

Identification and Quantification of Capsaicin from *Capsicum Annuum* L. by RP-HPLC

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Characterization and extraction of capsaicin from different plant species is in continuous expansion due to its curative and preventive effects in many diseases.

*In this study, two varieties of *Capsicum annuum* L. were investigated for their capsaicin content using a simple RP-HPLC method. Capsaicin extraction was accelerated by ultrasonication. The highest content was found in dried green pepper (85.262 mg/L) followed by dry red peppers that were previously removed the seeds and ribs (75.457 mg/L).*

Keywords: capsaicin, RP-HPLC, ultrasonication extraction, *Capsicum annuum* L

Capsaicin was used from ancient times by humans as a spice or as medicine, and has been the subject of thousands of research papers [1,2].

From the structural point of view it is a fat and alcohol soluble alkaloid (fig. 1), which is found in the fruit ribs of *Capsicum* genus.

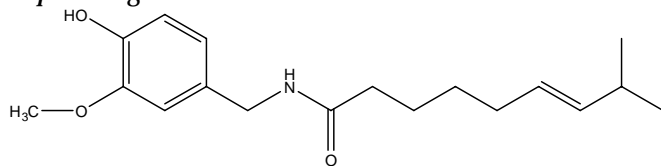


Fig. 1. Capsaicin structure

Topical application of capsaicin as an alcoholic extract of chili pepper was first used in the treatment of burns. The pharmacological activity of capsaicin and its primary receptors were analyzed at large scale, capsaicin being an exogenous agonist highly selective and potent for TRPV1 receptor, a transmembrane ion-receiver channel offering integrated responses to temperature, pH and endogenous lipids.

Formula optimization for topical application of capsaicin containing products or other analgesics to relieve pain, but with the fewest side effects is not limited only to vary the concentration of active substances.

Creams, lotions and patches, containing up to 0.1% of capsaicin applied over a period of 2 to 6 weeks are used in several countries for the treatment of neuropathic and musculoskeletal pain caused by osteoarthritis [3], post herpetic neuralgia or painful diabetic neuropathy [4].

Other pharmaceutical uses of capsaicin include stimulating the immune system, decrease in body weight, treating certain respiratory diseases, vasodilator effect, lower cholesterol [5] or cancer prevention [6] etc.

High performance liquid chromatography is a method applied successfully coupled with UV detection or MS in quantifying plant secondary metabolites [7,8].

In recent years, the capsaicin content of pepper belonging to the *Capsicum* genus was determined by various HPLC methods. Thus, Thapa et al. (2009)

determined capsaicin from 16 kinds of capsicum fruit by HPLC with UV detection after solid phase extraction procedure [9].

Experimental part

Plant material

The raw material was collected from the cultivated flora. The fruits of *Capsicum annuum* were identified and marked with two voucher specimens. After harvesting, samples were cleaned and rinsed with bidistilled water and the damaged portions were removed. A part of the raw material was air-dried in a dark room at constant humidity and temperature. After drying, the samples were grounded to fine powder with the help of a stainless steel grinder.

Capsaicin extraction from dried and raw material

For a quantitative extraction of capsaicin, 1 g of sample (dried red pepper and dried or raw green peppers) was subjected to ultrasonic-assisted extraction using ethanol as solvent. The used extraction ratio was of 1:20 (w/v). Extraction was conducted at a temperature of 60 °C for one hour. Previously dried product was finely grounded while green product was transformed into paste with a disintegrator.

Equipment

It was used a Thermo-Surveyor HPLC liquid chromatograph with the following characteristics:

- Finnigan Surveyor Plus LCPMPQ quaternary pump with degasser;
- Finnigan Surveyor Plus ASP autosampler, Peltier thermostat;
- Surveyor detector "Diode Array" PDA5P,
- 250 x 4.6 mm, 5µm Hypersil HyPurity column;
- Chrom Quest 4.2 software for device control, data acquisition and processing.

Experimental procedures

The operational parameters of the established method are: temperature: 25 0C; isocratic elution mode; mobile

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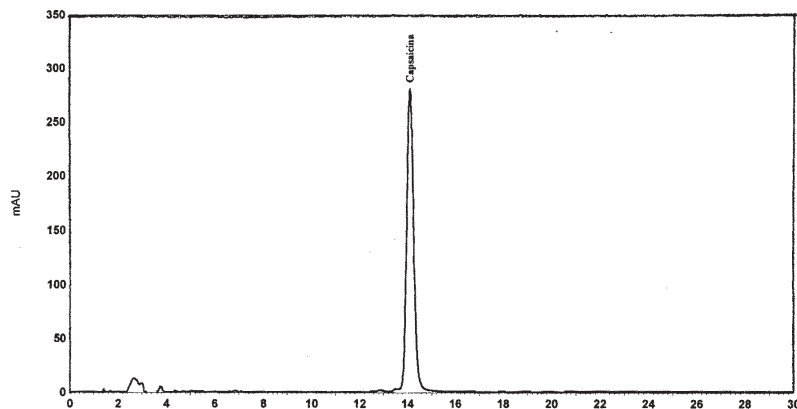


Fig. 2. Chromatogram obtained for a standard solution of capsaicin

phase: water: acetonitrile 50:50; mobile phase flow rate - 1 mL/min; Injection volume - 5 mL; UV-VIS detection, $\lambda=205$ nm, $\lambda=228$ nm, $\lambda=278$ nm.

All used chemicals were of analytical grade.

Results and discussions

Materials and methods

Although the synthesis of structurally similar compounds (capsaicinoids and capsinoids) [10,11] with no spicy taste was exponentially developed, however the healing properties of capsaicin could not be matched.

Sample preparation and extraction has a crucial role in obtaining reproducible results [12]. Fruit harvesting was made the 40th day of development when capsaicin reach maximum concentration [13].

In order to establish the retention time and wavelength for the identification and quantification of capsaicin, the chromatogram for a standard solution (at a concentration of 75 mg/L) was drawn (fig. 2).

The retention time of the capsaicin for up to 15 min an advantage because it gives a short analysis time.

In the spectrum of the pure compound (75 mg/L) were observed three peaks at $\lambda=204$ nm, $\lambda=227$ nm, $\lambda=280$ nm (fig. 3).

The calibration curve used in determining capsaicin of the vegetal extracts is shown in figure 4. The used method has a very good accuracy (recovery vary between 96-103%) and the calibration curve has a good linearity.

The results of HPLC determination of capsaicin in red and green pepper are resumed in table 1.

The highest content was found in dried green pepper (85.262 mg/L) followed by dry red peppers that were previously removed the seeds and ribs (75.457 mg/L). The results are consistent with other results in the literature. For example, Musfiroh et al. [14] found the same surprising result, capsaicin is found in greater quantity in the green peppers than in the red ones, from the same species

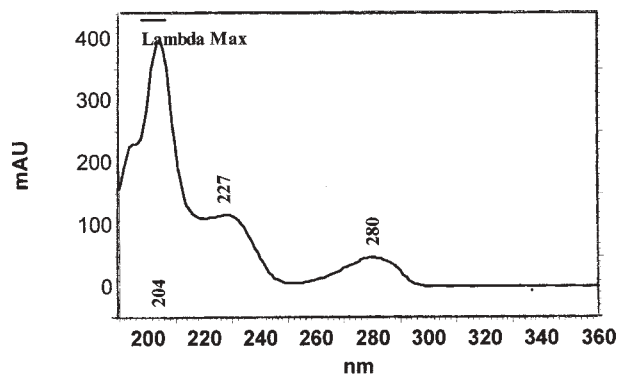


Fig. 3. UV-Vis spectrum of pure capsaicin

Peak: Capsaicin -- ESTD -- PDA-205nm

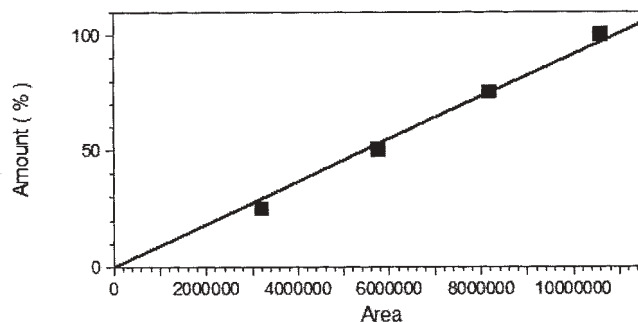


Fig. 4. The obtained calibration curve for HPLC determination of capsaicin. After plotting the calibration curve, the obtained extracts were subjected to HPLC analysis.

(*Capsicum frutescens*). Ultrasonic-assisted extraction helped to the extraction of large quantities of the compound, as reported for other compounds in the literature [15].

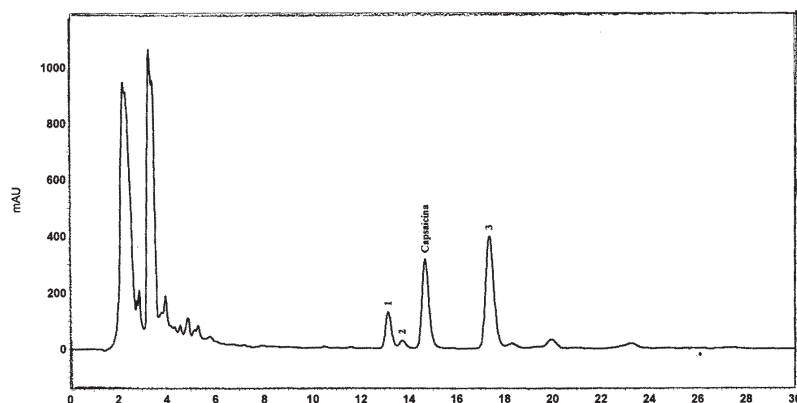


Fig. 5. The chromatogram of red pepper extract (seedless, several ribs)

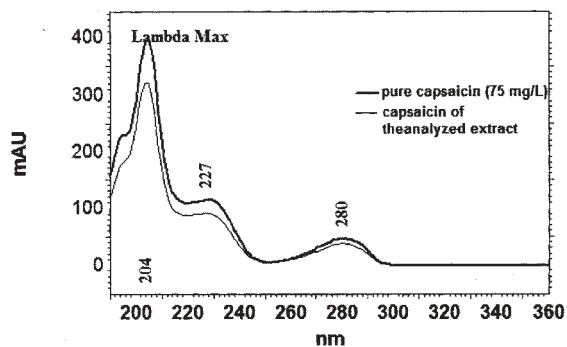


Fig. 6. UV-Vis overlapped spectra of the pure compound and of the extracted capsaicin

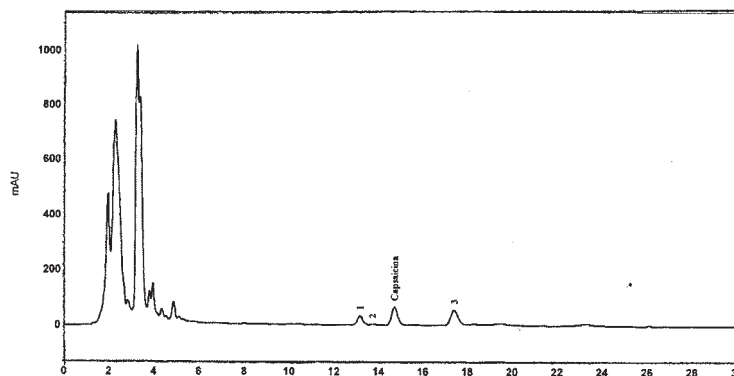


Fig. 7. The chromatogram of green pepper extracts (raw material)

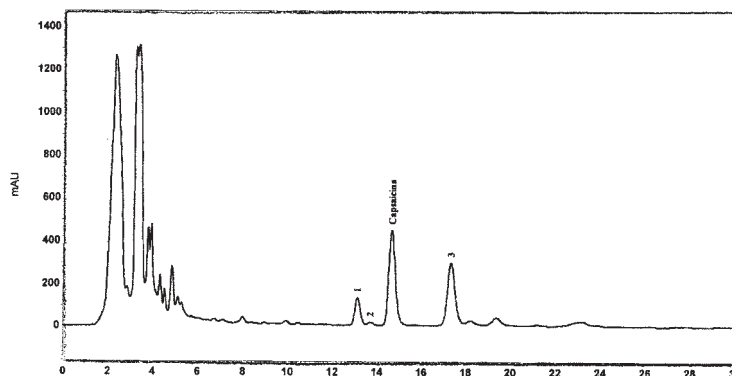


Fig. 8. The chromatogram of green pepper extract (dried material)

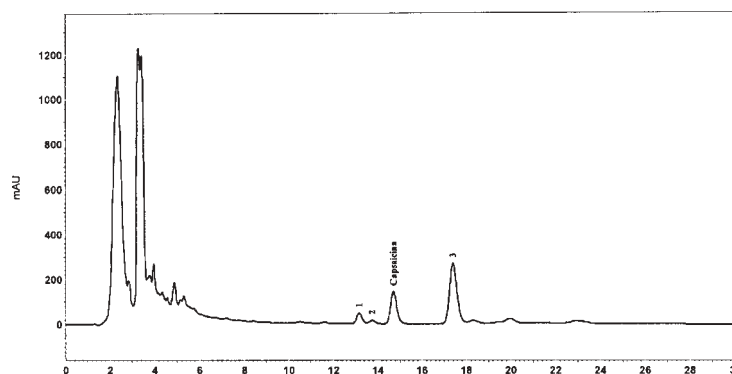


Fig. 9. The chromatogram of red pepper extracts (pulp with seeds and ribs)

Table 1
CAPSAICIN CONTENT IN *CAPSICUM ANNUUM*

Sample	Capsaicin (mg/L)
red pepper extract - pulp	75.457
red pepper extract (pulp with seeds and ribs)	32.736
green pepper extract (raw material)	11.22
green pepper extract (dried material)	85.262

Conclusions

The active secondary metabolite capsaicin was determined in *Capsicum annuum* by RP-HPLC. Although limited by its burning pungency, the large amount of capsaicin of the extract enables pharmaceutical applications.

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