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Pea protein isolate - soluble soybean polysaccharides electrostatic assembly: Effect of pH, biopolymer mass ratio, and heat treatment

Running title: Pea protein isolate - soluble soybean polysaccharides electrostatic complexes

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Abstract

Background: In past years, thousands of protein-polysaccharide complexes have been studied to modify protein characteristics and functionality in food systems. However, the interaction between pea protein isolate (PPI) and soluble soybean polysaccharide (SSPS) has not been thoroughly characterized yet.

Results: In the present work, the phase behavior of PPI and SSPS mixtures was analyzed as a function of PPI:SSPS mixing ratio (1:1 to 1:0.10) and pH (7.0 to 2.0), showing that these biopolymers could be electrostatically assembled at 1:1 to 1:0.25 mixing ratios and 4.0 to 3.0 pH values. Then, the characteristics of the PPI-SSPS complexes were studied before and after heating (90 °C, 30 min) by ζ-potential, surface hydrophobicity, protein solubility, particle size distribution, and physical stability for 56 days. By lowering the pH and PPI:SSPS mixing ratio, the complexes showed increased solubility, changed ζ-potential, and higher physical stability. By heating, the complexes presented increased hydrophobicity and physical stability.

Conclusion: Overall, PPI-SSPS complexes increased the protein solubility, reduced the particle size, and changed both the ζ-potential and the surface hydrophobicity with respect to PPI control, allowing stabilization of the colloidal system and broadening the possible applications of these high-quality proteins in acidic food systems.

Keywords: Protein-polysaccharide interactions; Soluble complexes; Plant proteins; Functional properties; Acidic food systems.

1. INTRODUCTION

During the last few years, the food industry has been looking for plant-derived protein sources as an alternative to replace animal-derived ones such as meat, milk, and egg proteins. In this sense, proteins derived from yellow pea (*Pisum sativum L.*) have become an attractive resource due to their health benefits, well-balanced amino acid profile, low allergenic and gluten-free properties, relatively low price, and great environmental sustainability [1]. Pea proteins mainly include an albumin fraction (10%-20%) and a globulin fraction (70%-80%). The latter fraction is composed of legumin, vicilin, and convicilin. Legumin (11S) is a hexameric (320—410 kDa) globulin bridged by inter-disulfide bonds, while vicilin (7S) and convicilin (8S) are trimeric (150 and 180-210 kDa, respectively) globulins assembled by non-covalent interactions [1–3].

Pea protein isolates (PPIs) have been explored since they are a high-quality plant protein resource obtained as a by-product of pea starch production. Nevertheless, the broad utilization of PPIs in acidic foods is partially hampered due to their limited solubility and functional properties [4,5]. In particular, PPIs have poor water solubility because of the rigid structures of legumin subunits, low surface charge, and hydrophobic structure [5]. In addition, commercial PPIs obtained by spray drying present poor water solubility because of the protein-protein aggregation induced during the drying process [6].

One strategy to overcome these limitations is to induce the formation of protein-polysaccharide electrostatic-assembled complexes. The interaction with polysaccharides could lead to the formation of complexes with improved functionalities to act as emulsifiers, modifiers of food texture and biomaterials, or carrier vehicles in the protection and delivery of bioactive compounds [7]. The induction of electrostatic assembly is generally allowed when the biopolymers present opposed net charges. Also, the obtained complexes could be soluble or insoluble, leading to the formation of one- or two-phase systems respectively [8–10]. Moreover, the characteristics of complexes are also determined by biopolymer characteristics (molecular weight, charge density, and rigidity), environmental conditions (pH and ionic strength), biopolymer ratio, biopolymer total concentration, and additional treatments (heat or mechanical treatments) [11–15].

Soluble soybean polysaccharides (SSPS) are a soluble dietary fiber and functional ingredient that present low bulk viscosity, high-temperature stability, pH stability, and water solubility [16,17]. Chemically, SSPS are rhamnogalacturonan backbones with branches of β-1,4-galactan, α-1,3 or α-1,5-arabinan, and protein moieties [18,19]. Furthermore, SSPS are a by-product of the tofu, soy milk, and soy protein industries. Many studies have been conducted on the interaction of PPI with different polysaccharides, including tragacanth gum [7], carboxymethylcellulose [20], gum arabic [21], and pectin with different degree and pattern of methoxylation [22–24]. However, as far as we know, only two previous studies analyzed the interaction with SSPS. On the one hand, Yin et al. (2015) studied the interaction of PPI aggregates with SSPS in one mixing ratio (~1:4 PPI:SSPS) and two pH conditions (3.25 or 7.0) and, then, the oil-water interfacial activity of the resultant complexes. These authors concluded that PPI aggregates can be used alone or by complexation with SSPS to produce stable emulsions [25]. On the other hand, Zhan et al. (2019) studied the interaction of PPI with SSPS in three mixing ratios (10:1, 10:2, and 10:3 PPI:SSPS) and one pH condition (2.5) during the freeze-drying process. They showed that SSPS induced the interconnection of the modified PPI particles during freeze-drying, resulting in the improvement of rheological properties [26].

Up to now, the interaction between SSPS and PPI has not been thoroughly characterized as a function of pH, PPI:SSPS mixing ratio, and other treatments (such as heating). Hence, this work aimed to determine the

pH and PPI:SSPS mixing ratio conditions that allow obtaining PPI-SSPS electrostatic-assembled complexes and characterize them before and after heating by ζ-potential, surface hydrophobicity, protein solubility, particle size distribution, and physical stability. It is believed that this work provides useful information for promoting the application of PPI-SSPS complexes in acidic food systems.

2. MATERIALS AND METHODS

2.1. Materials

Pea protein isolate (PPI, Emulprot arv+80%) was donated by Saporiti, S.A. (Buenos Aires, Argentina). According to the datasheet, the PPI composition was 800 g kg^{-1} crude protein (N×6.25), 40 g kg^{-1} total dietary fiber, 50 g kg^{-1} total fat, 50 g kg^{-1} carbohydrates, and 6 g kg^{-1} salts. PPI solid powder was obtained by spray drying technique. Soluble soybean polysaccharides (SSPS, Soyafibe-SCA100) were donated by Fuji Oil Co. Ltd (Osaka, Japan). According to the datasheet, the SSPS composition was 751 g kg^{-1} total dietary fiber, 78 g kg^{-1} crude protein (N×6.25), 58 g kg^{-1} moisture, and 78 g kg^{-1} crude ash. PPI and SSPS powders were used without further purification. *N,N*-Dimethyl-6-propionyl-2-naphthylamine (PRODAN) was purchased from Santa Cruz Biotechnology (Texas, USA), while 2,2′-Biquinoline-4,4′-dicarboxylic acid disodium salt (bicinchoninic acid, BCA) and bovine serum albumin (BSA) were supplied by Sigma Aldrich (Missouri, USA). All the other chemicals were of analytical grade and purchased from local distributors (Buenos Aires, Argentina).

2.2 Dispersion of PPI

Commercial protein isolates obtained by spray drying tend to be difficult to disperse since large protein aggregates are formed during drying. So, to obtain the PPI dispersion, a combination of pH-shifting and ultrasound treatments was performed according to Jiang et al. (2017), with some modifications. Briefly, an initial dispersion (25 g kg^{-1}) was prepared by mixing the solid PPI powder in double-distilled water under magnetic mixing at room temperature (25 ± 2 °C) [27]. Then, the pH was adjusted to 10.3 with 1 mol L^{-1} NaOH solution, and the dispersion was mixed for 2 h. The pH was monitored by C861 Consort pH/mV meter with a PY-P10-25 Sartorius electrode. Subsequently, an ultrasound treatment was applied for 150 s (30 s on/30 s off intervals, 100% amplitude) using a Sonics Vibra Cell VCX750 ultrasound homogenizer (Sonics & Materials Inc., USA) with the 28 mm diameter tip immersed 1/3 in a glass beaker. The increase in temperature during sonication was avoided by putting the beaker in a water-ice bath. After that, the pH was adjusted to 7.0 with 1 mol L^{-1} HCl solution, and the sample was mixed overnight to allow full hydration.
The protein concentration in PPI dispersion was determined by the bicinchoninic acid (BCA) method first described by Smith et al. (1985) with some modifications [28]. Reagent A was composed of 110 g kg^{-1} BCA, 20

g kg^{-1} Na_2CO_3, 4.0 g kg^{-1} NaOH, 1.6 g kg^{-1} sodium tartrate, and 9.5 g kg^{-1} $NaHCO_3$ (pH 11.25). Reagent B was composed of 40 g kg^{-1} $CuSO_4$-$5H_2O$. To obtain reagent C, reagents A and B were mixed at 50:1 A:B ratio on the day of the experiment. Next, in each well of a 96-well plate, 25 µL of protein sample was mixed with 200 µL of reagent C. These mixtures were incubated at room temperature for 20 min and then the absorbance at 562 nm was determined using the Cytation 5 microplate reader (BioTek Instruments, USA). The protein concentration was determined by comparison with a calibration curve performed with bovine serum albumin (BSA) in double-distilled water.

Finally, the concentration of the PPI dispersion was adjusted to 20 g kg^{-1} and sodium azide was added at 0.2 g kg^{-1} final concentration to prevent microbial growth. The PPI dispersion was stored at room temperature and protected from light until use.

2.3 Dispersion of SSPS

SSPS dispersion at 40 g kg^{-1} was prepared by mixing the solid powder in double-distilled water under magnetic mixing for 2 h at room temperature (25 ± 2 °C). Sodium azide was added at 0.2 g kg^{-1} final concentration to prevent microbial growth. The SSPS dispersion was stored at room temperature and protected from light until use.

2.4 Preparation and characterization of PPI-SSPS mixtures

2.4.1 Preparation of PPI-SSPS mixtures

PPI and SSPS were combined in different weight mixing ratios (1:1, 1:0.50, 1:0.25, 1:0.17, 1:0.125, or 1:0.1 PPI:SSPS) and pH-conditions (from 7.0 to 2.0, every 0.5 pH-units). Binary mixtures were obtained by weighing appropriate proportions of PPI and SSPS dispersions, adjusting the pH using 1 mol L^{-1} HCl solution, and mixing for 1 h at room temperature. In all the mixtures, the PPI final concentration was maintained at 10 g kg^{-1}, and the SSPS concentration was kept between 1.0 and 10 g kg^{-1}. Individual PPI and SSPS dispersions at 10 g kg^{-1} were used as control samples.

2.4.2 Characterization of PPI-SSPS mixtures

The ζ-potentials of PPI and SSPS control dispersions at pH values between 7.0 and 2.0 were determined using a Zetasizer Nano ZSP ZEN 5600 analyzer (Malvern Instruments, UK). The determinations were performed at room temperature. To avoid multiple light scattering effects, mixtures were diluted tenfold with double-distilled water previously adjusted at each pH with 1 mol L^{-1} HCl solution. The refractive index values were 1.54 for biopolymers and 1.33 for the dispersant.

The protein solubility in PPI control and PPI-SSPS mixtures was determined by relating the soluble protein concentration before and after centrifugation at 3340 xg for 10 min at room temperature. The protein concentrations were determined using the BCA method, as explained in section 2.2.

The state diagrams of PPI, SSPS, and PPI-SSPS mixtures at different pH values and PPI:SSPS mixing ratios were analyzed by visual observation on days 1 and 7 [10,29]. The samples were statically stored upright at room temperature and protected from light during the study. For each day, the samples were classified into five groups based on the turbidity of the suspension and the presence/absence of precipitate (Fig. 1).

Fig. 1

2.5 Preparation and characterization of PPI-SSPS complexes

2.5.1 Preparation of PPI-SSPS complexes

From the analysis of the results obtained so far, three PPI:SSPS mixing ratios (1:1, 1:0.50, and 1:0.25) and three pH conditions (4.0, 3.5, and 3.0) were chosen to obtain PPI-SSPS complexes. Hence, the PPI-SSPS complexes were prepared as in section 2.4.1, by mixing appropriate proportions of both biopolymers dispersion and adjusting the pH to the desired value. The PPI final concentration was 10 g kg^{-1}, and the SSPS final concentration was kept between 10 and 2.5 g kg^{-1}. In addition, to study the effect of heating, aliquots of each sample (35.0 g) were heated in a water bath at 90.0 ± 1.0 °C for 30 min, then cooled with running tap water to room temperature (25 ± 2 °C). As the water was evaporated during heating by roughly 2%, the weight of each sample (35.0 g) was restored by adding double-distilled water. Individual PPI and SSPS dispersions at 10 g kg^{-1} before and after heating were used as control samples.

2.5.2 Characterization of PPI-SSPS complexes

The ζ-potential and protein solubility of PPI-SSPS complexes were determined as explained in section 2.4.2.

The protein surface hydrophobicity values (S_0) of PPI-SSPS complexes were determined using the fluorescent PRODAN probe, according to the previously reported method [30]. For this, the samples were serially diluted in double-distilled water at the corresponding pH to obtain protein concentrations ranging from 1.25 to 0.078 g kg^{-1}.

The particle size distributions of PPI-SSPS complexes were determined by static light scattering using a Mastersizer 2000E analyzer, equipped with a Hydro 2000MU wet dispersion unit (Malvern Instruments, UK). The selected refractive indexes were 1.54 for complexes and 1.33 for the dispersing medium.

The physical stability of PPI, SSPS, and PPI-SSPS complexes was monitored by multiple light scattering using a Turbiscan Lab® analyzer (Formulaction, France). Dispersions without dilution were placed in cylindrical glass tubes, and transmission and backscattering profiles were determined on the sample up to day 56. The samples were statically stored at room temperature and protected from light during this period. The stability of the dispersions was analyzed based on their Turbiscan stability index (TSI), a relative number without units that allows comparison of the stability of different samples based on the variations of transmission and backscattering profiles of each one over time. Thus the larger the TSI, the lower the stability of the sample [31]. The TSI was calculated by TurbiSoft software (Formulaction, France).

2.6 Statistical analysis

All the preparations and characterization assays were conducted at least in triplicate. The acquired data were grafted and statistically analyzed using the Graph Pad Prism v8.0 software. In graphs, the results were expressed as mean ± standard deviation. Also, different letters were used to represent significant differences ($p<0.05$) according to Two-way ANOVA followed by Tukey's multiple comparisons post-test. Lowercase letters represent significant differences between different samples in the same pH conditions, whereas uppercase letters represent significant differences between the same sample in different pH conditions.

3. RESULTS AND DISCUSSION

3.1 Characterization of PPI-SSPS mixtures and selection of conditions for complexation

First, the ζ-potentials of PPI and SPPS control dispersions at pH values between 7.0 and 2.0 were determined to understand the driving force for electrostatic interactions (Fig. 2a). Then, PPI, SSPS, and PPI-SSPS mixtures in a wide range of pH conditions (7.0 to 2.0 every 0.5 pH-units) and PPI:SSPS mixing ratios (1:1 to 1:0.1) were analyzed by protein solubility (Fig. 2b) and state diagram (Fig. 2c). The physical dispersion state was determined by visual observations at day 0, 1, and 7 (Figs. S1, S2, and S3, respectively).

Fig. 2

The PPI control presented ζ-potential varying between -19.5 ± 0.9 mV at pH 7.0 and +22.5 ± 2.1 mV at pH 2.5, with the isoelectric point (pI) at 4.25 (Fig. 2a). This result agrees with previous works [7,23,25,32]. In addition to changes in ζ-potential values, a reduction in PPI solubility (Fig. 2b) and the precipitation even on the day of preparation (Figs. 2c and S1) were observed at pH ranging from 5.5 to 3.0. In a similar way, Carpentier et

al. (2021) showed that PPI solubility decreases from 22% at pH 7.0 to 3% at pH 4.5 [7]. These results are justified by the lack of electrostatic repulsion between protein particles at pH close to pI, which leads to protein aggregation, becoming the major challenge of including PPI in acid foods [20]. Moreover, the low PPI solubility in pH far away from pI may be justified by their elevated surface hydrophobicity that allows the formation of large protein aggregates [25].

The SSPS control presented negative ζ-potentials in the entire pH range, varying from –21.5 ± 0.6 mV at pH 5.5 to –0.9 ± 0.2 mV at pH 2.0 (Fig. 2a). This result agrees with those previously reported [33]. Also, the presence of galacturonic acid as the main sugar of this polysaccharide justified the obtained results. The SSPS formed stable and translucent dispersions throughout the studied pH range (Figs. 2c and S3). According to these results, PPI and SSPS might have an electrostatic assembly at 2.0 < pH < 4.25, since opposite net charges are necessary for electrostatic attraction.

The PPI-SSPS mixtures presented a different behavior depending on the pH and PPI:SSPS mixing ratio. On the day of preparation, all the samples were turbid and showed to be more stable than the PPI control (Fig. S1). After 24 h of storage, the mixtures with 1:0.10 and 1:0.125 mixing ratios showed precipitation at pH values between 5.5 and 3.0, while mixtures with 1:0.17 mixing ratios showed precipitation at pH values between 4.5 and 3.0 (Figs. 2c and S2). After 7 days of storage, the mixtures with 1:0.10, 1:0.125, and 1:0.17 mixing ratios strengthened the precipitation and clarification of upper suspension (Figs. 2c and S3). Through these results, we cannot confirm if the observed precipitates are formed by pea proteins only or by insoluble PPI-SSPS coacervates. However, we can confirm that a low concentration of SSPS did not increase the suspension stability of PPI. In addition, these mixing ratios (1:0.10 to 1:0.17) did not significantly ($p>0.05$) improve the solubility of pea proteins at a pH close to pI (Fig. 2b), showing that low SSPS concentrations are not adequate to form soluble PPI-SSPS complexes or maintain the PPI stable in suspension.

Otherwise, after 24 h of storage, the mixtures with 1:0.25, 1:0.5, and 1:1 mixing ratios remained as cloudy suspensions without precipitates in the entire pH range (Figs. 2c and S2). After 7 days of storage, only samples with a 1:0.25 mixing ratio presented cloudy suspensions with precipitates at pH values between 4.0 and 3.0 (Figs. 2c and S3). Similar state diagrams were previously reported for mixtures of PPI with sugar beet pectin, high methoxyl pectin, and low methoxyl pectin [3,10]. In these previous works, the authors sought to optimize the formation of coacervates, so they continued working with low concentrations of polysaccharides. In the present work, we seek to optimize the formation of soluble complexes that remain stable in suspension, so high polysaccharide concentrations showed to be more appropriate. Moreover, the mixtures with 1:1, 1:0.5, and 1:0.25 mixing ratios significantly ($p<0.05$) increased the protein solubility at a pH

close to PPI pI (Fig. 2b). In a similar profile, the presence of tragacanth gum (TRAG) increased the PPI protein solubility at pH 4.5 from 2.5% (control PPI) to 14.6% (2:1 PPI:TRAG mixing ratio) [7]. The stabilization effect of high polysaccharide concentrations could be explained by the formation of protein-polysaccharide complexes with smaller particle sizes than protein aggregates and by the reduction of PPI self-aggregation. These results allow us to infer that those high SSPS concentrations permitted the formation of soluble PPI-SSPS complexes with higher stability than the PPI control. Wei et al. (2020) reported that low concentrations of carboxymethyl cellulose were less efficient in protecting PPI molecules from precipitation during the acidification process and PPI-carboxymethyl cellulose soluble complexes were formed only in the presence of high concentrations of this polysaccharide [34].

From the results obtained so far, the conditions were chosen to assure the formation of soluble PPI-SSPS complexes. Specifically, pH 3.0, 3.5, and 4.0 conditions were selected since attractive electrostatic interactions are allowed in that range, as can be seen in the results of ζ-potential (Fig. 2a) and through solubility results (Fig. 2b). Besides, the 1:1, 1:0.5, and 1:0.25 PPI:SSPS mixing ratios were selected because significant improvements in dispersions stability were observed at those SSPS concentrations (Fig. 2c).

3.2 Characterization of PPI-SSPS complexes

PPI, SSPS, and PPI-SSPS complexes were prepared in the selected pH and PPI:SSPS mixing ratio conditions with and without heating (90 °C, 30 min). The heat treatment was performed to understand the impact of protein denaturalization on the characteristics of PPI-SSPS electrostatic complexes. Then, samples were characterized by ζ-potential (Fig. 3), surface hydrophobicity (Fig. 4), protein solubility (Fig. 5), particle size distribution (Fig. S4), volume-weighted mean diameter (Fig. 6), and Turbiscan Stability Index (TSI, Figs. 7 and S5).

Fig. 3

According to ζ-potential characterization (Fig. 3), a net positive charge was observed in the PPI controls, whose module significantly increased as the pH reduced and moved away from the pI. Otherwise, a net negative charge was observed in the SSPS controls, whose module significantly reduced as the pH lowered and moved close to the pKa. These opposite net charges allow the formation of PPI-SSPS complexes with intermediate ζ-potential values between those of the two separate controls. Carpentier et al. (2021) observed the same behavior for PPI-tragacanth gum complexes and assumed that a charge neutralization existed

since the addition of anionic polysaccharides is known to induce cationic protein adsorption via an electrostatic attraction [7]. In addition, Ardestani et al. (2022) observed similar behavior in the complex coacervation of sodium caseinate and high methoxyl pectin, where charge neutralization resulted in phase separation [35]. The ζ-potential values of PPI-SSPS complexes became more positive as the pH decreased (from 4.0 to 3.0), since proteins contribute with a higher amount of positive charge and polysaccharides with a lesser amount of negative charge. These results agree with those previously reported for whey protein isolate-SSPS [30] and grass pea (*Lathyrus sativus*) protein isolate-*Alyssum homolocarpum* seed gum [36]. Moreover, the ζ-potential values of PPI-SSPS complexes became more positive as the SSPS concentration decreased (from 1:1 to 1:0.25 PPI:SSPS mixing ratios) since a lesser amount of negatively charged polysaccharide chains was available to neutralize positively charged proteins. Similar trends were reported for PPI-sugar beet pectin interaction in different mixing ratios [3]. Regarding the heat treatment, only PPI at pH 3.5 presented a significant ($p<0.05$) increase of ζ-potential, indicating that heating in this pH condition could induce the unfolding and the exposure of charged groups on the surface of protein and protein aggregates. In brief, pH and PPI:SSPS mixing ratio but not heating modified the ζ-potential values of PPI-SSPS complexes.

Fig. 4

Concerning surface hydrophobicity (S_0) characterization (Fig. 4), a reduction of PPI and PPI-SSPS S_0 values was observed as the pH was reduced. These results could be explained by the increment of positive charge on the protein surface as the pH moved away from protein pI. Besides, the S_0 values of PPI-SSPS increased as the SSPS concentration decreased, since a lesser amount of hydrophilic polysaccharide was present to generate a hydrophilic surface. Similar results were previously reported for freeze-dried modified PPI-SSPS complexes [26] and for PPI-carboxymethyl cellulose complexes [20]. Regarding the heat treatment, the S_0 value of all samples significantly ($p<0.05$) increased after heating. These results could be justified by the alteration of the protein's secondary structure and exposure of hydrophobic regions on the surface of the protein aggregates or the PPI-SSPS complexes [37]. In summary, the surface hydrophobicity of PPI-SSPS complexes was modified by pH, PPI:SSPS mixing ratio and heating.

Fig. 5

Regarding protein solubility characterization (Fig. 5), an increment in protein solubility was observed for PPI and PPI-SSPS complexes as the pH was reduced. These results could be explained by both the increment of positive charge on the protein surface and the reduction in S_0-values as the pH moved away from protein pI. Besides, the protein solubility of PPI-SSPS increased as the SSPS concentration increased, since a higher amount of hydrophilic polysaccharides was available to interact with the proteins, improving the solubility and reducing the physical destabilization. Regarding the heat treatment, only PPI at pH 3.0 presented a significant ($p<0.05$) increase in protein solubility after heating, showing that this heat treatment did not modify the solubility of PPI-SSPS complexes. Overall, the pH and PPI:SSPS mixing ratio rather than heat treatment modified the protein solubility of PPI-SSPS complexes.

Fig. 6

According to particle size characterization (Figs. 6 and S4), the PPI control presented large protein aggregates with mean diameter D[4,3] higher than 5 μm. These results were expected given the proximity between pH and pI and the type of isolate (obtained by spray drying). Similarly, previous work reported a population size of 1952 ± 200 nm for PPI at pH 3.5 [34]. The PPI particle sizes were smaller as the pH was reduced. As seen in ζ-potential and S_0 measurements, PPI presented less superficial charge and more hydrophobicity at pH 4.0, justifying the protein interaction in large aggregates that also reduced the protein solubility. Besides, the PPI particle size did not show any changes after heating at pH 4.0 and 3.5, but significantly ($p<0.05$) changed at pH 3.0, agreeing with the previously obtained results of ζ-potential and solubility. On the other hand, the PPI-SSPS complexes showed D[4,3] close to 0.7 μm, independently of pH, PPI:SSPS mixing ratio, or performing a heat treatment. These results demonstrated that the interaction with SSPS reduced the PPI aggregation process, stabilizing the particles in a submicronic size. In a similar way, it was previously reported that high methoxy pectin reduced the PPI-PPI aggregation, decreasing the particle size and forming PPI-pectin complexes with D[4,3] < 1 μm [22]. Furthermore, it was reported that higher concentrations of high methoxyl pectin safeguard the sodium caseinate aggregates from further aggregation [35]. Also, other reports showed that the interaction of pea proteins with either corn fiber gum or konjac glucomannan allowed the reduction of particle size [34]. Likewise, it was informed that apple pectin interacted with PPI and formed particles with D[4,3] between 25 and 81 μm depending on PPI:pectin ratio. So the increment in pectin concentration allowed the formation of complexes with reduced size [23].

Fig. 7

The physical stability was analyzed by TSI during the 56 days of storage (Fig. S5). For contrast, the final TSI obtained on day 56 of storage was compared (Fig. 7). The SSPS control remained stable during storage, whereas the PPI control presented a rapid destabilization. This destabilization was evidenced by the precipitation of protein aggregates, which increased the backscattering at the bottom of the tube, and the clarification of suspensions, which increased the transmission at the upper zone of the tube. Both processes led to a rapid TSI increment. At pH 3.0, the rate of PPI destabilization was reduced with respect to pH 4.0 and 3.5 both before and after heating. This result could be explained by the increased solubility and ζ-potential and the reduced S_0 and particle size of pea proteins at this pH condition. Otherwise, PPI-SSPS complexes showed greater stability than PPI control. Also, the stability was higher as the SSPS concentration increased, showing that complexes at 1:1 and 1:0.5 mixing ratios were more stable than those obtained at 1:0.25 mixing ratio. In addition, heating also raised the stability of PPI-SSPS complexes showing a slower increase of TSI values during storage. Similar results were previously reported for PPI-propylene glycol alginate complexes, which showed lower TSI than the respective protein control [38]. In addition, using other equipment, it was shown that the physical instability index of pea proteins decreased as the concentration of either corn fiber gum or konjac glucomannan increased [34].

4. CONCLUSION

The interaction between pea protein isolate (PPI) and soluble soybean polysaccharides (SSPS) at specific pH and PPI:SSPS mixing ratio conditions allowed obtaining electrostatic-assembled soluble complexes. These PPI-SSPS complexes presented higher protein solubility and smaller particle size than the PPI control and induced changes in both ζ-potential and surface hydrophobicity. Moreover, the PPI-SSPS complexes showed to be more stable than the PPI control, delaying the sedimentation processes during 56 days of storage. The present study also demonstrated PPI-SSPS complexes presented increased surface hydrophobicity and physical stability after heating, whereas the particle size was not affected. The knowledge generated in this work is useful to design soluble PPI-SSPS complexes with specific characteristics (size, charge, and hydrophobicity) to be applied in food systems with an acidic environment. Although a deep characterization was performed in the present work, the techno-functional properties and stability against simulated gastrointestinal digestion of PPI-SSPS complexes must be studied.

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AUTHOR CONTRIBUTIONS

Daniela E. Igartúa: Conceptualization, Data curation, Formal analysis, Supervision, Investigation, Funding acquisition, Project administration, Methodology, Writing - original draft. Agustina Balcone: Data curation, Formal analysis, Investigation, Methodology. Fedra A. Platania: Data curation, Formal analysis, Investigation, Methodology. Dario M. Cabezas: Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing. Gonzalo G. Palazolo: Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.

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FIGURE LEGENDS

Fig. 1 – Classification of physical dispersion state. The state corresponds to translucent suspension (Δ), cloudy suspension (○), precipitate with translucent suspension (▲), or precipitate with cloudy suspension (●). Upon characterization of the PPI-SSPS mixtures, we selected the PPI:SSPS mixing ratios and pH conditions at which soluble PPI-SSPS electrostatic complexes were obtained.

Fig. 2 – Characterization of PPI-SSPS mixtures. (a) ζ-potential of PPI and SSPS dispersions as a function of pH. **(b)** Protein solubility in PPI control and PPI-SSPS mixtures as a function of pH. **(c)** State diagram of PPI, SSPS, and PPI-SSPS mixtures on days 1 and 7. The state corresponds to translucent suspension (Δ), cloudy suspension (○), precipitate with translucent suspension (▲), or precipitate with cloudy suspension (●).

Fig. 3 – ζ-potential characterization. ζ-potential of PPI, SSPS, and PPI-SSPS complexes at different pH and PPI:SSPS mixing ratio conditions before **(a)** and after **(b)** heating. Lowercase letters represent significant differences ($p<0.05$) between different samples in the same pH conditions, whereas uppercase letters represent significant differences ($p<0.05$) between the same sample in different pH conditions according to Two-way ANOVA followed by Tukey's multiple comparisons post-test.

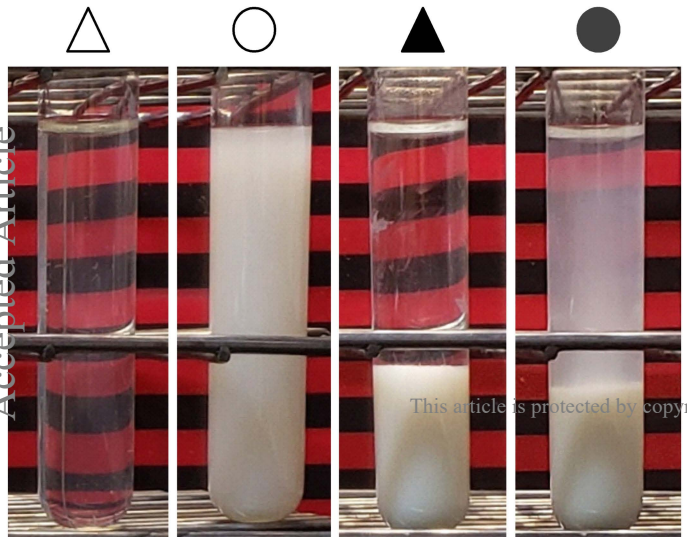
Fig. 4 – Surface hydrophobicity (S_0) characterization. S_0 of PPI and PPI-SSPS complexes at different pH and PPI:SSPS mixing ratio conditions before **(a)** and after **(b)** heating. Lowercase letters represent significant differences ($p<0.05$) between different samples in the same pH conditions, whereas uppercase letters represent significant differences ($p<0.05$) between the same sample in different pH conditions according to Two-way ANOVA followed by Tukey's multiple comparisons post-test.

Fig. 5 – Protein solubility characterization. Protein solubility of PPI and PPI-SSPS complexes at different pH and PPI:SSPS mixing ratio conditions before **(a)** and after **(b)** heating. Lowercase letters represent significant differences ($p<0.05$) between different samples in the same pH conditions, whereas uppercase letters represent significant differences ($p<0.05$) between the same sample in different pH conditions according to Two-way ANOVA followed by Tukey's multiple comparisons post-test.

Fig. 6 – Particle size characterization. Volume-weighted particle size diameter (D[4,3]) of PPI and PPI-SSPS complexes at different pH and PPI:SSPS mixing ratio conditions before **(a)** and after **(b)** heating. Lowercase letters represent significant differences ($p<0.05$) between different samples in the same pH

conditions, whereas uppercase letters represent significant differences ($p<0.05$) between the same sample in different pH conditions according to Two-way ANOVA followed by Tukey's multiple comparisons post-test.

Fig. 7 – Physical stability characterization. Turbiscan stabilization index (TSI) on day 56 during storage of PPI, SSPS, and PPI-SSPS complexes at different pH and PPI:SSPS mixing ratio conditions before **(a)** and after **(b)** heating. Lowercase letters represent significant differences ($p<0.05$) between different samples in the same pH conditions, whereas uppercase letters represent significant differences ($p<0.05$) between the same sample in different pH conditions according to Two-way ANOVA followed by Tukey's multiple comparisons post-test.



△ Translucent suspension

○ Cloudy suspension

▲ Precipitate with translucent suspension

● Precipitate with cloudy suspension