

EXTRACTION AND ANALYTICAL METHODS OF CAPSAICINOIDS - A REVIEW

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Abstract

Bioactive natural products are a main source of new drugs, functional foods and food additives. Chilli pepper is a very important plant used worldwide as a vegetable, a spice and an external medicine. Capsaicin, the pungent principle of *Capsicum* sp. (69%) is one of the best-known natural compounds. The extraction of the capsaicinoids can be made in many ways, with different types of organic solvents, but the yield varies with peppers variety and the conditions of extraction processing. The use of supercritical fluids, especially carbon dioxide, in the extraction of oleoresins, has increased during the last two decades, because it is a simple, inexpensive, fast, effective and solvent-free sample pretreatment technique in comparison with traditional methods (maceration, Soxhlet). Also, the level of capsaicinoids in the *Capsicum* fruits can be quantified by organoleptic, spectrophotometry, thin-layer chromatography, gas chromatography and high-performance liquid chromatography methods. Of these, high-pressure liquid chromatography (HPLC) is considered the most reliable and rapid method available for the identification and quantification of capsaicinoids.

Key words: organic solvents, supercritical fluid extraction, HPLC, peppers.

INTRODUCTION

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives. The production of valuable secondary metabolites is an attractive alternative to the that of whole plant extract (Namdeo et al., 2007).

GENUS *CAPSICUM*

The most important components in the group of secondary metabolites are derived from the biologically active components of the *Capsicum* species. (Tilahun et al., 2013).

The genus *Capsicum*, which originates from tropical and humid zones of Central and Southern America, belongs to the *Solanaceae* family and includes peppers of important economic value.

More than thirty of *Capsicum* species exist, five of which are domesticated and these are *C. pubescens*, *Capsicum baccatum*, *C. annuum*, *Capsicum chinense*, *Capsicum frutescens*, the last three species being widely spread and having the most pungent fruits.

Pepper pungency is measured in Scoville Heat Units (SHU). This measurement is the highest dilution of a chile pepper extract at which heat can be detected by a taste panel (Bosland et al., 2007).

Capsicum is the only genus known to produce capsaicinoids and capsaicin is the major and the most active, pungent compound of chilli peppers (Yamaguchi et al., 2010).

It is represented by 69%, dihydrocapsacin by 22%, nordihydrocapsacin by 7% homocapsacin and homodihydrocapsacin takes only 1% in the group of capsaicinoids (Gudeva et al., 2013) (Table 1).

According to most reports, capsaicinoids accumulate in the epidermis of the placenta are stored in vesicles on the surface of this tissue, while seeds occasionally absorb capsaicinoids because of their proximity to the placenta and the content in fruit flesh is very low (Moreno et al., 2012).

Recent studies showed that the concentration of capsaicin varies with taxa and genotype, the geographical origin and the climatic conditions (Cisneros-Pineda et al., 2007). The only *Capsicum* which don't contain capsaicin is the sweet pepper.

Table 1: Chemical structure of different capsaicinoids

Compounds	Structure
Capsaicin	
Dihydro-capsaicin	
Homo-capsaicin	
Nordihydro-capsaicin	
Homodihydro-capsaicin	

The structural characteristics of capsaicinoids that determine their spicy properties are associated with the presence of an amide bond connecting a vanillyl ring and an acyl chain. Capsaicin was first crystallized in 1876 by Tresh, who named it and capsaicin's molecular structure was resolved by Nelson and Dawson in 1919, with the following structural formula:

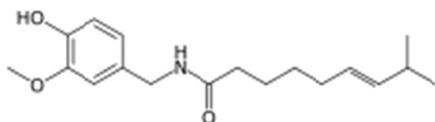


Figure 1: (E)-N-[(4-Hydroxy-3-methoxyphenyl)methyl]-8-methylnon-6-enamide)

The physico-chemical properties of capsaicin are presented in Table 2 (Arora et al., 2011).

Table 2: Physico-chemical properties of capsaicin

Properties	Value
Molecular weight	305.41 g/ mol
Melting point	62 to 65 °C (144 to 149 °F; 335 to 338 °K)
Boiling point	210 to 220 °C (410 to 428 °F; 483 to 493° K) 0.01 Torr
Flash point	113°C
Stability	Stable. Incompatible with strong oxidizing agents.
Solubility	H ₂ O- insoluble; alcohols and organic solvents-soluble
UVmax.	227 - 281 nm

CAPSAICINOID EXTRACTION

Capsaicinoid extraction from peppers is typically performed using organic solvents and the extraction efficiencies can vary with peppers, their parts and pre-extraction processing.

Attuquayefio and Buckle (1987) have determined the extraction of capsaicinoids from *Capsicum* fruits and oleoresins using solvents such as acetone, chloroform, methanol, acidified methanol and acetonitrile and they mentioned that acetone resulted in the highest capsaicinoid yields from dehydrated ground *Capsicum*. Later, in another study, Collins et al. (1995) suggested extraction with acetonitrile at reflux, but Barbero et al. (2006) found that acetonitrile is a fairly efficacious solvent, but less than ethanol and methanol. Chinn et al. (2011) studied the effects of solvent type (ethanol, acetone and acetonitrile) on capsaicinoid extraction. Ethanol and acetonitrile were better solvents for capsaicin extraction from fresh samples, while acetone was better for dried pepper parts.

In contrast, Nwokem et al., 2010 chose methanol for obtaining capsaicin, because of high extraction efficiency resulting with reduced amounts of pigments and oils extracted together with capsaicin, as compared to other suitable solvents, like acetone. The highest concentration of capsaicin was 9.177mg/g. Rafajlovska et al., (2011) using methanol, ethanol and n-hexane (ratio 1:20 w/v) for extraction of capsaicinoids from red pungent dried paprika fruits, methanol and ethanol being confirmed to be superior and selected as future extraction solvents.

Haejin et al., (2012) studied the presence of capsaicinoids in samples from different cultivars of hot pepper. Levels of capsaicinoid extraction were observed in the following order: hexane > EtOAc > acetone > MeOH. The maximum amounts of capsaicin and dihydrocapsaicin were extracted in hexane, ranging from 35.1 to 2495 µg/g and 16.8 to 1016 µg/g respectively. So, their study confirmed the use of hexane for extraction of capsaicinoids, using a Soxhlet method.

But, from all organic solvents utilized in extraction process, ethanol was the single non-toxic solvent. So, in a recent study, Xinrong et al. (2014) chose ethanol as solvent for

obtaining red pigment and capsaicinoids from peppers, 90°C, 4 ml/g solvent ratio and 120 minutes extraction time, without remain of toxic solvent.

The microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) are new techniques that combine microwave and ultrasound treatments, respectively, with traditional solvent extraction, for extraction of capsaicinoids.

The ultrasound-assisted extraction of capsaicinoids procedure used by Barbero et al., (2008) allowed extraction of the capsaicinoids present in peppers, in a short time (10 minutes), employing methanol as extraction solvent. They concluded that the developed method can be applied for the routine analysis of capsaicinoids in peppers. Barbero et al., (2014) extracted capsaicinoids using methanol, ultrasound power 360 W and 25 ml solvent/0.2 grams, during 15 minutes, at 50°C. The content of capsaicinoids was 1789 $\mu\text{mol/kg}$ fresh weight.

Chuichulcherm et al. (2013) studied capsaicinoids extraction by microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), compared with traditional Soxhlet method, using dried chilies (*Capsicum frutescens* L.). They concluded that the optimum method for extraction of capsaicinoids from *C. frutescens* was UAE method. So, although the capsaicinoids from MAE and UAE were 5.28 and 4.01 mg/g dried chili, respectively, UAE required minimum energy consumption and was evaluated as the most suitable method.

Another extraction techniques involving supercritical fluids have been investigated extensively over the last decades due to the numerous advantages offered in comparison with the conventional techniques of extraction. Apart from having relatively low critical pressure (74 bar), temperature (32°C) and favorable solubility, CO₂ is relatively non-toxic, non-flammable, available in high purity at relatively low cost, and is easily removed from the extract.

The aim of study proposed by Duarte et al., 2004 was to assess supercritical fluid extraction (SFE) of red pepper (*Capsicum frutescens* L.) oleoresins. The influence of pressure and superficial velocity of supercritical CO₂ at

313°K, on the *Capsicum frutescens* oleoresins yield and capsaicinoids content was studied. They found that the supercritical carbon dioxide can be used as solvent to obtain extracts from *Capsicum frutescens* and the highest extraction yields in oleoresins and capsaicinoids were obtained at pressures around 20-22MPa.

More recent, the objective of the study proposed by de Aguiar et al., (2013) was to select a variety of pepper with high concentration of capsaicin and subject it to supercritical fluid extraction (SFE), in order to determine the best conditions of temperature (40°C-60 °C) and pressure (15, 25 and 35 MPa). The conditions that presented numerically higher concentration of capsaicinoids extracted per mass of the sample were 15 MPa and 40 °C, which being considered the most suitable for extraction of capsaicinoids.

Rocha-Urbe et al., (2014) concluded in their study that oleoresin may be extracted from habanero chili powder using supercritical CO₂, providing capsaicinoids and carotenoids free of organic solvents that may be used in food industry for human consumption.

Santos et al. (2015) extracted capsaicinoids from *Malagueta* pepper (*Capsicum frutescens* L.) using supercritical fluid extraction (SFE) assisted by ultrasound, with carbon dioxide as solvent at 15 MPa and 40°C. The SFE global yield increased up to 77% when ultrasound waves were applied, and the best condition of ultrasound-assisted extraction was 360 W ultrasound power applied for 60 minutes. So, the use of ultrasound represented an efficient manner of producing small scale agitation, enhancing mass transfer on supercritical fluids (SF) extraction processes.

ANALYTICAL METHODS

In the last decade, there has been an increasing demand for new analytical methods that are more reliable and accurate, with short operational time and reduced cost, as well as with minimized use and generation of hazardous substances (Pena-Alvarez et al., 2009). Conventional methods used in determining the level of pungency or capsaicin

concentration are using a panel of tasters (Scoville Organoleptic test method).

High-Performance Liquid Chromatography (HPLC) method is considered the most reliable and accurate method for determining capsaicinoids. Preliminary purification of the extract has been applied before HPLC analysis of capsaicinoids. Thin Layer Chromatography (TLC) and Column Chromatography (CC) methods are also used. Gahungu et al., (2011) extracted capsaicinoids from *Scotch Bonnet* variety using column chromatography on silica gel and then quantitatively evaluated with a reverse phase-high performance liquid chromatography/photodiode array detection (RP-HPLC/PAD). Capsaicin (47.632 mg/g) and dihydrocapsaicin (23.096 mg/g) were the major found capsaicinoids.

Al Othman et al. (2011) chose HPLC method for separation, identification and quantification of capsaicin and dihydrocapsaicin of hot chillies, red chillies, green chillies, green peppers, red peppers and yellow peppers (*Capsicum annum* L). The results showed that hot chillies contained the highest concentration of capsaicin (4249 µg/g) and the highest pungency level (67984.60 SHU), whereas green chilli showed the lowest detected concentration (1 µg/g), green peppers, red peppers and yellow peppers were non pungent. Later, Chen et al., (2013) analysed by HPLC–UV the presence of capsaicin in the samples of red pepper extracts. The HPLC-UV chromatogram showed that capsaicin content was 9.48 mg/g dry weight.

Also, Barbero et al., (2014) identified in their study the five major capsaicinoids present in peppers using HPLC-MS. Ida Musfiroh et al., (2013) extracted various fruits of *Capsicum* and analyzed the extracts using high performance liquid chromatography. The optimum condition of analysis was attained using a reversed phase system, with a mobile phase of acetonitrile – acetate acid 2% (6:4), a flow rate of 1.0 ml/minute and a detection wavelength of 280 nm using UV detector.

In another study, Zamora et al., (2015) investigated direct spectrophotometric determination of capsaicinoids content in *Chiltepin* pepper as a possible alternative to HPLC analysis. The absorbance of the samples was observed at 215-300 nm and monitored at

280 nm. The results showed that comparative data determined spectrophotometrically and by HPLC on samples ranging from 29.55 to 129mg capsaicinoids/g, so spectrophotometric method can be also, routinely used with a correlation of 0.91 for total capsaicinoid analysis and quality control in pharmaceutical analysis.

UTILISATIONS

Capsaicinoids are important in the food and pharmaceutical industries.

Capsaicin is used in the development of new drugs because it has many beneficial properties, such as antioxidant, antimicrobial, anti-inflammatory and antitumor activities, and contributes to the control of diabetes and pain relief.

It is also used for the anti-convulsive and sedative properties and because stimulate the cardiovascular and respiratory systems. Moreover, capsaicinoids are also utilized as natural inhibitor of pathogenic microorganisms in food industry due to their antimicrobial properties.

CONCLUSIONS

Because of wide spread application of capsaicinoids, techniques have been developed with the objective of reducing extraction time, consumption of the solvent, pollution in analytical laboratories, and sample preparation costs.

Taking into account their benefits, there is a great interest in developing new technologies to obtain concentrated extracts.

So, plants will continue to provide novel products as well as chemical models for new drugs in the coming decades, because the chemistry of the majority of plant species is yet to be characterized.

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