Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.84622

CC BY

Effects of phospholipid type and concentration on the emulsion stability and in vitro digestion behaviors of fish oil-loaded emulsions

Mingyu YIN[1], Min CHEN[1], Ryosuke MATSUOKA[2], Yinci XI[1], Xichang WANG[1]*

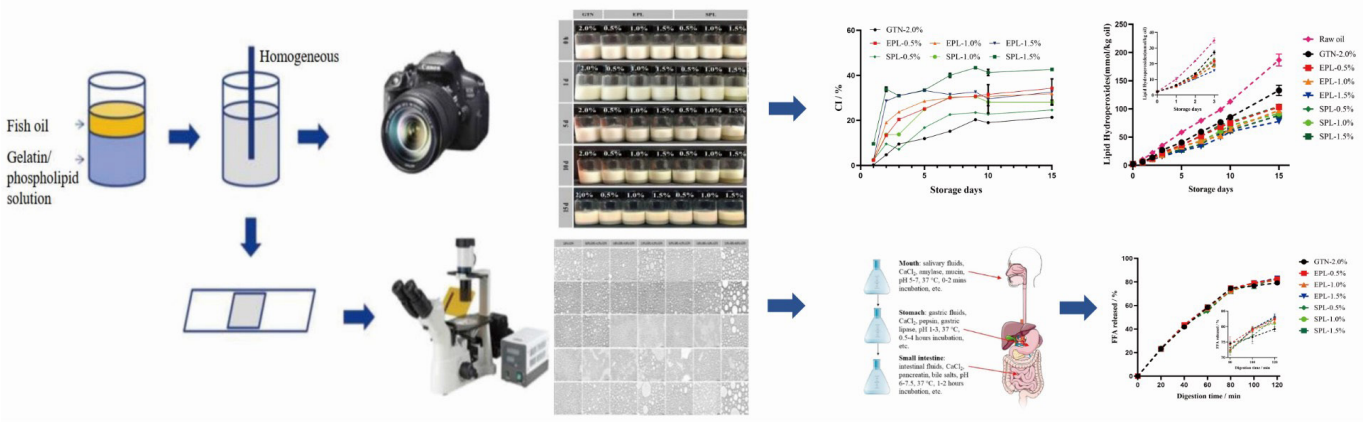
Abstract

Phospholipid (PL) have favorable emulsification ability. The simultaneous application of PL and protein is one of the research hotspots. In this work, the impact of egg yolk PL (EPL), soyabean PL (SPL) and their concentrations on the fish oil-loaded PL/gelatin (GTN)-stabilized emulsions were studied. The results suggested that SPL/GTN-stabilized emulsions comprised bimodal of droplets, and monomodal was observed in EPL/GTN, and GTN-stabilized emulsions. The related droplet sizes were as follows: 1.5% EPL/GTN < 1.0% EPL/GTN < 0.5% EPL/GTN < 2.0% GTN < 0.5% SPL/GTN < 1.0% SPL/GTN < 1.5% SPL/GTN.; and (4) Conclusion: The concentration of PL had significant effect on the droplet size and emulsion stability of the emulsions. The 1.0% SPL/GTN-stabilized emulsions demonstrated the optimal stability and droplet size when stored at room temperature for 15 days than emulsion contained PL. The addition of PL inhibited lipid oxidation and increased the rate of fatty acid release in *vitro* digestion. This work illustrated that PL/GTN-stabilized emulsions could potentially be a targeted delivery medium for nutrients or drugs.

Keywords: phospholipids; emulsification; stability; lipid oxidation; *vitro* digestion.

Practical Application: New technology for high quality fish oil processing.

Graphical Abstract



1 Introduction

Fish oil (FO) has become the new consumer product of the 21st century and is increasingly popular worldwide (Hashim et al., 2021). The FO, unlike other animal fats, is rich in polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), arachidonic acid (ARA), linoleic acid (LA), and linolenic acids, which are the main components of FO (Nikonova et al., 2020). High quality or refined FO is primarily used in food, health products and pharmaceuticals (Ashfaq et al., 2020; Malinowski et al., 2019). FO has a fishy odor (Güner et al., 2019) and is susceptible to oxidation (Ramos et al., 2021; Shehzad et al., 2021), thus limiting its application to some extent. Recently, these limitations have been overcome by microencapsulation (Encina et al., 2016), emulsification, and addition of masking agents (Wardhani et al., 2021).

Phospholipids (PL) are a special class of natural small molecule surfactants, usually derived from the cell membranes of plant, animals or microbial tissues, and are composed of glycerol and phosphate groups (Yin et al., 2021). Phosphate groups are usually

Received 01 June, 2022
Accepted 23 Aug., 2022
[1] *College of Food Science and Technology, Shanghai Ocean University, Shanghai, China*
[2] *Japan Kewpie Corporation, Tokyo, Japan*
*Corresponding author: xcwang@shou.edu.cn

in stereo specifically numbering (Sn)-3 position and two fatty acid chains in Sn-1 and Sn-2 positions, respectively. The differences in the relative position, chain length and unsaturation of the fatty acid chains are determined by the biological origin of the PL. Previous studies have shown that soy bean PL (SPL) could chelate metals to protect oils from oxidation (Cengiz et al., 2019). PL liposomes are easier to digested and absorbed in the gastrointestinal tract (Jiang et al., 2020). The addition of phospholipids in the avocado pulp promotes the emulsion to display a gel-like consistency and strong intermolecular forces (Züge et al., 2017). As surfactants, PL have potential and protective effect to fish oils. The hydrophobic part of a surfactant usually consists of a hydrocarbon group and the hydrophilic part is called the head group. The electrostatic and hydrophobic interactions between surfactants and proteins significantly change the nature of the adsorption layer between the interfaces of the two molecules, affecting the stability of dispersion systems such as emulsions and foams.

Gelatin, a natural amphiphilic macromolecule, has been widely used as a foam stabilizer, emulsifier (Chen et al., 2020) and wetting agent (Casoli et al., 2014). Protein and surfactants are physically blended to improve emulsion stability. Synergistic interfacial adsorption of caseinate and sodium stearoyl lactate (Chambi & Grosso, 2011), phospholipids and sucrose esters (Johnson et al., 2014) have been reported.

Understanding the digestion of these bioactive lipids throughout the gastrointestinal tract (GIT) and the release of free fatty acids (FFA) therein has become a research hotspot (Cheng et al., 2022; Li et al., 2022b; Huang et al., 2020). When the emulsion is ingested, it is exposed to the digestive liquid and enzymes present in the GIT, resulting in physicochemical changes. The bioavailability of omega-3 PUFAs may be affected by the type and concentration of emulsifiers (Infantes-Garcia et al., 2022; Udomrati et al., 2020). Therefore, the bioavailability of fish oil can be improved by using reasonable concentrations of emulsifiers. In this study, fish oil-loaded PL/gelatin (GTN)-stabilized emulsions were made and the effects of PL concentration and type on the emulsion droplets and stability of emulsions under 15 days storage, and in *vitro* fatty acid released ratio were investigated.

2 Materials and methods

2.1 Materials

EPLs (purity 99.69%, 63.21% Phosphatidylcholine, 12.11% Phosphatidylethanolamine) was presented by Kewpie Co., Ltd. (Tokyo, Japan). SPLs (purity 97.32%,42.19% Phosphatidylcholine, 19.27% Phosphatidylethanolamine) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The specific composition of the two PLs was shown in Table S1. GTN granules from bovine skin (type B, ~225 g bloom, Solarbio. Industrial Corp., Shanghai, China) were stored at 4 °C. And other common chemicals (analytical reagent grade) were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China and were stored at room temperature. Deep sea fish oils (FO, food grade, DHA + EPA ≥ 70%, Xi'an LvTeng Biological Technology Co., Ltd, Shaanxi Province, China) were stored at −40 °C. Deionized water was prepared by Milli,Q Reference system in this work. GTN pellets in bovine skin (type B, approx. 225 g) was purchased in Solarbio. Industrie (Shanghai, China). Fish oil (FO, n-3 polyunsaturated fatty acid ≥ 72.0%) was got from Xi'an Green Vine Biotechnology company (Shaanxi Province, China). Deionised water in this work was prepared by the Milli,Q reference system.

2.2 Fish oil-loaded PL/GTN-stabilized emulsions preparation

The 4.0% GTN solution was prepared by incubating the GTN in deionized water at room temperature for 30 minutes. Then the solution was incubated for 30 minutes by shaking (180 rpm), constant temperature (45 °C) water bath (model SW22, Julabo, Germany). The GTN solution was diluted, EPL and SPL were added to prepare PL/GTN solutions with phospholipid concentrations of 0.5%, 1.0%, and 1.5%, respectively, with a total emulsifier concentration of 2.0%. Due to the amphiphilic nature of phospholipids, when the phospholipids were added to the gelatin solution, the solution was incubated at 40 °C for 20 minutes, and then homogenized for 15 minutes at 1000 rpm. The pH was adjusted to 7.0 by 6 mol/L HCl solution or 6 mol/L NaOH solution. Then, the 5 mL FO was added into the 5 mL PL/GTN solutions. The 10 mL solution was homogenized (homogenization time 60 s, repeated twice; homogenization speed 1.0×10^4 r/min; room temperature (20 °C ± 2 °C). The obtained FO-loaded PL/GTN stabilized emulsion was photographed using a camera.

2.3 Optical microscopy measurements

The 4.0 μL FO-loaded PL/GTN-stabilized emulsions were added onto glass microslides, and then square cover glasses (22 × 22 mm) were covered to the emulsions. The inverted optical MS 600F microscope (Minz Precision Instruments Co. Ltd., Shanghai, China) was used to observed the samples.

2.4 Gaussian fitting

An optical microscope was used to observe the microscopic state of each sample. The droplet size was measured by Meizsmcs 6.0 software (Shanghai, China). The size of the droplets in the images were measured for subsequent analysis. For each emulsion, approximately 800-3000 droplets of size were obtained from three different images. Later, statistical analysis of the droplet size frequency distribution was performed. Bin sizes were chosen based on the distribution of droplet sizes and adjusted to ensure that the number of bins was 10-30, showing sufficient distribution for further analysis. Linear equation fit was applied to fit the curves of the peak values vs PL concentration.

2.5 The Creaming Index (CI)

The images of FO-loaded PL/GTN-stabilized emulsions were obtained by a digital camera. The creaming index (CI) was calculated as follows (Equation 1)

$$CI\ (\%) = Hs / Ht\ x\ 100\% \quad (1)$$

Where Hs is the height of the serum layer (the sum of the transparent and/or the turbid layers at the bottom of the vials and Ht is the total height of the FO-loaded PL/GTN-stabilized emulsions).

2.6 Determination of lipid oxidation in emulsions

Based on a previous work (Xu et al., 2022), The concentration of lipid hydrogen peroxide in the emulsion was measured during a 15-day storage period to reflect the extent of lipid oxidation. Briefly, 1.0 mL of the sample to be tested was mixed with the lipid extract solution (CH_3OH:$CHCl_3$; 2:1, V/V), pipetted repeatedly and left to stand for 10 min. The 0.2 mL $CHCl_3$ layer liquid was mixed with 2.8 mL of CH_3OH/n-$C_3H_7CH_2OH$ (2:1, V/V. 15 μL of 3.94 M NH_4SCN and 15 μL of working liquid (0.144M $FeSO_4$ and 0.132 M $BaCl_2$) were added to the assay system solution.

2.7 Free fatty acid release ratio of emulsions

The amount of free fatty acids (FFA) released from the emulsion during digestion was measured titrimetric ally, following the INFOGEST *vitro* digestion method (Beltrán et al., 2019). Briefly, Briefly, 0.5 mL of sample was diluted 10-fold using ultrapure water, then 5 mL of simulated saliva (SSF) was added. The pH was adjusted to 7.0 with 1 M NaOH and 1 M HCl and shaken at a constant temperature of 37 °C (180 r/min, 2 min). The above digest was mixed with 10 mL of simulated gastric fluid (SGF) and then the pH was adjusted to 3.0, shaken at 37 °C (180 r/min, 120 min) and the acidity values were corrected every 8 minutes (pH 3.00 ± 0.04). Simulated intestinal fluid (SIF) 20 mL was mixed with the upper post-digestion fluid, pH adjusted to 7.0 and shaken at 37 °C (180 r/min, 120 min). SSF, SGF and SIF formulations refer to previous methods (Beltrán et al., 2019), and all solutions must be pre-warmed at 37 °C before use.

The pH of the mixture was monitored during the 2 h small intestine digestion and the volume of NaoH consumed to stabilise pH 7.0 was recorded. The decrease in pH during digestion is due to the release of fatty acids in the free state. At this point the FFA content can be converted by recording the amount of NaOH consumed through the principle of acid-base neutralisation according to the formula. Assuming that each triacylglycerol produces two molecules of FFA, which was calculated according to the following Equation 2:

$$\%\ FFA = \left(100 * V_{NaOH} * m_{NaOH} * M_{Lipid}\right) / \left(2 * W_{Lipid}\right) \quad (2)$$

Where V_{NaOH} is the volume of NaOH required to neutralize the produced FFA, m_{NaOH} is the molar concentration of NaOH used (mol/L), W_{Lipid} is the total weight of fish oil, and M_{Lipid} is the molecular weight of fish oil (868 g/mol).

2.8 Statistical analysis

Statistical analysis was performed using software SPSS statistics 26.0 (IBM, USA). Differences were tested by using one-way ANOVA followed by Duncan's test. A value of $P < 0.05$ was considered to be statistically significant. The figures were drawn using Prism 8.0 (GraphPad, CA).

3 Results and discussion

3.1 Effect of PL type and concentration on the initial droplet microstructure of fish oil-loaded PL/GTN-stabilized emulsion

The FO-loaded PL/GTN-stabilized emulsions were photographed by digital camera and optical microscopy (Figure 1). The macro electron graph (Figure 1A) and the microscope image (Figure 1B) showed that the emulsion could be formed with different concentrations (0.5%, 1.0%, 1.5%) of EPL and SPL.

This also accorded with earlier observations, which showed that the minimum amount of PL for stable emulsion formation was 0.8%~1.3% (Lu et al., 2012). The emulsions formed by PL with different concentrations were milky white. It could be observed that the emulsion became slightly yellow, as the concentration of SPL increased. Figure 2B showed that solutions consisted of droplets, whose shape resembled a spherical shape. The droplets in the 1.0% EPL/GTN-stabilized, 0.5%, 1.0% SPL/GTN-stabilized emulsions were regular spherical shapes. In the 1.5% EPL/GTN-stabilized and 1.5% SPL/GTN-stabilized emulsions, the large droplets were irregularly spherical and the small droplets were regular spherical.

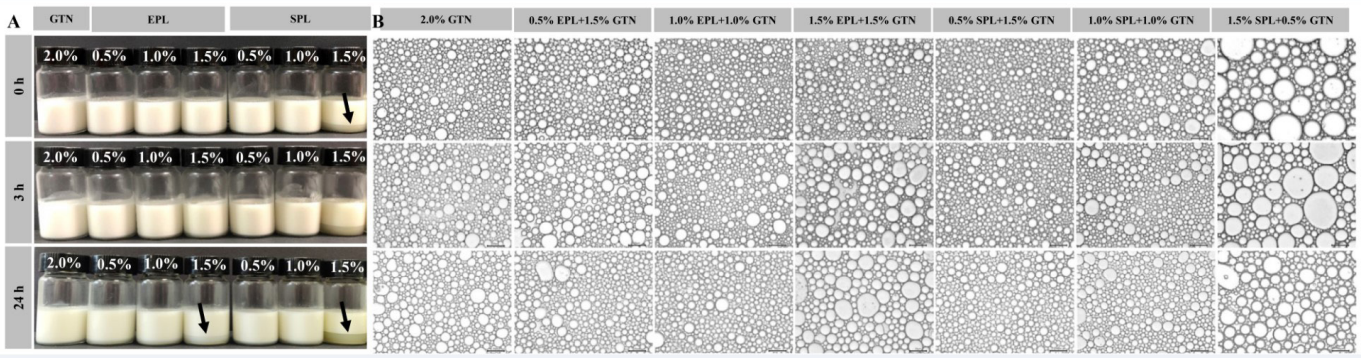


Figure 1. Stability of freshly prepared fish oil PL/GTN-stabilized emulsions and storage at room temperature for one day with different PL concentrations (0%, 0.5%, 1.0% and 1.5%). (A) Photograph; (B) Optical microscope image. Scale bars indicate 50 μm. EPL: egg yolk phospholipid; SPL: soy bean phospholipid; GTN: gelatin.

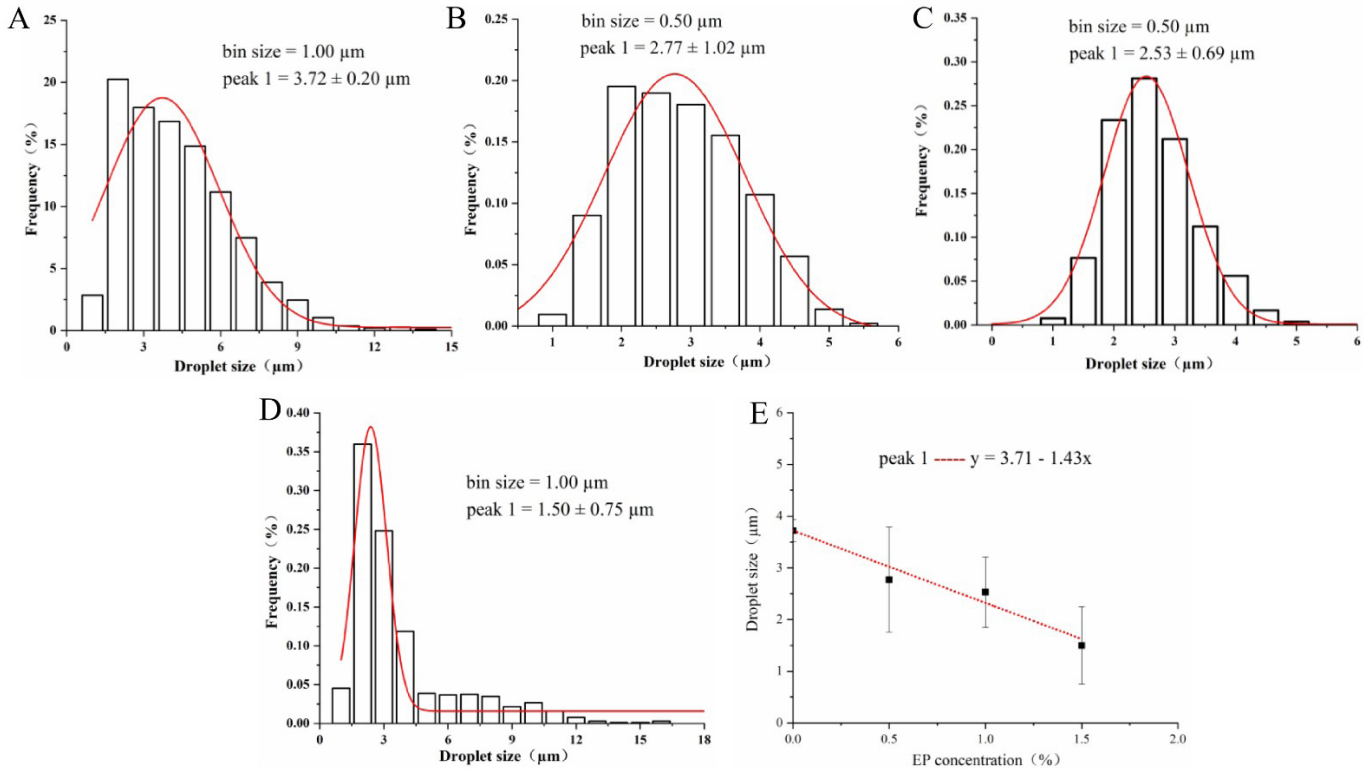


Figure 2. Representative droplet size distributions of freshly prepared fish oil-loaded EPL/GTN-stabilized emulsions at different PL concentrations. (A) 0%; (B) 0.5%; (C) 1.0%; (D) 1.5%. Red (peak 1); (E) Dynamic droplet size spectra of the most probable sizes as a function at different EPL concentrations. The data points for peak 1 were fitted to a linear equation and were shown in the upper right.

The initial droplet size distribution of the FO-loaded PL/GTN-stabilized emulsions at different PL concentrations were analyzed (Figures 2-3). EPL and SPL had a significant effect on the initial droplet microstructure of the emulsion. The FO-loaded GTN-stabilized and EPL/GTN-stabilized emulsions only consisted of monomodal (peak 1 in Figure 2), whereas SPL/GTN-stabilized emulsions were composed of bimodal droplets with different sizes (peak 1 and peak 2 in Figure 3), indicating the coexistence of EPL and GTN reduced the droplet size. In addition, the optical microscope image of the freshly prepared emulsion (Figure 2E) showed that the droplet size decreased with the increase of the EPL concentration. Unlike EPL, the droplet size of the emulsion increased as the concentration of SPL increased, showing a positive correlation. The quantitative analysis of the droplet size distribution as a function of the PL concentration (Figures 2-3) suggested that the fish oil-loaded EPL/GTN-stabilized emulsion was mainly composed of droplets of one size, and the size decreased from 3.72 ± 0.02 μm to 1.50 ± 0.75 μm linearly with the increase of the EPL concentration of the solution; the fish oil-loaded SPL/GTN-stabilized emulsion was mainly composed of 2 types, and the peak 2 droplet mean size decreased from 6.21 ± 2.14 μm to 3.53 ± 0.56 μm linearly with the increase of the SPL concentration of the solution. The droplets were in regular spherical shapes when its sizes were lower than 8.43 ± 1.35 μm. It might be ascribed to the stronger motility of fish oil (than water in O/W solution), the larger the size of the droplet, the less likely the droplet maintain a regular shape (Gao et al., 2019).

The initial droplet size produced by homogenization depends mainly on the properties of the emulsifier used for stabilizing the emulsion (GTN and PL in this work) and pH value (Hoffmann & Reger, 2014). Our lab previous studies have found that coexistence of GTN and SPL had synergistic effect on the stability of emulsion (Zhang et al., 2020a, b). The interaction between GTN and PL of different concentration and type affected the droplet size of emulsion. In this study, the homogenization conditions, added fish oil amount and the preparation of pH were the same. The difference of droplet size in PL/GTN-stabilized emulsion might depend on the nature of phospholipids in different concentrations of emulsion (steric properties) (Lam & Nickerson, 2013). As a small molecule surface active substance, PL has a much higher ability to adsorb at the oil-water interface to reduce interfacial tension than larger protein emulsifiers. there is a difference in the fatty acyl groups (hydrophobic groups) attached to the glycerol backbone between SPL and EPL. Therefore, when mixed with GTN, there are different interfacial effects. PL had a phosphate group (hydrophilic structure) and two fatty acid chains (hydrophobic structure) (Li et al., 2020). PL and GTN might have different electrical and steric properties at the water/oil interface: the hydrophobic part of PL and GTN enters the fish oil core, and the fatty acid chain of PL may be located on the surface of the fish oil; the phosphoric acid group in the phospholipid is hydrophilic and distributed on the water surface to form a relatively stable O/W structure. As the carbon content of fatty acids increases, the hydrophobic effect of PL increases (Hubbe et al., 2020; Mezzetta et al., 2019). The content of LA in

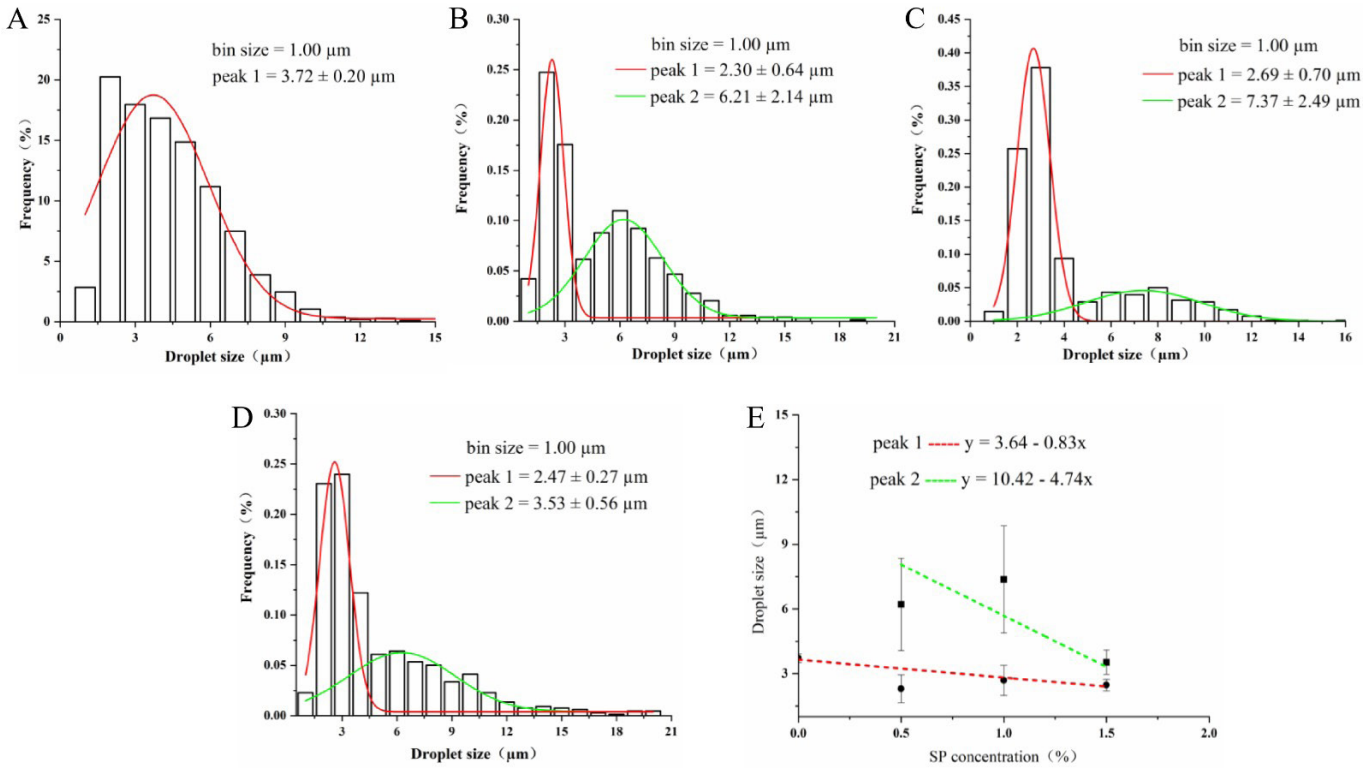


Figure 3. Representative droplet size distribution of freshly prepared SPL/GTN-stabilized emulsions loaded with fish oil at different PL concentrations. (A) 0%; (B) 0.5%; (C) 1.0%; (D) 1.0%; (E) 1.5%. Red (peak 1), green (peak 2). (E) Dynamic droplet size spectrum as a function of the most probable size at different SPL concentrations. The data points for peak 1, peak 2 were fitted as a linear equation and were shown on the upper right.

SPL (54.55 ± 0.06%) was significantly higher than that of EPL (15.14 ± 0.42%) ($P < 0.01$), and the content of palmitic acid in SPL (15.46 ± 0.71%) was significantly lower than EPL (29.87 ± 0.20) %) ($P < 0.01$). The initial particle size of the emulsion stabilized by PL/GTN was: SPL/GTN-stabilized emulsion > EPL/GTN-stabilized emulsion under the same PL concentration.

3.2 Effect of PL type and concentration on the microstructure of droplets of fish oil PL/GTN stabilized emulsion storage

The freshly prepared FO-loaded PL/GTN-stabilized emulsions were stored at room temperature, photographed and analyzed by optical microscope (0 h, 3 h, 1 d, 5 d, 10 d and 15 d). Different levels of emulsion were observed during the storage of 15 d. According to Figures 1, 4, the freshly prepared fish oil-loaded contained 1.5% SPL/GTN-stabilized emulsions came emulsification. 0.5% EPL/GTN-stabilized emulsion showed a slight milking phenomenon at 24 h, while 1.0% and 1.5% EPL/GTN-stabilized emulsions appeared creaming at 3 h and occurred grease precipitation at 24 h. The 0.5% and 1.0% SPL/GTN-stabilized emulsions appeared slight creaming at 24 h. EPL/GTN-stabilized and SPL/GTN-stabilized emulsions remained liquid even after 1 days of storage (Figure 2A). As shown in Figure 4A, the 1.5% SPL/GTN-stabilized emulsion showed the presence of a dark brown layer on the 5th day and turned into an emulsion gel, which was a soft solid material, both an emulsion and a gel (Nasirpour-Tabrizi et al., 2020). The droplets of the 1.5% SPL/GTN-stabilized emulsion and the 0.5% EP/GTN-stabilized emulsion showed shape deformation on the 15th day, and the increase of droplet size might be due to the aggregation of droplets. As illustrated in Figure 5, 1.0% EPL/GTN stabilized, (CI: 28.58 ± 0.03%) and 1.0% SPL/GTN-stabilized emulsions (CI: 24.98 ± 0.02%) showed emulsion layering on the 5th day. It further confirmed the co-adsorption of PL and GTN. The results were consistent with previous study (Zhang et al., 2020a).

The previous analysis results showed that during the preparation of fish oil emulsion, cooperative adsorption (PL/GTN) occurred at the oil/water interface, and the adsorption capacity increased or decreased respectively. The moving speed of an isolated spherical drop in an ideal liquid is formulated by Stokes' law (Equation 3):

$$V_{stokes} = 2gr_2(\rho_1 - \rho_2)/9\eta_1 \quad (3)$$

Where g is the acceleration of gravity, r is the droplet radius, ρ2 is the density of the dispersed phase (fish oil droplets in this study), ρ1 is the density of the continuous phase (water in this study), and η1 is the shear viscosity. According to Stokes' law, as the droplet size and density contrast increased, and the continuous phase viscosity decreased, as well as the speed of

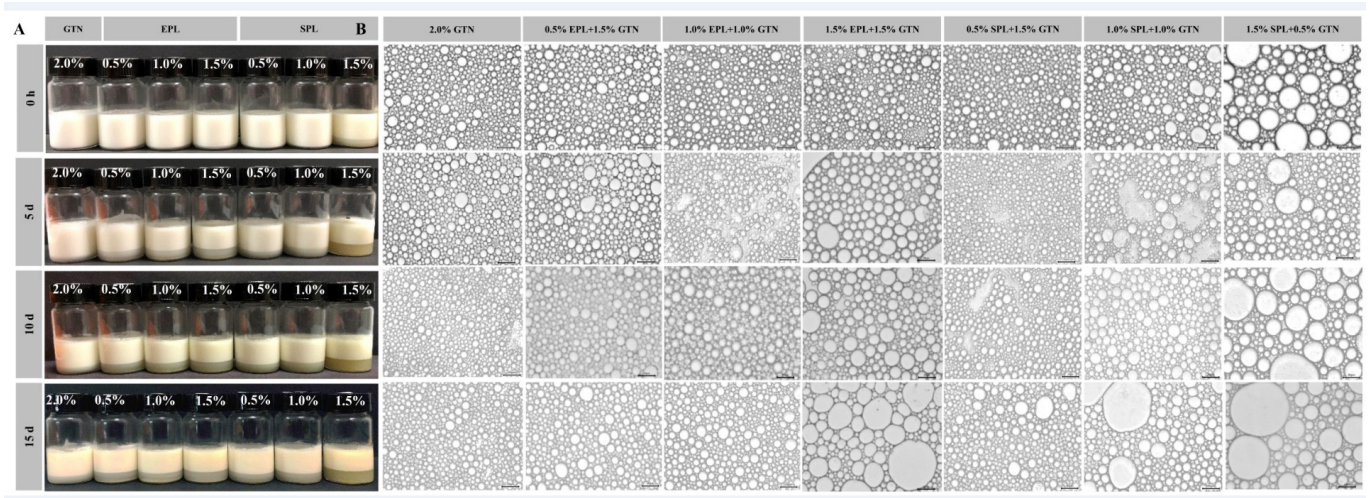


Figure 4. Stability of freshly prepared fish oil PL/GTN-stabilized emulsions and storage at room temperature for 15 days with different PL concentrations (0%, 0.5%, 1.0% and 1.5%). (A) Photograph; (B): Optical microscope image. Other emulsions are in liquid form. Scale bars indicate 50 μm. EPL: egg yolk phospholipid; SPL: soy bean phospholipid; GTN: gelatin.

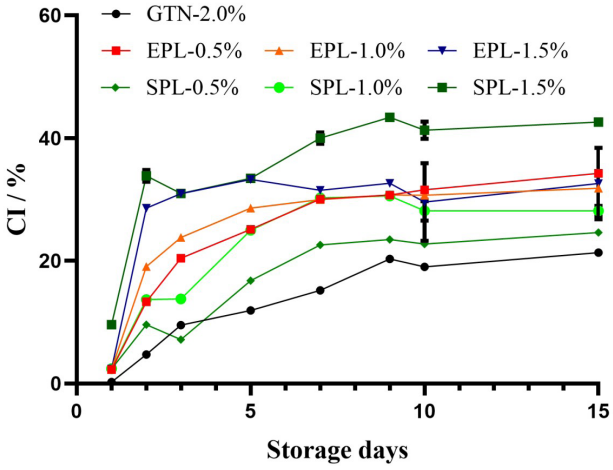


Figure 5. Creaming stability of fish oil-loaded PL/GTN-stabilized emulsions. EPL: egg yolk phospholipid; SPL: soy bean phospholipid; GTN: gelatin; CI: Creaminess index.

creaming increased. The radius of small size droplet of the SPL/GTN-stabilized emulsion was similar to that of the EPL/GTN-stabilized emulsion under same concentration; the radius of large droplet in the SPL/GTN-stabilized emulsion was 2~3 times than that of EPL/GTN-stabilized emulsion. The number of droplets increased with the increase of concentration. Therefore, the size of these droplets depended on the type and concentration of PL. As the droplet size increased, their motion accelerated, forming larger droplets, thus reducing the stability of emulsion.

The CI of SPL/GTN-stabilized emulsion was generally less than that of EPL/GTN-stabilized emulsion. Meanwhile, as the storage time went on, the CI of various products declined gradually: 1.5% SPL/GTN > 0.5% EPL/GTN > 1.5% EPL/GTN > 1.0% EPL/GTN > 1.0% SPL/GTN > 0.5% SPL/GTN > 2.0% GTN. It could be concluded that the stability of SPL/GTN-stabilized emulsion with appropriate concentration was higher than that of SPL/GTN-stabilized emulsion at the same concentration.

3.3 Lipid oxidation in emulsions

Lipid hydroperoxide is the main oxidation product of lipid oxidation (Rontani & Belt, 2020). Therefore, changes in lipid hydrogen peroxide can reflect the lipid oxidation behavior of emulsions. Lipid oxidation of the emulsion was monitored by measuring the lipid hydrogen peroxide content during the storage of the emulsion for 15 days (Figure 6). Compared with pure fish oil, gelatin and phospholipid stabilized emulsion reduced lipid peroxidation value. This suggested that the emulsion form can delay the oxidation of oils, which is consistent with previous work (Shen et al., 2014). The effect of lipid hydrogen peroxide content during storage of emulsion at room temperature was as follows: 1.5% EPL/GTN < 1.5% SPL/GTN < 1.0% EPL/GTN < 1.0% SPL/GTN < 0.5% EPL/GTN < 0.5% SPL/GTN < 2.0% GTN < pure oil. Therefore, EPL had higher antioxidant capacity for emulsions than SPL and gelatin at room temperature. Furthermore, lipid hydrogen peroxide content decreased with increasing PL concentration.

PL is an important emulsifier in food development, PL also has antioxidant activity (Rokosik et al., 2020; Biswas et al., 2021). Recent studies have found that the synergistic phenomenon of PL and tocopherol at the micellar interface layer can eliminate the detrimental effect of auto-oxidative products on antioxidant effectiveness (Biswas et al., 2021). Therefore, PL might act as an antioxidant in GTN-stabilized emulsions (in this work). Antioxidant effect was positively correlated with the concentration of phospholipids. It was reasonably proposed that the hydrophilic part of the emulsion interfacial layer was larger than the hydrophobic part due to the relative hydrophilicity of the gelatin molecules. Therefore, the gelatin molecules in the hydrophilic part of the emulsion interface layer might be much lower than the phospholipid molecules. Considering that the antioxidant capacity depended not only on the phospholipid

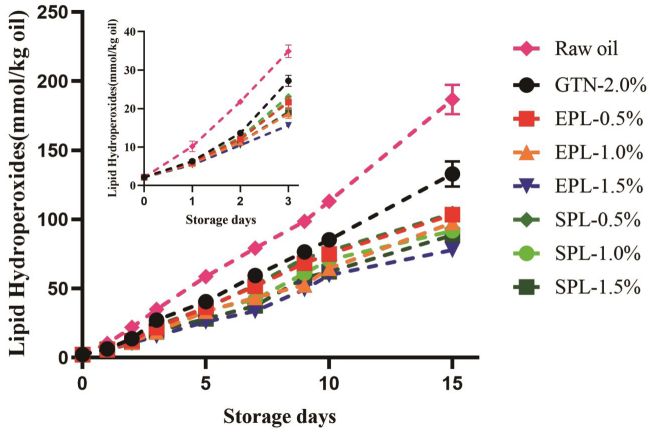


Figure 6. Lipid peroxidation values of fish oil-loaded PL/GTN-stabilized emulsions. EPL: egg yolk phospholipid; SPL: soy bean phospholipid; GTN: gelatin.

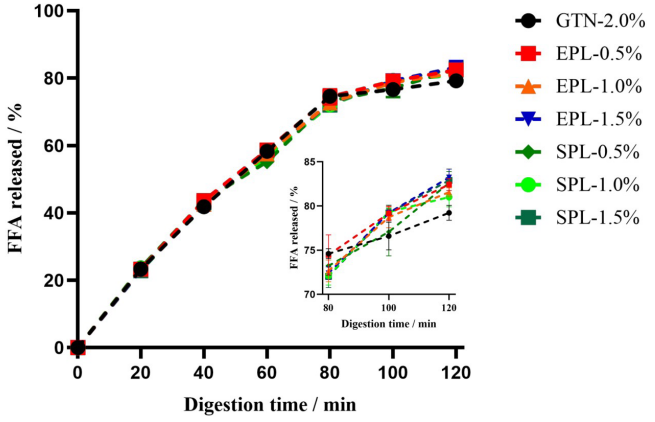


Figure 7. Simulates the release of free fatty acids (FFA) during intestinal processes. EPL: egg yolk phospholipid; SPL: soy bean phospholipid; GTN: gelatin.

molecules of the interface layer, but also on the unsaturation of the phospholipid molecules (Li et al., 2022a), it is necessary to pay attention to explore the phospholipid molecular structure and oxidation kinetics in the follow-up research. It was worth noting that even a low concentration of EPL could achieve a lipid oxidation inhibition rate of more than 50.0%, suggesting that EPL might be a suitable antioxidant choice for GTN-stabilized emulsions stored at room temperature to achieve a low-carbon economy.

3.4 In vitro digestion of emulsions

Lipid digestion is an interfacial process because lipases, bile acids, microorganisms, etc. must be adsorbed on the surface of oil droplets to catalyze lipid hydrolysis (Cheng et al., 2022; Li et al., 2022b; Huang et al., 2020). Therefore, the size and aggregation state of the droplets are important factors that have a great influence on the bioacceptability of omega-3 PUFAs (Infantes-Garcia et al., 2022; Udomrati et al., 2020). Fatty acid release from GTN-stabilized emulsions was analyzed by PL type and concentration in digestive systems (SSF, simulated gastric fluid (SGF), and simulated intestine fluid (SIF) that simulate the human gastrointestinal tract. The FFA released ratio during the SIF process increased with time, which was consistent with GTN-stabilized emulsions (Xu et al., 2022). 80.3%-82.3% of free fatty acids were released from PL-GTN-stabilized emulsions after 120 min of the SIF process (Figure 7). 2.0% GTN-stabilized emulsions had a lower fatty acid release rate ($P > 0.05$). The results suggested that PL-GTN might be an ideal choice for effective delivery of fish oil to the intestine compared to gelatin. EPL and SPL increased the release of FFA from the emulsions. At the process of SIF, the effect was enhanced by an increase in PL concentration after 120 min of action. Based on the above analysis, the stability of the emulsion droplets in the SIF phase might be the main factor influencing the release of FFA (Figure 8). This also suggested that phospholipids may be an effective measure to load fish oil and improve its fatty acid release rate and bioaccessibility.

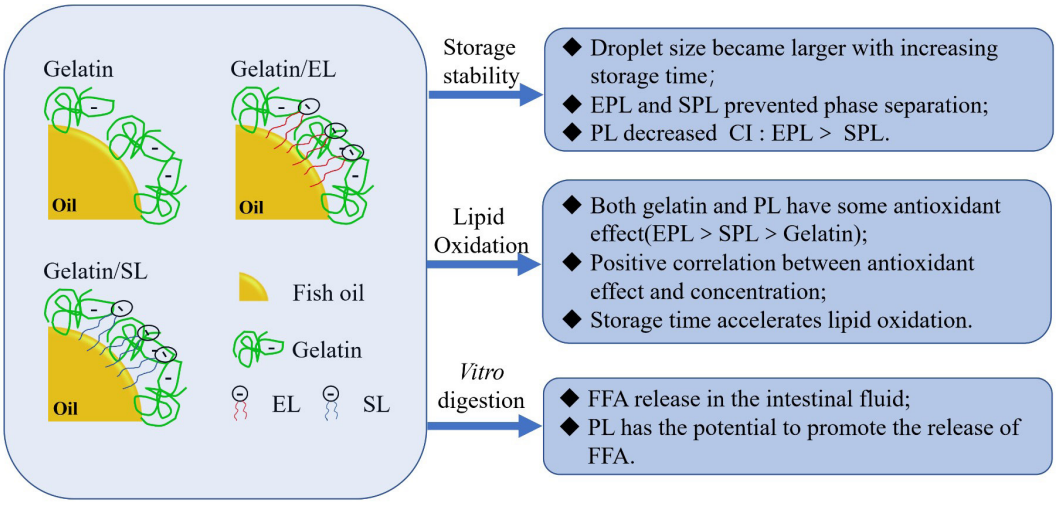


Figure 8. Schematic illustration of the applied mechanism for the stability of fish oil PL/GTN stabilized emulsion and the release of fatty acids from in vitro digestion. EPL: egg yolk phospholipid; SPL: soy bean phospholipid; GTN: gelatin.

4 Conclusion

In this study, the effects of different concentrations of EPL and SPL on the droplets and emulsion stability of fish oil-loaded PL/GTN-stabilized emulsions were studied under room temperature storage. These results indicated that the fish oil-loaded PL/GTN-stabilized emulsion exhibited different emulsification, liquid-gel phase transition, droplet coalescence behaviors and lipid oxidation during storage for 15 days at room temperature. These differences the way they coexisted with GTN at the oil/water interface (synergistic or competitive adsorption). In addition, the same 1.0% PL/GTN-stabilized emulsions had more stable emulsion droplets than the 0.5% and 1.5% PL/GTN-stabilized emulsions. PL/GTN-stabilized emulsions reduced lipid oxidation and increased the rate of fatty acid release. The droplets of the SPL/GTN-stabilized emulsions of the same concentration were more diverse than those of the EPL/GTN-stabilized emulsions. This study provided ideas for the preparation and industrialization of stabilized functionalized emulsions.

Conflict of interest

The authors declare no conflict of interest.

Funding

This study was supported by grant from National Key R&D Program of China (2018YFD0901006).

References

Ashfaq, W., Rehman, K., Siddique, M., & Khan, Q. (2020). Eicosapentaenoic acid and docosahexaenoic acid from fish oil and their role in cancer research. *Food Reviews International*, 36(8), 795-814. http://dx.doi.org/10.1080/87559129.2019.1686761.

Beltrán, J., Sandoval-Cuellar, C., Bauer, K., & Quintanilla-Carvajal, M. (2019). In-*vitro* digestion of high-oleic palm oil nanoliposomes prepared with unpurified soy lecithin: physical stability and nanoliposome digestibility. *Colloids & Surfaces A: Physicochemical and Engineering Aspects*, 578, 123603. http://dx.doi.org/10.1016/j.colsurfa.2019.123603.

Biswas, S., Mukherjee, P. K., Kar, A., Bannerjee, S., Charoensub, R., & Duangyod, T. (2021). Optimized piperine-phospholipid complex with enhanced bioavailability and hepatoprotective activity. *Pharmaceutical Development and Technology*, 26(1), 69-80. http://dx.doi.org/10.1080/10837450.2020.1835956. PMid:33059499.

Casoli, A., Isca, C., Iasio, S., Botti, L., Iannuccelli, S., Residori, L., Ruggiero, D., & Sotgiu, S. (2014). Analytical evaluation, by GC/MS, of gelatine removal from ancient papers induced by wet cleaning: a comparison between immersion treatment and application of rigid Gellan gum gel. *Microchemical Journal*, 117, 61-67. http://dx.doi.org/10.1016/j.microc.2014.06.011.

Cengiz, A., Kahyaoglu, T., Schröen, K., & Berton-Carabin, C. (2019). Oxidative stability of emulsions fortified with iron: the role of liposomal phospholipids. *Journal of the Science of Food and Agriculture*, 99(6), 2957-2965. http://dx.doi.org/10.1002/jsfa.9509. PMid:30471119.

Chambi, H., & Grosso, C. (2011). Effect of surfactants on the functional properties of gelatin-polysaccharide-based films. *European Food Research and Technology*, 232(1), 63-69. http://dx.doi.org/10.1007/s00217-010-1361-0.

Chen, S., Shen, X., Tao, W., Mao, G., Wu, W., Zhou, S., Ye, Y., & Pan, H. (2020). Preparation of a novel emulsifier by self-assembling of proanthocyanidins from Chinese bayberry (*Myrica rubra Sieb. et Zucc.*) leaves with gelatin. *Food Chemistry*, 319, 126570. http://dx.doi.org/10.1016/j.foodchem.2020.126570. PMid:32172049.

Cheng, L., Ye, A., Hémar, Y., & Singh, H. (2022). Modification of the interfacial structure of droplet-stabilised emulsions during in vitro dynamic gastric digestion: impact on in vitro intestinal lipid digestion. *Journal of Colloid and Interface Science*, 608(Pt 2), 1286-1296. http://dx.doi.org/10.1016/j.jcis.2021.10.075. PMid:34758419.

Encina, C., Vergara, C., Giménez, B., Oyarzún-Ampuero, F., & Robert, P. (2016). Conventional spray-drying and future trends for the microencapsulation of fish oil. *Trends in Food Science & Technology*, 56, 46-60. http://dx.doi.org/10.1016/j.tifs.2016.07.014.

Gao, Y., Li, J., Chang, C., Wang, C., Yang, Y., & Su, Y. (2019). Effect of enzymatic hydrolysis on heat stability and emulsifying properties of egg yolk. *Food Hydrocolloids*, 97, 105224. http://dx.doi.org/10.1016/j.foodhyd.2019.105224.

Güner, M., Yılmaz, E., & Yüceer, Y. (2019). Off-odor removal from fish oil by adsorbent treatment with selected metal-organic frameworks. *Flavour and Fragrance Journal*, 34(3), 163-174. http://dx.doi.org/10.1002/ffj.3489.

Hashim, N. A., Mudalip, S. K. A., Sulaiman, S. Z., & Shaarani, S. (2021). Nutritional values and microencapsulation techniques of fish oil from different sources: a mini review. *Materials Today: Proceedings*, 42(Pt 1), 222-228.

Hoffmann, H., & Reger, M. (2014). Emulsions with unique properties from proteins as emulsifiers. *Advances in Colloid and Interface Science*, 205, 94-104. http://dx.doi.org/10.1016/j.cis.2013.08.007. PMid:24161225.

Huang, Z., Brennan, C. S., Mohan, M. S., Stipkovits,, L., Zheng, H., Kulasiri, D., Guan, W., Zhao, H., & Liu, J. (2020). Milk lipid in *vitro* digestibility in wheat, corn and rice starch hydrogels. *International Journal of Food Science & Technology*, 55(11), 3361-3371. http://dx.doi.org/10.1111/ijfs.14607.

Hubbe, M. A., McLean, D. S., Stack, K. R., Lu, X., Strand, A., & Sundberg, A. (2020). Self-assembly of alkyl chains of fatty acids in papermaking systems: a review of related pitch issues, hydrophobic sizing, and pH effects. *BioResources*, 15(2), 4591-4635. http://dx.doi.org/10.15376/biores.15.2.Hubbe.

Infantes-Garcia, M. R., Verkempinck, S. H. E., Castillo-Santaella, T., Maldonado-Valderrama, J., Hendrickx, M. E., & Grauwet, T. (2022). In vitro gastric lipid digestion of emulsions with mixed emulsifiers: correlation between lipolysis kinetics and interfacial characteristics. *Food Hydrocolloids*, 128, 107576. http://dx.doi.org/10.1016/j.foodhyd.2022.107576.

Jiang, S., Zhang, T. T., Cong, P. X., Xu, J., Xue, C. H., Jiang, X. M., & Wang, Y. M. (2020). Comparative study on the digestion and absorption characteristics of n-3 LCPUFA-enriched phospholipids in the form of liposomes and emulsions. *Food Research International*, 137, 109428. http://dx.doi.org/10.1016/j.foodres.2020.109428. PMid:33233110.

Johnson, R., Nawroth, T., Khoshakhlagh, P., Langguth, P., Schmueser, L., Hellmann, N., Decker, H., & Szekely, N. K. (2014). Amphotericin B microparticles 'AmbiShell' from phospholipid and gelatin: development and investigation by combined DLS and SANS resolves the core-shell structure. *European Journal of Lipid Science and Technology*, 116(9), 1167-1173. http://dx.doi.org/10.1002/ejlt.201400069.

Lam, R. S. H., & Nickerson, M. T. (2013). Food proteins: a review on their emulsifying properties using a structure-function approach. *Food Chemistry*, 141(2), 975-984. http://dx.doi.org/10.1016/j.foodchem.2013.04.038. PMid:23790876.

Li, J., Nan, J., Wu, H., Park, H. J., Zhao, Q., & Yang, L. (2022a). Middle purity soy lecithin is appropriate for food grade nanoliposome: preparation, characterization, antioxidant and anti-inflammatory ability. *Food Chemistry*, 389, 132931. http://dx.doi.org/10.1016/j.foodchem.2022.132931. PMid:35500405.

Li, S., Wu, L., Zhang, X., & Jiang, X. (2020). The structure of water bonded to phosphate groups at the electrified zwitterionic phospholipid membranes/aqueous interface. *Angewandte Chemie*, 132(16), 6689-6692. http://dx.doi.org/10.1002/ange.202000511. PMid:32011767.

Li, X., Huang, Q., Wang, X., Zhang, M., Quan, S., Geng, F., Chen, H., & Deng, Q. (2022b). Exploration of suitable in vitro simulated digestion model for lipid oxidation of flaxseed oil emulsion during digestion. *Journal of the Science of Food and Agriculture*, 102(12), 5495-5501. http://dx.doi.org/10.1002/jsfa.11904. PMid:35355275.

Lu, F., Nielsen, N., Baron, C., Jensen, L., & Jacobsen, C. (2012). Physico-chemical properties of marine phospholipid emulsions. *Journal of the American Oil Chemists' Society*, 89(11), 2011-2024. http://dx.doi.org/10.1007/s11746-012-2105-z.

Malinowski, S. S., Barber, K. E., Kishk, O. A., Mays, A. A., Jones, S. R., Turner, A. L., & Riche, D. M. (2019). Effect of fish oil supplement administration method on tolerability and adherence: a randomized pilot clinical trial. *Pilot and Feasibility Studies*, 5(1), 3. http://dx.doi.org/10.1186/s40814-018-0387-0. PMid:30637118.

Mezzetta, A., Łuczak, J., Woch, J., Chiappe, C., Nowicki, J., & Guazzelli, L. (2019). Surface active fatty acid ILs: influence of the hydrophobic tail and/or the imidazolium hydroxyl functionalization on aggregates formation. *Journal of Molecular Liquids*, 289, 111155. http://dx.doi.org/10.1016/j.molliq.2019.111155.

Nasirpour-Tabrizi, P., Azadmard-Damirchi, S., Hesari, J., Khakbaz Heshmati, M., & Savage, G. P. (2020). Production of a spreadable emulsion gel using flaxseed oil in a matrix of hydrocolloids. *Journal of Food Processing and Preservation*, 44(8), 1-10. http://dx.doi.org/10.1111/jfpp.14588.

Nikonova, A. A., Shishlyannikov, S. M., Shishlyannikova, T. A., Avezova, T. N., Babenko, T. A., Belykh, O. I., Glyzina, O. Y., Obolkin, V. A., Pavlova, O. N., Smagunova, A. N., Sukhanova, E. V., Tikhonova, I. V., Khanaeva, T. A., & Khutoryansky, V. A. (2020). Determination of free and esterified fatty acids in hydrocoles of different content of polyunsaturated fatty acids by gas–liquid chromatography. *Journal of Analytical Chemistry*, 75(10), 1310-1321. http://dx.doi.org/10.1134/S1061934820100093.

Ramos, F. M., Silveira, V. Jr., & Prata, A. S. (2021). Impact of vacuum spray drying on encapsulation of fish oil: oxidative stability and encapsulation efficiency. *Food Research International*, 143, 110283. http://dx.doi.org/10.1016/j.foodres.2021.110283. PMid:33992383.

Rokosik, E., Siger, A., Rudzińska, M., & Dwiecki, K. (2020). Antioxidant activity and synergism of canolol and α-tocopherol in rapeseed oil is affected by the presence of phospholipid association colloids. *Lebensmittel-Wissenschaft + Technologie*, 133, 110095. http://dx.doi.org/10.1016/j.lwt.2020.110095.

Rontani, J.-F., & Belt, S. T. (2020). Photo- and autoxidation of unsaturated algal lipids in the marine environment: an overview of processes, their potential tracers, and limitations. *Organic Geochemistry*, 139, 103941. http://dx.doi.org/10.1016/j.orggeochem.2019.103941.

Shehzad, Q., Rehman, A., Jafari, S. M., Zuo, M., Khan, M. A., Ali, A., Khan, S., Karim, A., Usman, M., Hussain, A., & Xia, W. S. (2021). Improving the oxidative stability of fish oil nanoemulsions by co-encapsulation with curcumin and resveratrol. *Colloids and Surfaces B: Biointerfaces*, 199, 111481. http://dx.doi.org/10.1016/j.colsurfb.2020.111481. PMid:33360079.

Shen, Z., Bhail, S., Sanguansri, L., & Augustin, M. A. (2014). Improving the oxidative stability of krill oil-in-water emulsions. *Journal of the American Oil Chemists' Society*, 91(8), 1347-1354. http://dx.doi.org/10.1007/s11746-014-2489-z.

Udomrati, S., Pantoa, T., Gohtani, S., Nakajima, M., Uemura, K., & Kobayashi, I. (2020). Oil-in-water emulsions containing tamarind seed gum during in vitro gastrointestinal digestion: rheological properties, stability, and lipid digestibility. *Journal of the Science of Food and Agriculture*, 100(6), 2473-2481. http://dx.doi.org/10.1002/jsfa.10268. PMid:31960429.

Wardhani, D. H., Ulya, H. N., Kumoro, A. C., & Aryanti, N. (2021). Alginate modification for stabilizing fish oil emulsion. *IOP Conference Series: Materials Science and Engineering*, 1053, 012051. http://dx.doi.org/10.1088/1757-899X/1053/1/012051.

Xu, J., Huang, S., Zhang, Y., Zheng, Y., Shi, W., Wang, X., & Zhong, J. (2022). Effects of antioxidant types on the stabilization and in vitro digestion behaviors of silver carp scale gelatin-stabilized fish oil-loaded emulsions. *Colloids and Surfaces B: Biointerfaces*, 217, 112624. http://dx.doi.org/10.1016/j.colsurfb.2022.112624. PMid:35728370.

Yin, M., Zhang, L., Tang, S., Matsuoka, R., Xi, Y., Tao, N., & Wang, X. (2021). Egg yolk phospholipids modulate microbial imbalance in the intestinal tract of rats on a high-fructose diet. *European Journal of Lipid Science and Technology*, 124(3), 2100131.

Zhang, T., Ding, M., Tao, N., Wang, X., & Zhong, J. (2020a). Effects of surfactant type and preparation pH on the droplets and emulsion forms of fish oil-loaded gelatin/surfactant-stabilized emulsions. *LWT*, 117, 108654. http://dx.doi.org/10.1016/j.lwt.2019.108654.

Zhang, T., Ding, M., Zhang, H., Tao, N., Wang, X., & Zhong, J. (2020b). Fish oil-loaded emulsions stabilized by synergetic or competitive adsorption of gelatin and surfactants on oil/water interfaces. *Food Chemistry*, 308, 125597. http://dx.doi.org/10.1016/j.foodchem.2019.125597. PMid:31648095.

Züge, L. C. B., Maieves, H. A., Silveira, J. L. M., Silva, V. R., & Scheer, A. P. (2017). Use of avocado phospholipids as emulsifier. *LWT*, 79, 42-51. http://dx.doi.org/10.1016/j.lwt.2017.01.013.

Supplementary Material

Supplementary material accompanies this paper.

Table S1. The composition and content of egg yolk phospholipids (EPL) and soybean phospholipids (SPL) (%).

This material is available as part of the online article from https://doi.org/10.1590/fst.84622