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# Production and structural characterization of solid lipid microparticles loaded with soybean protein hydrolysate

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ABSTRACT

The aims of this study were to produce and evaluate solid lipid microparticles (SLMs) loaded with soy protein hydrolysate (HP). The SLMs were produced by spray chilling with an active material and carrier ratio of 1:5 and 1:10 and in two feed preparations: emulsion and suspension. The rheological parameters of the feeds produced by emulsions were studied, morphological characteristics of the SLMs were examined via scanning electron microscopy (SEM) and confocal microscopy, the particle size and distribution were measured via laser light diffraction, and the structural properties of the SLMs were characterized via infrared (FTIR) spectroscopy and X-ray diffraction (XRD). SEM images showed that SLMs were spherical and agglomerated. The analysis of X-ray diffraction indicated that the microparticles after 90 days of storage had β polymorphic form. The preparation methods for feeds, emulsion and suspension, had no influence on the rheological parameters, and the median particle size of the SLMs and interactions between the ingredients were not detected via FTIR spectroscopy; however, the SLMs prepared by emulsion contained pores and had a higher incorporation efficiency of HP. The spray chilling technique is suitable method for microencapsulation of soy protein hydrolysate. So, this technique could be useful for attenuating HP unpleasant taste, for its protection and also for promoting its release in the intestine, during fat digestion.



## 1. Introduction

Due to digestion and easier absorption of protein or free amino acid, protein hydrolysates can be incorporated into products intended for people who have difficulties in protein absorption: the elderly, premature infants and children with diarrhea (Rocha, Trindade, Netto, & Favaro-Trindade, 2009). Protein hydrolysates can also be used for the following purposes: 1) incorporation into products for those who have allergies to milk and soy protein, for example; 2) supplementation for athletes because it is a good source of nitrogen; and 3) treatment for those who have enzyme deficiencies that lead to disorders in amino acid metabolism, such as phenylketonuria (Clemente, 2000; Freitas et al., 1993; Gozáles-Tello, Camacho, Jurado, Páez, & Guadix, 1994; Rocha et al., 2009).

Despite the importance and numerous applications of protein hydrolysates, factors exist that hinder their use, e.g., some have an intense bitter taste associated with the release of the hydrophobic groups that were within the molecule (Rocha et al., 2009) and protein hydrolysates can be, reactive and hygroscopic (Ortiz et al., 2009). Besides, the hydrolysates are susceptible to digestion in the stomach, losing their therapeutic effects.

Microencapsulation has been used for attenuating the drawbacks of the application of hydrolysates. In previous works by this research group, casein hydrolysate was successfully encapsulated using spray drying with different carrier agents (Favaro-Trindade, Santana, Monterrey-Quintero, Trindade, & Netto, 2010; Ortiz et al., 2009; Rocha et al., 2009; Subtil et al., 2014) and via complex coacervation (Mendanha et al., 2009).

Spray chilling is already widely used for microencapsulation by the pharmaceutical and veterinary industries. In the food sector, interest in spray chilling has grown in accordance with the needs of new applications, especially for functional ingredients (Okuro, Matos, & Favaro-Trindade, 2013).

In the literature, there are at least four denominations for the same technique of encapsulation: spray chilling, spray cooling, spray congealing and prilling. However, independent of the name, this technology is based on the atomization of a mixture containing an active agent and one molten carrier in a chamber, whose temperature is below the melting point of the carrier. Thus, the carrier solidifies, and a powder comprised of microparticles is obtained.

The spray chilling method favors the production of microspheres; characterized by being spherical, solid particles, in which the active compound is uniformly distributed throughout its volume (Ilić et al., 2009).

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The carriers usually applied in the technique are fats, vegetable oils and their derivatives with a high melting point (Desai & Park, 2005; de Vos, Faas, Spasojevic, & Sikkema, 2010). The release mechanisms of active compound from the microparticles produced with triacylglycerols occur possibly by mechanical or thermal break (Favaro-Trindade, Pinho, & Rocha, 2008), and digestion of the carrier via digestive enzymes (Okuro, Matos, et al., 2013).

Spray chilling can be efficient in many applications, such as odor and flavor masking, improved appearance, increased stability, controlled release and protection of active compounds against injurious conditions (such as pH, light, and the action of proteases and oxygen) (Ilić et al., 2009; Matos-Jr, Di Sabatino, Passerini, Favaro-Trindade, & Albertini, 2015; Okuro, Matos, et al., 2013; Okuro, Thomazini, Balieiro, Liberal, & Favaro-Trindade, 2013; Pedroso, Thomazini, Heinimann, & Favaro-Trindade, 2012; Pedroso, Dogenski, Thomazini, Heinemann, & Favaro-Trindade, 2013).

The spray chilling process has many advantages in comparison to other microencapsulation technique, such as the following: low-cost and simplicity; a continuous and scalable process of high productivity; a clean (not requiring solvents) and mild process (not requiring high temperatures); and the ability to produce SLMs that can release their contents at the intestine via the actions of lipases.

In this context, the aims of this research were to investigate the feasibility of producing by spray chilling of SLMs containing soybean protein hydrolysate, the effect of feed (emulsion and suspension) in the production of SLM and the characterization of the microparticles produced. The production of SLM loaded with HP offer more opportunities to HP applications in different products and the information about the effect of feed type to spray chilling expands the knowledge about this encapsulation technique.

## 2. Material and methods

### 2.1. Materials

The carrier consisted of vegetable fat made with partially hydrogenate cotton seed oil, TRI HS-48 (Triangulo Alimentos Ltda, Itápolis, São Paulo, Brazil). Its melting point is 51 °C and it has in their composition a variety of saturated and unsaturated fatty acids, with a predominance of oleic, stearic and palmitic. As active material was used the hydrolysate produced by the soybean protein (Fuji Oil Ltd., Osaka, Japan), that is water soluble, has low molecular weight (<500 Da) and mean particle size estimated to be 7 μm. The surfactants for the feeds produced by the emulsion were evaluated: polyglycerol polyricinoleate (PGPR) (Danisco, Denmark), Tween 80 (Merck, Brazil) and soy lecithin (Pantec, Brazil).

### 2.2. Methods

#### 2.2.1. Feed preparation by emulsion and suspension

To evaluate the differences among the feed preparation methods, the HP was incorporated into the molten fat in two ways: emulsion and suspension. The feeds were maintained and atomized into the spray chiller at three different temperatures (60, 70, or 80 °C), so each one of the treatments was prepared in these three temperatures.

To prepare the suspensions, first, the fat was molten at the temperature of interest, and then the HP was added into the molten fat; the mixture was homogenized using an Ultra-Turrax (model IKA®T25, Staufen, Germany) apparatus set, stirring for 30 s at 3600 rpm under constant heating. The suspensions were prepared using proportions of HP:fat of 1:5 and 1:10.

In addition, to prepare the emulsion feeds, initially, the lipid carrier was molten at the temperature of interest. Next, HP (in water solution of 40%) and the surfactant (1%) were added into the molten carrier under constant heating, followed by homogenization using an Ultra-Turrax (IKA®T25, Staufen, Germany) apparatus set at predetermined values of speed and time. The proportions to HP:fat were the same as those for the suspensions, 1:5 and 1:10; however, in this case, the HP was in water solution and was also added to the surfactant. The HP concentration was increased by adjusting the solution volume.

*2.2.1.1. Emulsion stability.* To define which parameters would be used to produce the emulsions, its stability was previously evaluated. This analysis was carried out in duplicate. The effects of three variables were determined on the emulsion stability: surfactant type, time and stirring rates. The method used in preparing the emulsions was always the same, except for the variable under study. After preparation, the emulsions were placed in tubes and kept in a water bath. The stability of the emulsions was observed visually by evaluating the phase separation over 60 min at a temperature above to the lipid melting point.

To evaluate the emulsion stability, three surfactants were studied, in different concentrations: soybean lecithin (at 2, 5 and 7%), Tween 80 (1%) and PGPR (1%). Emulsions were prepared with proportion of HP:fat of 1:10, the surfactant and were stirred for 5 min at 8000 rpm. After determination of the best surfactant performance, the effect on the emulsion stability of the stirring time (1, 5 and 7 min) was studied. Next, the effect of stirring speeds was also evaluated (6000, 8000 and 10,000 rpm) in emulsion stability. These variables chosen were applied too at emulsion prepared with ratio HP:fat 1:5 to assess the permanence of stability with these variables.

The formulations chosen for the study are presented in Table 1, each of which was prepared and atomized at three different temperatures (60, 70 and 80 °C). The atomization process was repeated three times for each treatment.

#### 2.2.2. Feed rheology

The rheological measurements were performed using a rotational rheometer AR2000 (TA Instruments). A coaxial cylinder (ri = 14 mm, re = 15 mm, h = 42 mm and gap 5920 μm) was used to analyze the suspension and emulsions (Table 1) immediately after their preparation. The measurements were performed in duplicate at 60, 70 and 80 °C, and these temperatures were also used during the atomization of the samples in the spray chiller. Flow curves (shear stress vs. shear rate) were obtained using an up–down-program; the shear rate varied between 0 and 300 $s^{-1}$ to evaluate and eliminate the tixotropy. The second flow curve data were fitted to a power law model, which is the typical equation to characterize shear-thinning fluids, where (Pa) is the shear stress, ($s^{-1}$) is the shear rate, (Pa·sn) is the consistency index and (dimensionless) is the flow behavior index (n < 1 for a shear thinning fluid, and n = 1 for a Newtonian fluid). The results were analyzed using the Rheology Advantage Data Analysis V.5.3.1 software (TA Instruments, New Castle, USA).

#### 2.2.3. Preparation of solid lipid microparticles (SLMs)

The suspensions and emulsions were kept in a water bath at the temperature of interest before atomization, and the suspensions were maintained under constant magnetic stirring to prevent sedimentation of the HP. The feeds were pumped into the spray chiller (Labmaq, Ribeirão Preto, Brazil) using a peristaltic pump (Masterflex, USA) at a flow rate of 45 mL/min and then atomized into a cold chamber at 15 ± 2 °C using a double fluid atomizer (Ø = 1.2 mm) at a pressure of 2.2 kgf/$cm^2$.

**Table 1**
Treatments studied.

| Nomenclature | HP:fat | PGPR (%) | Stirring rate (rpm) | Stirring time |
|---|---|---|---|---|
| E1 | 1:10 | 1 | 8000 | 5 min |
| E2 | 1:5 | 1 | 8000 | 5 min |
| E3 | 1:10 | 1 | 6000 | 5 min |
| E4 | 1:5 | 1 | 6000 | 5 min |
| S1 | 1:10 | – | 3600 | 30 s |
| S2 | 1:5 | – | 3600 | 30 s |

E and S refer to feed prepared by emulsion, and suspension, respectively.