

# Ultrasound-assisted extraction of capsaicinoids from peppers

G.F. Barbero, A. Liazid, M. Palma\*, C.G. Barroso

*Departamento de Química Analítica, Facultad de Ciencias, Universidad de Cádiz, P.O. Box 40, 11510 Puerto Real, Cádiz, Spain*

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## Abstract

The development of a rapid, reproducible and simple method of extraction of the majority capsaicinoids (nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin and homodihydrocapsaicin) present in hot peppers by the employment of ultrasound-assisted extraction is reported. The study has covered four possible solvents for the extraction (acetonitrile, methanol, ethanol and water), the optimum temperature for extraction (10–60 °C), the extraction time (2–25 min), the quantity of sample (0.2–2 g), and the volume of solvent (15–50 mL). Under the optimum conditions of the method developed, methanol is employed as solvent, at a temperature of 50 °C and an extraction time of 10 min. The repeatability and reproducibility of the method (R.S.D. < 3%) have been determined. The capsaicinoids extracted have been analysed by HPLC with fluorescence detection and using monolithic columns for the chromatographic separation. The method developed has been employed for the quantification of the various capsaicinoids present in different varieties of hot peppers cultivated in Spain.

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## 1. Introduction

Capsaicinoids are the compounds responsible for the hot, spicy flavour presented by many varieties of peppers. Among the many natural capsaicinoids found in hot chilli peppers, two compounds are predominant: capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-*N*-vanillylnonanamide) [1]; they represent around 90% of the total capsaicinoids present in the hot spicy varieties of peppers. In addition to these two major capsaicinoids, other minor capsaicinoids are found in hot peppers, including nordihydrocapsaicin, norcapsaicin, homocapsaicin I and II, homodihydrocapsaicin I and II, nornorcapsaicin, nornornorcapsaicin, and nonivamide, among others [2,3]. The structural characteristic of capsaicinoids that determines their spicy properties is associated with the presence of an amide bond connecting a vanillyl ring and an acyl chain [4].

Hot peppers are one of the most important species cultivated widely around the world. The properties of colour, aroma, flavour and pungency presented by these peppers account for their extensive usage. In addition to these culinary properties,

capsaicinoids present many biological activities. Among these activities, capsaicinoids act as powerful antioxidants [5], present anti-mutagenic and anti-tumoral properties [6,7], function as topical analgesics against pain [8], have anti-inflammatory properties [9] and stimulate the cardiovascular and respiratory systems [10].

Many different techniques have been employed for the extraction of capsaicinoids from pepper, such as maceration [11], magnetic stirring [12], enzymatic extraction [13], ultrasound-assisted extraction [14], Soxhlet [15], extraction by supercritical fluids [16], extraction by pressurized liquids [17] and microwave-assisted extraction [18,19]. The conventional extraction methods, like Soxhlet extraction, which have been employed for decades, need long extraction times and require relatively large quantities of solvent [20]. Recent years have seen increasing demand for extraction techniques that shorten extraction times and reduce the consumption of organic solvents. Among these more efficient extraction techniques are ultrasound-assisted extraction (UAE), microwave-assisted extraction, supercritical fluid extraction and accelerated solvent extraction. The UAE technique is particularly attractive because of its simplicity and low equipment cost; it is based on the employment of the energy derived from ultrasounds (sound waves with frequencies higher than 20 kHz) to facilitate the extraction of analytes from the solid sample by the organic

\* Corresponding author. Tel.: +34 956 016775; fax: +34 956 016460.  
E-mail address: [miguel.palma@uca.es](mailto:miguel.palma@uca.es) (M. Palma).

solvent, which is selected in function of the nature of the solutes to be extracted [21]. This technique has been employed to extract various organic compounds from different matrices, including phenolics in cosmetic creams [22], chlorinated pesticides in bird livers [23], organic acids in grapes [24], phenolic compounds from strawberries [25] or isoflavones from soybeans [26].

The enhancement of extraction efficiency of organic compounds by ultrasound is attributed to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave. Cavitation bubbles are produced and compressed during the application of ultrasound. The increase in the pressure and temperature caused by the compression leads to the collapse of the bubble. With the collapse of bubble, a resultant “shock wave” passes through the solvent enhancing the mixing [27].

Ultrasound also exerts a mechanical effect, allowing greater penetration of solvent into the sample matrix, increasing the contact surface area between solid and liquid phase. This, coupled with the enhanced mass transfer and significant disruption of cells, via cavitation bubble collapse, increases the release of intracellular product into the bulk medium. The use of higher temperatures in UAE can increase the efficiency of the extraction process due to the increase in the number of cavitation bubbles formed [27–29].

Although studies have been published on the employment of UAE for the recovery of capsaicinoids from peppers [14], these not have evaluated the influence of the extraction variables nor has a systematic study for the optimisation of the method been carried out; therefore no specific protocol for the UAE of capsaicinoids in peppers has been produced. Thus, the object of the work reported here is to perform the optimisation of the various extraction parameters, particularly the appropriate solvent, temperature, extraction time, quantity of sample, etc. It is also intended to utilise the method developed to quantify the capsaicinoids present in several varieties of hot peppers cultivated in Spain.

## 2. Experimental

### 2.1. Chemical and reagents

The solvents utilised: ethanol (Panreac, Barcelona, Spain), methanol, acetonitrile and glacial acetic acid (Merck, Darmstadt, Germany), are of HPLC grade. The water was obtained by a Milli-Q water purification system, from Millipore (Bedford, MA, USA). The capsaicinoid standards: capsaicin (97%) and dihydrocapsaicin (90%), and the internal standard 2.5 dihydroxybenzaldehyde utilised were obtained from Sigma–Aldrich (Steinheim, Germany).

### 2.2. Plant material

The hot Cayenne pepper (*Capsicum frutescens* L.) was employed for the development of the ultrasound-assisted extraction method. They were obtained from local markets. The peppers were peeled, and the peduncle and seeds were separated. Only the pericarp and the placenta of the pepper were

studied. Both the pericarp and the placenta were triturated with a conventional beater, until a homogeneous sample was obtained for the analysis. The triturated sample obtained was conserved in a freezer at  $-20^{\circ}\text{C}$  until its analysis.

### 2.3. Extraction procedure

The extraction of capsaicinoids originating from peppers by means of ultrasound was performed employing various different extraction conditions—solvents: methanol, ethanol, acetonitrile and water; percentage of water in methanol: 0–100%; temperature:  $10\text{--}60^{\circ}\text{C}$ ; volume of solvent: 15–50 mL; quantity of sample: 0.2–2 g; extraction time: 2–25 min. A volume of 0.5 mL of internal standard was added to the extracts obtained (1300 ppm). The extracts were filtered through a  $0.45\ \mu\text{m}$  nylon syringe filter (Millex-HN, Ireland) before the chromatographic analysis.

The extraction by ultrasound was performed in an ultrasonic bath of 360 W (J.P. Selecta, Barcelona, Spain) coupled to a temperature controller, which allowed the water in the bath to be renewed.

### 2.4. HPLC-fluorescence analysis

The HPLC-fluorescence analysis was carried out in a Dionex chromatographic system (Sunnyvale, CA, USA), consisting of an automated sample injector (ASI-100), pump (P680), thermostated column compartment (TCC-100), a photodiode array detector (PDA-100), a fluorescence detector (RF 2000), a universal chromatography interface (UCI-50) and Chromeleon 6.60 software. Capsaicinoids were separated using a Chromolith Performance PR-18e (100 mm  $\times$  4.6 mm) monolithic column (Merck).

The chromatographic separation was performed with extracts of the hot Cayenne pepper (*C. frutescens* L.). The wavelengths employed for the detection were 278 nm (excitation) and 310 nm (emission).

The method of chromatographic separation utilised a gradient of two solvents: acidified water (0.1% acetic acid, solvent A) and acidified methanol (0.1% acetic acid, solvent B), working at a flowrate of 6 mL/min. The gradient method utilised is the following: 0 min, 10% B; 2 min, 50% B; 4 min, 50% B; 4.5 min, 55% B; 5.5 min, 55% B; 6 min, 60% B; 7 min, 60% B; 9 min, 70% B; 10 min, 100% B; 15 min, 100% B. The temperature of the column was held constant at  $30^{\circ}\text{C}$ . The chromatogram obtained by utilising this separation method is represented in Fig. 1.

### 2.5. Calibration

Using the method developed, calibration curves were prepared for capsaicin and dihydrocapsaicin, which are the two capsaicinoid standards commercially available. The results obtained are presented in Table 1. The limits of detection and quantification were calculated using the ALAMIN software [30].

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