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Enhanced thermal stability of potato protein-pectin emulsions: Synergistic effects of bulk and interfacial properties

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ABSTRACT

Proteins and polysaccharides have been used extensively in emulsion formulation, both individually and in conjunction. However, the mechanisms underlying the thermal stability of protein-stabilized emulsions in the presence of pectin require further elucidation. Here, we systematically investigate the effect of thermal processing on such emulsions (with protein-pectin mass ratio of 1:0, 10:1, 5:1, 2:1, 1:1, and 0:1). The thermal behavior (20–95 °C) of potato protein isolate (POPI) and pectin was studied through: (1) continuous-phase properties (2) interfacial characteristics, and (3) the thermal stability of 45 wt% oil-in-water (O/W) emulsions at pH 4. POPI-pectin mixtures at ratios of 2:1 and 1:1 exhibited smaller particle size and strongly negative zeta potentials compared to higher ratios (10:1, 5:1). Heating and subsequent cooling increased both continuous-phase viscosity and interfacial elastic modulus, most probably through hydrophobic interactions, with a 2:1 ratio achieving higher interfacial elasticity values that exceed those of protein interfaces. Emulsions stabilized by the 2:1 mixture were thermally stable, maintaining small droplet sizes (~3 μm; compared to ~8 μm for protein-stabilized emulsions) without aggregation, coalescence, or creaming over 5 days. The enhanced thermal stability is attributed to a synergistic mechanism, in which heating induces gelation in the continuous phase and enhances elastic film formation at the interface. The findings are expected to be relevant for plant protein-stabilized emulsions, of which stability can be improved by clever combination with polysaccharides.

1. Introduction

The shift toward plant-based alternatives for traditional animal-derived emulsifiers comes with challenges, especially in terms of thermal stability (Einhorn et al., 2021). The thermal stability of emulsions decreases near the iso-electric point (IEP) of proteins, where charge stabilization diminishes (Guldiken et al., 2023). To mitigate this, the combination of protein with polysaccharides has been suggested. Protein-pectin complexes can be formed via electrostatic interaction in the continuous phase, and their properties are strongly influenced by pH, biopolymer ratio, biopolymer concentration, and ionic strength, etc. (Lan et al., 2018, 2020; Yavuz-Düzgün et al., 2020). Alternatively, complexes can be formed through thermal treatment, which may lead to Maillard reaction products that improve emulsion stability from both physical and chemical perspectives (Feng et al., 2022, 2023). In literature, both enhanced continuous-phase and interfacial properties have been reported (Feng et al., 2021; Ma et al., 2019), mostly at room temperature. However, more fundamental understanding of how especially plant proteins interact with polysaccharides under thermal processing, an intrinsic part of food production, is needed for effective food emulsion design.

In the continuous phase, protein-polysaccharide interactions are enhanced at higher temperature, leading to increased viscosity and even the formation of heat-induced gels (Dickinson, 2008; Esfanjani et al., 2017; Jones & McClements, 2011). Upon cooling, additional stabilization can occur through hydrogen bonding, contributing to the formation of structured gel networks (Savadkoohi & Farahnaky, 2012; Zhang et al., 2024). These thermal responses are well studied in dairy protein-polysaccharide systems, including whey protein isolate (Kotchabhakdi & Vardhanabhuti, 2020; Zhang et al., 2012) and β-lactoglobulin (Hong & McClements, 2007). For plant proteins, studies have mostly focused on characterizing bulk properties at room temperature, showing that combinations such as soy protein-pectin (Ma et al., 2019; Yildiz et al., 2018), pea protein-pectin (Gharsallaoui et al., 2010; Guldiken et al., 2023; Lan et al., 2018, 2020; Yildiz et al., 2018), and hemp seed protein-pectin (Feng et al., 2021) enhance viscosity and storage modulus. Very few studies have examined the heat-induced thickening and gelation behavior of plant protein-pectin complexes

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(Zhang et al., 2024), which is critical for designing thermally stable emulsions.

At the oil-water interface, protein-polysaccharide complexes can act as effective emulsifiers by forming viscoelastic films (Feng et al., 2021; Guldiken et al., 2023). The adsorption of proteins and polysaccharides at the interface, and their subsequent intermolecular interactions, reduce the interfacial tension and strengthen the interfacial layer (Kim et al., 2005; Lam & Nickerson, 2013; Zhou et al., 2021). Protein-pectin complexation, in particular, has been shown to enhance interfacial rheological properties such as shear and dilatational elasticity, without compromising interfacial activity (Tamm & Drusch, 2017). Emulsions stabilized by complexes such as pea protein-pectin (Gharsallaoui et al., 2010) and lentil protein-fenugreek gum (Gadkari et al., 2019) exhibit improved interfacial rigidity. For proteins, it has been found that heat-induced conformational changes in adsorbed layers can enhance interfacial viscoelasticity, contributing to stronger interfacial films that resist coalescence (Zhou et al., 2021). A logical next step is to investigate protein-polysaccharide interactions in the continuous phase and at the interface, both at elevated temperature and upon cooling, and explore how these interactions jointly influence emulsion stability.

Among plant proteins, potato protein isolate (POPI) is a promising source due to its abundance, non-allergenic nature, and high nutritional value (Glusac et al., 2017; Tan et al., 2023). Unlike other plant proteins with more rigid globular structures, such as those from soy, pea, lentil, and faba bean, POPI stands out as a promising emulsifier due to the relatively low molecular weight of its main components (patatin: 40–45 kDa; protease inhibitor: 5–25 kDa), its high solubility, flexible conformation, and high surface activity (Tan et al., 2023). In addition, its ability to form a gel further enhances its utility in forming stable food matrices (Cao et al., 2024; Cao et al., 2025; Sreeprakash et al., 2025; O'Sullivan et al., 2017). In recent studies, Eichhorn et al. (2025) characterized the interfacial rheological properties of potato protein-pectin conjugates obtained via Maillard reaction, demonstrating that they form viscoelastic films at the oil-water interface, with stabilization behavior significantly influenced by pH and pectin type. Some investigations also suggest that these complexes may also improve sensory profiles, perhaps through bitterness masking (Yavuz-Düzgün et al., 2020). However, no studies have examined the interaction between POPI and pectin under thermal conditions. Particularly, it is unclear how temperature-induced changes in both continuous-phase and interfacial properties influence the overall thermal stability of emulsions stabilized by POPI-pectin mixtures. This represents a critical knowledge gap, especially given the relevance of heat treatment in realistic food emulsion production.

This study addresses these research gaps by systematically investigating how continuous-phase rheology and interfacial properties are affected by temperature treatment and how these aspects jointly determine the thermal stability of O/W emulsions stabilized by POPI and pectin at pH 4. We first characterize the POPI-pectin mixtures with different mass ratio (1:0, 10:1, 5:1, 2:1, 1:1, and 0:1) by measuring the zeta-potential and particle size. Next, we examine temperature-dependent changes (heating 20–95 °C, cooling 95 – 20 °C) in continuous-phase rheology (storage modulus, and viscosity) and in properties of oil-water interfaces, using rheometer and dynamic automated droplet tensiometry, respectively. Finally, we correlate these aspects to the thermal stability of O/W emulsions (45 wt% oil), providing mechanistic insights into how protein-pectin interactions enhance stability through synergistic effects in the continuous phase and at the interface.

2. Materials and methods

2.1. Materials

Potato protein isolate (protease inhibitor-rich protein fraction, POPI, ~90 % protein *w/w*) was provided by Royal Avebe (Veendam, The Netherlands). The protease inhibitory activity was already lost during the ingredient's production process prior to our experiments, and was not assessed in this study. Pectin from citrus peel (GA≥74.0 %, dried basis) was obtained from Sigma-Aldrich (Saint Louis, MO, USA), and the degree of esterification (DE) is ~55–65 %. Sunflower oil (Borges Agricultural & industrial edible oils, Tarrega, Spain) was bought from a local supermarket (Jumbo). Sodium phosphate monobasic, phosphoric acid, and sodium dodecyl sulfate (SDS) were from Sigma Aldrich (Saint Louis, MO, USA) and were at least of analytical grade. Sodium hydroxide and hydrochloric acid (37 %) were purchased from Supelco (EMD Millipore Corporation, Burlington, MA, USA) and VWR Chemicals BDH® (Fontenay-sous-Bois, France), respectively. All chemicals were used as received. Deionized water from a Milli-Q system (Millipore Corporation, Billerica, MA, USA) was used for all experiments.

2.2. Preparation of POPI-pectin mixtures

Protein-pectin mixtures were prepared in varying mass ratios (protein-pectin of 1:0, 10:1, 5:1, 2:1, 1:1, and 0:1), with a total constant concentration of the biopolymers (potato protein and pectin) at 0.6 wt% (~6 g/L), or 0.9 wt% (~9 g/L), according to previous studies with some modifications (Feng et al., 2021; Jones and McClements, 2011; Lan et al., 2018).

Potato protein and pectin were separately dissolved in the same volume of phosphate buffer (pH 4) at 0.6 or 0.9 wt%. Each solution was stirred for 1 h using a magnetic stirrer (IKA C-MAG HS 7, IKA-Werke GmbH & Co. KG, Staufen, Germany). The POPI and pectin solutions were then mixed (volume ratio 1:1) and stirred for another hour. Next, the mixtures were stored in the fridge (4 °C) overnight (~15 h) for complete hydration. The next day, the pH of the solutions was readjusted to pH 4 using 0.5 M NaOH or 0.5 M HCl before the measurement.

2.3. Characterization of protein, pectin, and their mixtures

2.3.1. Zeta potential and particle size measurements

POPI, pectin, and POPI-pectin mixtures (~6 g/L) were diluted 60 times using phosphate buffer (pH 4) before measurement, to obtain 0.1 g/L. The zeta potential and average size were measured in a folded capillary cell (DTS 1070, Malvern Instruments, Worcestershire, UK) by dynamic light scattering (Zetasizer Ultra, Malvern Panalytical Ltd., Almelo, The Netherlands). The refractive indices were set at 1.360 for the POPI, 1.55 for the pectin and POPI-300-Pectin mixtures, and 1.33 for water, following the method described by Cao et al. (2024). Three measurements were taken at pH 4 and analyzed with ZS Xplorer 3.0.0.53.

2.4. Continuous-phase properties

Rheological properties (storage modulus, and viscosity) of POPI, pectin, and POPI-pectin mixtures (6 g/L) were measured using an Anton Paar Rheometer MCR 702 (Anton Paar, Breda, The Netherlands). A concentric cylinder geometry CC17 measuring system with a diameter of 16.66 mm and a length of 24.858 mm was used for 5 mL sample, as reported by Cao et al., 2025.

2.4.1. Viscosity at 20 °C

Continuous shear tests were conducted across shear rates ranging from 1 to 100 s^{-1} at 20 °C (Cao et al., 2024; Li et al., 2024; Lomolino et al., 2022), and the apparent viscosity of the sample was determined as a function of the shear rate.

2.4.2. Network formation

To determine the influence of temperature on gel network formation in the bulk, an oscillatory measurement was performed, using a temperature ramp (heating and cooling). Measurement started with a 5 min equilibration period at 20 °C. Next, the samples were heated from 20 to

95 °C at a heating rate of 1.5 °C/min, then kept at 95 °C for 10 min and subsequently cooled to 20 °C at a cooling rate of 3.75 °C/min. At last, the temperature was held at 20 °C for 5 min (Li et al., 2024; Tanger et al., 2022). A fixed oscillation frequency of 1 Hz and 0.2 % strain were used throughout these experiments. Storage modulus, and viscosity were determined throughout the described temperature ramp.

2.5. Oil-water interface properties

The TRACKER™ Automatic Drop Tensiometer (ADT) with Dilatational Interfacial Rheometer (Teclis, Civrieux-d'Azergues, France) was used to study the interfacial properties in an oil/water system. The interfacial tension (γ_0), dilatational interfacial elasticity, and Lissajous plot were determined according to the methods used by Shen et al., 2025, Hinderink et al. (2021), and Corstens et al. (2017).

2.5.1. Preparation of the continuous phase

Before measurement, all protein, pectin, and mixture solutions (initial concentrations of 6 g/L or 9 g/L) were diluted in a pH 4 phosphate buffer to obtain final total concentrations of 0.1 g/L or 0.15 g/L. Subsequently, 30 mL solution was transferred into a cuvette that was placed in a desiccator, which was evacuated to the lowest achievable pressure (targeting pressures below 20 mbar) to avoid air bubbles during the heating process. The sample was kept under vacuum for at least 5 min to ensure maximum removal of dissolved gases.

2.5.2. Adsorption behavior and dilatational rheology

A sunflower oil droplet was formed at the tip of a J-shaped syringe (SGE Syringe, 500 μL Luer Lock, Trajan Scientific Australia Pty Ltd, Ringwood, Australia), and immersed into a cuvette containing the continuous phase. To control the temperature of the bulk phase and syringe, a water bath was connected to the Automatic Drop Tensiometer. The densities of the oil droplet and continuous phase were set as 0.921 g/cm^3 and 1.000 g/cm^3 respectively.

The interfacial tension (γ_0) was monitored for 3 h to reach a rather constant value at 20 °C, using a drop area of 30 mm^2. Following this adsorption period, oscillatory dilatational deformations were applied at a constant frequency of 0.1 Hz at 20 °C, with deformation amplitudes of 5 and 30 %, to determine the interfacial elasticity modulus. Next, the temperature of the bulk phase was increased stepwise to 40, 50, 60, and 70 °C by adjusting the water bath temperature, with the same oscillatory measurements performed at each step. After reaching 70 °C, the temperature was decreased in reversed order (60, 50, 40, and back to 20 °C), again performing oscillations at each temperature. Due to heat loss, the actual temperatures were slightly lower than that of the water bath, approximately 20, 40, 51, 63, and 73 °C, respectively. The oscillating interfacial tension signal was analyzed with a Fast Fourier transform, and the intensity and phase of the first harmonic were used to calculate the elasticity modulus.

To further understand the non-linear viscoelastic behavior of the interfacial layers, Lissajous plots were made by plotting interfacial pressure changes ($\Delta\pi$) against the relative oscillating deformation of the interface ($\Delta A/A_0$), where $\Delta\pi = \gamma - \gamma_0$, $\Delta A = A - A_0$, with γ and γ_0 referring to the interfacial tensions of the deformed and undeformed oil-water interface, respectively; while A and A_0 refer to the interfacial area of the deformed and undeformed interface, respectively (Kempen et al., 2013; Sagis & Scholten, 2014).

2.5.3. Correction of the elasticity modulus

To identify the emulsifier's contribution at the interface, the elasticity modulus values were adjusted by compensating for the effects related to differences in viscosity and interfacial tension of a bare oil-water interface at different temperatures.

2.5.3.1. Correction for viscosity effects. Equation (1) is used to determine the elastic modulus by removing viscous effects.

$$E'_{elastic} \approx E' - E'' \cdot \tan\delta \quad (1)$$

Here, E' is the elastic modulus ("RealPart (mN/m)"), E'' is the viscous modulus ("Imaginary part (mN/m)"), and the phase shift (δ) is a critical parameter that quantifies the time lag between the applied oscillatory deformation.

2.5.3.2. Correction for interfacial tension effects. In the range investigated here, interfacial tension of the bare oil-water interface linearly decreases with increasing temperature (Bui et al., 2021), and the elasticity modulus values obtained from the Automatic Drop Tensiometer were corrected accordingly. Previous studies reported the interfacial tension of the hexadecane-water interface decreases by about 0.07 mN/m per °C (Deng et al., unpublished results; *under revision*). Similarly, the surface tension of vegetable oil-air interfaces was found to decrease by approximately 0.07 mN/m per °C (Sahasrabudhe et al., 2017). Based on these findings, the temperature dependency of the bare sunflower oil-water interfacial tension was estimated using Equation (2). Subsequently, the effective interfacial elasticity modulus (E'_{eff}) of emulsifier-stabilized interface is calculated by Equation (3).

$$\gamma_0\,(T) = \gamma_{0,\ 20^\circ C} - kT\ (k \approx 0.07\ \text{mN/m per}\ ^\circ\text{C}) \quad (2)$$

$$E'_{eff} = E'_{elastic} \cdot \frac{\gamma_{0,20^\circ C}}{\gamma_0(T)} \quad (3)$$

Here, $\gamma_{0,20^\circ C}$ and $\gamma_0\,(T)$ is the equilibrium interfacial tension of bare oil-water interface at 20 °C and other temperatures (Table S1), respectively. $E'_{elastic}$ is defined in (Equation (1)).

2.6. Emulsion preparation and characterization

2.6.1. Preparation of O/W emulsions

Based on the results obtained, POPI-pectin mixtures with different mass ratios (1:0, 10:1, 2:1 and 0:1) were tested on their emulsification properties. After preparation of the protein-pectin mixture solutions as described in Section 2.2, a two-step homogenization process was used to prepare the O/W emulsions containing 45 wt% sunflower oil at pH 4.0 following the method described by Cao et al. (2024). In short, all solutions were exposed to a high-speed disperser (S18N-19G, Ultra-turrax IKA T18 digital, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 3000 rpm for 90 s, after which the sunflower oil was added during mixing at 6000 rpm. A coarse premix emulsion was obtained by increasing mixing to 12000 rpm for 40 s, after which the pH was adjusted to 4.0. Then, the homogenization took place using a lab-scale colloid mill (IKA Magic Lab, Staufen, Germany) with a gap width of 0.32 mm at 17000 rpm for 4 min.

Fresh emulsions were heated in a water bath at 95 °C for 30 min and then cooled to room temperature in the refrigerator. The droplet size was measured on the day of preparation (day 1) and day 5 using the methods described next.

2.6.2. Droplet size

The droplet size distribution was measured by static light scattering using a Master-sizer 3000 (Malvern Instruments Ltd.; Worcestershire, UK). The refractive index was set at 1.465 for the dispersed phase (sunflower oil) and 1.330 for the dispersant (water). An absorption index of 0.01 was applied. For measurements, emulsions were diluted (1:10, *v/v*) with deionized water or 1 wt% SDS solution prior to measurement to break down possible aggregates and distinguish this effect from droplet size increase due to coalescence (Hammouda, 2013; Hinderink et al., 2019). Droplet size was reported as the average Sauter mean diameter ($d_{3,2}$) of at least two independently prepared emulsions, each of which were measured three times.

2.7. Statistical analysis

All protein and pectin solutions and emulsions were prepared in at least in duplicate, and each droplet size measurement was performed in triplicate to ensure reliability and reproducibility. The data were analyzed by variance (ANOVA) and Duncan test from the SPSS 30.0. Differences were considered statistically significant at $p < 0.05$, with different letters in the figures indicating significant differences between groups. Data were expressed as mean ± standard deviation (SD). Measurements on the bulk and interfacial rheology were also done at least as independent duplicates.

3. Results and discussion

3.1. Characterization of protein, pectin, and their mixtures

Fig. 1 shows the zeta-potential and average particle size of POPI, pectin, and their mixtures at varying ratios (10:1, 5:1, 2:1, and 1:1) at pH 4, at a constant total concentration of 0.1 g/L. Protein-pectin ratio plays a critical role in the extent of aggregation (Ye, 2008). The POPI used in this study is a protease inhibitor (without any residual activity) with an iso-electric point (IEP) of approximately 7 (Cao et al., 2024), therefore, at pH 4, POPI has a positive zeta-potential (+12 mV), whereas pectin is negatively charged (−26 mV). These opposite charges create favorable conditions for electrostatic attraction. Accordingly, the zeta-potential of a mixture shifts toward more negative values with increasing pectin concentration (Fig. 1). This shift reflects protein-to-pectin charge neutralization, followed by a transition to pectin-dominated surface charge at higher ratios through the abundance of carboxylic groups in pectin (Yavuz-Düzgün et al., 2020).

The particle size of POPI alone (~209 nm) is larger than would be expected for monomeric potato proteins (typically <10 nm), indicating the presence of native supramolecular aggregates. Pectin alone (~971 nm), is as expected for a long, flexible polysaccharide (Fig. 1). For POPI-pectin mixtures, protein-to-pectin ratio strongly influences aggregation. The particle size of mixtures at higher protein-pectin ratios (2:1 and 1:1) is 354 nm and 402 nm, respectively; while at low pectin ratios (10:1 and 5:1) aggregates are much larger (4031 nm and 3154 nm, respectively) (Fig. 1), likely due to extensive aggregation of pectin and POPI molecules at practically neutral zeta potential (Yavuz-Düzgün et al., 2020). Under such condition, pectin interacts with multiple positively charged POPI aggregates, promoting bridging flocculation (Gharsallaoui et al., 2010).

The zeta potential and particle size of the samples may affect the bulk and interfacial properties. Subsequent sections explore the influence of temperature on the continuous phase and interfacial properties, which in the last section is linked to emulsion stability.

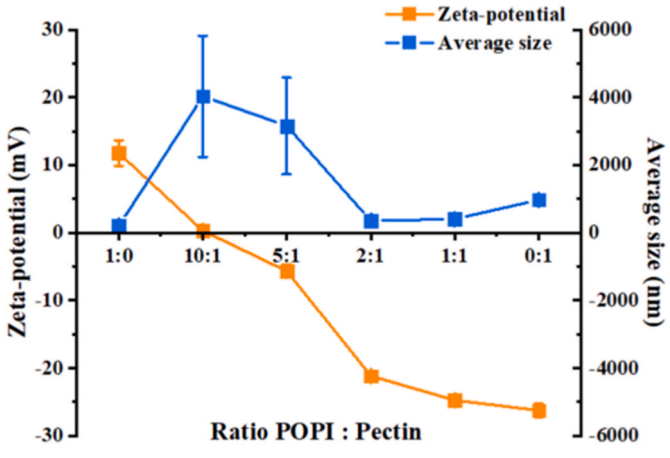

Fig. 1. Zeta potential (orange) and particle size (blue) of POPI, pectin, and POPI-pectin mixtures with different ratios (10:1, 5:1, 2:1, and 1:1) at pH 4. The total concentration of the protein and pectin is 0.1 g/L for all the samples. POPI: Potato protein isolate.

3.2. Continuous-phase properties

We discuss the influence of temperature on the rheological properties of protein, pectin, and POPI-pectin mixtures with different ratios (5:1, 2:1, and 1:1). The total concentration of POPI and pectin was 6 g/L. First, we characterize the viscosity of the samples at 20 °C. Subsequently, the viscosity and storage modulus are investigated during controlled heating and cooling cycles.

3.2.1. Viscosity at 20 °C

Fig. 2 presents the apparent viscosity of POPI, pectin, and their mixtures at 20 °C as a function of shear rate. The viscosity of pectin is the highest, and for POPI the lowest. The high viscosity of pectin arises from the extended polysaccharide chains (Kaya et al., 2014). They entangle and thereby enhance solution resistance to flow. The viscosity of the mixtures increased with increasing amount of pectin in the solutions, while at 10:1 and 5:1 ratio this effect is minimal. The mixtures with higher pectin proportions (2:1 and 1:1) had higher viscosity, albeit still below that of pectin only, which is understandable given the lower effective pectin concentrations present at the same overall concentration.

This concentration-dependent behavior aligns with previous observations in plant protein-pectin mixtures, where increasing polysaccharide concentration was reported to intensify molecular interactions through hydrogen bonding and electrostatic attraction, thus raising solution viscosity (Albano et al., 2019; Renard et al., 2006). For instance, the incorporation of high methoxyl pectin into pea protein dispersions has been shown to increase apparent viscosity more strongly at 2:1 ratio compared to 5:1 ratio (Lan et al., 2018), while potato protein-chitosan (Hu & Xiong, 2022) and potato protein-β-glucan (Li et al., 2024) systems display similar concentration-dependent viscosity behavior. Besides, the shear-thinning behavior observed here is consistent with other protein-polysaccharide solutions, such as pea/soy protein with modified starch or gum Arabic (Yildiz et al., 2018), which has been attributed to the disentanglement of biopolymer networks under

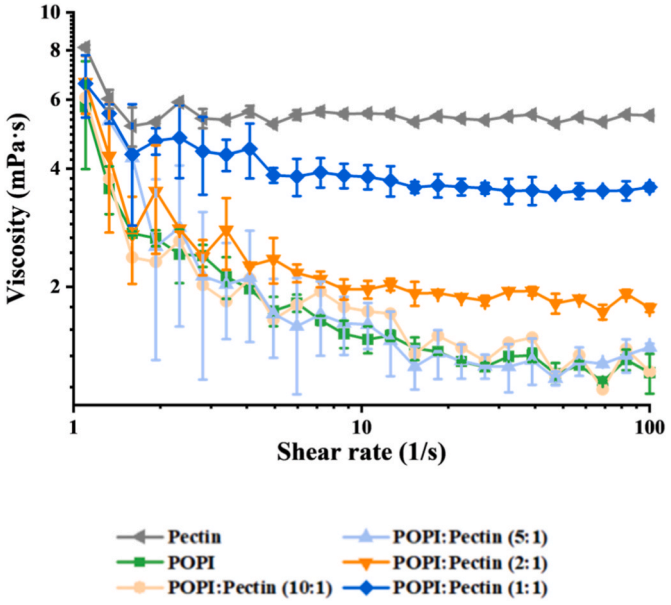

Fig. 2. Viscosity as a function of the applied shear rate for POPI, pectin, and POPI-pectin mixtures of different ratios (5:1, 2:1, and 1:1) at pH 4. The total concentration of protein and pectin is 6 g/L. POPI: Potato protein isolate.

shear.

3.2.2. Temperature-induced gelation in the continuous phase

Fig. 3 presents the rheological properties of POPI, pectin, and their mixtures during heating (20–95 °C) and cooling (95 – 20 °C). During heating, POPI and POPI-pectin mixtures showed an increase in storage modulus and viscosity at the gelation temperature of POPI (60–70 °C), corresponding to the thermal denaturation temperature (T_d) of POPI (Cao et al., 2025). Heating induces unfolding of proteins, exposing hydrophobic groups (confirmed by surface hydrophobicity of POPI after heating; Fig. S1) that were previously buried (Savadkoohi and Farahnaky, 2012). These changes promote hydrophobic interaction and intermolecular disulfide crosslinking, leading to protein gelation (Einhorn et al., 2021). Pectin alone kept a constant storage modulus throughout heating, which was slightly lower than most mixtures.

Upon cooling, all samples exhibited further increases in storage modulus and viscosity, with the most pronounced effects observed in the POPI-pectin mixtures. The final viscosity followed the order of POPI-pectin mixtures > POPI alone > pectin alone (Fig. 3B), indicating clear synergistic behavior. For the 1:1 mixture, the highest final modulus and viscosity were found, consistent with the temperature dependence of non-covalent interactions. More specifically, hydrogen bonding becomes more favorable at lower temperatures (Albano et al., 2019; Einhorn et al., 2021), and the close association between pectin chains and partially unfolded POPI enables extensive hydrogen bond formation, which strengthens the network. Pectin, with high degree of esterification, has also been suggested to provide steric stabilization and additional chain entanglement, thus further promoting network development (Wei et al., 2020). Together, hydrogen bonding and steric stabilization drive the formation of a denser and more elastic gel network during cooling (Xu et al., 2021).

Comparable increases in storage modulus and viscosity have been reported for other plant protein-polysaccharide systems. For instance, pea protein combined with low methoxyl pectin developed stronger viscoelastic gels during heating and cooling which was explained by intensified intermolecular associations (Zhang et al., 2024). In plant-based egg yolk systems containing potato protein and β-glucan, heating and holding at a high temperature (e.g., 90 °C), leads to further increase in gel strength (Li et al., 2024). Such a strengthened microstructure correlates with improved emulsion stabilization, as will be discussed in the last section.

3.3. Oil-water interface properties

To investigate temperature effects on interfacial properties, we worked with 2:1 POPI-pectin ratio at two concentrations, taking the two separate ingredients as references.

3.3.1. Adsorption behavior

Time-dependent interfacial tension measurements were carried out at pH 4.0 and 20 °C (Fig. 4). As expected, the interfacial tension decreased rapidly and subsequently levelled off for POPI (Fig. 4A; full curves: Fig. S2). Pectin hardly lowered interfacial tension on its own, suggesting poor interfacial activity, consistent with its large and hydrophilic polysaccharide structure (Albano et al., 2019).

For the POPI-pectin mixture (2:1 ratio), the interfacial tension showed a two-step process (Figs. 4A & 5C). Initially, the interfacial tension shows a similar trend as POPI alone at the same protein concentration (step 1, Fig. 4A), indicating that POPI dominates the early interfacial adsorption. POPI is the smaller molecule (5–25 kDa) (Fig. 1) and diffuses faster than pectin, allowing it to adsorb fast to the oil-water interface and act as a "bridge head". After the first step, a more pronounced and rapid decrease in interfacial tension occurs (step 2, Fig. 4A), exceeding the rate observed for protein alone. This suggests the subsequent adsorption of pectin or small POPI-pectin complexes. Pectin and POPI-pectin complexes in the continuous phase, can interact with the positively charged POPI already present at the interface. These processes result in the formation of a thicker layer that decreases the interfacial tension beyond what the protein alone can achieve (see also interfacial rheology section). In addition, the accelerated adsorption for mixtures at the oil-water interface was particularly evident at higher POPI concentrations (0.1 g/L), which also resulted in lower equilibrium interfacial tension values (Fig. 4B).

Depending on the POPI and pectin ratios (1:0, 5:1, 2:1, 1:1, 0:1), adsorption varied at a fixed total concentration (0.1 g/L) (Fig. S3). With an increasing fraction of pectin in the mixtures, the interfacial tension decreased slower, leading to higher equilibrium values. This is in line with the findings for Fig. 4. The concentration dependency on POPI may be an indication for the first explanation in which protein forms a 'bridge head' for further effects to take place, and not necessarily related to the complex as a whole. Others have explained similar effects based on hydrophobicity effects of potato protein-chitosan complex (Hu and Xiong, 2022) and lentil protein isolate-fenugreek gum complex (Gadkari et al., 2019).

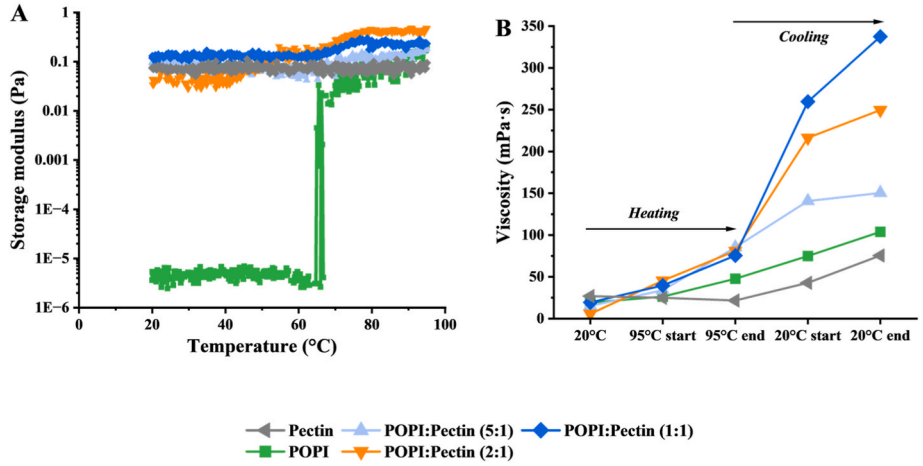

Fig. 3. Storage modulus (A) and viscosity (B) as a function of temperature for POPI, pectin, and POPI-pectin mixtures with different ratios (5:1, 2:1, and 1:1) at pH 4. The total concentration of the protein and pectin is 6 g/L for all the samples. We did at least two independent replicates, and one of the lines was used in the figure of the storage modulus. POPI: Potato protein isolate.

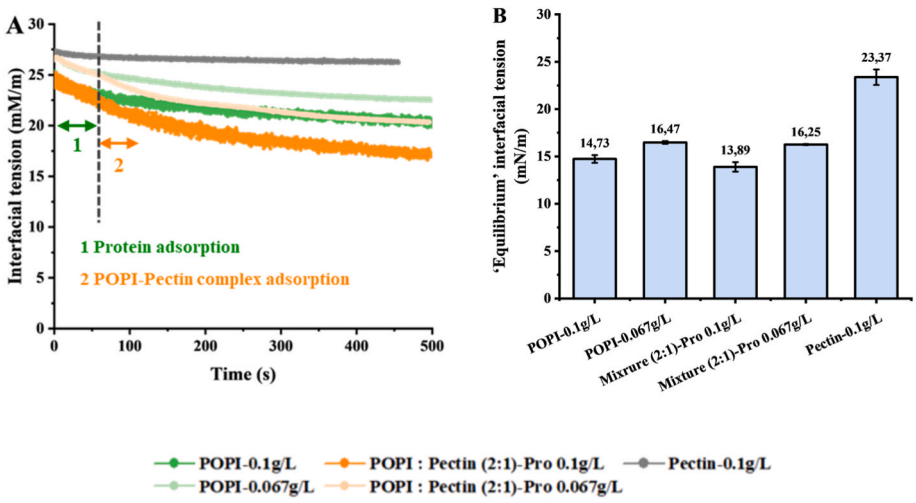

Fig. 4. Interfacial tension of oil-water interfaces stabilized by POPI (green), pectin (gray), and 2:1 POPI-pectin mixtures (orange) at pH 4.0 during adsorption within 500s (A) and at 'equilibrium' after 10000s of adsorption (B). We did at least two independent replicates, and one representative curve is shown in figure A to illustrate typical behavior. POPI: Potato protein isolate.

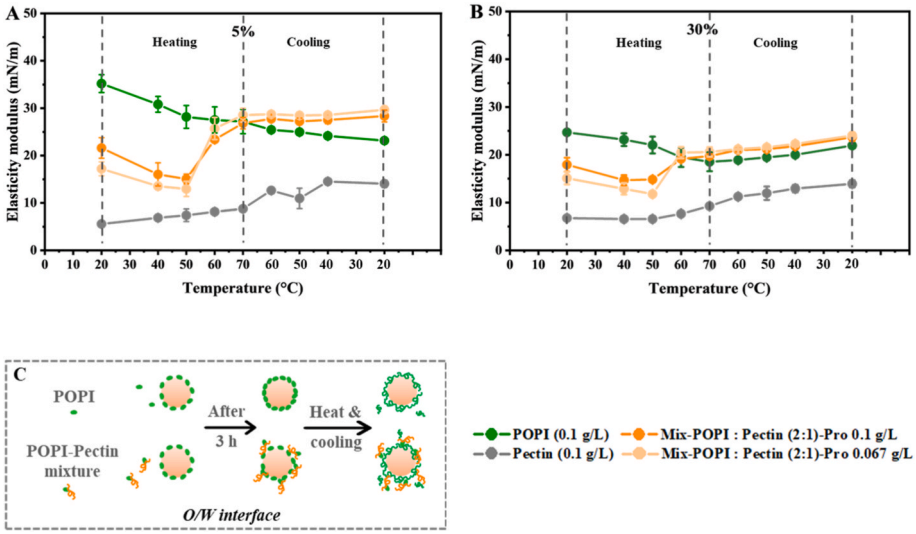

Fig. 5. The effective elastic modulus (E'_{eff}) at 5 % (A) and 30 % (B) dilatational deformation for oil-water interfaces stabilized by POPI (green), pectin (gray), and POPI-pectin mixtures (orange) at pH 4.0 during heating and cooling (20 °C–70 °C). (C) Schematic representation of the protein and pectin adsorption at the interface and formation of the interfacial layer. POPI: Potato protein isolate.

3.3.2. *Dilatational rheology*

3.3.2.1. Elasticity modulus. Interfacial dilatational rheology was carried out at temperatures ranging from 20 to 70 °C, followed by cooling to 20 °C (Fig. 5). For POPI-stabilized interfaces, the elastic modulus decreased at higher temperatures. This reduction is attributed to changes in the interactions between proteins at the interface, likely resulting from irreversible structural changes (Cao et al., 2024). During the subsequent cooling, a modest recovery in elasticity modulus was observed, particularly at higher amplitude (30 %, Fig. 5B), which supports that irreversible changes have taken place.

Pectin-stabilized interfaces demonstrated a gradual increase in elasticity modulus throughout heating, and this effect even became more pronounced during the cooling phase (Fig. 5). At all temperatures, the E' values are lower than for protein, pointing to the low surface activity and interfacial interactions of pectin. Albeit that, the elasticity of pectin-stabilized interface increases after heat treatment, unlike protein. Although pectin has negligible affinity for the interface, its temperature-dependent gel formation (Schmidt & Schütz, 2016) slightly strengthens the system upon cooling.

The POPI-pectin mixture (ratio 2:1) shows a more complex but synergistic behavior. Initially, the elastic modulus lies between those of protein and pectin only (Fig. 5). Upon heating to 50 °C, the modulus further decreased, as was seen for protein only. At temperatures above the denaturation temperature (60–70 °C), a significant increase in elasticity modulus occurred. At such high temperatures, proteins (partly) unfold, and more hydrophobic or charged residues are exposed as discussed in section 3.2.2. This enhances further interactions between the pectin or complexes in the continuous phase and POPI at the interface. These interactions facilitate the formation of a more elastic film. After cooling from 70 °C back to 20 °C, the elastic modulus keeps increasing, surpassing the initial values at 20 °C (Fig. 5C), indicating the formation of a stronger cooling-induced interfacial network via hydrogen bonds.

Although few studies directly measured the interfacial elasticity modulus at various temperatures as we did, some research provides indirect insights through rheological measurements of emulsions. For

example, similar viscoelastic reinforcement was reported in heated WPI-pectin (Kotchabhakdi & Vardhanabhuti, 2020) and SPI-peach gum (Chen et al., 2024) systems. These studies showed that heating enhanced interactions (hydrophobic and electrostatic) between protein and polysaccharide, resulting in the rearrangement of the complexes at the oil-water interface and the formation of highly viscous emulsions.

We also conducted phase-exchange experiments (Fig. S4), which were designed to test whether bulk components contribute to the overall effects measured at the interface. After exchanging the continuous phase (POPI-pectin mixture) with buffer (Fig. S4), the elasticity modulus consistently decreased, indicating that excess pectin and protein-pectin complexes present in the continuous phase play a role in reinforcing the interface. When the protein and pectin were adsorbed sequentially (layer-by-layer), an even higher elasticity modulus was observed (Fig. S5). The trends were similar to those found for the protein-pectin mixture, which points to the importance of interactions of protein and pectin to consolidate elasticity upon heating (Fig. S5). These additional results further support the importance of POPI-pectin interactions at the oil-water interface, a phenomenon investigated here for the first time.

As mentioned in the methods section, the E' values were corrected for temperature related effects on viscosity and interfacial tension. While viscosity had minimal impact (Fig. S6), interfacial tension significantly influenced values at higher temperatures (Fig. S7).

3.3.2.2. Lissajous plots. Lissajous plots provide insights into the nonlinear viscoelastic behavior of the interfacial layers under deformation. We start by explaining the behavior at 20 °C (Fig. 6), and next comparing to the behavior upon heating and cooling (Fig. 7).

At 20 °C and small deformation (5 % amplitude), all interfaces showed linear Lissajous plots, indicating elastic behavior. POPI-stabilized interface showed the steepest slope (Fig. 6A_1), corresponding to the highest interfacial stiffness and elasticity. Pectin-stabilized interfaces displayed a much lower slope (Fig. 6C_1), suggesting weak interfacial elasticity and minimal resistance to deformation. The POPI-pectin mixture showed a small, enclosed symmetric area (Fig. 6B_1), indicating linear viscoelastic response. This suggests that at low strain, pectin contributes to moderate stabilization without disrupting the protein network. This is in line with previous studies, showing narrow ellipses or linear behavior that were indicative of intact viscoelastic networks for proteins and protein-polysaccharide conjugates (Eichhorn et al., 2025).

At 30 % amplitude, all interfaces showed nonlinear viscoelastic responses. The POPI interface (Fig. 6A_2) showed pronounced asymmetry, characterized by strain softening during expansion and a pointed shape in the lower-left part of the plot, suggesting partial network disruption and weak in-plane interactions (Cao et al., 2024). Similar features were previously observed for interfaces stabilized by potato protein (Ikenaga & Sagis, 2024) and pea protein isolate (Hinderink et al., 2020). The pectin-stabilized interface (Fig. 6C_2) remained nearly linear and showed very low stiffness even at large deformation, which is indicative of poor ability to form a structured interfacial layer. For the POPI-pectin mixture (Fig. 6B_2), the interface showed moderate nonlinearity, with some strain softening, of which the extent was less pronounced than in the pure POPI system. This further indicates that pectin restricts the mobility of POPI at the interface, resulting in a less elastic interfacial layer than that formed by POPI alone (Fig. 5A). This decreased elasticity is consistent with pectin chains interconnecting with the protein network through electrostatic and hydrogen-bond interactions as discussed earlier.

To better understand the temperature-dependent interfacial behavior, Lissajous plots at 30 % deformation were analyzed during heating and cooling (20 → 70→20 °C) (Fig. 7). Both the potato protein (Fig. 7A_1) and mixed (Fig. 7A_2) interfaces exhibited nonlinear viscoelastic behavior at 20, 50, and 70 °C. For the POPI interface, the slope of the plots decreased with increasing temperature, indicating decreased elasticity (Fig. 5). This is most probably due to partial thermal denaturation and weakened interfacial protein-protein interactions. The POPI-pectin mixture showed an increase in slope at 70 °C, suggesting the formation of a more viscoelastic layer at higher temperature, as discussed in section 3.3.2.1. Pectin-stabilized interfaces (Fig. 7A_3) remained nearly linear with a shallow slope, indicating weak and largely temperature-insensitive elasticity.

Upon cooling from 70 to 20 °C, both the protein (Fig. 7B_1) and mixed (Fig. 7B_2) interfaces showed increased slopes in the Lissajous plots during expansion. This indicates strain hardening due to network reformation, which can be attributed to proteins, as well as synergistic effects with pectin. In compression, the pointed shape in the lower left

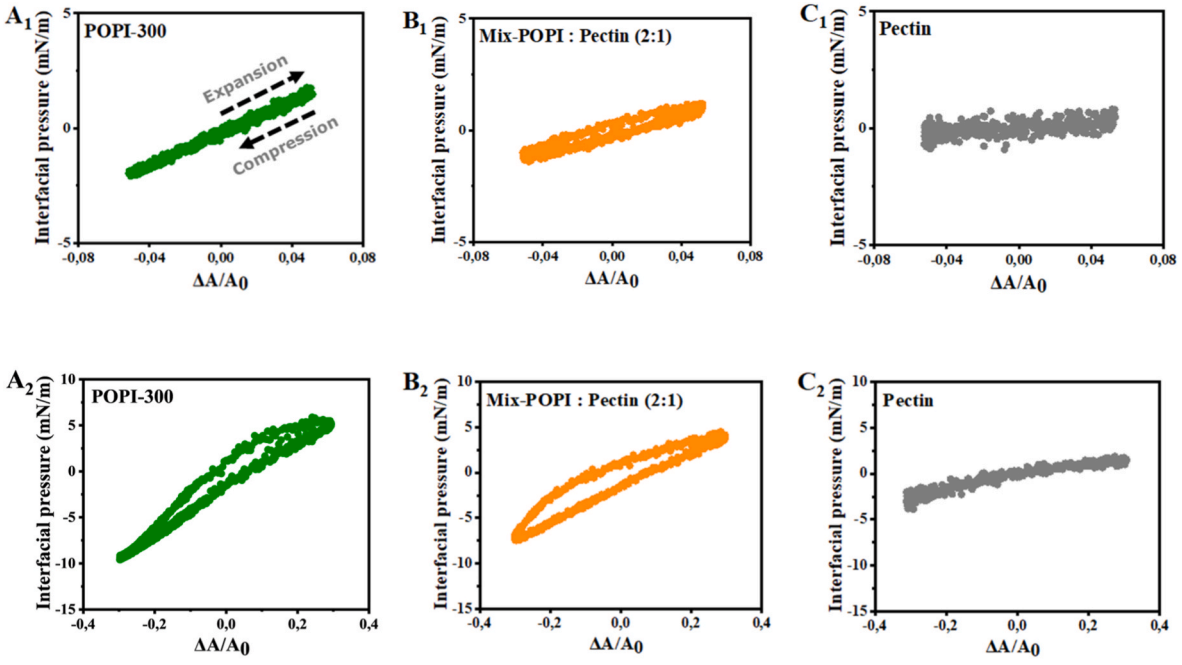

Fig. 6. Lissajous plots at 5 % and 30 % deformation of oil-water interfaces stabilized by POPI (A), POPI-pectin mixture (2:1) (B), and pectin (C) at pH 4.0 at 20 °C. A_1-C_1: 5 % amplitude; A_2-C_2: 30 % amplitude. For clarity, one representative plot or curve was displayed for each condition. POPI: Potato protein isolate.

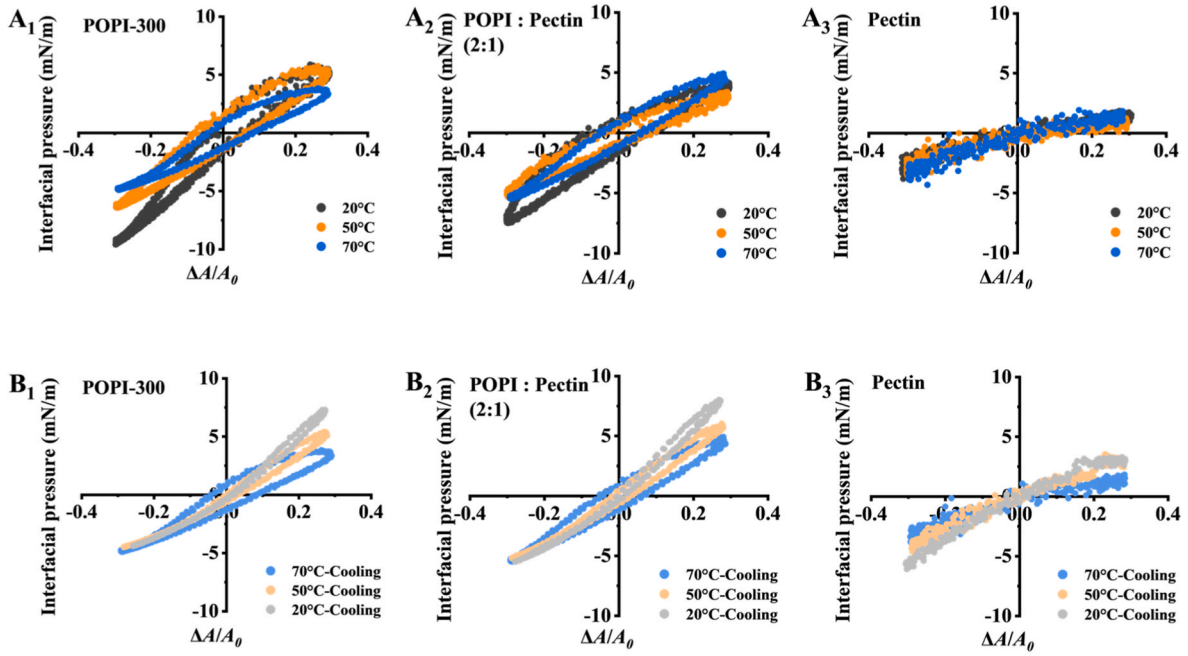

Fig. 7. Lissajous plots at 30 % deformation of oil-water interfaces stabilized by POPI, pectin, and POPI-pectin mixture (2:1) at pH 4.0 during heating (A) and cooling (B) (20 °C–70 °C). For clarity, one representative plot or curve was displayed for each condition. POPI: Potato protein isolate.

quadrant suggests in-plane interactions. The pectin interface showed only a slight increase in slope (Fig. 7B$_3$), confirming its consistently weak elasticity and low temperature responsiveness.

To summarize, the elasticity modulus and Lissajous plots indicate that POPI contributes to interfacial elasticity. Pectin and POPI-pectin complexes present in the continuous phase interact with POPI at the interface to form a viscoelastic, thicker interfacial film. POPI-pectin mixed interfaces are temperature- and strain-responsive interfacial networks strengthened by hydrophobic interactions and hydrogen bonding during heating and cooling.

In the next section, we discuss how the protein, pectin, and their mixtures influence emulsion stability through their behavior in the bulk and at the interface (as discussed above).

3.4. Emulsion stability

The thermal stability of O/W emulsions stabilized by POPI, pectin, and their mixtures at ratio 2:1 (Fig. 8) and 10:1 (Fig. S8) was investigated through droplet size analysis at constant total stabilizer concentration, and variation thereof. At 6 g/L (concentration in the continuous phase), POPI-stabilized emulsions maintained consistent droplet sizes ($d_{3,2}$–5 μm) before and after heat treatment with no significant aggregation or coalescence ($p > 0.05$) (Fig. 8 & Fig. S9), which suggests good emulsion stability due to heat-induced gelation (high bulk viscosity, Fig. 3). Pectin-stabilized emulsions made at 6 g/L had larger initial droplet size at 20 °C ($d_{3,2}$–12 μm), and pronounced coalescence occurred upon heating ($d_{3,2}$–28 μm) (Fig. 8). Although pectin may undergo minor conformational adjustments (e.g., coil-helix transitions) in response to temperature (Ye, 2008), these changes do not enhance interfacial adsorption, nor strengthen the interfacial films upon heat treatment (Figs. 4 and 5), thus explaining the weak thermal stability. Similar results were found in earlier research with pectin-only emulsions, which showed rapid destabilization during heating (55 °C) or over accelerated storage (Feng et al., 2021).

The emulsions stabilized by POPI-pectin mixture (ratio 2:1) had lower droplet size ($d_{3,2}$–3 μm) than those stabilized by POPI alone, both at 6 and 4 g/L. No aggregation, coalescence, or creaming were observed after heating and during 5 days of storage (Fig. 8). This can be attributed

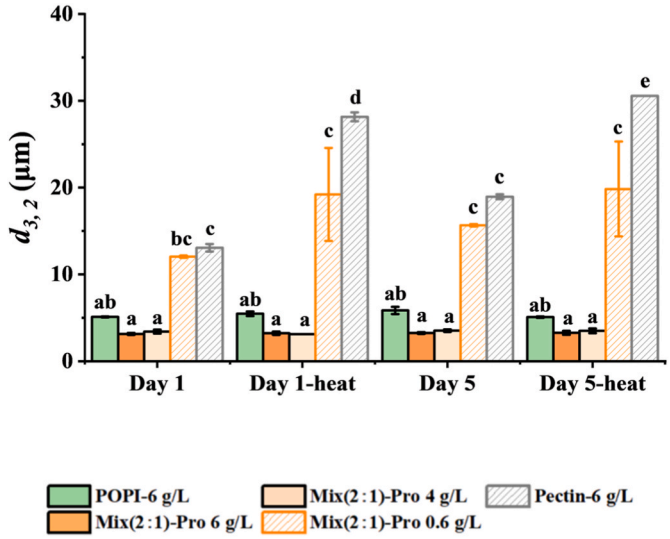

Fig. 8. Droplet size ($d_{3,2}$) of emulsions stabilized by POPI (green), pectin (gray), and POPI-pectin mixtures (ratio 2:1; orange) at pH 4 without and with heat treatment on Day 1 and Day 5. Different letters indicate significant difference ($p < 0.05$). POPI: Potato protein isolate.

to the formation of a stronger interfacial network after heating and subsequent cooling (Fig. 5), as discussed in section 3.3.2. In addition, high viscosity (Fig. 3) in the continuous phase further strengthens the connections between protein, pectin, protein-pectin complexes, and droplets. At a lower total concentration of 0.6 g/L (2:1 ratio), the initial droplet size was significantly bigger ($p < 0.05$), and droplet coalescence after heating occurred, suggesting that a critical interface coverage is required for stability.

The enhanced performance of the POPI-pectin system is due to synergistic interactions between the protein and polysaccharide during heating and cooling, both in the continuous phase and at the interface. Importantly, cooling further strengthens their interactions through hydrogen bonding, thus preventing coalescence further. This is

consistent with previous reports on protein-pectin complexes, where the combination of a surface-active protein and a sterically stabilizing polysaccharide produces multilayered interfacial structures that resist coalescence during heating, or under acidic conditions (Albano et al., 2019). Previous studies found that pea protein-pectin emulsions exhibit much greater resistance to thermal destabilization near the isoelectric point compared to pea protein alone (Gharsallaoui et al., 2010). In addition, soy protein-pectin complexes significantly reduce creaming and aggregation by forming thicker interfacial layers (Ma et al., 2019). The improved stability in our POPI-pectin system aligns with results for heat-treated whey protein isolate-pectin emulsions, for which the use of preheated proteins leads to smaller droplets and improved creaming stability (Kotchabhakdi and Vardhanabhuti, 2020). This further confirms that heat-induced changes in protein can enhance their interactions with polysaccharides.

4. Conclusion

This study demonstrates that the thermal stability of potato protein-pectin emulsions is driven by a synergistic interplay between continuous-phase and interfacial processes. In the continuous phase, pectin gelation increases viscosity and restricts droplet movement. Whereas at the oil-water interface, a sequential adsorption process occurs: proteins adsorb first, followed by pectin or protein-pectin complexes that lower interfacial tension further. Although these complexes initially form a less elastic layer than protein alone, thermal treatment and subsequent cooling strengthens the interfacial network through hydrophobic interactions and hydrogen bonding, ultimately surpassing the elasticity of single-protein interfaces. Together, these effects stabilize emulsions, enhancing resistance to creaming, aggregation, and coalescence, with the 2:1 mixture showing the highest stability. These findings directly address a critical knowledge gap and can be used in the design of thermally stable protein-stabilized emulsions.

In this study, droplet size was used as the primary stability indicator, as it is one of the most sensitive and widely used methods for assessing (early-stage) emulsion destabilization. This is sufficient to identify viable options that would need to be investigated further in longer-term storage studies. Direct microstructural visualization (e.g., CLSM) can be instrumental in providing additional validation, particularly for elucidating destabilization mechanisms that take place in food applications requiring extended shelf-life, which is part of future work.

CRediT authorship contribution statement

Jiarui Cao: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Meinou N. Corstens:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Karin Schroën:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2026.119050.

Data availability

Data will be made available on request.

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