

## SCALE-UP OF SFE FROM *Capsicum* PEPPER USING SUPERCRITICAL CARBON DIOXIDE

Luiz P. S. Silva<sup>(1)</sup>, Ana C. de Aguiar<sup>(1)</sup>, Gerardo F. Barbero<sup>(2)</sup> and Julian Martínez<sup>(1)\*</sup>

(1) Food Engineering Department, Food Engineering College  
University of Campinas (UNICAMP)  
R. Monteiro Lobato 80, P.O. Box:6121, 13083-862, Campinas, SP, Brazil

(2) Department of Analytical Chemistry  
University of Cádiz  
Avda. República Saharaui, s/n, 11510 Puerto Real, Cádiz, Spain

Email: julian@fea.unicamp.br

**Abstract.** The challenge of finding scale-up criteria that allow reproducing laboratory data at higher scales is extremely important for the industrial supercritical fluid extraction (SFE) processes. The objective of this work is to investigate the scale-up of SFE from *Capsicum frutescens* pepper using supercritical CO<sub>2</sub>, and to evaluate the extraction kinetic curves of oleoresin and capsaicinoids of the large scale extraction. Extractions were performed at laboratory and pilot scale at 15 MPa and 40 °C, and their curves are compared in terms of yield and kinetics, also using mathematical modeling. The scale-up criterion adopted in this study was to keep constant the ratio between solvent flow rate and substrate feed ( $Q/F$ ), considering that diffusion would be the controlling mechanism. The broken and intact cell model of Sovová (1994) was applied to fit the extraction curves. The applied model fitted well to experimental data, nevertheless the values of the modeled parameters for small and large scale curves reveal differences between both processes. The most outstanding differences appear in the constant extraction period ( $t_{CER}$ ) and in the convective mass transfer coefficient ( $k_f$ ). The possible reasons that led to those differences can be related to SFE bed packing procedure, different separation methods of the small and large scale equipment and extraction bed geometry. The yields for the small and large scale were 3.97(±0.09) and 4.18(±0.04), respectively. By analyzing the kinetic extraction curve in the large scale, it can be observed that at 135 min of extraction, more than 85 % of total capsaicinoids were obtained.

**Keywords:** SFE extraction, Scale-up, Kinetics, Capsaicinoids.

### 1. Introduction

*Capsicum* peppers are used as natural condiments in food and as raw material for pharmaceutical industries, due to the presence of bioactive compounds [1]. Pungency is one of the main attributes of the *Capsicum* genus and the compounds responsible for this characteristic are the capsaicinoids [2]. The most representative capsaicinoids are capsaicin (C) and dihydrocapsaicin (D) (80 to 90 %).

Oleoresins from peppers are commonly extracted by traditional methods using organic solvents, such as cyclohexane, acetone [3] and n-hexane [4]. Such extractions, using organic solvents, have limitations in obtaining solvent-free extracts, which reduce the quality of the final product.

Supercritical fluid extraction (SFE) has been receiving great attention as an alternative method, due to the advantages that it presents over conventional techniques, especially when carbon dioxide (CO<sub>2</sub>) is used as solvent. Among the advantages of SFE with CO<sub>2</sub> it is important remark the use of moderate temperatures, efficient solvent use with recycling, low energy consumption, prevention of undesirable reactions such as oxidation, operational flexibility through process parameter tuning, and enhanced product quality due to the complete separation between solute and solvent [5-7].

Hundreds of materials, procedures and process parameters are reported in published books, articles and patents, based on results obtained at laboratory extraction procedures. On the other side, few results are found for pilot plant scale processes, and even less in industrial scale. Studying scale-up criteria for

SFE is important to establish a methodology that allows predicting the behavior of the process at industrial scale from laboratory data, considering the differences observed in processes conducted in equipments of significantly different sizes. Moreover, the works that explore the relationships between small and large scale extraction processes are, in most cases, limited to specific raw materials, products, and process conditions, which make unviable their generalization.

Thus, the objective of this work is to investigate the scale-up of SFE from *Capsicum frutescens* peppers using supercritical CO<sub>2</sub>, and to evaluate the extraction kinetic curves of oleoresin and capsaicinoids of the large scale extraction.

## 2. Material and methods

### 2.1 Materials

Fruits of *Capsicum frutescens* peppers (malagueta) were purchased in a local market in Campinas, Brazil. The fruits were cleaned, oven-dried at 65 °C for 22 hours, and ground in a knife mill. Carbon dioxide with 99.9 % purity (White Martins, Campinas, Brazil) was used as solvent. The SFE pressure and temperature were of 15 MPa and 40 °C, respectively.

### 2.2 SFE experiments

Two SFE units were used. A Spe-ed SFE unit (Applied Separations, Allentown, PA) with a 300 cm<sup>3</sup> column was employed for the laboratory (small scale) extractions. This unit contains a back pressure valve, a CO<sub>2</sub> pump, an extraction vessel, which is placed in an oven. For the scale-up experiments, a pilot unit (Thar Technologies, model SFE-2X5LF-2-FMC, Pittsburgh, PA) with a 5150 cm<sup>3</sup> column was employed. As well as the Spe-ed SFE unit, this equipment has a back pressure valve, an extraction vessel, which is involved by a heating mantle at the required temperature. Solvent flow rate is regulated by the pump which is controlled by software. A set of three separators connected in series is used to separate the extract from the CO<sub>2</sub>. The first separator worked at 8 MPa bar and 40 °C, the second one at 5 MPa and 30 °C and the last one at 3 MPa and 30 °C. The extracts were collected at defined times, and each sample was weighed in order to build the SFE curves, representing yield versus time. Both laboratory and pilot scale extractions were performed in duplicates.

Table 1 shows the process parameters for laboratory and pilot extractions, needed to define the scale-up criteria and to apply the mass transfer model to fit the SFE curves. All these data were determined experimentally, except CO<sub>2</sub> density, which was obtained from the literature [8].

**Table 1.** Process parameters for laboratory and pilot scale SFE of *Capsicum frutescens* pepper at 15 MPa and 40 °C.

	Laboratory	Pilot
Mass substrate, $F$ (kg)	0.10001 ± 0.00001	1.99 ± 0.01
Particle diameter, $d_p$ (mm)	0.34 ± 0.02	0.34 ± 0.02
Extractor height, (cm)	12.54	61.4
Bed height filled with glass spheres (cm)	3.34	15.0
Bed height, $L$ (cm)	9.2	46.4
Bed diameter, $D$ (cm)	5.42	10.34
$L/D$	1.7	4.5
Extractor volume (cm <sup>3</sup> )	289.33	5155.84
Bed volume (cm <sup>3</sup> )	212.26	3879.47
Solvent flow rate, $Q$ (kg/s)	(1.65 ± 0.02) × 10 <sup>-4</sup>	0.033
Solid density, $\rho_s$ (kg/m <sup>3</sup> )	1320 ± 10	1320 ± 10
Supercritical solvent density, $\rho$ (kg/m <sup>3</sup> )	780.23	780.23
Solubility, $Y^*$ (kg solute/kg CO <sub>2</sub> )	0.003166	0.003166
Global yield, $X_0$ (kg solute/kg feed)	0.069 ± 0.003	0.069 ± 0.003

The scale-up criterion adopted in this study was to keep constant the ratio between solvent flow rate and substrate feed ( $Q/F$ ), considering that diffusion would be the controlling mechanism of this process. From the data of Table 1 we can calculate that ratio, which is of 0.0165 (kg CO<sub>2</sub>)/(kg solid.s).

### 2.3 Mathematical Model

The broken and intact cell model of Sovová [9] was applied to fit both laboratory and pilot scale extraction curves. A multiple-fitting approach was adopted to fit simultaneously each pair of duplicates, leading for a unique set of parameters for each scale. A derivative-free routine [10] was used to fit the model to the experimental data. The objective function ( $f$ ) to be minimized was the sum of squared errors.

### 2.4 Extracts evaluation

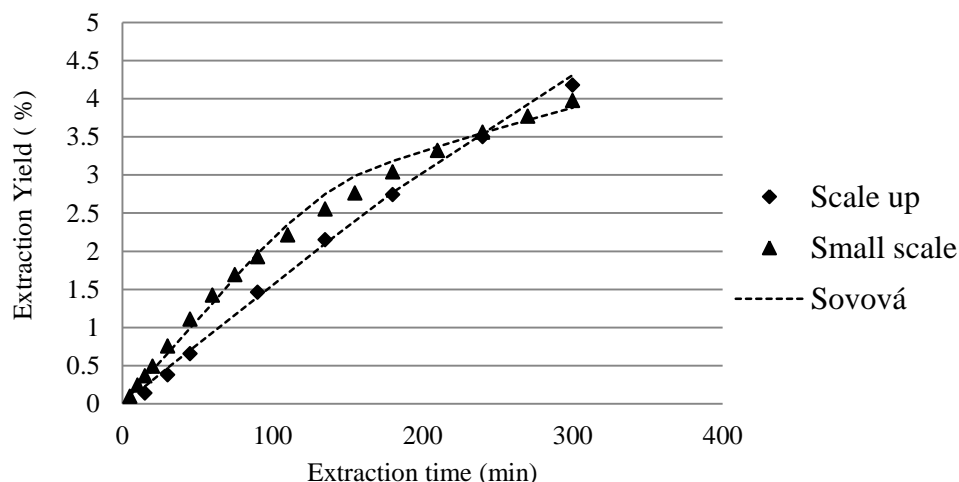
The HPLC-PDA analysis: The HPLC-PDA analysis was carried out in a Dionex chromatographic system (Sunnyvale, CA, USA), consisting of an automated sample injector (ASI-100), pump (P680), thermostatic column compartment (TCC-100), a photodiode array detector (PDA-100), a universal chromatography interface (UCI-50) and Chromeleon 6.60 software. Capsaicinoids were separated using a LiChrospher RP-18e (250 mm × 4 mm, 5 μm i.d., Merck). The wavelength employed for the detection was 280 nm. The method of chromatographic separation utilized 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 flowrate 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, 100% B. The temperature of the column was held constant at 25 °C.

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 coefficient ( $r^2$ ) (0.9997 for capsaicin and 0.9999 for dihydrocapsaicin), limits of detection (9.76 mg L<sup>-1</sup> for capsaicin and 4.10 mg L<sup>-1</sup> for dihydrocapsaicin) and quantification (32.55 mg L<sup>-1</sup> for capsaicin and 13.67 mg L<sup>-1</sup> 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.

## 3. Results and discussion

Figure 1 present the experimental curves for the SFE at laboratory and pilot scale, respectively, as well as the modeled curves. In Table 2 the values of the objective function and the adjusted model parameters for both scales are reported.



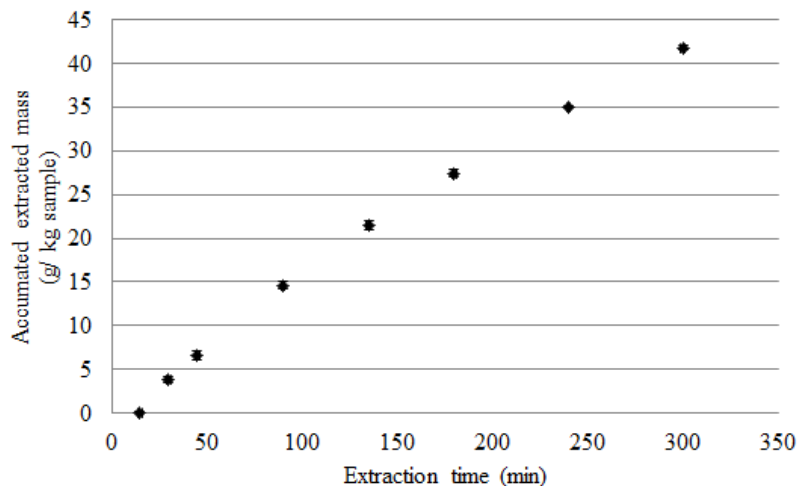
**Figure 1.** Experimental and modeled SFE curves from *Capsicum frutescens* pepper at 15 MPa and 40 °C at small and scale up scales.

In a scale-up procedure one expects to achieve at large scale the same process behavior obtained in laboratory. By observing Figure 1 we note that the scale-up did not succeed to achieve such goal, since the SFE curves obtained at laboratory and pilot scale are quite different. Moreover, the values of the modeled parameters shown in Table 2 also reveal some differences between both processes. In this sense, we can recall that, for both scales, the applied model fitted very well to experimental data, so we can discuss the values of the adjusted parameters. Among those parameters, the most outstanding differences appear in the constant extraction period ( $t_{CER}$ ) and in the convective mass transfer coefficient ( $k_f$ ). On the other side, the values of  $X_k$ , the solute ratio inside the cells, are similar for both scales, as expected since this value depends only of the SFE pressure and temperature, particle diameter and pre-treatment procedures, which were equal.

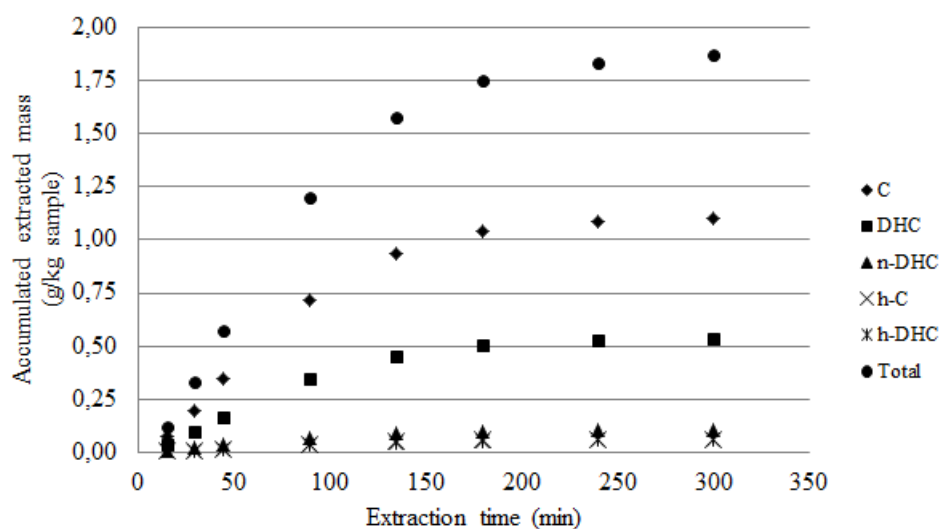
**Table 2.** Model parameters and objective function for the SFE from *Capsicum frutescens* pepper at 15 MPa and 40 °C in laboratory and pilot scales

Parameter	Laboratory	Pilot
$t_{CER}$ (min)	82	145
$X_k$ (kg solute/kg feed)	0.0413	0.0400
$k_f$ (s <sup>-1</sup> )	1.08e-3	6.95e-4
$k_s$ (s <sup>-1</sup> )	1.90e-5	4.30e-5
$f$	3.93e-7	2.51e-5

As observed in Table 2,  $t_{CER}$  is considerably higher at the pilot extraction. Physically, this means that more time was spent to extract all the easily available solute. Since this step is controlled only by convection, the convective coefficient would be expected to be lower at pilot scale, and it effectively is. The possible reasons that led to those results may be the differences in the extractor size, since bigger extraction beds require additional care on packing to avoid the formation of preferential paths to the solvent. In this case, the solvent would be unable to reach all the easily available solute and as a result, the extraction rate is lower than expected, reflecting in the higher  $t_{CER}$  and lower  $k_f$ , when comparing to the laboratory extraction. The separation methods of the equipments are also different. The complexity of the separation procedure at the pilot extraction may lead to higher extract losses, even of pepper oleoresin that remains stuck on the separators, or evaporation of volatile compounds together with ethanol. Thus, the recovery of extract may have occurred in rates lower than those potentially feasible at the performed conditions, leading to the differences in the SFE curves,  $t_{CER}$  and  $k_f$ . And finally, another scale-up criterion to be considered is the extraction bed geometry. For industrial extraction,  $L/D$  ratios from 5 to 7 are usually recommended in order to achieve the best yields and rates. In Table 1 the ratios of both laboratory and pilot scale are shown, and they are of 1.7 and 4.5, respectively. Then, the pilot scale configuration is closer to the recommended for industrial processes, and we should find what worked better at pilot SFE to relate with such difference. We observe, then, in Table 2 that the solid phase mass transfer coefficient,  $k_s$ , is higher in the pilot extraction. Indeed, in Figure 1 we notice a clear trend to achieve higher yield at pilot scale than in laboratory, if we extrapolate both curves. In this sense, high  $L/D$  ratio seems to be positive for the diffusive period of extraction, where radial diffusion should be prevented. Moreover, in such configuration the enhanced contact between solvent and substrate may have helped to accelerate diffusion.



**Figure 2.** Experimental SFE curve from *Capsicum frutescens* pepper at 15 MPa and 40 °C at pilot scale



**Figure 3.** Kinetics extractions curves for capsaicin (C), dihydrocapsaicin (DHC), nordihydrocapsaicin (n-DHC), homocapsaicin (h-C), homodihydrocapsaicin (h-DHC) and total capsaicinoids (Total) at pilot scale.

Figure 3 shows the extraction kinetics of capsaicinoids. The curves from accumulated recovery of capsaicinoids didn't follow the same trends as those for oleoresin recovery (Figure 2). We can clearly see that extraction curve of capsaicinoids has already achieved the diffusion-controlled period; while the extraction curve of yield did not. This fact shows that the capsaicinoids would be one of the priority substances in extraction process. In the first 30 min of extraction, approximately 18 % of total capsaicinoids were extracted, while for the extraction yield of oleoresin at 30 min the percentage recovery is 9 %. This may be related to location of these compounds in the pepper fruit, which are mainly on the surface of the placenta [11], readily available for extraction with supercritical CO<sub>2</sub> by convection. The curves also indicate that the capsaicinoids have a higher solubility than the oleoresin under the supercritical CO<sub>2</sub> 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<sub>2</sub>. They concluded that the extraction rate of capsicum oleoresin was slightly slower than that of capsaicinoids at the operation conditions (40 °C and 12 MPa) due to their main components, the triglycerides, are much less soluble than capsaicin under those conditions.

#### 4. Conclusion

For the system *Capsicum frutescens* and supercritical dioxide carbon at 15 MPa and 40 °C, the criteria of scale up keeping constant the ratio between solvent flow rate and substrate feed ( $Q/F$ ) did not achieved its goals. In other words, it was not able to see the same process behavior obtained in laboratory scale at the pilot scale. The differences among the beds geometry and the way they were packed may have influenced these observed differences. The extraction kinetics of capsaicinoids at pilot scale has shown that these substances are extracted priority.

#### 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.

#### References

- [1] S. Surassmo, S. G. Min, P. Bejrappa, M. J. Choi, Effects of surfactants on the physical properties of capsicum oleoresin-loaded nanocapsules formulated through the emulsion–diffusion method, *Food Research International*, 43 (2010) 8-17.
- [2] P. W. Bosland, E. J. Votava, *Pepper: Vegetable and Spice Capsicum*, CABI. 2000, p. 248.

- [3] H. G. Daood, V. Illés, M. H. Gnayfeed, B. Mészáros, G. Horváth, P. A. Biacs, Extraction of pungent spice paprika by supercritical carbon dioxide and subcritical propane, *Journal of Supercritical Fluids*, 23 (2002) 143–152.
- [4] J. P. Fernández-Trujillo, Extracción convencional de oleoresina de pimentón dulce y picante I. Generalidades, composición, procesos e innovaciones y aplicaciones, *Grasas y aceites*, 58 (2007) 252–263
- [5] A. Jouyban, M. Rehman, B. Y. Shekunov, H. K. Chan, B. J. Clark, P. York, Solubility prediction in supercritical CO<sub>2</sub> using minimum number of experiments, *Journal of Pharmaceutical Sciences*, 91 (2002) 1287-1295.
- [6] G. Brunner, *Gas extraction*, Springer. New York, 1994, p. 5-28.
- [7] E. Reverchon, I. De Marco, Supercritical fluid extraction and fractionation of natural matter, *The Journal of Supercritical Fluids*, 38 (2006) 146-166.
- [8] NIST (2012). <http://webbook.nist.gov/chemistry/fluid/> (accessed in August, 2012)
- [9] H. Sovová, Rate of vegetable oil extraction with supercritical CO<sub>2</sub> - I. Modeling of extraction curves, *Chemical Engineering Science*, 49 (1994) 409-414.
- [10] M. J. D. Powell. *Subroutine BOBYQA*, Department of Applied Mathematics and Theoretical Physics, Cambridge University, 2009.
- [11] M. L. Reyes-Escogido, E. G. Gonzalez-Mondragon, Chemical and Pharmacological Aspects of Capsaicin, *Molecules*, 16 (2011) 1253-1270.
- [12] J. M. del Valle, M. Jiménez, J. C. de la Fuente, Extraction kinetics of pre-pelletized Jalapeño peppers with supercritical CO<sub>2</sub>, *Journal of Supercritical Fluids*, 25 (2003) 33-44.