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Supercritical carbon dioxide extraction of capsaicinoids from malagueta pepper (*Capsicum frutescens* L.) assisted by ultrasound

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ABSTRACT

Extracts from malagueta pepper (*Capsicum frutescens* L.) were obtained 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 ultrasound power of 360 W applied during 60 min. Four capsaicinoids were identified in the extracts and quantified by high performance liquid chromatography. The use of ultrasonic waves did not influence significantly the capsaicinoid profiles and the phenolic content of the extracts. However, ultrasound has enhanced the SFE rate. A model based on the broken and intact cell concept was adequate to represent the extraction kinetics and estimate the mass transfer coefficients, which were increased with ultrasound. Images obtained by field emission scanning electron microscopy showed that the action of ultrasonic waves did not cause cracks on the cell wall surface. On the other hand, ultrasound promoted disturbances in the vegetable matrix, leading to the release of extractable material on the solid surface. The effects of ultrasound were more significant on SFE from larger solid particles.

1. Introduction

Hot peppers (*Capsicum* sp.) are rich in capsaicinoids, which are substances responsible for the pungency of the fruit. Among capsaicinoids, capsaicin is the most representative [1,2]. Capsaicin is currently used in the development of new drugs due to its beneficial properties, such as antioxidant, antimicrobial, anti-inflammatory and antitumor activities, also contributing to the control of diabetes and pain relief [3]. Taking into account the benefits of capsaicinoids, there is great interest in developing new technologies to obtain extracts concentrated in such compounds. According to Aguiar et al. [4] malagueta pepper (*Capsicum frutescens* L.) has the highest levels of capsaicin and dihydrocapsaicin, when compared to other Brazilian red native peppers, such as dedo-de-moça (*Capsicum baccatum*) and bode-amarela (*Capsicum chinense*).

Extraction of active compounds from vegetal raw materials is a promising area in the food, chemical and pharmaceutical industries. On the other hand, the recovery of these compounds is a complex task because, in most cases, they are oxidative or thermolabile substances. Furthermore, severe legal restrictions have been adopted to avoid the use of organic solvents in industrial extraction processes. Therefore, there is considerable interest in replacing traditional procedures for the recovery of active compounds, such as steam distillation and organic solvent extraction [5].

Supercritical fluid extraction (SFE) has been applied as an alternative to traditional methods for the extraction and fractionation of active compounds. Carbon dioxide (CO_2) is the most commonly used supercritical solvent in these processes, due to its advantages, which are: low cost, nontoxicity, non-flammability, inertness and good extraction capacity [5–7]. Indeed, the critical properties of CO_2 ($P_c = 7.38$ MPa, $T_c = 304.2$ K) are moderate when compared to other green solvents, allowing SFE to be carried out with low energy cost for pressurization, and at temperatures that do not damage the target compounds.

Generally, in a SFE unit, one can change the temperature, pressure, extraction bed size, solvent flow rate, among others, in order to maximize the extraction rate and the yield of a specific compound [8,9]. The morphology of the solid substrate particle can also influence the extraction efficiency, since the solvent must

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cross diffusive paths inside the solid particle, in order to extract specific compounds [5,10]. Moreover, the SFE process capacity may be improved by using combined extraction techniques, such as the use of different co-solvents and ultrasonic waves [11].

The ultrasound technique is based on the formation of high frequency ultrasonic waves, which are capable of causing cavitation due to the expansion and contraction cycles that the material goes through when submitted to ultrasound. These cycles disrupt the cell walls of the vegetable matrix, favoring the penetration of the solvent and the mass transfer, thus increasing the extraction rate and yield [12]. Low pressure extraction assisted by ultrasound with different solvents has been applied to different vegetable substrates, such as grape [13], pomegranate [14], rosemary [15], tomato [16] and pepper [17,18]. However, there are few published works that focus on ultrasound-assisted extraction at high pressure, such as SFE [11,19–21].

The objective of this work was to evaluate the effects of ultrasonic waves on the supercritical fluid extraction (SFE) of malagueta pepper (*C. frutescens* L.), as well as to evaluate the capsaicinoid and total phenolic content in the extracts. A mathematical model, based on the broken and intact cell concept, was fitted to the extraction curves, and the influence of particle diameter on the extraction kinetics was verified. Besides, field emission scanning electron microscopy (FESEM) was used to analyze the effects of ultrasonic waves on the structure of pepper particles.

2. Materials and methods

The work was carried out in the Laboratory of Supercritical Technology: Extraction, Fractionation and Identification of Vegetal Extracts – LASEFI-DEA/FEA-UNICAMP. The raw material was malagueta pepper, which is a Brazilian native variety of hot pepper (*C. frutescens* L.), purchased at the "Central de Abastecimento de Campinas/SP (CEASA)", a local market in Campinas, southeastern Brazil.

2.1. Sample preparation

The fruits with good physical integrity were selected, washed with running water and stored under refrigeration (≈4 °C) for further procedures. The raw material was oven-dried at 70 °C for 12 h, according to the methodology used by Aguiar et al. [4]. After drying, the samples were ground in a knife mill (Marconi, model MA 340, Piracicaba), in order to homogenize the substrate and decrease the resistance to mass transfer during the later stages of extraction.

The solid pepper particles were separated according to their size in a vertical vibratory sieve shaker (Bertel Metallurgic Ind. Ltda., SP, Brazil) Tyler series (Wheeling, USA) system (Bertel, model 1868, Caieiras, SP, Brazil) with sequential openings of 12, 16, 24, 32, 48 and 80 mesh. The mean particle diameter was calculated according to ASAE Standards [22]. To check the influence of particle diameter on the kinetics of ultrasound-assisted SFE, samples were classified in three groups: 1. Larger particles, formed by particles retained in a sieve of 16 mesh (1.18–1.68 mm, mean particle diameter (dp) of 1.43 ± 0.35 mm); 2. Smaller particles, formed by the material retained in a sieve of 48 mesh (0.177–0.342 mm, dp = 0.23 ± 0.16 mm); and 3. Particles of mean diameter, formed by the material retained in all sieves. The density of the particles was measured by helium pycnometer (Quantachrome Instruments, Ultrapyc 1200e, Boynton Beach, USA), whereas bulk density was measured by weighing a known volume of the solid material. Both solid and bulk densities data needed to apply the broken and intact cell model to the SFE kinetic curves.

2.2. Soxhlet extraction

The Soxhlet method was selected as a conventional extraction technique, in order to determine the total capsaicinoid content of the samples and to compare them with the results of SFE. Soxhlet extraction was performed using four solvents, with different polarities [23]: hexane, dichloromethane, ethyl ether and ethyl acetate. Each extraction was carried out using 0.15 L of solvent and 5.0 g of dried sample packed inside filter paper. The reflux of boiling solvent was kept for 6 h. Then, the liquid extract was recovered by solvent evaporation under vacuum (at 25 °C), weighed and stored under freezing (−18 °C) for further analyses. The capsaicinoid and the total phenolic content in the extracts were determined according to the methodologies presented in Sections 2.4.1 and 2.4.2, respectively. The Soxhlet extractions were performed in triplicates.

2.3. Supercritical fluid extraction (SFE) experiments

The conditions of the SFE experiments were fixed at pressure of 15 ± 0.5 MPa and temperature of 40 ± 3 °C, which were optimized by Aguiar et al. [4] for supercritical CO_2 extraction from malagueta pepper. Experiments were carried out using constant substrate mass, and aiming to obtain enough extract to perform the subsequent chemical analyses. In global yield experiments, the ratio between solvent and feed (S/F) was kept constant at 600 ± 2 kg CO_2/kg feed. This value of S/F is high when compared those used by Duarte et al. [24], Daood et al. [25], and Perva-Uzunalic et al. [8], which were 170, 30 and 120 kg of solvent per kg of pepper, respectively. The high applied value of S/F guarantees the solute exhaustion in the vegetal matrix and the consequent achievement of SFE global yield at the performed condition. Finally, the influence of particle size in the kinetics of

Table 1
Experimental conditions of SFE from malagueta pepper (*Capsicum frutescens* L.) with and without ultrasound on global yield and kinetics experiments.

Global yield (X_0)

Method	Solvent	Polarity[1]
Soxhlet	Ethyl acetate	0.0
	Dichloromethane	3.1
	Ethyl ether	4.4
	Hexane	0.0

	Power (W)	Time (min)[2]	Energy (kJ/cm^2)
SFE (40 °C/15 MPa)	–	–	–
SFE + US	360	240	223.0
SFE + US	360	150	139.4
SFE + US	360	60	55.7
SFE + US	280	240	173.5
SFE + US	280	150	108.4
SFE + US	280	60	43.3
SFE + US	200	240	123.1
SFE + US	200	150	77.4
SFE + US	200	60	31.0

Kinetics Experiments

	dp (mm)	Power (W)	Time (min)[2]	Energy (kJ/cm^2)
SFE	0.94 ± 0.03	–	–	–
SFE	1.43 ± 0.35	–	–	–
SFE	0.23 ± 0.16	–	–	–
SFE + US	0.94 ± 0.03	360	60	55.7
SFE + US	1.43 ± 0.35	360	480	446.1
SFE + US	0.23 ± 0.16	360	480	446.1

SFE – supercritical fluid extraction; US – ultrasound; X_0 – global yield (g of extract/g of raw material).

[1] Source: Byers [23].

[2] Irradiation time expressed in minutes; dp – particle diameter (mm).