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Proceeding Paper

In Vitro Digestion of Chia Seed Oil Nanoemulsions †

Luciana Julio [1,*], Greilis Quintero-Gamero [2], Estefanía Guiotto [2] and Vanesa Ixtaina [2,3]

[1] Instituto de Investigaciones para la Industria Química (INIQUI) (CONICET Salta-Jujuy, UNSa), Av. Bolivia 5150, Salta 4400, Salta Province, Argentina
[2] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (CONICET La Plata, FCE-UNLP, CICBA), Calle 47 y 116, La Plata 1900, Buenos Aires, Argentina; greilisquintero@quimica.unlp.edu.ar (G.Q.-G.); esnagui@hotmail.com (E.G.); vanesaix@hotmail.com (V.I.)
[3] Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata (FCAyF-UNLP), Calle 60 y 119, La Plata 1900, Buenos Aires, Argentina
* Correspondence: luci_julio86@hotmail.com
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Abstract: Oil-in-water (O/W) nanoemulsions offer significant potential for protecting and delivering sensitive ingredients such as chia seed oil, which is rich in ω-3 fatty acids (approximately 64% α-linolenic acid, ALA). This research work aimed to study the in vitro fat digestibility of chia O/W nanoemulsions (Cas1000) with 10% (*w/w*) of chia oil and 2% (*w/w*) of sodium caseinate prepared by microfluidization (1000 bar, 3 passes) and characterized through their droplet size, superficial droplet charge, and global stability. In terms of the in vitro fat digestibility, three different matrices were studied: a water solution of sodium caseinate, a chia O/W nanoemulsion, and a bulk chia oil. The particle size distribution, mean diameter, and microstructure were evaluated after in vitro stomach and small intestine simulation according to the INFOGEST method. Free fatty acids (% FFA) produced during lipolysis were quantified at the end of digestion through their neutralization by acid-base volumetric assay. The droplet size of the Cas1000 had slight changes during the gastric phase while a significant variation of this parameter was observed at the end of the intestinal phase. A higher %FFA was obtained in Cas1000 compared to bulk chia oil with values of 58.26 and 38.13%, respectively. The ALA content in the lipid phase was quantified at the end of the gastrointestinal digestion process. The results indicated no significant changes compared to the initial oil, suggesting no losses of active compounds during digestion.

Keywords: free fatty acids; INFOGEST; lipolysis; microfluidization; omega-3 fatty acids

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1. Introduction

The ω-3 and ω-6 fatty acids are essential for human health, but modern diets often have an imbalance, with higher omega-6 intake, such as linoleic acid (LA), compared to ω-3 like α-linolenic acid (ALA). This imbalance can lead to negative health effects, making it important to improve the ω-3/ω-6 ratio in the diet. Chia (*Salvia hispanica* L.) is a rich source of ALA, contributing about 64% of the total fatty acids in its oil, which helps increase ω-3 intake and rebalance this ratio, making it a valuable functional ingredient for better health outcomes [1,2].

ALA is highly susceptible to oxidation, which comprises its stability during food storage and processing. Additionally, its bioavailability in the body is limited, as it can be degraded before being effectively absorbed and utilized. These challenges underscore the need for efficient delivery systems, such as nanoemulsions, that protect ALA from oxidation and enhance its absorption in the body, ensuring that consumers can fully benefit from its health-promoting properties [1].

Emulsions can be tailored with various compositions and techniques to achieve different physicochemical and functional properties. Nanoemulsions, with droplet sizes of 20 to 200 nm, provide enhanced stability and bioavailability of lipophilic compounds, reducing

gravitational separation and droplet aggregation compared to conventional emulsions, thus extending food shelf life [3].

In vitro digestibility is a key tool for assessing the breakdown and absorption of essential fatty acids, like ALA, in the gastrointestinal tract. This method simulates nutrient release and availability under controlled conditions, providing valuable insights into digestion and utilization efficiency [4].

This study investigated the in vitro digestibility of chia oil-in-water nanoemulsions, formulated with sodium caseinate as the emulsifier and microfluidization for the emulsification process.

2. Materials and Methods

2.1. Materials

Chia oil (CO) was purchased from Solazteca SDA S.A. (Buenos Aires, Argentina). Sodium caseinate (Cas), pepsin, pancreatin, and bovine bile were obtained from Sigma-Aldrich (Steinheim, Germany), and ALA and LA methyl ester standards were from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were analytical grade.

2.2. Emulsion Preparation

O/W nanoemulsions (Cas1000) were prepared with 10% *w/w* of chia oil and 2% *w/w* of sodium caseinate. Pre-emulsions were formed by homogenizing CO and Cas solution with an Ultraturrax T-25 (Janke and Kunkel GmbH, Staufen, Germany) at 10,000 rpm for 2 min, then further processed using a microfluidizer (LM10, Microfluidics, Westwood, LA, USA) at 1000 bar for 3 cycles. Nanoemulsions were treated with 12 ppm nisin and 1000 ppm of potassium sorbate to inhibit microbial growth.

2.3. Emulsion Characterization

2.3.1. Particle Size and ζ-Potential

Particle size was measured in triplicate using static light scattering with a Malvern Mastersizer 2000E (Malvern Instruments Ltd., Worcestershire, UK) according to Julio et al. [3]. The ζ-potential was determined using a Zeta Potential Analyzer (Brookhaven 90 Plus/BiMAS, Holtsville, NY, USA) [3].

2.3.2. Physical Stability

The physical stability of emulsions was assessed with a Vertical Scan Analyzer (Quick Scan, Coulter Corp., Miami, FL, USA) during 50 d [3].

2.3.3. Microscopic Analysis

The microstructure was analyzed with a Leica DMLB optical microscope (Leica Microscopy Systems Ltd., Heerbrugg, Switzerland), using 0.2% Nile red fluorescent dye to stain the oil phase. Micrographs were captured at 63× magnification at room temperature (25 ± 1 °C).

2.4. In Vitro Digestion

In vitro digestion of chia O/W nanoemulsion (Cas1000) was performed using the static INFOGEST protocol [5], with continuous phase (Cas) and bulk chia oil (CO) as controls. Simulated gastric fluids included pepsin (2000 U/mL), and intestinal fluids contained pancreatin (100 U/mL of trypsin) and bovine bile (10 mmol/mL).

2.4.1. Particle Size and Microscopic Analysis

The size and microstructure of the particles in each system subjected to in vitro digestion at the end of the gastric and intestinal phases were evaluated as detailed in Section 2.3.

2.4.2. Extent of Lipolysis

The degree of lipolysis was determined by measuring the amount of free fatty acids (FFA) produced in CO and Cas1000 immediately after intestinal digestion according to Pinheiro et al. [6].

2.4.3. Fatty Acid Composition

The relative percentages of LA and ALA in chia oil and Cas1000 oil before and after in vitro digestion were determined by gas chromatography using an Agilent Technologies 7890 A gas chromatograph (Santa Clara, CA, USA) with a flame ionization detector and a DB-23 column. For oil extraction, 25 mL of a 1:3 isopropanol/isooctane mixture was added to 40 mL of the emulsion or digestate, vortexed, and centrifuged at 3000 rpm for 2 min. The organic phase was collected, and the solvent was evaporated using a Büchi B-480 rotary evaporator (Büchi, Switzerland) at 100 bar and 50 °C.

2.5. Statistical Analysis

The results were analyzed using ANOVA ($p \leq 0.05$) with Statgraphics Centurion XV.II software (StatPoint Technologies, Warrenton, VA, USA). Multiple comparisons were conducted with the Tukey test ($p \leq 0.05$) at a 95% confidence level.

3. Results and Discussion

3.1. Emulsion Characterization

The particle size distribution (PSD) of Cas1000 showed a bimodal pattern (Figure 1) with a narrow peak around 0.134 µm and a secondary peak at 0.63 µm. $D_{4,3}$ value was 0.15 ± 0.1 µm. At pH~6.5, the droplets had a negative surface charge of −41 ± 1 mV. Cas1000 remained physically stable, maintaining its BS profiles without significant changes over 50 d. These results indicate that the microfluidization process and emulsifier were effective in forming chia O/W nanoemulsions.

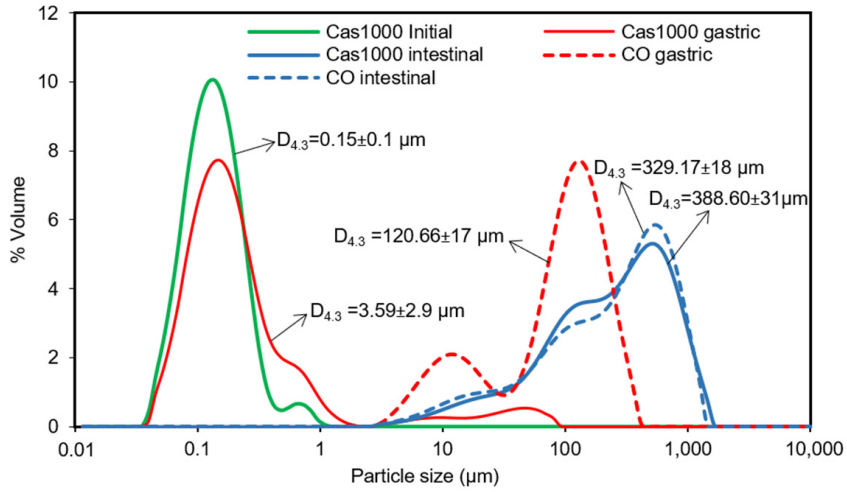

Figure 1. The particle size of Cas1000 (solid line) and CO (dotted line) at the initial time (—), after gastric (—), and intestinal (—) stages of the in vitro digestion assay. CO: chia oil; Cas1000: chia O/W nanoemulsions.

3.2. In Vitro Digestion

3.2.1. Particle Size and Microscopic Analysis

The size of oil droplets can indicate the efficiency and rate of lipolysis [7]. Figure 1 shows the PSD of Cas1000 before digestion and of both CO and Cas1000 at the end of the gastric and intestinal phases. The PSD of Cas1000 after the gastric phase was similar to the initial system (Cas1000 Initial). However, after the intestinal stage, the PSD of Cas1000 changed significantly, displaying a broad range of particle sizes and shifting towards larger

sizes. Regarding CO, it exhibited bi and trimodal PSD after the gastric and intestinal phases, respectively. By the end of digestion, CO and Cas1000 had similar PSD.

The mean particle size of Cas1000, expressed as $D_{4,3}$, remained similar after exposure to the simulated gastric conditions ($p > 0.05$), indicating the stability of this system. However, this diameter significantly increased ($p \leq 0.05$) after intestinal digestion. On the other hand, CO had a significantly larger $D_{4,3}$ ($p \leq 0.05$) than Cas1000 at the end of the gastric phase. By the end of the intestinal phase, there were no significant differences ($p > 0.05$) between the two systems for this parameter (Figure 1).

Confocal microscopy images of the CO and Cas1000 after both gastric and intestinal digestion stages are shown in Figure 2. While no significant changes in particle size were observed between the initial Cas1000 and after gastric digestion using the light scattering method, the microscopy images revealed some degree of flocculation and early signs of coalescence (Figure 2). In the gastric phase, the high ionic strength and acidification of the medium (pH 3.0), passing through the isoelectric point of the proteins (~4.6), may be responsible for these changes [8]. Under these conditions, the protein layer loses sufficient repulsive forces, leading to droplet aggregation. Furthermore, confocal microscopy showed that most lipids were digested by the end of the intestinal phase since few droplets remained after this stage. Consistent with the particle size results, aggregates of various sizes observed at the intestinal stage suggest the presence of micelles, liposomes, insoluble calcium soaps, and protein complexes.

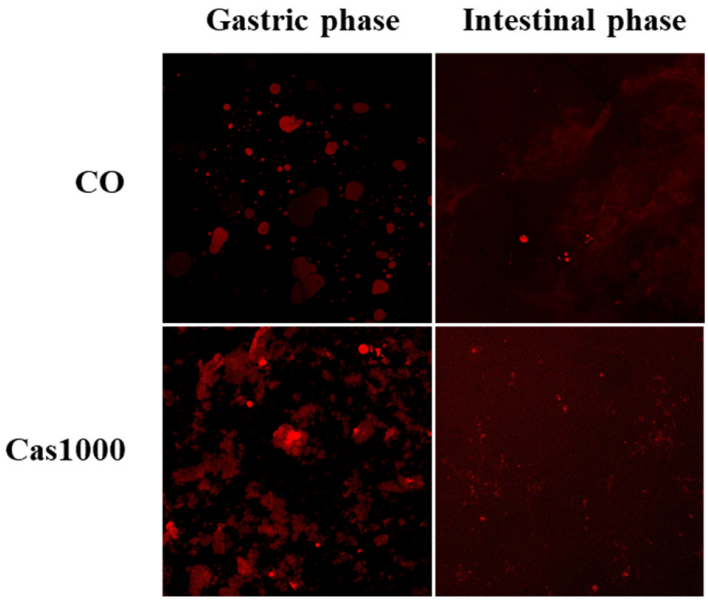

Figure 2. Confocal images (63×) of CO and Cas1000 after in vitro digestion. CO: chia oil; Cas1000: chia O/W nanoemulsions.

3.2.2. Extent of Lipolysis and Fatty Acid Composition

Lipid digestion begins in the gastric phase, and lipolysis in the intestinal phase occurs due to enzymatic action. The extent of lipolysis was assessed by measuring free fatty acids (FFA) produced during intestinal digestion. Significant differences were observed ($p \leq 0.05$), with a mean of 38.13% for CO and 58.26% for CAS1000. The higher FFA from Cas1000 suggests that emulsifying the oil droplets with protein enhances their resistance to enzymatic and acidic degradation in the gastric phase. This allows more oil to reach the intestinal phase, where lipolysis results in increased production of low molecular weight fatty acids. This behavior is consistent with that reported by Timilsena et al. [7].

Regarding fatty acid composition, CO and Cas1000 showed significant differences ($p < 0.05$) from the initial chia oil in terms of LA after the intestinal phase (Table 1). For ALA, Cas1000 did not differ significantly ($p > 0.05$) from CO, but both systems exhibited

significant differences compared to the initial chia oil. These findings suggest that the digestion process impacts the fatty acid profile of the oil, likely due to changes in the emulsion stability and the extent of lipolysis.

Table 1. Fatty acid composition of initial chia oil and post-digestion OC and Cas1000.

Fatty Acid	Initial Chia Oil	Intestinal Phase	
		CO	Cas1000
Linoleic acid (%)	18.30 ± 0.50 [a]	20.88 ± 0.08 [b]	21.67 ± 0.01 [c]
α-linolenic acid (%)	67.00 ± 1.30 [b]	58.28 ± 0.09 [a]	58.48 ± 0.30 [a]

Average values ± standard deviations (n = 2). Different letters in the same row indicate significant differences ($p \leq 0.05$) between systems according to the Tukey test. CO: chia oil; Cas1000: chia O/W nanoemulsions.

4. Conclusions

Cas1000 nanoemulsions maintained high stability and consistent particle size distribution over 50 days. During digestion, Cas1000 was stable in the gastric phase but showed increased particle sizes in the intestinal phase, while CO displayed more significant changes. Cas1000 also had higher lipolysis than CO, indicating better resistance to gastric degradation and improved availability for intestinal digestion. Both systems showed changes in fatty acid composition from the initial chia oil, highlighting the impact of digestion on oil stability and bioavailability. These results underscore Cas1000's potential to enhance oil stability and bioavailability, supporting its use as a functional ingredient. Given these characteristics, Cas1000 nanoemulsions could be effectively applied in developing nutraceuticals, functional foods, and supplements where improved delivery and bioavailability of omega-3 fatty acids are crucial. Additionally, they may be valuable in formulating specialized dietary products aimed at optimizing lipid intake and digestion.

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