

Microencapsulation of Chia Seed Oil (*Salvia hispanica* L.) in Spray and Freeze-Dried Whey Protein Concentrate/Soy Protein Isolate/Gum Arabic (WPC/SPI/GA) Matrices [†]

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Abstract: Microencapsulation by different drying methods protects chia seed oil (CSO) against oxidative degradation, and ultimately facilitates its incorporation in certain foods. The aim of this work was to analyze the influence of freeze or spray drying, as well as of the coacervation phenomena in a ternary wall material blend—whey protein concentrate/soy protein isolate/gum arabic (WPC/SPI/GA)—on the physico-chemical properties of microencapsulated CSO. Differential scanning calorimetry studies indicated that the onset, peak, and end set temperatures for denaturation events shifted from 72.59, 77.96, and 78.02 to 81.34, 86.01, and 92.58 °C, respectively, in the ternary blend after coacervation. Oxidative stability indexes (OSI) of powders were significantly higher ($p < 0.05$) for both drying methods after inducing coacervation—from 6.45 to 12.04 h (freeze-drying) and 12.05 to 15.31 h (spray drying)—which was possibly due to the shifted denaturation temperatures after biopolymer interaction. It can be concluded that the ternary WPC/SPI/GA blend constitutes an adequate matrix to encapsulate CSO.

Keywords: chia seed oil; complex coacervation; freeze and spray drying; microencapsulation; oxidative stability; ternary wall material blend

1. Introduction

Chia seeds constitute a vegetable source rich in essential fatty acids given that, out of 30–40% total lipid (chia seed oil, CSO), 85% is composed of polyunsaturated fatty acids including Omega-3 (~66%) and Omega-6 (~20%). This composition entails a technological disadvantage due to its high susceptibility to oxidative degradation [1]. Therefore, suitable protection and delivery systems must be designed in order to preserve the functionality and organoleptic attributes of CSO within different formulated products [2].

Among microencapsulation methods, complex coacervation constitutes a great alternative for lipid cores due to the high encapsulation efficiency achieved [3]. It is an associative phase separation phenomenon where a protein and a polysaccharide exert strong attractive interactions between each other, mostly of an electrostatic nature [2]. Complex coacervates can be converted into powder either by freeze or spray drying, the latter being the most widespread technology in the microencapsulation field due to its low cost, flexibility and scalability [2,3]. Rapid water removal results in increased product shelf-life and provides the consumer with a stable product [4].

Soy protein isolate (SPI) has become one of the most utilized plant origin-emulsifiers, probably owing to the extensive production of this legume worldwide [5]. Nonetheless, it has been established that the emulsions formulated with these proteins alone are not very stable [2]. Therefore, coacervation between SPI and anionic polysaccharides has been studied to enhance the stability of emulsions [5]. In the present work, gum arabic (GA) was selected as the polysaccharide source for the emulsion formulation, mostly because of its dual role as both surfactant and drying matrix [4]. In addition, whey protein concentrate (WPC), besides its well-known emulsion capacity, contributes with an inherent antioxidant activity, owing to the formation of thick films at droplet interfaces and the chelation of prooxidant metals [4].

Based on the above exposed exposé, the present work aimed to analyze the influence of two different drying methods (freeze or spray drying) and the presence (or absence) of complex coacervation phenomena in a ternary wall material blend—whey protein concentrate/soy protein isolate/gum arabic (WPC/SPI/GA)—as an encapsulant matrix for CSO.

2. Materials and Methods

2.1. Materials

Chia seed oil (CSO) was extracted from seeds coming from the Salta province (Nutracéutica Sturla SRL, Argentina), as described by Martínez et al. [6], in a pilot plant screw press (Komet Model CA 59 G, IBG Monforts, Mönchengladbach, Germany). Soy protein isolate (SPI) SUPRO E with 90% protein on fat free basis was purchased from The Solae Company (Argentina); gum Arabic (GA) (Alland & Robert, Port-Mort, France) and acid whey protein concentrate (WPC) with 80% protein were purchased from local distributors (Distribuidora NICCO and Natural Whey, respectively; both from Argentina).

2.2. Preparation of WPC/SPI/GA Dispersions

Wall material powders were re-dispersed in Milli-Q water for 1 h with gentle stirring according to González et al. [1] and kept overnight at 4 °C for complete hydration. The necessary quantity of powder was weighted in order to yield a 30% (*w/v*) total solid concentration in dispersions, with a WPC/SPI/GA ratio (weight basis) of 8/1/1.

2.3. Zeta Potential and Turbidity Measurements

The charge density of the individual biopolymers' dispersions and their mixture (0.1% *w/v* total concentration) was determined in order to find the optimum pH (pH_{opt}), where the coacervate yield reaches its highest value and the net charge on the system is zero [2]. The measurements were performed at 25 °C (Nano Zetasizer, Worcestershire, Malvern Instruments, UK).

In addition, the turbidity of dispersions was measured at 600 nm by spectrophotometry in order to follow the formation of complex coacervate particles as a function of pH, according to Timilsena et al. [2].

2.4. Differential Scanning Calorimetry of Dispersions

Thermal analyses of individual biopolymers' dispersions and their mixture (with or without complex coacervation induced) were performed with a DSC823e Calorimeter (Columbus, OH, USA)

according to Blanco-Canalis et al. [7]. Specific enthalpies (J/g dispersion), as well as onset, peak, and end set temperatures for protein denaturation events were informed.

2.5. Emulsion Preparation and Characterization

Coarse emulsions were prepared by the high speed homogenization of CSO and a mixture of WPC/SPI/GA (15,000 rpm, 2 min, Ultraturrax homogenizer IKA T18, (Janke & Kunkel GmbH, Staufen, Germany); WPC, SPI and GA were present in an 8/1/1 ratio (weight basis) in emulsions, which were formulated with wall material and CSO contents of 30 and 15% (*w/v*), respectively. The coarse emulsions were further homogenized in a high-pressure valve homogenizer at 700 bar (1 cycle, Emulsi Flex C5, Avestin, Ottawa, ON, Canada). Subsequently, fine emulsions were either dried (by spray or freeze-drying) or subjected to pH adjustment (pH = 4.0) in order to induce complex coacervation among biopolymers before drying. That pH value was determined as described in Section 2.3. The coacervation reaction was completed with stirring at room temperature. Particle size distribution of emulsions was determined according to Us-Medina et al. [8] and with a LA 950V2 Horiba (Kyoto, Japan) analyzer. Time-dependent steady shear properties of emulsions were evaluated using a controlled-stress rheometer MCR 301 Anton Paar, equipped with a plate-cone geometry (50 mm diameter) and working with a 0.05 mm gap [9]. The rheological data were fitted to the power law and the hysteresis area was calculated.

2.6. Spray and Freeze-Drying Processes

Spray drying of chia-oil-in-water emulsions was performed as described by González et al. [1] in a laboratory-scale spray dryer, Büchi B-290 (Büchi Labortechnik AG, Flawil, Switzerland) equipped with a two-fluid nozzle atomizer. The drying conditions were the following: 1050 L/h; drying air inlet temperature: 130 ± 1 °C; atomization air flow rate: 538 L/h; pump setting (feed volumetric flow rate)—20% (approximately 5.6 mL/min); aspirator setting (drying air volumetric flow rate)—100% (38 m³/h). The emulsions were stored at -70 °C for 48 h before freeze-drying. This operation was performed in a laboratory bench-top freeze-dryer (L-T8, Rificor, Buenos Aires, Argentina) operated with a condenser at -50 °C and a vacuum of 0.1 mbar for 2 cycles of 24 h.

2.7. Powder Analysis

The powders obtained with both drying methods were characterized in terms of moisture content (MC), water activity (a_w), and particle size distribution (d_{43} volume-based mean diameter) as described by Us-Medina et al. [8]. The aggregation index (AI) of microparticles after drying of chia-oil-in-water emulsions was determined according to Ma et al. [10]. Color measurements were performed with a CM600d spectrophotometer (Konica Minolta, Tokyo, Japan) according to González et al. [1]. The whiteness (WI) and yellowness (YI) indexes were estimated as described by Rodríguez et al. [11]. Surface fat (SF) and encapsulation efficiency (EE) were determined according to González et al. [1]. The powder flowability was evaluated using the Carr's Index (CI) and the Hausner Ratio (HR) as described by Rodríguez et al. [11]. Finally, the morphology of complex coacervates and reconstituted emulsions was assessed with an optical microscope (Leica DM5000 B, Leica Microsystems, Wetzlar, Germany) operated in a dark field mode. The morphology of powders was evaluated by scanning electron microscopy (SEM, LSM5 Pascal; Zeiss, Oberkochen, Germany) according to González et al. [1].

3. Results and Discussion

The determination of zeta potential as a function of pH is a commonly used approach to study complex coacervation, given that the pH range at which the phenomenon occurs can be easily identified [1]. For the WPC/SPI/GA ternary blend, a rapid phase separation occurred (0.1%*w/v* total solids) at pH = 4.0, for which the charge density determined by zeta potential measurements was in the range of 0.659–1.100 mV, reaching practically zero net charge for the system. This was in consonance with turbidity determinations, for which a peak in absorbance values at 600 nm (1.360–

1.362) was observed at pH = 4.0, indicating the formation of insoluble complexes upon pH adjustment in a clear initial system (neutral pH, recorded absorbance values of 0.256–0.304). These complexes grow in size and can be readily identified as round-shaped entities by optical microscopy (Figure 1). Finally, an additional evidence of the interaction among biopolymers in the ternary blend was given by DSC studies (Table 1). The informed temperatures were in accordance with values reported elsewhere for whey proteins [12]. As can be seen, the onset, peak and end set temperatures for WPC denaturation shifted to higher ($p < 0.05$) values after complex coacervation, compared with a WPC dispersion and a WPC/SPI/GA mixture without induced coacervation. This may be related to the preferred biopolymer–biopolymer over biopolymer–water interactions during complex coacervation, preventing the complete hydration of proteins, and thus preserving them from denaturation [12].

Table 1. DSC parameters for wall material dispersions.

	ΔH J/g	T Onset °C	T Peak °C	T Endset °C
HAP-treated WPC ^A	1.10–1.30	71.30–72.40	76.50–77.70	81.30–81.50
WPC/SPI/GA mixture	0.74–0.96	71.60–72.60	77.40–78.00	84.00–84.30
WPC/SPI/GA mixture-c.c ^B	0.53–0.67	81.30–81.50	85.90–86.00	91.30–92.60

^A HAP-treated WPC (WPC suspension homogenized at 700 bar in a high pressure valve homogenizer);

^B cc (complex coacervation); ΔH (DSC enthalpy, J/g dispersion); T onset, T peak, T endset (°C).

Many authors pointed out the influence of parent emulsion characteristics on the final properties of powders [8], which are useful for tracking changes caused due to processing transformations. As regards particle size distribution, the emulsions showed d_{43} initial values of 5.00–5.40 μm and 12.10–12.80 μm after complex coacervation, which agrees with the droplet flocculation phenomena that take place during the multinucleated capsule formation. The rheological behavior data were fitted to the power law ($R^2 > 0.94$). Flow index (n) values of 0.61–0.67 were found for parent emulsions, indicating a shear-thinning behavior. The consistency index (k) values were 0.25–0.58 Pa s^n (0.1–300 s^{-1} shear rate range). Finally, the viscosity values at 100 s^{-1} (η_{100}), typical of many food processes, were 0.0613–0.1190 Pa s .

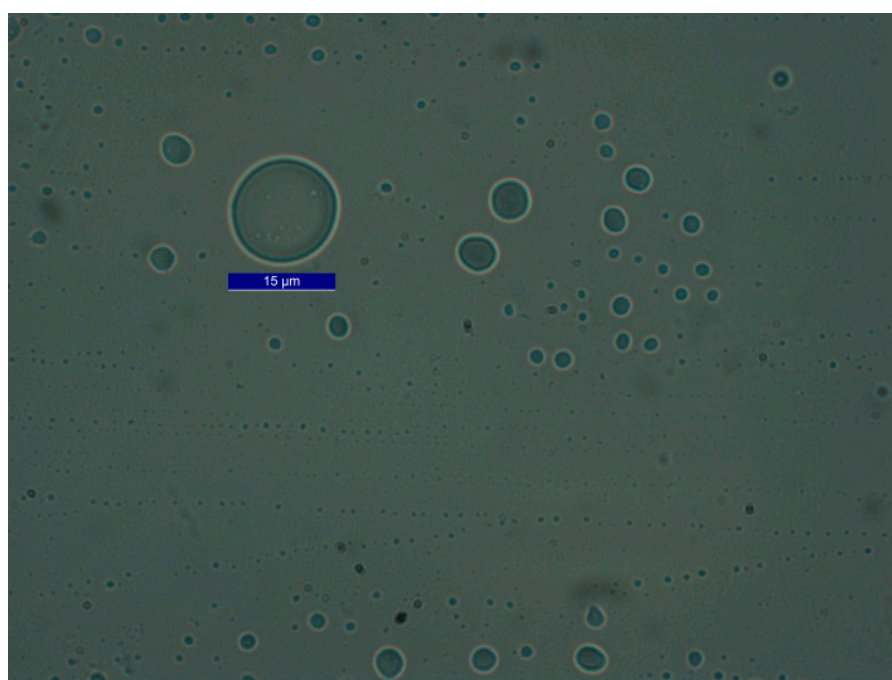


Figure 1. Optical microscopy images of whey protein concentrate/soy protein isolate/gum arabic (WPC/SPI/GA) complex coacervates.

The physico-chemical properties of powders can be found in Table 2. Values of d_{43} for reconstituted emulsions varied significantly ($p < 0.05$) according to the drying method: 51.60–61.20 μm (freeze drying) and 2.40–22.10 μm (spray drying). A significant correlation ($p < 0.05$, $r = 0.7810$) was found between d_{43} and AI, for which the highest values were observed in powders obtained by freeze drying.

This is in agreement with the fission forces that take place during atomization processes, reducing the particle size for enhanced heat and mass transfer [4] and yielding tiny solid particles (Figure 2). As expected, strong correlations ($p < 0.05$) were found for AI-WI ($r = -0.9656$) and AI-YI ($r = 0.9694$).

Table 2. Powder physico-chemical properties.

	MC	a_w	AI	EE	WI	YI	OSI
	% Wet Basis			% Dry Basis			h
FD ^A	3.70–4.30	0.216–0.220	17.00–39.70	65.60–77.70	18.70–31.40	30.30–36.70	6.45–12.04
SD ^B	2.91–4.01	0.214–0.257	0.14–0.16	71.30–80.07	61.30–64.10	15.20–16.22	12.05–15.31

^A FD (freeze drying); ^B SD (spray drying); MC (moisture content, % wet basis); a_w (water activity); AI (aggregation index); EE (encapsulation efficiency, % dry basis); WI (whiteness index); YI (yellowness index); OSI (oxidative stability index, h).

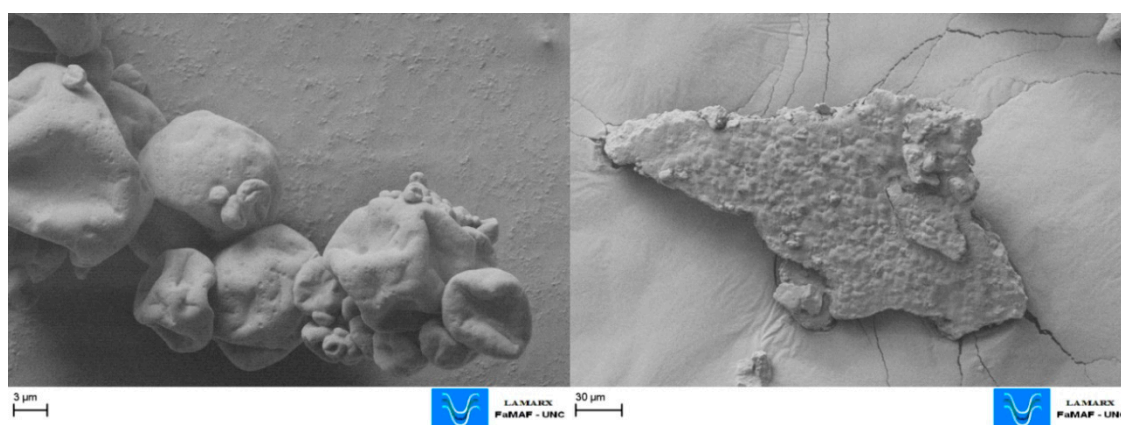


Figure 2. Scanning electron microscopy (SEM) images for microencapsulated CSO. (Left) spray drying; (Right) freeze drying.

As regards the flowing properties, $CI > 25\%$ and $HR > 1.4$ were observed, evidencing a poor flowability and strong inter-particle forces [11].

Significant changes in OSI values were observed ($p < 0.05$) according to the drying method and the interactions among biopolymers—from 6.45 to 12.04 h (freeze-drying) and from 12.05 to 15.31 h (spray drying) after inducing coacervation—which were possibly associated with the shifted denaturation temperatures (Table 1).

The porous, irregular and flake-like structure (Figure 2) of freeze-dried powders allows the diffusion of oxygen more rapidly than in spray-dried powders, which would explain the lower OSI values [1]. Nonetheless, higher OSI values than bulk CSO (3.00–3.33 h) were obtained with both drying methods.

4. Conclusions

The physico-chemical properties of microencapsulated CSO proved to be greatly affected by the drying method and the interactions among biopolymers in the ternary WPC/SPI/GA blend. Enhanced OSI values for microencapsulated CSO could be obtained by spray drying, through the formation of less porous solid microparticles compared with freeze-dried powders. In addition, even higher OSI values could be observed after coacervation in the ternary blend, possibly due to shifted WPC

denaturation temperatures. It could be concluded that the ternary WPC/SPI/GA wall material blend constitutes an adequate matrix to encapsulate CSO.

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