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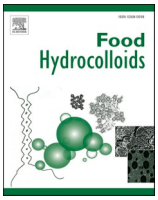

High internal phase emulsions and edible films with high methoxyl pectin and pea protein isolate or sodium caseinate

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ABSTRACT

Structuring of sunflower oil by forming HIPEs in the presence of high methoxyl pectin (HMP) and pea protein isolate (PPI) or sodium caseinate (SC) was evaluated first. Initially, emulsions with protein: polysaccharide ratios of 2:1 and 6:1 and two different pectin concentrations (0.5 and 1% wt) were formed and compared with emulsions with just protein, with the former exhibiting greater viscosity and stability. All emulsions exhibited pseudoplastic behaviour with the higher biopolymer concentrations leading to increased emulsion viscosity. The type of protein was significant for stability only for the initial pectin concentration of 0.5% wt. Then, SC and PPI emulsions with a protein: polysaccharide ratio of 6:1 for both pectin concentrations were selected for HIPE formation. The formed HIPE for both proteins had a great oil loss (83–95%), showing that the used combination of biopolymers did not lead to the formation of a structured system. Subsequently, edible films formed by drying the aqueous dispersions of the previously selected protein-polysaccharide binary systems were studied. All films had the same density. The greater total biopolymer concentration led to thicker, heavier, stronger and stiffer films with greater water vapour permeability and opacity. SC films were thicker, heavier, stronger, stiffer and brighter than PPI films, with lower moisture content and opacity. Overall, the used protein affected the studied properties of the films, indicating differences in the formed network.

1. Introduction

Food structure is very important as it can be used for controlling texture, oral processing and perception and functionality. Proteins and polysaccharides are among the basic ingredients affecting food structure by presenting certain properties such as hydration and water binding, viscosity, gelation, emulsification and foaming ability (Goff & Guo, 2019). Their simultaneous presence in a solution leads to their interaction. Interactions may be associative or segregative and lead to one or two-phase systems due to three possible outcomes: miscibility, association or segregation (de Kruif & Tuinier, 2001).

The type and concentration of the biopolymers as well as their mixing ratio and the pH are among the major factors affecting protein-polysaccharide interactions. The various interactions are utilized for the formation of many different colloidal structures such as particles, emulsions, gels, oleogels, edible films and foams (Weiss, Salminen, Moll, & Schmitt, 2019), which can be exploited by the food industry for a variety of important applications like their use as encapsulation matrices and packaging materials, for the creation of reduced fat products, etc.

As the relationship between health and food is of great importance, the fat content of a food becomes significant. Saturated and *trans*-fats, apart from providing the desired functionality, texture and taste of food, they are associated with cardiovascular diseases and other health problems (Zeng et al., 2017). This has led to the introduction of new legislation on reducing or banning the use of these fats in food products (Vélez – Erazo, Bosqui, Rabelo, Kurozawa, & Hubinger, 2020). Therefore, there is a growing interest for oil structuring alternatives for processed foods high in fat. Oleogels are novel structures exhibiting solid-like properties while incorporating a large amount of liquid oil (usually> 90%) in a physical network (Wijaya et al., 2019). The emulsion-templated method is used for oleogel formation. In this method, an emulsion is initially formed and then, its water is removed by drying. High internal phase emulsions (HIPEs) are another interesting alternative as they are highly concentrated and viscous. Moreover, they exhibit a gel-like structure and excellent resistance to oxidation (Li, Xiong, Wang, Zhang, & Luo, 2023). The formation and stabilisation of HIPEs and oleogels by the use of proteins and polysaccharides are becoming of great interest as these biopolymers are

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considered Generally Recognized as Safe (GRAS) by the Food and Drugs Administration (FDA), have a low cost and they are easy to find (Vélez – Erazo, Bosqui, Rabelo, & Hubinger, 2021). In these systems, proteins by being surface actives act as emulsifiers whereas polysaccharides, due to their hydrophilic character, act as thickening agents (Wijaya et al., 2019).

Another topic of interest for the food industry is the development of edible films as alternatives to plastic packaging. Edible films are preformed, thin edible layers placed on a food. They are made from natural polymers of animal and vegetable origin and as such, proteins and polysaccharides are among their main constituents (Drakos, Pelava, & Evageliou, 2018). Edible films are biodegradable and environmentally friendly packaging that improve product life, quality and safety of a food product; thus, supporting the evolving and changing role of packaging in food protection (Pascall, De Angelo, Richards, & Arensberg, 2022).

Based on the above, the present work aimed to utilise protein-polysaccharide mixtures for both the structuring of sunflower oil by forming emulsions and HIPEs and the formation of edible films. HIPEs and films were formed in the presence of high methoxyl pectin (HMP) in combination with either pea protein isolate (PPI) or sodium caseinate (SC). Pea Protein isolate (PPI) is a plant protein receiving a growing interest over the last years, especially due to its low cost and allergenicity, as well as its potential health benefits (Zha, Gao, Rao, & Chen, 2021). PPI mainly consists of globulins and albumins (70–80% and 10–20% of PPI, respectively) (Zha, Dong, Rao, & Chen, 2019). Sodium caseinate (SC) is a well-known random coil animal protein, which mainly consists of 4 types of phosphoproteins (a_{s1}, a_{s2}, β-and κ-caseins). High methoxyl pectin (HMP) is extracted mainly from citrus peels and apple pomace. It is an anionic heteropolysaccharide, that mainly consists of about 200–1000 (1 → 4)-linked α-D-galacturonic acid units (do Nascimento Oliveira et al., 2018).

In the present study, emulsions of the two proteins at protein: polysaccharide ratios of 2:1 and 6:1 and two different pectin concentrations (0.5 and 1% wt) were formed and their stability and viscosity was measured and compared with emulsions with just the protein. Four of these emulsions (two per protein), which were formed in the presence of specific protein: polysaccharide mixtures, were selected for HIPE formation. The oil loss of the formed HIPEs was determined. As a further step, the aqueous dispersions of the previously selected protein-polysaccharide binary systems were used for the formation of edible packaging films. Several properties of the films were studied, i.e. weight, thickness, moisture content, water vapour permeability, colour, mechanical strength, turbidity and density. To the best of our knowledge, the use of these binary systems under the experimental conditions of the present work has not been studied.

2. Materials and methods

2.1. Materials & chemicals

High methoxyl pectin from apple (HMP, with 50–75% esterification; 93854), was obtained from Sigma-Aldrich (Steinheim, Germany). Sodium caseinate (SC, Excellion® EM-7, 93% protein on dry basis) and yellow pea protein isolate (PPI, NUTRALYS® F85F, 84% protein on dry basis), were kindly donated by Alteco S.A. Food Ingredients (Athens, Greece) and Roquette (Lestrem, France), respectively. Sunflower oil (SANOLA, Kore, Koropi, Greece) was purchased locally. Sodium chloride (NaCl) was from Panreac Quimica S.A. (Barcelona, Spain), glycerol from Merck (Darmstadt, Germany), whereas all the remaining reagents from Sigma-Aldrich (Steinheim, Germany). Distilled water was used throughout.

2.2. Preparation of pectin and protein stock solutions

Protein stock solutions (12% wt) were prepared by dissolving protein powder in distilled water and stirring for 4h, at room temperature. Pectin stock solutions (2% wt) were prepared by gradually adding pectin powder in distilled water at 90 °C, while stirring. All solutions were stored at 4 °C overnight, for complete hydration.

2.3. Preparation of protein-pectin and single protein aqueous dispersions

Protein-pectin mixtures were prepared by mixing the appropriate amounts of the corresponding stock solutions prepared in §2.2 in order to achieve protein: pectin ratios of 2:1 and 6:1. Two groups of mixtures were prepared. The first had a constant pectin concentration of 0.5% wt whereas the second of 1% wt. Solutions of just the protein at a concentration equal to the total biopolymer concentration of each ratio, were also prepared. The pH of all solutions was set to 6, which was roughly the natural pH of both the mixtures and the single protein solutions, with the addition of 0.1N HCl.

2.4. Preparation and characterization of emulsions

Emulsions were prepared by dispersing 60% wt sunflower oil in a 40% wt protein: pectin mixture or protein solution using a high-energy dispersing unit (CAT X 120, M. Zipperer GmbH, Germany) at 18000 rpm for 5 min at room temperature (Tavernier, Patel, Van der Meeren, & Dewettinck, 2017). Table 1 presents the type and the concentration of the biopolymers used for the formation of the emulsions along with the corresponding sample names.

Emulsion stability was estimated by storage assessment based on the method proposed by Huang, Kakuda, and Cui (2001). Each emulsion (10 mL) was placed in 15 mL vials, sealed tightly and stored at 4°C for 7 days. Phase separation of the emulsions is possible over time, during which a transparent layer at the bottom and an opaque layer at the top can be observed. The initial height of the emulsions before storage (H_0) and the height of the remaining emulsified layer after storage ($H_{storage}$) were recorded daily. Two vials were measured for each emulsion and the emulsion stability (ES, %) was calculated using the following equation:

$$ES\ (\%) = \frac{H_{storage}}{H_0} \times 100 \quad (1)$$

Emulsion viscosity was measured using a rotational viscometer (Viscolead One, Fungilab S.A., Barcelona, Spain) at rotational speed from 1 to 100 rpm, at room temperature.

Table 1
Type and concentration of the biopolymers present in the emulsions along with the corresponding sample names [SC: Sodium caseinate; PPI: Pea protein isolate].

Protein	Pectin (% wt)	Protein (% wt)	Protein: pectin ratio	Emulsion sample names
SC	0.5	1.0	2:1	PSC(0.5–1)
	0.5	3.0	6:1	PSC(0.5–3)
	–	1.5	–	PSC(0–1.5)
	–	3.5	–	PSC(0–3.5)
	1.0	2.0	2:1	PSC(1–2)
	1.0	6.0	6:1	PSC(1–6)
	–	3.0	–	PSC(0–3)
	–	7.0	–	PSC(0–7)
PPI	0.5	1.0	2:1	PPPI(0.5–1)
	0.5	3.0	6:1	PPPI(0.5–3)
	–	1.5	–	PPPI(0–1.5)
	–	3.5	–	PPPI(0–3.5)
	1.0	2.0	2:1	PPPI(1–2)
	1.0	6.0	6:1	PPPI(1–6)
	–	3.0	–	PPPI(0–3)
	–	7.0	–	PPPI(0–7)

2.5. Preparation and characterization of high internal phase emulsions (HIPE)

For the formation of HIPE, two emulsions per protein were selected. The selected emulsions were formed in the presence of protein: pectin mixtures with a ratio of 6:1 for both HMP concentrations (i.e. 0.5 and 1.0% wt). The emulsions (30 g per Petri dish) were dehydrated in an oven (Memmert, Schwabach, Germany) at 60°C for 18h. The water content (%) of the dried product was determined gravimetrically. Then, the dried product was sheared using a laboratory mill (3 cycles of 10 s each one) to obtain the HIPE. Oil loss of HIPE was determined with the method proposed by Vélez – Erazo et al. (2020). Aliquots of HIPE were weighed (m_i) and then, subjected to centrifugation at 6000 rpm for 30 min at 5°C using a centrifuge (Z 326 K, Hermle Labortechnik GmbH, Wehingen, Germany). After that, the free oil was removed and the remaining mass was weighed (m_f). Oil loss (OL) was calculated using Equation (2).

$$OL = \frac{m_i - m_f}{m_i} \times 100 \quad (2)$$

2.6. Preparation and characterization of films

2.6.1. Preparation of films

Film-forming solutions (FFS) for each protein were prepared by the aqueous dispersions of protein: pectin mixtures with a ratio of 6:1 for both 0.5 and 1.0% wt HMP concentrations mixed with glycerol (30% wt biopolymers), which was used as a plasticizer. Then, 30 g of each FFS were poured onto sterile glass Petri dishes (diameter 9 cm) and dried in an oven (Memmert, Schwabach, Germany) at 50 °C for 24 h. Finally, the dried films were peeled off and kept in a desiccator with silica gel until analysis. Three Petri dishes per formulation were studied.

2.6.2. Characterisation of films

2.6.2.1. Physicochemical properties. The weight of the films was measured by means of an analytical balance (AE 200, Mettler-Toledo, USA) (Drakos et al., 2018).

Thickness was measured at five random positions on the film surface using a micrometer (Holex, Munich, Germany) with accuracy of 0.01 mm (Zioga, Chroni, & Evageliou, 2022).

For film density, film samples were cut into squares with an area (A) of 4 cm^2, and weighted (m_1). They were then dried in a hot-air oven (Memmert, Schwabach, Germany) at 105 °C for 18 h and weighted again (m_2). Film density and moisture content were calculated form the following equations (Zioga et al., 2022):

$$Film\ Density = \frac{m_1}{x \times A} \quad (3)$$

$$Moisture\ Content\ (\%) = \frac{m_1 - m_2}{m_1} \times 100 \quad (4)$$

x is the film's thickness.

2.6.2.2. Optical properties. Colour parameters [L*], [α*] and [b*] of the CIELAB system were measured by a spectrocolorimeter (LC 100, Lovibond, Dortmund, Germany). Measurements were carried out at five random positions of each film (Zioga et al., 2022)

For opacity, a rectangular film strip (1 cm × 4 cm) was placed directly in a spectrophotometer cell (UV1800, Shimadzu Europa GmbH, Duisburg, Germany) and the spectrum (400–800 nm) of absorbance was recorded, using an empty spectrophotometer cell as reference. The area under the recorded curve was defined as opacity (Drakos et al., 2018)

2.6.2.3. Barrier properties. Water vapour permeability was determined gravimetrically according to the method proposed by Gontard, Guilbert, and Cuq (1993) based on the ASTM E96 test (ASTM Standard, 1989), with slight modifications. The test cups were filled with 3 g of silica gel (desiccant) to produce a 0% RH below the film. The films were sealed on the cups (2.9 cm height, 3 cm diameter) using a waterproof adhesive (paraffin), to ensure humidity migration only through the film, and the air gap was at approximately 2 cm between the film surface and the desiccant. A thin coating of paraffin was applied around the cup circumference to prevent water vapour transfer through the sealant area. The cups were placed in a desiccator with distilled water to provide 100% RH. After the test began, steady state was attained in almost 2 h, and after that, the cup weights were measured at specific time intervals (4 h, 24 h, 48 h, 5 d). Water vapour permeability (WVP, 10^{-8} g mm/h cm^2 Pa) was calculated with the following equation:

$$WVP = \frac{m}{tA} \frac{x}{\Delta P} \quad (6)$$

where x is the average thickness of the edible films (μm), A is the permeation area (7.065 cm^2), ΔP is the difference of partial vapour pressure of the atmosphere with the silica gel and pure water (2642 Pa, at 22°C), and the term m/t was calculated by linear regression from the points of weight gain and time, in the constant rate period. Films free of any defects such as pinholes, air bubbles and cracks were used for WVP determinations. Each of the tests was replicated three times.

2.6.2.4. Mechanical properties. The stress at puncture point (maximum force) and the elastic modulus (Young's modulus) were measured by an Instron Universal machine (Instron 1011, Norwood, Massachusetts, USA) equipped with a 50 N load cell and a cylindrical probe (3 mm diameter). The movement of the probe had a constant speed of 1 mm/s while being perpendicular to the film surface. Measurements were conducted on the fifth day of storage at nine random points of the films. Three films per formulation were measured (Drakos et al., 2018).

2.7. Statistical analysis

Analysis of variance (ANOVA) and least significant difference tests (LSD) were carried out on the data in order to determine significant differences among the samples. The significant level was $P < 0.05$ throughout the study. Analysis of data was carried out with statistical software package Statistica v.8.0 for Windows.

3. Results & discussion

3.1. Emulsions and high internal phase emulsions (HIPE)

Initially, protein-pectin mixtures of both proteins were used as the aqueous phase in the formation of emulsions. All combinations for both proteins are shown in Table 1. The viscosity and the stability of the formed emulsions were studied and presented in Fig. 1. For comparison reasons the corresponding values for the emulsions with just the protein at a concentration equal to the total biopolymer concentration of each ratio (i.e. 1.5, 3.5, 3 and 7% wt), are also presented. Table 1 presents the composition of the aqueous phase of all the formed emulsions along with their sample name.

Regarding the viscosity of the emulsions with SC or SC-HMP mixtures when 0.5% wt HMP concentration was used (Fig. 1a), it is clearly seen that all emulsions exhibited pseudoplastic behaviour. The emulsions with the mixtures [i.e. PSC(0.5–1) and PSC(0.5–3)] showed greater viscosity values compared to the ones with just protein [i.e. PSC(0–1.5) and PSC(0–3.5)]. Moreover, the total biopolymer concentration was important as emulsions PSC(0.5–3) and PSC(0–3.5) (with a total biopolymer concentration of 3.5% wt) presented higher viscosity values compared to PSC(0.5–1) and PSC(0–1.5), respectively (with a total biopolymer concentration of 1.5% wt). The same pattern was reported for the viscosity of the emulsions when 1% wt HMP concentration was

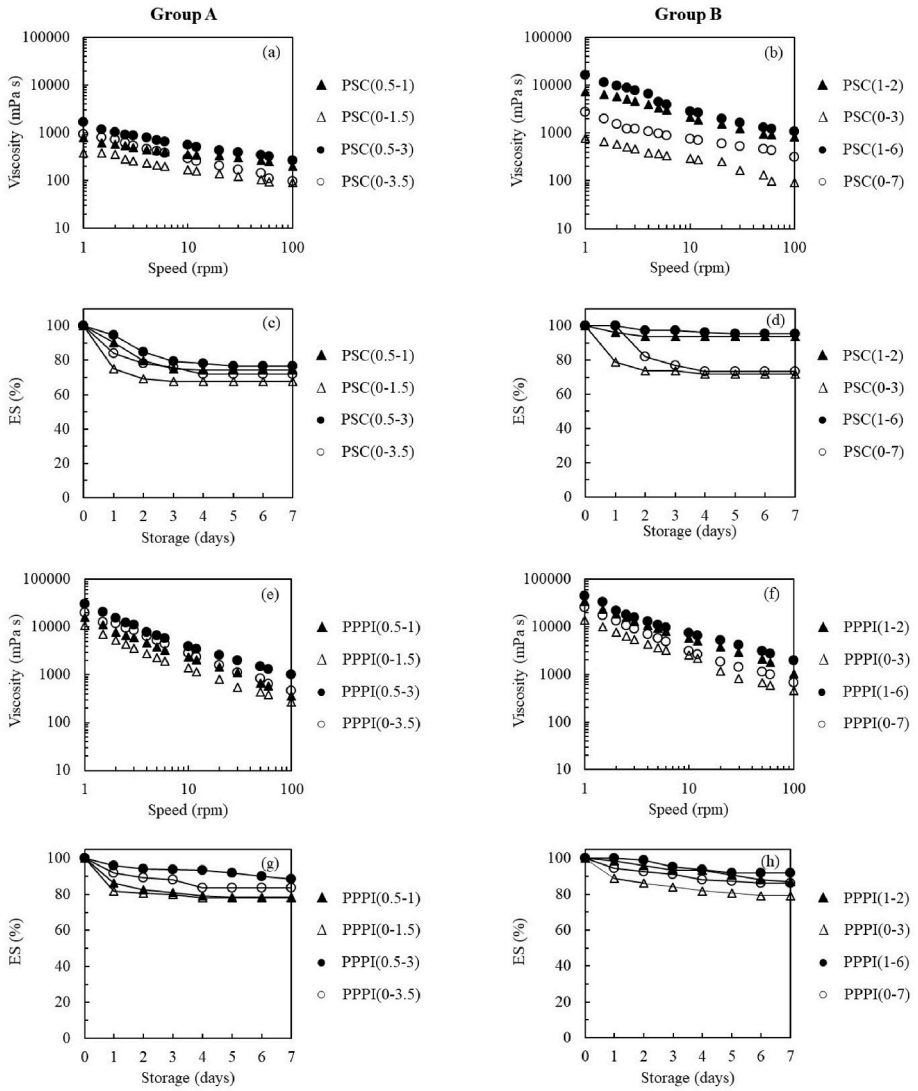


Fig. 1. Viscosity (mPa s) and emulsion stability (ES,%) of emulsions with SC (a–d) or PPI (e–h) in the presence and absence of HMP at two concentrations; 0.5% wt (Group A) and 1.0% wt (group B).

used (Fig. 1b). The emulsions with the highest final concentration of biopolymers (3% or 7% w/w, for protein: pectin ratios of 2:1 or 6:1, respectively), had the greatest viscosity values. For the shear rate of 100 rpm, viscosity ranged from 90 to 266 and 93–1093 mPa s for the emulsions with 0.5% and 1% wt HMP, respectively.

The stability over a one week period was also evaluated and the values for the emulsions with SC or SC-HMP mixtures are shown in Fig. 1 (c and d). For both the emulsions with 0.5% and 1% wt HMP, the presence of protein-pectin mixtures imparted greater stability to the emulsion than protein. Emulsions PSC(0.5–3) and PSC(1–6) with the greater protein: pectin ratio (6:1) had the greatest stability among the emulsions with the same HMP concentration, showing an ES(%) of ~77 and 95%, respectively, whereas PSC(0–1.5) and PSC(0–3) with just protein were the least stable with an ES(%) of ~68 and 72% at the end of storage. Overall, PSC(1–2) and PSC(1–6) emulsions were the most stable of all the studied emulsions.

Fig. 1e-h presents the viscosity and the stability of the emulsions with PPI or PPI-HMP mixtures. More or less, the same pattern seen for SC emulsions is repeated for PPI, with the mixtures exhibiting greater viscosity and stability than the emulsions with just protein. The emulsions with 1% wt HMP were more viscous than those with 0.5% wt HMP. For the shear rate of 100 rpm, viscosity ranged from 268 to 1012 and 460–1946 mPa s for the emulsions with 0.5% and 1% wt HMP, respectively. Once again, the emulsions with the greater protein: pectin ratio (6:1) [i.e. PPPI(0.5–3) and PPPI(1–6)] had the greatest stability among the emulsions with the same HMP concentration, showing an ES (%) of ~89 and 92%, respectively, whereas PPPI(0–3) along with PPPI (0.5–1)and PPPI(0–1.5) were the least stable with an ES(%) of ~79 and 78% at the end of storage, respectively.

Based on the above, the type of protein was important for the observed values of both properties. PPI gave more viscous emulsions than SC. For 0.5% wt HMP concentration, the PPI emulsions were more stable than the SC ones whereas for 1% wt HMP concentration, the emulsions showed comparable values for both proteins. Our findings are further confirmed by the photos of the PSC(0.5–3), PSC(1–6), PPPI (0.5–3) and PPPI(1–6)] emulsions shown in Fig. 2.

PPI is considered as promising alternative to animal proteins. However, several studies report that it presents inferior functional properties, e.g. water holding, foaming, and emulsifying properties, compared to other proteins like whey and soy (e.g. Cheng et al., 2022). The present study deviates from these reports as PPI resulted in more viscous and stable emulsions compared to SC, probably due to its different source and treatment. Regarding the effect of biopolymer concentration on the parameters, our observations in the viscosity measurements were expected due to the greater number of molecules present which perturb the solvent's flow. Similarly, as emulsifier acting proteins adsorb to the

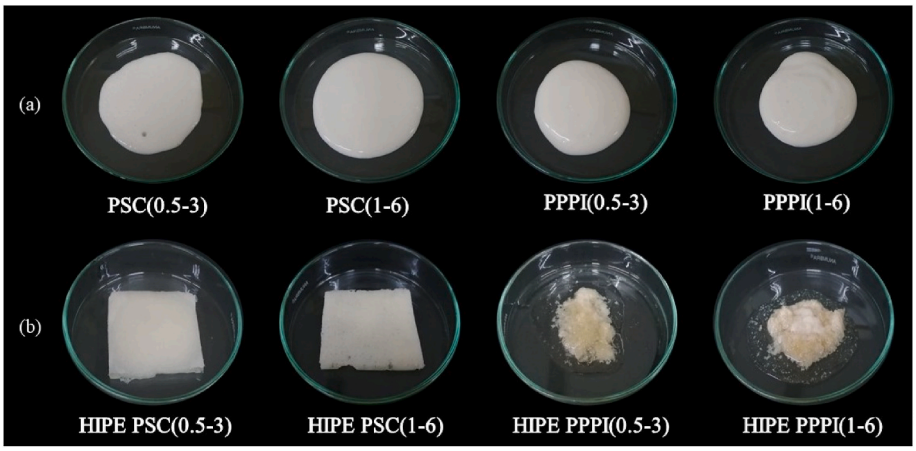


Fig. 2. Macroscopic appearance of (a) emulsions and (b) dried products (HIPE) formed in the presence of HMP and either SC or PPI with a protein: pectin ratio of 6:1 (Sample names as defined in Table 2) [HMP: High methoxyl pectin; SC: Sodium caseinate; PPI: Pea protein isolate].

surface of the oil droplets and form a protective coating that inhibits droplet aggregation, the higher concentrations are needed for the oil droplets' stabilisation due to the increase in their specific surface area (Panagopoulou et al., 2015). Furthermore, a stabilising effect of pectin was also reported as mixtures performed better than proteins, which can be attributed to the pectin's well known performance as a thickener which also possesses emulsifying properties (e.g. Kpodo et al., 2017).

An attempt to exploit the emulsions for the formation of oleogels via the emulsion-template method took place then. However, no complete drying of the systems was achieved and HIPE were obtained instead. Based on our previous findings, the emulsions of both proteins with either 0.5 or 1% wt HMP concentration and a protein: pectin ratio of 6:1 were selected for this step and thus, HIPE PSC(0.5–3), HIPE PSC(1–6), HIPE PPPI(0.5–3) and HIPE PPPI(1–6) were formed (Table 2). The water and oil content as well as the oil loss of the produced HIPE were determined and also presented in Table 2. Photos of the dried emulsions (HIPE) are shown in Fig. 2. HIPE PSC(0.5–3) and HIPE PSC(1–6) presented firmer structures than the corresponding PPI systems. Moreover, they had a lower oil loss (~83% compared to ~93% for PPI systems). However, as all systems had a great oil loss, it is clear that the used combination of biopolymers did not lead to the formation of a structured system.

In literature, depending on the stabilisers and surfactants used, as well as their ratio and the pH, various levels of stability are reported. Meng et al. (2018a, b) reported HIPEs with oil loss above 6% and above 12% in the presence of 1% hydroxypropyl methyl cellulose (HPMC) or 0.6% HPMC and 0.3% Xanthan Gum (XG). A very stable HIPE with an oil loss ~0% was reported by Gaudino, Ghazani, Clark, Marangoni, and Acevedo (2018). They used soybean lecithin and stearic acid at quite high concentrations (~20 and 30%). When sodium caseinate and alginate mixtures were used for HIPE formation at various protein to polysaccharide ratios and pHs, oil loss varied from ~2 to 35%, with both parameters influencing the HIPE properties. HIPE from pea protein isolate mixtures with various polysaccharides at a fixed ratio (4:1) and total biopolymer concentration of 2%, were also studied (Vélez – Erazo et al., 2020). The different polysaccharides exhibited different oil structuring behaviours as oil loss varied from 0.5% up to ~75%.

In the present study, the same thickening polysaccharide (HMP) was used in mixtures with two different proteins. According to our findings, for both proteins, the thicker 1% wt HMP- emulsions kept more water during drying compared to the emulsions with 0.5% wt HMP (Table 2), suggesting, in good agreement with literature (e.g. Vélez – Erazo et al., 2020) that the more viscous emulsions led to HIPE with less water loss during drying. Moreover, despite the fact that all the studied emulsions had a great stability over storage, they did not result to a stable HIPE, which was also reported by Vélez – Erazo et al. (2020).

It seems that the structure of the emulsions prior to drying was significant for our observations. Structure is related to the interfacial behaviour of the protein-polysaccharide mixture used. In the present study the mixtures had a pH of 6, which is above both the pI of the proteins (~4.6) (Burger & Zhang, 2019) and the pKa of pectin (3.5) (Lan, Chen, & Rao, 2018), and complex formation is in the best case limited. However, as the total biopolymer concentration is 3.5 and 7%, for the systems with 0.5 and 1% wt HMP concentration, respectively, the possibility of thermodynamic incompatibility in the bulk phase exists, and both biopolymers can adsorb at the interface if sufficient space exists (Patino & Pilosof, 2011). In addition, the type of protein was important for HIPE formation with the SC HIPEs showing a firmer structure, suggesting that they withstood the drying and homogenization processes involved in their formation. According to Vélez – Erazo et al. (2021), this can relate to a more flexible structure of the system, which depends on the extent of the protein-polysaccharide interactions.

Moreover, as all produced HIPEs were unable to structure oil, it seems that the pectin molecules did not perform well in keeping the water into the system, which possibly destabilized the rather firm matrix observed for the HIPE, leading to phase separation after shearing.

3.2. Films

The next step of the current work involved the formation and study

Table 2
Water and oil content after drying of the emulsions (initial weight: 100 g) and oil loss (%) of HIPE formed in the presence of HMP and either SC or PPI at a protein: pectin ratio of 6:1 after centrifugation. [HMP: High methoxyl pectin; SC: Sodium caseinate; PPI: Pea protein isolate].

Sample name	Composition of the aqueous phase of the initial emulsion	Total mass	Water content		Oil content		Protein + HMP		Oil loss of HIPE
		g	g	%	g	%	g	%	%
HIPE PSC(0.5–3)	0.5% HMP +3% SC	62.60	1.20	1.92	60	95.84	1.40	2.24	84.43
HIPE PSC(1–6)	1% HMP +6% SC	65.10	2.30	3.53	60	92.16	2.80	4.30	82.33
HIPE PPPI(0.5–3)	0.5% HMP +3% PPI	62.63	1.23	1.96	60	95.79	1.40	2.24	95.52
HIPE PPPI(1–6)	1% HMP +6% PPI	66.00	3.20	4.85	60	90.91	2.80	4.24	91.80

of edible films. For their formation aqueous dispersions of both proteins with either 0.5 or 1% wt HMP concentration and a protein: pectin ratio of 6:1 were mixed with glycerol and dried. As a result F PSC(0.5–3), F PSC(1–6), F PPPI(0.5–3) and F PPPI(1–6) films were formed (Table 3). All films were peelable and their surface had no bubbles and cracks. As edible films are meant to be used as packaging materials, several important properties were measured. Table 3 presents the values for weight, thickness, density, moisture content, water vapour permeability (WVP) as well as the mechanical properties.

Weight values ranged from ~1 to ~3 g and thickness from 107 to ~278 μm. For both proteins, films with 1% wt HMP [i.e. F PSC(1–6) and F PPPI(1–6)] were heavier and thicker than those with 0.5% wt HMP [i.e. F PSC(0.5–3) and F PPPI(0.5–3)]; most probably due to their greater total biopolymer concentration. As films were prepared by casting the same amount of film forming solutions, the increased total biopolymer concentration enhanced the dry mass (Eghbal et al., 2017). Furthermore, thickness is affected by the plasticizer's concentration as its molecules disperse in the film matrix and thus, increase the interstitial space between the polymer chains of the matrix (Farhan & Hani, 2017). In the present study, F PSC(1–6) and F PPPI(1–6) films contain a greater glycerol content than F PSC(0.5–3) and F PPPI(0.5–3). The type of protein used was also important for both weight and thickness as, for the same total biopolymer concentration, films with SC had greater weight and thickness than those with PPI; thus, reflecting differences in the formed network during drying. All films, regardless the protein and the biopolymer concentration, had the same density (~1.4 g/cm^3) suggesting a similar compact structure.

Moisture content is another important parameter affecting the functional properties of films. According to Table 3, for the same protein, regardless the HMP concentration, films had the same moisture content i.e. ~13 and 14.5%, for SC and PPI films, respectively, showing that the type of protein is important for moisture content. The biopolymer mixtures used for film formulation consist of an amphiphilic protein (SC or PPI) that induce hydrophobicity to the films, and HMP, which is more hydrophilic than the proteins (Eghbal et al., 2016). As all films share the same protein: pectin ratio, i.e. 6:1, it seems that ratio and not the total biopolymer concentration correlates with moisture content as it determines film hydrophobicity and thus, its water uptake (Eghbal et al., 2017).

Water vapour permeability was evaluated then. As seen in Table 3, for both proteins, films with 1% wt HMP had greater WVP than those with 0.5% wt HMP, with the type of protein not being statistically significant for our findings. WVP depends on the diffusivity and solubility of water molecules in the film matrix (Chakravartula et al., 2019). In our case, the total biopolymer concentration is a critical factor for WVP as the greater concentration enhances the interaction with water and favours the transmission of water vapour. The latter is also enhanced by the increase in the concentration of glycerol.

The maximum force and Young's modulus that correlate to the strength and stiffness of the films, respectively, were also determined and the corresponding values are shown in Table 3. Maximum force ranged from ~11.5–44 N whereas the modulus from ~273 to 714 kPa. According to literature, the film's mechanical behaviour depends on the type and concentration of its constituents and it is affected by the film-forming network (Talón et al., 2017). As seen from Table 3, films with the greater total biopolymer concentration for both proteins [i.e. F PSC (1–6) and F PPPI(1–6)] were stronger and stiffer than those with the lower concentration, as expected. Moreover, SC films for both total biopolymer concentrations were stronger and stiffer. It seems that the greater water content of the PPI films may contribute to these findings as that excess water can act as a plasticizer and thus, leading to more flexible films.

The film's optical properties are important for the acceptance of the product by the consumers. As such, in Fig. 3, the photos of the studied films are shown along with the values of the three colour parameters and opacity. [L*] values ranged from ~73 to 86, with the F PSC(0.5–3) film being the brighter. The least bright was the F PPPI(1–6) film. Overall, SC films were brighter than PPI films whereas F PSC(0.5–3) and F PPPI (0.5–3) were brighter than their counterparts with the higher HMP concentration. The [α*] values were positive for all films. SC films showed the same value (~1.25) which was lower than the values of ~3 and 7 for the F PPPI(0.5–3) and F PPPI(1–6) films, respectively. [b*] values were also positive for the films, with the SC (3.3 & 11, for F PSC (0.5–3) and F PSC(1–6), respectively) showing lower values compared to the PPI ones (16.5 & 33 for F PPPI(0.5–3) and F PPPI(1–6), respectively). The colour of the individual biopolymers significantly contribute to these observations as the values for the colour parameters are ~70, 90 and 79 for [L*], ~9, −0.4, 7 for [α*] and ~20, 10, 22 for [b*], for HMP, SC and PPI, respectively.

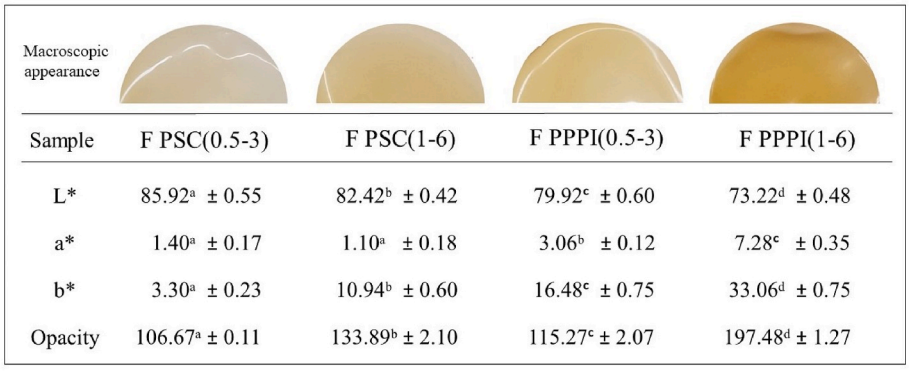
Regarding opacity, its values ranged from ~106 to 198. Within the same protein, films with 1% wt HMP [i.e. F PSC(1–6) and F PPPI(1–6)] were more opaque than those with 0.5% wt HMP [i.e. F PSC(0.5–3) and FE PPPI(0.5–3)]. Opacity is related to thickness with thicker films being more opaque (e.g. Andrade-Mahecha, Tapia-Blácido, & Menegalli, 2012). In the present study, within the same protein, a positive thickness – opacity correlation is reported. Furthermore, for a given total biopolymer concentration, the PPI films were more opaque than the SC ones.

Overall, the used protein affected the studied properties of the films, indicating differences in the formed network. These differences may arise from various factors. Regarding HMP-protein electrostatic interactions, as already mentioned, at the experimental conditions (pH = 6, protein: pectin ratio = 6:1) of the present study, only a limited number of them may occur, with their strength and density differing for the two proteins. The lower protein concentration (84%) in PPI compared to SC (93%) can be another contributing factor for our observations. However,

Table 3
Physicochemical properties of edible films formed in the presence of HMP and either SC or PPI at a protein: pectin ratio of 6:1. [HMP: High methoxyl pectin; SC: Sodium caseinate; PPI: Pea protein isolate].

Sample name	Formulation	Weight (g)	Thickness (μm)	Density (g/cm^3)	Moisture content (%)	WVP (10^{-8} g mm/h cm^2 Pa)	Maximum force (N)	Young's Modulus (kPa)
F PSC (0.5–3)	0.5% HMP - 3% SC + glycerol (30% wt biopolymers)	1.50^a ± 0.09	190.00^a ± 14.14	1.32^a ± 0.03	13.06^a ± 0.13	10.17^a ± 0.82	37.98^a ± 1.28	632.03^a ± 13.88
F PSC(1–6)	1% HMP - 6% SC + glycerol (30% wt biopolymers)	2.94^b ± 0.10	277.87^b ± 12.00	1.36^a ± 0.11	13.06^a ± 0.01	14.30^b ± 0.74	43.73^b ± 1.37	714.43^b ± 21.39
F PPPI (0.5–3)	0.5% HMP - 3% PPI + glycerol (30% wt biopolymers)	0.93^c ± 0.03	107.63^c ± 5.00	1.40^a ± 0.10	22.33^b ± 1,07	7.52^a ± 0.97	11.52^c ± 0.93	273.46^c ± 14.70
F PPPI (1–6)	1% HMP - 6% PPI + glycerol (30% wt biopolymers)	1.92^d ± 0.04	210.70^d ± 9.00	1.5^a ± 0.21	20.05^b ± 1,92	14.15^b ± 0.49	16.04^d ± 0.61	332.23^d ± 12.88

*: Values with different superscripts for each property are significantly different ($p < 0.05$).



Sample	F PSC(0.5-3)	F PSC(1-6)	F PPPI(0.5-3)	F PPPI(1-6)
L*	85.92^{a} ± 0.55	82.42^{b} ± 0.42	79.92^{c} ± 0.60	73.22^{d} ± 0.48
a*	1.40^{a} ± 0.17	1.10^{a} ± 0.18	3.06^{b} ± 0.12	7.28^{c} ± 0.35
b*	3.30^{a} ± 0.23	10.94^{b} ± 0.60	16.48^{c} ± 0.75	33.06^{d} ± 0.75
Opacity	106.67^{a} ± 0.11	133.89^{b} ± 2.10	115.27^{c} ± 2.07	197.48^{d} ± 1.27

Fig. 3. Photos, colour parameters and opacity of films formed in the presence of HMP and either SC or PPI with a protein: pectin ratio of 6:1 (Sample names as defined in Table 3) [HMP: High methoxyl pectin; SC: Sodium caseinate; PPI: Pea protein isolate].

we must not forget that the kinetics of the drying procedure for film formation are significant for the film's structure and properties (Kokoszka, Debeaufort, Lenart, & Voilley, 2010). For example, in regard to PPI, structural changes and intermolecular interactions of the polypeptide chains in the protein network are reported during drying (Gueguen, Viroben, Noireaux, & Subirade, 1998). Thus, the variation of the interactions among the biopolymers and the network rearrangement during drying can affect the performed measurements.

4. Conclusions

Mixtures of high methoxyl pectin (HMP) and pea protein isolate (PPI) or sodium caseinate (SC) led to pseudoplastic emulsions with good stability over one week storage. When emulsions with selected protein-pectin mixtures were tested for structuring of sunflower oil by forming oleogels, no complete drying was achieved and HIPE were formed instead. Regardless the protein used, all formed HIPEs had a great oil loss (83–95%). Edible films formed by drying the aqueous dispersions of the same selected protein-polysaccharide mixtures had the same density. The greater total biopolymer concentration led to thicker, heavier, stronger and stiffer films with greater water vapour permeability and opacity. SC films were thicker, heavier, stronger, stiffer and brighter than PPI films, with lower moisture content and opacity. Overall, the studied SC/PPI-HMP mixtures did not perform well in sunflower oil structuring but they are good candidates for the formation of edible packaging material.

Author statement

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Declaration of competing interest

The authors declare no conflict of interest and no ethics issues. Moreover, we declare that the present manuscript has not been submitted or published elsewhere.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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