Characterization of Three-Layer Microcapsules of Chia Seed Oil Obtained for Electrostatic Deposition Technology †

Claudia N. Copado 1, Luciana M. Julio 1, Bernd W. Diehl 2, Vanesa Y. Ixtaina 1,3 and Mabel C. Tomás 1,*

1 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CCT La Plata (CONICET), Facultad de Ciencias Exactas (FCE), Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Universidad Nacional de La Plata (UNLP), calle 47 y 116, La Plata CP1900, Argentina
2 Spectral Service GmbH, Emil Hoffman Str. 33, 50996 Cologne, Germany
3 Facultad de Ciencias Agrarias y Forestales (FCyF), Universidad Nacional de La Plata (UNLP), calle 60 y 119, La Plata CP1900, Argentina
* Correspondence: mabtom@hotmail.com
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Abstract: Oils with high omega-3 fatty acids are known for their multiple health benefits. For this reason, new strategies have been developed to protect these fatty acids from lipid oxidation to incorporate them into foods. Microencapsulation is an attractive alternative for protecting and incorporating chia oil in food matrices. A three-layer microencapsulation process was performed using a layer-by-layer technique with the addition of sunflower lecithin, chitosan, and chia mucilage by spray-drying. The microcapsules obtained were studied and stored in darkness at a controlled temperature and relative humidity for 90 days. The ζ-potential evidenced the electrostatic deposition of the layers in powders through the change of the values in the electric charge. Microcapsules showed a high microencapsulation efficiency and low moisture content and water activity levels. Microencapsulated chia oil presented low oxidation values (<10 meq hydroperoxides/kg oil) and high omega-3 fatty acid content after storage. These results suggest that the three-layer microcapsules studied are suitable to provide high stability against the oxidative deterioration of functional lipid components in chia oil and constitute a promising application in the food industry.

Keywords: chia oil; α-linolenic acid; LBL technique; microcapsules; Omega-3

1. Introduction

Currently, oils rich in ω-3 fatty acids are known for their multiple health benefits. For this reason, new functional ingredients are being developed that allow their incorporation into foods and protect the oil from lipid oxidation. Microencapsulation represents an attractive alternative for protecting and incorporating chia oil in various food matrices [1]. One of the most commonly used encapsulation methods is spray-drying [2]. Obtaining stable emulsions with a subsequent proper drying process for conversion into powder material is essential in preparing microencapsulated oils. Different factors, such as the characteristics of the parent oil-in-water (O/W) emulsion, the total solid content, the type of wall and core material, and the microencapsulation processing conditions, can affect the physicochemical properties and the stability of the oil microparticles [2]. The layer-by-layer (LBL) technique is a strategy to improve the emulsion stability, which enables the creation of multiple layers of emulsifiers and polyelectrolytes around the oil droplets [1]. Sunflower lecithin can be used as an emulsifier and presents the advantage that it is a non-GMO additive, which some consumers appreciate. Chitosan soluble in acidic aqueous media presents a positive charge at pH values < 8, increasing its ζ-potential as the pH decreases. Chia mucilage, an anionic polymer within a pH range of 1.8–12.0, confers viscosity to dispersions in water [3].
The objective of this work was to obtain chia oil three-layer microcapsules by spray-drying using modified sunflower lecithins, chitosan, chia mucilage, and maltodextrin as wall materials, carrying out the respective physicochemical characterization.

2. Materials and Methods

2.1. Materials

Cold-pressed chia oil was provided by Solazteca SDA (Lobos, Buenos Aires, Argentina). The modified (deoiled (LD) and hydrolyzed (LH)) sunflower lecithins were provided by Lasenor Emul S.L. (Olesa de Montserrat, Barcelona, Spain). Chitosan (Q) was purchased from Sigma Chemical Company (St. Louis, MO, USA). Maltodextrin (Mxd) DE 13–17% was donated by INDECAR SAICyF (Ciudad Autónoma de Buenos Aires, Argentina). Chia mucilage was extracted from the seed, as described by Copado et al., 2021 [1].

2.2. Methods

2.2.1. Parent Emulsions: Preparation and ζ-Potential Determination

The stock dispersions to prepare the emulsions were: LD or LH 2.2% \( w/w \), Q 2.0% \( w/w \), and chia mucilage 11.9% \( w/w \) (pH 5). Pre-emulsification was made using an Ultraturrax homogenizer (IKA-Labortechnik, 130 GmbH & Co., Staufen, Germany), 2 min at 9500 rpm. Subsequently, a high-pressure valve homogenizer (Panda 2K GEA Niro Soavi, Parma, Italy) (1000 bar, 4 cycles) was used to obtain the primary. The chitosan dispersion addition using similar operating conditions allowed for obtaining the secondary emulsion. After that, chia mucilage was added through an ultrasonic processor (VCX 750, Sonics & Materials Inc. Newtown, CT, USA) (amplitude of 40% for 2 min). Finally, Mxd was incorporated. The total solid content was 26% \( w/w \) (final concentration in emulsion: 5% chia oil, 0.5% lecithins (LD or LH), 18.2% Mx, 0.3% chitosan, and 2% chia mucilage).

The ζ-potential after each step of the three-layer emulsion obtention was analyzed using a zeta potential analyzer (Brookhaven 90Plus/Bi-MAS, Holtsville, NY, USA) on the function of electrophoretic mobility at room temperature [4].

2.2.2. Microcapsule Preparation by Spray-Drying

The spray-drying of the emulsions was carried out in a Mini Spray Dryer B-290 (BÜCHI, Flawil, Switzerland) (0.5 mm diameter nozzle, 0.6 L/h of feed rate, and 170/75 °C of air inlet/outlet temperatures) [5].

The storage of the microcapsules was made at 33%RH, 25 ± 2 °C, in darkness for 90 d. Bulk chia oil stored under the same conditions was included as control system.

2.2.3. Microcapsule Characterization

Moisture Content and Water Activity (\( a_w \))

The moisture content and the \( a_w \) was evaluated, according to Copado et al. [1].

Microencapsulation Efficiency

It was calculated by the relation between the encapsulated and the total oil, both gravimetrically determined, according to Copado et al. [1] and Klinkesorn et al. [6], respectively. ζ-potential of the Reconstituted Emulsions

The powders were reconstituted in a ratio of 10% \( w/w \) with acetic acid/acetate buffer (100 mM) at a pH of 5, at ~25 °C, stirring for 30 min. Afterward, the ζ-potential was analyzed, according to the procedure detailed previously for parent emulsions.

Peroxide Value (PV)

The primary lipid oxidation products were evaluated, according to the method of Díaz et.al. (2003) [7].

Content of Omega-3 Polyunsaturated Fatty Acids (PUFAs)
It was analyzed by NMR spectroscopy using an Avance III 400 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany) with a 5 mm BBI probe (Eurofins, Hamburg, Germany) [8]. Spectroscopic measurements (1H NMR) were carried out in triplicate.

2.2.4. Statistical Analysis

To detect any significant difference between samples, data were subjected to analysis of variance (ANOVA) at a 95% confidence level ($p \leq 0.05$). Significantly different data sets were classified after post hoc comparison tests using Tukey’s honestly significant differences test (HSD, $p \leq 0.05$).

3. Results and Discussion

3.1. ζ-Potential of the Parent Emulsions

The deposition of layers was verified by the inversion of the electrical charge after each step of the parent emulsion preparation [9]. After the lecithin addition, the emulsions presented a negative ζ-potential (−10.39 and −6.43 mV for emulsion with LD and LH, respectively). Positive values of ζ-potential were recorded after the addition of Q (31.08 mV for LD, and 28.99 for LH). This fact shows the successful chitosan deposition on the layer of modified sunflower lecithins at a pH of 5, conferring to the particles a cationic character. Finally, a new change of ζ-potential towards negative values was recorded for the three-layer emulsions, caused by the interaction between the chitosan and the chia mucilage forming the third layer (−18.91 mV for LD; −18.87 mV for LH). These results are according to other research works, which reported changes in the electrical charges during the formulation of multilayered microparticles by the LBL electrostatic deposition technique [10,11].

3.2. Characterization of Multilayer Microcapsules

3.2.1. Moisture Content (MC%) and Water Activity ($a_w$)

The values of MC% and $a_w$ (Table 1) varied between 0.86–1.29% and 0.224–0.280, respectively, which are suitable values for powder products (3–4% d.b.) [1]. No significant differences ($p > 0.05$) were found between the microcapsules with different modified lecithin, whereas the $a_w$ of TLD was significantly ($p \leq 0.05$) higher than TLH. The $a_w$ values are in the range where the lipid oxidation is minimal (0.2–0.4) due to a delay in the decomposition of the hydroperoxides and a decrease in the pro-oxidant activity of metals that promotes the oxidation process [12].

Table 1. Physicochemical characterization of chia oil microcapsules at t = 0 and 90 d of storage at 25 ± 2 °C, 33% RH, darkness.

<table>
<thead>
<tr>
<th>Microcapsule</th>
<th>MC% (d.b.)</th>
<th>$a_w$ (25 °C)</th>
<th>ME</th>
<th>PUFAs Omega-3 Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLD</td>
<td>0.86 ± 0.16 a</td>
<td>0.280 ± 0.003 b</td>
<td>98.33 ± 0.51 a</td>
<td>60.40 ± 0.00 aA</td>
</tr>
<tr>
<td>TLH</td>
<td>1.29 ± 0.04 a</td>
<td>0.224 ± 0.015 a</td>
<td>98.16 ± 0.52 a</td>
<td>60.50 ± 0.00 aA</td>
</tr>
</tbody>
</table>

Mean values ± standard deviation ($n = 3$). The different letters in each column indicate differences ($p \leq 0.05$) between storage times (p ≤ 0.05) for each system, according to the Tukey test (HSD). Different capital letters in the rows indicate differences between storage times (p ≤ 0.05) for each system, according to the Tukey test (HSD). Moisture content (MC%), dry basis (d.b.), water activity ($a_w$), microencapsulation efficiency (ME), and polyunsaturated fatty acid content (PUFAs omega-3 content) at initial time (t = 0 d) and final time (t = 90 d). TLD: Three layer with LD, TLH: Three layer with LH.

3.2.2. Microencapsulation Efficiency (ME)

The studied systems presented high ME values (98.33–98.16%), indicating that the wall materials and the microencapsulation process were appropriate to encapsulate the lipid nucleus. No significant differences ($p > 0.05$) were found between the microcapsules with different modified lecithin. It could be related to the multiple layers of chitosan and mucilage that covered up any possible effect of the lecithin type.
3.2.3. ζ-Potential

The ζ-potential of the particles of the reconstituted emulsions was similar to the parent emulsions (TLD-15.45 mV and TLH-15.90 mV). These results suggest different layers in the powders showing that the microencapsulation process did not alter the electrical nature of the biopolymers.

3.2.4. Oxidative Stability: PV Values and PUFAs Omega-3 Content

At the initial time, the PV were 1.02, 1.06, and 2.02 meq of peroxide/kg of oil for TLD, TLH, and chia oil, respectively. The PV of the bulk chia oil increased faster than the microencapsulated systems during storage at 33% RH, 25 ± 2 ºC, in darkness, achieving a value of 18.99 meq of peroxide/kg of oil after 90 d. On the contrary, the microencapsulated oil did not present significant differences (p > 0.05) on storage time, with PV < 10 meq/kg oil being the maximum value allowed for the consumption of chia oil (Codex Alimentarius Commission, Standard CXS 19-1981, amended in 2019).

Regarding the ω-3 PUFAs of the microcapsules, they did not show significant variation between the initial and the final storage time. The content of these PUFAs was ~60%, without significant differences (p > 0.05) between the systems (Table 1). These results are analogous to the PV, suggesting that the three-layer microcapsules are efficient systems to protect chia oil against lipid oxidation. The chia muclilage addition would increase the thickness of the interfacial membrane, constituting an additional barrier to oxygen diffusion.

4. Conclusions

In the present research work, chia oil microcapsules were developed from the spray-drying of three-layer emulsions obtained by electrostatic deposition, using the LBL technique. The results allowed us to observe a high microencapsulation efficiency, suggesting the process that the materials used to form the wall were adequate to encapsulate the oil. The spray-drying conditions were appropriate, since the moisture and aw values are within the accepted limits for the stability of dehydrated foods. The electrical charge of the rehydrated systems allowed us to verify the different layers formed in the microcapsules. The peroxide values showed a marked increase in the oxidation of the unencapsulated oil, compared with those of the microencapsulated systems, which were below 10 meq of hydroperoxides/kg of oil. Therefore, the studied microcapsules were efficient protecting systems against the oxidation of chia oil-sensitive compounds. This protective effect can also be seen in the high omega-3 content of microcapsules at the end of storage.

These results suggest that the three-layer microcapsules are suitable for providing high oxidation stability of the functional lipid components in chia oil and are interesting for their application in the food industry for food enrichment.

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