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Ultrasound-assisted extraction of capsaicinoids from *Capsicum frutescens* on a lab- and pilot-plant scale

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ABSTRACT

The influence of operating parameters (solvent type, powder to solvent ratio and temperature) on the ultrasonically assisted extraction of capsaicinoids from dried *Capsicum frutescens* (fruit) was studied. From the economic perspective, the suitable condition for capsaicinoid extraction by indirect sonication in an ultrasonic bath with a working frequency of 35 kHz was at a ratio of 1 g of solid material: 5 ml of 95% (v/v) ethanol, 45 °C, where 85% of the capsaicinoids were removed from the raw material in 3 h. In an experimental pilot study in 20-l extraction tank at the fixed ultrasonic frequency of 26 kHz and 70 kHz, the recovery of capsaicinoids was 76% and 70%, respectively. It was shown that the ultrasonic extraction produced a significant reduction in extraction time at a lower operational temperature than under a conventional industrial hot maceration process.

1. Introduction

The chilli pepper is a popular plant found in many parts of the world. Chilli has been widely used as a food flavoring, a coloring agent, a feed additive in livestock and in food and pharmaceutical industries. Capsaicin and its analogues, called capsaicinoids are pungent compounds of *Capsicum* fruits. Capsaicin, the major pungent compound, is an amide derivative of vanillylamine and 8-methlnon-*trans*-6-enoic acid. Its hot flavor for about 95% of the pungency is caused by three major substances, capsaicin, dihydrocapsaicin and nordihydrocapsacin of capsaicinoids [1]. Capsaicinoids are known for their pharmacological properties for instance as chemoprotectors against mutagenesis or tumorigenesis [2], as antimicrobials [3], as antioxidants [4], for their anticancer effect [5], their analgesic effects [6], and their effect on the neuronal responsible for pain transmission and neurogenic inflammation [7]. The classical techniques for capsainoid extraction are maceration [8], magnetic stirring [9] and Soxhlet [10].

Ultrasonics has been proven to assist the solvent extraction of bioactive compounds from herbs [11–15]. The application of ultrasound-assisted extraction (UAE) offers many advantages including the reduction of solvents, temperature and the time for extraction, which is very useful for the extraction of thermolabile and in unstable compounds. The ultrasonic enhancement of supercritical extraction of pungent compounds from ginger owing to physical effects on the surface of particles was reported [16].

Recently, new techniques such as microwave-assisted extraction [17] and supercritical CO_2 extraction [18] have been applied to the extraction of capsaicinoids from chilli powder. However, relative to those techniques, the use of UAE is more convenient and affordable. In addition, UAE can be simply industrially employed in local companies. Owing to its cavitational phenomenon, the UAE can be applied in place of the boiling maceration method. While many papers have been published dealing with the UAE of different plant materials [11–16,19–21], only a preparative method of capsaicinoids for HPLC analysis by using ultrasonic extraction with acetone, methanol and acetonitrile as solvents has been reported [19]. In order to improve capsaicinoids extraction efficiency by UAE, the influence of operating parameters on a lab- and pilot-plant scale were examined in this study. The obtained results were compared with conventional extraction methods, maceration and Soxhlet.

2. Materials and method

2.1. Plant materials

Fruits of *Capsicum frutescens* were purchased from a local market in Mae Chan district, Chiangrai, Thailand in the form of dried chilies. For UAE lab study, the dried chilli pepper was grounded with a brabender (No. 1855334, type 880805, Germany) to obtain 3 mm particle size. For UAE pilot study, the sample size of dried

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chilli pepper was reduced to 13 mm by using Hammer mill Model H-15 Hosokawa Micron Corp. (Osaka, Japan). The ground samples were packed in plastic bags purged with nitrogen, and stored at 4 °C before use.

2.2. Soxhlet extraction

A classical Soxhlet apparatus was employed in which 25 g of the sample was placed into the cartridge with 200 ml of 95%v/v ethanol in 250 ml flask. Extraction at boiling point (78.1 °C) was carried out for 5 h.

2.3. Maceration

The experiment was performed in a 250 ml flask filled with 25 g of ground sample in 200 ml of 95% v/v ethanol. The extraction was carried out at 45 °C, 250 rpm for 15 h.

2.4. UAE lab study

The experiment was carried out in triplicate using an ultrasonic cleaning bath with a working frequency of 35 kHz and the power of 600 W (Bandelin Sonorex Super RK 1050, Germany). The bath was a rectangular container (50 cm × 60 cm × 20 cm) connected to a temperature-controlled bath (Polyscience 9610, USA). A set of 250 ml Erlenmeyer flasks filled with 25 g of ground samples of 3 mm particle size and assigned solvent was immersed into the ultrasonic bath under a controlled water level at about 10 mm from the bottom of the bath. The milling degree of the sample material played an important role in the extraction process; however, the reducing sizes of the material particles less than 3 mm particle size caused inconvenience in the filtration step.

2.5. UAE pilot study

The experiment was carried out in a 20-l extraction tank consisting of transducers bonded to walls with double output of ultrasonic frequencies (26 and 70 kHz) and ultrasound electric power of 1.08 kW (Jinning Sinobes Electronic, China). The stirrer speed was 16 rpm from an explosion proof motor. Three kilograms of course ground dried chilli pepper was employed for each batch with the selected conditions from the previous UAE lab study. The milling method for material preparation in the pilot study followed the conventional milling method of the industrial scale hot maceration. The sample size of dried chilli pepper was reduced to 13 mm particle size by using Hammer mill. The particle size was larger than that in the lab study in order to ease the filtration step.

2.6. Analytical method for capsaicinoids

The concentrations of capsaicinoids were determined following the method of the Association of Analytical Communities (AOAC) method 995.03 using a high-performance liquid chromatography (HPLC) system; Thermo Separation Product (TSP) consisting of the Spectra System P1000 solvent delivery and UV-6000LP diode array UV visible detector (Thermo Separation Product, USA). A column (150 × 4.6 mm ID) was packed with C18 reverse phase of 5 μm particle size. Isocratic 40% acetonitrile in 1% acetic acid (v/v) was used as a mobile phase with a flow rate of 1.5 ml/min. The injection volume was 20 μl and the UV wavelength was 280 nm. The concentration of capsaicinoids was a combination of capsaicin, dihydrocapsaicin and nordihydrocapsacin concentrations. Measured retention times and peak areas represented at least triplicate injections. The capsaicinoids concentration was quantified on the basis of a corresponding calibration curve using pure substances. For dry weight determination, 2 g of the sample in a small aluminum tray was placed in a vacuum oven at a pressure of 90 mbar and 105 °C for 4 h and then weighed.

3. Results and discussion

3.1. Effect of ultrasonication and its extraction time

Ground dried chilli peppers were added with a ratio of powder (g): 95% (v/v) ethanol (ml) of 1:8, in 250 ml Erlenmeyer flasks and ultrasonicated in an ultrasonic cleaning bath with a working frequency of 35 kHz at 45 °C for a period of 3 h. Extraction by maceration at 45 °C was used as the control system. The effect of ultrasonication and its extraction time on the recovery of capsaicinoids is shown in Fig. 1. The extraction rate of capsaicinoids was very high during the first 5 min of the extraction. After that, the recovery of capsaicinoids gradually increased with the extraction time.

Capsaicinoid recovery by UAE for 3 h was then compared to that of maceration (15 h) and Soxhlet (5 h) (Table 1). The percentage recovery of capsaicinoids by maceration, Soxhlet and UAE was 79.4, 92.0 and 87.4, respectively. UAE gave 10% greater capsaicinoid recovery compared with maceration but an approximately 5% lower yield compared with Soxlet extraction. The Soxhlet extraction gave the highest percentage recovery because the extraction was performed at boiling point temperature (78.1 °C) and the extraction was done all the time with fresh solvent, unloaded with substances, having therefore a greater concentration gradient as driving force, comparing with all other extractions.

UAE significantly improved the extraction yield. Since ultrasound could accelerate swelling and hydration and caused an enlargement in the pores of the plant cell walls, it resulted in a better mass transfer of solute constituents from the plant materials to solvent. The disruption of plant cells by microjet after the cavitation bubble collapsed could increase the rate of solvent penetration into plant tissue [20,21]. The release rate of capsaicinoids was very

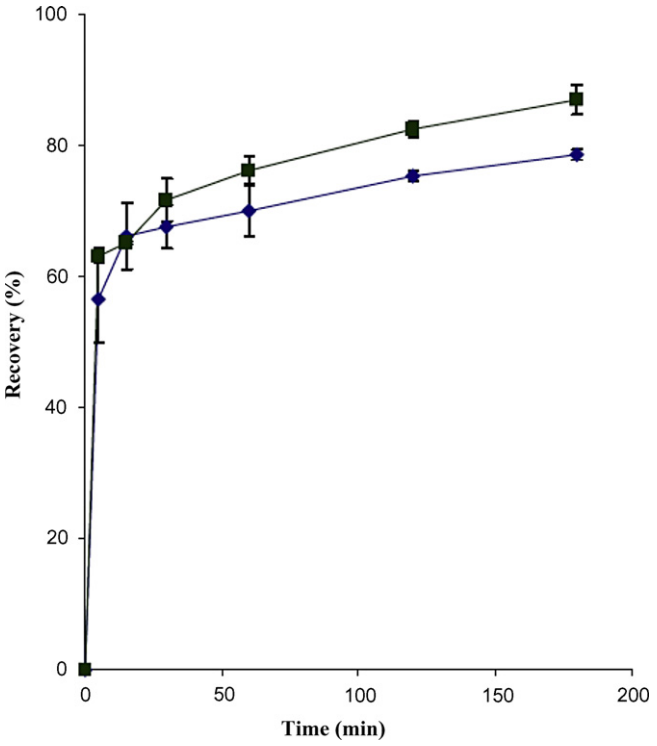

Fig. 1. Effect of ultrasonication and its extraction time on the extraction of capsaicinoids at 45 °C in 95% (v/v) ethanol: (■) UAE and (♦) maceration.