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Oleogel-structured composite for the stabilization of ω3 fatty acids in fish oil

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Abstract

This study examined the encapsulation and stabilization of ω3 in fish oil into a multi-compartment system consisting of β-cyclodextrins (β-CD) complexation within an oleogel structure, which was further coated with a layer of whey protein isolate (WPI). The formation of β-CD-fish oil complex was confirmed by thermogravimetric analysis and Fourier-transform infrared spectroscopy. Particle size and ζ-potential of the oleogel-based oil-in-water emulsion did not change significantly over a 28-day storage period under different pH conditions (pH 3.5-7) and NaCl concentrations (50-500 mg/L). The WPI-coated oleogel and β-CD-fish oil complex were subjected to UVC light exposure and quantified by their eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents. Results indicated that more than 50 wt% of both EPA and DHA were retained after a 4-h UV exposure period in the WPI-coated system. In addition, sensory evaluation results showed a decreased fish oil odor in the WPI-coated oleogel samples. Overall, the results from this study demonstrated that this oleogel-structured composite incorporating β-CD-fish oil complexes can be formed successfully to retain lipophilic components with decreased undesirable fish oil odor.

1. Introduction

Marine oils, including fish and algal oils, are consumed because of their high ω3 long chain polyunsaturated fatty acid (LCPUFA) content. When ω3 LCPUFA intake is low and imbalanced, neurodevelopmental deficits and cardiovascular disease risk is greater[1–3]; epidemiological studies are concordant[4]. However, intake of ω3 LCPUFA worldwide is well below recommended levels in part due to the current dietary habits[5] and the shortened shelf life of LCPUFA-rich products[6].

The main forms of ω3 LCPUFA found in marine oils are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, the incorporation of ω3 LCPUFA into foods is challenging because these fatty acids are highly unsaturated and subject to rapid attack by activated oxygen species via their bis allylic sites[7]. The formation of oxidation products results in the loss of functionality in foods and secondary oxidation products yield undesirable odors making the product unappealing to consumers[8,9]. Encapsulation of fish oil in an appropriate delivery systems can retard oxidation, as well as masking the off-flavor formed through oxidation by physical entrapments[10]. Several conventional delivery systems have been utilized to improve the stability of fish oil, in the form of nanoemulsions[11], nanoparticles[12], liposomes[13], and solid lipid nanoparticles[14,15]. These delivery systems can also be engineered by changing the thickness and composition of the external coating and internal structure to enhance the stability of the encapsulated materials. Nevertheless, challenges remain for encapsulation techniques to protect highly

oxidizable fish oil against destabilizing environments. First, current encapsulation methods have a low encapsulation efficiency, and residual fish oil on the surface of the particles can accelerate lipid oxidation[16]. Second, encapsulating materials themselves can induce fish oil oxidation; thus, suitable encapsulation materials, preferably natural materials with antioxidant capability, are highly sought after [11,17]. Last, encapsulates containing a single shell layer offer limited protection against oil oxidation[18–20].

In this context, a combination of multiple surface coatings and the internal structuring of delivery systems could be important factors to retard oil oxidation more efficiently than applying either encapsulation techniques alone[21–23]. Internal structuring of composites can be achieved using oleogels. Oleogels are delivery systems in which organogelators, such as waxes or solid structurants, are incorporated into liquid oils. This system offers a three-dimensional network that immobilizes the bioactive compounds. Wax-based oleogels have been used as vehicles to create semi-solid fillings in soft capsules to stabilize lipophilic drugs that are susceptible to hydrolysis and oxidation[24]. In addition, similar to nanostructured lipid carriers (NLC), oleogels can prevent potential expulsion of bioactive compounds during storage and influx of pro-oxidative compounds into the microcapsules[25].

In this study, we demonstrate the development of a novel hybrid system for fish oil encapsulation that incorporates the β-cyclodextrin (β-CD) solid core into nano-scale oleogel. β-CD has been demonstrated to form an inclusion complex with hydrophobic materials to improve solubility and stability, which decreases fish oil odor[26]. The insertion

of β-CD complexes into the oleogel can reinforce the internal structure of the particles, and improve oil oxidation stability. Furthermore, the surface of the oleogel was modified by forming an additional whey protein isolate (WPI) layer. The dense layer of WPI can function as a physical barrier against the penetration of destabilizing agents such as pro-oxidant and transition metals and enable powder formation during spray drying. The physicochemical stability of the formulated system was then evaluated by measurements of thermogravimetry behavior, Fourier transform infrared spectroscopy, particle size, and ζ-potential. The amount of EPA and DHA remained after an accelerated storage study was also investigated. Last, the odor of the encapsulated fish oil formulation was evaluated in a sensory panel to assess the system's effectiveness to mask the fish oil odor.

2. Material and Methods

2.1 Materials

Tween® 60, heptadecanoic acid standard, and Menhaden fish oil (triglyceride oil) were purchased from Sigma-Aldrich (St. Louis, MO, USA). According to the product information supplied, the fish oil is composed of 6-9% myristic acid (14:0), 15-20% palmitic acid (16:0), 9-14% palmitoleic acid (16:1), 3-4% stearic acid (18:0), 5-12% oleic acid (18:1), <3% linoleic acid (18:2), <3% linolenic acid (18:3), 2-4% octadecatetraenoic acid (18:4), <3% arachidonic acid (20:4), 10-15% eicosapentaenoic acid (20:5), and

8-15% docosahexaenoic acid (22:6). The total identified fatty acids consisted of 80% of the fish oil, whereas the remaining 20% of fish oil are other unidentified fatty acids. β-CD was obtained from Chem Center (La Jolla, CA, USA). Corn oil was purchased from a local market (Ithaca, NY, USA). Beeswax was kindly donated by Strahl & Pitsch, Inc. (West Babylon, NY, USA). WPI was kindly donated by Davisco Food International Inc. (Le Sueur, MN, USA). All other reagents were of analytical grade.

2.2 Preparation of fish oil and β-CD complexes (β-CD-FO)

The method for fish oil and β-CD complexation was derived from Choi et al. (2010), with some modifications. β-CD was dissolved in distilled water at a ratio of 1:100 (w/w) at 25 °C, followed by homogenization at 17500 rpm for 3 min (Model VWR200, Radnor, PA, USA). After homogenization, fish oil was added into the β-CD solution drop by drop at a β-CD:fish oil molar ratio of 1:1. This solution was homogenized again at 17500 rpm for 3 min. The homogeneous fish oil β-CD solution was then placed on a mechanical shaker and agitated for 4 h. After shaking, the mixture was stored at -80 °C freezer for 24 h prior to freeze-drying (Labconco FreeZone 2.5L system, Kansas City, MO, USA). The powdered mixture was stored at room temperature of 25 °C until characterization.

2.2.1 Determination of fish oil and β-CD complex formation

115 2.2.1.1 Thermogravimetric analysis (TGA)

116

117 The TGA curves were obtained using a thermogravimetric analyzer (TGA Q500,
118 TA Instruments, New Castle, DE, USA). The measurements were conducted under
119 nitrogen gas at a flow rate of 60 mL/min. Approximately 8 mg of the sample was loaded
120 onto the platinum pan and heated from 20 to 600 °C, at a rate of 10 °C/min. The
121 thermogravimetric curve was plotted as the derivative of mass loss percent (%) over
122 temperature (°C) *vs*. heating temperature (°C). The first derivative curve was plotted
123 using the Universal Analysis 2000 software (Version 4.5A, TA instruments, New Castle,
124 DE, USA).

125

126 2.2.1.2 Fourier-transform infrared spectroscopy (FTIR)

127

128 FTIR spectra of the samples were obtained using a FTIR spectrometer
129 IR-Affinity-1S (Shimadzu, Kyoto, Japan) equipped with a single-reflection attenuated
130 total reflectance (ATR) apparatus. Samples were scanned from 4000 to 400 cm^{-1}, using a
131 resolution of 4 cm^{-1} and 64 scans[28].

132

133 2.3 Preparation of oleogel carrier containing fish oil β-CD complexes (OG-β-CD-FO)

134

135 The oleogel mixture, which consisted of 65% (w/w) corn oil, 4% (w/w) beeswax,

and 31% (w/w) Tween® 60, was heated to 70 °C with constant stirring. The preformed β-CD-FO powder was added to the oleogel mixture and stirred for 3 min to achieve a homogeneous dispersion. After dispersion, double distilled water at 70 °C was added until the mass ratio of OG-β-CD-FO mixture to water reached 1:4. This mixture was further homogenized at 17500 rpm for 3 min at 70 °C. Immediately after homogenization, the solution was placed into an ice bath to cool down to reach 4 °C.

2.3.1 Physical Characterization of OG-β-CD-FO

To investigate the stability of the fish oil in OG-β-CD-FO under different conditions, the oleogel-based emulsions were subjected to different pH values (pH 3.5, 4, 5, 6, 7, by adjustment with NaOH or HCl solutions) or varying concentrations of sodium chloride (50, 100, 500 mg/L). The samples were stored in the absence of light at room temperature for 28 days, and analyzed for particle size and ζ–potential every 7 days.

2.3.1.1 Particle size measurement

The mean particle diameter of the OG-β-CD-FO emulsion was measured using dynamic light scattering zetasizer (Nano-ZS; Malvern Instruments, Worcester-shire, UK). This instrument measures the size of the droplets within an emulsion using an angular scattering pattern. The emulsions were diluted 20 times to prevent multiple scattering

effects. Each sample was measured every 7 days.

2.3.1.2 ζ–potential measurement

The electrical charge (ζ-potential) of the emulsion droplets was measured using particle electrophoresis (Zetasizer Nano-ZS; Malvern Instruments, Worcester-shire, UK). Similar to particle size analysis, the emulsions were diluted 20 times to prevent multiple scattering effects.

2.3.2 Nanoparticle structure analysis

Sample morphology were visualized using a field emission scanning electron microscope (SEM, LEO 1550 FESEM, Carl Zeiss, New York, USA) and a transmission electron microscope (TEM, FEI T12, Hillsboro, OR, USA). SEM images were taken with EHT at 18 keV, and an aperture size of 30 μm. For TEM, the samples were stained with 1.5 wt% uranium acetate and inspected at 120 kV, a LaB6 filament, SIS Megaview III CCD camera, and a STEM dark field and bright field detector.

2.4 Spray drying of the WPI-coated oleogel (WPI-β-CD-FO)

The oleogel-based emulsion prepared as described in section 2.3 was mixed with

178 a 2% (w/w) WPI solution at a ratio of 1:1 (v/v). The WPI solution was used to coat the
179 oleogel droplets and assist in the spray drying process. The mixtures were then spray
180 dried in a FT30MkIII-G Spray Dryer (Armfield Ltd., Hampshire, England), equipped
181 with an atomizer nozzle. The product feed temperature was kept at 4 °C, and inlet and
182 outlet temperatures were 150 °C and 50-55 °C, respectively. The dried powders were
183 collected and stored in a desiccator at 25 °C until characterization.
184
185 2.4.1 Chemical Characterization of WPI-β-CD-FO
186
187 Powdered WPI-β-CD-FO complexes were subjected to ultraviolet (UV) C light
188 exposure to assess the chemical stability of EPA and DHA in comparison to β-CD-FO as
189 the control. UVC radiation is not biologically relevant due to ozone filtration; however,
190 its effects in *in vitro* assays is analogous to UVB, in a considerably faster rate [29]. A UVC
191 lamp (254 nm) UVP UVLS-28 EL Series (Upland, CA, USA) with a measured fluence
192 rate of 6.9 mW/cm^2 was used, with an exposure distance of 2 cm. The stability of the
193 encapsulated oil in the control and WPI-β-CD-FO samples were evaluated in triplicate
194 after 0, 2, and 4 h of UVC light exposure by comparing the EPA and DHA
195 concentrations.
196
197 2.4.1.1 Quantitative determination of EPA and DHA by gas chromatography
198

To measure the fatty acid components, gas chromatography with a flame ionization detector (GC-FID) was used. Fatty acids were derivatized into the corresponding methyl esters (FAME) by a modified, acid-catalyzed methanolysis method by Garcés & Mancha (1993). Briefly, approximately 10 mg of the sample was measured into a glass tube to which 2 ml of heptane and internal standard (heptadecanoic acid, 17:0) were added. Next, reagent A (methanol:2,2-dimethoxypropane:H_2SO_4, 85:11:4, v/v/v) and reagent O (heptane: toluene= 63:37, v/v) were added into the sample sequentially at a ratio of 14:16 (v/v), to a total volume of 5 mL. Both reagents were prepared fresh at the day of sample processing. The inside and outside of each sample tube cap was sealed with Teflon tape and vortexed for 1 min, followed by incubation in a shaking dry bath at 80 °C for 2 h. After cooling to room temperature, 2 mL of saturated NaCl solution was added, followed by shaking using vortex. Next, the FAME mixtures were centrifuged at 959 × *g* for 10 min, and the top layers were transferred to new clean tubes. The extraction process was repeated by adding another 2 mL of heptane into the FAME mixture, vortexed, centrifuged, and decanted. The final top layers were combined, resulting in approximately 4 mL of the extracts. The extracts were dried under nitrogen and reconstituted with 2 mL of heptane. Finally, the samples were diluted 10 times with heptane into a GC vial. Separation and quantification of EPA and DHA were performed on a GC-FID 5890 Series II (Hewlett Packard, Bothell, WA, USA). One μL was injected into a BPX-70 column (0.2 mm × 25 m, SGE, Pflugerville, TX, USA). An equal percentage by weight standard mixture was used to calculate response factors (FAME

220 mixture 462A, Nu-Check Prep Inc., Waterville, MN, USA). All GC analyses were
221 performed in triplicate.
222
223 2.5 Sensory evaluation
224 Volunteers of 14 adult males (n=7) and females (n=7) aged ≥ 18 years were recruited
225 (Ithaca, NY, USA). The sensory evaluation studies were performed under the guidelines
226 of human participants policy and standard operating procedures, and the experiments
227 were approved by the ethics committee at the Cornell University (Protocol ID#
228 1804007947). Informed consents were obtained from panelists of this study. Panelists
229 were presented with one different (WPI-β-CD-FO) and two alike samples (fish oil)
230 throughout the sensory test, with same concentrations of fish oil contents, and avoided
231 with visual assessments. The samples were prepared a day before the evaluation and were
232 stored in a desiccator at 25°C until samples were evaluated. Just about right (JAR) tests
233 will be used to describe the WPI-β-CD-FO and fish oil by rating the fish oil odor in 6
234 scales, as being undetected, much too mild, slightly too mild, just about right, slightly too
235 strong or much too strong. In addition, odor comparison tests were conducted with
236 panelists being asked to identify the odd sample and group the three samples into two
237 groups based on the odor alikeness. The odor comparison results were scaled into 10
238 scales based on the fish oil odor intensity of each group.
239
240 2.6 Statistical analysis

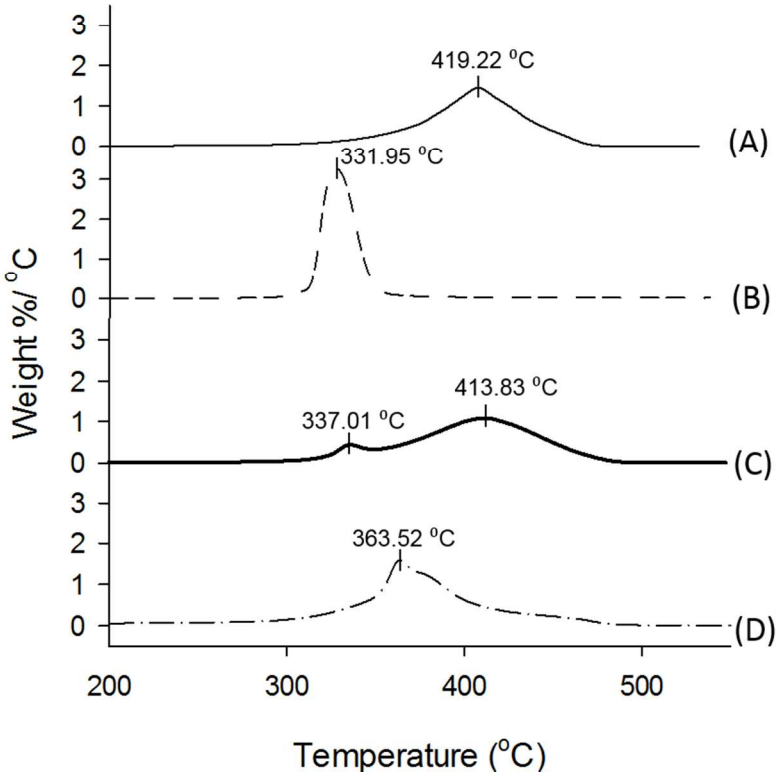
All experiments were carried out in triplicate and the results were reported as mean ± standard deviation. IBM SPSS® version 24.0 (Chicago, IL, USA) was used to determine statistical significance results. One-way ANOVA was employed for all tests, with Tukey's post-hoc test, and all results were considered significant at $P < 0.05$. For sensory evaluation, mean was used to express the results from both JAR test and odor comparison test. JAR and Odor comparison results were analyzed using independent t-test to obtain critical value.

3. Results and Discussion

3.1 Characterization of β-CD-FO complex formation

The β-CD-FO complexes was characterized by TGA and FTIR. For TGA analysis, samples of fish oil, β-CD, physical mixture of both components, and β-CD-FO were subjected to a heat ramp from 20 to 600 °C (Figure 1). For single components (Figure 1A-B), signals appeared at 419.22 °C for fish oil and 331.95 °C for β-CD. When the components were mixed, the curve exhibited two mass loss peaks at 337.01 °C and 413.83 °C, representing the un-complexed β-CD and fish oil, respectively (Figure 1C). These minor peak shift to lower temperatures indicated that the fish oil would be more susceptible to degradation when physically mixed with β-CD than alone[31]. This is

262 because the increased surface area created by β-CD in the mixture could have increased
263 the exposure of fish oil to the environment, resulting in a faster degradation of the fish oil.
264 For the β-CD-FO complex (Figure 1D), the curve presented a single intermediate peak
265 closer to the β-CD peak, at a temperature of 363.52 °C, while fish oil and β-CD cannot be
266 detected separately. Additionally, this result indicated that fish oil was covered, and thus
267 less likely to be degraded in the β-CD-FO complex[31].
268



269
270 Figure 1. First derivative TGA curves of (A) fish oil, (B) β-CD, (C) physical mixture
271 of β-CD and fish oil, and (D) β-CD-FO.
272

273 Figure 2 shows similar spectra between the samples of fish oil alone and physical

mixture. Interestingly, β-CD-FO spectra showed a similar peak profile to β-CD, with lower signals of fish oil. Detailed assignments of the FTIR peaks in fish oil have been reported previously in the literature [32,33]. Peaks at wavenumbers of 3010, 2954, and 2922-2852 cm^{-1} represented C-H stretching of *cis*-alkene, asymmetrical stretching of methyl group, and asymmetrical or symmetrical stretching of methylene group, respectively. The peak at 3010 cm^{-1} was reported to be indicative of the concentration of ω3 LCPUFA, including EPA and DHA, in the system. The band at 1743 cm^{-1} represents the stretching vibration of aldehyde or ester carbonyl groups (C=O), and can reflect the degree of unsaturation together with the band at 2922 cm^{-1}.

For β-CD samples, a broad peak from 3600 to 3200 cm^{-1} is associated with the stretching vibrations of hydroxyl groups from hydrogen bond. A broad range of peaks from 1500 to 1300 cm^{-1} indicates the presence of C-H deformation vibration from the primary or secondary alcohol group (Figure 2B). A peak at 1020 cm^{-1} represents the stretching vibration of C-OH bonding in alcohol group, and peaks from 950-600 cm^{-1} are associated with the vibration of glucopyranose cycles [34].

In addition, the β-CD peak at 3284 cm^{-1} shifted to 3313 cm^{-1} in β-CD-FO and had a slightly lower intensity. This decrease in peak intensity could be due to the complex formation with fish oil, which decreases the vibration of the hydroxyl group and creates a more hydrophobic environment. Overall, the β-CD peaks were more pronounced than the fish oil peaks in β-CD-FO samples, but with the absence of new peaks. These results demonstrated that β-CD-FO were physical complexes without any chemical bonding

involved. This suggests that β-CD partially covered the fish oil droplets, results are concordant with TGA curves.

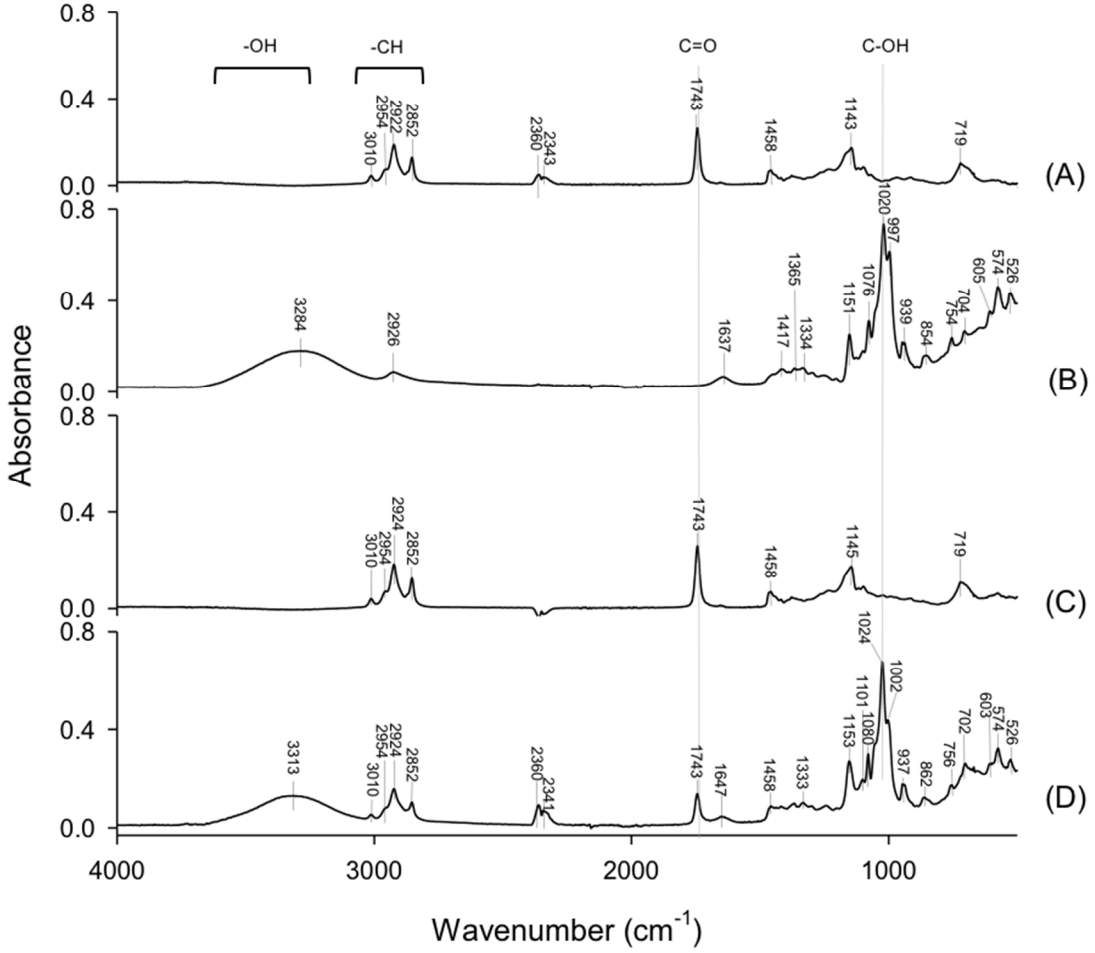


Figure 2. FTIR spectra of (A) fish oil, (B) β-CD, (C) physical mixture of β-CD and fish oil, and (D) β-CD-FO.

3.2 Physical stability of OG-β-CD-FO

305 To further stabilize the fish oil, we incorporated the β-CD-FO complexes into a
306 beeswax-based oleogel (OG-β-CD-FO). The particle size and ζ-potential of these
307 OG-β-CD-FO emulsions were measured immediately after preparation and during
308 storage (Figure 3). The OG-β-CD-FO formed an opaque emulsion upon production and
309 was stable to gravitational separation over the 28-day tested storage period at 25 °C. The
310 particle size and ζ-potential of the OG-β-CD-FO did not change significantly ($P > 0.05$)
311 during 28 days of storage nor under several acidic pHs and salt contents. These results
312 suggest that the loading of β-CD-FO aggregates into the oleogel did not destabilize the
313 emulsion. The OG-β-CD-FO had intermediate particle size and its high negative surface
314 charges could stabilize the emulsion system by electrostatic repulsion (Figure 3 C-D). In
315 addition, the stability of the emulsion may suggest that an adequate structure was
316 provided by the addition of the beeswax to prevent droplet disassociation over time.
317 When adding OG-β-CD-FO with a higher concentration of NaCl (500 mg/L), ζ-potential
318 decreased (Figure 3D), due to the electrostatic screening provided by the sodium ions in
319 the OG-β-CD-FO emulsion. This screening of the surface potential was reported
320 previously and can increase the tendency of the droplet aggregation to occur [20,35]. Despite
321 that, the system showed strong resistance towards high concentration of salt with only
322 negligible ζ-potential changes during storage. It is believed that the oleogel structure in
323 this system prevented the droplets from Ostwald ripening, which is a phenomenon where
324 larger droplets grow at the expense of smaller droplets.
325

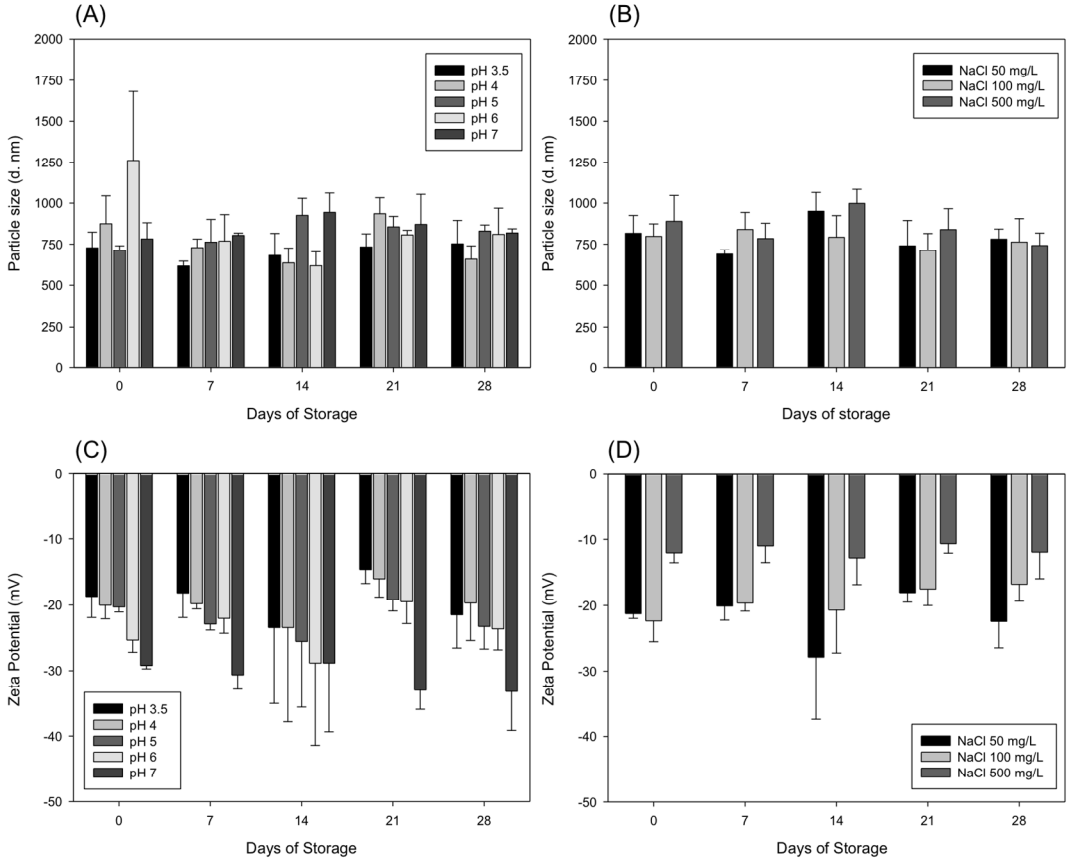


Figure 3. Particle size of OG-β-CD-FO emulsion under different pH (A) and NaCl concentrations (B); ζ-potential under different pH (C) and NaCl concentration (D) during 28 days of storage, with measurements made every 7 days. No significant differences ($P > 0.05$) were observed between the treatments.

3.3 Morphology of the OG-β-CD-FO and WPI-β-CD-FO

We further coated the OG-β-CD-FO with WPI prior to spray-drying. WPI is widely used as a coating material for encapsulation because of its high solubility in water and effective emulsifier ability for oil-in-water emulsions[36]. WPI can assist to form

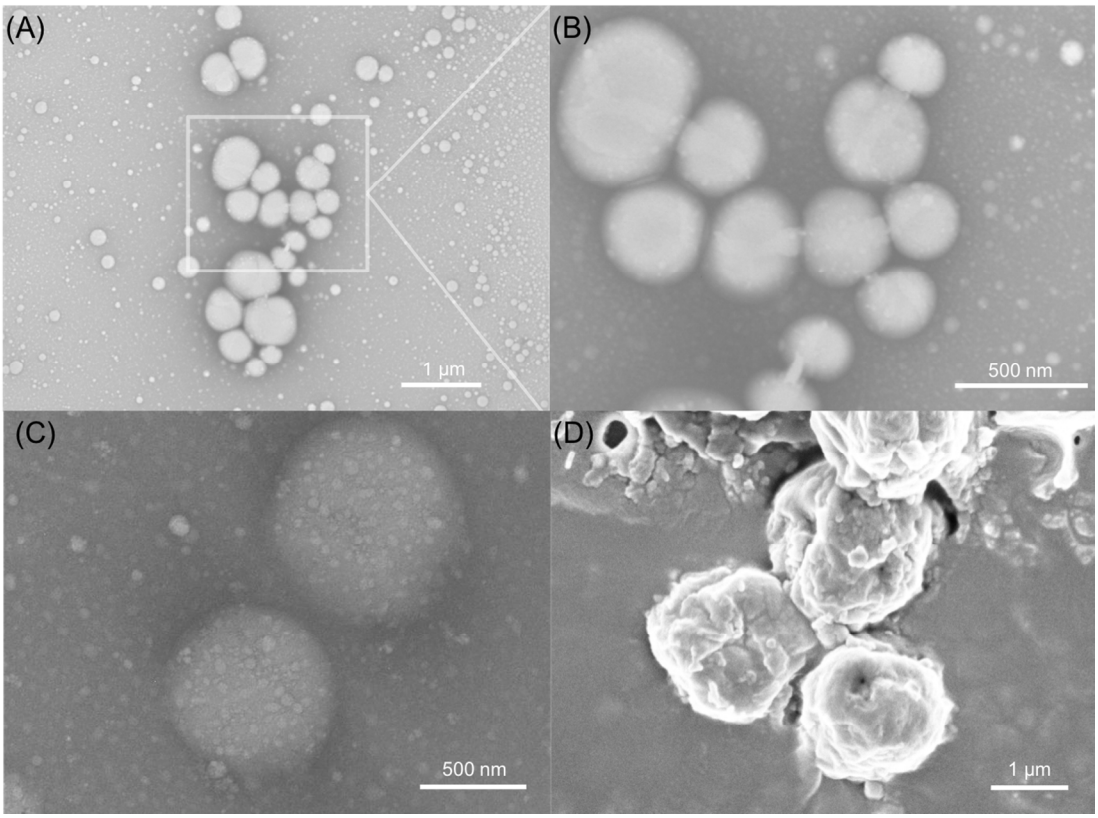
338 WPI-β-CD-FO powder during spray drying process, and the powder form of the
339 encapsulates are typically desirable for shelf-life extension. Prior to TEM visualization,
340 WPI-β-CD-FO was prepared by suspension of the spray-dried powder in water. For
341 comparison, we also inspected the structure of OG-β-CD-FO without WPI coating.

342 TEM images (Figure 4A-C) showed that multiple β-CD-FO aggregates located
343 within the oleogel droplets in OG-β-CD-FO samples (Fig. 4 A and B). The sizes of the
344 oleogel droplets were between 500-800 nm, in accordance with the dynamic light
345 scattering particle size measurements.

346 For WPI-β-CD-FO, multi-core structures of β-CD-FO aggregates were again
347 observed (Figure 4C). Spray-dried WPI-β-CD-FO samples had a small particle size of
348 around 500 nm and were spherical when dispersed in water. Spherical shape is desired
349 due to decreased surface area compared with "platelets" or irregular-shaped particles,
350 which reduces the surface contact during collision and increase the stability in water
351 (Mehnert & Mäder, 2001). In addition, homogenous distribution of multi-β-CD-FO core
352 in OG-β-CD-FO and WPI-β-CD-FO showed the possibility of fabrication of droplets with
353 sizes smaller than 1 μm.

354 SEM provided complementary information on the structure of WPI-β-CD-FO
355 (Figure 4D). The surface of the spray-dried particle was wrinkled, although maintained a
356 spherical shape, suggesting that a rigid internal structure was achieved and a dense layer
357 of WPI provided enough coverage on the surface. The SEM image shows the presence of
358 smaller particles adhering onto the surface of WPI-β-CD-FO. This could be explained by

359 the presence of some smaller spherical droplets in the emulsion prior to the spray-drying
360 process, which would merge smaller particles onto a bigger particle. According to
361 Rosenberg & Young (1993), the roughness and irregularity of the particles are attributed
362 to the mechanical stress during water evaporation at the atomizing process during
363 spray-drying[37]. When comparing the TEM and SEM images, the powdered form of
364 oleogels were mostly spherical, and was easily re-dispersable with the ability to
365 reconstruct spherical structures. Thus, both the OG-β-CD-FO and the WPI-β-CD-FO
366 proved to be appropriate delivery systems for fish oil.
367



368
369 Figure 4. TEM images of (A, B) OG-β-CD-FO and (C) WPI-β-CD-FO with both
370 images observed with high operating voltages (120 kV). (D) SEM images of
371 WPI-β-CD-FO.

372

373

374 3.4 Chemical Stability of WPI-β-CD-FO

375

376 One of the major challenges of incorporating fish oil into food products is the high
377 susceptibility of EPA and DHA to oxidation induced by heat, light, UV radiation, and
378 influx of transition metals [38]. Here, we studied the oxidative stability of WPI-β-CD-FO
379 for the potential to produce powdered formulations for food applications. β-CD-FO
380 complexes were used as a control instead of liquid fish oil. This is because the liquid oil
381 would have overall less surface exposure to the UV light, conversely creating a partial
382 protection to the oil against oxidation.

383 Table 1 shows that in the absence of WPI layer, β-CD-FO was very susceptible to
384 oxidation. After 2 hr of UVC light exposure, roughly 53% of the EPA and 56.4% of DHA
385 were lost. When the exposure time extended to 4h, EPA and DHA were not detectable.
386 These results suggest that β-CD cannot effectively protect EPA and DHA in fish oil
387 against oxidative degradation.

388 WPI-β-CD-FO were more stable to oxidation compared with β-CD-FO (Table 1).
389 After 4h of exposure, EPA and DHA were reduced by 33.7% and 33.9%, respectively,
390 demonstrating that the delivery system could protect fish oil from oxidation. Such
391 stabilizing effect could be attributed to several mechanisms. First, the combination of fish
392 oil and corn oil in oleogel matrix can enhance the overall oxidative stability. This was

supported by previous research that mixture of oils from different source can retard oxidation [39]. In addition, the creation of oleogel structures can provide a rigid oil layer around β-CD-FO, which can prevent oil diffusion and coalescence. Oleogel structures are also shown to assist in creating a multi-core structure in the WPI-β-CD-FO. The three-dimensional structure is expected to act as a physical barrier for the transition metals, free radicals, and pro-oxidants.

Table 1. Amount of EPA and DHA detected in β-CD-FO and WPI-β-CD-FO in a UVC light exposure study

	EPA content (mg/g fish oil)			DHA content (mg/g fish oil)		
	0 hr	2 hr	4 hr	0 hr	2 hr	4 hr
β-CD-FO	96.79 ± 0.75	44.96 ± 0.42	-	76.12 ± 1.29	33.17 ± 0.24	-
WPI-β-CD-FO	109.58 ± 0.85	96.44 ± 1.14	59.47 ± 2.23	96.22 ± 1.05	79.03 ± 1.10	46.39 ± 2.06

Results were expressed as mean ± standard deviations. All values were significantly different ($P < 0.05$).

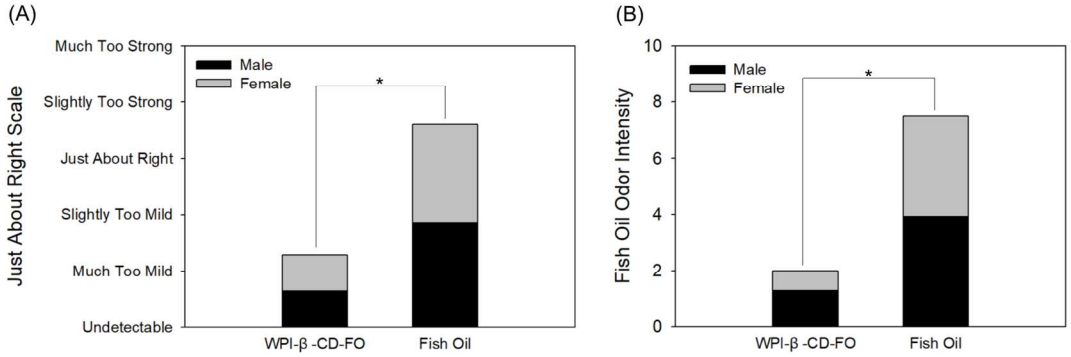
3.5 Sensory evaluation of fish oil and WPI-β-CD-FO

Extensive fish oil oxidation could cause undesirable fish oil odor [40]. During the oxidation of PUFA, volatiles are formed, resulting in the fish oil odor described as fishy [41]. Thus, these odor characteristics can be an indicator of lipid oxidation. Previous study demonstrated that fish oil odor can be reduced and become highly achievable after incorporated into β-CD [42]. In this case, we compared the odor evaluation of WPI-β-CD-FO with the bulk fish oil at the presence of same fish oil content. In the JAR

412 sensory evaluation test, majority of panelists agreed that the WPI-β-CD-FO has overall
413 lower fish oil odor than solely fish oil (Figure 5A). As result, the fish oil odor in
414 WPI-β-CD-FO was described as "much too mild", as opposed to the fish oil, which was
415 being described as stronger than just about right value and closer to descriptive value of
416 "slightly too strong". In figure 5, we've also incorporated the scoring breakdown
417 according to genders. Looking at the gender breakdown of Figure 5A, the ratings between
418 male and female showed similar scores, with no significant difference between ratings
419 from either group. Figure 5B shows the results from odor comparison sensory tests, all
420 panelists were able to differentiate the encapsulated WPI-β-CD-FO from bulk fish oil
421 according to its fish oil odor intensity (P<0.05). Furthermore, WPI-β-CD-FO again
422 showed overall lower fish oil intensity detection as compared to bulk fish oil. However,
423 in odor comparison tests, intra-population variation of scores between male and female
424 exists for WPI-β-CD-FO sensory ratings. Male in general suggested that the fish oil odor
425 was more intense and provided with higher scoring values and contributed to 64.3% of
426 the entire scores; while females contributed to 35.7 % of the scores and has consistent
427 results where fish oil odor was less intense (P<0.05). When looking at the fish oil ratings,
428 male and female agreed upon the intensity of fish oil odor, and that the intra-population
429 variation does not exist between male and female groups (P>0.05). Despite the scoring
430 variation within groups, overall scoring indicated that WPI-β-CD-FO can reduce the fish
431 oil odor up to 55%, and this result is concordant with the chemical stability assessment on
432 WPI-β-CD-FO described previously (section 3.4), as it can delay fish oil oxidation thus

433 resulted in less fishy odor. This implication of lower fish oil odor can allow
434 WPI-β-CD-FO to be used for functional food fortification purpose.

435



436

437 Figure 5. (A) Fish oil odor ratings of the WPI-β-CD-FO and fish oil samples in a just
438 about right sensory test. (B) The ratings of fish oil odor intensity from the grouping test.
439 The results are shown as mean values, symbol represents significant values between
440 samples ($P< 0.05$).

441

442

443 **4. Conclusion**

444

445 WPI-β-CD-FO systems were fabricated by creating multi β-CD-FO cores in the
446 oleogel system using beeswax, and further coating with WPI to create a spray-dried
447 powder. TGA and FTIR results confirmed successful β-CD-FO complex formation, and
448 TEM and SEM characterization of the OG-β-CD-FO and WPI-β-CD-FO suggest
449 successful fabrication of multi-core β-CD-FO into oleogel. The WPI-β-CD-FO system
450 showed great physical stability in storage studies with varying environmental factors (e.g.

451 pHs and salt concentrations). In addition, the fish oil in WPI-β-CD-FO had higher
452 chemical stability than those in β-CD-FO when exposed to UVC light. Overall, this study
453 showed that multi-core fabrication of β-CD-FO into an oleogel system and coating with a
454 WPI layer is highly effective in protecting fish oil from oxidation, and that the nutritional
455 value of EPA and DHA is retained in the system. Other methods to protect fish oil
456 oxidation and to prolong WPI-β-CD-FO powder shelf-life, such as the incorporation of
457 antioxidants and the selection of different encapsulation wall materials, are worthy of
458 investigation in the future. With the capability of production at large scale, this study on
459 such for nanocomposite systems in the field of fish oil encapsulation demonstrated a
460 potential application in the food industry.
461

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468

469 **6. Reference**
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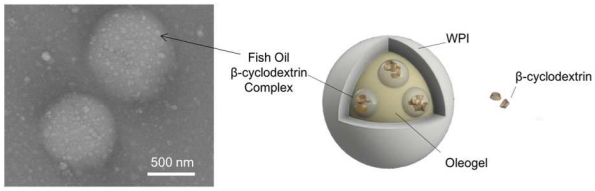
Graphical Abstract



The fish oil is encapsulated in a multi-compartment system featuring β-cyclodextrin complexation within whey protein isolate (WPI) coated oleogel particles.