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EXTRACTION METHODS OF CAPSAICIN

BY

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Abstract. Capsaicin is the active ingredients in chili peppers that give them their pungent and spicy taste. Various extraction methods of capsaicin from peppers have been used with high yields over the last few decades. Extraction is one of the most common methods for separating and purifying organic substances, but the method chosen must be fast, inexpensive, versatile and efficient with high performance (selection of appropriate solvent being of critical importance). This review analyses the most common methods used for capsaicin extraction from natural sources: solvent extraction (SE), microwave assisted extraction (MAE), solid phase microextraction (SPME), ultrasound-assisted extraction (UAE), SOX extraction, extraction with liquids under pressure (PLE), enzymatic treatment, aqueous two-phase system (ATPS), supercritical fluid extraction.

Keywords: capsaicin; microwave-assisted extraction; ultrasound-assisted extraction; solvent extraction; Soxhlet; HPLC.

1. Introduction

The hot sensation is a key characteristic associated with *Capsicum* members, as well as a significant feature of fruit quality (Reddy and Sasikala, 2013). Capsaicinoids are a group of phenolic alkaloids specific to peppers, and

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the most abundant of these components are capsaicin (8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (*N*-(4-Hydroxy-3-methoxybenzyl)-8-methylnonanamide), which account for about 90% (Reddy and Sasikala, 2013) of the heat sensation provided by peppers.

Hot peppers, which are members of the *Solanaceae* family and belong to the genus *Capsicum*, are primarily used as a food additive due to the hot sensation they can produce.

Capsaicin was discovered more than a century ago, with the chemical structure identified in 1930 (Kim *et al*., 2019). Capsaicin is used as an analgesic, as well as in the treatment of arthritis, due to its physico-chemical properties, antimicrobial, anti-inflammatory properties (Civan and Kumcuoglu, 2009), and therapeutic ability (Nigam *et al*., 2019), and several studies shown its effect in cardiovascular and gastrointestinal disorders (Santos *et al*., 2016). Capsaicin is also used in self-defense products (Bley *et al*., 2012) such as paralyzing sprays, as well as natural oral supplements, since it causes severe inflammation when it comes into contact with the ocular, nasal, or oral mucosa.

Extraction is one of the most basic operations in organic chemistry, since it is one of the most popular methods for separating and purifying organic substances (Berk, 2009). Extraction is the process of transferring one or more components of a phase (liquid or solid) to another phase (liquid) that is immiscible or partly miscible with the first. Capsaicin extraction can be done in a variety of ways, including: solvent extraction (SE), microwave assisted extraction (MAE), solid phase microextraction (SPME), ultrasound-assisted extraction (UAE), SOX extraction, extraction with liquids under pressure (PLE), enzymatic treatment, aqueous two-phase system (ATPS), supercritical fluid extraction.

2.1. Solvent Extraction, SE

Solvent extraction is a method of separating chemicals based on their relative solubilities. This procedure entails the use of a solvent, which is a liquid capable of dissolving another chemical. The touching (mixing) of the two phases (solvent and dried pepper) as well as the separation of the two phases are both part of a solid-liquid extraction process. Ethanol, acetone and acetonitrile can be used as solvents for the extraction of capsaicin

Chinn *et al.* (2011) used a biomass filler: solvent (15% (w/v) based on the initial moisture content of the pepper samples to extract capsaicin from oven-dried, fresh and lyophilized preparations (0.5 g dry weight). In 50 mL glass conical tubes, (Chinn *et al*., 2011) mixtures of samples and solvents were homogenized and mentained in a water bath with stirring (50°C), for 24 hour, with samples taken every 20 minutes. Capsaicin yields were not substantially different at times greater than 1 hour, according to preliminary research. Vacuum filtration (1.6 m Whatman GF/A glass fiber filters) was used to prepare the samples, which were then stored at -20°C (2 mL aliquots) until HPLC analysis.

Across all solvents tested: ethanol, acetone and acetonitrile, the seeds yielded about 16 mg of capsaicin per dry gram (Chinn *et al*., 2011). Capsaicin recovery from whole peppers was 8 mg/dry gram, followed by 5 mg/dry gram from pepper shells.

2.2. Ultrasound-Assisted Extraction UAE

The cavitation phenomenon occurs when an ultrasonic wave passes through an organic solvent, producing energy to increase the mixing and penetration of the solvent into the sample matrix, making the UAE technique successful (Lu *et al*., 2017). The use of UAE has a number of benefits, including the reduction of solvents, temperature, and extraction time, all of which are critical for the extraction of thermolabile and unstable compounds (Lu *et al*., 2017).

Barbero *et al*. developed a swift and repeatable water method for capsaicinoids from three different pepper varieties in Spain, using 25 mL of methanol as a solvent at 50°C for 10 minutes (Barbero *et al*., 2008).

The capacity of the extraction solvent to absorb and transmit the energy of the ultrasounds will dictate the outcome of ultrasound-assisted extraction. The capsaicinoids used in the extraction should be soluble in the solvent. Methanol, ethanol, acetonitrile, and water are the four solvents that have been tested for extracting capsaicinoids from the matrix of the sample. Because methanol is more compatible (Barbero *et al*., 2008) with the solvents used in the chromatographic method, it was chosen as the solvent for the development of the extraction procedure. Capsaicin was extracted with an 85.26 ± 1.35% (Lu *et al*., 2017) yield using ultrasound-assisted extraction.

2.3. SOX Extraction

When the targeted compound has reduced solubility in a solvent and impurities are insoluble in this solvent, the SOX process is a conventional procedure that is commonly used to remove oil from the organic matrix (Lu *et al*., 2017).

Bajer *et al.* (2015) used the SOX method with methanol as solvent and a 2 hour extraction period to extract capsaicinoids from a variety of chili samples. The same SOX method was used in a study by Liu *et al*. (2013), in which a 1 g sample of *Capsicum annuum* was extracted for 2 hours with 50 mL methanol. While SOX is the most common extraction method, it has drawbacks such as a longer extraction time, higher energy consumption, and lower capsaicinoids yields when compared to other extraction methods.

2.4. Extraction with Liquids under Pressure, PLE

The PLE procedure is often carried out at high temperatures and pressures, allowing for high compound solubility in the solvent while holding the solvent below its boiling point, resulting in high solvent penetration into the sample matrix (Lu *et al*., 2017).

Barbero *et al*. developed a PLE system using methanol and ethanol as the solvents for extracting water at a temperature of 200°C and a pressure of 100 atm. Capsaicin, along with two other capsaicinoids, was extracted from dry samples of *C. annuum* using the PLE method with methanol as the solvent; temperature at 100°C and pressure at 1500 psi, according to Liu *et al*. (2013). Bajer *et al*. (2015) extracted capsaicinoids from 10 chili samples using the pressurized hot water extraction process. Water was used as an ecological solvent in this experiment, and it was heated to 200°C at a pressure of 20 MPa.

2.5. Microwave Assisted Extraction, MAE

Microwave extraction is combined with conventional solvents in the MFA method, which uses the energy produced by microwave radiation to heat the solvents and improve extraction kinetics. Many studies have used MAE to extract capsaicinoids from peppers (Lu *et al*., 2017).

According to Williams *et al*. (2017), the MAE process doubled the yield of capsaicinoids and cut the extraction time in half as compared to conventional reflux and stirring flask extraction methods. Barbero *et al*. (2016) optimized the MAE conditions for extracting capsaicinoids from fresh pepper samples. The extraction conditions: 125°C - the extraction temperature, 0.5 g of crushed pepper in 25 mL solvent (ethanol), 500 W and 5 minutes of extraction time were found to be the most reliable parameters for the extraction. The authors also compared the extraction efficiency of effective approaches, such as magnetic stirring, and found that MAE is significantly faster (Lu *et al*., 2017).

2.6. Solid Phase Microextraction, SPME

SPME allows for multiple sampling, sample retention, reduces the possibility of sample contamination due to the technique's simplicity, is also quicker than conventional techniques, and can be easily automated (Schmidt and Podmore, 2015). Furthermore, the lower detection limits provided by SPME allow confirmation of previously undetected positive samples. Another advantage of SPME is the lack of solvents, which can save money (Spencer and Almirall, 2005) and minimize or eliminate the chance of exposing analysts to hazardous compounds.

To find the best effective solvent for extracting capsaicin and dihydrocapsaicin from cotton swabs, Spencer and Almirall (2005) tested methanol, ethylacetate, chloroform, and methylene chloride as extraction solvents. Twelve previously spiked cotton swabs containing 10 g of capsaicin and dihydrocapsaicin were made in triplicate for each solvent utilized. Individually, the swabs were inserted in 13 mm x 100 mm test tubes. Each solvent was introduced to the test tube in two milliliter increments and sonicated for 20 minutes. The solvent was decanted, and the cotton swabs were pushed through the tip with a pipette tip to express any leftover solvent from the swabs. As an internal standard (Spencer and Almirall, 2005), 100µL of 250 ng/µL nonivamide was added to each test tube (IS). After being dried by a stream of nitrogen, the extracts were reconstituted with 100 µL of methanol. Methanol yielded the highest capsaicin recovery (50–60%), followed by ethylacetate (44%), chloroform (35%), and methylene chloride (30%).

2.7. Enzymatic Treatment

During fruit extraction, enzymatic processes have been suggested to improve yield and selectivity. Various commercially available enzymes were used to soften the tissues of *Capsicum spp*. in a study by Santamaria *et al*. (2000) Using peppers, researchers were able to increase the extraction yield by 7%, resulting in an 80% capsaicinoids recovery. OLIVEX (mainly pectinase), CELLUCLAST (mainly celurase), VISCOZYME L (mainly carbohydrase), and PECZYMA 5XAL were among the enzymes used in this study (mainly pectinesterase and arabanase). The procedure was carried out at 50°C, with 7 hours of stirring in a rotary shaker at 120 rpm and a 1:50 ratio of chili powder to water. EXTRASIM (mainly pectinase and multiple carbohydrase) and ENERGEX (mainly glucanase) were then used to test a related treatment process, which increased the capsaicinoid extraction yield by 32%. In this case, the temperature was held at 3°C for 12 hours, with a 1:1 ratio of chili powder to water (Lu *et al*., 2017).

Salgado-Roman *et al.* (2008) proposed a non-commercial enzymatic treatment based on the treatment methods described above, using enzymatic extracts extracted from *Rhizopus nigricans*. The chili fruit was dehydrated in a vacuum oven after enzymatic degradation and then ground. The powder samples were then collected with tetrahydrofuran in a SOX device at 60°C. Capsaicinoids had an extraction yield of more than 85%, indicating that this non-commercial enzyme extract had a higher cellulose activity to soften cell walls and promote cell degradation.

2.8. Aqueous Two-Phase System, ATPS

A purification stage must complete the extraction to increase the purity of the final product, namely aqueous two-phase systems (ATPS), which have been well defined and studied in various processes in recent decades (Santos, 2016). Due to its ability to fractionate a variety of compositions, from the easiest to the most complex matrices, this form of liquid-liquid extraction is commonly used as a purification technology. Due to its higher water content, which allows this device a favorable environment for biomolecules to preserve their chemical structure and key activities, it is usually considered a biocompatible technique, as it is a more continuous extraction and purification process (Santos, 2016).

Cienfuegos *et al*. (2017) used a microwave reactor (CEMDiscover S System) to extract capsaicin from pepper biomass in one experiment. The extraction was carried out with the aid of a microwave. A 70% (v/v) aqueous ethanol solution was used with a mass to volume ratio of 0.2 for 1 minute at 125°C, irradiated at 250 W, and without stirring.

Then, according to the optimized conditions, ATPS are made as follows: biphasic systems were made in graduated centrifuge tubes (20 mL) by weighing the required quantities of ethanol (20-25 wt%) and sodium-salt (20-35 wt%). Pure capsaicin was used in all systems at a concentration of around 60 mg/L (when the complete mixture was taken into account). From *C. chinense var. chinense*, the volume of separated and distilled capsaicin was 5.23 ± 0.12 μmol / kg, with an extraction efficiency of 85.6 ± 0.4%.

2.9. Supercritical Fluid Extraction

Supercritical fluids are non-polar compounds that are heavy solvents at pressures and temperatures above their critical values (Lu *et al*., 2017). The supercritical fluids can return to the gaseous phase and evaporate without leaving any solvent traces after the pressure is adjusted. During the extraction of bioactive compounds, supercritical fluid extraction has been used as an alternative to conventional extraction methods, with the advantages of mild temperatures, low energy consumption, and high purity extracts (Lu *et al*., 2017). Because of its low cost, non-toxicity, non-inflammatory properties, inertia, and high extraction power, carbon dioxide (CO_2) is commonly used (Lu *et al*., 2017) as a supercritical solvent for the extraction of capsaicinoids.

Santos *et al*. (2015) used CO_2-assisted ultrasonic SFE as a solvent to extract capsaicinoids from malagueta pepper (*Capsicum frutescens L.*) at 15 MPa, 40°C, and 1.673 x 10^{-4} kg/s, respectively. When the ultrasound power was increased to 360 W for 60 minutes, the SFE rate improved. Dias *et al*. (2016) conducted a similar SFE test on the *dedo de moça pepper* in 2016 (25 MPa, 40°C, 600 W, 80 min) and without (25 MPa, 40°C) ultrasonic use. The CO_2 flow rate was maintained at 1.7569 x 10^{-4} kg/s during the experiment. The findings revealed that SFE's overall performance has improved significantly. In short, using ultrasound to increase the SFE yield of capsaicinoids in peppers may be a viable alternative to the conventional extraction methods currently in use.

2.10. Combination of Tunable Aqueous Polymer-Phase Impregnated Resin (TAPPIR) Extraction and Chromatography Technology

Yanmin Lu and Bo Cui (Lu and Cui, 2019) used aqueous two-phase extraction technology combined with chromatography to prepare high-purity capsaicin. The high water concentration in these systems (between 65 and 90% by mass) provides a mild and biocompatible atmosphere for the extraction target molecules. However, due to the ATPS's low interfacial stress, small density differences, and high viscosities, phase separation can take a long time. As a result, this operation is extremly important, auxiliary equipment and additional energy are needed to accelerate it.

A new technology was developed by immobilizing one phase of ATPS in porous particles. When the extraction method contains 0.25 g capsicum oleoresin, the yield of capsaicin reaches 95.82% (Lu and Cui, 2019) under ideal conditions. The polymer step of an aqueous two-phase system containing 18.5 percent PEG6000, 15% sodium citrate, and 10% [Emim] [OAc] at pH 6.5 was the best impregnated phase. Capsaicin recovery and purity were both at 85% and 92% (Lu and Cui, 2019), respectively, in the extraction process. The findings revealed that a higher molecular weight of Polyethylene glycol (PEG) and 1-ethyl-3-methyl imidazolium acetate is better for increasing capsicin yield (Lu and Cui, 2019).

3. Conclusions

The yields of the extraction methods described above are shown in Table 1.

Table 1
Yields of the Extraction Methods of Capsaicin

Method	Solvent	Yield	Ref.
Solvent extraction	Acetonitrile; Ethanol; Acetone	≈16 mg/dry g	(Chinn *et al.*, 2011)
UAE	Methanol	85.26 ± 1.35%	(Lu *et al.*, 2017)
SOX	Methanol; Ethylacetate; Ethyl eter; Hexane	88.31±1.03%	
PLE	Methanol; Ethanol; Water	98.31 ± 1.46%	
MAE	Ethanol	86.36 ± 1.12%	
TAPPIR	Polyethylene glycol (PEG); 1-ethyl-3-methyl imidazolium acetate	85-92%	(Lu and Cui, 2019)
SPE + US		58.8-60.8%	(Santos *et al.*, 2015)
Enzymatic treatment	Enzymes and tetrahydrofuran	>85%	(Salgado-Roman, *et al.*, 2008)

ATPS	Ethanol:salt	85.6 ± 0.4%.	(Cienfuegos *et al*., 2017)
SPME	Methanol Ethylacetate Chloroform Methylene chloride	50-60% 44% 35% 30%	(Spencer and Almirall, 2005)

Soxhlet extraction is the traditional extraction method, however it has drawbacks such a longer extraction time, higher energy consumption, and lower capsaicinoids yields when compared to alternative extraction methods as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and extraction with liquids under pressure (PLE).

Following the steps outlined above for performing each type of extraction and comparing the results, the method with the highest yield, extraction with liquids under pressure, PLE, is also the most viable in terms of the variety of solvents employed, parameters, and resources available.

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METODE DE EXTRACȚIE PENTRU CAPSAICINĂ

(Rezumat)

Capsaicina este unul dintre ingredientele active din ardeiul iute, care le conferă acestora gustul înțepător și picant. În ultimele decenii, s-au folosit diverse metode de extracție a capsaicinei din ardei cu randamente ridicate. Extracția este una dintre cele mai utilizate metode de separare și purificare a substanțelor organice, dar metoda aleasă trebuie să fie rapidă, ieftină, versatilă și eficientă, cu performanțe ridicate (alegerea solventului adecvat fiind de o importanță critică). Acest studiu analizează cele mai frecvente metode utilizate pentru extracția capsaicinei din surse naturale: extracția cu solvent (SE), extracția asistată de microunde (MAE), microextracția în fază solidă (SPME), extracția asistată de ultrasunete (UAE), extracția Soxhlet, extracția cu lichide sub presiune (PLE), extracția combinată cu tratament enzimatic, extracția în sisteme bifazice apoase (ATPS), extracție cu fluide supercritice.