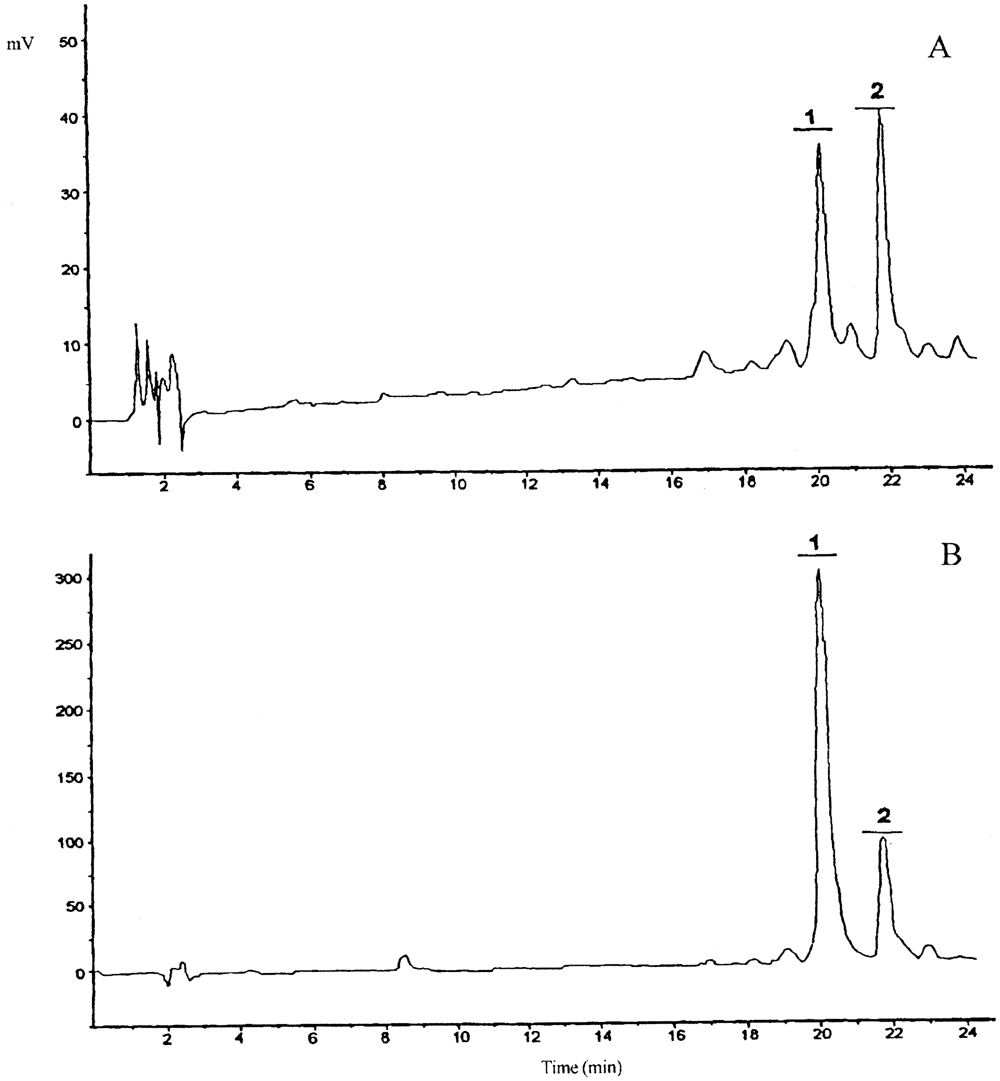

Fig. 2. HPLC Chromatogram A of extract of hot pepper fruits, B of standard solution, 1 — capsaicin, 2–dihydrocapsaicin.

quantification, the standard curve of 0.2, 0.4, 0.6 ml of standard solution (0.2 mg/ml) was developed on TLC and prepared in a similar way to the extracts.

For HPLC determination the Knauer chromatograph equipped with a computer control system, data processor and UV detector was used. Separations were performed on Eurospher 80 (C18) column and 1 ml/min solvent delivery. The linear gradient of 100% A (10% acetonitrile in water) and 100% B (90% acetonitrile) lasting 30 min was used as a solvent system. The concentration of capsaicinoids was calculated from the reference capsaicin and dihydrocapsaicin (Fig. 2) for which a standard curve was calculated.

2.4. Results and discussion

In the present study a mixture of acetone and petroleum ether was used for extracting capsaicinoids (Fig. 1). Earlier reports by other authors on the subject of extraction of capsaicinoids from hot pepper fruit say that the most often used dissolvents were:acetone (Krajewska & Powers, 1987; Saria et al., 1981; Sass et al., 1977; Weaver & Awde, 1986), chloroform (Jentzsch et al., 1969; Spanyar & Blazovich, 1969), ethyl acetate (Attila, 1989; Bajaj, 1980), 50% methanol (Andre & Mile, 1975), or 80% ethanol (Sudhakar et al., 1992).

Most researchers additionally purified the obtained extracts by alkalization and removing colloidal compounds. Some of them used alkaline diethyl ether in methanol, or ethyl acetate for extraction (Krajewska & Powers, 1987; Sass et al., 1977).

In the present study the process was simplified (Fig. 1). Besides capsaicinoids the extract also contained carotenoids. Separation of these two groups of compounds was done by the method of thin layer chromatography with the use of the system of dissolvents suggested by

Table 2
Capsaicin, dihydrocapsaicin and capsaicinoid contents in *Capsicum annuum* L. fruit (mg/g.w)

Sample no.	HPLC method capsaicin + dihydrocapsaicin total	Spectrophatometric method capsaisinoids	Correlation coefficient
1	0.737	0.724	
2	0.622	0.698	
3	0.737	0.715	
4	0.67 1	0.690	
5	0.765	0.750	
Mean	0.706	0.715	0.933
S.D.	0.0 14	0.020	

Davis et al. (1970) for determination of carotenoids — benzene:ethyl acetate:methanol (75:20:5) which apart from carotenoids also separates capsaicinoids. Use of this system seems to be interesting because of the possibility of simultaneous determination of the content of β-carotene and the main, red pigments of hot pepper: capsantine and capsorubine, whose R_f values are presented in Table 1. The results of RI$_f$ for particular carotenoid bands agree with the ones presented in the literature (Davis et al., 1970) and make it possible to identify and to quantitatively determine these compounds by the densitometric or spectrophotometric method (Perucka, 1996b).

Determination of capsaicinoids content in fresh fruit of hot pepper var. Bronowicka Ostra was carried out by the spectrophotometric method and the HPLC. The results of the data analysis describing the mean measure error and the correlation coefficient refer to the study carried out in 5 replicates and determined parallel by two methods and they are presented in Table 2. The obtained results confirm the proposition that the spectrophotometric method of determining capsaicinoids after their isolation on TLC may by successfully applied when it is enough to determine the capsaicinoids total, for the contents of capsaicin and dihydrocapsaicin determined by the HPLC are highly correlated with the capsaicinoids total determined by the spectrophotometric method.

3. Conclusions

1. Spectrophotometric determination of isolated capsaicinoids by the TLC is comparable to determination by the HPLC. The correlation coefficient for these two methods is high (0.93).
2. Besides capsaicinoids this method allows in a simple way to determine the level of carotenoids in fresh and powdered hot pepper fruit and can be successfully used in laboratories that are not so well equipped.

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