LWT - Food Science and Technology 190 (2023) 115580

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of enzymatically produced tuna oil acylglycerol on the characteristics of gelatin O/W emulsion during microencapsulation using complex coacervation

Junyong Xuan [a], Qiuyu Xia [a,b,**], Yinyi Tu [a], Tingyu Luo [a], Qingya Mao [a], Zongyuan Han [a], Colin J. Barrow [d], Shucheng Liu [a,b,e,*], Bo Wang [c]

[a] College of Food Science and Technology of Guangdong Ocean University, Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety, Guangdong Provincial Engineering Technology Research Center of Seafood, Guangdong Province Engineering Laboratory for Marine Biological Products, Key Laboratory of Advanced Processing of Aquatic Product of Guangdong Higher Education Institution, Zhanjiang, Guangdong, 524088, China
[b] Guangdong Laboratory of Southern Marine Science and Engineering (Zhanjiang), Zhanjiang, Guangdong, 524088, China
[c] School of Behavioural and Health Sciences, Australian Catholic University, Sydney, 2060, Australia
[d] Centre for Sustainable Bioproducts, Deakin University, Waurn Ponds, VIC, 3217, Australia
[e] Collaborative Innovation Center of Seafood Deep Processing, Dalian Polytechnic University, Dalian, Liaoning, 116034, China

ARTICLE INFO

Keywords:
Diacylglycerol
Monoacylglycerol
Oil-in-water emulsion
Interfacial properties
Macromolecule interaction

ABSTRACT

Complex coacervation is an effective process to deliver ingredients for functional food applications. A stable oil-in-water (O/W) emulsion with desired characteristics significantly affects the complex coacervation and the quality of final microcapsules. In this study, tuna oil was partially hydrolyzed using TL100 and ADL lipases to produce acylglycerols TL100-AC and ADL-AC, respectively. These lipids were subsequently stabilized by gelatin in the O/W emulsion, followed by the complex coacervation with sodium hexametaphosphate. The effect of lipids on emulsion properties, such as interfacial properties, rheological properties, protein conformation and microcapsule formation during complex coacervation, was investigated. Compared with tuna oil-based emulsion, acylglycerol-based ones exhibited reduced droplet size (<600 nm), zeta-potential, interfacial tension (<8 mN/m) and interfacial protein concentration (about 80%). The gelatin in the acylglycerol-based emulsions had reduced β-sheet and slightly increased random coil contents. Compared with the one containing tuna oil, the ones containing acylglycerol possessed higher complex coacervation yields (>75%). These changes were beneficial to the formation of coagulant and flocculant so that gelatin-stabilized acylglycerol-based O/W emulsion resulted in improved complex coacervation between gelatin and sodium hexametaphosphate. This study provides a scientific basis for designing specific gelatin O/W emulsions and microencapsulation for the stabilization and delivery of omega-3 fatty acids.

1. Introduction

Fish oil has various health benefits such as anti-inflammatory, brain development and cardiovascular disease prevention, primarily attributed to long-chain omega-3 lipids in the molecule (Chatterjee & Judeh, 2016). However, it is easily oxidized during processing and/or storage, hindering its applications in food products. Microencapsulation technology is used to partially stabilize fish oil and convert it into a formulatable powder. Complex coacervation has been commercially used for this purpose (Eratte, Dowling, Barrow, & Adhikari, 2018). The fish oil microcapsules stabilized using this technique show high oil loading capacity, low surface oil content, high encapsulated efficiency and high oxidation stability compared with the ones produced via the spray drying method (Barrow, Nolan, & Holub, 2009; Wang, Adhikari, & Barrow, 2014).

A stable oil-in-water (O/W) emulsion is a prerequisite to fabricate

* Corresponding author. College of Food Science and Technology of Guangdong Ocean University, Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety, Guangdong Provincial Engineering Technology Research Center of Seafood, Guangdong Province Engineering Laboratory for Marine Biological Products, Key Laboratory of Advanced Processing of Aquatic Product of Guangdong Higher Education Institution, Zhanjiang, Guangdong, 524088, China.
** Corresponding author. 1 Haida Road, Mazhang District, Zhanjiang, Guangdong, China.
E-mail addresses: qiuyu@gdou.edu.cn (Q. Xia), lsc771017@163.com (S. Liu).

https://doi.org/10.1016/j.lwt.2023.115580
Received 9 August 2023; Received in revised form 18 November 2023; Accepted 20 November 2023
Available online 23 November 2023

microcapsules via complex coacervation. Emulsion stability is affected by the emulsion type, emulsifier concentration and the interaction between the interfacial film and lipid (Guo, Huang, Wu, Qi, & Mao, 2019; Zou & Akoh, 2013). Emulsion characteristics such as the emulsifier's emulsifying activity, interfacial behavior and rheological properties significantly affect the complex coacervation process and the quality of final microcapsules (Liu, Wang, Barrow, & Adhikari, 2014). Although various animal- and plant-based proteins have been used to fabricate microcapsules, the correlation between emulsion characteristics and complex coacervation remains unknown.

Fish oil can be partially hydrolyzed to alter lipid structure, change physicochemical properties, modify metabolic properties and improve nutritional value (Martinez-Galan et al., 2021; Xia, Akanbi, et al., 2019). During hydrolysis, a large amount of DAG and MAG are formed and the lipases significantly affect the lipid composition of acylglycerol (Xuan et al., 2022). A previous study reported that acylglycerol-based microcapsules had a denser encapsulated structure than fish oil-based ones stabilized by gelatin-based complex coacervate. Finally, acylglycerol-based microcapsules were more oxidatively stable than fish oil-based ones (Xia, Akanbi, et al., 2019; Xia et al., 2020). It was hypothesized that amphiphilic macromolecules such as DAG and MAG affected the formation and structure of O/W emulsion, complex coacervation and microcapsules. As a result, the acylglycerol-based microcapsules exhibited smooth surfaces and dense structures (Xia et al., 2017; Xia, Akanbi, et al., 2019; Xia et al., 2020). However, there is still research paucity in investigating the correlation between emulsion characteristics and microcapsule properties in the acylglycerol-based microcapsules. In an O/W emulsion stabilized by protein, it has been commonly accepted that the interaction between lipid phase and protein affects emulsion formation and stability (Shao, Zou, Xu, Wu, & Zhou, 2011). Some key properties, such as structural characteristics and rheological properties of O/W emulsions, play important roles in microcapsule formation via complex coacervation (Fuhrmann, Sala, Stieger, & Scholten, 2019; Liu et al., 2014). However, few studies so far investigated the effect of modified lipid by lipase on the characteristics of gelatin-based O/W emulsions during microencapsulation using complex coacervation.

In this paper, tuna oil was partially hydrolyzed using *Thermomyces lanuginosus* lipase (TL100) and *Candida antarctica* lipase (ADL) lipases to produce acylglycerols. Subsequently, these acylglycerols were used as the lipid phases to prepare gelatin-stabilized O/W emulsion. Finally, complex coacervation between gelatin at the O/W interface and sodium hexametaphosphate was induced to fabricate the microcapsules.

The effect of acylglycerol characteristics on the protein adsorption at the O/W interface and complex coacervation was investigated. This study aims to interpret macromolecule interaction mechanisms during complex coacervation and provide insights to future researchers on developing novel acylglycerol-based microcapsules.

2. Materials and methods

2.1. Materials

Tuna oil was donated by Nu-Mega Co., Ltd (Melbourne, Victoria, Australia); lipases *Thermomyces lanuginosus* (TL100) and *Candida antarctica* (ADL), were purchased from Novozymes Co., Ltd. Pork skin gelatin (VetecTM reagent, type A, ~300 g bloom) and α-tocopherol (97%) were purchased from Sigma-Aldrich Co., Ltd and McLean Biochemical Technology Co., Ltd (Shanghai, China). All other reagents used were of analytical grade.

2.2. Preparation of acylglycerol

Acylglycerol was prepared from tuna oil using the previously reported method (Xia et al., 2020) with some modifications. Briefly, lipases TL100 or ADL were added to 20 g of tuna oil at 2% (w/w). The mixture was incubated at 40 °C under mild agitation (350 r/min) for 2 and 6 h, respectively. These enzymatic treatment conditions were optimized to ensure a 30% hydrolysis degree. Subsequently, the hydrolysis was stopped by adding anhydrous ethanol and acylglycerols were separated using n-hexane to perform the following study. These acylglycerols are named TL100-AC and ADL-AC in the following content.

2.3. Lipid class analysis

The lipid class of fish oil and acylglycerols, in terms of triacylglycerol (TAG), free fatty acid (FFA), diacylglycerol (DAG) and monoacylglycerol (MAG), were determined using thin-layer chromatography (SF-2020 system, Shandong Zibo Shanfen Analysis Instrument Co., Ltd, Zibo, Shandong, China) according to a previous method (Xia et al., 2017; Xia, Akanbi, et al., 2019; Xia et al., 2020) with minor modifications. Briefly, 10 μL of the sample was mixed in 4 mL of n-heptane. Subsequently, 1 μL of the mixture was spotted onto the rods and developed for 20 min in a solvent tank containing 50 ml n-hexane/ether/formic acid (60:17:0.2, v/v/v), followed by a 5 min drying at room temperature and then scanning using MD-FID detector. The lipid classes in the sample were separated and contents calculated by the software of Sf-800 (Sf-800, Shandong Zibo Shanfen Analysis Instrument Co., Ltd, Zibo, Shandong, China)

2.4. Preparation of tuna oil and acylglycerol O/W emulsion

Tuna oil or acylglycerol (16 g) containing 40 mg α-tocopherol/100 g, were mixed with 120 g of gelatin solution (8 g/100 g) individually and emulsified at 1200 r/min for 5 min using an overhead digital stirrer (MS-40, Hangzhou, China). Gelatin solution was prepared ahead and incubated at 50 °C for 20 min until a clear solution was obtained. A fine O/W emulsion was obtained by emulsifying the pre-emulsified samples at 20,000 r/min for 15 min using a high-speed homogenizer (WIGGENS D-5000, Wiggens Technology, Beijing, China).

2.5. Emulsion characteristics

2.5.1. Emulsifying activity index (EAI) and emulsifying stability index (ESI)

The EAI and ESI of tuna oil and acylglycerol-based O/W emulsions were determined according to Diao, Guan, Zhao, Chen, and Kong (2016) with minor modifications. Briefly, the emulsion was diluted 5 times using ultrapure water. 0.5 mL of fine emulsion was further diluted 100 times using 100 mg/100 g sodium dodecyl sulfonate (SDS) solution. The absorbance of the diluted sample was measured at a wavelength of 500 nm using a UV–Vis spectrophotometer (UV-2550, Shimadzu, Co., Ltd, Kyoto, Japan) and recorded as A0, with SDS solution as the blank. After 12 h storage, the fine O/W emulsion was diluted using the same protocol to determine its absorbance (A12). The EAI (m^2/g) and ESI (%) of the emulsions were calculated using Equations (1) and (2), respectively:

$$EAI\left(\frac{m^2}{g}\right)=\frac{2\times 2.303\times A_0\times \mathrm{D}}{\mathrm{C}\times(1-\varphi)\times 10000} \quad (1)$$

$$ESI(\%)=\frac{A_{12}}{A_0}\times 100 \quad (2)$$

Where A0 is the absorbance value of diluted emulsion at 0 and 12 h, respectively; D is the dilution factor (100 ×); C is the mass fraction of protein (g/mL); and φ is the volume fraction of the oil phase (2.35%, w/w).

2.5.2. Average particle size and Zeta-potential of emulsion droplets

The fresh emulsions were diluted 400 times with ultrapure water to measure their average particle size and zeta-potential using a NanoZS

Zetasizer (Malvern Instruments Ltd, Worcestershire, UK).

2.6. Emulsion interfacial properties

2.6.1. Interfacial protein concentration

The protein concentration at the O/W interface was determined according to Cao et al. (2022) with minor modifications. Briefly, the fresh fine O/W emulsion was centrifuged at 10,509×*g* for 30 min at 4 °C. The clear supernatant was carefully collected using a syringe and passed through a 0.45 μm membrane filter (ANPEL Laboratory Technologies, Shanghai) to remove impurities from the serums, which contained non-adsorbed proteins. Lowry method was used to measure the protein content in the initial O/W emulsions and recovered supernatants to calculate the interface protein adsorption (AP, g/100 g) using Equation (3):

$$AP(g/100\ g) = \frac{C_{INI} - C_{SER}}{C_{INI}} \times 100 \quad (3)$$

Where C_{INI} and C_{SER} are protein content (mg/mL) in the initial O/W emulsions and supernatants, respectively.

2.6.2. Interfacial tension

The interfacial tension between various lipids and gelatin solutions was determined according to Liu et al. (2014) with a contact angle/-surface tensiometer (LSA100, Lauda Scientific, Lauda-Königshofen, Germany). Lipids were injected into the gelatin solution from a syringe at 40 °C. The morphology of the oil droplet was captured by a camera and analyzed by the software using the Young–Laplace equation to determine the interfacial tension.

2.7. Rheological behavior

2.7.1. Dynamic shear test

Fresh O/W emulsion (0.5 mL) was allocated on the Peltier plate of a rheometer (HAAKE MARS III, Thermo Fisher Scientific, Shanghai, China). After equilibrating for 5 min, its apparent viscosity was measured in the shear rate range of 0.1–100 s^{-1} at 25 °C. A P35 probe was used in the test with a measurement gap of 0.5 mm.

2.7.2. Oscillation frequency sweep test

Based on a dynamic strain sweep test at the frequency of 10 rad/s, the linear viscoelastic region (LVR) of emulsions was found at strain value from 1% to 10%. Therefore, a 1% strain value was used in the oscillation frequency sweep test. Various emulsion samples were allocated using the protocol described in section 2.7.1. After equilibrating for 5 min, storage modulus (G′) and loss modulus (G″) of the emulsions were measured using a rheometer in the oscillation frequency range of 0.1–10 Hz.

2.8. Protein conformation

2.8.1. Secondary structure

After diluting the fine emulsion by 100 times with ultrapure water, 200 μL of the diluted sample was transferred to a quartz absorption cell to determine its secondary structure using a circular dichroism spectrometer (Chirascan V10, Applied Photophysics Ltd., Leatherhead, England). The scanning wavelength range was 180–260 nm, while the scanning speed was controlled at 100 nm/min. Three scans were performed per sample to report the average value, and the content of various protein secondary structures was calculated using CDNN software (Version 2.1, Copyright© Gerald Bohm, Institut für Biotechnologie, Martin-Luther Universit at Halle-Wittenberg).

2.8.2. Tertiary structure

The tertiary structure of the protein in various emulsions was determined according to Xiao et al. (2021) with minor modifications. Fine emulsions made from different lipid phases were diluted 1000 times with ultrapure water, followed by an endogenous fluorescence measurement using a fluorescence spectrometer (RF-5301pc, BRUKER, Karlsruhe, Germany). The following parameters were used: excitation wavelength = 280 nm, emission wavelength = 300–400 nm, emission slit width = 5 nm and increment = 10 nm. Each sample was measured three times to report the average value.

The emulsion was diluted to 62.5, 125, 250, 500, and 1000 times to measure its exogenous fluorescence according to Dong et al. (2021) with modifications. 1-anilino-8-naphthalenesulfonic acid (ANS) was used as the fluorescent probe. Briefly, 20 μL of 8 mmol/L ANS in 0.1 M PBS phosphate buffer (pH 7.0) was mixed with 4 mL of diluted O/W emulsion. The mixture was stored in darkness for 30 min to allow complete binding between the hydrophobic groups in the protein molecules and ANS. Then the fluorescence intensity (FI) of the sample was measured using a fluorescence spectrometer. The excitation and emission wavelengths were 390 and 470 nm, respectively.

The relative fluorescence intensity (RFI) of diluted emulsions were calculated using Equation (4):

$$RFI = \frac{F_s - F_0}{F_0} \quad (4)$$

Where Fs and F_0 are the fluorescence intensity of the protein-ANS conjugate and the fluorescence intensity of ultrapure water, respectively, the surface hydrophobicity is expressed as the initial slope of RFI as a function of protein concentration (mg/mL).

2.9. Complex coacervation between gelatin at O/W interface and sodium hexametaphosphate

2.9.1. Complex coacervation

The complex coacervation between gelatin in the O/W emulsion and sodium hexametaphosphate was performed according to Xia et al. (2017) with modifications. Briefly, 100 g of the gelatin-stabilized emulsion with various lipid phases was mixed with 100 g of sodium hexametaphosphate solution (530 mg/100 g) and 50 g of ultrapure water. Subsequently, the pH of the mixture was adjusted to 4.9 using 1% (w/w) phosphoric acid solution. The complex coacervation between gelatin in the emulsions and sodium hexametaphosphate was monitored using a microscope with a camera (OLYMPUS CX43, Oberkochen, Baden-Württemberg, Germany). During complex coacervation, the temperature was maintained at 50 °C.

2.9.2. Complex coacervation yield

The emulsions with complex coacervates formed at pH 4.9, as described in section 2.9.1 were used to determine the complex coacervation yield according to Wang, Li, Jiang, Qi, and Zhou (2014). The formed complex coacervates and the serums were separated at 10,509×*g* for 20 min after stewing for stratification at room temperature. The separated complex coacervates were dried at 105 °C until constant weight. The complex coacervation yield was calculated using Equation (5).

$$CY(\%) = \frac{m_i}{m_0} \times 100 \quad (5)$$

Where CY is the yield of complex coacervation yield (%), m_i is the mass of dried complex coacervate (g), and m_0 is the total mass of gelatin, oil sample and sodium hexametaphosphate (g).

2.10. Statistical analysis

All experiments were performed in triplicates and the results were expressed as "mean ± standard deviation". One-way analysis of variance (ANOVA) and Tukey's HSD multiple comparisons were used to

determine the difference at a confidence level of 95% with JMP® Pro 13.0.0 software (SAS, Cary, North Carolina, USA). Origin Pro 2022 software was used to plot the graphs.

3. Results and discussion

3.1. Lipid class and major fatty acids in tuna oil and acylglycerols

The lipid class of tuna oil and its two acylglycerols obtained after hydrolysis by lipase TL100 and ADL is shown in Table 1. DAG and MAG were observed in the acylglycerols due to lipid hydrolysis, with a decreased TAG content. The DAG content in both acylglycerols was significantly higher than MAG ($p < 0.05$). The content of DAG was relatively high, and the content of DAG and MAG in TL100-AC was higher than that in ADL-AC. Compared with TL100-AC, the lower content of saturated fatty acids and the higher content of EPA were found in ADL-AC. Thus, it can be seen that two acylglycerols had distinct lipid composition and structure (Xuan et al., 2022).

3.2. O/W emulsion particle size, zeta potential and surface hydrophobicity

The average particle size and polydispersity index (PDI) of various gelatin-stabilized O/W emulsions with different lipid phases are shown in Table 2. The acylglycerols-based emulsions had reduced droplet size and PDI value, compared with the tuna oil-based one ($p < 0.05$). This is because DAG and MAG in the acylglycerols participated in the emulsification process as effective surfactants. Because of their superior emulsification and stability properties, DAG and MAG are widely used in the fields of emulsifying stability and physical property improvement of protein emulsion (Liu et al., 2021; Loi, Eyres, & Birch, 2019).

The zeta potential value of the two acylglycerol-based emulsions was significantly lower than that of the tuna oil-based one ($p < 0.05$). The surface hydrophobicity of the acylglycerol-based emulsions was significantly higher than that of the tuna oil emulsion ($p < 0.05$). It suggested that more hydrophobic groups in protein molecules were exposed in the acylglycerol-based emulsion. Xia, Akanb, et al. (2019) reported that the decreased zeta potential value and increased surface hydrophobicity of the acylglycerol emulsion might be due to the reduced amount of positively charged gelatin at the droplet surface due to the presence of MAG and DAG. Wang, Pan, Chiou, Li, and Ding (2022) found that the exposure of hydrophobic groups in the blend dispersion of whey protein and nanoliposomes changed the structure of whey protein, leading to dissociation of protein subunits and expansion of peptide chains, thereby improving protein emulsifying properties and adsorption behavior on the O–W interface.

Table 1
Lipid class and main fatty acid profile of tuna oil and its acylglycerols hydrolyzed by lipase *Thermomyces lanuginosus* (TL100) and *Candida antarctica* (ADL), respectively.

		Normalized Percentage/%		
		Tuna oil	TL100-AC	ADL-AC
Lipid class	Triacylglycerol (TAG)	97.86 ± 0.79^{a}	59.48 ± 7.60^{c}	83.66 ± 2.35^{b}
	Free fatty acid (FFA)	0.28 ± 0.28^{c}	2.40 ± 0.58^{a}	0.97 ± 0.26^{b}
	Diacylglycerol (DAG)	0.51 ± 0.32^{c}	26.45 ± 6.43^{a}	12.78 ± 1.35^{b}
	Monoacylglycerol (MAG)	1.35 ± 0.19^{b}	11.69 ± 0.60^{a}	2.60 ± 1.25^{b}
Characteristic fatty acids	C14:0	4.12 ± 0.43^{a}	3.41 ± 0.12^{a}	2.18 ± 0.29^{b}
	C16:0	20.97 ± 0.52^{a}	17.21 ± 0.08^{b}	11.50 ± 1.89^{c}
	C16:1 n^{7}	5.06 ± 0.43^{ab}	4.00 ± 0.42^{b}	5.36 ± 0.33^{a}
	C18:0	5.84 ± 0.02^{a}	4.37 ± 0.22^{b}	3.41 ± 0.48^{c}
	C18:1 n^{9}	15.34 ± 0.22^{b}	12.81 ± 0.67^{c}	17.62 ± 0.62^{a}
	C20:5 n3	7.06 ± 0.12^{b}	7.76 ± 0.27^{b}	9.33 ± 0.78^{a}
	C22:5 n3	1.84 ± 0.32^{a}	2.26 ± 0.39^{a}	2.54 ± 0.14^{a}
	C22:6 n3	27.54 ± 0.65^{b}	37.24 ± 0.46^{a}	36.50 ± 2.60^{a}

Note: Mean values in the same row with different superscripts are significantly different ($p < 0.05$).

Table 2
Particle size, zeta-potential and surface hydrophobicity of gelatin-stabilized O/W emulsions formed from tuna oil, lipase *Thermomyces lanuginosus* (TL100) modified acylglycerol (TL100-AC) and lipase *Candida antarctica* (ADL) modified acylglycerol (ADL-AC).

Lipid	Particle size (nm)	PDI	Zeta-potential (mV)	Surface hydrophobicity
Tuna oil	1799 ± 337^{a}	0.81 ± 0.12^{a}	15.07 ± 0.54^{a}	8.57 ± 0.23^{c}
TL100-AC	575 ± 21^{b}	0.51 ± 0.01^{b}	13.53 ± 0.05^{b}	11.58 ± 0.16^{b}
ADL-AC	597±7^{b}	0.50 ± 0.09^{b}	13.47 ± 0.61^{b}	12.30 ± 0.25^{a}

Note: Mean values in the same column with different superscripts are significantly different ($p < 0.05$).

3.3. EAI and ESI of O/W emulsions with different lipids

The EAI and ESI of the gelatin-stabilized O/W emulsions with different lipid phases are shown in Fig. 1. Tuna oil-based emulsion had the highest EAI value, followed by the ones containing ADL-AC and TL100-AC. It might be due to the reduced particle size of the acylglycerol-based emulsions. As discussed in section 3.2, the gelatin content on the individual acylglycerol emulsion droplet might also be reduced due to the competitive adsorption of MAG and DAG at the O/W interface. Ultimately it caused a reduced EAI. Fan et al. indicated that EAI of emulsion represented protein's relative surface adsorption capacity on the oil droplet (Fan, Peng, Pang, Wen, & Yi, 2021). On the other hand, TL100-AC and ADL-AC had FFA (Table 1), these FFA molecules might hinder protein adsorption at the O/W interface (Waraho, McClements, & Decker, 2011). As shown in Table 1, TL100-AC had more

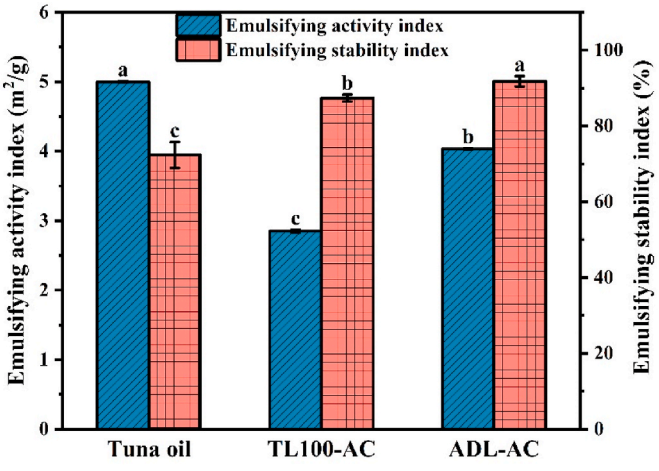

Fig. 1. Emulsifying activity index and emulsifying stability index of gelatin-stabilized O/W emulsions formed from tuna oil, lipase *Thermomyces lanuginosus* (TL100) modified acylglycerol (TL100-AC) and lipase *Candida antarctica* (ADL) modified acylglycerol (ADL-AC).

FFA than ALD-AC. Therefore, the EAI in emulsion with TL100-AC as lipid phase was lower than that made from ADL-AC.

The ESI of the emulsions showed an opposite trend to the EAI. The acylglycerol-based emulsion had significantly higher ESI values than those containing tuna oil ($p < 0.05$). The amphiphilic nature of DAG and MAG in the acylglycerol exhibited high surface activity and emulsifying properties (Blankart, Oellig, Averweg, Schwack, & Hinrichs, 2020; Kowalska, Zbikowska, Wozniak, & Kucharczyk, 2017). During the emulsification, DAG and MAG reduced the interfacial tension and formed emulsions with improved stability, compared with tuna oil as the lipid phase. Similarly, Chang et al. reported that O/W emulsions prepared from algal oil rich in DAG and MAG had a higher ESI than those prepared from oils in the TAG form (Chang & Lee, 2020). It is worth noting that an emulsion made with ADL-AC showed a slightly but significantly higher ESI value than one containing TL100-AC ($p < 0.05$). As shown in Table 1, the combined MAG and DAG content in TL100-AC and ADL-AC was approximately 38 and 15%, respectively. Compared with proteins, MAG/DAG are low-molecular weight surfactants which produce droplets without electrostatic charge and reduced steric hindrance effect. Thus, the emulsion stability may be compromised with changed environmental conditions (Dickinson, 2019). Furthermore, the higher content of DAG and MAG in the lipid phase may indicate that more DAG and MAG are involved in the construction of the interfacial film, resulting in insufficient protein to saturate the interface, reducing the electrostatic repulsion among droplets and physical stability of the emulsion.

3.4. Interfacial properties of O/W emulsions

The protein adsorption at the O/W interface in various emulsions is shown in Fig. 2. The tuna oil-based emulsion had the highest interfacial protein concentration of 96.52 g/100 g among the samples. This result agrees well with its highest EAI value (Fig. 1). It has been accepted that this interfacial film made by proteins is highly viscoelastic and effective in stabilizing the emulsion. In the emulsions with acylglycerol as the lipid phase, significantly reduced interfacial protein concentrations were observed. DAG and MAG are effective surfactants due to their hydrophilic hydroxyl groups and low molecular weights (Shimada & Ohashi, 2003). During emulsification, they readily migrated to the O/W interface, hindering protein adsorption. Similar competitive adsorption between monoacylglycerols and small peptides as well as subunits was also reported by Chen et al. (2019). The O/W interfacial film formed by the proper volume of DAG or MAG usually exhibited compromised mechanical properties compared with the protein one. Therefore, these acylglycerol-based emulsions were more unstable or prone to aggregation under certain circumstances (Li, Wang, Li, Jiao, & Chen, 2019).

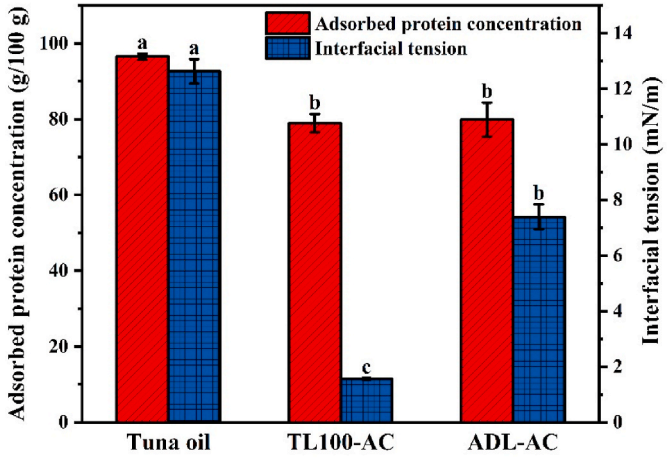

Fig. 2. Adsorbed protein concentration and interfacial tension of gelatin-stabilized O/W emulsions prepared from tuna oil, lipase *Thermomyces lanuginosus* (TL100) modified acylglycerol (TL100-AC) and lipase *Candida antarctica* (ADL) modified acylglycerol (ADL-AC).

The interfacial tension results of various emulsions are presented in Fig. 2. The acylglycerols-based emulsions showed significantly reduced interfacial tension compared to the tuna oil-based one. It was due to the competitive adsorption between MAG/DAG and gelatin at the O/W interface. Among the emulsions, the one with TL100-AC as the lipid phase had the lowest interfacial tension, possibly due to its highest MAG and DAG content (Table 1). It is worth noting that both acylglycerols-based emulsions had similar interfacial protein concentrations, although the interfacial tension values were significantly different. This result indicates that the concentration of MAG and DAG in the emulsions was above the critical micelle concentration. In both acylglycerols-based emulsions, the O/W interfaces were dominated by MAG and DAG. A similar competitive adsorption phenomenon was observed in the emulsion stabilized by sucrose ester and sodium caseinate (Zhao et al., 2014).

3.5. Rheological properties of O/W emulsions

The apparent viscosity of various O/W emulsions with different lipid phases is shown in Fig. 3A. Briefly, the viscosity of the emulsion decreased gradually with the increased shear rate in the range of 0–100 s^{-1}. It indicates the emulsions were typical shear-thinning pseudoplastic non-Newtonian fluids. The apparent viscosity of the acylglycerol-based emulsions was significantly lower than that of the tuna oil-based one. It might be due to the reduced particle size and narrowed distribution.

Meanwhile, the reduced interfacial protein concentration (Fig. 2) in the acylglycerol-based emulsions decreased repulsion and steric hindrance effects among the droplets. Ultimately, it reduced the emulsion viscosity (Chen, Qiu, Chen, Li, & Liang, 2020). The low viscosity of the acylglycerol-based emulsions facilitated the aggregation of droplets, which favored microcapsule formation during the subsequent complex coacervation (Fig. 5).

The storage (G′) and loss modulus (G″) of various emulsions are shown in Fig. 3B and C. The G′ value of all the samples was significantly higher than their G″ value. It indicates that the viscoelasticity of the emulsion was dominated by elasticity, with a certain degree of rigidity. Among the emulsions, the tuna oil-based one had the highest G′ value due to its highest interfacial protein concentration (Fig. 2). Meanwhile, this emulsion had the largest droplet size (Table 1), which enhanced the friction among the emulsion droplets when an external shear was applied (Fuhrmann et al., 2019).

3.6. Conformation of interfacial protein in various O/W emulsions

The circular dichroism spectrum of emulsions is shown in Fig. 4A. All the samples had a characteristic negative absorption peak at approximately 200 nm (Fig. 4A) due to the helical structure of gelatin (Raja, Duraipandi, Kiran, & Fathima, 2016). The tuna oil-based emulsion had the highest intensity in the entire circular dichroism spectrum which may be due to its highest adsorbed protein concentration (Fig. 3). The relative content of each secondary structure of gelatin in these emulsions are presented in Table 3. Compared with tuna oil emulsion, the relative content of α-helix and random coil structure in the acylglycerol emulsion increased, while the relative content of β-sheet (β-Sheet) structure decreased significantly by 2.15% and 0.81%, respectively ($p < 0.05$) (Table 3). The reduction of β-sheet structure content may be due to the interaction between acylglycerol and gelatin protein, during which internal structure of the protein was partially destroyed, leading to the exposure of hydrophobic amino acids that were kept in the internal structure. Wang et al. found that the β-sheet structure in proteins was a secondary structure connected by intermolecular or intramolecular hydrogen bonds, and the main function of which was to maintain the hydrophobic amino acids inside the protein structure, when the internal

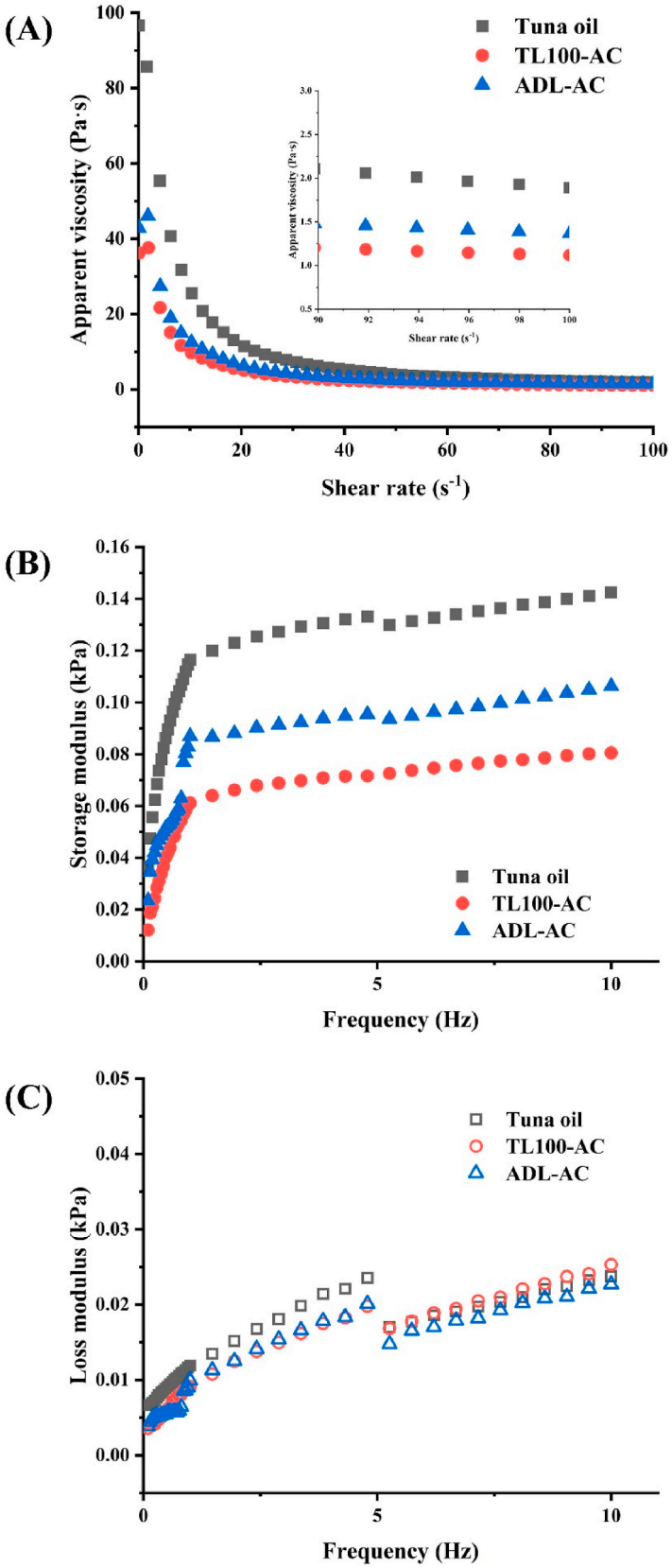

Fig. 3. Rheological properties of gelatin-stabilized O/W emulsions prepared by tuna oil, lipase *Thermomyces lanuginosus* (TL100) modified acylglycerol (TL100-AC) and lipase *Candida antarctica* (ADL) modified acylglycerol (ADL-AC) tested by Rheometer. (A) Apparent viscosity of the emulsion of tuna oil, TL100-AC and ADL-AC. (B) Storage modulus of the emulsion of tuna oil, TL100-AC and ADL-AC (G′). (C) Loss modulus of the emulsion of tuna oil, TL100-AC and ADL-AC (G″).

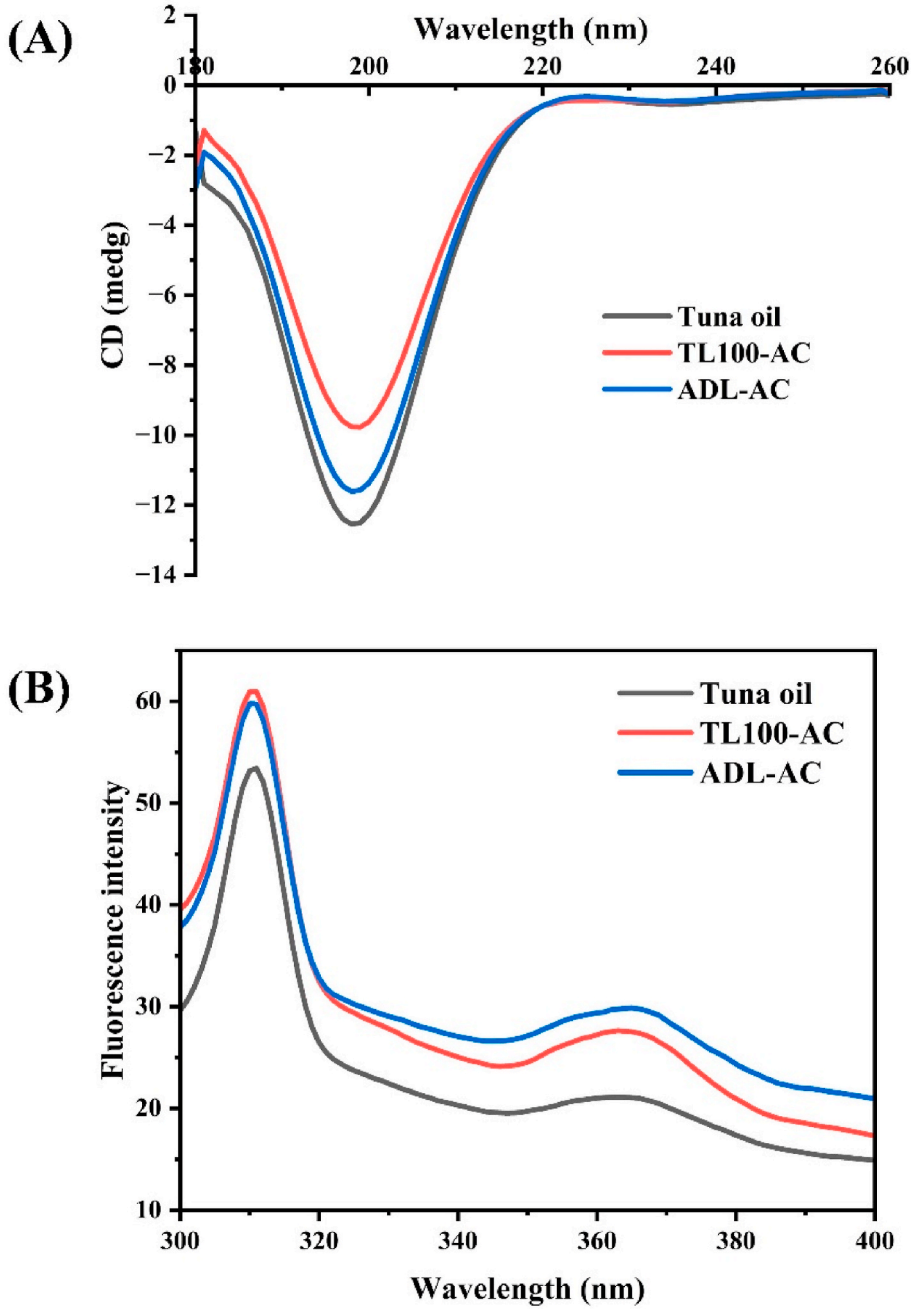

Fig. 4. Protein conformation analysis for gelatin-stabilized O/W emulsions of tuna oil, lipase *Thermomyces lanuginosus* (TL100) modified acylglycerol (TL100-AC) and lipase *Candida antarctica* (ADL) modified acylglycerol (ADL-AC). (A) Secondary structure tested by Circular dichroism. (B) Tertiary structure tested by Fluorescence spectrometer.

structure of the protein was partially destroyed, the hydrophobic amino acids would be exposed (Wang, Li, et al., 2014).

The endogenous fluorescence analysis result of the emulsions is presented in Fig. 4B to investigate the tertiary structure of gelatin in the emulsions. Generally, the endogenous fluorescence of a protein is usually affected by its hydrophobic residuals such as tyrosine (Tyr), tryptophan (Trp) and phenylalanine (Phe) and their interactions. As shown in Fig. 4B, the maximum absorption peak of all samples appeared at approximately 310 nm due to the Tyr residual. Compared with the tuna oil-based emulsion, the peak intensity of acylglycerol-based ones at this wavelength was significantly higher. It indicates facilitated exposure of hydrophobic Tyr at the O/W interface during the emulsification in these emulsions. The DAG and MAG in acylglycerols might stretch the gelatin molecules to a loose and disordered state, slightly increasing random coil content and facilitating protein unfolding (Table 3). Ultimately, hydrophobic groups initially buried inside the protein molecules were exposed towards the surface.

3.7. Complex coacervation

The formation of complex coacervates between the gelatin film of the emulsion droplets and sodium hexametaphosphate in the acidic environment is shown in Fig. 5. Briefly, the interaction between two polymers was driven by electrostatic force and hydrophobic interactions (Ach et al., 2015). The complex coacervation yields of the three systems were 67.03 ± 2.03, 77.40 ± 0.86 and 75.11 ± 1.00% for emulsions with tuna oil, TL100-AC and ADL-AC as the lipid phase, respectively. As shown in Table 2 and Fig. 3, compared with tuna oil-based emulsion, the

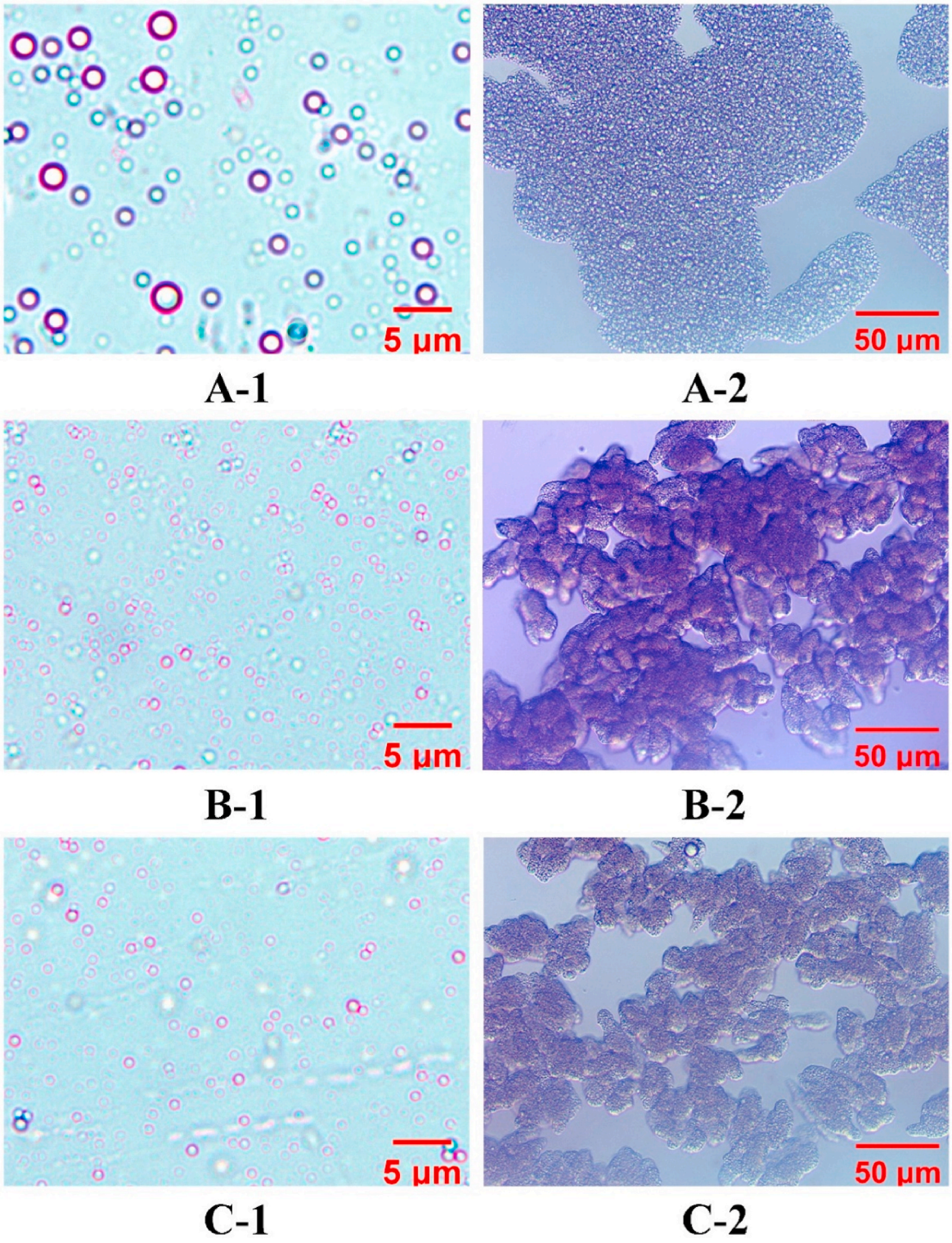

Fig. 5. Morphology of the complex coacervates of three kinds of emulsion using optical microscope; (A-1) morphology of tuna oil-based emulsion; (A-2) morphology of the complex coacervate of tuna oil-based emulsion; (B-1) morphology of lipase *Thermomyces lanuginosus* (TL100) modified acylglycerol (TL100-AC-based) emulsion; (B-2) morphology of the complex coacervate of lipase *Thermomyces lanuginosus* (TL100) modified acylglycerol (TL100-AC-based) emulsion; (C-1) morphology of lipase *Candida antarctica* (ADL) modified acylglycerol (ADL-AC-based) emulsion; (C-2) morphology of the complex coacervate of lipase *Candida antarctica* (ADL) modified acylglycerol (ADL-AC-based) emulsion; The magnification of optical microscope used to observe emulsions is 100x and the scale is 5 μm; The magnification of optical microscope used to observe complex coacervates is 40x and the scale is 50 μm.

Table 3
Relative content of protein secondary structure various gelatin-stabilized O/W emulsions formed from tuna oil, lipase *Thermomyces lanuginosus* (TL100) modified acylglycerol (TL100-AC) and lipase *Candida antarctica* (ADL) modified acylglycerol (ADL-AC).

	Normalized percentage/%			
	α- Helix	β-Sheet	β-Turn	Random coil
Tuna oil	9.34 ± 0.15^{c}	54.15 ± 0.20^{a}	17.01 ± 0.03^{a}	19.49 ± 0.02^{b}
TL100-AC	10.13 ± 0.07^{a}	52.00 ± 0.78^{b}	16.93 ± 0.15^{a}	20.94 ± 0.65^{a}
ADL-AC	9.75 ± 0.07^{b}	53.34 ± 0.12^{a}	17.04 ± 0.05^{a}	19.87 ± 0.20^{ab}

Note: Mean values in the same column with different superscripts are significantly different ($p < 0.05$).

acylglycerol-based ones had reduced size and zeta potential, increased surface hydrophobicity and decreased apparent viscosity. Therefore, the acylglycerol emulsion droplets readily formed aggregates, driven by the hydrophobic force during cooling.

The complex coacervates formed by tuna oil-based emulsion and sodium hexametaphosphate were larger overall, with loose structure, irregular shape, blurry boundary and uneven size. However, the complex coacervate formed by the acylglycerol-based emulsion and sodium hexametaphosphate reacted more intensely, and the coacervation phenomenon was more significant. It shows that the acylglycerol-based emulsion was subject to a stronger driving force, which was more favorable for the occurrence of complex coacervation. The differences in intensity and yield of complex coacervation were consistent with the changes in emulsion particle size, zeta potential, surface hydrophobicity, emulsifying properties, rheological and interfacial properties, and protein conformation and other parameters analyzed above. It shows that the relevant force driving the complex coacervation of the acylglycerol-based emulsion is stronger, which promotes more intense complex coacervation behavior, which is conducive to the formation of complex coacervation microcapsules with a denser encapsulated structure (Xia, Akanbi, et al., 2019; Xia et al., 2020).

4. Conclusions

In this paper, the effects of the acylglycerols obtained from the hydrolysis of tuna oil by TL100 and ADL respectively on the emulsifying properties, physicochemical properties and interfacial properties of the gelatin O/W emulsion were studied. The results showed that the lipid composition and structure of the oil phase of gelatin O/W emulsions, especially the contents of DAG, MAG and FFA, were significantly related to the physicochemical characteristics of the emulsion. The TL100-modified acylglycerol-based emulsion had a smaller droplet size, interfacial protein adsorption and apparent viscosity. The ADL-modified acylglycerol-based emulsion had higher emulsifying stability and surface hydrophobicity, and lower zeta potential. Compared with tuna oil-based emulsion, acylglycerols-based emulsions obtained higher complex coacervation yield. The lower O–W interfacial tension, smaller EAI and interfacial protein concentration indicated that the acylglycerol interacted with gelatin and competed for the O–W interface adsorption sites, which changed the properties of the interfacial protein membranes. Decreased surface charge and increased surface hydrophobicity of emulsions may indicate weakened electrostatic repulsion and enhanced hydrophobic interactions among droplets. These improved properties and interfacial behavior made the acylglycerol-based gelatin-stabilized O/W emulsion more prone to intense complex coacervation reactions, which helped to form complex coacervation microcapsules with a denser encapsulated structure. This paper provides a new reference for the stable delivery of bioactive substances such as fish oil and the research and development of related products.

CRediT authorship contribution statement

Junyong Xuan: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Qiuyu Xia:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Yinyi Tu:** Data curation, Investigation. **Tingyu Luo:** Data curation, Investigation. **Qingya Mao:** Data curation, Investigation. **Zongyuan Han:** Methodology, Software. **Colin J. Barrow:** Writing – review & editing. **Shucheng Liu:** Funding acquisition, Project administration, Resources. **Bo Wang:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

The authors acknowledge the financial support from the National Natural Science Foundation of China (32172252), Key Field Project Supported by Educational Commission of Guangdong Province (Biological Medicine and Health) (2022 ZDZX2028), National Natural Science Foundation of China (Youth Program) (32202086), Guangdong Innovation Team of Seafood Green Processing Technology (2019KCXTD 011) and the Program for Scientific Research Start-up Funds of Guangdong Ocean University (R20077).

References

Ach, D., Briancon, S., Dugas, V., Pelletier, J., Broze, G., & Chevalier, Y. (2015). Influence of main whey protein components on the mechanism of complex coacervation with Acacia gum. *Colloids and Surfaces A-Physicochemical and Engineering Aspects, 481*, 367–374. https://doi.org/10.1016/j.colsurfa.2015.06.006

Barrow, C. J., Nolan, C., & Holub, B. J. (2009). Bioequivalence of encapsulated and microencapsulated fish-oil supplementation. *Journal of Functional Foods, 1*(1), 38–43. https://doi.org/10.1016/j.jff.2008.09.006

Blankart, M., Oellig, C., Averweg, S., Schwack, W., & Hinrichs, J. (2020). Effect of storage at high temperature on chemical (composition) and techno-functional characteristics of E471 food emulsifiers applied to aerosol whipping cream. *Journal of Food Engineering, 277*(C), Article 109882. https://doi.org/10.1016/j.jfoodeng.2019.109882

Cao, W. H., Gao, R., Wan, X., He, Z. Y., Chen, J., Wang, Y. S., … Li, W. W. (2022). Effects of globular and flexible structures on the emulsifying and interfacial properties of mixed soy proteins. *Food Hydrocolloids, 127*, Article 107539. https://doi.org/10.1016/j.foodhyd.2022.107539

Chang, H. J., & Lee, J. H. (2020). Emulsification and oxidation stabilities of DAG-rich algae oil-in-water emulsions prepared with the selected emulsifiers. *Journal of the Science of Food and Agriculture, 100*(1), 287–294. https://doi.org/10.1002/jsfa.10037

Chatterjee, S., & Judeh, Z. M. A. (2016). Impact of encapsulation on the physicochemical properties and gastrointestinal stability of fish oil. *LWT - Food Science and Technology, 65*(1), 206–213. https://doi.org/10.1016/j.lwt.2015.08.010

Chen, W. P., Liang, G. J., Li, X., He, Z. Y., Zeng, M. M., Gao, D. M., … Chen, J. (2019). Impact of soy proteins, hydrolysates and monoglycerides at the oil/water interface in emulsions on interfacial properties and emulsion stability. *Colloids and Surfaces B-Biointerfaces, 177*, 550–558. https://doi.org/10.1016/j.colsurfb.2019.02.020

Chen, X., Qiu, Q. Y., Chen, K. W., Li, D., & Liang, L. (2020). Water-soluble myofibrillar protein-pectin complex for enhanced physical stability near the isoelectric point: Fabrication, rheology and thermal property. *International Journal of Biological Macromolecules, 142*, 615–623. https://doi.org/10.1016/j.ijbiomac.2019.10.003

Diao, X. Q., Guan, H. N., Zhao, X. X., Chen, Q., & Kong, B. H. (2016). Properties and oxidative stability of emulsions prepared with myofibrillar protein and lard diacylglycerols. *Meat Science, 115*, 16–23. https://doi.org/10.1016/j.meatsci.2016.01.001

Dickinson, E. (2019). Strategies to control and inhibit the flocculation of protein-stabilized oil-in-water emulsions. *Food Hydrocolloids, 96*, 209–223. https://doi.org/10.1016/j.foodhyd.2019.05.021

Dong, Y.b., Lan, T., Huang, G., Jiang, L. Z., Zhang, Y., & Sui, X. N. (2021). Development and characterization of nanoparticles formed by soy peptide aggregate and epigallocatechin-3-gallate as an emulsion stabilizer. *LWT - Food Science and Technology, 152*, Article 112385. https://doi.org/10.1016/J.LWT.2021.112385

Eratte, D., Dowling, K., Barrow, C. J., & Adhikari, B. (2018). Recent advances in the microencapsulation of omega-3 oil and probiotic bacteria through complex coacervation: A review. *Trends in Food Science & Technology, 71*, 121–131. https://doi.org/10.1016/j.tifs.2017.10.014

Fan, Y. T., Peng, G. F., Pang, X., Wen, Z., & Yi, J. (2021). Physicochemical, emulsifying, and interfacial properties of different whey protein aggregates obtained by thermal treatment. *LWT - Food Science and Technology, 149*, Article 111904. https://doi.org/10.1016/j.lwt.2021.111904

Fuhrmann, P. L., Sala, G., Stieger, M., & Scholten, E. (2019). Clustering of oil droplets in o/w emulsions: Controlling cluster size and interaction strength. *Food Research International, 122*(C), 537–547. https://doi.org/10.1016/j.foodres.2019.04.027

Guo, Y. L., Huang, W. C., Wu, Y. F., Qi, X. M., & Mao, X. Z. (2019). Conformational changes of proteins and oil molecules in fish oil/water interfaces of fish oil-in-water emulsions stabilized by bovine serum albumin. *Food Chemistry, 274*, 402–406. https://doi.org/10.1016/j.foodchem.2018.08.122

Kowalska, M., Zbikowska, A., Wozniak, M., & Kucharczyk, K. (2017). Long-term stability of emulsion based on rose oil. *Journal of Dispersion Science and Technology, 38*(11), 1563–1569. https://doi.org/10.1080/01932691.2016.1262777

Liu, N., Li, N., Faiza, M., Li, D. M., Yao, X. L., & Zhao, M. M. (2021). Stability and in vitro digestion of high purity diacylglycerol oil-in-water emulsions. *LWT - Food Science and Technology, 148*, Article 111744. https://doi.org/10.1016/j.lwt.2021.111744

Liu, H. H., Wang, B., Barrow, C. J., & Adhikari, B. (2014). Relating the variation of secondary structure of gelatin at fish oil-water interface to adsorption kinetics, dynamic interfacial tension and emulsion stability. *Food Chemistry, 143*, 484–491. https://doi.org/10.1016/j.foodchem.2013.07.130

Li, W. W., Wang, Y. S., Li, J. C., Jiao, Y., & Chen, J. (2019). Synergistic and competitive effects of monoglycerides on the encapsulation and interfacial shear rheological behavior of soy proteins. *Food Hydrocolloids, 89*, 631–636. https://doi.org/10.1016/j.foodhyd.2018.11.023

Loi, C. C., Eyres, G. T., & Birch, E. J. (2019). Effect of mono- and diglycerides on physical properties and stability of a protein-stabilised oil-in-water emulsion. *Journal of Food Engineering, 240*, 56–64. https://doi.org/10.1016/j.jfoodeng.2018.07.016

Martinez-Galan, J. P., Ontibon-Echeverri, C. M., Costa, M. C., Batista-Duharte, A., Batista, V. G., Mesa, V., … Baviera, A. M. (2021). Enzymatic synthesis of capric acid-rich structured lipids and their effects on mice with high-fat diet-induced obesity. *Food Research International, 148*, Article 110602. https://doi.org/10.1016/j.foodres.2021.110602

Raja, I. S., Duraipandi, N., Kiran, M. S., & Fathima, N. N. (2016). An emulsion of pigmented nanoceria as a medicinal cosmetic. *RSC Advances, 6*(103), 100916–100924. https://doi.org/10.1039/c6ra15816a

Shao, J. H., Zou, Y. F., Xu, X. L., Wu, J. Q., & Zhou, G. H. (2011). Evaluation of structural changes in raw and heated meat batters prepared with different lipids using Raman spectroscopy. *Food Research International, 44*(9), 2955–2961. https://doi.org/10.1016/j.foodres.2011.07.003

Shimada, A., & Ohashi, K. (2003). Interfacial and emulsifying properties of diacylglycerol. *Food Science and Technology Research, 9*(2), 142–147. https://doi.org/10.3136/fstr.9.142

Wang, B., Adhikari, B., & Barrow, C. J. (2014). Optimisation of the microencapsulation of tuna oil in gelatin–sodium hexametaphosphate using complex coacervation. *Food Chemistry, 158*, 358–365. https://doi.org/10.1016/j.foodchem.2014.02.135

Wang, Z. J., Li, Y., Jiang, L. Z., Qi, B. K., & Zhou, L. Y. (2014). Relationship between secondary structure and surface hydrophobicity of soybean protein isolate subjected to heat treatment. *Journal of Chemistry, 2014*, 1–10. https://doi.org/10.1155/2014/475389

Wang, Q., Pan, M. H., Chiou, Y. S., Li, Z. S., & Ding, B. M. (2022). Surface characteristics and emulsifying properties of whey protein/nanoliposome complexes. *Food Chemistry, 384*, Article 132510. https://doi.org/10.1016/j.foodchem.2022.132510

Waraho, T., McClements, D. J., & Decker, E. A. (2011). Impact of free fatty acid concentration and structure on lipid oxidation in oil-in-water emulsions. *Food Chemistry, 129*(3), 854–859. https://doi.org/10.1016/j.foodchem.2011.05.034

Xia, Q. Y., Akanbi, T. O., Wang, B., Li, R., Liu, S. C., & Barrow, C. J. (2020). Investigation of enhanced oxidation stability of microencapsulated enzymatically produced tuna oil concentrates using complex coacervation. *Food & Function, 11*(12), 10748–10757. https://doi.org/10.1039/d0fo02350g

Xia, Q. Y., Akanbi, T. O., Wang, B., Li, R., Yang, W. R., & Barrow, C. J. (2019). Investigating the mechanism for the enhanced oxidation stability of microencapsulated omega-3 concentrates. *Marine Drugs, 17*(3), 143. https://doi.org/10.3390/md17030143

Xia, Q. Y., Akanb, T. O., Li, R., Wang, B., Yang, W. R., & Barrow, C. J. (2019). Lipase-catalysed synthesis of palm oil-omega-3 structured lipids. *Food & Function, 10*(6), 3142–3149. https://doi.org/10.1039/c9fo00668k

Xiao, N. H., He, W., Zhao, Y., Yao, Y., Xu, M. S., Du, H. Y., … Tu, Y. G. (2021). Effect of pH and xanthan gum on emulsifying property of ovalbumin stabilized oil-in water emulsions. *LWT - Food Science and Technology, 147*, Article 111621. https://doi.org/10.1016/j.lwt.2021.111621

Xia, Q. Y., Wang, B., Akanbi, T. O., Li, R., Yang, W. R., Adhikari, B., et al. (2017). Microencapsulation of lipase produced omega-3 concentrates resulted in complex coacervates with unexpectedly high oxidative stability. *Journal of Functional Foods, 35*, 499–506. https://doi.org/10.1016/j.jff.2017.06.017

Xuan, J. Y., Wang, Z. F., Xia, Q. Y., Luo, T. Y., Mao, Q. Y., Sun, Q. X., … Liu, S. C. (2022). Comparative lipidomics profiling of acylglycerol from tuna oil selectively hydrolyzed by *Thermomyces lanuginosus* lipase and *Candida Antarctica* lipase A. *Foods, 11*(22). https://doi.org/10.3390/foods11223664

Zhao, Q. Z., Liu, D. L., Long, Z., Yang, B., Fang, M., Kuang, W. M., et al. (2014). Effect of sucrose ester concentration on the interfacial characteristics and physical properties of sodium caseinate-stabilized oil-in-water emulsions. *Food Chemistry, 151*, 506–513. https://doi.org/10.1016/j.foodchem.2013.11.113

Zou, L., & Akoh, C. C. (2013). Characterisation and optimisation of physical and oxidative stability of structured lipid-based infant formula emulsion: Effects of emulsifiers and biopolymer thickeners. *Food Chemistry, 141*(3), 2486–2494. https://doi.org/10.1016/j.foodchem.2013.05.029