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Vitamin D and E-loaded Pickering emulsion stabilized with the insoluble fraction of Persian gum and polyphenols: chemical stability and in vitro gastrointestinal digestion

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Vitamin D and E-loaded Pickering emulsion stabilized with the insoluble fraction of Persian gum and polyphenols: chemical stability and *in* vitro gastrointestinal digestion

Abstract

The present study examines the oxidative stability and digestion behavior of vitamin D_3 and E (α-tocopherol)-loaded flaxseed oil-in-water Pickering emulsions (PEs) stabilized with the insoluble fraction of Persian gum (IFPG) and polyphenolic compounds (quercetin and curcumin). The results of the oxidative stability measurements, which monitored changes in the levels of primary (lipid hydroperoxide, LH) and secondary (malondialdehyde, MDA) oxidation products, showed that IFPG-stabilized PEs were significantly more stable than non-emulsified oil during a 28-day storage period under both dark and light conditions. The incorporation of polyphenolics further enhanced the oxidative stability of the emulsions. Additionally, loading D_3 and E vitamins into PEs reduced their degradation rate, with retention levels exceeding 80% after 28 days of storage. The presence of polyphenols also decreased the degradation rate of the vitamins, resulting in higher retention, potentially at the expense of their decomposition. *In vitro* gastrointestinal digestion studies demonstrated that IFPG-stabilized emulsions maintained their structural integrity, modulating the degree of lipolysis and bioaccessibility, with polyphenols playing a more significant role in this effect. Quantitatively, bioaccessibility of α-tocopherol and vitamin D_3 reached 29.4% and 58.9%, respectively, in PE systems, while lower values were observed in PECQ (18.1% and 26.5%), consistent with the reinforced interfacial structure and moderated lipolysis. These findings highlight the potential of IFPG-stabilized polyphenol-loaded Pickering emulsions as natural carriers for fat-soluble vitamins in functional foods, offering enhanced chemical protection, antioxidant activity, and controlled release, particularly when combined with polyphenols.

Keywords: Insoluble fraction of Persian gum, Pickering emulsion, *In vitro* digestion, Fat-soluble vitamins, Chemical stability.

1. Introduction

Food manufacturers are increasingly focused on developing functional foods enriched with bioactive compounds to meet consumer demand for health-promoting and clean-label products [1,2]. However, the incorporation of lipophilic nutraceuticals into aqueous food systems remains challenging due to their poor water solubility, chemical instability, and low bioavailability. As a result, the development of effective and natural delivery systems for fat-soluble bioactives has attracted considerable research interest. Vitamin D and vitamin E are essential fat-soluble vitamins with well-established roles in bone health, immune function, antioxidant defense, and

prevention of chronic diseases [3]. Despite their physiological importance, both vitamins suffer from limited stability and bioavailability when incorporated directly into food matrices. Vitamin D, particularly vitamin D_3, is highly sensitive to light, heat, and acidic conditions, while vitamin E is prone to oxidative degradation and exhibits limited gastrointestinal absorption due to its high lipophilicity. These limitations significantly restrict their application in functional food formulations.

Emulsion-based delivery systems have been widely explored to improve the dispersibility, stability, and bioaccessibility of lipophilic compounds [4]. Among them, Pickering emulsions (PEs) stabilized by solid particles have gained increasing attention due to their superior physical stability, reduced need for synthetic surfactants, and strong interfacial adsorption. Solid particles form a rigid and thick interfacial layer around oil droplets, which limit droplet coalescence and protects encapsulated bioactives from environmental stresses and oxidative degradation [5]. Consequently, oil-in-water PEs represent a promising clean-label strategy for delivering fat-soluble vitamins [6].

Recently, natural biopolymer-based particles have been extensively investigated as Pickering stabilizers. In this context, the insoluble fraction of Persian gum (IFPG) has demonstrated excellent potential for stabilizing PEs due to its partial wettability, intrinsic anionic nature, and anisotropic structure. IFPG particles irreversibly adsorb at the oil–water interface, imparting electrostatic repulsion and enhanced capillary forces, which collectively improve emulsion stability. These properties make IFPG a promising natural stabilizer for food-grade PEs [7,8].

From a nutritional perspective, flaxseed oil is a rich source of long-chain ω-3 polyunsaturated fatty acids, which are associated with cardiovascular, cognitive, and anti-inflammatory benefits. However, its high unsaturation degree renders it susceptible to oxidative degradation [9]. The incorporation of polyphenolic compounds alongside flaxseed oil can enhance oxidative stability while providing additional health benefits, making such systems attractive for multifunctional food applications.

Despite significant advancements in the field, there is a lack of research on the oxidative stability, digestibility and bioaccessibility of co-emulsified fat-soluble vitamins and essential fatty acids in colloidal particles-stabilized PEs. It is particularly important to study the simultaneous delivery of vitamins D and E, as they have complementary physiological roles and vitamin E acts as and antioxidant to protect lipid components and vitamin D_3 from oxidative degradation. Therefore, the purpose of this study was to develop flaxseed oil-in-water PEs stabilized by IFPG particles for the co-emulsification of vitamins D and E, and to systematically evaluate their chemical stability, oxidative resistance, and behavior during *in vitro* gastrointestinal digestion behavior. This study introduces a new, novel clean-label IFPG-based Pickering system and provides valuable insights into the co-delivery performance of lipid-soluble vitamins in functional food applications.

2. Materials and Methods

2.1. Materials

Persian Gum (PG) was purchased from a local market and the flaxseed oil (fatty acid composition: 51.5% 18 C:3, 16.9% 18 C:2, 19.7% 18 C:1, 6.1% 16 C:0, and 5.1% C18:0) was extracted from dry flaxseed through cold pressing. Quercetin, curcumin, mucin (from porcine stomach, M2378), pepsin (from porcine gastric mucosa, P7125), bile-extract porcine, lipase (from porcine pancreas, L3126, with an enzymatic activity of 100−500 units/mg protein), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous sulfate, and ammonium thiocyanate were provided from Sigma-aldrich. α-tocopherol (95%), and vitamin D_3 (99%) were provided by fisher scientific company.

2.2. Preparation of IFPG-stabilized Pickering emulsions

First, the IFPG aqueous solution was prepared at a constant concentration of 2.5% (w/w) and sheared by Ultra Turrax at 24000 rpm for 5 min. The Pickering emulsions were prepared by mixing the IFPG dispersion with flaxseed oil (FO) at a mass fraction of 20% w/w. Quercetin and curcumin (at a ratio of 1:1, 0.5 mg/g oil) were added to 20% w/w oil phase (flaxseed oil+ α-tocopherol; 2% w/w+ vitamin D_3; 0.1% w/w). In brief, the mixture was sheared with a high-speed rotor stator mixer (Ultra Turrax, T25, IKA Werke, Germany) at 24000 rpm for 10 min. The coarse PEs were further homogenized using a microfluidizer (M110S, Microfluidics, USA) at a pressure of 280 bar for 1 min under continuous recirculation mode, corresponding to multiple passes, to obtain fine emulsions. The IFPG-stabilized PEs -with and without polyphenols- were stored in capped plastic jars for 28 days at ambient temperature and evaluated every 7 days to ascertain the content of encapsulated vitamins, polyphenols as well as chemical stability.

2.3. Lipid oxidation measurements

The protection effects of IFPG stabilized PEs against the lipid oxidation were studied through the primary and secondary lipid oxidation products. For this purpose, 10 g of PEs were placed in screw-cap plastic tubes and incubated at the ambient temperature under light as well as a dark place for up to 28 days. Samples were sporadically collected in order to track changes in the lipid hydroperoxide (LH) and malondialdehyde (MDA), as the sign of primary and secondary products of lipid oxidation, respectively. IFPG-stabilized PEs containing fat-soluble components, with or without phenolic compounds, and the bulk oil were compared to each other.

To determine LH, 0.3 mL samples was combined with 1.5 mL of isooctane/isopropanol (3:1 v/v) and then the samples were centrifuged at 10000 g for 5 min. Subsequently, 0.2 mL of supernatant was mixed with 2.8 mL of methanol/n-butanol mixture solution (1:2 v/v), 50 µL of 3.94 M ammonium thiocyanate, and 50 µL of ferrous iron solution (0.144 M FeSO4 and132 M BaCl2, 1:1). Samples were maintained in a dark place for 20 min, followed by reading their absorbance at 510 nm. Finally, the standard curve of hydrogen peroxide was used for determination of the LH content **[**10].

Using the thiobarbituric acid reactive substances test (TBARS), MDA in PEs was tracked. To that end, 0.1 g of sample was dispersed in 1.9 mL of deionized water. Next, 4.0 mL of TBA solution was added to the dispersion and the obtained solution was incubated in boiling water for 15 min. To prepare the TBA solution, 15 g of TCA and 0.375 g of TBA were dissolved in 100 mL of 0.125 M HCl. Sample was then centrifuged for 20 min at 4000 g, and the absorbance was read at 532 nm. The standard curve of 1,1,3,3-tetramethoxypropane was used to calculate the content of MDA **[**11]. According to the following schematic formula one molecule TEP can form one molecule MDA.

2.4. Stability determination of fat-soluble vitamins

A UV-visible technique was used to measure fat-soluble vitamins including α-tocopherol and D_3 in accordance with Walia & Chen, (2020). In order to determine the amount of free fat-soluble vitamins (α-tocopherol and D_3) present in the IFPG-stabilized PEs, 0.4 mL of the samples were combined with 3.6

mL of hexane, vortexed for 1 min, then centrifuged for 10 min at 8000 g to produce a distinct hexane layer. After gathering the supernatant, the spectrophotometer was used to measure the absorbance of D_3 and α-tocopherol at 270 and 290 nm, respectively. Utilizing a standard curve, the quantity of vitamins was calculated. On days 1, 7, 14, 21, and 28, the PEs were analyzed in triplicate while being stored at ambient condition at dark and under light. The maintained vitamins in the systems were determined using the following formula:

Embedding rate (%) = (total vitamin–free vitamin)/total vitamin × 100

2.5. Measurement of phenolic compounds retention

The quantity of quercetin and curcumin in IFPG-stabilized PEs was extracted using methanol, due to its high polarity and strong solubilizing capacity for both curcumin and quercetin, as well as its ability to efficiently disrupt the emulsion matrix and release encapsulated phenolic compounds (Huang et al., 2017; Lee et al., 2021; Zeng et al., 2017), and its concentration was measured at 369 nm and 424 nm, respectively, using an ultraviolet-visible (UV) spectrophotometer (UV-1600PC, VWR, PA, USA). Briefly, 100 µL samples were mixed with 1 mL methanol and then centrifuged at 13200 g for 10 min. Encapsulated polyphenols were identified as the quercetin or curcumin concentration in the supernatant using suitable standard calibration curves.

The following Equations were used to evaluate entrapment efficiency (EE).

EE (%) = (Weight of encapsulated phenols/ Total weight of phenols in sample) × 100

2.6. *In vitro* digestion studies

The *in vitro* gastrointestinal digestion studies were performed following the standardized INFOGEST protocol with slight modifications [14]. To prepare the simulated saliva fluid (SSF) for the mouth stage, 0.03 g/mL mucin was dissolved in distilled water at pH 6.8. 20 mL of SSF was preheated at 37 ℃ for 2 min and then mixed with 20 mL of initial PEs. The samples were incubated with continuous shaking (100 rpm) for 10 min at 37^{o}C.

For the gastric stage, simulated gastric fluid (SGF) was supplied by adding 0.2 g of NaCl and 0.32 g of pepsin to100 mL deionized water, and then the pH of fluid was adjusted to pH 2.5 using 1 M HCl. Next, prepared SGF was mixed with samples from oral digestion at a volume ratio of 1:1, followed by incubating at 37^{o}C for 2 h under continuous shaking to simulate the gastric digestion.

After simulated gastric phase, digested samples were mixed with simulated intestinal fluid (SIF) at the volume ratio of 1:1. SIF contained 20 mg/mL bile salt and 0.8 mg/mL lipase at pH 7. For small intestinal digestion, the samples were incubated at 37 ℃ for 2 h under continuous shaking.

The degree of lipolysis in Pickering emulsions was calculated by the amount of free fatty acids (FFA) released. It was supposed that when 1 mol of triglycerides is digested, 2 mol of FFA will be released and 2 mol of NaOH is required to neutralize the FFA **[**15]. The amount of FFA released was measured according to the following Equation.

$$FFA\ (\%) = \frac{V_{NaOH} \times m_{NaOH} \times MW_{TG}}{w_{TG} \times 2} \times 100$$

Where V_{NaOH} stands for the volume of NaOH consumed, m_{NaOH} considered as the molarity of NaOH, MW_{TG} and w_{TG} were the average molecular mass ($C_{18}H_{34}O_2$, 282.46 g/mol) and total mass of flaxseed oil.

2.6.1. Microstructure observation

The appearance microstructure of the PEs was assessed using optical microscopy. For this purpose, samples were diluted with distilled water 10-fold. Next, small aliquots (10 μL) of the diluted PEs were placed on a glass slide and covered with a glass slip, and the images were observed with a 100×objective lens.

2.7. Bioaccessibility determination of fat-soluble vitamins

The percentage of vitamin present in the mixed micelle phase relative to the digesta is known as the bioaccessibility [16]. 10 mL of samples resulted from the simulated small intestinal digestion were centrifuged for 30 min at 8000 g at room temperature. After centrifugation, the samples were separated into two layers: an opaque sediment layer at the bottom and a clear yellow micelle layer at the top. To extract the vitamins, 2 mL of the clear yellow color micelle layer was mixed with a 10 mL solution of isooctane and ethyl alcohol (1:3), vortexed, and then centrifuged for an additional 20 min at 2000 g. The clear supernatant was collected and the content of fat-soluble components was recorded by using a UV-Vis spectrophotometer at 270 nm and 290 nm for vitamin D_3 and α-tocopherol, respectively. The bio-accessibility of fat-soluble components was calculated according to the following Equation.

Bio-accessibility (%) = Content of fat-soluble vitamins in supernatant/ Content of fat-soluble vitamins in the digesta × 100

2.8. Statistical analysis

All experiments were carried out in triplicate and experimental data were expressed as mean ± standard deviation (SD). All results have been analyzed by Minitab 17.0 for variance analysis and significance among data was defined at $p < 0.05$.

3. Results and Discussion

3.1. Lipid Oxidation

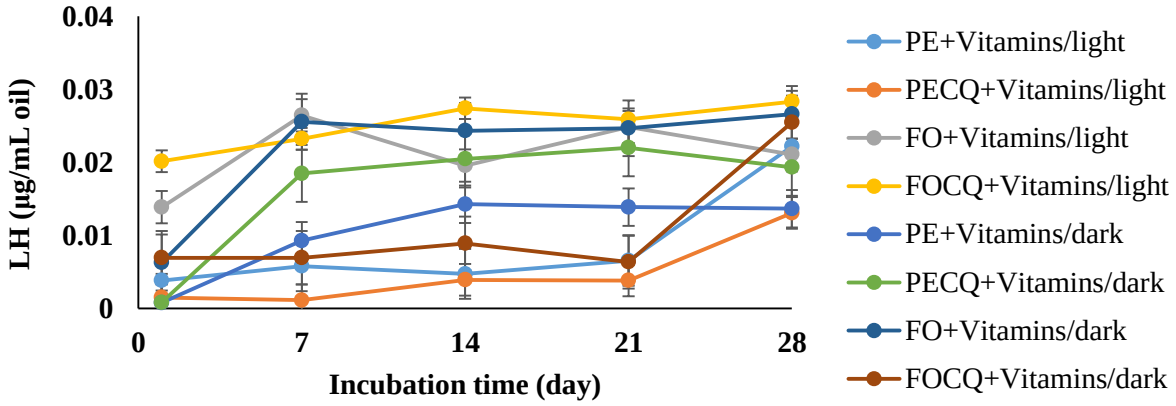
Lipid oxidation is a major factor limiting the quality and shelf life of lipid-based food systems. To evaluate the oxidative stability of flaxseed oil-in-water PEs stabilized by IFPG, primary and secondary oxidation products, namely lipid hydroperoxides (LH) and malondialdehyde (MDA), were monitored during 28 days of storage in the presence and absence of polyphenols. As shown in Fig. 1a, LH values of all samples remained below 0.04 μg/mL oil throughout storage, which is substantially below the commonly accepted oxidation threshold of 10 mEq/kg oil [17]. IFPG-stabilized PEs exhibited significantly lower LH formation compared with bulk flaxseed oil (FO), indicating the protective role of the particle-stabilized interfacial layer. Among the emulsions, polyphenol-loaded PEs (PECQ) showed the lowest LH levels after 28 days, demonstrating superior resistance to primary oxidation.

Secondary oxidation was evaluated by measuring MDA formation using the TBARS assay (Fig. 1b). A gradual increase in MDA concentration was observed in all samples during storage, following trends similar to those observed for LH. After 28 days, MDA levels increased from 2.9 to 13.5 μmol/L oil in PE and from 3.1 to 9.7 μmol/L oil in PECQ, whereas higher values were observed in bulk flaxseed oil (FO: 15.2 μmol/L oil) and FO containing polyphenols (FOCQ: 14.8 μmol/L oil). These results indicate that emulsification markedly retarded lipid oxidation, and that the incorporation of quercetin and curcumin further enhanced oxidative stability. The lower MDA content in IFPG-stabilized PEs compared with bulk oil can be attributed to the formation of a rigid and thick interfacial particle layer, which limits the diffusion of oxygen and pro-oxidants into the oil phase. The additional reduction in oxidation observed in PECQ is likely due to the antioxidant activity of polyphenols, which can scavenge free radicals and

inhibit the decomposition of hydroperoxides into secondary oxidation products. The interaction between IFPG particles and polyphenols at the water-oil interface results in the accumulation of these antioxidants in this region leading to the formation of a protecting barrier scavenging oxygen, transition metals, and other pro-oxidants, thereby preventing them from diffusing into the oil phase, a mechanism which is probably missing in the bulk oil (FOCQ). This efficiently reduces the rate of lipid peroxidation and thus the generation of primary and secondary oxidation products.

Moreover, possible interactions between IFPG particles and polyphenols at the oil–water interface may lead to the formation of a more compact interfacial network, further restricting mass transfer of pro-oxidants. The increased viscosity induced by polyphenol incorporation may also contribute to reduced diffusion of reactive species [18]. Consistent with previous studies, curcumin- and quercetin-containing emulsions exhibited enhanced oxidative stability compared with polyphenol-free systems [12,13]. Storage under dark conditions resulted in lower oxidation levels compared with light exposure, confirming the light sensitivity of PUFA-rich flaxseed oil systems. Overall, the lowest MDA content was observed in PECQ, while the highest oxidation occurred in bulk FO, highlighting the synergistic protective effect of IFPG-based Pickering stabilization and polyphenol co-encapsulation.

a)



b)

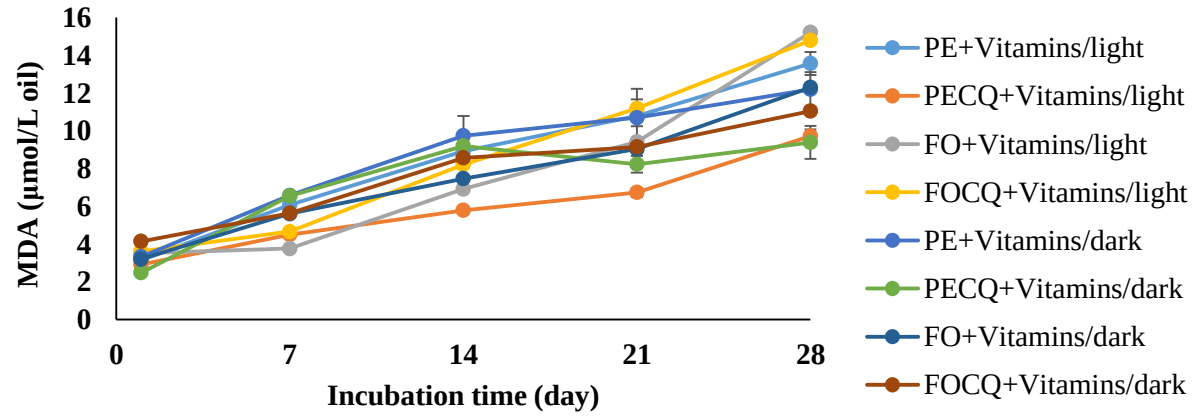


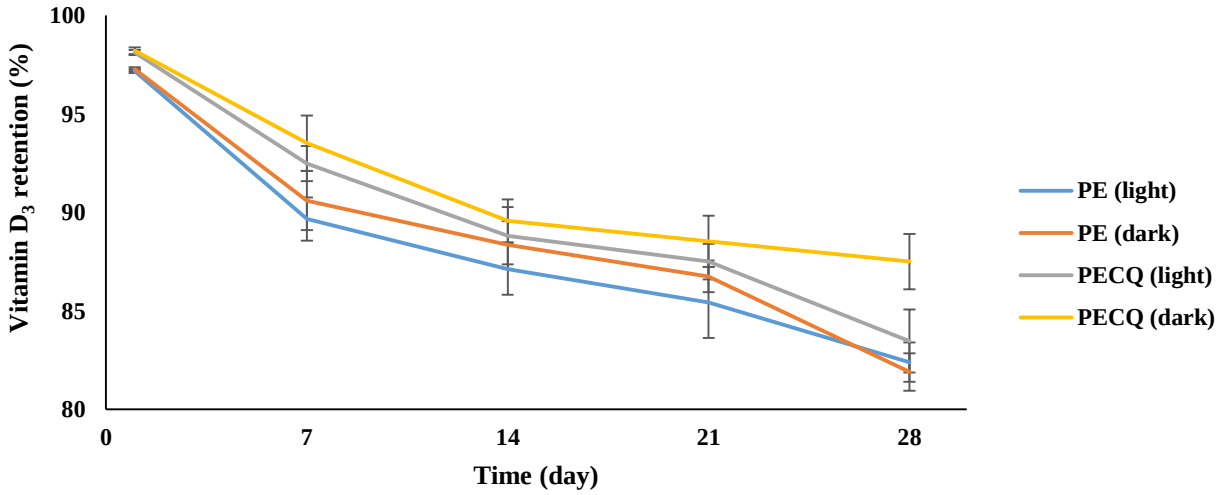
Fig. 1. Time evolution of LH (a) and MDA (b) in Pickering emulsions stabilized with IFPG particles, either alone (PE) or in combination with a mixture of curcumin and quercetin (PECQ), as well as in the bulk oil (FO) during incubation at ambient temperature under light and dark conditions.

3.2. Stability of Fat-Soluble Vitamins

The stability of fat-soluble vitamins encapsulated within IFPG-stabilized PEs was evaluated by monitoring vitamin retention during storage under light and dark conditions (Fig. 2). Although a slight decrease in vitamin content was observed in all formulations over time, more than 85% of the encapsulated vitamins were retained in both PE and polyphenol-loaded PE (PECQ) throughout the storage period. Notably, PECQ consistently exhibited higher vitamin retention than IFPG-stabilized emulsions without polyphenols, indicating an additional protective role of quercetin and curcumin. The encapsulation efficiency of vitamin D_3 was particularly high, reaching approximately 97% and 98% in PE and PECQ, respectively (Fig. 2a), demonstrating the strong affinity of fat-soluble vitamins for the oil phase and the effectiveness of IFPG particles in forming a stable interfacial barrier. Specifically, α-tocopherol exhibited excellent retention (>88%) over 28 days in both PE and PECQ, highlighting the strong protective effect of IFPG-stabilized emulsions and the additional benefit of polyphenol incorporation (Fig. 2b).

The enhanced stability of vitamins in IFPG-stabilized PEs can be attributed to multiple protective mechanisms. The vitamins were confined within the oil phase, thereby limiting direct exposure to environmental stressors such as light and oxygen. Furthermore, the densely packed IFPG particle layer at the oil–water interface acted as a physical shield, reducing mass transfer and photodegradation reactions. Storage under dark conditions resulted in higher vitamin retention compared with light exposure, confirming the light sensitivity of fat-soluble vitamins. The presence of polyphenols further improved vitamin stability, likely due to their antioxidant activity and potential interactions with IFPG particles at the interface, which may reinforce the interfacial structure and suppress oxidative degradation pathways. Similar stabilizing effects of particle-based and biopolymer-stabilized delivery systems on vitamin retention have been reported previously [16,19,20], supporting the suitability of IFPG-based PEs as efficient carriers for fat-soluble vitamins in functional food applications.

a)



b)

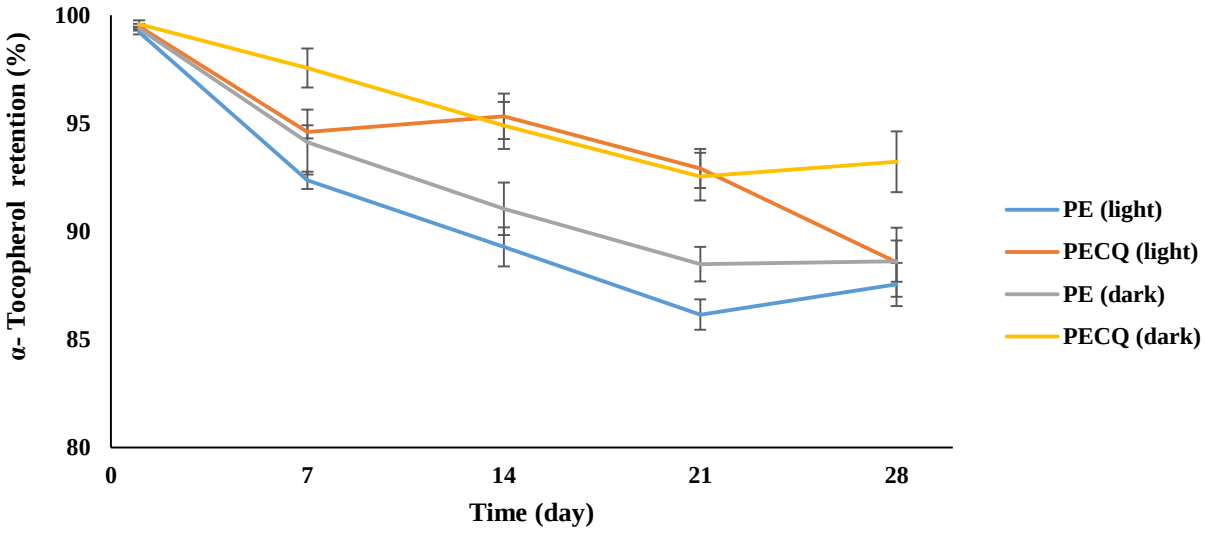


Fig. 2. Changes in the retention percentage of vitamin D_3 and α-tocopherol in Pickering emulsions stabilized with IFPG particles, either alone (PE) or in combination with a mixture of curcumin and quercetin (PECQ), during storage at ambient temperature under light and dark conditions.

3.3. Entrapment Efficiency of Polyphenols

The entrapment efficiency and stability of curcumin and quercetin in IFPG-stabilized PEs were evaluated during 28 days of storage under light and dark conditions. A day after preparation, high retention of both polyphenols was observed in PECQ stored in the dark, with residual ratios of approximately 95% for curcumin and 97% for quercetin. As storage progressed, polyphenol retention gradually decreased, with a pronounced effect of light exposure (Fig. 3). After 28 days, the retained amounts of curcumin and quercetin in light-exposed samples declined to approximately 45% and 62%, respectively, indicating the photosensitive nature of these phenolic compounds. In contrast, samples stored in the dark maintained slightly higher encapsulation efficiencies.

The lower retention of curcumin compared to quercetin can be attributed to its unique conjugated structure, which is comprised of two methoxy phenols and an enol form of β-diketone, enabling it to act as a more potent antioxidant and hence more efficiently trapping free radicals and breaking chain reactions which in turn leads to a significantly higher reduction in its content during storage **[**21]. However, as highlighted above the protective capacity of PEs markedly prevented the oxidative degradation of polyphenols as well as the emulsified FO. This is mainly due to IFPG particles that formed a dense and rigid interfacial layer around the oil droplets, which limited the diffusion of oxygen and other pro-oxidants and thus slowed down the rate of oxidation reactions. However, partial degradation of polyphenols during storage may be associated with their antioxidant role within the system, as they actively scavenge free radicals generated during lipid oxidation and vitamin degradation. Moreover, the co-presence of curcumin and quercetin may provide synergistic antioxidant effects **[**22], enhancing overall oxidative protection while gradually consuming the phenolic compounds themselves. These results demonstrate that IFPG-stabilized PEs offer effective encapsulation and controlled retention of polyphenols, with storage conditions playing a critical role in preserving their functional integrity.

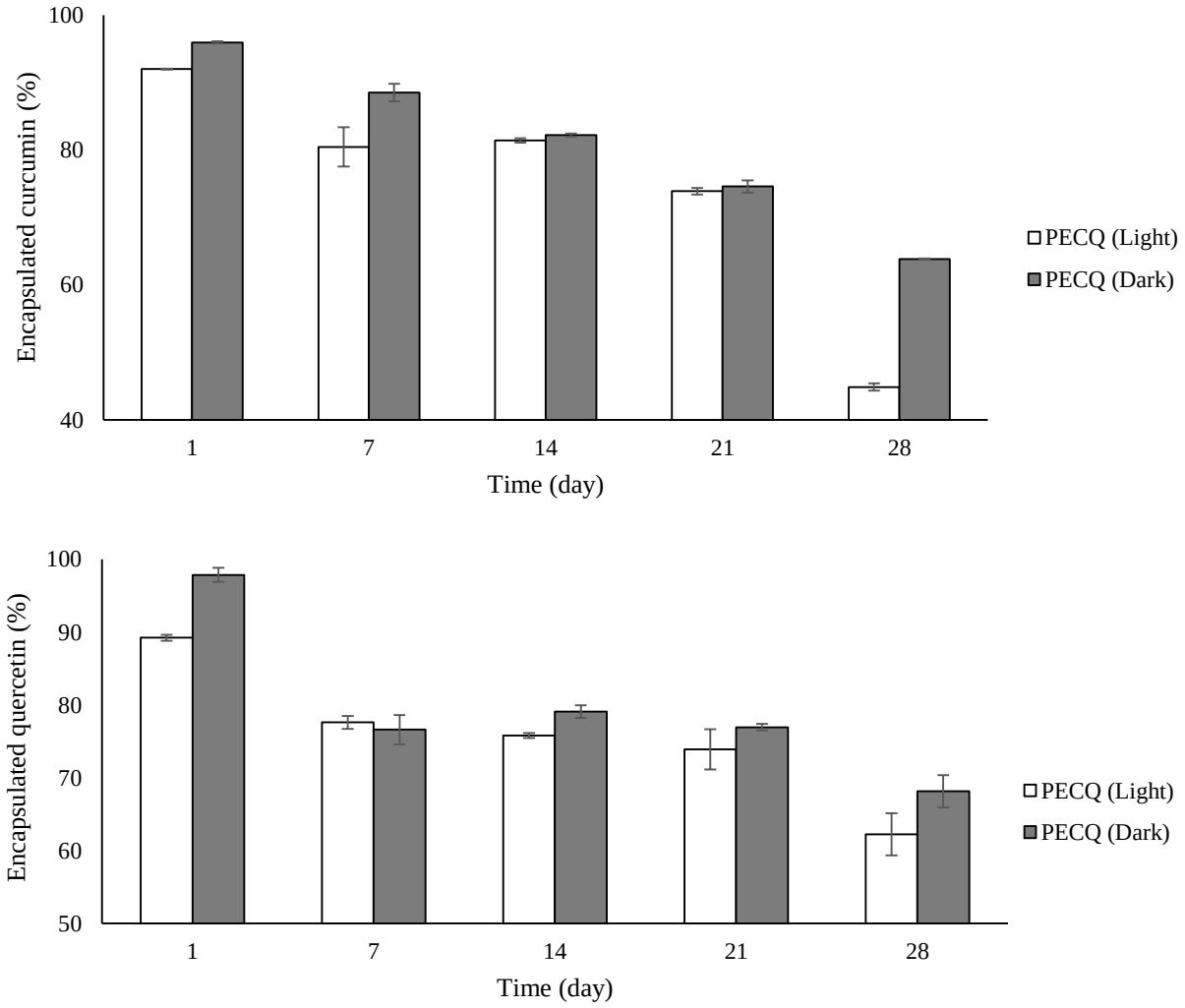


Fig. 3. Changes in the encapsulated curcumin and quercetin content (%) of IFPG-stabilized Pickering emulsions (PECQ) during storage at ambient temperature under light and dark conditions.

3.4. Effect of polyphenols on *In Vitro* Digestion Behavior of IFPG-Stabilized Pickering Emulsions

The development of nanoscale and colloidal delivery systems has attracted considerable attention as an effective strategy to enhance the bioavailability of lipophilic bioactives. However, the fate and functionality of such systems under gastrointestinal conditions remain a critical concern **[**23]. During digestion, emulsions are exposed to dynamic physicochemical environments involving enzymatic activity, pH variation, and mechanical stresses, which can significantly alter droplet structure and interfacial properties [24]. Therefore, monitoring changes in structure is essential for understanding digestion behavior and lipid bioaccessibility. In the present study, IFPG-stabilized PEs were investigated as potential carriers for fat-soluble vitamins under simulated oral, gastric, and intestinal conditions.

The digestion behavior of PEs co-loaded with vitamin D_3 and α-tocopherol was first examined under simulated oral conditions. Microstructural observations revealed a noticeable increase in droplet size after the mouth phase (Fig. 4), indicating partial droplet aggregation. This phenomenon is likely associated with the presence of salivary mucin, which can induce flocculation through depletion and bridging

mechanisms. Interestingly, a higher mucin concentration than that typically found in human saliva was required to trigger pronounced aggregation in the in vitro model. This suggests that salivary components other than mucin may also contribute to emulsion destabilization through yet unidentified mechanisms. Despite this initial aggregation, the emulsions maintained their overall integrity, highlighting the resistance of the IFPG-based interfacial layer to mild oral conditions.

Upon transition to simulated gastric conditions, further droplet aggregation was observed. However, the droplets maintained their integrity. In the intestinal phase, emulsions encountered bile salts, pancreatic lipase, and elevated pH and ionic strength, leading to more pronounced structural transformations. Bile salts are known to displace surface-active materials from droplet interfaces, facilitate lipase adsorption, and accelerate lipid hydrolysis. Concurrently, lipolysis generates additional surface-active species, such as free fatty acids, monoacylglycerols, and diacylglycerols, resulting in complex competitive adsorption processes at the interface. The final interfacial composition and emulsion stability are therefore governed by the dynamic exchange of these molecules **[**25], a process that remains incompletely understood.

Lipid digestion kinetics were evaluated by monitoring the volume of NaOH solution used during the intestinal stage to keep the pH constant, which is an indication of the amount of free fatty acids (FFA) released. PEs showed a delayed lipid digestion due to the diffusion barrier imposed by the IFPG particles-stabilized interfacial layer. Among the two emulsions studied, PE exhibited higher lipolysis rate than PECQ with a total FFA release of 61% compared to 54% for the latter at the end of digestion period. This different digestion behavior can be related to the difference in their droplet size and the composition of their interfacial film **[**26]. Although PECQs exhibited smaller droplets and thus larger surface areas, the presence of co-encapsulated polyphenols likely strengthened the structure of the interfacial film and reduced lipase accessibility, resulting in a slower FFA release rate compared to PE. This apparent discrepancy can be explained by the reinforced interfacial architecture formed through the combined presence of IFPG particles and polyphenolic compounds. The adsorption of polyphenols at or near the oil–water interface likely enhanced interfacial cohesion and reduced the effective accessibility of pancreatic lipase to triglyceride substrates. Consequently, despite the increased interfacial area, enzymatic hydrolysis was partially hindered, leading to a moderated FFA release rate. Moreover, the formation of droplet aggregates under intestinal conditions further limited enzyme diffusion and interfacial renewal, thereby contributing to the observed controlled digestion and sustained release of fat-soluble vitamins. These findings highlight that lipid digestion kinetics in PEs are governed not solely by droplet size, but by the physicochemical properties and dynamic rearrangement of the interfacial layer. Collectively, these results demonstrate that IFPG-stabilized PEs provide effective structural resistance during gastrointestinal transit and enable controlled digestion and release of fat-soluble vitamins.

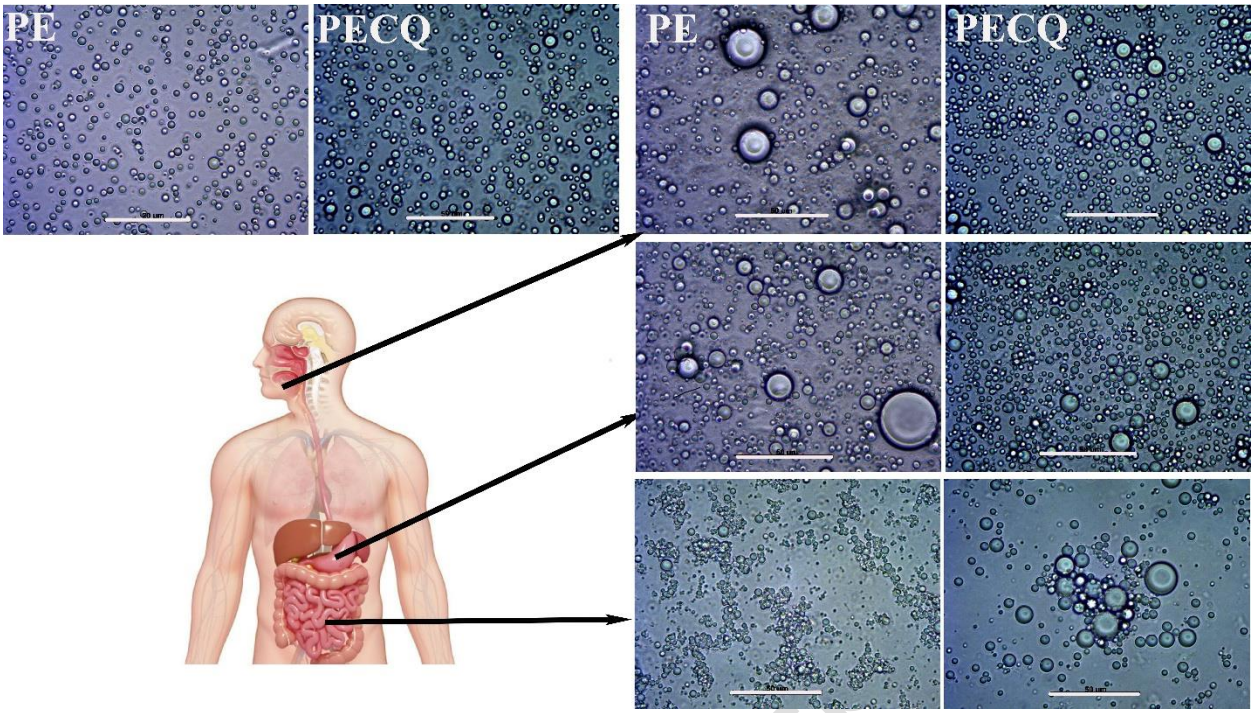


Fig. 4. Changes in the microstructure of Pickering emulsions stabilized with IFPG particles, either alone (PE) or in combination with a mixture of curcumin and quercetin (PECQ), after simulated oral, gastric, and small intestinal digestion (100× magnification). The two images on the left show the microstructure of intact initial emulsions. Scale bars represent 50 μm.

3.5. Bioaccessibility of Fat-Soluble Vitamins

The bioaccessibility of fat-soluble vitamins represents the fraction of encapsulated compounds released from the emulsion matrix and incorporated into mixed micelles, making them available for intestinal absorption. Efficient delivery systems must protect bioactive components during storage and ensure their controlled release under gastrointestinal conditions. In the present study, both vitamin D_3 and α-tocopherol were released from IFPG-stabilized PEs during simulated digestion, and their bioaccessibility was influenced by droplet size, interfacial properties, and the presence of polyphenolic compounds. Smaller droplets provide a larger specific surface area, facilitating lipase adsorption and enhancing hydrolysis, whereas a robust interfacial layer formed by IFPG particles, particularly when combined with curcumin and quercetin, can slow enzymatic access and modulate the release kinetics.

Quantitative analysis revealed that bioaccessibility of vitamin D_3 and α-tocopherol was generally higher in PE compared to PECQ (Fig. 5), reflecting a balance between interfacial stability and enzymatic hydrolysis. The hydrolysis of FO generated FFAs and monoacylglycerols, which contributed to the formation of mixed micelles and increased surface anion density, enhancing micelle-mediated transport. Despite the slightly lower bioaccessibility of vitamins entrapped in PECQ, it can be potentially advantageous for their sustainable release during intestinal digestion. These findings highlight that IFPG-stabilized PEs provide a tunable platform for the delivery of lipophilic vitamins, where interfacial architecture and polyphenol incorporation jointly regulate lipid hydrolysis and micellar solubilization, ultimately influencing intestinal absorption efficiency.

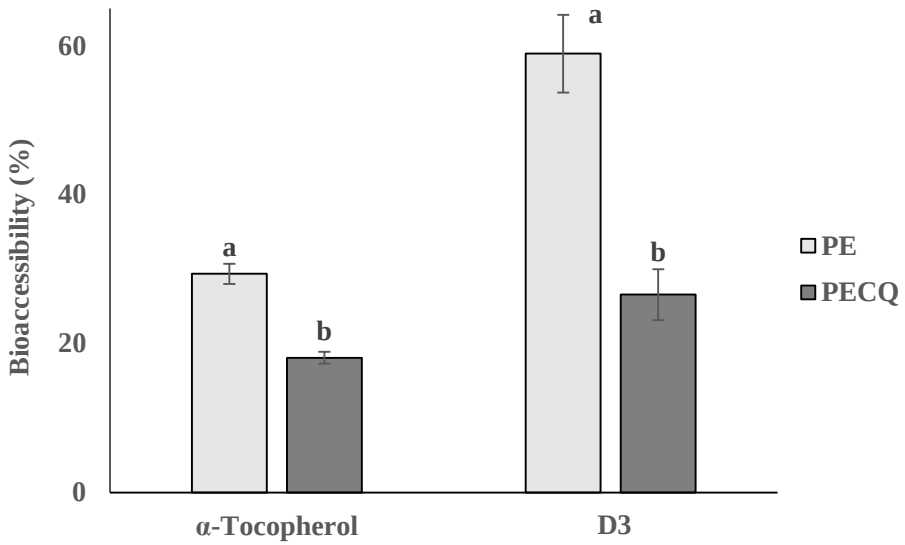


Fig. 5. Bioaccessibility of fat-soluble vitamins D_3 and α-tocopherol entrapped in the Pickering emulsions stabilized with IFPG particles, either alone (PE) or in combination with a mixture of curcumin and quercetin (PECQ). The bars bearing different letters indicate that the bioaccessibility values of the corresponding vitamin are significantly different ($p < 0.05$).

4. Conclusion

In this study, flaxseed oil-in-water Pickering emulsions stabilized with the insoluble fraction of Persian gum (IFPG) were successfully developed as a food-grade delivery system for fat-soluble vitamins D_3 and α-tocopherol. Incorporation of polyphenolic compounds, namely curcumin and quercetin, significantly enhanced the chemical stability, antioxidant capacity, and encapsulation efficiency of the vitamins. IFPG-stabilized emulsions effectively protected the lipids from oxidation and aggregation, while the polyphenols contributed to prolonging shelf life and resistance to processing conditions. Spectrophotometric analyses confirmed that polyphenol-loaded emulsions exhibited higher radical scavenging activity, suggesting synergistic protective effects on both lipids and vitamins. During simulated gastrointestinal digestion, IFPG-stabilized emulsions almost maintained their structural integrity and showed modulated lipolysis as well as controlled bioaccessibility which can be potentially advantages for sustainable release. Overall, IFPG-stabilized Pickering emulsions containing curcumin and quercetin demonstrated excellent physical and chemical stability, high vitamin encapsulation efficiency, and enhanced antioxidant properties, highlighting their potential as functional food ingredients and efficient carriers for lipophilic bioactive compounds. However, their *in vivo* behavior as well as the possible impacts they may impose on the textural and sensory attributes of the real food matrices in which they are incorporated are among the issues yet to be addressed, which could be the focus of future studies.

Declaration of conflict of interest

No conflict of interest has been declared by the authors.

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