PRECIPITATION OF CAPSICUM PEPPER ETHANOLIC EXTRACT AND POLY(L-LACTIC ACID) BY SUPERCRITICAL CO_2 ANTISOLVENT PROCESS

A. C. de AGUIAR[1], F. M. BARRALES[1], C. A. REZENDE[2], G. F. BARBERO[3], J. MARTÍNEZ[1]

[1] University of Campinas, College of Food Engineering
[2] University of Campinas, Institute of Chemistry
[3] University of Cádiz, Department of Analytical Chemistry
Contact email: julian@fea.unicamp.br

ABSTRACT - Biquinho pepper is rich in capsinoids, which present strong pharmacological effects on health. The lack of pungency assigned to capsinoids make them interesting for the application in pharmaceutical and food industries. The aim of this work was to evaluate the encapsulation of biquinho extracts by supercritical antisolvent technique using Poly(L-lactic acid) as the coating material. A lab-scale apparatus that consists of a CO_2 supply system, solution and CO_2 injection unit (coaxial nozzle with an internal diameter equal to 127 µm), and a high pressure column was used. The process parameters were: temperature and CO_2 flow rate were fixed at 40 ˚C and 20.4 g/min respectively, pressure varied from 8 to 12 MPa, and solution flow rate varied from 0.5 and 1.0 mL/min. The morphology of particles was analyzed using a scanning electron microscope. The micrographies showed that the geometry of the particles was greatly influenced by pressure and solution flow rate. At the conditions of 10 MPa and 0.75 mL/min solution flow rate, small spherical particles (diameter of approximately 5-10 µm) were observed.

1. INTRODUCTION

Hot cultivars of *Capsicum* peppers are rich in capsaicinoids, which are the compounds responsible for the spicy flavor imparted by many peppers. Capsaicinoids have strong pharmacological effects on health, which may be used in pain relief, cancer prevention, and weight reduction (Luo *et al.*, 2011).

A similar group of compounds named capsinoids (naturally occurring in some varieties of sweet peppers) seem to have similar effects to those of capsaicinoids, without presenting pungency (Hursel and Westerterp-Plantenga, 2010). These compounds have an ester bond instead of the amide bond between the vanillyl moiety and fatty acid chain normally found in capsaicinoids (Kobata *et al.*, 1998). Known capsinoids include capsiate (CTE), dihydrocapsiate (DHCTE), and nordihydrocapsiate (n-DHCTE). Capsinoids are more unstable than the capsaicinoids: they are labile in polar solvents and probably tend to decompose in protic solvents, such as ethanol and water (Sutoh *et al.*, 2001). Accordingly, the microencapsulation of these compounds can be an alternative to increase stability and ensure protection of their properties. Microparticle drug delivery systems have attracted

great attention in recent years, since they allow increasing bioavailability, provide sustained release and reduce the side effects of drugs (Hoffman, 2008).

Traditional encapsulation methods include the emulsification/solvent evaporation (Rogers *et al.*, 2002), jet milling (Schlocker *et al.*, 2006), spray drying (Vehring, 2008) and freeze drying (Semyonov *et al.*, 2010). However, these methods may present limitations, such as relatively large particle size, wide particle size distribution, degradation of the product and difficulties in complete recovery of organic solvents (Wang *et al.*, 2013). The use of supercritical CO_2 can be an alternative to overcome the drawbacks of conventional encapsulation methods, and presents many advantages for different applications, such as near ambient operating temperatures, efficient separation, very low or no organic solvent residue, and being environmentally safe solvent (Hakuta *et al.*, 2003, Jung and Perrut, 2001).

As described by Cocero *et al.* (2009), supercritical antisolvent processes (SAS) are based on bringing into contact a solution of the solutes of interest (eg. drug and polymer) in a conventional liquid solvent with a supercritical fluid. Upon mixing, the supercritical fluid saturates the liquid solvent and depletes it by extraction. The saturation of the liquid solvent causes the precipitation of the solute by an antisolvent effect, resulting in the particle precipitation.

To the best of our knowledge, the co-precipitation study involving extracts from *Capsicum* peppers by supercritical antisolvent extraction has not been previously reported in the literature. Therefore, the aim of this work is to co-precipitate the alcoholic extract of biquinho pepper (BPE), a Brazilian sweet pepper with capsinoids in its composition, and Poly(L-lactic acid) (PLLA) by the SAS process using ethyl acetate as co-solvent and CO_2 as antisolvent.

2. MATERIAL AND METHODS

2.1. Chemicals and samples

Approximately 5 kg of ripe Biquinho pepper were purchased at a local market in Campinas, Brazil. The antisolvent used in the SAS process was CO_2 (White Martins, Campinas, SP, Brazil) with 99.0 % purity. PLLA was purchased from Sigma Aldrich (Saint Louis, USA). All the other solvents and chemicals were of analytical grade.

2.2. Preparation of the ethanolic extract from Biquinho pepper (BPE)

The ethanolic extract of biquinho pepper (BPE) was obtained by ultrasound-assisted extraction. The extraction process consisted of transferring 25 g of lyophilized and ground pepper into 500 mL of ethanol. The mixture was then subjected to an ultrasonic probe and processed under ultrasonic power of 360 W for 10 minutes. The ultrasonic system used (Unique Group, model DES500, Campinas, Brazil) is composed by a transducer unit with frequency of 20 kHZ and a variable output power controller. The mixture resulting from the extraction process was separated by filtration and was subsequently employed in the precipitation assays by SAS.

2.3. Apparatus and procedure for particles formation

The experimental SAS apparatus shown schematically in Figure 1 consists of a CO_2 supply system, a liquid injection unit, and a high pressure stainless steel column. Briefly, CO_2, previously cooled in a thermostatic bath (MA184, Marconi, Campinas, Brazil) at -10 °C, was pressurized using a pneumatic pump (PP 111-VE MBR, Maximator, Nordhausen, Germany) and subsequently heated to operating temperature in a heating bath (MA184, Marconi, Campinas, Brazil). Then, CO_2 was injected into the high pressure column with flow rate controlled by a heated micrometer valve coupled to a rotameter used as gas flow meter. The internal temperature of the column was maintained by using the heating bath. After temperature and pressure were stabilized, the operation solution (pure PLLA or PLLA+ BPE solubilized in ethyl acetate) was introduced to the high pressure column using a HPLC pump (PU-2080, Jasco, Tokyo, Japan) through a coaxial nozzle with internal diameter of 127 µm. At the column inlet, previously saturated with supercritical CO_2, rapid diffusion occurs at the cosolvent ethyl acetate and supercritical CO_2. After the injection of the solution (40 mL), the system was kept under the same operation conditions to remove the residual organic solvent from the particles for 30 min. Afterwards, the vessel was slowly depressurized and then the particles were collected in petri plates, sealed and stored under refrigeration.

First, PLLA was precipitated without addition of BPE, at the pressures of 8 and 12 MPa and flow rates of feed solution (Q_{sol}) of 0.5 and 1 mL/min. The other parameters (temperature (40 °C), PLLA solution concentration (0.5 %, w/v) and CO_2 flow rate (20.4 g/min)) were kept constant. Then, the precipitation of PLLA with BPE was conducted at the pressures and flow rates of feed solution presented in Table 1. The other process parameters were kept constant at the same levels applied for precipitation of pure PLLA.

Table 1 - Experimental condition for SAS process

Exp	Pressure (MPa)	Flow rate of feed solution (mL/min)
1	8	0.5
2	8	1.0
3	12	0.5
4	12	1.0
5	10	0.75
6	10	0.75
7	10	0.75

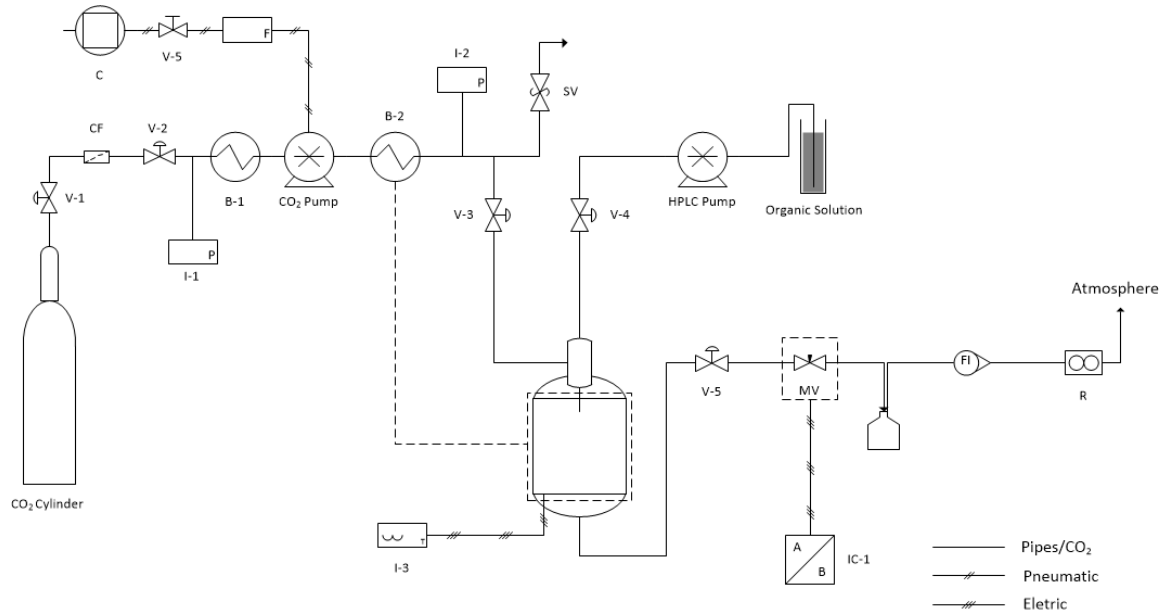

Figure 1 - Experimental apparatus in SAS configuration.
V-1, V-2, V-3, V-4 and V-5 – Control valves; MV – Micrometer valve; SV – Safety valve; C- Compressor; F- Compressed air filter; CF – CO_2 Filter; B1 –Cooling bath; B2 – Heating bath; I-1 e I-2 – Pressure indicators; I-3 – Temperature indicator; IC-1 – Indicators and controllers of temperature of micrometer valve, FL – Rotameter; R – Gas totalizer.

2.4. Particle characterization

The particles morphology were determined according to the analisys described below:

Morphology: The morphology of the particles was analyzed using a scanning electron microscope equipped with a field emission gun (FESEM - FEI Quanta 650). Prior to analysis, the samples were coated with gold in a SCD 050 sputter coater (Oerlikon-Balzers, Balzers, Liechtenstein). Both equipments were available at the National Laboratory of Nanotechnology (LNNano, Campinas-SP, Brazil). Analyses of the sample surfaces were performed under vacuum, using a 5 kV acceleration voltage and a large number of images were obtained on different areas of the samples to assure the reproducibility of the results.

3. RESULTS AND DISCUSSION

From an initial visual observation, all the precipitation assays (for pure PLLA and PLLA + BPE mixture) resulted in the formation of powder, with different colors and degrees of agglomeration, depending on the operating conditions employed.

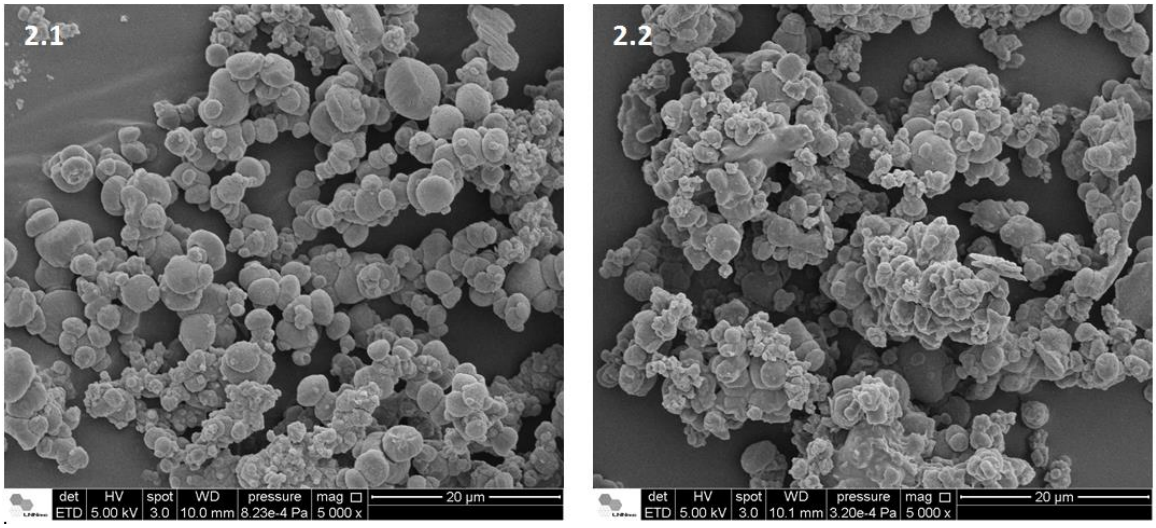

Figure 2 - FESEM micrographs (5000x magnitude) of PLLA pure particles, (2.1) P = 12 MPa, Q_{sol} = 0.5 mL/min, (2.2) P = 8 MPa, Q_{sol} = 1.0 mL/min.

Regarding the precipitation of pure PLLA, according to the micrographs shown in Figure 2 (2.1 and 2.2) it was found that in both operating conditions, the particles formed exhibited spherical geometry and slightly irregular and porous surface. Under the conditions of pressure of 8 MPa and flow rate of the feed solution of 1.0 mL/min (Figure 2.2), the presence of agglomerated and non-homogenously distribution of particle size was observed. The combined low pressure and high flow rate may have contributed to the incomplete removal of the organic solvent present in the particles by the supercritical fluid, possibly providing supersaturation of the ethyl acetate in the vessel, which in turn may have disturbed the process of regular particle size nucleation and growth, as previously reported by Sacchetin *et al.* (2013).

The same behavior of the pure PLLA particles was observed in particles of PLLA + BPE. In Figure 3 (3.1 and 3.2), it is found that at the lower operating pressure, 8 MPa, the particles have become more irregular and agglomerated (Figure 3.2), unlike at the greater pressure of 10 MPa (Figure 3.1), where one can verify that the particles are more regular, spherical and less prone to agglomeration. Possibly, the presence of residual ethyl acetate leads to this particle conformation, and further analysis to determine the content of residual solvent can confirm this tendency. For both conditions studied, a reasonable particle size distribution was observed, ranging from 0.5 to 10 µm.

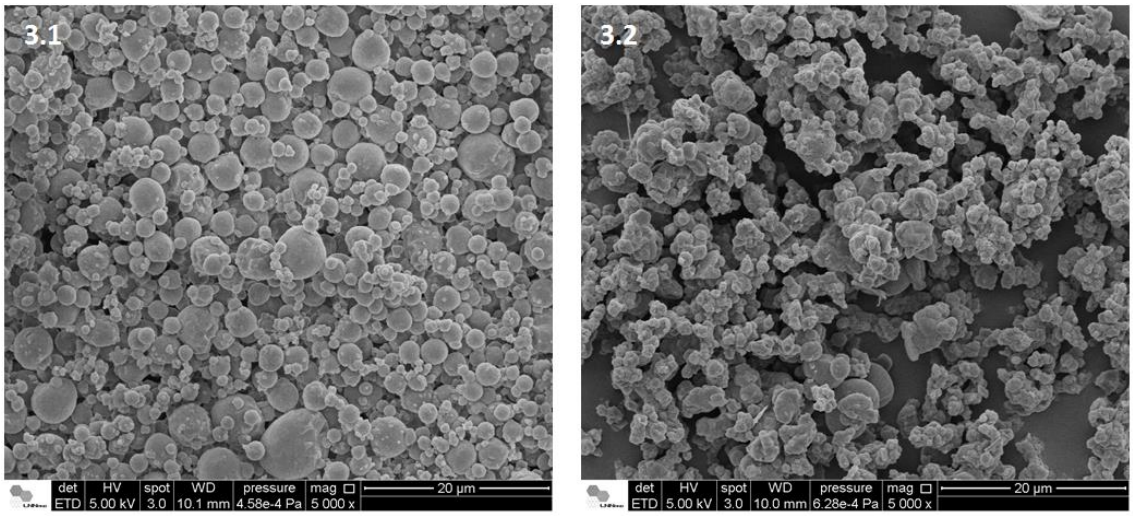

Figure 3 - FESEM micrographs (5000x magnitude) of PLLA + BPEE particles, (3.1) P = 10 MPa, Q_{sol} = 0.75 mL/min, (3.2) P = 8 MPa, Q_{sol} = 0.5 mL/min.

4. CONCLUSIONS

The precipitation process ethanolic extract of biquinho pepper with PLLA as polymer by SAS technique was successfully applied and resulted in particles of spherical morphology and porous surface. The degree of particle agglomeration (pure PLLA and PLLA + BPE particles) was higher for the particles obtained at pressure of 8 MPa.

5. ACKNOWLEDGEMENTS

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