



Multiscale characterization of citrate-acidified hawthorn pectin emulsion gels: Structure, storage behavior, and phenanthroline-detectable ferrous iron in digestion supernatants

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Abstract

Fortifying lipid-containing foods with ferrous iron remains challenging because Fe^{2+} is readily oxidized and can promote matrix destabilization and lipid oxidation. In this study, hawthorn pectin (HP) was used to construct Fe^{2+} -loaded emulsion gels, and five systems were compared: HP-Fe, HP-O, HP-Fe-O, HP-Fe-O-CA, and commercial low-methoxyl pectin (LMP)-Fe-O-CA. A multiscale characterization strategy, including apparent particle/aggregate size, ζ -potential, rheology, texture, low-field nuclear magnetic resonance (LF-NMR), Fourier transform infrared spectroscopy (FTIR), microscopy, storage behavior, apparent oxidation-related indices, and INFOGEST static digestion, was used to relate gel structure to phenanthroline-detectable Fe^{2+} retention and a nominal-volume-normalized supernatant Fe^{2+} index during static *in vitro* digestion. The citrate-containing acidified HP emulsion gel showed an intermediate apparent droplet/aggregate size ($D[4,3]=4.86\pm0.21\ \mu\text{m}$), a lower absolute ζ -potential ($-8.63\pm0.28\ \text{mV}$), and the highest hardness ($153.6\pm6.5\ \text{g}$), indicating a more deformation-resistant gel-like matrix. During storage, this formulation exhibited the lowest separated-phase release, the highest assay-detectable Fe^{2+} retention, and lower reagent-blank-corrected apparent peroxide value (POV) and thiobarbituric acid-reactive substances (TBARS) signals than HP-Fe-O under the defined assay conditions. During *in vitro* digestion, HP-Fe-O-CA showed a lower early-stage nominal-volume-normalized supernatant Fe^{2+} index and the highest final intestinal index under the defined digestion and assay conditions. These results support the potential of HP-Fe-O-CA as a hawthorn-pectin-based matrix for Fe^{2+} -fortified lipid-containing systems under the defined storage and digestion-assay conditions.

Keywords Pectin network · Ferrous retention · Apparent oxidation indices · Rheology · LF-NMR · Static *in vitro* digestion

Introduction

Iron deficiency remains one of the most common nutrition-related disorders worldwide, and insufficient dietary iron intake is a primary contributor to anemia [1, 2]. Although non-heme iron is the predominant form in plant-derived foods, its absorption can be limited by phytates, polyphenols, calcium, and certain dietary proteins [3]. Soluble ferrous salts, especially ferrous sulfate, are widely used in supplements and fortified foods due to their relatively high bioavailability; however, they can cause gastrointestinal discomfort and may promote undesirable interactions in complex food matrices, particularly in lipid-containing systems [4, 5]. In lipid-containing fortified systems, this challenge is particularly important because soluble Fe^{2+} may accelerate

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lipid oxidation while also undergoing oxidation, precipitation, or matrix-dependent changes in extractability. These limitations have increased interest in food-compatible delivery matrices that can retain ferrous iron during processing and storage while allowing its release during digestion [6]. Emulsion gels are relevant in this context because oil droplets can be immobilized within a gelled biopolymer network, thereby combining the loading capacity of emulsions with the physical stability of gels [7]. In pectin-based systems, molecular structure, interfacial adsorption, carboxyl groups, degree of esterification, molecular weight, and pH/cation responsiveness can jointly influence emulsification, gelation, and release behavior [8]. These properties make pectin-based emulsion gels suitable carriers for the co-delivery of hydrophilic and lipophilic components, including mineral/lipid co-delivery systems [9]. Recent studies further indicate that pectin fine structure can modulate the microstructure, rheology, stability, and release behavior of Ca^{2+} -pectin emulsion gels, highlighting the importance of linking pectin molecular descriptors with emulsion-gel functionality [10, 11]. In parallel, studies on polysaccharide-containing emulsion and interfacial systems have shown that biopolymer structure and interfacial organization can markedly affect emulsion stability and macroscopic mechanical properties [12–14]. These findings suggest that the performance of pectin-based emulsion gels should be interpreted not only from bulk gelation, but also from the coupled effects of pectin structure, ion-mediated association, proton-mobility behavior, and interfacial organization.

Hawthorn pectin (HP) is a food-derived acidic polysaccharide recovered from hawthorn fruit residues and processing by-products. Recent studies have shown that its extraction route and fractionation history markedly affect its physicochemical and techno-functional properties, including viscosity, emulsifying performance, emulsion stability, and gel-forming behavior [15–18]. In addition to these interfacial and rheological attributes, HP has also shown relevance to iron-associated delivery systems, as evidenced by ferrous-cross-linked HP/SPI hydrogel beads and HP–iron complexes with promising anti-anemic potential [19–21]. Collectively, these findings position HP not merely as a conventional gelling or emulsifying polysaccharide, but as a structurally distinctive food macromolecule of interest for iron-fortified colloidal systems in which matrix stability and post-digestion Fe^{2+} detectability must be considered simultaneously.

However, pectin emulsion gels, HP gels, and pectin–iron complexes have mostly been studied separately. Less is known about lipid-containing Fe^{2+} -fortified matrices in which pectin network formation, droplet organization, apparent oxidation-related behavior, and digestion-stage changes in phenanthroline-detectable Fe^{2+} occur concurrently. This

knowledge gap is important because soluble Fe^{2+} can influence lipid-containing matrices during storage, while matrix compactness may affect the distribution of assay-detectable Fe^{2+} in digestion supernatants. Therefore, the central formulation challenge is to retain Fe^{2+} in a detectable form during storage while maintaining a favorable final intestinal nominal-volume-normalized supernatant Fe^{2+} index.

The specific objectives of this study were: (i) to construct Fe^{2+} -loaded HP systems with or without oil incorporation and a citrate-containing acidic medium; (ii) to compare their initial colloidal properties, rheological behavior, texture, proton-relaxation distribution, FTIR profiles, and microstructure; (iii) to evaluate storage behavior, separated-phase release, assay-detectable Fe^{2+} retention, and apparent oxidation-related indices; and (iv) to compare the nominal-volume-normalized supernatant Fe^{2+} index among formulations during static *in vitro* digestion. The novelty of this work lies in the integrated evaluation of a hawthorn-pectin-based Fe^{2+} emulsion gel by linking multiscale matrix structure, storage behavior and digestion-stage changes in phenanthroline-detectable Fe^{2+} in the supernatant within the same lipid-containing formulation. A commercial low-methoxyl pectin (LMP)-based Fe^{2+} emulsion gel was included as an acidified reference system. Because the present formulation design did not independently separate citrate complexation, pH, and pectin structural effects, the citrate-acidified systems are interpreted as integrated formulation systems rather than as evidence for citrate effects alone.

Materials and methods

Materials and reagents

HP was extracted from hawthorn residue using ultrasound-assisted extraction in the ionic liquid 1-butyl-3-methylimidazolium hydrogen sulfate ([Bmim][HSO₄]). The hawthorn residues were provided by the College of Food Science and Engineering, Shandong Agricultural University (Tai'an, China). In brief, the [Bmim][HSO₄] concentration was 0.6 mol/L, the solid-liquid ratio was 1:10 (w/v), the ultrasonic power was 360 W, the ultrasonic temperature was 60 °C, and the extraction time was 60 min. After extraction, the mixture was centrifuged at 4500 rpm for 15 min, corresponding to $4860 \times g$ for the rotor used in this study. The supernatant was precipitated with ethanol, redissolved, freeze-dried, sealed, and stored at 4 °C until use [22]. Commercial low-methoxyl citrus pectin (LMP) was obtained from Andre Pectin Company (Yantai, Shandong, China) and used as the reference pectin. Because the HP used in this study was also a low-methoxyl pectin, commercial LMP was selected as

a commercial low-methoxyl reference rather than a universal commercial pectin control. The source information and physicochemical characteristics of HP and LMP are summarized in Table S1 (Supplementary Material). Olive oil was purchased from Xiyan (Qingdao Tianxiang Foods Group, China). Vitamin E acetate (96%) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Pepsin from porcine gastric mucosa (P7000, ≥ 250 U/mg solid), pancreatin from porcine pancreas (P7545, 8 $\times$ USP specifications), pancreatic lipase from porcine pancreas (L3126, Type II; 30–90 U/mg protein using triacetin as the substrate), and porcine bile extract (B8631) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Supplier-specified activities were used as the nominal basis for enzyme dosing. Unless otherwise specified, all reagents were of analytical grade, and all solutions were prepared using deionized water.

Preparation of pectin-based systems

Pectin-based systems were prepared using HP or commercial low-methoxyl pectin (LMP) as the matrix, with slight modifications based on previously reported pectin emulsion-gel methods [23, 24]. HP or LMP was dispersed either in deionized water or in a 10 mM citric-acid solution adjusted to pH 4.0 with 1.0 M NaOH, stirred at room temperature for 3 h, and then hydrated at 4 °C for 12 h before use to obtain 3.0% (w/v) pectin stock solutions. Vitamin E acetate was added to the oil phase at 1.0 mg per mL of oil before emulsification [25, 26].

All formulations were standardized to a final pectin concentration of 2.0% (w/v). For oil-containing systems, the oil accounted for 20% (v/v) of the final formulation. For iron-containing systems, freshly prepared 45.0 mM $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ solution was added to obtain a final Fe^{2+} concentration of 6.0 mM. A 45.0 mM $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ solution was freshly prepared immediately before use in deoxygenated water under dim-light conditions, and the vessel was protected from light with aluminum foil during preparation and addition to minimize Fe^{2+} oxidation. Deionized water or 10 mM citric-acid solution adjusted to pH 4.0 was added when necessary to equalize the final volume among formulations. The oil phase was first homogenized with the pectin phase at 14,000 rpm for 10 min using an XHF-DY high-speed disperser (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) to obtain a primary emulsion. For iron-containing formulations, the FeSO_4 solution was then added dropwise under gentle stirring [27]. The resulting systems were stored at 4 °C for 4 h before further analysis. The final formulation characteristics of the pectin-based hydrogel, emulsion, and emulsion-gel systems are summarized in Table 1.

Physicochemical and structural characterization

Apparent pH measurement

pH values of the samples were determined with slight modifications to previously reported methods [28, 29]. 1.0 g sample was accurately weighed and mixed with 9 mL of deionized water, followed by brief homogenization for 1 min to obtain a uniform dispersion. The pH was then measured at 25 °C using a calibrated digital pH meter. The electrode was rinsed with deionized water after each measurement. To

Table 1 Final formulation characteristics of HP/LMP-based hydrogel, emulsion, and emulsion-gel systems

Sample	HP-Fe	HP-O	HP-Fe-O	HP-Fe-O-CA	LMP-Fe-O-CA
Dispersion medium	Water	Water	Water	10 mM citric-acid solution adjusted to pH 4.0	10 mM citric-acid solution adjusted to pH 4.0
Final pectin concentration (% w/v)	2.0	2.0	2.0	2.0	2.0
Oil fraction (% v/v)	0	20.0	20.0	20.0	20.0
Fe^{2+} concentration (mM)	6.0	0	6.0	6.0	6.0
System type	HP/ Fe^{2+} hydrogel-like system	HP oil-in-water emulsion	Fe^{2+} -loaded HP emulsion gel	Citrate-acidified Fe^{2+} -loaded HP emulsion gel	Citrate-acidified Fe^{2+} -loaded LMP emulsion gel
Comparative role	Fe^{2+} -containing non-oil reference	Iron-free oil-containing reference	Non-acidified Fe^{2+} emulsion-gel reference	Target citrate-acidified HP formulation	Acidified commercial LMP reference

HP, hawthorn pectin. O denotes the olive-oil phase containing vitamin E acetate; Fe denotes 6.0 mM Fe^{2+} ; CA denotes the citric-acid-based acidic medium used for pectin dispersion, prepared as a 10 mM citric-acid solution adjusted to pH 4.0. HP-Fe and HP-O were included as non-oil Fe^{2+} and iron-free oil-containing references, respectively. HP-Fe-O was used as the non-acidified Fe^{2+} emulsion-gel reference, while LMP-Fe-O-CA was used as the acidified commercial low-methoxyl pectin reference. The final apparent pH values of the diluted systems are reported in Table 2

ensure consistency, the apparent pH of the diluted dispersions was used for comparative evaluation across all formulations. The diluted-dispersion pH values are reported only as apparent formulation indicators.

Apparent particle/aggregate size and ζ potential

Based on a previously reported method, with slight modifications [30], the apparent particle/aggregate size distribution of the samples was determined using a laser diffraction particle-size analyzer (Mastersizer 3000 + Ultra, UK). For the oil-containing systems, the reported D [3, 4] value reflects apparent droplet/aggregate size, whereas for HP-Fe it reflects the apparent size of dispersed aggregates in the diluted system. The apparent size was expressed as the volume-weighted mean diameter, D [3, 4], calculated as follows:

$$D [4, 3] = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (1)$$

where n_i is the number of particles with diameter d_i . The ζ -potential of the diluted samples was measured separately by electrophoretic light scattering using a Zetasizer Advance system (Malvern, UK) at 25 °C [31].

Dilution may alter weakly associated gel structures; therefore, the D [3, 4] and ζ -potential values are interpreted as comparative colloidal indicators rather than as direct in situ structural parameters of the intact gels.

Rheological properties

Rheological properties of the samples were measured using a controlled-stress rotational rheometer (AR2000ex, TA Instruments, USA) equipped with a 20 mm parallel plate geometry [32]. All measurements were performed at 25 °C. After loading, excess sample was trimmed, the edge was covered with low-viscosity silicone oil to minimize evaporation, and the sample was equilibrated for 5 min before measurement. Steady-shear tests were conducted over a shear-rate range of 0.1–100 s⁻¹ to determine the apparent viscosity of the samples. Frequency sweep measurements were performed over an angular frequency range of 0.1–100 rad/s at an oscillatory strain of 0.5%, and the storage modulus (G') and loss modulus (G'') were recorded to characterize the viscoelastic behavior of the gel systems.

Texture measurement

Texture properties were measured using a texture analyzer (TA.XT Plus, Stable Micro Systems, UK) with slight modifications to a reported method [28]. Samples were

transferred into cylindrical containers and stored at 4 °C for 4 h before measurement to ensure consistent gel setting. A P/0.5 cylindrical probe was used for compression at a pre-test speed of 1.0 mm/s, a test speed of 0.5 mm/s, and a trigger force of 5 g. The maximum force during compression was defined as hardness. All measurements were performed at room temperature, with at least three samples analyzed per formulation.

Proton relaxation distribution by low-field NMR

Proton relaxation distributions of the samples were analyzed using a low-field nuclear magnetic resonance (LF-NMR) analyzer [33, 34]. The samples were transferred into glass tubes and inserted into the LF-NMR instrument for measurement. The experimental parameters were set as follows: echo time, 0.5 ms; waiting time, 4000 ms; number of echoes, 4000; and scan repetitions, 8. The resulting T_2 relaxation curves were fitted to multi-exponential models using MultiExp Inv Analysis software. The relaxation times and corresponding relative peak areas were used to characterize the relaxation-associated proton populations in the samples; because oil-only and aqueous-matrix controls were not included, these populations were not assigned exclusively to specific water states.

Fourier transform infrared spectroscopy (FTIR)

Samples were freeze-dried, ground into powders, and analyzed using an FTIR spectrometer (Nexus 670, Thermo Fisher Scientific, USA). The spectra were recorded over 4000–600 cm⁻¹ at a resolution of 4 cm⁻¹ with 64 scans.

Optical microscopy

Sample microstructure was observed using an inverted optical microscope (IX73, Olympus, Japan). A small amount of sample was evenly spread onto a microscope slide and carefully covered with a coverslip to minimize air bubble formation. The samples were examined using a 40× objective, and the images were captured using Cap Studio software [17].

Confocal laser scanning microscopy (CLSM)

Following Arain et al. [12], with slight modifications, sample microstructure was observed using a confocal laser scanning microscope (FV3000, Olympus, Japan). Fluorescein isothiocyanate (FITC, 1 mg/mL in dimethyl sulfoxide) and Nile Red (1 mg/mL in ethanol) were used to visualize fluorescence associated with the continuous aqueous/polar phase and the oil phase, respectively. The samples were

incubated with the dyes in the dark for 30 min at a final dye concentration of 0.1% (w/w) and then observed using a 100× objective. The excitation/emission wavelengths of FITC and Nile Red were 488/500 nm and 543/605 nm, respectively. Because FITC was added directly rather than covalently conjugated to pectin, the green fluorescence was not interpreted as a pectin-specific signal.

Storage behavior and apparent oxidation-related measurements

Appearance changes during storage

The samples were transferred into sealed transparent glass vials (20 mL glass vials containing 10.0 g of sample, with a headspace of 10 mL) and stored at 25 °C in the dark for 28 days. Appearance changes were documented by digital photography on days 0, 7, 14, 21, and 28. The self-supporting capacity, structural collapse, visible separated phase release, and exudation of the samples were visually evaluated. Separate independent vials were prepared for separated-phase release, assay-detectable Fe²⁺ retention, POV, and TBARS measurements at each formulation and storage time point. The vials assigned to separated-phase release were kept undisturbed and used only for gravimetric collection of the separated phase. No separated phase was removed from the vials assigned to Fe²⁺, POV, or TBARS analysis. Before collecting the 0.50 g, 50 mg, or 100 mg analytical portions, the entire contents of each corresponding vial were thoroughly remixed until visually homogeneous to ensure representative sampling.

Determination of separated phase release

At each storage time point, the samples were visually examined for the presence of a separated phase. Separated phase release was evaluated by gravimetrically collecting the clearly separated upper or lower phases after standardized standing without agitation. The collection criterion and collection procedure were identical across all formulations and time points. The released oil, aqueous phase, or mixed separated phase was carefully collected and weighed to evaluate the overall extent of separated phase release during storage [35, 36]. The separated phase release was calculated according to the following equation:

$$\text{Separated phase release (\%)} = \frac{M_s}{M_0} \times 100 \quad (2)$$

where m_0 is the initial sample mass and m_s is the mass of the visually separated phase after storage.

Assay-detectable Fe²⁺ retention during storage

Assay-detectable Fe²⁺ content of the iron-containing samples was determined on days 0, 7, 14, 21, and 28 using the 1,10-phenanthroline spectrophotometric method [37]. Briefly, 0.50 g of sample was mixed with 10 mL of 0.01 M HCl, vortexed for 1 min, and centrifuged at 5000 rpm, corresponding to $6000 \times g$, for 15 min under dim-light conditions. Then, 1.0 mL of the supernatant was mixed with 1.0 mL of 5 mM 1,10-phenanthroline solution and allowed to react in the dark for 10 min. The absorbance was measured at 510 nm, and the Fe²⁺ content was calculated from a ferrous sulfate standard curve. To evaluate matrix effects on the 1,10-phenanthroline assay, the four undigested Fe-containing formulations and representative oral, gastric (G120), and intestinal (I120) supernatants from HP-Fe-O and HP-Fe-O-CA were tested. Fe²⁺ spike recovery was evaluated at approximately 50% of the native detectable concentration. Sample-specific blanks were prepared by replacing the 5 mM 1,10-phenanthroline solution with an equal volume of deionized water, and the blank absorbance was subtracted from the reaction absorbance. The final reaction pH was measured after 10 min without additional buffering. All measurements were performed in triplicate, and recovery was calculated as:

$$\text{Recovery} = \frac{C_{\text{spiked}} - C_{\text{unspiked}}}{C_{\text{added}}} \times 100 \quad (3)$$

where C_{spiked} and C_{unspiked} are the blank-corrected Fe²⁺ concentrations in the spiked and unspiked samples, respectively, and C_{added} is the theoretical Fe²⁺ concentration added. The validation results are presented in Table S3.

In this study, “assay-detectable Fe²⁺” refers to the ferrous fraction detectable by the 1,10-phenanthroline assay under the defined extraction, centrifugation, and reaction conditions. This value should not be interpreted as total iron content, total iron retention, or absolute iron bioaccessibility because the assay does not distinguish total iron, oxidized iron species, precipitated iron, and non-extractable iron. Assay-detectable Fe²⁺ retention during storage was calculated as follows:

$$\text{Fe}^{2+} \text{ retention (\%)} = \frac{C_t}{C_0} \times 100 \quad (4)$$

where C_0 is the initial assay-detectable Fe²⁺ content and C_t is the assay-detectable Fe²⁺ content after storage for time t . C_0 and C_t were expressed in the same concentration unit after the same extraction and dilution correction before calculating the retention percentage.

Determination of apparent lipid-oxidation-related indices

Apparent lipid-oxidation-related behavior during storage was evaluated using peroxide value (POV), an indicator associated with primary lipid oxidation products, and thiobarbituric acid-reactive substances (TBARS), an indicator associated mainly with malondialdehyde-related secondary oxidation products [38]. For POV determination, 50 mg of sample was mixed with 5 mL of chloroform/methanol (7:3, v/v), followed by the addition of 25 μ L of 30% (w/v) potassium thiocyanate solution and 25 μ L of freshly prepared 20 mM FeCl₂ solution in 3.5% (v/v) HCl, with slight modifications to the method [38]. After standing at room temperature for 5 min, the absorbance was measured at 500 nm using a UV–Vis spectrophotometer, and the results are expressed as meq O₂/kg oil. For TBARS determination, 100 mg of sample was mixed with 2 mL of TBA reagent composed of 15% (w/v) trichloroacetic acid (TCA), 0.375% (w/v) thiobarbituric acid (TBA), and 0.25 M HCl. The mixture was heated in a boiling water bath for 30 min, cooled to room temperature, and centrifuged at 2000 rpm, corresponding to 960 $\times$ g, for 15 min. The absorbance of the supernatant was recorded at 532 nm, and the TBARS content was calculated from a standard curve prepared from 1,1,3,3-tetraethoxypropane (TEP). TEP was used only to prepare the TBARS calibration curve and was not added to the analyzed samples. For POV analysis, the reagent blank contained 5 mL of chloroform/methanol (7:3, v/v), 25 μ L of 30% (w/v) potassium thiocyanate solution, and 25 μ L of freshly prepared 20 mM FeCl₂ solution in 3.5% (v/v) HCl. For TBARS analysis, the reagent blank contained 2 mL of the complete TBA reagent and was subjected to the same heating, cooling, and centrifugation procedures as the samples. The corresponding reagent-blank absorbance was subtracted before calculation. The reagent-blank-corrected POV and TBARS values were used as comparative apparent oxidation-related indices under identical analytical conditions. POV results are expressed as meq O₂/kg oil, and TBARS results are expressed as mg MDA/kg sample. The analytical correction applied in this study was reagent-blank subtraction. Formulation-dependent contributions from endogenous iron, sample color, residual turbidity, and light scattering were therefore retained within the apparent analytical signals. The resulting values were used for comparative evaluation among formulations analyzed under identical assay conditions.

In vitro digestion and calculation of nominal-volume-normalized supernatant Fe²⁺ indices

Static *in vitro* gastrointestinal digestion was performed according to the standardized INFOGEST 2.0 static digestion protocol, with slight modifications for the present emulsion gel systems [39]. The compositions of the simulated salivary fluid (SSF), simulated gastric fluid (SGF), and

simulated intestinal fluid (SIF) are listed in Table S2 (Supplementary Material).

For each digestion trial, 5.0 g of sample was mixed with 4.000 mL of 1.25 $\times$ SSF, 25 μ L of 0.3 M CaCl₂, and 0.975 mL of deionized water. Salivary α -amylase was omitted because the samples contained no added starch. The oral phase was incubated at 37 $^{\circ}$ C for 2 min under orbital shaking at 100 rpm, giving a final volume of 10.0 mL. For gastric digestion, 8.000 mL of 1.25 $\times$ SGF, 5 μ L of 0.3 M CaCl₂, and 0.667 mL of pepsin stock solution (60,000 U/mL, prepared in deionized water) were added to the oral bolus, providing a nominal final pepsin activity of 2,000 U/mL in the 20.0 mL gastric digesta. The pepsin stock concentration was calculated from the supplier-specified activity of P7000. The pH was adjusted dropwise to 3.0 with 5 M HCl, after which deionized water was added to obtain a final volume of 20.0 mL. The acid addition was controlled by the target pH to accommodate formulation-dependent differences in buffering capacity. Gastric lipase was omitted from the gastric phase. Gastric digestion was performed at 37 $^{\circ}$ C for 2 h under orbital shaking at 100 rpm. For intestinal digestion, the gastric chyme was mixed with 8.000 mL of 1.25 $\times$ SIF containing pancreatic lipase, 5.000 mL of pancreatin suspension (4.0 mg/mL), 3.000 mL of porcine bile-extract suspension (200 mg/mL), and 40 μ L of 0.3 M CaCl₂. The pancreatin addition corresponded to 20.0 mg per digestion vessel and a final mass concentration of 0.50 mg/mL in the 40.0 mL intestinal digesta. Pancreatin dosing was expressed on a mass basis according to the supplier's 8 $\times$ USP product specification. Pancreatic lipase (L3126, Type II) was added at 80,000 U per intestinal digestion vessel and incorporated into the 8.000 mL SIF portion immediately before intestinal digestion, corresponding to a nominal supplier-based triacetin activity of 10,000 U/mL in the SIF portion and 2,000 U/mL in the final 40.0 mL intestinal digesta. This activity basis is distinct from the tributyrin pH-stat activity recommended in the standardized INFOGEST protocol. The porcine bile-extract addition corresponded to 600 mg per digestion vessel and a final mass concentration of 15 mg/mL. The pH was adjusted dropwise to 7.0 with 5 M NaOH, after which deionized water was added to obtain a final volume of 40.0 mL. The base addition was controlled by the target pH to accommodate formulation-dependent differences in buffering capacity. Intestinal digestion was performed at 37 $^{\circ}$ C for 2 h under orbital shaking at 100 rpm.

To monitor assay-detectable Fe²⁺ in the digestion supernatants, samples were collected at the end of the oral phase and at 30, 60, and 120 min during the gastric and intestinal phases. Independent digestion vessels were prepared for each formulation and each sampling time point to avoid volume-depletion effects caused by repeated sampling. Aliquots were immediately cooled in an ice bath to minimize

further digestion and then centrifuged at 10,000 rpm, corresponding to $24,000 \times g$, for 15 min at 4 °C. At the end of the intestinal phase, the digesta were further centrifuged at 10,000 rpm, corresponding to $24,000 \times g$, for 30 min at 4 °C, and the clear supernatant was collected as the final intestinal supernatant fraction. The assay-detectable Fe^{2+} content in digestion supernatants was determined using the 1,10-phenanthroline reaction described in [Assay-detectable \$\text{Fe}^{2+}\$ retention during storage](#), with the centrifuged supernatants analyzed directly or after appropriate dilution and representative oral, gastric (G120), and intestinal (I120) supernatants from the citrate-free HP-Fe-O and citrate-containing HP-Fe-O-CA formulations included in the matrix-effect validation.

The nominal-volume-normalized supernatant Fe^{2+} index at digestion stage t (I_t) and the final intestinal index (I_{IS}) were calculated as follows:

$$I_t (\%) = \frac{C_t V_t}{C_0 m_0} \times 100 \quad (5)$$

$$I_{IS} (\%) = \frac{C_{IS} V_{IS}}{C_0 m_0} \times 100 \quad (6)$$

where I_t and I_{IS} are the nominal-volume-normalized supernatant Fe^{2+} indices at digestion stage t and at the final intestinal stage, respectively; C_0 is the assay-detectable Fe^{2+} content of the undigested sample (mg/g sample); m_0 is the initial sample mass used for digestion (5.0 g); C_t and C_{IS} are the assay-detectable Fe^{2+} concentrations in the corresponding digestion supernatants (mg/mL); and V_t and V_{IS} are the nominal total digestion volumes used as consistent normalization factors. Nominal oral, gastric, and intestinal digestion volumes of 10.0, 20.0, and 40.0 mL, respectively, were applied. The resulting indices were used for comparative evaluation of formulation- and digestion-stage-dependent supernatant Fe^{2+} behavior.

Statistical analysis

All experiments were performed in triplicate using independently prepared samples, and the results are presented as mean $\pm$ standard deviation (SD). For storage experiments,

separate independent vials were prepared for each formulation $\times$ time $\times$ analytical-endpoint combination, with three independent vials used for each measurement. For single-time-point variables, including pH, apparent particle/aggregate size, ζ -potential, and hardness, statistical differences among formulations were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. For storage and *in vitro* digestion data, statistical analysis was performed using two-way ANOVA, with formulation and time as fixed factors. When a significant formulation $\times$ time interaction was detected, simple-effect comparisons were then performed using Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS Statistics 26.0.

Results and discussion

Initial physicochemical properties and colloidal characteristics of the emulsion gel systems

Significant differences were observed among the pectin-based systems with respect to apparent pH, apparent particle/aggregate size, ζ -potential, and hardness (Table 2). Because the pH values were measured in diluted dispersions, they should be interpreted as apparent pH values for formulation comparison rather than as the true internal pH of the intact gels. HP-O showed the highest pH (4.02) and the largest absolute ζ -potential value (-18.94 mV). In contrast, HP-Fe-O-CA and LMP-Fe-O-CA exhibited lower apparent pH values in the diluted dispersions together with lower absolute ζ -potential values. These concurrent differences are consistent with formulation-dependent changes in the acid-base and ionic environment, including altered carboxyl-group ionization and charge screening in the presence of Fe^{2+} . This pattern agrees with the expected behavior of pectin systems as pH decreases and cation binding increases [40].

With respect to apparent particle/aggregate size, HP-Fe-O, HP-Fe-O-CA, and LMP-Fe-O-CA exhibited larger D [3, 4] values than HP-O, indicating that Fe^{2+} incorporation promoted droplet association and/or flocculation in

Table 2 Initial physicochemical properties and hardness of different pectin-based systems

	HP-Fe	HP-O	HP-Fe-O	HP-Fe-O-CA	LMP-Fe-O-CA
pH	3.74 ± 0.03^b	4.02 ± 0.02^a	3.66 ± 0.04^c	3.06 ± 0.03^c	3.21 ± 0.02^d
$D[4,3]$ (μm)	0.91 ± 0.46^c	2.98 ± 0.12^d	7.42 ± 0.29^a	4.86 ± 0.21^c	6.71 ± 0.25^b
ζ -potential (mV)	-12.48 ± 0.31^c	-18.94 ± 0.42^c	-13.57 ± 0.37^d	-8.63 ± 0.28^b	-7.52 ± 0.25^a
Hardness (g)	34.8 ± 2.2^d	11.7 ± 0.9^c	126.3 ± 5.8^b	153.6 ± 6.5^a	98.4 ± 4.7^c

Values are expressed as mean $\pm$ SD ($n = 3$). Different superscript letters within the same row indicate significant differences among formulations ($p < 0.05$). The pH values were measured in diluted dispersions (1:10, w/v). D [3, 4] represents the apparent particle/aggregate size of the diluted samples

the oil-containing systems. Among the oil-containing systems, HP-O showed the smallest apparent size together with the highest absolute ζ -potential, suggesting a relatively more dispersed initial state. In contrast, the lower absolute ζ -potential values of the Fe^{2+} -containing systems suggest weakened electrostatic stabilization after Fe^{2+} addition, particularly in the citrate-containing acidic medium. This trend is consistent with the reported effects of cations, pH, and dispersed phases on pectin emulsification properties [27, 40]. For HP-Fe, however, the measured D [3, 4] represents the apparent size of dispersed aggregates in the diluted system and should not be directly compared with the apparent droplet/aggregate size of the oil-containing systems.

In terms of hardness, HP-Fe-O-CA showed the highest value (153.6 ± 6.5 g), followed by HP-Fe-O and LMP-Fe-O-CA, whereas HP-Fe and HP-O showed much lower values. These results suggest that the combined presence of Fe^{2+} , oil, and the citrate-containing acidic medium was associated with a mechanically stronger gel-like structure in the HP system. This interpretation is consistent with previous reports showing that pectin emulsion-gel properties are closely related to gel-network formation, pH/ion-mediated association, and microstructural organization [23, 24, 27]. Nevertheless, because the citrate-containing formulations also differed in apparent pH from the non-acidified formulations, the higher hardness and altered colloidal indicators of HP-Fe-O-CA should not be attributed to citrate alone. In addition, HP and LMP differed in galacturonic acid (GalA) content, degree of esterification (DE), molecular weight, and homogalacturonan/rhamnogalacturonan-I (HG/RG-I) distribution (Table S1, Supplementary Material). Therefore,

the observed differences in deformation resistance are more appropriately interpreted as the combined outcome of HP structure, Fe^{2+} addition, oil incorporation, lower apparent pH, and the citrate-containing acidic medium.

Gel network formation and multiscale structural characteristics

Rheological properties and texture characteristics

Steady-shear results showed that the apparent viscosity of all samples decreased with increasing shear rate, indicating typical shear-thinning behavior (Fig. 1a). Such behavior is common in pectin-based emulsions and emulsion gels [22]. Among the tested samples, HP-Fe-O-CA exhibited the highest viscosity over the entire shear-rate range, followed by HP-Fe-O and LMP-Fe-O-CA, whereas HP-O reached the lowest viscosity. This result suggests that HP-Fe-O-CA formed a more viscous internal structure than the other tested systems.

Frequency sweep data showed that G' remained higher than G'' throughout the measured frequency range for all samples, indicating predominantly elastic responses under the selected testing conditions. HP-O displayed relatively low G' and G'' values, whereas HP-Fe-O-CA and HP-Fe-O showed higher G' values and weaker frequency dependence, suggesting more developed gel-like structures [41]. Hardness followed the same overall trend as G' , namely HP-Fe-O-CA > HP-Fe-O > LMP-Fe-O-CA > HP-Fe > HP-O. This consistency between rheological and textural results indicates that HP-Fe-O-CA had the most deformation-resistant structure among the tested formulations [42].

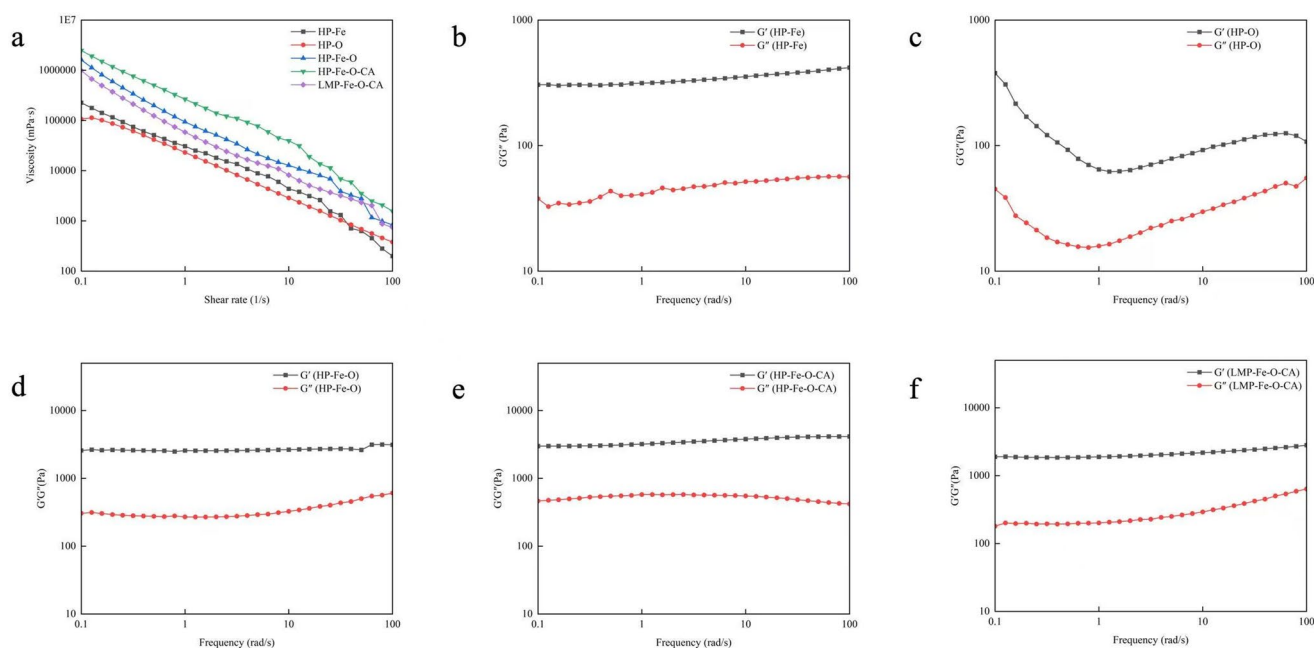


Fig. 1 Steady-shear viscosity (**a**) and frequency-sweep rheological profiles (**b–f**) of different pectin-based systems

Proton relaxation distribution by LF-NMR

T_2 relaxation profiles in Fig. 2a differed markedly among the systems and could be divided into three relaxation-associated proton populations, namely the short- T_2 (T_{21}), intermediate- T_2 (T_{22}), and long- T_2 (T_{23}) populations. HP-Fe showed the highest relative contribution of the long- T_2 population, with T_{23} and T_{22} accounting for approximately 85.6% and 13.3% of the total fitted peak area, respectively, indicating a proton-relaxation distribution dominated by the long- T_2 population. In contrast, the oil-containing systems were dominated by the intermediate- T_2 proton population. The relative T_{22} peak-area contributions were approximately 98.5%, 97.7%, 93.5%, and 88.0% for HP-O, HP-Fe-O, HP-Fe-O-CA, and LMP-Fe-O-CA, respectively. The marked decrease in the relative T_{23} peak area after oil incorporation indicates that emulsion-gel formation substantially altered the overall proton-relaxation distribution compared with the Fe^{2+} hydrogel-like system. Fe^{2+} addition and acidification did not simply increase the intermediate- T_2 peak-area contribution; instead, they appeared to alter the organization of the continuous phase and droplet-associated network.

HP-Fe-O-CA did not show the highest T_{22} peak-area contribution, but it showed a lower long- T_2 peak-area contribution than LMP-Fe-O-CA, together with the highest hardness and a stronger viscoelastic response. Therefore, the improved stability of HP-Fe-O-CA is best described by its integrated matrix features, including its proton-relaxation behavior, droplet/aggregate immobilization, and higher deformation resistance. Shorter T_2 relaxation times are generally associated with more restricted proton mobility [32, 33]. However, because LF-NMR detects protons from both aqueous and lipid components and oil-only and aqueous-matrix controls were not included, lipid-proton relaxation may overlap with the fitted populations. Therefore, the present results demonstrate differences in overall proton-relaxation behavior but do not independently establish absolute water fractions or superior water-retention capacity.

Functional-group environment by FTIR

FTIR spectra in Fig. 2c showed that all five samples retained the main absorption bands characteristic of pectic systems, suggesting that the fundamental polysaccharide framework

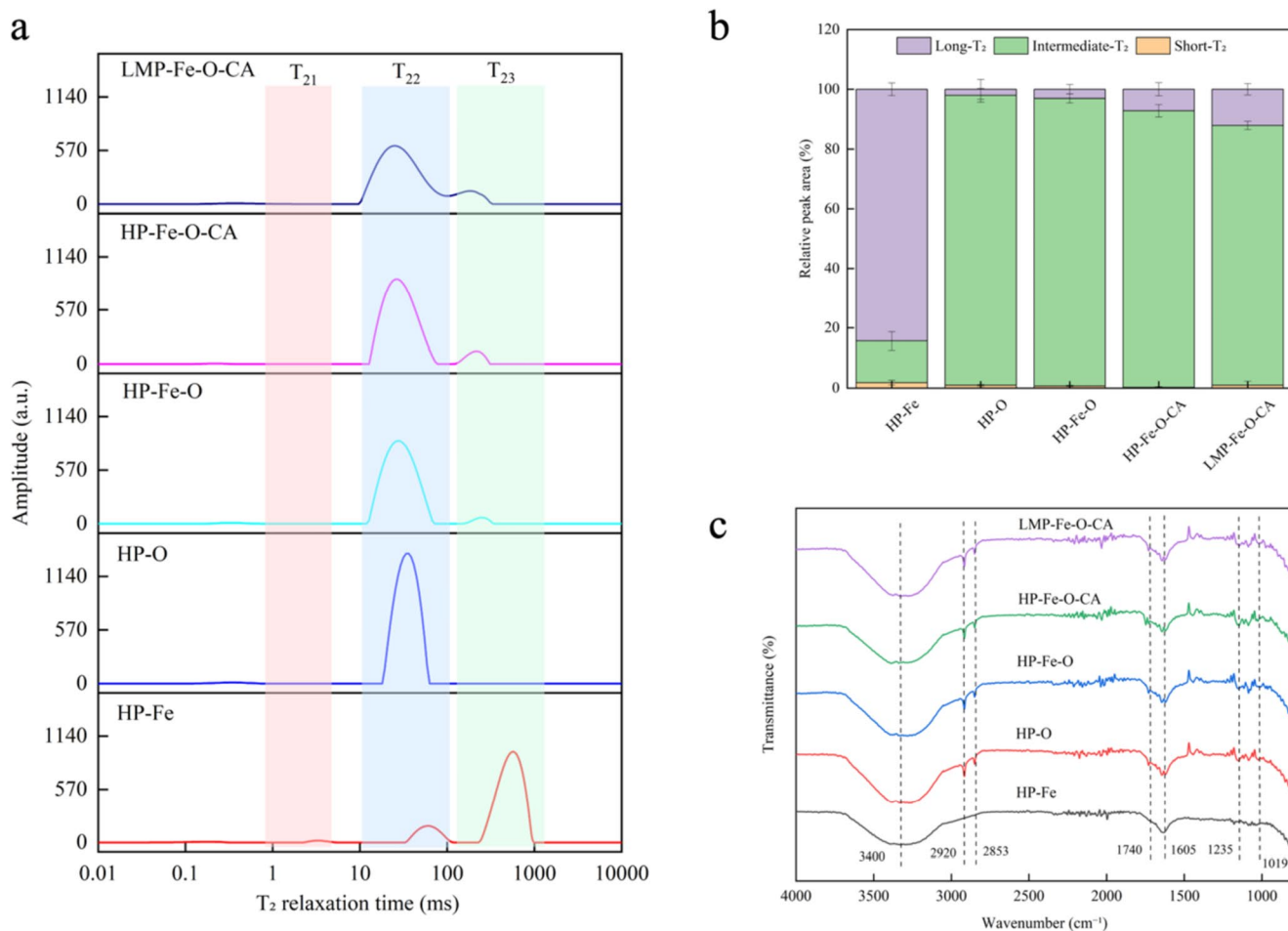


Fig. 2 Low-field NMR T_2 relaxation distributions (a), relative peak-area distributions of the short-, intermediate-, and long- T_2 relaxation-associated proton populations (b), and Fourier transform infrared spectra (c) of different pectin-based systems

was largely preserved after formulation. The broad band around 3400 cm^{-1} was assigned to O–H stretching vibrations. The bands near 2920 and 2853 cm^{-1} were assigned to C–H/ CH_2 stretching vibrations, while the band around 1740 cm^{-1} was attributed to ester carbonyl stretching, which may arise from esterified pectin groups and, in oil-containing systems, lipid ester groups. The peaks at 1235 and 1019 cm^{-1} were attributed to C–O, C–O–C, and pyranose-ring skeletal vibrations, which are typical FTIR features of pectin [43, 44].

Relative to HP-O, the Fe^{2+} -containing systems, particularly HP-Fe-O, HP-Fe-O-CA, and LMP-Fe-O-CA, showed changes in band shape and relative intensity in the O–H and carboxyl-related regions. These spectral variations suggest changes in the local hydrogen-bonding environment and in the local environment of pectin functional groups after Fe^{2+} addition and acidification. However, FTIR provides only indirect evidence for such interactions, and the present spectra should not be interpreted as definitive proof of Fe^{2+} –carboxyl coordination. The absence of new characteristic bands indicates that the formulation process was mainly governed by non-covalent interactions and physical network organization rather than by the formation of new covalent bonds [23, 45, 46]. Therefore, the FTIR results are used here as supporting evidence for changes in the functional-group environment, in combination with rheology, texture, LF-NMR, and microscopy, rather than as a standalone chemical-state analysis.

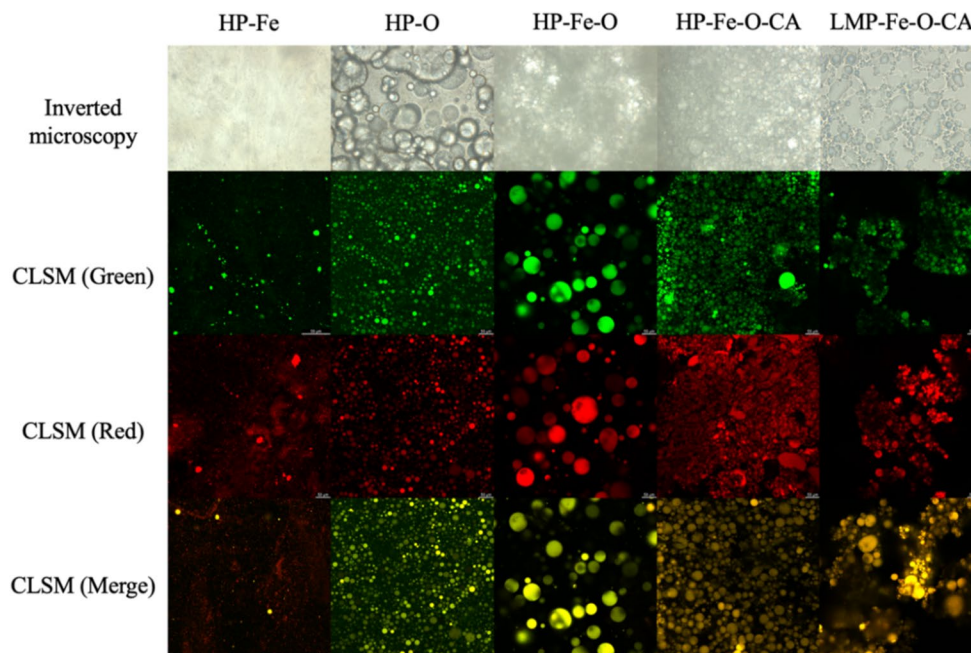
Microstructure by optical microscopy and CLSM

Microscopic observations revealed clear differences in microstructure among the pectin-based systems (Fig. 3). No typical droplet structure was detected in HP-Fe,

suggesting that it mainly existed as a continuous Fe^{2+} -containing matrix. In contrast, HP-O exhibited numerous droplets with relatively clear contours and a comparatively uniform distribution, suggesting the formation of a more dispersed emulsion. Compared with HP-O, HP-Fe-O displayed larger droplets and a broader size distribution, implying increased droplet association after Fe^{2+} incorporation. HP-Fe-O-CA showed a denser and more integrated microstructure, whereas LMP-Fe-O-CA also formed an aggregated network but still exhibited local clusters and heterogeneous regions. Previous studies have shown that the microstructure of pectin emulsions and emulsion gels is related to pectin molecular characteristics, interfacial adsorption behavior, and continuous-phase viscosity [22, 23]. The micrographs qualitatively showed that HP-Fe-O-CA had a more uniformly distributed continuous-phase fluorescence and a more integrated droplet–matrix organization than the other Fe^{2+} -containing formulations.

Rheological, textural, LF-NMR, and microscopic results indicate that HP-Fe-O-CA formed a more integrated gel-like matrix than the other Fe^{2+} -containing formulations [23, 33, 41, 42]. Although this formulation showed neither the highest absolute ζ -potential nor the smallest apparent droplet/aggregate size, it exhibited greater deformation resistance, distinct proton-relaxation behavior, and a more integrated droplet–matrix organization. These results suggest that its structural stability was mainly associated with matrix reinforcement and droplet/aggregate immobilization, rather than electrostatic stabilization alone [23, 27, 40, 48].

Fig. 3 Inverted optical microscopy and CLSM images of different pectin-based systems. In the CLSM images, green fluorescence was associated with the continuous aqueous/polar phase, whereas the oil phase was visualized with Nile Red. Scale bar = $50\text{ }\mu\text{m}$



Storage behavior, assay-detectable Fe^{2+} retention, and apparent oxidation-related indices

Appearance changes during storage

Figure 4 shows that the pectin-based systems differed markedly in appearance stability during storage. HP-Fe displayed a weak hydrogel-like character and could not maintain its original shape after inversion, consistent with limited structural integrity. HP-O also showed flowability and visible separated phase release during storage, suggesting limited stability of the emulsion system alone. In contrast, HP-Fe-O, HP-Fe-O-CA, and LMP-Fe-O-CA all displayed better shape retention. Among them, HP-Fe-O-CA maintained the most intact appearance and exhibited the weakest visible separated phase release throughout storage, whereas HP-Fe-O and LMP-Fe-O-CA had some local heterogeneity, slight exudation, and discoloration at later stages. These observations likely reflect differences in overall matrix integrity and droplet distribution [47].

Separated phase release

Data in Fig. 4b indicate that the separated-phase release of all samples showed an overall increasing numerical trend during storage. HP-Fe showed the highest separated phase release, suggesting that the pectin/ Fe^{2+} hydrogel network alone was insufficient to resist relaxation and gravitational

separation during storage. HP-O showed the second-highest separated phase release, suggesting that the emulsion system alone also provided limited resistance to separated phase release. In contrast, HP-Fe-O, HP-Fe-O-CA, and LMP-Fe-O-CA all gave markedly lower separated phase release, among which HP-Fe-O-CA consistently exhibited the lowest values throughout storage. These results indicate that HP-Fe-O-CA maintained its structure more effectively during storage than the other systems [10].

Assay-detectable Fe^{2+} retention during storage

During 28 d of storage, assay-detectable Fe^{2+} retention showed an overall decreasing numerical trend in all iron-containing samples, but clear formulation-dependent differences were observed (Fig. 4c). HP-Fe-O-CA consistently showed the highest retention, followed by HP-Fe-O and LMP-Fe-O-CA, whereas HP-Fe showed the lowest values. This pattern suggests that the more deformation-resistant and less phase-separated HP-Fe-O-CA matrix was associated with better preservation of Fe^{2+} in a phenanthroline-detectable form under the defined extraction and assay conditions. This result should be interpreted as analytical Fe^{2+} preservation rather than total iron retention. The advantage of HP-Fe-O-CA became more pronounced at later storage stages, indicating that the integrated emulsion-gel structure contributed to maintaining assay-detectable Fe^{2+} over time.

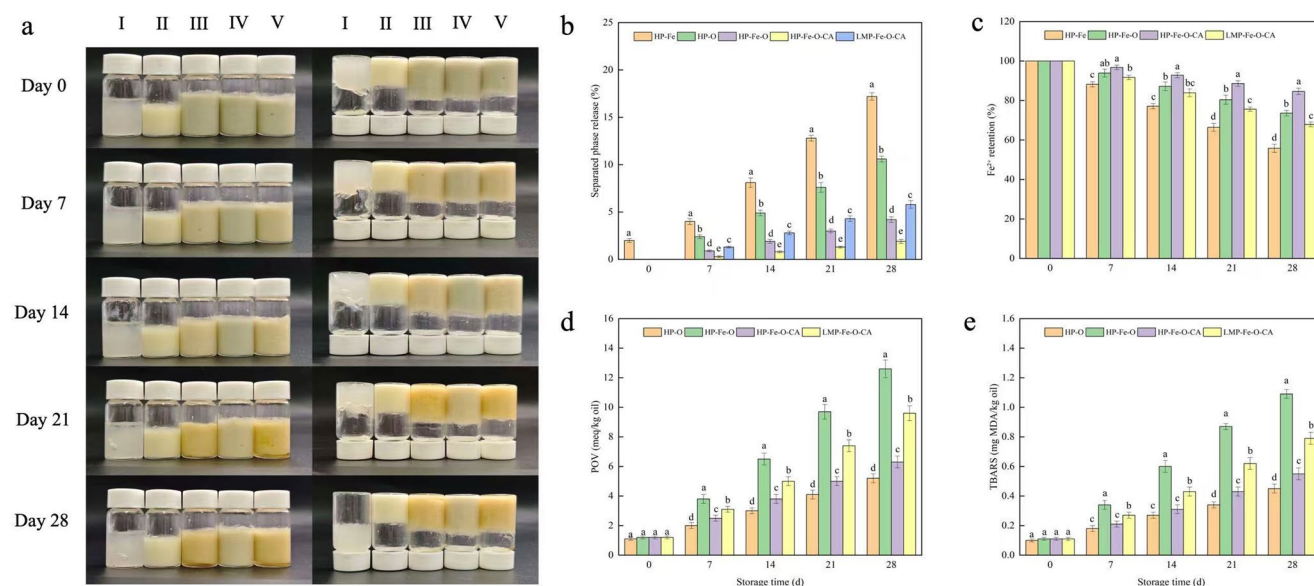


Fig. 4 Storage behavior of different pectin-based systems during 28 d of storage. (a) Representative appearance images in the upright (left) and inverted (right) states on days 0, 7, 14, 21, and 28. I, HP-Fe; II, HP-O; III, HP-Fe-O; IV, HP-Fe-O-CA; V, LMP-Fe-O-CA. (b) Separated phase release. (c) Assay-detectable Fe^{2+} retention. (d) Reagent-

blank-corrected apparent POV signal. (e) Reagent-blank-corrected apparent TBARS signal. Values are expressed as mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among formulations at the same storage time ($p < 0.05$)

Higher assay-detectable Fe^{2+} retention in HP-Fe-O-CA was consistent with its stronger gel structure, lower separated phase release, and more integrated matrix organization [24, 48]. Previous studies have shown that acid- or ion-induced pectin emulsion gels can affect the retention and release of encapsulated components by regulating network compactness and interfacial organization, while structural differences among low-methoxyl pectins may further influence gel performance [48]. Fe^{2+} retention during storage was more likely related to the overall emulsion-gel architecture and pectin–cation interactions than to a single chemical factor [40, 48]. However, because the present assay only quantified phenanthroline-detectable Fe^{2+} under defined extraction conditions, further total iron determination and $\text{Fe}^{2+}/\text{Fe}^{3+}$ speciation are needed to clarify whether the decrease in detectable Fe^{2+} resulted from oxidation, precipitation, stronger matrix association, or reduced extractability.

A focused matrix-effect validation was performed to assess the comparability of the 1,10-phenanthroline response among the formulation extracts and representative digestion supernatants (Table S3). The final reaction pH ranged from 2.54 to 6.82, and the sample-specific blank absorbance at 510 nm ranged from 0.016 to 0.063. Recoveries ranged from 95.62% to 100.62%, with RSD values below 5.06%. These results indicate broadly comparable analytical recovery across the tested matrices and support the comparative interpretation of the assay-detectable Fe^{2+} results.

Apparent lipid-oxidation-related indices during storage

During storage, the reagent-blank-corrected apparent POV and TBARS signals showed overall increasing numerical trends in the oil-containing formulations (Fig. 4d and e). HP-Fe-O consistently showed the highest signals, whereas HP-O showed the lowest. Among the Fe^{2+} -containing emulsion gels, HP-Fe-O-CA exhibited lower apparent POV and TBARS signals than HP-Fe-O, while LMP-Fe-O-CA showed intermediate values. These results indicate that HP-Fe-O-CA displayed a more favorable apparent oxidation-related response than the corresponding non-acidified Fe^{2+} emulsion gel under the defined analytical conditions.

The lower apparent oxidation-related signals of HP-Fe-O-CA occurred together with higher hardness, lower separated-phase release, distinct proton-relaxation behavior, and a more integrated microstructure. The combined results support an integrated formulation effect involving matrix integrity, phase organization, proton-relaxation characteristics, and the local environment of Fe^{2+} [49, 50]. The

POV and TBARS values are therefore interpreted as comparative reagent-blank-corrected apparent signals reflecting the response of each complete formulation under the same assay conditions.

In vitro digestion behavior and nominal-volume-normalized supernatant Fe^{2+} indices

Nominal-volume-normalized supernatant Fe^{2+} index during *in vitro* digestion

The nominal-volume-normalized supernatant Fe^{2+} index showed an overall increasing numerical trend as digestion proceeded, although distinct formulation-dependent patterns were observed (Fig. 5a). HP-Fe showed higher apparent values during the oral and gastric phases. In contrast, HP-Fe-O-CA showed lower early-stage values followed by a pronounced numerical increase during the intestinal phase, eventually reaching the highest final value, followed by LMP-Fe-O-CA and HP-Fe-O. These results indicate that HP-Fe produced a higher early-stage nominal-volume-normalized supernatant Fe^{2+} index, whereas the citrate-acidified emulsion-gel system showed lower early-stage but higher final intestinal values under the present static digestion conditions [23]. The comparable spike recoveries obtained for the representative oral, gastric, and intestinal supernatants indicated that the observed digestion-stage differences were unlikely to result primarily from systematic matrix-dependent enhancement or suppression of the phenanthroline response.

Low early-stage and high late-stage nominal-volume-normalized supernatant Fe^{2+} index values were consistent with pH-responsive reorganization of the matrix during sequential digestion. Under gastric conditions, the acidic environment can reduce carboxylate ionization and help maintain a compact emulsion-gel matrix, thereby limiting the early transfer of detectable Fe^{2+} into the supernatant. After transition to the intestinal phase, the increase in pH can enhance carboxylate ionization, promote network swelling or loosening, and reduce the strength of some Fe^{2+} -associated interactions [24, 40, 51]. At the same time, exposure to porcine bile extract and pancreatic enzyme preparations may have contributed to matrix reorganization and changes in the partitioning of digestion products, which may facilitate the transfer of Fe^{2+} into the aqueous supernatant. Under the INFOGEST conditions used here, the intestinal increase in the nominal-volume-normalized supernatant Fe^{2+} index is best described as digestion-stage redistribution of phenanthroline-detectable Fe^{2+} within a progressively reorganized emulsion-gel matrix, rather than as direct evidence of enhanced iron absorption.

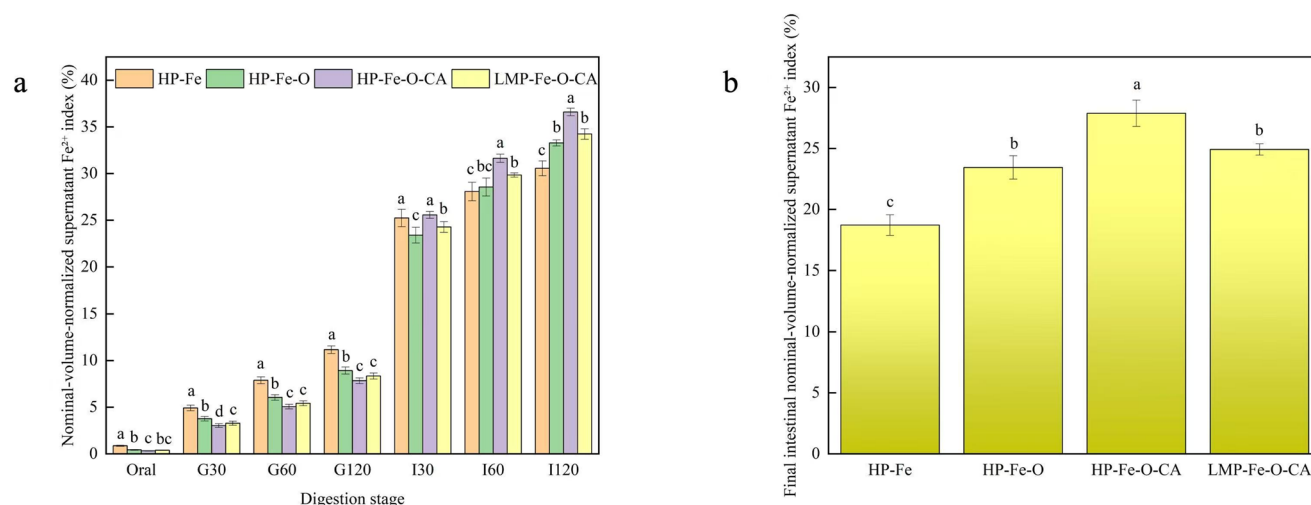


Fig. 5 Nominal-volume-normalized supernatant Fe²⁺ indices of different pectin-based systems during *in vitro* digestion. **(a)** Digestion-stage nominal-volume-normalized supernatant Fe²⁺ index. Oral denotes the end of the oral phase; G30, G60, and G120 denote 30, 60, and 120 min of the gastric phase, respectively; and I30, I60, and I120 denote 30, 60, and 120 min of the intestinal phase, respectively. **(b)** Final intestinal nominal-volume-normalized supernatant Fe²⁺ index.

Final intestinal nominal-volume-normalized supernatant Fe²⁺ index

Final intestinal nominal-volume-normalized supernatant Fe²⁺ indices differed among formulations. HP-Fe-O-CA showed the highest value, followed by LMP-Fe-O-CA and HP-Fe-O, whereas HP-Fe showed the lowest value. Together with the matrix-validation results, this finding indicates that HP-Fe-O-CA combined higher assay-detectable Fe²⁺ retention during storage with a higher final intestinal nominal-volume-normalized supernatant Fe²⁺ index under the defined analytical conditions. A higher early-stage index did not necessarily correspond to a higher final intestinal index, indicating that formulation-dependent redistribution remained important during digestion [51].

A higher final value for HP-Fe-O-CA may be related to digestion-induced reorganization of the emulsion-gel matrix. Potential contributors include pH-dependent changes in carboxylate ionization, partial weakening of Fe²⁺-associated interactions, and matrix disruption by bile salts and digestive enzymes [24, 40, 51]. However, this endpoint quantifies only phenanthroline-detectable Fe²⁺ recovered from the final intestinal supernatant under the specified centrifugation and assay conditions. It should therefore be regarded as a comparative digestion-stage supernatant Fe²⁺ index, rather than as total iron bioaccessibility or direct evidence of *in vivo* iron absorption. Further work using pH-matched controls, total iron determination, and Fe²⁺/Fe³⁺ speciation would help clarify the individual roles of pH, citrate complexation, and pectin structure.

tinal nominal-volume-normalized supernatant Fe²⁺ index. Nominal oral, gastric, and intestinal digestion volumes were used as consistent normalization factors. Values are expressed as mean ± SD (*n* = 3). Different lowercase letters indicate significant differences among formulations at the same digestion stage or time point (*p* < 0.05).

Conclusion

This study used multiscale characterization to evaluate the relationships among matrix structure, storage behavior, and digestion-stage supernatant Fe²⁺ characteristics in Fe²⁺-loaded HP/LMP pectin systems prepared with oil incorporation and a citrate-containing acidic medium. The results showed that HP-Fe-O-CA exhibited the highest *G'* and hardness, the lowest separated-phase release, a distinct proton-relaxation profile, and the highest assay-detectable Fe²⁺ retention. Compared with HP-Fe-O, HP-Fe-O-CA showed lower reagent-blank-corrected apparent POV and TBARS signals under the defined analytical conditions. During static *in vitro* digestion, HP-Fe-O-CA showed a lower early-stage nominal-volume-normalized supernatant Fe²⁺ index and the highest final intestinal index. Overall, among the tested formulations, HP-Fe-O-CA combined greater matrix integrity and higher assay-detectable Fe²⁺ retention with favorable apparent oxidation-related signals and a higher final intestinal nominal-volume-normalized supernatant Fe²⁺ index under the defined experimental conditions.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1007/s11694-026-04827-4>.

Author contributions Hao Zhang: Investigation, Methodology, Data curation, Validation, Writing – original draft; Xiaosheng Feng: Data curation, Writing – original draft; Weijia Zhu: Investigation, Methodology, Writing – original draft; Christophe Blecker: Methodology, Supervision, Writing – review & editing; Chuanhe Zhu: Conceptualization, Project administration, Funding acquisition, Supervision, Visualization, Writing – review & editing.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval Not applicable.

Consent for publication Not applicable.

Consent to participate Not applicable.

Competing interests 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.

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