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# Determination of the best interaction of inulin with different proteins by using interfacial rheology: the relationship with the emulsion activity and stability in emulsion systems

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**Abstract:** This study aimed to develop functional emulsions with dietary fibre/proteins and to examine the role of interfacial rheological properties on the emulsion stability. Emulsions with inulin and various animal/vegetable proteins were prepared, and their emulsifying and interfacial rheological properties were appraised for their possible applications in stabilizing oil-in-water emulsions. Interfacial measurements including the frequency, time and strain sweep test were determined depending on the protein differences. The results revealed that the adsorption behaviour of proteins at the two interfaces was quite different. The apparent viscosity ( $\eta_{50}$ ) of the emulsions ranged between 0.006 and 0.037 Pa s. The highest interfacial viscosity ( $\eta_i$ ) values at low shear rates were determined in the mixture of egg protein-inulin at the oil/water interface. In particular, the interfacial properties of egg protein were not similar to those of other proteins. This study indicated that interfacial rheological properties and emulsifying properties of the proteins were influenced by the presence of inulin which contributes to the existing body of knowledge on the preparation of the prebiotic emulsions with proteins.

**Keywords:** colloid; emulsion; interfacial rheology; inulin; protein.

## 1 Introduction

Based on consumer demands, proteins are gaining interest in food industries as they are considered as sources of natural emulsifiers and effectivity in stabilizing emulsions by successfully decreasing interfacial tension [1]. Adsorbed proteins at the oil/water (O/W) interface could form a viscoelastic film thanks to the coexistence of both hydrophilic and hydrophobic amino acids in their structures [2]. Furthermore, proteins are important for food production. First, proteins are considered as the main sources of essential amino acids and the proteins function as stabilizers for food systems involving foams and emulsions [3]. Proteins can change the rheology of the aqueous phase and the interfacial properties that contribute to colloid-chemical stability considered in this second function [4]. Since proteins are effective absorbers at the interfaces, they tend to act as emulsifiers.

Inulin viewed as a valuable food ingredient given its major health benefits and its non-digestible prebiotic structure [5, 6]. Inulin, consisting of prebiotic dietary fibre and carbohydrate-based fat substitute, is utilized in numerous food product formulations because of its high water-holding capacity. Inulin also offers a unique combination of important technological and health benefits [7]. Moreover, it significantly affects the physicochemical properties, stability and especially rheological and textural structure of the food products by interacting with other food components such as water, protein and fat. Since inulin shows diverse interactions such as hydrogen and disulphide bonding, hydrophobic and electrostatic interactions [8] and van der Waals interactions [9] with various proteins, it is necessary to determine the appropriate type of protein in the food product formulations. It is quite critical to determine the rheological properties at the interface, to provide the correct interactions and to reflect the results on food formulations in terms of food prescription costs, shelf life, product quality and stability.

Food components might interact with each other during processing. These interactions could change the bioavailability of nutrients, the flavour or texture of the

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product [10]. Moreover, biopolymers are widely utilized in food processing as they provide food products with desirable structure. An increase in emulsion stability of protein-polysaccharide complexes can be attributed to electrostatic interactions in their structures [11]. The controlled use of protein-polysaccharide mixtures contributes to emulsion stability and prevents phase separation during the shelf-life [11]. When thoroughly mixed with water, a gel-like structure with insoluble crystals is formed [7]. Structural properties of inulin-based particles are similar to those of oil droplets in oil-in-water emulsions [12]. Inulin gels are formed by bonding microcrystals to form water-retaining networks [13]. Inulin has been used as fat replacer in low-or zero-fat food products, which also indicates that rheological properties of the food products are similar to oil crystals in the oil [14]. Inulin gel with its three-dimensional gel network is made up of insoluble sub-micron crystalline inulin particles capable of immobilizing water molecules. This mechanism is similar to oil structuring in oil-in-water emulsions [15]. And thus, promotes the water-holding capacity of the emulsion-based food products. Therefore, it is necessary to investigate the adsorption behaviour of proteins and inulin at oil/water interfaces and the effects on the emulsifying activity of proteins. Of the fundamental functions of emulsifiers is to assist the process of stabilization that delays the coalescence of the droplets once they are formed. One of the mechanisms is that due to the presence of emulsifiers in the interface, the gradients in the interface tension can arise which enable the interface to resist tangential stresses in adjacent flowing liquids [16].

As direct means to quantify steady and dynamic interfacial properties of adsorbed layers of dietary fibre and protein with different structural stability may shed light on the relationship between dietary fibre-protein interactions and their role in emulsification. Protein adsorption at interfaces is a problematic phenomenon for food processing. Since, a viscoelastic multilayer is formed by protein adsorption to the oil/water interface. Due to the flexibility and aggregation of proteins. Moreover, this protein adsorption can not only reduce the stability of the protein, as well as the therapeutic efficacy, but also can reduce the stability of the protein [17]. Interfacial rheological interactions of proteins with other food ingredients are of great interest due to their stable emulsifying properties and the advantage of cost-effectiveness. The interactional process between protein-polysaccharide molecules is known to be complex which makes it difficult to determine the significant protein attributes at the interfaces. Besides, there is still an urgent need to offer a versatile model for proteins and

dietary fibre that could predict their interfacial behaviours, including rapid expansion under separation conditions. Therefore, the major objective of this study is to contribute to the existing literature providing a comprehensive and systematic investigation of dynamic behaviours of proteins and dietary fibre. Second, this study intends to gain in-depth understandings of the role of proteins-inulin in emulsion preparation and subsequent stabilization of these model systems.

## 2 Materials and methods

### 2.1 Materials

Four different types of protein including gelatin (protein content 57.7%), pea (protein content 12.9%), whey (protein content 18.43%) and egg protein (protein content 48.0%) were used. To investigate the effect of protein types on the emulsification and rheological properties, emulsions were prepared with a control sample containing only inulin. Protein ratios were adjusted to be the same in the final product to enable objective comparison of emulsions. All the proteins used in this study was obtained from Alfasol, Istanbul, Türkiye. Inulin as dietary fibre was obtained from Orafit Food Ingredients (High-Performance Inulin HP, Belgium) while the sunflower oil used to form the oil phase of the emulsions and interface for oil/water was obtained from a local market.

### 2.2 Measurement of emulsifying properties

The emulsion activity index (EAI) and emulsion stability index (ESI) of the emulsion were determined with the spectrophotometric method of Manoi and Rizvi [18]. For the analysis of creaming index, 10 mL of emulsion was filled into a test tube (1.5 cm inner diameter  $\times$  12 cm height) and was monitored 14 days and the height of serum layer was recorded on the 1st, 7th and 14th day of the storage [19].

### 2.3 Measurements of rheological properties

Oil-in-water emulsions were prepared with inulin and proteins (0.5–0.5%, w/w) using a high-speed mixer (IKA, T25, Germany) at 9000 rpm for 5 min. Rheological measurements were conducted on emulsions with steady and dynamic rheological properties. The steady shear rheological measurements of emulsions were performed by a Peltier temperature-controlled rheometer (Thermo-HAAKE, Mars III, Karlsruhe, Germany) with plate-plate geometry. The flow behaviour index ( $n$ ) and consistency coefficient ( $K$ ) values were obtained from Ostwald de Waele model. The frequency sweep test was carried in the range of 0.1–10 Hz in the linear viscoelastic region at 25 °C.

### 2.4 Measurement of interfacial rheological properties

The interfacial rheological properties of emulsions were performed with the rheometer (Thermo-HAAKE, Mars III, Karlsruhe, Germany)

via a BiCone probe (BC 68/5Ti). Prior to interfacial measurements, the aqueous phase (containing 0.5–0.5% inulin/protein) with high density was filled up to the specified line determine the gap height after performing probe and micro stress calibrations. The sunflower oil was carefully added on top of the aqueous phase (Figure 1). Dynamic shear interfacial rheology analyses were carried out with different tests including time sweep, strain sweep and frequency sweep tests. Dynamic time sweep tests were conducted with a strain amplitude of  $\gamma = 0.1\%$  and angular frequency of  $\omega = 1 \text{ rad s}^{-1}$  for 1 h at 25 °C. Dynamic frequency sweep tests were performed in the range of  $\omega = 0.1\text{--}10 \text{ rad s}^{-1}$  and strain amplitude of  $\gamma = 0.1\%$  in a linear region. Dynamic strain sweep tests were conducted in the range of  $\gamma = 0.01\text{--}100\%$  and  $\omega = 1 \text{ rad s}^{-1}$ .

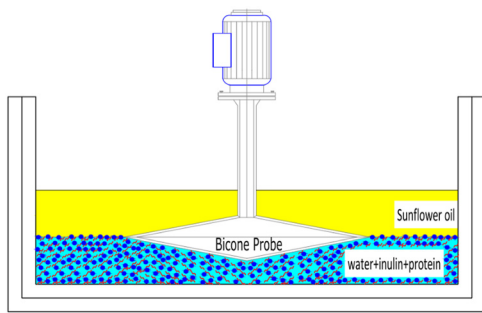
## 2.5 Statistical analysis

For the statistical analysis of the data, SPSS Statistics 17.0 package program was employed. A significance level of 95% or  $p \leq 0.05$  was used to interpret the statistical differences between the samples.

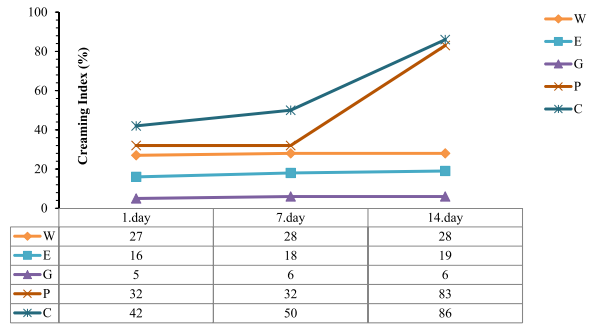
# 3 Results and discussion

## 3.1 Emulsifying properties of emulsions

The emulsifying properties of inulin and proteins were determined with the creaming index, ESI and EAI. Figure 2 indicated the creaming index of emulsion samples. Visual examination of the emulsion after seven and 14 days stored at 4 °C and on the day of 14, the height of serum layers and emulsion were recorded to calculate the creaming index (Figures 2 and 3). The most drastic change for creaming index value was observed in the control sample emulsion while the minimal difference was recorded in gelatine-stabilized samples at the end of the 14 days. It was also obvious in Figure 2 that the creaming index value of the control sample was higher than that of other emulsions containing various proteins. Therefore, it would not be wrong to assume that the proteins in the emulsions had a positive impact on the stability of the emulsions.

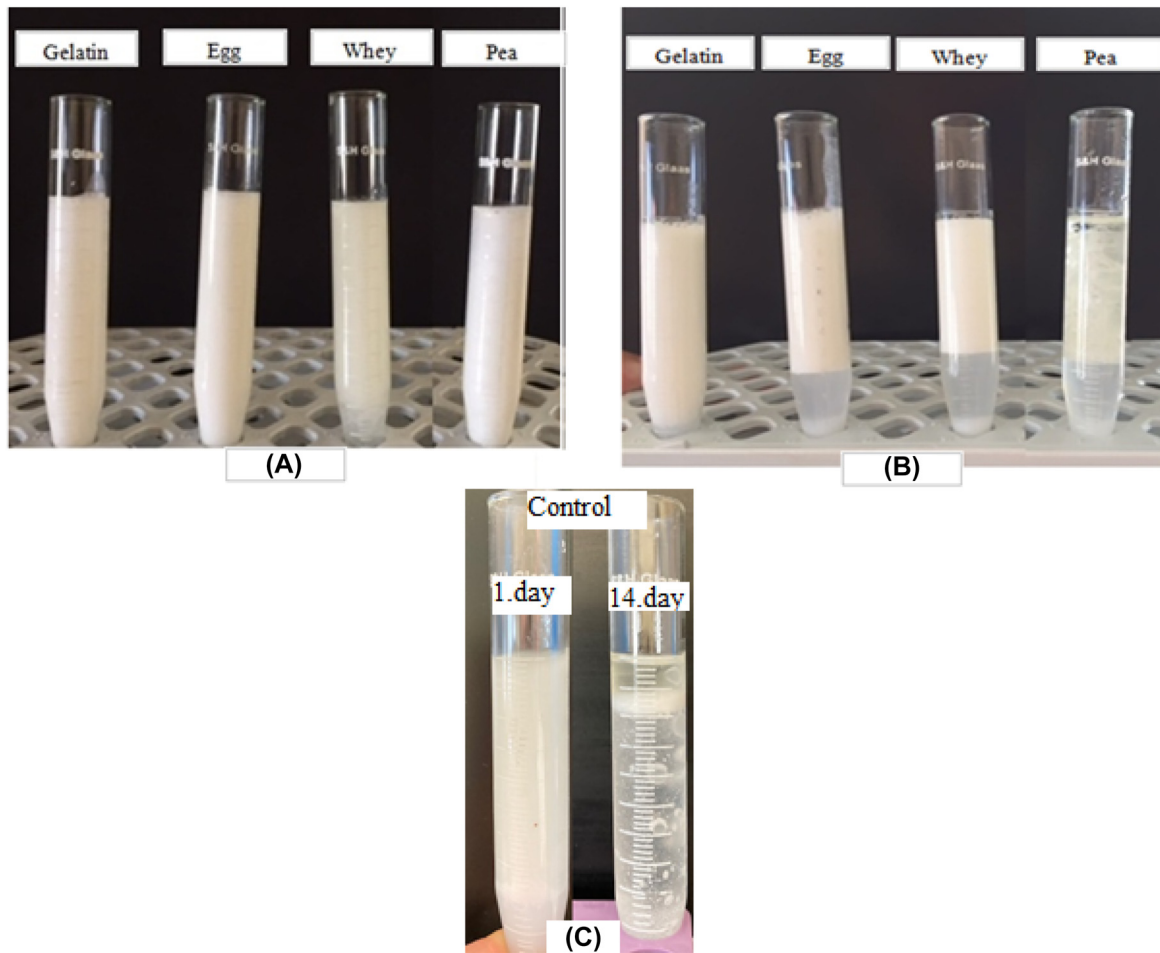


**Figure 1:** The schematic representation of the interfacial rheology experiment.

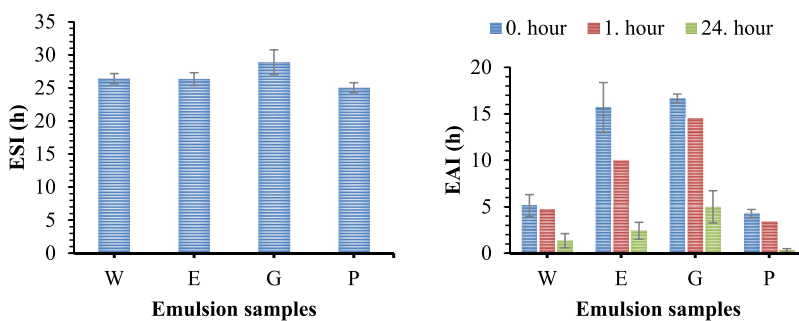


**Figure 2:** The creaming index values of emulsion samples. G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.

Furthermore, proteins would prevent the creaming throughout the shelf life. Food emulsions tend to be destabilized and after sufficient time, the two phases will collapse as food emulsions attempt to minimize the contact area [20]. In the relevant literature, some scholars have used emulsion stability to measure the degree of creaming in an emulsion [21]. Creaming occurs due to the difference in density between the two phases under the effect of gravity [22]. Creaming index related with emulsion stability and help to predict the degree of droplet in an emulsion [23]. In this regard, it can be concluded that if the creaming index is lower, the emulsion is more stable to phase separation throughout the shelf life. Thus, it may be assumed that the gelatine was found to be more effective when preparing stable emulsions with inulin considering the shelf life and phase and/or gravitational separation. The prepared protein-stabilized emulsions subjected to quantitative analysis with the ESI measurement, which gives more insight on the emulsifier ability to prevent droplets from aggregating over time. Thus, a higher emulsion stability index value indicates a lesser degree of phase separation [24]. As illustrated in Figure 4, ESI of samples prepared with pea protein was  $25.03 \pm 0.75 \text{ h}$  and slightly increased with the usage of gelatine in the formulation to the  $28.87 \pm 1.88 \text{ h}$ . Generally, the emulsifying characteristics of different origin proteins are distinct due to structural features of the protein that can stabilize an emulsion by forming a thin layer adsorbed at the oil-water interface. It ensures emulsions stability against flocculation and coalescence via covalent bonding [25]. It was found that the variation in the origin of protein affected the ESI of the emulsion and the maximum stability was achieved by the gelatine-inulin stabilized emulsions in terms of ESI. This result could be attributable to the differences in tension between oil/water interface and inulin-gelatine interaction. Since the different influences which affect the emulsion stability such as



**Figure 3:** Images of creaming index analysis. (A) 1st day, (B) 14th day of protein samples and (C) 1st and 14th day of control samples.



**Figure 4:** EAI and ESI values of protein-stabilized emulsions. G, gelatine; W, whey protein; E, egg protein; P, pea protein.

particle size distributions, protein-polysaccharide interaction, rheological properties, flocculation and interfacial tension [26], the ESI analysis was conducted. As a result of ESI analysis, it was revealed that protein-polysaccharide interaction may also affect emulsifying properties of emulsions. Similar conclusions have been reported in previous studies. The emulsifying characteristics of sugar beet pectin [27] and corn fibre gum [28] are improved with protein by covalent bonding. The value of interfacial area per gram of

emulsifier was calculated by the emulsion activity index (EAI). The development of the emulsifier activity index by Pearce and Kinsella [29] was a useful step in the science of comparing and evaluating emulsifiers. Figure 4 showed the effect of different originated protein on the EAI values of emulsion samples. The maximum value for EAI ( $16.66 \pm 0.47 \text{ g/m}^2$ ) occurred in gelatine stabilized protein while the minimum value ( $4.26 \pm 0.45 \text{ g/m}^2$ ) appeared at the pea protein stabilized emulsion at the beginning of the analysis. The

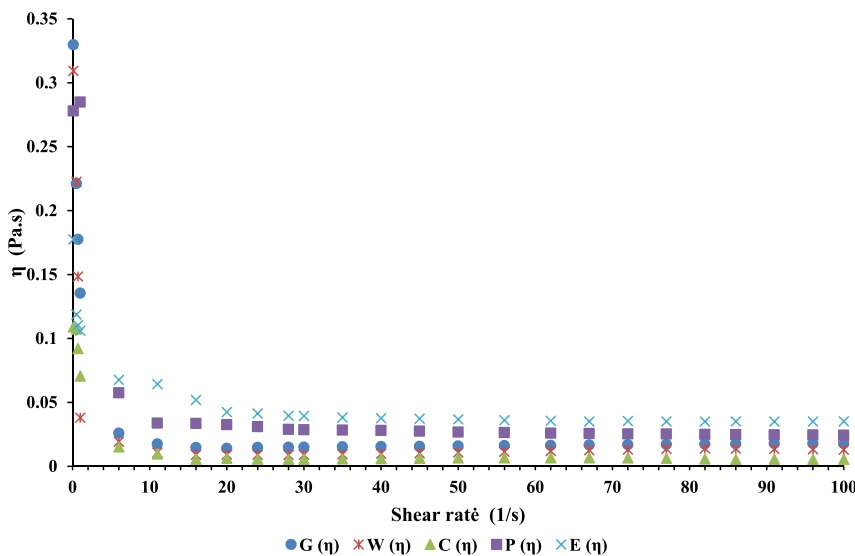
EAI values of the emulsion samples stabilized with different proteins and inulin during the whole analyses procedure (0, 1, 24 h) were statistically different ( $p < 0.05$ ). EAI of the gelatine stabilized emulsion was relatively higher than others. This may be due to the solubility of gelatine. Because, in general, the peptides or proteins with higher solubility exhibits greater values for EAI [30]. Combined with the ESI, the EAI also showed higher performance when the ESI was relatively high, throughout the analysis. A similar result has also been reported in a previous study [31]. Moreover, the differences between the first and last values of EAI calculated at the 0 and 24 h were lower as compared to other samples. This is because of the effect of oil/protein ratio, surface and structure hydrophobicity of the protein, which are the major parameters influencing the emulsifying properties [25]. Therefore, it might be concluded that the gelatine stabilized emulsion will be more stable throughout the shelf life.

## 3.2 Rheological properties

### 3.2.1 Steady shear rheological properties

Figure 5 showed the effect of protein differentiation on the apparent viscosity of inulin-protein stabilized emulsions measured at 25 °C. The increasing shear rate values led to a decrease in apparent viscosity values of the emulsions, this results indicated shear thinning flow behaviour [32]. This was consistent with a previous study in which the shear thinning behaviour was reported for the emulsions and was stated that thinning behaviour was associated with the incorporation of oil droplets [24]. This conclusion generally

the case with the coalesced emulsions or those to which a thickener was added [24]. Also, in the whole shear rate range, all protein stabilized emulsions and control sample exhibited pseudoplastic flow behaviour, indicating that the associated droplets are agglomerated in the emulsions [33]. The lowest apparent viscosity value was recorded in control sample. Furthermore, emulsions prepared with whey protein had lower viscosity compared to the other protein containing emulsions and the differences between the viscosity of the emulsions were statistically significant ( $p < 0.05$ ). The reason for the variation on the viscosities of the emulsions may be related to the interfacial area, protein-inulin interactions or coverage and the surface hydrophobicity of the used protein. On the other hand, as the shear rate increases sufficiently to overcome the Brownian motion, the emulsion droplets become more uniform along with the flow area and exhibit less resistance to flow and therefore lower viscosity [34]. Moreover, with increasing shear rate, emulsion droplets and polymer chains are further aligned along the flow direction, which reduces resistance to flow, is observed as a lower viscosity [35]. The apparent viscosity ( $\eta_{50}$ ) of the emulsions ranges between 0.006 and 0.037 Pa s and the maximum value was observed in the egg protein stabilized the emulsion. The differentiation in protein type was affected by the apparent viscosity due to their characteristic nature. The results of the apparent viscosity are consistent with the emulsifying properties of emulsions. These findings indicated that the flocculation could attenuate the emulsion flow by resistance force between the particles and thereby increase the viscosity of the emulsions [35]. The shear stress exhibited a dependence on the shear rate for



**Figure 5:** The effect of different protein on the apparent viscosity of emulsions. G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.



the ranges of the shear rate used in the study. Power Law model has defined the flow curves and the parameters were illustrated in Table 1. The power-law model provided a good fit for experimental data ( $R^2 > 0.989$ ). It can be inferred that the egg and pea-protein-stabilized emulsions behaved like a near-Newtonian fluid. The flow behaviour index and consistency index are parameters that represents the internal structure of the foods [36]. The variations in protein type had a significant effect on the flow behaviour index. The flow behaviour index ( $n$ ) of emulsions was approximately ranged between 0.8 and 1.2. The consistency coefficient ( $K$ ) values which are the indicator of viscous nature of emulsion [37] was recorded to increase from  $0.004 \text{ Pa s}^n$  to  $0.088 \text{ Pa s}^n$  with the addition of whey protein and egg protein to the formulation, respectively (Table 1). When the result of  $K$  values of was associated with the emulsifying properties of emulsion, it may not be wrong to suggest that the formation of non-spherical aggregations stems from coalescence increased apparent dispersed phase, both factors contribute to increasing in emulsion consistency [38]. Furthermore, the interface membrane has natural viscoelasticity and may explain this condition in the interface [39]. Membrane formed around oil droplets can be caused by egg protein. Since this membrane delays the permeability of the tangential shear stress from the continuous phase to the droplets, it prevents the flow of liquid within the droplet [40].

### 3.2.2 Dynamic oscillatory shear test

In order to measure the frequency dependence of the storage modulus ( $G'$ ) and loss modulus ( $G''$ ), the dynamic frequency sweep tests were conducted in the linear viscoelastic region. The dynamic rheological measurement of  $G'$  and  $G''$  indicates whether the emulsion system is weakly or strongly flocculated [41]. Figure 6 depicted the mechanical

spectra of control sample and different protein-stabilized emulsions to describe the viscoelastic behaviour at  $25^\circ\text{C}$ . The magnitudes of both the loss and storage modulus of gelatine and whey protein stabilized emulsion increased with frequency and were almost frequency-dependent. Both  $G'$  and  $G''$  values of pea protein stabilized emulsion was decreased with the increasing frequency range. Most of the previous studies on the frequency dependence revealed that the  $G''$  value was over  $G'$ , which indicates the emulsions behaved liquid-like. According to the Steffe [42], this behaviour concerns with the characteristics of viscoelastic fluids. When  $G''$  is much higher than  $G'$  at low frequencies, the energy is dispersed viscously that used to deform the material and the sample behaviour shows liquid-like property [43] due to hydrophilic properties polysaccharides which are not surface active [44]. Given the results of the oscillatory rheological measurements of storage and loss modulus, it was demonstrated that the addition of inulin as a polysaccharide to the emulsion formulation affected the viscoelastic characteristic of the system. The polysaccharides addition to the emulsion system can alter the rheological behaviour of emulsions and greatly impacts on protein absorption at the interface [45]. The addition of polysaccharides to the medium promotes the concentration of whey proteins and then affects the gel formation process [46]. In other words, polysaccharides could change the modify the gelling properties of whey protein. It is important to note that the interaction between the two biopolymers accelerates the gelation process by reducing the protein concentration during gel formation process [47].

## 3.3 Interfacial rheological properties

The interaction between the different types of protein with inulin leads to the formation of protein/polysaccharide complexes in the bulk aqueous phase. The protein-polysaccharide interaction different from the bulk solution at the interface results from the conformation of the proteins at the interface [48]. Surface stabilization is achieved through polysaccharide/protein combination at fluid interfaces [44]. In fact, the stability is formed through the polysaccharide-protein interaction at their interfaces. Food polysaccharides reduce the thinning behaviour of films by increasing the thickness of the protein layer and controlling the rheology of the aqueous phase. Thus, surface layer stability is greatly enhanced in the presence of protein-polysaccharide at the interface [48]. Interfacial rheological measurements were performed at the interface and were prepared with inulin dietary fibre together with

**Table 1:** The rheological properties of prepared emulsions.

Samples	Consistency coefficient $K$ ( $\text{Pa s}^n$ )	Flow behavior index ( $n$ )	Apparent viscosity/ $n_{50}$ ( $\text{Pa s}$ )
Gelatin	$0.008 \pm 0.00^{c,d}$	$1.174 \pm 0.05^a$	$0.015 \pm 0.00^c$
Whey protein	$0.004 \pm 0.00^d$	$1.283 \pm 0.07^a$	$0.010 \pm 0.00^{c,d}$
Egg protein	$0.088 \pm 0.00^a$	$0.838 \pm 0.61^b$	$0.037 \pm 0.00^a$
Pea protein	$0.049 \pm 0.00^b$	$0.804 \pm 0.09^b$	$0.027 \pm 0.01^b$
Control	$0.009 \pm 0.00^c$	$0.879 \pm 0.01^b$	$0.006 \pm 0.01^d$

Letters in the same column show the differences between the groups ( $p < 0.05$ ) mean  $\pm$  standard deviation.

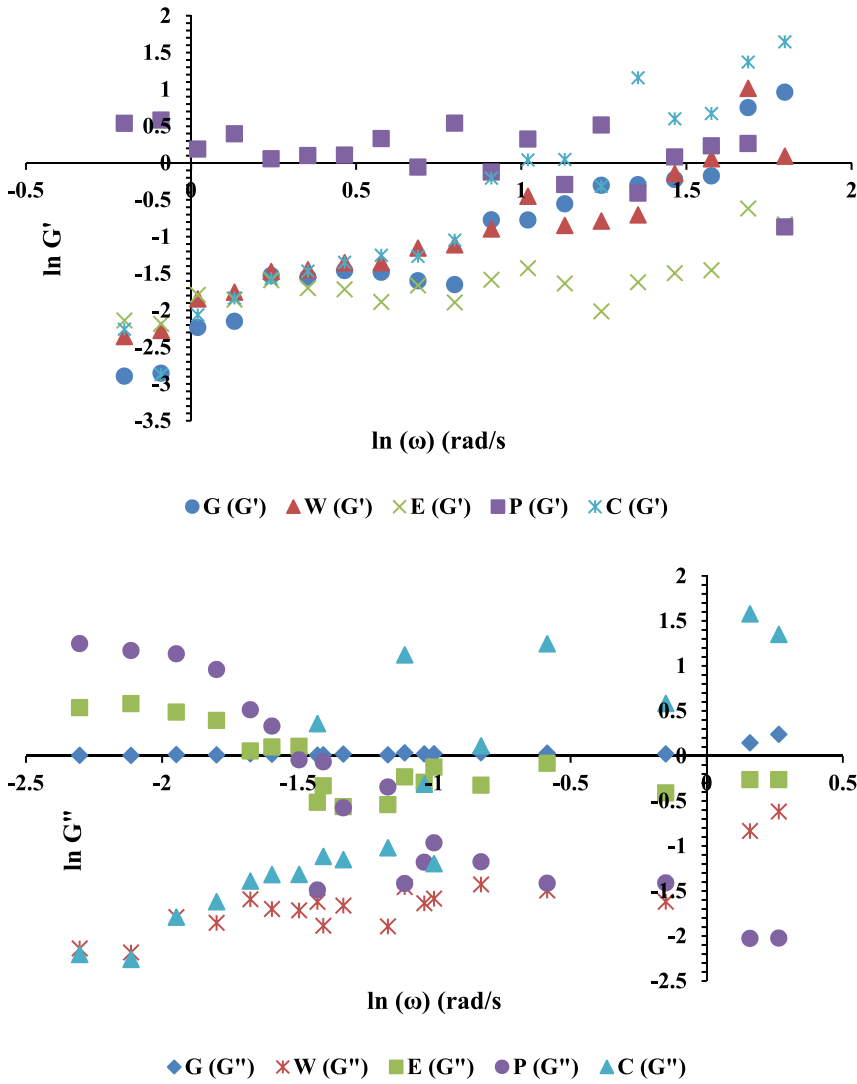


Figure 6: The mechanical spectra of emulsions at increasing frequency level. G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.

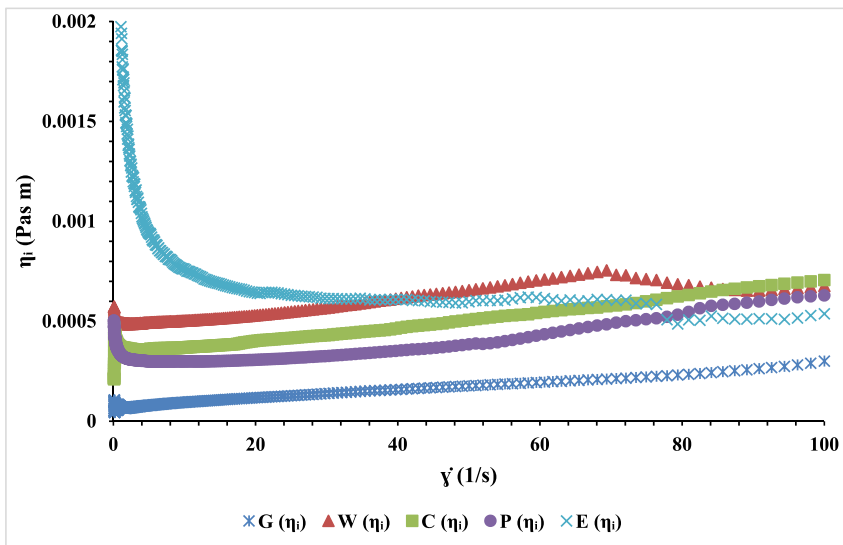


Figure 7: Steady interfacial properties of protein-inulin interactions. G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.

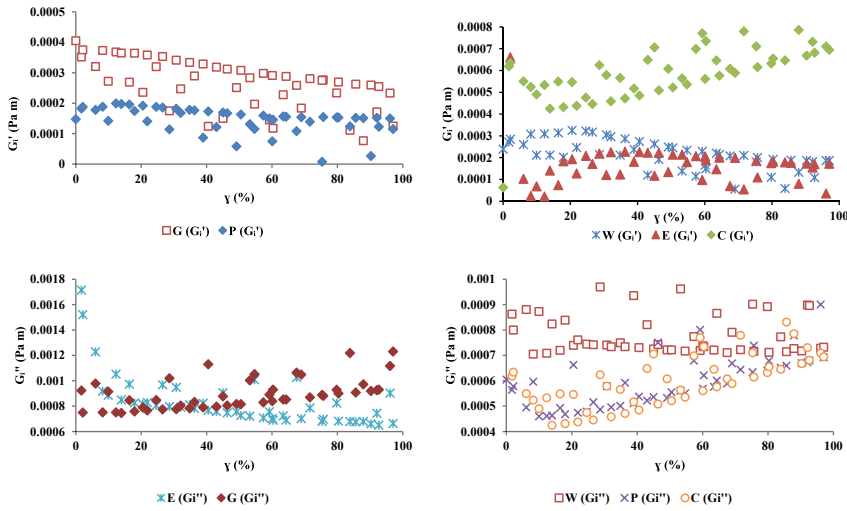


Figure 8: Strain sweep dynamic interfacial properties of protein-inulin interactions. G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.

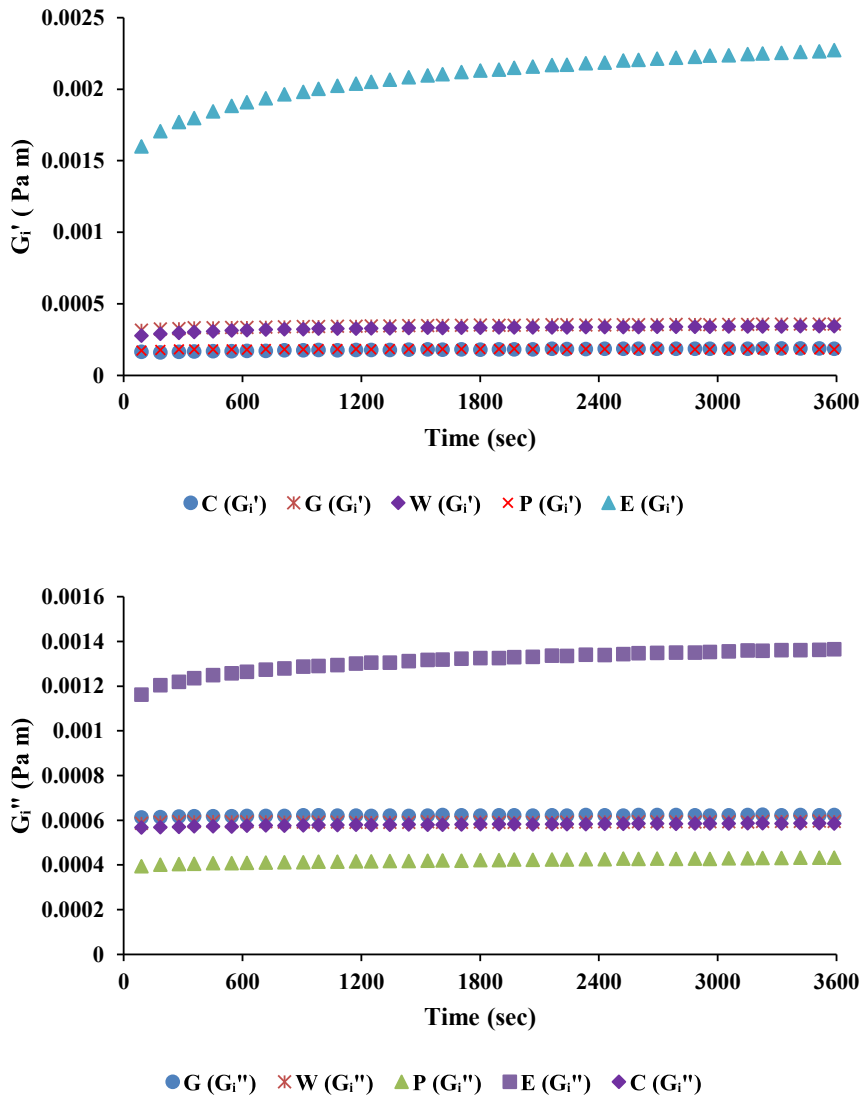
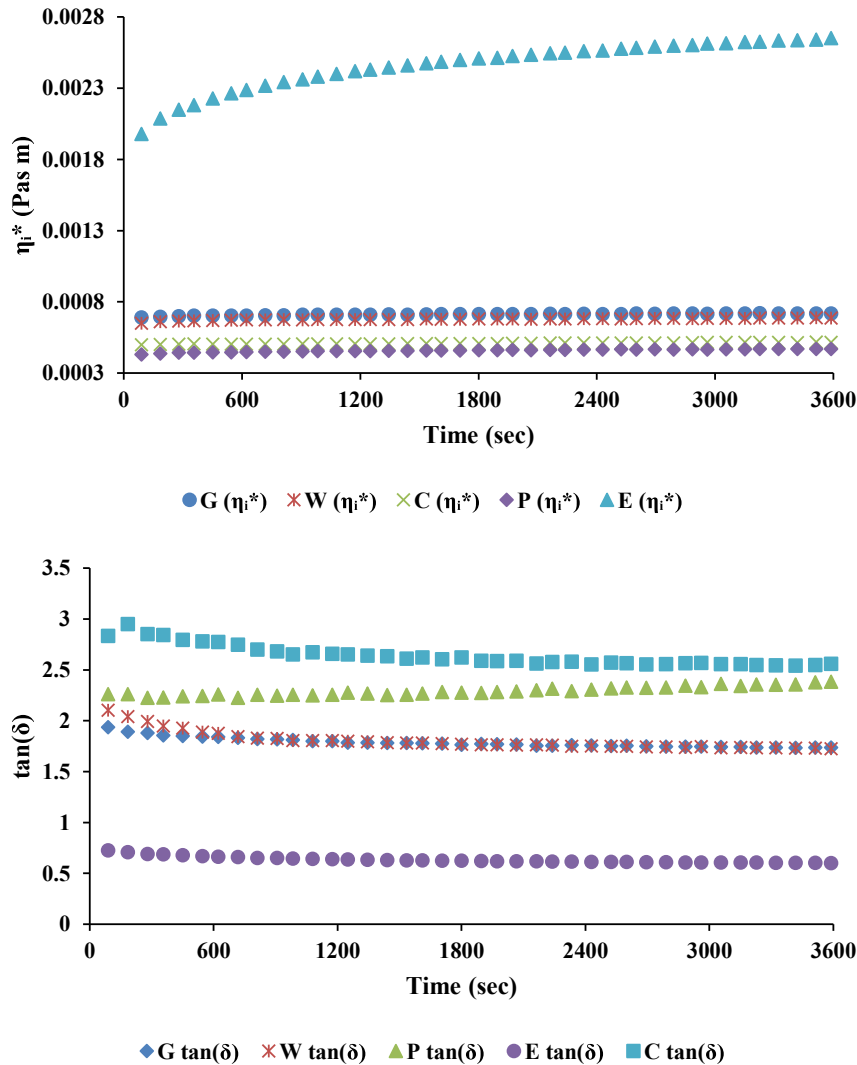


Figure 9: Time sweep dynamic interfacial properties of protein-inulin interactions for  $G'$  and  $G''$ . G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.



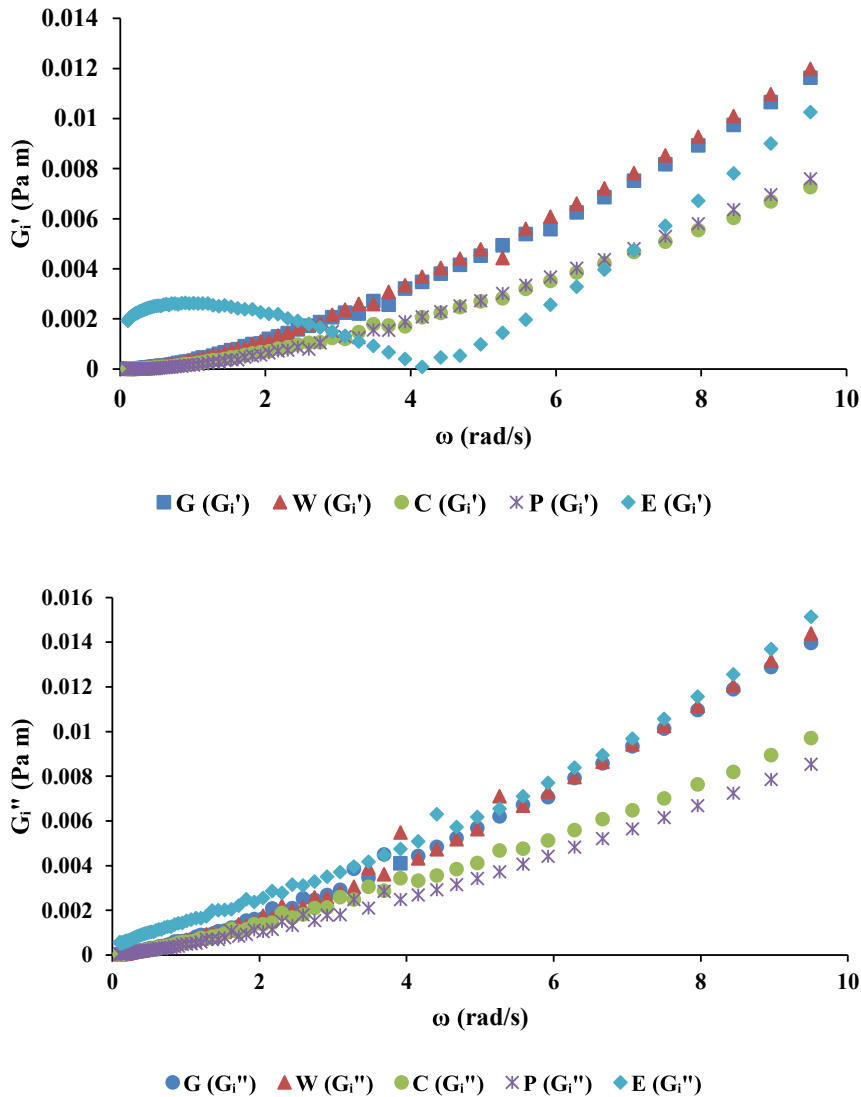


**Figure 10:** Time sweep dynamic interfacial properties of protein-inulin interactions for  $\eta_i^*$  and  $\tan(\delta)$ . G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.

four varieties of different proteins. The results of steady interfacial tests were illustrated in Figure 7. The lowest viscosity value was obtained in the gelatine-inulin mixture by increasing the shear rate. The highest interfacial viscosity ( $\eta_i$ ) values at low shear rates were determined in the mixture of egg protein inulin at oil/water interface. While the whey protein-inulin mixture tends to decrease after the increase in  $\eta_i$ , interfacial viscosity of the control sample, pea protein-inulin and gelatine-inulin samples were increased with the increasing shear rate. The interfacial viscosity values of the samples and changing led by the different in shear rate as a result of the interaction of different proteins with inulin. Adsorption of proteins at the interface is a complex process which is also evidenced by diverse properties of proteins such as hydrophobicity, charge, molecular weight and secondary structure [49]. A

high-molecular-weight polysaccharide has a large impact on not only the increase of the viscosity but also the balance at the interface [48].

The strain sweep test was performed for the structural fracture mechanism in the adsorption layer. Interfacial storage modulus ( $G_i'$ ) and interfacial loss modulus ( $G_i''$ ) values of the samples were determined at strain amplitude range of  $\gamma = 0.01$ –100% and the results were illustrated in Figure 8. Protein adsorption shows viscoelastic multilayer property formed as a result of the flexibility and aggregation property of proteins at the oil-water interface. However, the protein-inulin association at the interface may alter this effect.  $G_i'$  showed a tendency to decrease in all protein-inulin interaction systems at oil/water interface. The loss modulus value decreased in egg protein-inulin interface, while the other samples increased against the

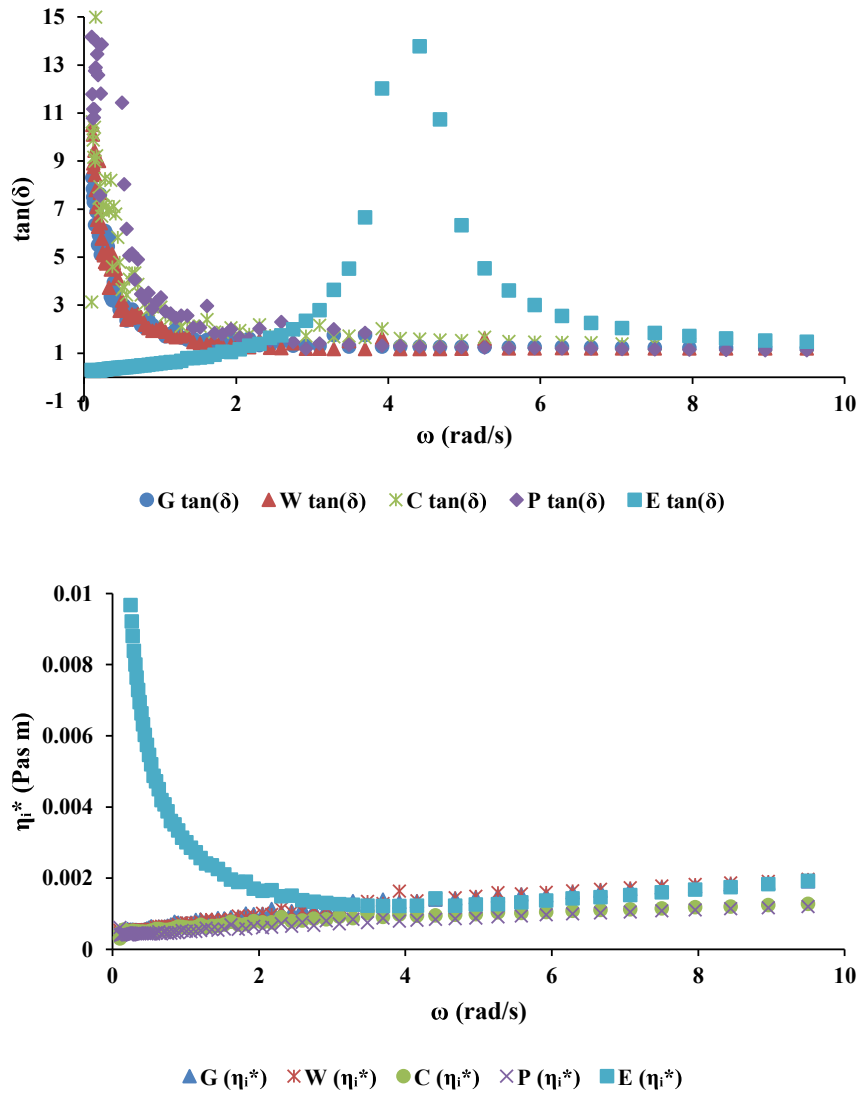


**Figure 11:** Frequency sweep dynamic interfacial properties of protein-inulin interactions for  $G_i'$  and  $G_i''$ . G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.

strain amplitude. The loss modulus values were found to be higher than the storage modulus. This result indicated that the interfacial layers behaved as fluid-like.

In the presence of protein and inulin, the interfacial time sweep test was performed at 3600 s at the oil-water interface. Interfacial storage modulus ( $G_i'$ ) and interfacial loss modulus ( $G_i''$ ) values were measured as shown in Figure 9. While  $G_i'$  and  $G_i''$  values of egg at protein-inulin mixture interface increased, other protein inulin mixtures showed a constant trend in time. An increase in the interfacial loss modulus was measured because of the formation of inter-protein linkages by hydrophobic interactions and probably included the formation of multiple layers during adsorption [50]. The lowest interfacial storage and loss modulus were specified for the interface of pea

protein-inulin mixture.  $G_i''$  was larger than  $G_i'$  for protein inulin mixture except for egg protein, indicating that the loss modulus dominated the adsorbed layer. Accordingly, Felix, Romero [51] demonstrated that  $G_i''$  and  $G_i'$  values of Faba bean protein increased with time at oil-water interface. Therewithal, in another study, it was stated that the  $\beta$ -casein behaved as fluid-like at the hexadecane-water interface [50]. Time sweep experiments are predictive for protein adsorption at the interface [52]. The interfacial viscoelasticity is formed in the time sweep tests in three ways. First, there is a surface-active diffusion from the bulk phase, while the second step is unfolding and adsorption, and finally network is formed due to the association of the molecules [53]. The elastic modulus shows a monotonous shot in systems containing pure protein [54]. On the



**Figure 12:** Frequency sweep dynamic interfacial properties of protein-inulin interactions for  $\eta_i^*$  and  $\tan(\delta)$ . G, gelatine; W, whey protein; E, egg protein; P, pea protein; C, control.

other hand, in complex systems like food emulsions, the increases might occur as a function of time [53].

Interfacial complex viscosity ( $\eta_i^*$ ) and  $\tan(\delta)$  values are indicators of time sweep dynamic properties was shown in Figure 10. When the value of  $\tan(\delta)$  was examined, it can be seen that  $\tan(\delta)$  decrease against time in all protein-inulin mixtures. Stabilization is affected by adsorption rate in protein stabilized emulsions and generally, the adsorption rate is generally estimated based on hydrophobicity [55].

Frequency sweep tests were conducted at  $\gamma = 0.1\%$  at 25 °C at the frequency range of 0.1–10 rad s<sup>-1</sup>, and the results were presented in Figures 11 and 12. Furthermore, the  $G_i'$  and  $G_i''$  values were frequency dependent. It was observed that all samples showed increasing storage and

loss modulus values with the increasing frequency values, except the media prepared with egg protein. The  $G_i''$  was larger than  $G_i'$  throughout the experiment with frequency change. The results were in agreement with Felix, Romero's [51] study. They found that loss modulus of Faba bean protein was larger than storage modulus as well as the  $G_i'$  and  $G_i''$  values raised by increasing frequency values at the oil-water interface. While the  $\eta_i^*$  measurement results were evaluated, a decrease with increasing frequency was observed. Moreover, the  $\tan(\delta)$  value of egg protein-inulin mixture increased before about 5 rad/s then decreased. This results were in line with another study in which the protein layer turned into the viscoelastic structure as a result of pectin and protein interactions containing emulsions at the interface [48].

## 4 Conclusions

The present work indicated that the interfacial, dynamic and steady rheological properties along with the emulsifying properties of protein-stabilized emulsions were affected by the different types of proteins with inulin in the formulation. The existence of inulin in oil-in-water emulsions containing food protein modified their physical-emulsifying characteristics. Thickening effect of polysaccharide in aqueous phase and inulin-protein interaction at the interface are the parameters that affect this results respectively. Inulin is used as a prebiotic in commercial foods products. However, the results supported the use of inulin in food emulsions, highlighting the importance of the interactions with the used protein and its role as a thickener. Overall, the results of this study underpins the significance of determining the interfacial characteristics of protein-stabilized emulsions because they can be used to predict the stability of emulsion throughout the shelf-life and to gain a deeper understanding on the effects of the dietary fibre fortification on the emulsion efficiency in food products. The findings are of great importance in understanding the stability and behaviour of protein-stabilized emulsions and in the improvement of gelled emulsions with potential use as a carrier system for active lipid-soluble components. In our follow-up research, we hope to prepare multiple emulsions and analyse in order to determine whether the protein-inulin interaction might affect the interfacial and emulsifying properties when the second interface is present.

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## References

1. Araiza-Calahorra A, Sarkar A. Designing biopolymer-coated Pickering emulsions to modulate in vitro gastric digestion: a static model study. *Food Funct* 2019;10:5498–509.
2. Lu J, Xu X, Zhao X. Interfacial rheology of alkali pH-shifted myofibrillar protein at O/W interface and impact of Tween 20 displacement. *Food Hydrocolloids* 2022;124:107275.
3. Bouyer E, Mekhloufi G, Rosilio V, Grossiord J-L, Agnely F. Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: alternatives to synthetic surfactants in the pharmaceutical field? *Int J Pharm* 2012;436:359–78.
4. Hailing PJ, Walstra P. Protein-stabilized foams and emulsions. *Crit Rev Food Sci Nutr* 1981;15:155–203.
5. Kelly G. Inulin-type prebiotics—a review: part 1. *Alternative Med Rev* 2008;13:315–29.
6. Kolida S, Tuohy K, Gibson GR. Prebiotic effects of inulin and oligofructose. *Br J Nutr* 2002;87(2 Suppl):S193–7.
7. Franck A. Technological functionality of inulin and oligofructose. *Br J Nutr* 2002;87(2 Suppl):S287–91.
8. Xu Q, Qi B, Han L, Wang D, Zhang S, Jiang L, et al. Study on the gel properties, interactions, and pH stability of pea protein isolate emulsion gels as influenced by inulin. *Lebensm Wiss Technol* 2021;137:110421.
9. Karimi R, Azizi MH, Ghasemlou M, Vaziri M. Application of inulin in cheese as prebiotic, fat replacer and texturizer: a review. *Carbohydr Polym* 2015;119:85–100.
10. Fernández-García E, McGregor JU. Fortification of sweetened plain yogurt with insoluble dietary fiber. *Z Lebensm -Forsch A* 1997;204:433–7.
11. Tran T, Rousseau D. Stabilization of acidic soy protein-based dispersions and emulsions by soy soluble polysaccharides. *Food Hydrocolloids* 2013;30:382–92.
12. Bot A, Erle U, Vreeker R, Agterof WG. Influence of crystallisation conditions on the large deformation rheology of inulin gels. *Food Hydrocolloids* 2004;18:547–56.
13. Nourbehesht N, Shekarchizadeh H, Soltanizadeh N. Investigation of stability, consistency, and oil oxidation of emulsion filled gel prepared by inulin and rice bran oil using ultrasonic radiation. *Ultrason Sonochem* 2018;42:585–93.
14. Barclay T, Ginic-Markovic M, Cooper P, Petrovsky N. Inulin—a versatile polysaccharide with multiple pharmaceutical and food chemical uses. *J Excipients Food Chem* 2016;1:1132.
15. Alvarez-Sabatel S, de Marañón IM, Arbolea J-C. Impact of high pressure homogenisation (HPH) on inulin gelling properties, stability and development during storage. *Food Hydrocolloids* 2015;44:333–44.
16. Benjamins J. Static and dynamic properties of proteins adsorbed at liquid interfaces: sn. Wageningen University and Research; 2000.
17. Baldursdottir SG, Fullerton MS, Nielsen SH, Jorgensen L. Adsorption of proteins at the oil/water interface—observation of protein adsorption by interfacial shear stress measurements. *Colloids Surf, B* 2010;79:41–6.
18. Manoi K, Rizvi SS. Emulsification mechanisms and characterizations of cold, gel-like emulsions produced from texturized whey protein concentrate. *Food Hydrocolloids* 2009; 23:1837–47.
19. Firebaugh J, Daubert C. Emulsifying and foaming properties of a derivatized whey protein ingredient. *Int J Food Prop* 2005;8: 243–53.
20. Rousseau D. Fat crystals and emulsion stability—a review. *Food Res Int* 2000;33:3–14.
21. Dhungana P, Truong T, Bansal N, Bhandari B. Apparent thermal and UHT stability of native, homogenized and recombined creams with different average fat globule sizes. *Food Res Int* 2019;123:153–65.
22. Loi CC, Eyres GT, Birch EJ. Effect of milk protein composition on physicochemical properties, creaming stability and volatile profile of a protein-stabilised oil-in-water emulsion. *Food Res Int* 2019;120:83–91.

23. Qayum A, Chen W, Ma L, Li T, Hussain M, Bilawal A, et al. Characterization and comparison of  $\alpha$ -lactalbumin pre-and post-emulsion. *J Food Eng* 2019;269:109743.
24. Dammak I, do Amaral Sobral PJ. Investigation into the physicochemical stability and rheological properties of rutin emulsions stabilized by chitosan and lecithin. *J Food Eng* 2018; 229:12–20.
25. Sharif HR, Williams PA, Sharif MK, Abbas S, Majeed H, Masamba KG, et al. Current progress in the utilization of native and modified legume proteins as emulsifiers and encapsulants—a review. *Food Hydrocolloids* 2018;76:2–16.
26. Kunarayakul S, Thaiphanit S, Anprung P, Suppavorasatit I. Optimization of coconut protein deamidation using protein-glutaminase and its effect on solubility, emulsification, and foaming properties of the proteins. *Food Hydrocolloids* 2018;79: 197–207.
27. Chen H, Ji A, Qiu S, Liu Y, Zhu Q, Yin L. Covalent conjugation of bovine serum albumin and sugar beet pectin through Maillard reaction/laccase catalysis to improve the emulsifying properties. *Food Hydrocolloids* 2018;76:173–83.
28. Liu Y, Qiu S, Li J, Chen H, Tatsumi E, Yadav M, et al. Peroxidase-mediated conjugation of corn fiber gum and bovine serum albumin to improve emulsifying properties. *Carbohydr Polym* 2015;118:70–8.
29. Pearce KN, Kinsella JE. Emulsifying properties of proteins: evaluation of a turbidimetric technique. *J Agric Food Chem* 1978; 26:716–23.
30. Mustapha NA, Ruttarattanamongkol K, Rizvi SS. The effects of supercritical fluid extrusion process on surface hydrophobicity of whey protein concentrate and its relation to storage and heat stability of concentrated emulsions. *Food Res Int* 2012;48: 470–7.
31. Ai M, Tang T, Zhou L, Ling Z, Guo S, Jiang A. Effects of different proteases on the emulsifying capacity, rheological and structure characteristics of preserved egg white hydrolysates. *Food Hydrocolloids* 2019;87:933–42.
32. Yilmaz MT, Karaman S, Kayacir A, Dogan M, Yetim H. Construction of predictive models to describe apparent and complex viscosity values of O/W model system meat emulsions using adaptive neuro – fuzzy inference system (ANFIS) and artificial neural networks (ANN). *Food Biophys* 2012;7:329–40.
33. Liu F, Tang C-H. Emulsifying properties of soy protein nanoparticles: influence of the protein concentration and/or emulsification process. *J Agric Food Chem* 2014;62:2644–54.
34. Khalloufi S, Alexander M, Goff HD, Corredig M. Physicochemical properties of whey protein isolate stabilized oil-in-water emulsions when mixed with flaxseed gum at neutral pH. *Food Res Int* 2008;41:964–72.
35. Xiong W, Ren C, Tian M, Yang X, Li J, Li B. Emulsion stability and dilatational viscoelasticity of ovalbumin/chitosan complexes at the oil-in-water interface. *Food Chem* 2018;252:181–8.
36. Guo T, Zhao K, Zhang Z, Gao X, Qi X. Rheology study on low-sugar apple jam by a new nonlinear regression method of Herschel-Bulkley model. *J Food Process Preserv* 2017;41:e12810.
37. Dogan M, Aslan D, Gurmeric V. The rheological behaviors and morphological characteristics of different food hydrocolloids ground to sub-micro particles: in terms of temperature and particle size. *J Food Meas Charact* 2018;12:770–80.
38. Erçelebi EA, Ibanoglu E. Rheological properties of whey protein isolate stabilized emulsions with pectin and guar gum. *Eur Food Res Technol* 2009;229:281–6.
39. Dickinson E. Food emulsions and foams: stabilization by particles. *Curr Opin Colloid Interface Sci* 2010;15:40–9.
40. Dammak I, Lourenço RV, do Amaral Sobral PJ. Active gelatin films incorporated with Pickering emulsions encapsulating hesperidin: preparation and physicochemical characterization. *J Food Eng* 2019;240:9–20.
41. Torres LG, Iturbe R, Snowden MJ, Chowdhry BZ, Leharne SA. Preparation of o/w emulsions stabilized by solid particles and their characterization by oscillatory rheology. *Colloids Surf A Physicochem Eng Asp* 2007;302:439–48.
42. Steffe JF. *Rheological methods in food process engineering*. East Lansing, USA: Freeman Press; 1996.
43. Rao MA. *Rheology of fluid and semisolid foods: principles and applications*. New York, USA: Springer Science & Business Media; 2010.
44. Dickinson E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids* 2003;17: 25–39.
45. Murray BS. Interfacial rheology of food emulsifiers and proteins. *Curr Opin Colloid Interface Sci* 2002;7:426–31.
46. Spotti MJ, Santiago LG, Rubiolo AC, Carrara CR. Mechanical and microstructural properties of milk whey protein/espina corona gum mixed gels. *LWT—Food Sci Technol* 2012;48:69–74.
47. Khalesi H, Emadzadeh B, Kadkhodaei R, Fang Y. Effect of Persian gum on whey protein concentrate cold-set emulsion gel: structure and rheology study. *Int J Biol Macromol* 2019;125: 17–26.
48. Piazza L, Dürr-Auster N, Gigli J, Windhab EJ, Fischer P. Interfacial rheology of soy proteins—high methoxyl pectin films. *Food Hydrocolloids* 2009;23:2125–31.
49. Haynes CA, Norde W. Globular proteins at solid/liquid interfaces. *Colloids Surf, B* 1994;2:517–66.
50. Freer EM, Yim KS, Fuller GG, Radke CJ. Interfacial rheology of globular and flexible proteins at the hexadecane/water interface: comparison of shear and dilatation deformation. *J Phys Chem B* 2004;108:3835–44.
51. Felix M, Romero A, Carrera-Sanchez C, Guerrero A. Assessment of interfacial viscoelastic properties of Faba bean (*Vicia faba*) protein-adsorbed O/W layers as a function of pH. *Food Hydrocolloids* 2019;90:353–9.
52. Li W, Wang Y, Zhao H, He Z, Zeng M, Qin F, et al. Improvement of emulsifying properties of soy protein through selective hydrolysis: interfacial shear rheology of adsorption layer. *Food Hydrocolloids* 2016;60:453–60.
53. Piazza L, Bulbarelo A, Gigli J, editors. *Rheological interfacial properties of espresso coffee foaming fractions*. In: 13th World Congress of Food Science & Technology; 2006, vol 2006.
54. Roberts SA, Kellaway IW, Taylor KM, Warburton B, Peters K. Combined surface pressure– interfacial shear rheology study of the effect of pH on the adsorption of proteins at the air–water interface. *Langmuir* 2005;21:7342–8.
55. Delahaije RJ, Gruppen H, Giuseppin ML, Wierenga PA. Towards predicting the stability of protein-stabilized emulsions. *Adv Colloid Interface Sci* 2015;219:1–9.