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Proceeding Paper

Molecular Encapsulation of Hydrolyzed Chia Seed Oil by Ultrasonically Treated Amylose Inclusion Complexes †

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Abstract: Chia (*Salvia hispanica* L.) seed oil is a naturally rich source of α-linolenic (~65%) and linoleic (~20%) essential fatty acids, which are known for their beneficial effects on health. However, they are highly susceptible to oxidative deterioration. Amylose, the linear component of starch, has the ability to form inclusion complexes with hydrophobic molecules (ligand), which may act as delivery systems of sensitive bioactive compounds, including essential omega-3 and omega-6 fatty acids. In the present work, the hydrolytic effectiveness of *Candida rugosa* and porcine pancreatic lipases to obtain chia seed oil-free fatty acids was compared, which were complexed with high-amylose starch through the alkaline method with and without the incorporation of ultrasonic treatment. The highest level of free fatty acids released (>80%) was reached with *Candida rugosa* lipase. The inclusion complexes formed with this hydrolysate displayed a typical V-type X-ray diffraction pattern (peaks at ~7.5, 13, and 20° (2θ)), which confirmed an effective complexation. Moreover, ultrasonically treated complexes displayed a small peak at ~21°, from crystallized saturated fatty acids. Through attenuated total reflectance Fourier-transform infrared spectroscopy, the presence of typical bands of fatty acids in the complexes was verified, whose intensity increased after the application of ultrasonic treatment. The dissociation temperature determined using differential scanning calorimetry was >90 °C. According to this, *Candida rugosa* lipase showed better hydrolytic effectiveness on chia seed oil, and the fatty acids released were able to form amylose inclusion complexes with high thermal stability, whose properties varied after ultrasonic treatment.

Keywords: amylose inclusion complex; chia seed oil; enzymatic hydrolysis; α-linolenic acid; linoleic acid; ultrasound

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1. Introduction

Chia (*Salvia hispanica* L.) seed oil is a rich source of α-linolenic (C18:3, ~60%) and linoleic (C18:2, ~18%) fatty acids (FAs). Their multiple health benefits and susceptibility to oxidative deterioration have promoted the development of delivery systems. Amylose, the linear component of starch, interacts with hydrophobic molecules forming inclusion complexes (ICs), a helical structure that incorporates the guest (ligand) inside its inner cavity, which may potentially act as carrier agents of bioactive compounds [1]. The complexation of triacylglycerols is restricted by steric hindrance effects and low water solubility [2], while the amylose interaction with free fatty acids may be enhanced through the application of ultrasonic treatment (UT) [3].

The aim of the present work was to compare the effectiveness of porcine pancreatic (PP) and *Candida rugosa* (CR) lipases to hydrolyze chia seed oil and to study the physicochemical

properties of amylose–chia seed oil fatty acid ICs formed with and without the application of ultrasonic treatment. It will contribute to the development of ICs as potential delivery systems of chia oil essential fatty acids.

2. Materials and Methods

2.1. Materials

High-amylose corn starch (>70% amylose) was kindly provided by Ingredion Inc. (Westchester, IL, USA). Chia oil (fatty acid composition: α-linolenic 63.5%, linoleic 18.2%, palmitic 9.4%, oleic 5.7%, and stearic 3.2% acids) was purchased from Solazteca SDA S.A. (Buenos Aires, Argentina). Porcine pancreatic (type II, enzymatic activity 100–400 U/mg) and *Candida rugosa* (type VII, enzymatic activity ≥700 U/mg) lipases were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade.

2.2. Chia Seed Oil Enzymatic Hydrolysis

The hydrolytic reaction of chia oil with porcine pancreatic and *Candida rugosa* lipases was performed and monitored according to the procedures previously described [4], i.e., o/w emulsion 20% *w/w*, 37 °C, pH = 7, free fatty acid (FFA, %) measurement by titration with 0.1N NaOH. Then, the fatty acids released (hydrolysate) were extracted from the reaction mixture that reached the highest FFA content, by acidification to pH < 2 with 4N HCl, followed by centrifugation (4000× *g*, 20 min).

2.3. Formation of Untreated and Ultrasonically Treated Inclusion Complexes

The inclusion complex formation was performed following the procedures previously described [4], with a crystallization step of 2 h at 90 °C. Ultrasonically treated complexes were obtained under the same protocol, but subjecting the starch–hydrolysate mixture to ultrasound (1 min, 30% amplitude, pulsed on/off for 5 s) before their acidification to pH~4.7, using a VCX 750 ultrasonic processor (Sonics & Materials Inc., Newtown, CT, USA).

2.4. Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR spectra of powdered ICs were directly measured and recorded in the wavenumber range of 500 to 4000 cm^{-1} under 4 cm^{-1} spectral resolution accumulating 16 scans per spectra, using an ATR-FTIR Thermo Nicolet iS10 spectrometer (Thermo Scientific, Waltham, MA, USA). The results were analyzed using OMNIC software (version 8.3, Thermo Scientific, MA, USA).

2.5. X-ray Diffraction (XRD)

The X-ray diffraction measurements of powdered ICs were carried out using a PANalytical X'Pert Pro diffractometer (Panalytical, Netherlands). Operating conditions: CuKα radiation (λ = 1.5403 Å), current 40 mA, voltage 40 kV, 5–35° (2θ) range, steps of 0.02°/s. The diffractograms were analyzed using the PeakFit v4.12 software (SeaSolve Software Inc., San José, CA, USA) and the crystallinity (%) was calculated as the ratio of the crystalline to the total (crystalline + amorphous) peak areas [4].

2.6. Differential Scanning Calorimetry (DSC)

The endothermic transitions of untreated and ultrasonically treated ICs previously suspended distilled water (1/4 *w/w* IC/water) were registered according to the procedure described by [4] (~8 mg of sample in hermetically sealed aluminum pans, 20–150 °C range at 5 °C/min) with a differential scanning calorimeter Q100 (TA Instruments, New Castle, DE, USA). A sealed empty pan was used as the reference. Thermograms were analyzed with the TA Instruments Universal Analysis 2000 software (TA, New Castle, DE, USA).

2.7. Statistical Analysis

ANOVA and Tukey ($p \leq 0.05$) tests were used to establish significant differences between means, and performed using the Statgraphics Centurion XV.II software (StatPoint Technologies, Warrenton, VA, USA).

3. Results and Discussion

3.1. Enzymatic Hydrolysis of Chia Seed Oil

Figure 1 shows the free fatty acids (%) released by *Candida rugosa* and porcine pancreatic lipases. As can be seen, in both cases, a rapid increase in FFA was observed at the beginning of the reaction, followed by a plateau in which the FFA remained almost constant over time. The maximum values reached by CR and PP lipases after 5 h of reaction were 82 and 24% FFA, respectively, indicating that CR had better hydrolytic effectiveness than PP lipase (Figure 1). The microbial enzyme catalyzes the hydrolysis of triacylglycerols randomly and is, therefore, able to release all types of acyl chains, regardless of their position in the glycerol molecule. The animal enzyme is a typical sn-1,3-specific lipase, i.e., the hydrolysis of triacylglycerols occurs in both the *sn*-1 and *sn*-3 positions of the glycerol backbone, while the fatty acid esterified in the *sn*-2 position remains non-hydrolyzed [5]. This may explain the better effectiveness of CR lipase than PP during the chia seed oil hydrolysis. Based on these results, the hydrolyzed lipid fraction obtained using the enzyme from CR was chosen to be used as a ligand during the following complex formation.

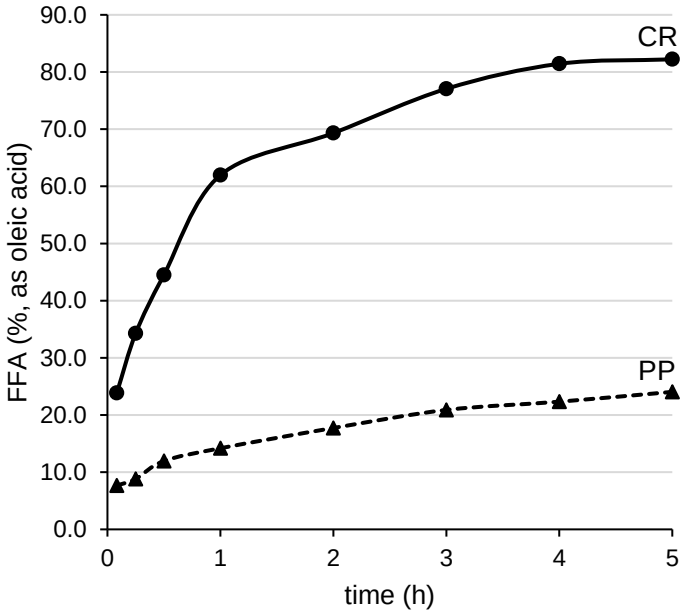

Figure 1. Free fatty acids (%, as oleic acid) released by enzymatic hydrolysis of chia seed oil with *Candida rugosa* (CR) and porcine pancreatic (PP) lipases.

3.2. Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (ATR-FTIR)

The original (non-hydrolyzed) chia seed oil displayed a high-intensity band at ~1742 cm^{-h} (Figure 2a), from the stretching of the ester bonding between the glycerol and fatty acids in the triacylglycerol molecule. This band was not observed in the hydrolysate spectrum, but a new high-intensity band appeared at ~1707 cm^{-1} (Figure 2b), which originated from the stretching vibration of the carbonyl (–C = O) of the acid functional group of FAs released by the enzymatic hydrolysis. Moreover, the = C – H vibration of cis double bonds of unsaturated FAs originated a medium-intensity band at ~3010 cm^{-1} in both the oil and hydrolysate (Figure 2a,b). These samples also showed bands at 2852 and 2924 cm^{-1}, corresponding to the symmetric and asymmetric vibration of the CH_2 groups from the fatty acid alkyl chains, respectively (Figure 2a,b).

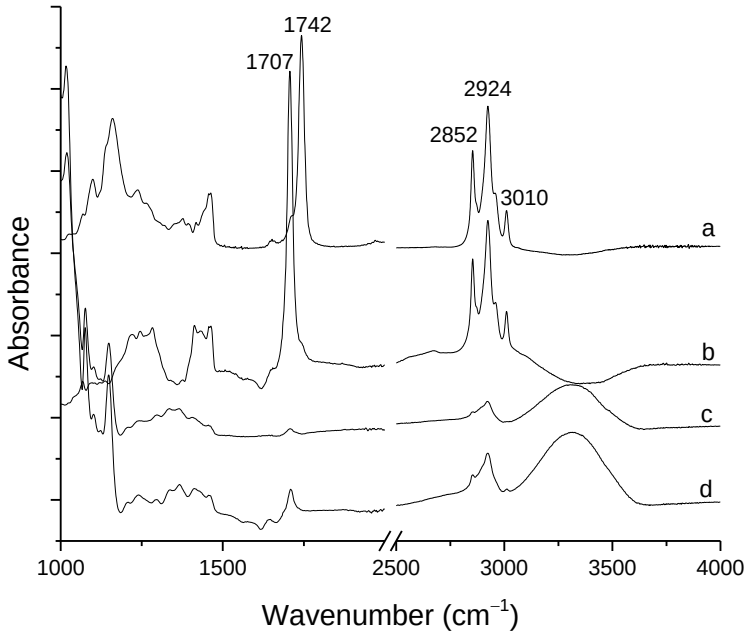

Figure 2. ATR-FTIR spectra of: (a) chia seed oil, (b) enzymatically hydrolyzed chia seed oil, (c) untreated inclusion complexes, and (d) ultrasonically treated inclusion complexes.

The typical bands from the hydrolysate previously mentioned were also present with lower intensity in the spectra of ICs, especially those at 1707, 2852, and 2924 cm^{-1} (Figure 2c,d). It confirms the presence of guest FAs in the ICs formed under the different conditions studied. The peak intensity in the ultrasonically treated ICs was higher than in the untreated ones, suggesting higher retention of FAs after ultrasonic treatment.

3.3. X-ray Diffraction

The crystalline structure of the powdered ICs obtained after freeze-drying was characterized by XRD. Both untreated and ultrasonically treated complexes displayed a semi-crystalline V-type diffraction pattern with two main reflections at ~13 and 20° (2θ) and a small reflection at ~7.5° (Figure 3), confirming an effective complexation of chia oil fatty acids. In addition, the ultrasonically treated samples displayed a higher degree of crystallinity (~37%) than the untreated ones (~31%) ($p \leq 0.05$) and a small peak at ~21° from crystallized saturated fatty acids. According to this, sonication promoted the complexation of FAs, in agreement with the results found by ATR-FTIR and with a previous work [3]. This finding may be attributed to the cavitation effect produced by ultrasound that disrupts the starch granules favoring the release of amylose and also improves the FA dispersibility in the starch solution, which increases the amylose–FA interaction to form ICs [3].

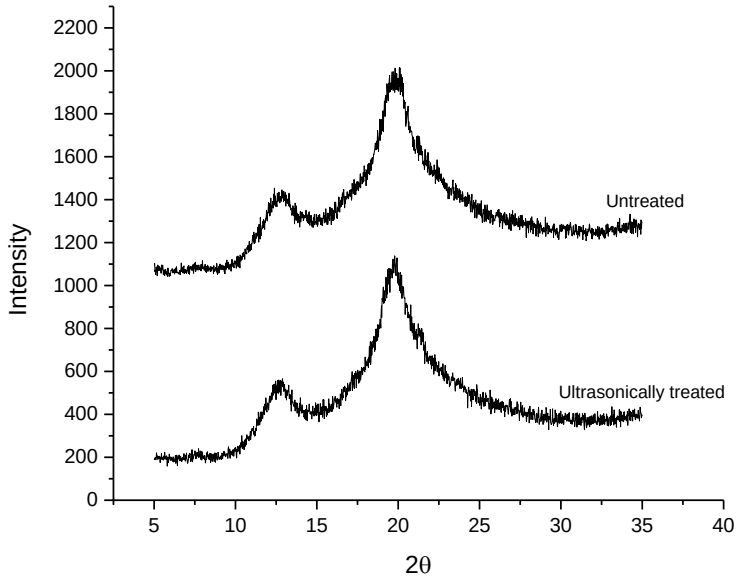

Figure 3. X-ray diffraction patterns of untreated and ultrasonically treated inclusion complexes of high-amylose corn starch with chia seed oil fatty acids.

3.4. Differential Scanning Calorimetry

Through DSC, it was observed that the ICs displayed a broad endothermic transition (Figure 4), which could be associated with the amylose–ligand dissociation. This confirms the effective complexation of chia oil fatty acids, in agreement with the XRD results. Both the untreated and ultrasonically treated ICs showed high-temperature stability (peak temperature (T_p) >90 °C) and a melting enthalpy (ΔH) of ~6 J/g (d. b.). No significant differences were found in the thermal parameters of ICs ($p \geq 0.05$), indicating that sonication did not have an effect on their thermal behavior. Since the ligand used in this work is formed by a mixture of fatty acids, the thermal transitions observed may be the result of several overlapped individual endotherms, thus yielding a broad dissociation range (Figure 4).

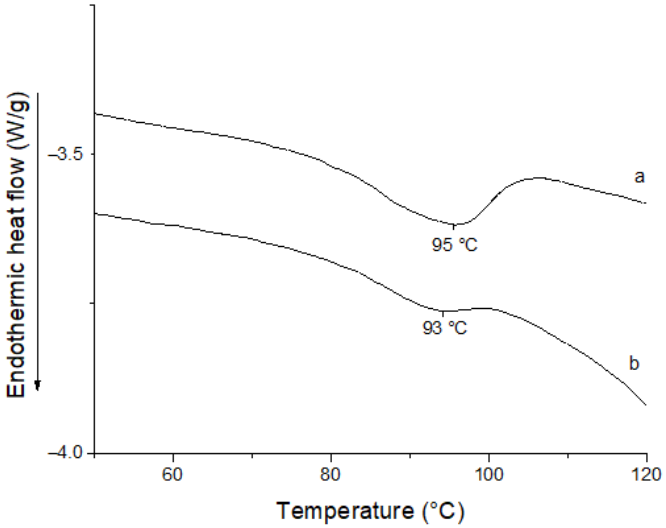

Figure 4. DSC thermograms of (a) untreated and (b) ultrasonically treated inclusion complexes of high-amylose corn starch with chia seed oil fatty acids.

4. Conclusions

From the results of the present work, it can be concluded that CR lipase had a better performance than PP to hydrolyze chia seed oil. The free fatty acids obtained after this

reaction have demonstrated to successfully form V-type ICs with high-amylose corn starch, as verified by complementary XRD, DSC, and ATR-FTIR. The incorporation of ultrasonic treatment to the formation process promoted the complexation of these FAs, as inferred from the increased crystallinity and higher band intensity observed in the XRD and ATR-FTIR analysis, respectively. The high T_p of ICs (>90 °C) would suggest that this system may potentially act as vehicle of chia seed oil fatty acids in thermally treated foods.

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Conflicts of Interest: The authors declare no conflict of interest.

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