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Development of low oil emulsion gels by solidification of oil droplets and determination of their rheological properties

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Abstract

This study aims to develop low-fat emulsion gels by physically solidifying oil droplets using a combination of pectin, soy protein, and bovine gelatin, and to investigate the rheological properties of these emulsion gels. The emulsion gels were formulated with different combinations of these biopolymers [PSG30 (pectin + soy protein + gelatin + 30% oil), PS30 (pectin + soy protein + 30% oil), P30 (pectin + 30% oil), G30 (gelatin + 30% oil)] and compared with commercially available low-fat mayonnaise (DYM40, 40% oil), mayonnaise (TM80, 80% oil), and spreadable fat (SY59, 59% oil) samples. The consistency index (K, Pa.s^n) of the emulsion gels ranged from 1.903 to 150.739 Pa.s^n, with PSG30 and PS30 formulations exhibiting higher K values than the commercial samples. The highest structural recovery percentage was observed in the SY59 sample at 114.91%. Thermal stability tests demonstrated that PSG30 and PS30 maintained their viscosity and storage modulus (G') values over a wide temperature range. Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed significant hydrogen bonding and cross-linking interactions between pectin, soy protein, and gelatin. Microstructural imaging showed that PSG30 had the most homogeneous structure, consistent with its superior rheological performance. Molecular docking analysis determined the binding energy between gelatin and pectin to be -6.40 kcal/mol. Interaction between pectin (Arg-522 residue) and soy protein (11S globulin TGT) was facilitated by salt bridge formation. The developed formulations of pectin, soy protein, and gelatin demonstrate potential for producing low-fat emulsion gels with acceptable texture and stability properties for various food applications.

Keywords Low-fat, Emulsion gels , Pectin, Soy protein, Gelatin

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INTRODUCTION

There is a growing interest in developing emulsion gels with low oil content. However, there is limited literature on how to obtain such emulsions by changing the oil phase and solidifying the oil droplets by physical methods. Emulsion gels have attracted attention with their properties such as reducing trans fat and saturated fat (Montes de Oca-Ávalos et al., 2016), improving food structures (Foegeding et al., 2017) and increasing bioavailability (Lee et al., 2019) by exhibiting both emulsion and gel properties.

Emulsion gels are complex colloidal systems that combine the properties of emulsions and gels, characterized by a three-dimensional network structure that incorporates dispersed lipid droplets within a continuous gel matrix (Fontes-Candia et al., 2020). These emulsions develop the characteristics of soft solid materials with remarkable physical stability and viscoelastic capabilities due to their network topologies, which contribute to their high mechanical properties (Chen et al., 2022). In particular, cold-setting emulsion gels have outstanding potential for food applications in reducing fat content in foods and encapsulating bioactive compounds (Souza Paglarini et al., 2021; Muñoz-González et al., 2021). Fat-reduced products are of considerable interest in the food industry due to the increasing consumer demand for healthier options. Emulsion gels play an important role in the development

of reduced fat products as effective fat replacers while maintaining the desired sensory properties and structural integrity of foodstuffs (Dickinson, 2012). By incorporating emulsion gels into fat-reduced formulations, the total fat content and energy density of products can be reduced, thereby improving their nutritional profile (Ren et al., 2022). Emulsion gels, when used as fat substitutes in meat products, effectively reduce product fat content while maintaining desirable textural and sensory properties (Kim et al., 2018). Emulsion gels prepared with ingredients such as chia mucilage and olive oil have been used in the development of reduced fat burger formulations and have been shown to be an alternative for healthier food options (Liu et al., 2022). Emulsion gels have outstanding potential as fat substitutes to achieve desired structural properties in low-fat products without compromising taste or quality (Silva, Ferdaus, Foguel, & da Silva, 2023).

Pectin and gelatin are two popular biopolymers that are used in the food industry. Pectin, a natural polysaccharide that is predominantly derived from the cell walls of fruits, particularly citrus fruits and apples, is known for its gelatinization, thickening, and stabilizing properties. It is particularly advantageous in the production of jams, jellies, and other structured food products due to its capacity to form gels in the presence of sugar and acid (Robledo & Vázquez, 2019). In addition, pectin is acknowledged as a source of dietary fiber, which has been associated with potential cholesterol-lowering effects and enhanced digestive health (Blanco-Pérez et al., 2021). Gelatin possesses a distinct amino acid content and is a naturally occurring hydrocolloid that is produced by the hydrolytic breakdown of collagen (Alipal et al., 2021). In applications of oil-in-water emulsions such as confectionary, low-fat margarine, and milk cream, gelatin serves as an emulsifying agent due to its amphoteric properties and hydrophobic regions in the peptide chain (Aewsiri et al., 2009).

Proteins and polysaccharides are safe additives that can improve the stability of emulsion gels while producing physically stable emulsions (Chityala et al., 2016). The characterization, mechanical properties, and applicability of emulsion gels stabilized with soy protein-xanthan gum complexes in plant-based processed meat products were investigated (Funami et al., 2023). Ren et al. (2022) used gelatin and pectin in their study to formulate emulsion gels for three-dimensional food printing using a 3D printer. Li et al. (2020) created food-grade emulsions and emulsion gels using soy protein-pectin complex nanoparticles and glycyrrhizic acid nanofibrils, demonstrating the interaction between soy protein isolate and pectin. Souza et al. (2021) used an emulsion gel high in fiber that had inulin, soy protein isolate, and soybean oil instead of animal fat in bologna-style sausages with less salt and fat. This was done to make healthier meat products using emulsion gels. No study revealed the interaction of pectin, soy protein, and gelatin to determine a fat-reduced emulsion gel formulation with strong rheological properties.

The aim of this study is to develop emulsion gels with reduced fat content by combining pectin, soy protein, and gelatin, and to investigate the interactions between these components to obtain strong rheological properties. Within the scope of the study, low-fat emulsion gel structures will be developed in different combinations of pectin, soy protein, and gelatin, and their structures will be examined by detailed rheological analyses. The emulsion gels obtained will be compared rheologically with the target products of commercial spreadable oil, mayonnaise, and reduced-fat mayonnaise products. In addition, pectin-gelatin and pectin-soy protein interactions will be revealed by Fourier transform infrared (FTIR) spectroscopy and molecular docking. This research aims to provide value-added products to industries producing healthy food products by developing fat-reduced, strong emulsion structures and low-calorie alternatives to fats and oils.

MATERIALS AND METHODS

Materials

Pectin (E440, apple pectin with medium methoxyl), soy protein (81.7%), and bovine gelatin (220 Bloom, E441) Kimbiotek Kimyevi Maddeler San. Tic. A.Ş. (Istanbul, Turkey). Sunflower oil, low-fat mayonnaise (40% fat), commercial mayonnaise (80% fat), and spreadable oil (59% fat) were purchased from a national market in Turkey.

Preparation of Emulsion Gels

Emulsion gels were formed by combining pectin (P), soy protein (SP), gelatin (G), and sunflower oil (O) in varying proportions (Table 1). The ratios of P, SP, G, and O were determined as a result of preliminary trials.

According to the % ratios specified in the formulation, gelatin was first completely dissolved in pure water at 50–55ºC with a magnetic stirrer (Heidolph Instruments GmbH & Co., P/N: 506-11100-00, Germany). Then pectin was added, and the dissolution process was continued until a homogeneous mixture was obtained. After obtaining a homogeneous solution, soy protein was added and stirred for another 10 minutes. The mixture was cooled to 25ºC and then kept at 4ºC refrigerator temperature for 20 hours. Low-oil emulsions were obtained by adding 30% oil phase to the solution phase containing the P-SP-G mixture to form emulsions. A homogenizer (Ultraturrax T18, IKA, Germany) was used at 13,500 rpm for 70 seconds to solidify the oil droplets. The prepared emulsions were rested at 4 °C for 24 hours and then analyzed (Kamer, 2024).

Table 1. Emulsion gel formulations

Sample Code	Pectin (%)	Soy Protein (%)	Gelatin (%)	Sunflower oil (%)
PSG30	5	1	0.5	30
PS30	5	1	0	30
P30	5	0	0	30
G30	0	0	0.5	30

* Commercial control samples were coded with the abbreviations DYM40 for low fat mayonnaise (40% fat), TM80 for commercial mayonnaise (80% fat), and SY59 for spreadable oil (59% fat).

Figure 1. Emulsion gels with low oil content produced in different formulations

Rheological Characterisation of Emulsion Gels

The rheological properties of low oil content emulsion gels and commercial products were carried out using two different deformation tests, constant shear and dynamic shear, using a temperature controlled (peltier system) Discovery Hybrid Rheometer-2 (TA Instruments New Castle, USA). TRIOS Software (V3.0) was used for equipment control and obtaining rheological parameters. The data obtained were visualised using OriginPro 2016 software (OriginLab Corporation, USA).

Steady shear flow behaviour measurements

During the flow behavior analysis of emulsion gels, the samples were placed between parallel plates (measurement range 1 mm; diameter 40 mm), and a total of 100 data points were collected at 10-second intervals. The constant shear test was performed at 20 °C with a shear rate of 0.1–300 s^{-1}. Data collected included shear rate, shear stress, and apparent viscosity (Kamer, 2024). Flow behavior, coefficient of consistency (K, Pa.s^n.), and flow behavior index (n) values were determined using the power-law model with the highest coefficient of determination (R^2).

Power law (Ostwald de -Waele equation) model: $\sigma=K(\gamma)^n$ (Eq. 1)

For the Power law model, σ is the shear stress (Pa) and γ is the shear rate (s^{-1}). K is the consistency index (Pa. s^n) and n is the flow behaviour index.

Structural recovery - thixotropy test

To determine the structural recovery, or thixotropy, an oscillation time scan was performed at 1 Hz with three intervals. In the first stage, the specimens were subjected to a 0.1% strain for 120 s. In the second stage, 20% strain (above the LVR) was applied for 300 s to simulate structural failure conditions. In the third stage, the strain was restored to the first stage (0.1% strain) and reapplied for 120 s. The recovery was calculated as a percentage by dividing the storage modulus at the beginning of the thixotropy (G'^1) by the storage modulus at the end of the thixotropy (G'^3) to obtain three thixotropy ranges (3-ITT) (Abdolmaleki et al., 2020).

The effect of temperature on the rheological parameters of emulsion gels

The effect of temperature on the apparent viscosity of emulsion gels was determined by a heating (5 °C/min, 1 Hz) test between 5 and 60 °C. Temperature screening for thermo-viscoelasticity properties was carried out by heating from 5 to 45 °C under constant shear (f = 1 and y = 1%) (Rao, 2010).

Dynamic shear rheological measurements

The linear viscoelastic region (LVR) of emulsion gels and commercial samples was determined by a scanning test (0.1%–100%, 1 Hz, 20 °C) using a parallel plate geometry with a measurement range of 1 mm and a diameter of 40 mm. The modulus of accumulation (G′) and loss (G″) were recorded as a function of frequency through a

dynamic oscillation measurement at 0.1% strain, with a frequency sweep ranging from 0.1 to 100 rad/s. Using the Bohlin model, the frequency dependence of G′ and G″ was investigated (Abdolmaleki et al., 2020).

$G' = A'(\omega')^{b'}$ (Eq. 2)

The dynamic rheology parameters G′ and G″ determined against angular frequency values were subjected to linear regression analysis and intercept values (A′), slope values (b′) and R^2 values were calculated using the above equations. Here ω is the angular frequency (rad/s).

Fourier transform infrared spectrometry (FTIR) analysis

The molecular behavior of the emulsion gels was analyzed using ATR-FTIR spectroscopy (Pintado et al., 2015). For this purpose, a Nicolet iN5 infrared microscope (Thermo Fisher Scientific, Waltham, MA, USA) was used with a 500–4000 cm^{-1} survey range, transmission mode (2 cm^{-1} resolution by accumulating 128 scans), and post-processing of spectra in Omnic 9 software (Thermo Fisher Scientific, Waltham, MA, USA). The data obtained were visualized with OriginPro 2016 software (OriginLab Corporation, USA).

Investigation of Pectin-Soy Protein and Pectin-Jelatin Interaction by Molecular Docking Method

AutoDock Vina, Pyrex, Discovery Studio, and the molecular graphics program UCSF Chimera (University of California, San Francisco) were used as virtual screening software for docking simulation in this study. In this study, 7S (PDB ID: 3AUP) and 11S globulin (PDB ID: 1OD5) (Zhang et al., 2023), which are the main components of soya protein, and type I collagen (PDB ID: 5CTI) protein models with heterotrimer structure for bovine gelatin were used. These proteins were obtained from the Protein Data Bank (https://www.rcsb.org) of the Research Collaboratory for Structural Bioinformatics (Fu et al., 2023). Galacturonan (tetragalacturonic acid, CID 5459352) (Wang et al., 2024) was chosen as a representative ligand because the pectin molecule was too large to simulate docking. PyMol software performed the visualization of the binding sites. The 2D diagram was generated by Proteins Plus (DoG Site Scorer) (https://proteins.plus/) (Schöning-Stierand et al., 2020).

Statistics

The statistical significance of the proposed models was evaluated using a one-way analysis of variance (ANOVA). Experiments were carried out in three replicates, and differences between samples were evaluated using Duncan's multiple range tests with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The statistical significance level was 95%.

RESULTS AND DISCUSSION

Rheological Properties of Emulsion Gels

In order to reveal the rheological characterization of the emulsion gels, the viscosity and shear stress values in steady shear were first investigated at 20 °C. Figure 2A shows the change in shear stress as a function of shear rate for various formulations. In all samples, the apparent viscosity decreases with an increase in shear rate. This indicates that the emulsions exhibit shear-thinning behavior. As the shear rate increased, PSG30 and PS30 formulations exhibited the highest shear stress values. This indicates that the combination of pectin, soy protein, and gelatin leads to a significant increase in viscosity and provides a strong and homogeneous texture. The P30 formulation exhibited high shear stress values but was lower compared to PSG30 and PS30. This indicates that pectin alone can generate significant viscosity even in the absence of soy protein and gelatin. The G30 formulation showed significantly lower shear stress values compared to the other formulations. This indicates that gelatine-based emulsion alone is insufficient to increase viscosity. Commercial mayonnaise samples, DYM40 and TM80, exhibited comparable patterns in terms of shear rate, but PSG30 showed lower shear stress values compared to PS30 and P30 formulations. The rheological properties of emulsion gels, such as consistency and viscosity, are critical to determining their sensory qualities, flow behavior during processing, structural and physical stability, and optimizing formulation and processing conditions (Patel et al., 2014). Xu et al., (2021) demonstrated the versatility of pectin in food formulations by detailing the preparation of a fat substitute based on high-methoxyl pectin from citrus and its application to croissant crust. Wang et al. (2014) and Albano & Nicoletti (2018) investigated the use of gelatin as a fat substitute in various food products. Gelatin's ability to provide sensory properties similar to those of fat makes it an ideal ingredient for low-fat formulations.

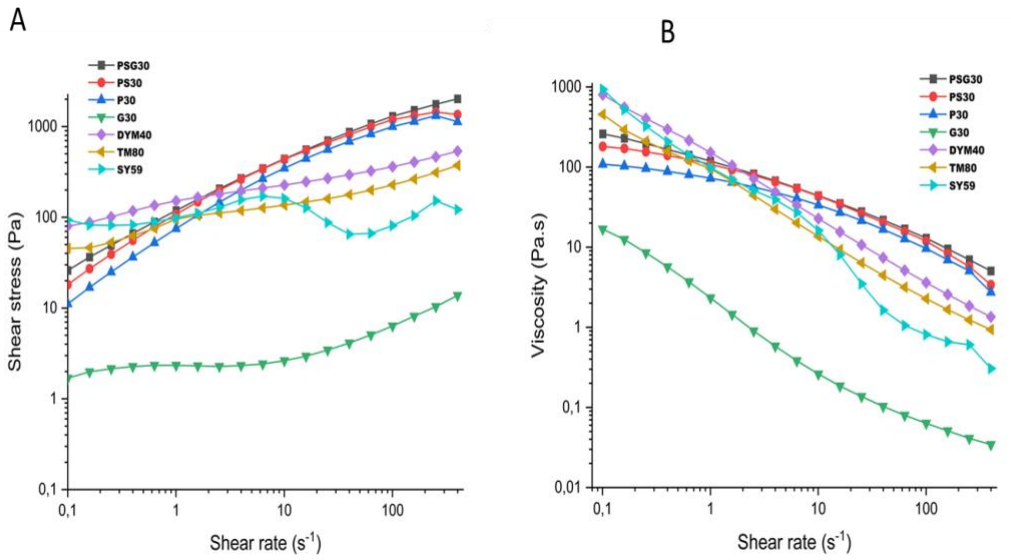

Figure 2. Shear stress and viscosity rheograms of emulsions in the range of 1-100 s^{-1}

The coefficient of consistency and flow behavior index values for the Power-Law (PL) model parameters of the emulsions are given in Table 2. The consistency index K (Pa.s^n) values ranged from 1.903 to 150.739 Pa.s^n, with the PS30 formulation achieving the highest consistency and the G30 formulation demonstrating the lowest consistency. The consistency index values of the samples differed significantly ($p<0.05$). The PSG30 and PS30 emulsion gel formulations yielded more viscous emulsions in comparison to commercial products. This indicates that the developed oil-reduced formulations may have potential for use in different food formulations, such as mayonnaise and spreadable oil. The flow behavior index (n) values of the samples were in the range of 0.019–0.466, and the difference between the samples was found to be statistically significant ($p<0.05$). All samples showed a flow behavior index below 1, and an increase in shear rate resulted in a decrease in the viscosity value. This indicates that emulsions have pseudoplastic and time-independent non-Newtonian behavior. Nasrolahi et al. (2023) obtained similar findings in a study on the rheological properties of emulsions prepared with modified starch, corn oil, and resin gum. Although the PSG30 formulation has n <1, it appears to be the sample with the highest n value. This can be attributed to the presence of gelatin in combination with pectin and soy protein, forming a more complex network with additional cross-linking points and providing additional flexibility. Gelatin interacted with both pectin and soy protein, resulting in a potentially stronger but more shear-thinning network. Hydrocolloids that exhibit shear thinning are widely used to improve or modify food texture. A lower solution viscosity makes it easier to process during high shear processes like pumping and filling. On the other hand, a high apparent viscosity makes the product feel good in the mouth (Marcotte et al., 2001). The flow curves show similar behavior, in agreement with other studies on mayonnaise rheology (Moros et al., 2002; Peressini et al., 1998). As the shear rate increases, the agglomerated droplets deform and distort, leading to the shear-thinning effect (McClements, 2004).

Table 2. Power-Law (PL) model parameters of emulsion gels

Sample codes	*K* (Pa.s^n)	*n*	R^2	Recovery(%)
PSG30	147.483±1.55^a	0.466±0.08^a	0.98±0.005^a	63.51
PS30	150.739±2.55^a	0.441±0.04^b	0.98±0.01^a	87.69
P30	112.731±1.08^c	0.464±0.05^a	0.98±0.01^a	63.19
G30	1.903±0.05^f	0.249±0.05^c	0.84±0.005^b	58.84
DYM40	142.742±0.55^b	0.202±0.01^e	0.99±0.005^a	94.09
TM80	82.650±0.61^e	0.219±0.01^d	0.98±0.01^a	83.88
SY59	104.165±0.99^d	0.019±0.02^f	0.72±0.01^c	114.91

* PSG; pectin + soy protein + gelatin + 30% fat, PS30; pectin + soy protein + 30% fat, P30; pectin + 30% fat, G30; gelatin + 30% fat, DYM40; low fat commercial mayonnaise (40% fat), TM80; commercial mayonnaise (80% fat), SY59; spreadable oil (59% fat). a, b, c : Means with different letters in the same column are different from each other ($p<0.05$)

Structural recovery and thixotropy properties of emulsion gels

A thixotropy test was carried out to determine the ability of emulsion gels to be exposed to a low rotational force followed by a high force and to recover when returned to a low force. Especially in squeezed products such as mayonnaise, the ability to recover is very important. The structural recovery values of the emulsion gels given in Table 2 are generally lower than those of the commercial samples. The lower structural recovery values exhibited in the emulsion gels, as compared to commercial samples, may be attributed to the inclusion of various gums or thickeners in commercial mayonnaise, particularly those with low fat content. Commercial formulations often incorporate a blend of hydrocolloids, including xanthan gum, guar gum, or modified starches, with a specific objective of increasing viscosity, stabilizing emulsions, and enhancing the textural characteristics of the product (Blok et al., 2023). The presence of these components enhances the thixotropic behavior by promoting faster recovery following the removal of shear pressures, therefore enabling the product to regain its structure and consistency with greater efficiency. However, it is seen that the PS30 sample with a recovery of 87.69% is higher than the commercial mayonnaise (TM80) sample with a very high oil content. The fact that the thixotropic behavior, which is defined as the process of reconstruction of the molecular structure, is the lowest in the G30 sample shows that gelatin alone cannot provide sufficient elasticity at low concentrations. This can be explained by the poor ability of the gel to trap water at low gelatin concentrations and, consequently, the weak gel network structure of the emulsion (Zeng et al., 2023).

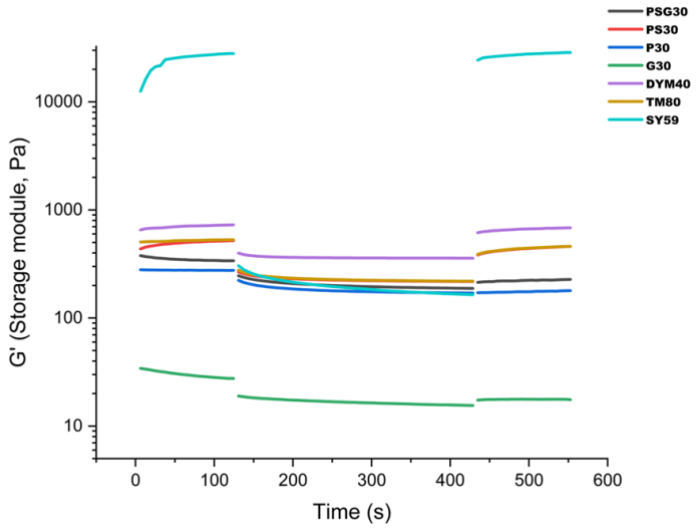

Figure 3. Structural recovery behaviour of emulsion gels

When the structural recovery behaviors of commercial samples and emulsion gels are compared in Figure 3, it is seen that the modulus of deposition is highest in spreadable oil, followed by low-fat mayonnaise, full-fat mayonnaise, and PS30 samples. The significant decrease in the deposition modulus with increasing shear rate in the SY59 sample indicates the pseudoplastic behavior of the sample (Mohammadi et al., 2021). The recovery value of PS30 was found to be 25% higher than that of P30. This is thought to be due to the synergistic effect between soy protein and pectin. In addition, the negative charge of pectin and the positive charge of soy protein may have contributed to the development of stronger emulsion structures by forming electrostatic complexes (Albano and Nicoletti, 2018). Emulsion gels that exhibit shear-thinning behavior, have sufficient elastoplastic behavioral ability to flow, and have a sufficiently high modulus of deposition to maintain their shape during flow show excellent performance in terms of both fluidity and structure stability in the 3D printing process (Feng et al., 2016). The PS30 formulation exhibits the characteristics of an emulsion gel with these qualities.

Thermorheological Properties of Emulsion Gels

The viscosity of different emulsion gel formulations is shown in Figure 4, with temperature being the independent variable. The viscosity properties of the samples provide important information about their capacity to maintain their structural integrity and thermal stability at different temperature settings. Both PSG30 and PS30 formulations showed a consistent viscosity over a wide temperature range, demonstrating a strong and stable structure. In these samples, the viscosity tended to decrease slightly as the temperature increased (above 30 °C), indicating a slight shear thinning behavior. However, the overall viscosity remained high, indicating strong thermal stability. The P30 formulation also exhibited a consistent viscosity at low temperatures. However, there was a gradual decrease in viscosity as the temperature increased. The lack of soy protein and gelatin may have resulted in a slightly less stable network compared to PSG30 and PS30. However, it still has a considerable viscosity, emphasizing the effectiveness of pectin in forming a stable gel matrix. The G30 formulation showed a significant decrease in viscosity with increasing temperature, indicating poorer thermal stability compared to the pectin-

containing formulations. This indicates that gelatin alone is not as effective in maintaining viscosity at high temperatures. Gelatin derived from bovine skin is known to exhibit melting temperatures ranging from 33.07 °C to 34.51 °C (Samatra, Noor, Razali, Bakar, & Shaarani, 2022). As expected, the gelatin-based emulsion formulation (G30) also showed a melting tendency in the range of 25–30 °C (Figure 4A). DYM40 and TM80 commercial mayonnaise samples showed a more stable structure as the temperature increased. The specific stabilizers used in their commercial production likely created these formulations to withstand moderate temperature changes. The viscosity of the SY59 spreadable oil sample decreased significantly as temperature increased, particularly when compared to the other samples. This significant decrease indicates that the formulation may not be able to withstand higher temperatures and is not stable. The temperature stability of emulsion-type food products such as mayonnaise is critical for both storage and shelf life, as well as for use in various dishes such as hamburgers, burgers, and sandwiches. These applications often expose the delicate emulsion to high temperatures, such as in meatloaf. In addition, it is important to consider the stability of the emulsion to ensure that it can withstand temperature fluctuations during transport and retail storage, especially in tropical climates. This is crucial for the commercial success of the product (Rudra et al., 2020). This supports the high commercial utilization potential of the PSG30 and PS30 formulations. These formulations maintain high G' values throughout the temperature range and show only a slight decrease as the temperature increases. This indicates that the gels have strong elastic properties and are able to maintain their structure under thermal stress, suggesting a synergistic effect of pectin, soy protein, and gelatin in increasing gel strength. The viscoelastic properties of the emulsions were analyzed at temperatures ranging from 5 to 60 °C at a heating rate of 5 °C per minute. The results are shown in Figure 4B. At temperatures above 40 °C, an increase in the modulus of accumulation of PSG30 and DYM40 samples was observed, which can be attributed to the interaction between soya proteins and other components in the emulsion at high temperatures (Rudra et al., 2020). Chang et al. (2017) observed similar results and attributed this behavior to the enhanced interaction between protein granules, pectin molecules (the hydrocolloid they focus on), and oil droplets. This interaction leads to a small increase in elasticity.

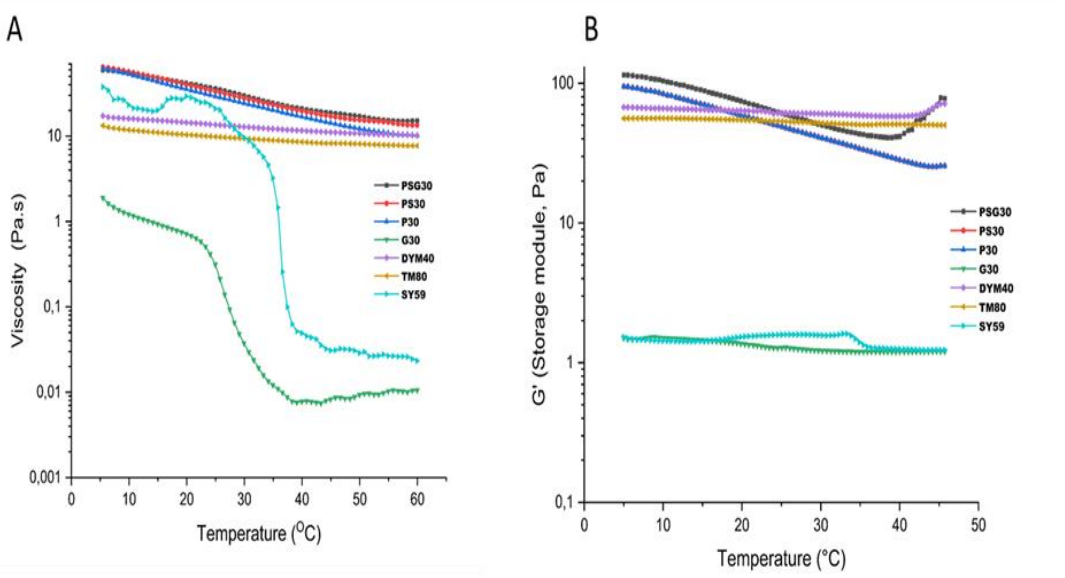

Figure 4. Temperature dependent changes in viscosity and storage modulus of emulsion gels.

Viscoeleastic properties of emulsion gels

The frequency dependence of the G' (storage modulus) and G" (loss modulus) values of the emulsions is given in Figure 5, and their viscoelastic rheological properties according to the Bohlin model are given in Table 3. A dynamic frequency sweep revealed the elasticity and viscosity of the emulsions (Figures 5A–B). G′ (storage modulus) shows the elastic properties of the emulsions, and G″ (loss modulus) shows the viscous properties of the emulsions. In all of the emulsions, the storage modulus was clearly greater than the loss modulus. This indicates that the emulsions exhibit solid-like behavior. As the angular frequency increased to 10 rad/s, the commercial samples showed stable behavior, while the accumulation modulus of the emulsion gels increased. When the angular frequency increased above 10 rad/s, all samples showed an increase in mechanical strength. This shows the tendency toward solid-like behavior of the samples (Han et al., 2024; Ma et al., 2023).

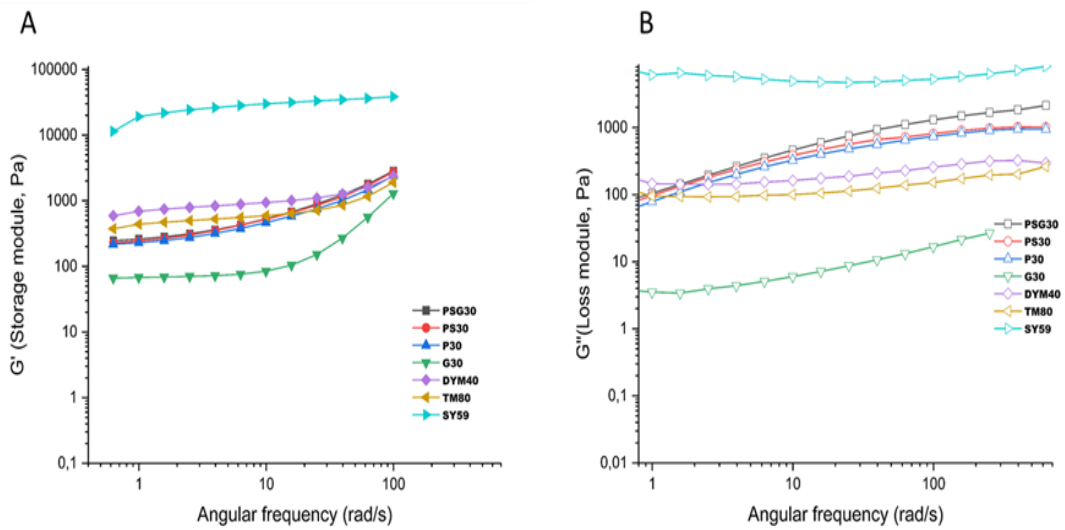

Figure 5. Storage modulus (G') and loss modulus (G'') rheograms of emulsion gels in the range of 1-100 rad/s angular velocity

The highest storage modulus was found in commercial spreadable oil, while the lowest was found in emulsion gel containing 0.5% gelatin. The structural firmness and stability of the emulsion gels were compared by calculating the A' and b' values with the Bohlin model over the storage modulus. Here, the A' value is a coefficient indicating the strength of the bonds in the emulsion, while the b value provides information about their interactions (Xu et al., 2021). The b' values of the samples vary between 0.16 and 1.61. It was seen that the frequency-dependent changes of emulsion gels other than G30 were less. PSG30 emulsion gel has the highest A' value. Pectin has a greater effect on the A' value than soy protein and gelatin. There was a statistical increase in both the A' and b' values in the PSG30 sample compared to the P30 sample. The increase in the b' value indicates an increase in the interaction sites of the complexes between the oil droplet surface and the aqueous phase, while the increase in the A' value indicates a significant increase in the interactions between the sites (Wei et al., 2017). The more complex structure of the PSG30 sample suggests a higher number of interaction sites between oil droplets and complexes.

Table 3. Bohlin model fitting of storage and loss modulus of emulsion gels and equation parameters.

Samples	G' (Storage Modulus)		
	A' (intercept)	*b'(slope)*	R^2
PSG30	96.90±21.28^{b}	0.521±0.05^{b}	0.99
PS30	90.63±21.40^{b}	0.527±0.03^{b}	0.99
P30	85.39±20.69^{b}	0.509±0.05^{b}	0.99
G30	0.767±0.481^{b}	1.61±0.13^{a}	0.99
DYM40	535.611±72.87^{b}	0.281±0.03^{cd}	0.99
TM80	273.458±60.38^{b}	0.376±0.05^{bc}	0.99
SY59	19684±1105^{a}	0.156±0.01^{d}	0.99

a, b, c : Means with different letters in the same column are different from each other ($p<0.05$)

Fourier transform infrared spectrometer (FTIR) Analysis Results

The FTIR spectra of the emulsions are shown in Figure 6. All of the samples showed a strong absorption band around ≈3000-3340 cm^{-1} resulting from the stretching vibrations of the O-H bond. This phenomenon is caused by intramolecular and intermolecular hydrogen bonding. This broad peak that was seen in all the samples is caused by O-H stretching vibrations, which show that hydrogen bonds are present and there is water in the gels (Singh et al., 2013).The peak intensity is highest in sample G30, indicating a higher degree of hydrogen bonding or water content in this formulation. The peaks observed around 2925-2853 cm^{-1} are indicative of the presence of lipid components in the samples. These peaks are associated with C-H stretching vibrations of methyl and methylene groups commonly found in lipids and fatty acids (Forfang et al., 2017). The band near 2907 cm^{-1} corresponds to symmetric stretching of methylene chains in membrane lipids or proteins, while the peak near 2957 cm^{-1} is attributed to asymmetric stretching of methylene groups, further supporting the presence of lipids or proteins (Calabrò et al., 2013). In addition, the high-intensity bands at 2957 and 2907 cm^{-1} are characteristic of the C-H stretching vibrations of lipids and correspond specifically to the methyl and methylene groups of fatty acid chains

(Pereira et al., 2019). The shift of the band from 2108 cm^{-1} to 2173 cm^{-1} in the PSG30 sample indicates a change in the molecular interactions within the emulsion gel. The shift to a higher wavenumber (2173 cm^{-1}) suggests a change in the hydrogen bonding environment. This could mean stronger hydrogen bonds or altered molecular interactions between pectin, soy protein, and gelatin in the PSG30 sample compared to other formulations in emulsion gels. The band around 2108 cm^{-1} is usually associated with C≡C or N≡C stretching vibrations in proteins or lipids. Stronger interactions or changes in the conformation of protein-lipid complexes in the gel can explain the shift to a higher wavenumber. This may be due to the specific combination of pectin, soya protein and gelatin in the PSG30 formulation. Peaks around 1235-1105 cm^{-1} are probably related to C-O stretching vibrations arising from pectin components (Makebe et al., 2020). PSG30 and PS30 samples show different peaks in this region, indicating the presence of pectin and its interaction with proteins. The micrographs on the right side of Figure 6 show the microstructures of samples G30, P30, PS30, and PSG30. The G30 micrograph showed a relatively coarse and less homogeneous structure, which is consistent with the weaker hydrogen bonding and less stable gel network shown by FTIR analysis. P30 similarly showed a weaker structure. PS30 exhibited a more uniform and fine structure, indicating a well-formed gel matrix with strong interactions between pectin and soy protein. This is consistent with the distinct peaks observed in the FTIR spectrum, reflecting strong molecular interactions. PSG30 showed the most uniform and smooth structure, resulting in the most stable and well-integrated gel network among the formulations. The combination of pectin, soy protein, and gelatin showed a robust gel structure, as supported by the FTIR spectrum showing significant peaks for the O-H, C-H, and amide I bands.

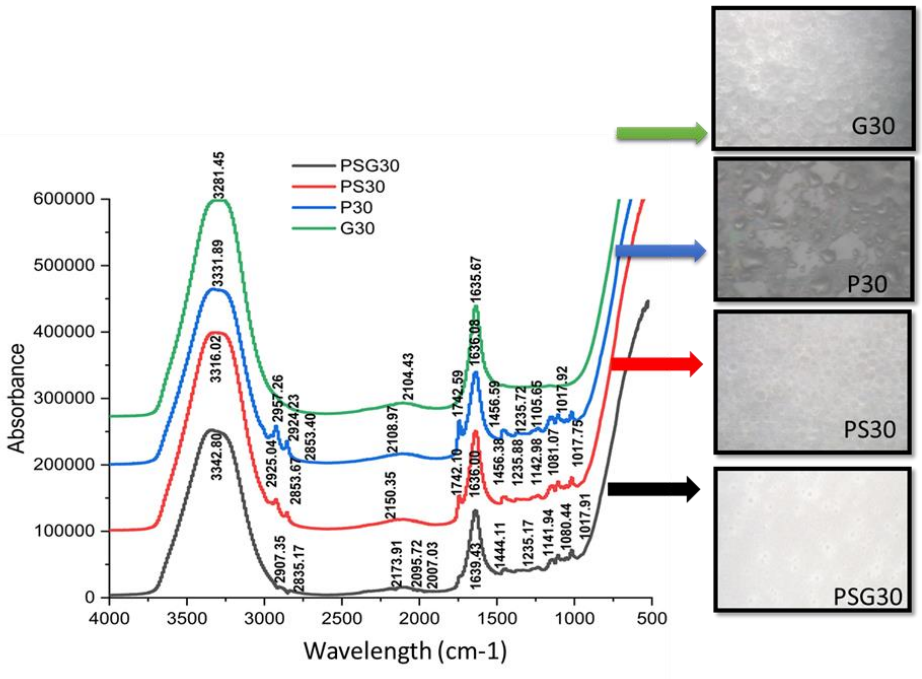

Figure 6. FTIR spectra of emulsion gels.

Molecular Docking Results

Molecular docking is applied to determine the appropriate binding sites between the target protein molecule and the ligand and to obtain information about the binding affinities of the molecules (Kınaytürk, 2023). Accordingly, the binding energies between 7S and 11S globulin, which are the main components of soya protein, and pectin were determined to be -8.97 and -7.74 kcal/mol, respectively. 2D and 3D images of these bonds are given in Figures 7B and C. The repeating heterotrimer structures of glycine (Gly), proline (Pro), and alanine (Ala) amino acids responsible for the triple helix structure of gelatin were simulated with type I collagen, and the binding energy with tetra-galacturonic acid (TGT), the main component of pectin, was determined to be -6.40 kcal/mol. The binding energies of all proteins that interacted with pectin were lower than -5.00 kcal/mol. This indicates that protein binding with pectin is relatively stable (Zhang et al., 2023). It can be suggested that the most stable binding is with 7S globulin. Tetra-galacturonic acid with 7S was linked via hydrogen bonding with the amino acid and bond position: Leu-956, Gln-957, Asp-1157, Asn-1159, Asn-1161, Asn-1161, Thr-1191, His-1192, Gln-1193, Gly-1211, Met-1213, Thr-1215, Thr-1215, Gln-1220, and Arg-1441.The bonding distances of these hydrogen bonds were determined as 2.84, 2.76, 2.94, 2.35, 2.62, 2.44, 2.26, 3.24, 3.34, 2.72, 3.14, 3.53, 3.42, 3.33, and 2.88 Å, respectively. In addition, His-1162 (5.20 Å), Arg-1141 (4.22 Å), and Arg-1441 (5.15 Å) amino acid and binding positions were determined in electrostatic interactions. Arginine (Arg) may contribute to emulsion stability by exhibiting high binding affinity through both electrostatic and hydrogen bonds (Cao et al., 2022). 11S globulin

TGT and residual amino acids Tyr-438, Val-523, Glu-533, His-534, Thr-537, and Gly-542 and their binding sites were hydrogen bonded with a distance of 2.26, 2.15, 2.37, 2.78, 1.82, and 2.99 Å, respectively. It was observed to have a more basic structure compared to 7S, with a closer bonding distance. A salt bridge represents non-covalent interactions combining electrostatic attraction and hydrogen bonding. They are stronger than hydrogen bonds and play an important role in maintaining protein stability (Spassov et al., 2023). 11S globulin also binds with TGT via a salt bridge with the residual amino acid Arg-522 at a distance of 5.12 Å. We examined TGT binding with gelatin (Type 1 collagen) (Figure 7A) and found nine amino acid residues in the hydrogen bond interaction. These are Glu-120 (2.98 Å), Gln-121 (2.27 Å), Gln-121 (3.10 Å), Ser-189 (2.28 Å), Ser-189 (2.98 Å), Glu-190 (3.66 Å), Glu-190 (3.14 Å), Ser-207 (1.99 Å), and Ser-207 (2.24 Å). A salt bridge was also detected between the carboxyl group of amino acid Arg-181 and TGT at a distance of 5.31 Å. The binding between gelatin and TGT may be suppressed by the interaction between 7S and 11S and TGT. This may explain the lower stability of sample PSG30 (Zhang et al., 2023). Furthermore, these findings are in agreement with the results obtained from FTIR analysis.

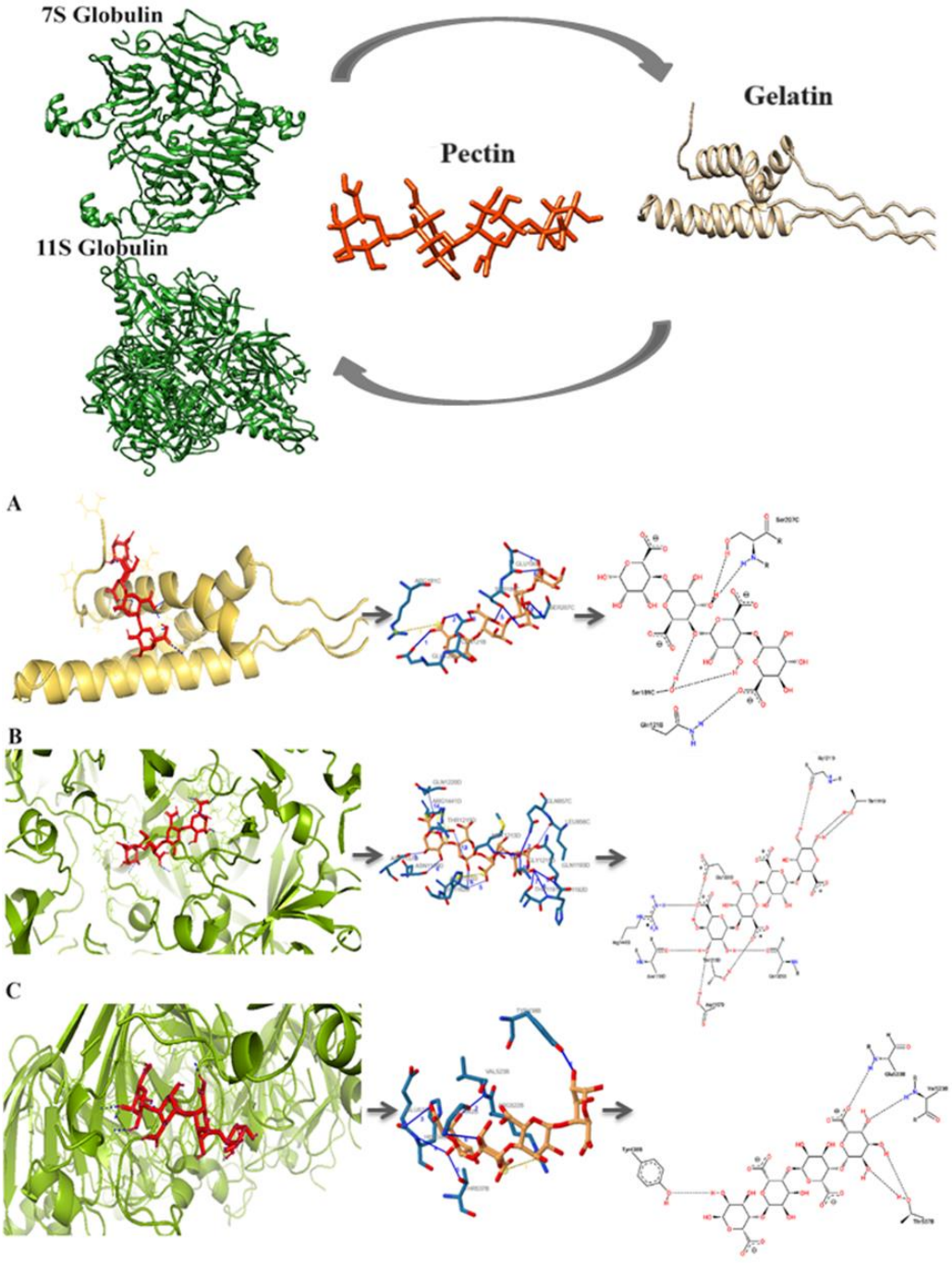

Figure 7. 2D and 3D molecular simulation of the interaction of soya proteins (7S and 11S globulin), gelatin and pectin. A: Gelatin and pectin, B: 7S globulin and pectin, C: 11S globulin and pectin.

CONCLUSION

This study investigated the effects of the combination of pectin, soy protein, and gelatin on consistency and viscosity properties in developing low-fat gel emulsions. PSG30 and PS30 formulations formed thicker emulsions compared to commercial products. Thermorheological analyses revealed that PSG30 and PS30 formulations demonstrated consistent viscosity across a wide temperature range and high thermal stability. The gel structure in PSG30 was the most stable. It had stronger hydrogen bonds or different molecular interactions between pectin, soy protein, and gelatin. It was found that the binding energy between soy protein and pectin was more stable than that of gelatin. This study reveals the synergistic effects of pectin, soy protein, and gelatin in the development of low-fat and high-viscosity food products. The findings provide an important basis for the development and optimization of low-fat emulsion products, especially in the food industry. These formulations have a lot of potential for commercial use. Especially for its high thermal stability and desirable rheological properties.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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