EFFECT OF PRESSURE ON THE SC-CO_2 EXTRACTION KINETICS IN TERMS OF GLOBAL YIELD AND CAPSAICINOIDS CONCENTRATION OF *Capsicum* PEPPER

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Abstract. Hot cultivars of pepper are rich in capsaicinoids that are the compounds responsible for the spicy flavor imparted by many peppers. Capsaicinoids have strong pharmacological effects on health and can be regarded as functional food ingredients. The use of supercritical CO_2 is an interesting alternative for oleoresin extraction due to many advantages over the conventional extraction techniques that use toxic organics solvents. The objective of this study is to evaluate the effect of pressure on the supercritical CO_2 kinetics extraction curves of oleoresin and five capsaicinoids: nordihydricapsaicin (n-DHC), capsaicin (C), dihydrocapsaicin (DHC), homocapsaicin (h-C) and homohydrocapsacin (h-DHC). The raw material used was lyophilized and ground fruits of malagueta pepper (*Capsicum frutescens*). The kinetic SFE experiments were performed at 150, 250 and 350 bar, at 50 °C and CO_2 flow rate of 0.98 10^{-4} kg/s. The extraction curves indicated that with increasing pressure, there is an increase in extraction global yield as well as a reduction in process time. The global yields were 10.3(±0.4), 11.44(±0.09) and 12.1(±0.3) % at 150, 250 and 350 bar respectively. The major capsaicinoids in the extracted material at all pressures were C and DHC. In the first 5 minutes of extraction was observed the highest extraction yield of total capsacinoids: 88%, 86% and 65% for pressures of 350, 250 and 150 bar, respectively. Moreover, the concentration of total capsaicinoids at 150 bar in the first 5 minutes of extraction (205 mg total capsaicinoids/g extract) was about 3 times higher than the values obtained at pressures of 250 and 350 bar.

Keywords: Pepper oleoresin, Supercritical fluid extraction, Kinetics, Capsaicinoids.

1. Introduction

Hot or spicy peppers (*Capsicum* spp.) are widely used as fresh fruit and savory food additives in many parts of the world due to their attributes of color, pungency, and aroma. Hot cultivars are rich in capsaicinoids that are the compounds responsible for the spicy flavor imparted by many peppers. These compounds have been described in only the fruits of plants of the *Capsicum* genus. The two major capsaicinoids present in most varieties of hot peppers are capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-*N*-vanillylnonanamide [1]. Capsaicinoids have also strong pharmacological effects on health, which may be used in pain relief, cancer prevention, and weight reduction, besides providing gastrointestinal and cardiovascular benefits [2].

Accordingly, *Capsicum* oleoresin can be regarded as a functional food ingredient [3]. Its composition is mainly of lipid matter, carotenoids and capsaicinoids [4]. Parameters as coloring capacity and pungency are quality factors closely related to its commercial value and are consequence of raw material characteristics and extraction technique [4].

The traditional process of oleoresin extraction consists in the extraction of the dried and ground *Capsicum* fruits by organic solvents, especially n-hexane [5], whose residual content in the final product is governed by strict food regulations due to undesirable effects on human health [6].

The 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. Under supercritical conditions, the extraction process is suitable to decrease volatility and thermal degradation [7]. Additionally, supercritical carbon dioxide (CO_2) has been extensively used as the "green solvent" for many extraction processes [8, 9]. The objective of this study is to evaluate the effect of pressure on the supercritical CO_2 kinetic extraction curves of oleoresin and five capsaicinoids: nordihydricapsaicin (n-DHC), capsaicin (C), dihydrocapsaicin (DHC), homocapsaicin (h-C) and homohydrocapsacin (h-DHC) from freeze-dried fruits of malagueta pepper (*Capsicum frutescens*).

2. Material and methods

2.1 Chemicals

The solvent used in supercritical extractions was CO_2 (White Martins, Campinas, SP, Brazil) with 99.0 % purity. For chromatography analysis, the capsaicin and dihydrocapsaicin standards were purchased from Cayman Chemical (Cayman Chemical, USA. Purity > 95 %). All the others solvents and chemicals were of analytical grade.

2.2 Sample preparation

Approximately 0.5 kg of malagueta pepper (*Capsicum frutescens*) (Figure 1) was purchased at a local market in Campinas, Brazil. The fresh pepper was freeze-dried in a bench lyophilizer (L101-LioTop/LIOBRÁS, SP, Brazil) for 72 hours. After drying, the samples were ground in a knife mill (Marconi, mod. 340, SP, Brazil) in order to homogenize them and reduce the resistance to mass transfer during the later stages of extraction.

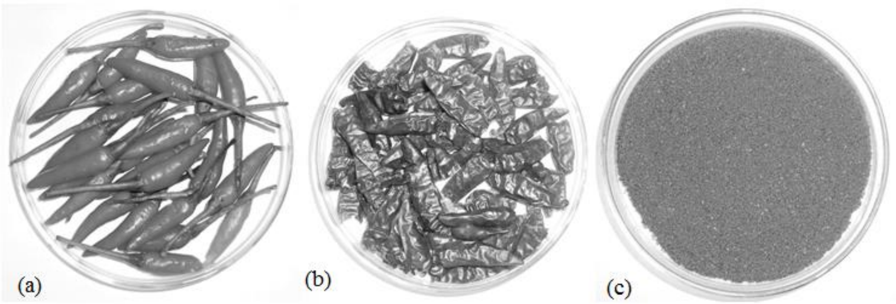

Figure 1. Malagueta pepper sample under different stages of processing: (a) fresh pepper, (b) freeze-dried pepper and (c) ground pepper.

The pepper solid particles were characterized by size classification in a vertical vibratory sieve shaker (Bertel Metallurgic Ind. Ltda., SP, Brazil), and the mean average superficial particle diameter ($\bar{d}_s$) was calculated using the Equations 1 and 2, as proposed by Gomide [10].

$$\Delta l_i = \frac{m_i}{M} \tag{1}$$

$$\bar{d}_s = \sqrt{\frac{\sum_{i=1}^{n} \frac{\Delta l_i}{\bar{d}_i}}{\sum_{i=1}^{n} \frac{\Delta l_i}{\bar{d}_i^{\,3}}}} \tag{2}$$

Where, $\bar{d}_s$ average superficial particle diameter (mm); m_i, mass of the sample retained on the sieve i (g); M, total mass of sample (g); $\bar{d}_i$ diameter of the sieve i (mm); n, total number of fractions.

The solid density of material was measured by helium pycnometer, whereas bulk density was measured by weighing a known volume of solid material.

2.3 Supercritical CO_2 extraction experiments

The supercritical CO_2 extraction experiments were performed at least in duplicates, using the dynamic method that consists of the continuous flow of solvent through a fixed solid bed placed inside the extraction column. Experiments were performed in a dynamic extraction unit (Applied Separations, Spe-ed, Allentown, PA) (Figure 2). Approximately 4.0 g of sample was used, forming a fixed bed inside a 5.6 mL stainless steel column. The extracts were collected in glass flasks and weighed in analytical balance.

The kinetic experiments were performed at constant temperature of 50 °C, and pressures of 150, 250 and 350 bar. The CO_2 flow rate used was of 1.98 10^{-4} kg/s, and the mean particle diameter of the material was of 0.44 (±0.4) mm. In these experiments extraction curves were obtained by determining the accumulated extract mass as function of time. The capsaicinoids content in the extract was determined according to the methodology presented in Section 2.4.

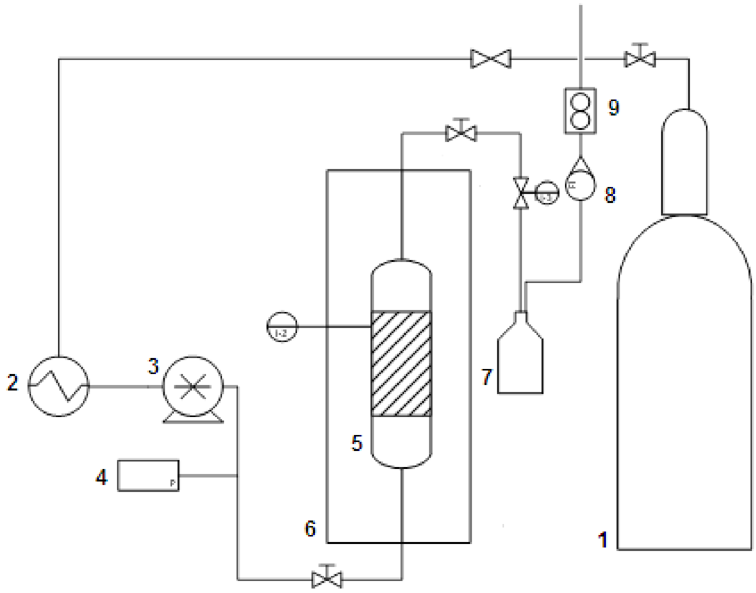

Figure 2. Diagram of the SFE equipment: (1) CO_2 cylinder; (2) heat exchanger; (3) CO_2 pump; (4) manometer; (5) extraction vessel; (6) vessel oven; (7) collection flask; (8) flow meter and (9) CO_2 totalizer.

2.4 Extract evaluation

Capsaicinoids analysis The HPLC-PDA analysis was carried out in a Dionex chromatographic system (Sunnyvale, CA, USA), consisting of an automated sample injector (ASI-100), pump (P680), thermostatic column compartment (TCC-100), a photodiode array detector (PDA-100), a universal chromatography interface (UCI-50) and Chromeleon 6.60 software. Capsaicinoids were separated using a LiChrospher RP-18e (250 mm × 4 mm, 5μm i.d., Merck). The wavelength employed for the detection was 280 nm.

The method of chromatographic separation used 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 flow rate of 1 mL/min. The gradient method utilized is the following: 0 min, 0 % B; 2 min, 55 % B; 6 min, 55 % B; 7 min, 60 % B; 12 min, 60 % B; 14 min, 65 % B; 15 min, 65 % B; 20 min, 70 % B; 25 min, 70 % B; 27 min, 10 0% B. The temperature of the column was held constant at 25 ºC.

HPLC Calibration The HPLC method was used to prepare calibration curves for capsaicin and dihydrocapsaicin (y = 2272x for capsaicin and y = 2235x for dihydrocapsaicin), which are the two commercially available capsaicinoid standards. Regression equations and the correlation coefficients (r^2) (0.9997 for capsaicin and 0.9999 for dihydrocapsaicin), limits of detection (9.76 mg L^{-1} for capsaicin and 4.10 mg L^{-1} for dihydrocapsaicin) and quantification (32.55 mg L^{-1} for capsaicin and 13.67 mg L^{-1} for dihydrocapsaicin) were calculated using Microsoft Office 2010 software.

Quantification of the capsaicinoids Capsaicin and dihydrocapsaicin were quantified from the calibration curves obtained from the standard solutions. Since there are no commercial standards for nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin, these compounds were quantified from the calibration curve of dihydrocapsaicin (for nordihydrocapsaicin and for homodihydrocapsaicin) and from the calibration curve of capsaicin (for homocapsaicin), given the structural similarities between these molecules and taking into account their molecular weights. All analyses were run in triplicate.

2. Results and discussion

Figure 3 shows the supercritical CO_2 extraction curves at a constant temperature of 50 °C and pressures of 150, 250 and 350 bar. The extraction curves indicate that with increasing pressure, there is an increase in extraction global yield as well as a reduction in process time. The global yields were 10.3(±0.4), 11.44(±0.09) and 12.1(±0.3) % at 150, 250 and 350 bar respectively.

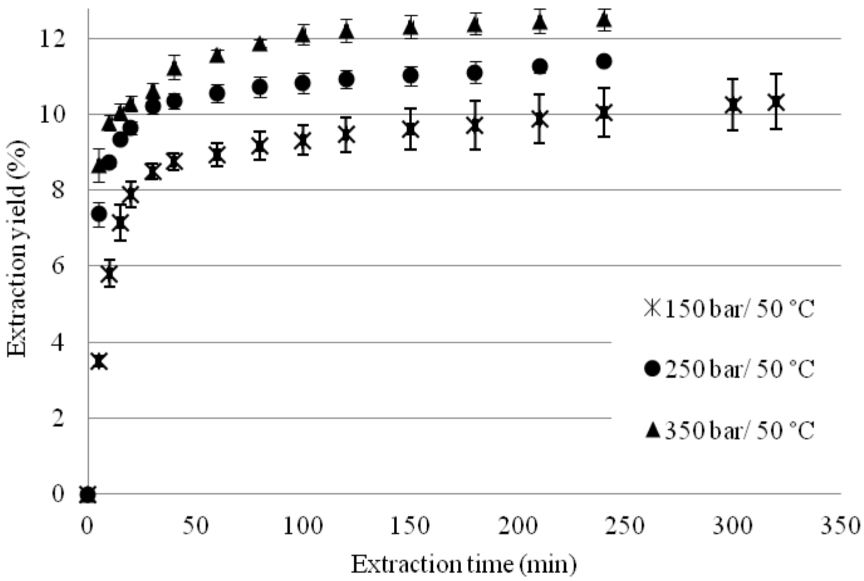

Figure 3. Kinetic extraction curves (oleoresin) for freeze-dried malagueta pepper at pressures of 150, 250 and 350 bar and fixed CO_2 flow rate and temperature of 1.98 10^{-4} kg/s and 50 °C, respectively.

These results are well in accordance with the concept that, generally, the power of solvent increases with increasing density. Duarte et al. [11] evaluated the influence of pressure and superficial velocity of supercritical CO_2 at 40 °C, on the *C. frutescens* oleoresin extraction yield. The results showed that the percentage of oleoresin extracted by SFE increases with increasing pressure. Higher pressures increase the solubility of oleoresin in supercritical dioxide carbon which means that, for a same amount of solvent more solute will be extracted.

According to Del Valle et al. [12], in the initial stages of the extraction process, the easily accessible solute is dissolved by the supercritical solvent. If the loaded supercritical phase reaches an equilibrium condition with the solid matrix in its initial conditions, there is no transfer of solute to the loaded supercritical phase starting at some position along the extractor, moving the position of the saturation front. This situation prevails until the saturation front reaches the end of the extractor. In this study, as expected, it was observed that the higher the operating pressure, the less time required to the saturation front reaches the end of the extractor. For the pressure 150 bar, 250 bar and 350 bar this time was ≈ 20, ≈ 10, and ≈ 5 min, respectively.

Figure 4 shows the extraction kinetics of capsaicinoids: nordihydricapsaicin (n-DHC), capsaicin (C), dihydrocapsaicin (DHC), homocapsaicin (h-C), homohydrocapsacin (h-DHC) and total capsaicinoids (Sum of n-DHC, C, DHC, h-C and h-DHC).

The curves of the accumulated recovery of capsaicinoids followed the same trends as those for oleoresin recovery (Figure 3). In the first 5 minutes of extraction, approximately 80 % of total capsaicinoids were extracted. This may be related to the location of these compounds in the pepper fruit, which are mainly on the surface of the placenta [13], readily available for extraction with supercritical CO_2 by convection. The curves also indicate that the capsaicinoids have higher solubility than the oleoresin in the supercritical CO_2 operating conditions used. Del Valle et al. [12] observed the same behavior studying the kinetics extraction of oleoresin and capsaicin of Jalapeño peppers with supercritical CO_2. They concluded that the extraction rate of capsicum oleoresin was slightly slower than that of capsaicinoids at the operation conditions (40 °C and 120 bar), since their main components, the triglycerides, are much less soluble than capsaicin under those conditions.

For all the capsaicinoids, (n-DHC, C, DHC, h-C and h-DHC), the extraction conditions of 150 bar and 50 °C provided the highest concentrations. Peusch et al. [14] and Perva-Unzunalic et al. [6] observed that an increasing density of CO_2 causes enhanced capsaicinoids yield in the SFE extracts, whereas values above 720 kg/m^3 did not lead to major changes. This behavior was not observed in this study, suggesting that other parameters (besides CO_2 density) are related to the capability of extracting capsaicinoids from malagueta pepper by SFE.

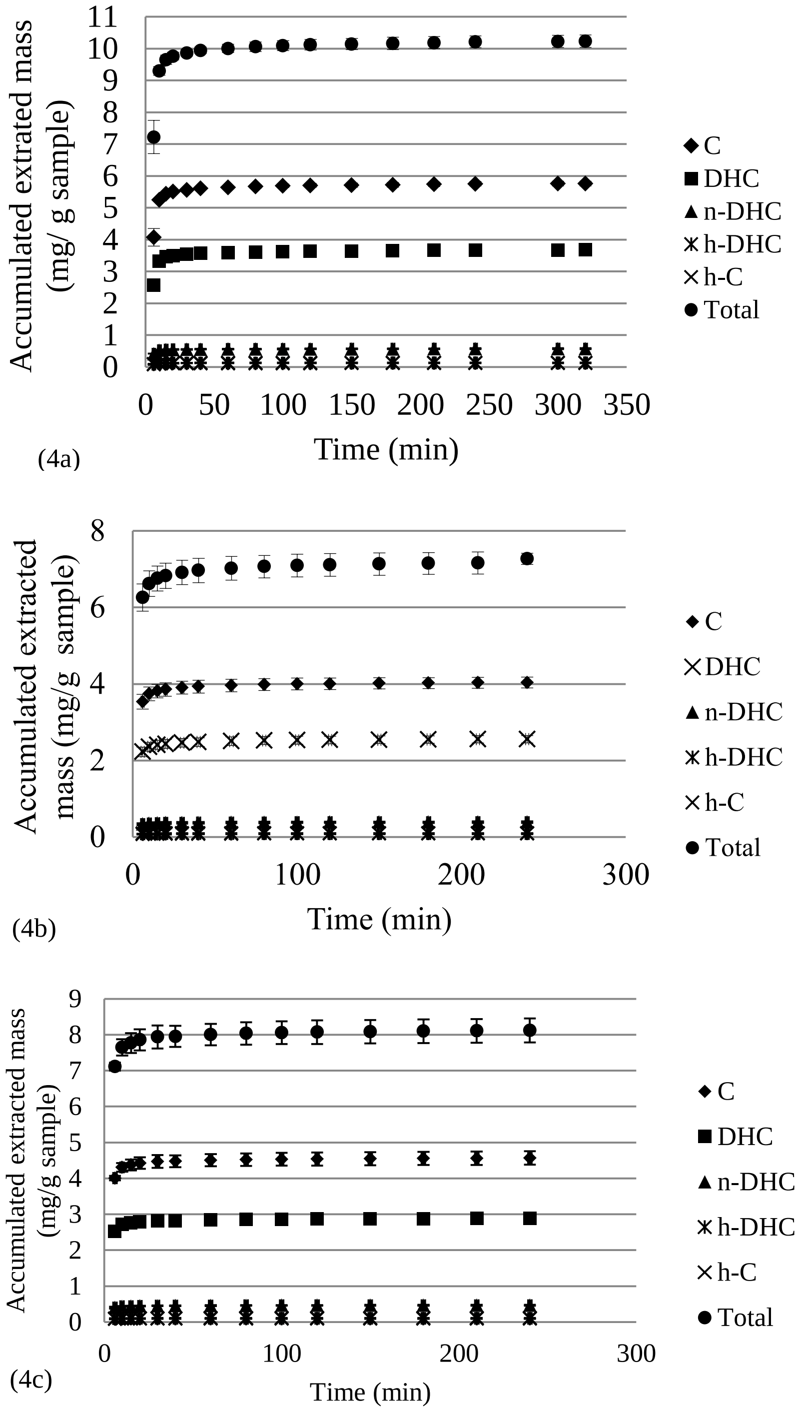

Figure 4. Kinetic extraction curves of capsaicin (C), dihydrocapsain (DHC), nordihydrocapsaicin (n-DHC), homodihydrocapsaicin (h-DHC), homocapsaicin (h-C) and total capsaicinoids (Total) for freeze-dried malagueta pepper at CO_2 conditions of 50 °C, fixed CO_2 flow rate of 1.98 10^{-4} kg/s and: (a)150 bar, (b) 250 bar and (c) 350 bar.

The major part of capsaicinoids in the extracted material at all pressures was the C and DHC. In the first 5 minutes of extraction the highest extraction yields of total capsacinoids were observed: 88%, 86%

and 65% for pressures of 350, 250 and 150 bar respectively. Moreover, the concentration of total capsaicinoids at 150 bar in the first 5 minutes of extraction (205 mg total capsaicinoids/g extract) was about 3 times higher than the values obtained at pressures of 250 and 350 bar. These results indicate that despite the higher yield obtained in oleoresin in higher operating pressures; the amount of capsaicinoids was higher for the smaller pressure due to the high solubility of capsaicinoids in supercritical CO_2. Another explanation is that at higher pressures, substances that were not soluble at low pressure would start competing with capsaicinoids in the extraction process.

The use of milder pressures can significantly reduce the cost of extraction on an industrial application; therefore the kinetic study of the influence of the different extraction pressures is important to verify the best extraction conditions.

3. Conclusion

Supercritical fluid extraction from *Capsicum frutescens* was carried out and it was able to observe that higher pressures produce higher extraction yields. Nevertheless, in terms of capsaicinoids it was observed that the highest concentrations were obtained at the lowest pressure, indicating that these compounds have their solubility decreased at higher pressures due to the extraction of other compounds from pepper.

Acknowledgements

The authors wish to thank CAPES, FAEPEX (451/11), CNPq (Project 473342/2011-1) and FAPESP (Project 2011/08656-7) for the financial support.

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