JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Interfacial and Oil/Water Emulsions Characterization of Potato Protein Isolates

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ABSTRACT: Interfacial and emulsifying properties of potato protein isolate (PPI) have been studied to evaluate its potential application to stabilize oil/water emulsions at two pH values (2 and 8). The amount, type, and solubility of proteins and the size of aggregates have been determined in aqueous dispersion. Air−water and oil−water interfacial properties (adsorption, spreading, and viscoelastic properties) have been determined as a function of concentration and pH using soluble phases of PPI. The behavior of PPI stabilized oil/water emulsions has been then analyzed by droplet size distribution measurements and interfacial concentration. PPI exhibits low solubility over a wide range of pH values, with the presence of submicrometer aggregates. The pH value exerts a negligible effect on interfacial tension (oil−water) or surface pressure (air−water) but displays very important differences in viscoelastic properties of the interfacial films formed between oil and water. In this sense, pH 8 provides a major elastic response at oil−water interfaces as compared to pH 2. In relation with this result, a much higher ability to produce fine and stable emulsions is noticed at pH 8 as compared to pH 2. Consequently, there is an evident relationship between the rheological properties of the oil−water interfacial films and the macroscopic emulsion behavior.

KEYWORDS: potato protein isolates, interfacial tension, surface pressure, interfacial rheology, emulsion

INTRODUCTION

Proteins are extremely effective in stabilizing dispersed systems like emulsions and foams by a fair reduction of interfacial tension and the formation of viscoelastic films.[1−3] The lowering of interfacial tension facilitates the breakup of droplets during emulsion formation. Moreover, the viscoelastic film helps to stabilize the newly formed droplets against immediate coalescence during emulsion formation or against coalescence during storage of the product. Therefore, interfacial rheology plays an essential role in maintaining this stability, and interfacial rheology parameters can be obtained by subjecting the interface to either dilatational or shearing deformation.[4] As an example, it has been demonstrated that, for short-term stability, interfacial rheology in compression/expansion is considered to be far more relevant, [5] whereas shearing deformation implies middle-term stability. Therefore, the contribution of interfacial properties is relevant to the behavior of oil/water−protein-stabilized emulsions.

Novel sourcing proteins have been explored from various raw agro-materials due to increasing market demands on protein ingredients.[6−8] Furthermore, due to a probable rarefaction of animal proteins near the half of the 21th century, it is imperative to study the reimplacement of animal proteins by other sources, the easiest being plant proteins. Among them, proteins from food industry wastes, that produce an undesirable environmental impact, are starting to attract interest. However, a novel protein has to possess desirable functional and nutritional qualities to be useful for food applications or at least has to provide functionalities similar to the one that it replaces.[9,10]

Potato proteins show a nutritional quality higher than most major plant proteins and close to that of egg proteins.[11] Potato proteins are regarded as a byproduct of potato starch manufacture that contains ca. 1.5% (w/v) soluble protein.[12,13] Several extraction processes of proteins from potato have been investigated. However, heat coagulation is the only one that may be used at an industrial scale.[14] Therefore, potato proteins from industrial wastes are generally subjected to extreme conditions so that an important denaturation takes place.[15] As a result, low protein solubility and a severe loss of their functional properties over the entire pH range typically occur.[16,17] Despite this fact, undenatured potato protein has exhibited some emulsifying [14,15] and foaming[18] ability, although only to a limited extent. Some alternatives such as hydrolysis to improve solubility of potato proteins and, therefore, other functional properties such as gelation, emulsification, or foaming in food systems are currently being studied.[19] In any case, potato industry is currently highly interested in the development of a new process that will result in significant improvements in environmental conditions (i.e., energy efficiency) and sustainability (i.e., by converting wastes into added value byproducts) along with improvement in the quality of potato protein.[12]

Other potential applications, as a functional ingredient in nutraceutical food products, derived from the high nutritional value of potato protein, and related to the above-mentioned functional properties improvement, could be also envisaged for potato proteins. Globally, previous studies[15,18] showed that emulsions stabilized with potato proteins presented a smaller droplet size but more important droplet aggregation at pH 7 as compared to pH 3, whereas pH 5 was damaging for the two parameters. Yet very few studies have attempted to connect interface characteristics (two dimensions) with emulsion properties (three dimensions).

Received: February 22, 2011
Accepted: August 1, 2011
Revised: July 29, 2011
Published: August 02, 2011

dx.doi.org/10.1021/jf2019853 | J. Agric. Food Chem. 2011, 59, 9466–9474

Consequently, the objectives of this work are to study the composition and structure of potato protein and its behavior either at the air−water or at the oil−water interfaces and to link these results to the corresponding potato-stabilized emulsions, in view to bring some bases about their uses in food emulsions. It should be taken into account that this study seeks to evaluate PPI under conditions at which its interfacial behavior is expected to show higher potentials, being aware that its use in food products would probably require an eventual modification of pH, and, consequently, further research should be performed previously to its application. This approach has been recently followed to enhance the stability of different protein-based emulsions.[20−23]

MATERIALS AND METHODS

Materials. Potato protein isolate (PPI) (ca. 80 wt %) was supplied by Protastar (Reus, Barcelona, Spain). All chemicals used were of analytical grade purchased from Sigma Chemical Co. (St. Louis, MO). Distilled water was used for the preparation of all solutions. Buffers at pH 2 and 8 were phosphate buffers prepared using phosphoric acid and NaH_2PO_4, respectively. Buffer concentration is 10 mM, and the ionic strength is controlled at 20 mM by sodium chloride for both buffers.

Chemical Composition of Protein Isolates. The protein contents were determined in quadruplicate as %N × 6.25 using a LECO CHNS-932 nitrogen micro analyzer (Leco Corp., St. Joseph, MI).[24] Lipid content was analyzed by Soxhlet extraction. Moisture and ash content of the isolate were determined in quadruplicate by AOAC, 1995 approved methods.

Electrophoresis. Protein isolate composition was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Continuous and stacking gels of 10% and 3.5% of acrylamide, respectively, were prepared. A buffer of 2 M Tris-base, pH 8.8 containing 0.15% SDS for the separating gel and 0.027 M Tris-base, 0.38 M glycine pH 8.3 with the addition of 0.15% SDS for the running buffer were used. Coomassie Brilliant Blue was used as colorant agent. Low molecular weight markers (Biorad SDS-calibration kit) used included phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa).

Determination of Protein Isoelectric Point (pI) and Solubility. For the determination of the pI, aqueous potato protein isolate dispersions (1 mg protein/mL) were prepared, and the pH of different aliquots was adjusted with 6 N NaOH and 2 and 6 N HCl to alkaline and acid pH values, respectively. These dispersions were equilibrated for 1 h at room temperature under continuous stirring. Four milliliters of each dispersion was taken for the determination of the initial protein content by means of the above-mentioned LECO nitrogen micro analyzer. The remaining dispersion was centrifuged for 20 min at 10 000*g*, at 10 °C. The supernatants were collected for protein content determination. Percentages of soluble protein (calculated as protein content of supernatant × 100/weight of PPI powder) were plotted versus pH to determine the pI. These supernatants were used for the rest of the experiments presented in this study. On the other hand, *z*-potential was measured using a "Zetasizer 2000" (Malvern Instruments, U.K.). Samples were measured in triplicate at 20 °C. The zeta potential was calculated from electrophoretic mobility using the Henry equation and the Smoluchowski approximation.

Atomic Force Microscopy (AFM). A drop of 1 g/L PPI solution was spread with a Pasteur pipet at the surface of a freshly cleaved mica sheet at each pH value. A compressed nitrogen stream then was used to dry the sample, achieving a homogeneous distribution on the mica plate. After that, the mica plate was kept in a desiccator overnight to achieve the complete drying of the sample.

AFM images are recorded using an AFM CP (Park Scientific Instruments, Sunnyvale, U.S.) in the air in the tapping mode using conventional pyramidal silicon nitride cantilevers (Digital Instruments, U.S.). In this mode, the cantilever oscillates at its resonance frequency, the tip coming into contact with sample only periodically at the lowest possible scanning force (less than 10 nN). An amplitude image corresponding to the oscillation amplitude of the tip is then recorded over a surface range of 4 μm^2.

Satistical data of images were obtained using WSxM 4.0 software developed by nanotec Electronica (www.nanotec.es). Five images of at least two replicates of each sample were analyzed. One of the most representative measruments was selected for the figures.

Air−Water Interface Properties. Measurements of the surface pressure (Π)−surface area (A) isotherms have been performed by compression−expansion cycles using the Wilhelmy plate method on an isolated and fully automated Langmuir-type film balance (KSV 300 V2, Helsinki, Finland). The balance consisted of two movable barriers with Wilhelmy plate as a tension captor. The area of the vessel, initially of 350 cm^2, was reduced to 40 cm^2 at the end of the compression. It was filled with each of the buffer systems employed for emulsion preparation, and the temperature was kept at 20 °C by water circulation from a thermostat Bioblock Ministat (Illkirch, France).

The cleanliness of the buffers was checked by a compression and expansion cycle using the buffer without protein injection, considering a cleaned interface when surface pressure rising was negligible (less than 0.05 mN/m). The barrier speed has been fixed at 40 cm^2/min. PPI was suspended in a concentration of 2 g/L in the two buffers and then centrifuged 30 min at 10 000*g*. The supernatant was diluted in different concentrations calculated to spread 100 μL of solution over the balance surface and obtain different quantities of protein spread in the surface: 10, 30, 50, and 60 μg. The deposition of the drops was performed with a micrometric syringe avoiding thick smears and located on the interface. To allow the process of the protein spreading, adsorption, and rearrangements, samples were allowed to stand 40 min before compression. The compression speed was maintained constant at 28.4 cm^2/min, which is sufficiently low to prevent secondary effects (shear gradient or material displacement) due to the barrier displacement. These measurements were performed at different protein concentrations and pH values (2 and 8). At least three isotherms were performed for each sample.

Oil−Water Interface Properties. Transient interfacial tension and interfacial dilatational parameters were carried out using a drop tensiometer from IT Concept (Longessaigne, France). An axisymmetric drop of cleaned sunflower oil was formed at the tip of the needle of a syringe whose verticality was controlled by a computer. The drop profile was digitized and analyzed through a CCD camera coupled to a video image profile digitizer board connected to a computer. The image was continuously visualized on a video monitor. Drop profiles were processed according to the Laplace equation.[25]

All of the experiments were carried out, at least in triplicate, in an optical glass cuvette (8 mL), containing the corresponding aqueous solution of protein. The system was thermostatted at 20 ± 0.1 °C. Interfacial tension kinetics were performed for different protein concentrations, from 0.1 to 1.0 g/L. On the other hand, the viscoelastic moduli of protein adsorption layers were determined after 500, 2500, 4500, 6500, and 11000 s at one frequency (0.02 Hz) and once the equilibrium was achieved at different frequencies (0.005−0.05 Hz). In this last case, the protein concentration was fixed at 1.0 g/L at which the protein layer was saturated. The dynamic surface viscoelastic parameters (E^*, E', E'', and tan δ) were determined from these experiments and followed the subsequent equations:

$$E^*_{eq} = d\sigma/(dA/A) = E' + iE'' \quad (1)$$

where $d\sigma$ is the change in interfacial tension and dA is the change in interfacial area.

$$\tan \delta = E''/E' \quad (2)$$

where E^* is the complex dilatational modulus, E' is the elastic modulus, E'' is the loss modulus, and tan δ is the tan of phase angle.

Table 1. Chemical Characterization of Potato Protein Isolate Expressed in Relative Percentage on the Fresh Weight Basis[a]

	% fresh weight
proteins	80.1 ± 2.3
lipids	3.1 ± 0.4
carbohydrates	5.9 ± 0.6
ashes	0.8 ± 0.1
moisture	10.1 ± 2.0

[a] All of the experiments were realized in quadruplicate.

Emulsion Processing. Different 50% (wt/vol) sunflower oil emulsions were prepared using 3 wt % potato protein isolate and water. Oil and water were homogenized for 1 min at 20 000 rpm using a polytron PT 3000 homogenizer (Kinematica, Switzerland) equipped with a 12 mm diameter head. Homogenization of the emulsion premix was then achieved with a two-stage high-pressure valve homogenizer (TC5, Stansted Fluid Power, UK) at 15 MPa for 5 min. The emulsion (40 mL) was left recirculating in the homogenizer for 3 min at a flow rate of 120 mL/min.

Droplet Size Distribution. Measurements of droplet size distribution were performed in a Saturn Digisizer 5200 from Micromeritics Instruments Corp. (U.S.). For this purpose, 0.5 mL of emulsion was taken and diluted in 11.5 mL of 0.05 M, pH 8 Tris-HCl buffer with 1% SDS, to facilitate disruption of the flocs.[26] Values of the volumetric mean diameter, $d_{4,3}$, which is inversely proportional to the specific surface area of droplets, and the uniformity ratio (U), which is an index of polydispersity of the different droplet sizes, were obtained.[27]

Interfacial Protein Concentration. Nonadsorbed proteins were washed from the oil droplets following a method adapted from the procedure described by Patton and Huston.[28] Each fresh emulsion (2 mL) was diluted in 2 mL of sucrose solution (500 mg/mL, with the same pH value as the aqueous phase of the emulsion: pH 2.0 and 8.0). This dilution (2 mL) was then carefully deposited at the bottom of a centrifuge tube containing 10 mL of a buffer solution with the same pH and NaCl concentration as the respective emulsion. The tubes were then centrifuged at 3000g for 2 h at 10 °C. After centrifugation, three phases were observed in the tubes: the creamed oil droplets at the top, the intermediate buffer solution, and a concentrated dispersion of protein deprived of oil droplets at the bottom. The tubes were frozen (at ca. −20 °C) and cut so as to recover the three phases. Proteins from the upper phase were the adsorbed proteins (AP), and those from the bottom phase are unadsorbed proteins. When the middle phase was turbid due to the presence of small oil droplets, the protein stabilizing those droplets was aggregated to adsorbed proteins from the upper layer. Protein concentration was assessed in the bottom of the tubes by the procedure of Markwell et al.[29] Protein concentration was referred to emulsion volume, and interfacial protein concentration (Γ, mg/m^2) was calculated as follows:

$$\Gamma = \frac{C_{ap}}{S_V} \quad (3)$$

where C_{ap} is the overall protein concentration (mg/mL emulsion), and S_V is the specific interfacial area (m^2/mL emulsion) according to the procedure of Martinet et al.[30]

Statistical Analysis. Three replicates of each measurement were carried out. Statistical analyses were performed using the t test and one-way analysis of variance (ANOVA, $p < 0.05$), and standard deviations from some selected parameters were calculated.

RESULTS AND DISCUSSION

Protein Characterization. *Chemical Composition of Potato Protein Isolate.* The composition of the PPI studied is shown in Table 1. The protein content is 80.1 ± 2.3% of fresh weight corresponding to 89.1% of the dry weight, which is not significantly different from the limit generally accepted for potato protein isolates.[31] Excluding moisture, carbohydrates are the second more abundant component in PPI, corresponding to starch that cannot be separated in the protein extraction stage. PPI also contains small amounts of lipids and ashes (3.1 ± 0.4% and 0.8 ± 0.1%, respectively).

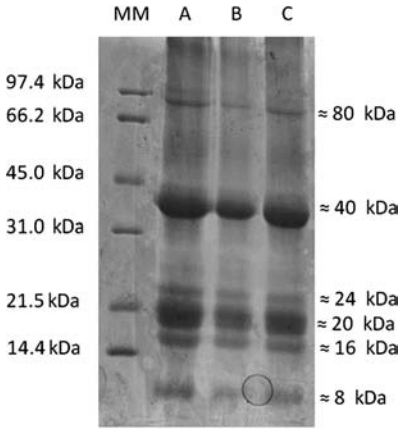

Figure 1. SDS-PAGE of PPI for different amounts of protein: A, 10 μg; B, 15 μg; and C, 20 μg of potato proteins. Patatin: 40 kDa. Protease inhibitors: 8−24 kDa. Phosphrylase: 80 kDa. MM: Molecular markers.

Electrophoresis. Figure 1 shows the results of a SDS-PAGE analysis for PPI for different amounts of proteins. Three main groups of proteins are observed in all of the electrophoresis tests performed. First, a rather intense band can be clearly noticed at around 40 kD. This band corresponds to patatin monomer, which is the most abundant potato protein, contributing 40−60% of the total protein fraction.[32] Patatin is a highly homologous group of isoforms consisting of 41 kDa glycoproteins, generally arranged in the form of dimers, with a storage function and lipid acyl hydrolase (LAH) activity.[33]

Second, protease inhibitors, which according to Pots et al.[32] represent 20−30% of the total protein, can be observed. Ralet and Gueguen[14] divided the protease inhibitor group into two subclasses depending on their molecular weights. Subclass I corresponds to 8−10 kDa and a much more abundant complex protein group (subclass II) of various molecular weights (16−25 kDa).[34] Both protease inhibitors groups may be clearly distinguished in Figure 1.

Finally, a further fraction was found at 80 kDa. This band was attributed to other high-molecular weight proteins (accounting for 20−30% protein) involved in starch synthesis such as an 80 kDa phosphorylase that must be mentioned.

pH−Solubility Curves. The solubility profile of PPI was obtained (Figure 2) to determine the pH at which the isoelectric point was reached. Thus, a minimum that corresponds to the IEP was detected at pH 4 (around 10% of solubility), and a maximum (around 35%) is obtained at pH 8. At pH 8, patatin chains contain more negative net charge influencing its structural stability giving rise to higher solubility.[35] We have measured at this pH a zeta-potential of −11.4 mV, whereas it was around 0.5 mV at pH 4 (results not shown). Similar results were obtained by others.[14,16] These last authors mainly attributed this minimum to the low solubility of the patatin fraction related to the presence of an acid-coagulable fraction. In fact, other authors improved the solubility by hydrolysis.[19] In our case and whatever the pH, protein solubility is very low, being always below 40 wt %, which

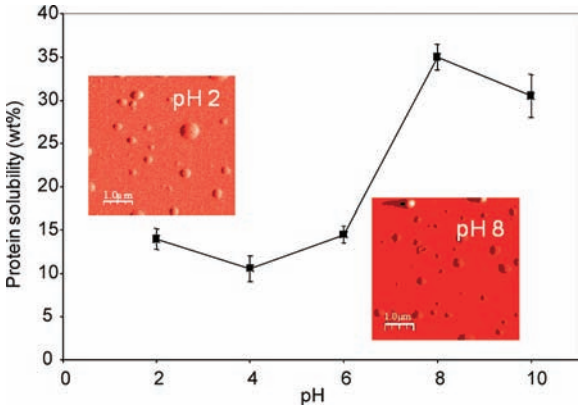

Figure 2. Solubility profile against pH of PPI for 1 mg/mL of PPI dipersions at pH 2 and 8. The experiments were made in triplicate. AFM images recorded in the air in the tapping mode and amplitude images corresponding to the oscillation amplitude of the tip are then recorded over a surface range of 4 μm^2.

may be probably due to a significant denaturation of these proteins during the previous extraction process.

To highlight this reduced solubility, we have imaged dispersions of PPI at the two pH values of our study (pH 2 and 8) by spreading a drop of each sample at the surface of a freshly cleaved mica sheet (Figure 2). As it may be deduced from all of the AFM images (we only gave a single example), aggregates of 50−500 nm size were observed at both pH values, without a difference in mean size between the two pH values. For this reason of low solubility, we have decided that the study concerning interfacial properties of PPI would be performed strictly with supernatants obtained after 10 000*g* centrifugation. We have verified that in the supernatant the same protein composition was conserved for the two pH values.

Interfacial Properties. *Air−Water Interface.* Figure 3A and B shows the $\Pi-A$ isotherms obtained as a function of air−water interface for two different pH values at 20 °C by double compression−expansion cycles, using the Langmuir trough method. These experiments allow the analysis of the behavior of potato proteins at a planar air−water interface.

We can observe that surface pressure (Π) is close to zero just before the compression (gas phase) and then increased until it reached a final pressure corresponding to the collapse of the interfacial film. At pH 8, two different concentration regimes are clearly identified: 10 μg and 30−60 μg, which clearly implies distinct behaviors. At 10 μg, the gas phase is very important, and the pressure just takes off at the end of the compression; for the higher concentrations, the pressure increases rapidly (from 250 cm^2). At pH 2, these behaviors were also observed with a gap toward smaller surfaces for the same pressure. Furthermore, for the highest concentrations (50 and 60 μg), the pressure rose very rapidly at pH 2, whereas a gas phase was observed before the pressure climb up at pH 8 (for the same concentrations). All of these results indicate that PPI tends to spread more efficiently at air−water interfaces at pH 2, although not significant differences between both pH values were found after saturation, in terms of surface pressure. Additionally, at high concentration regime, inflection points are noticed, signifying reorganizations and structural changes inside the interfacial film.

Whatever the pH value studied in our experiment, the protein film showed a collapse pressure ranging from 20 to 25 mN/m for the lowest concentrations (10−30 μg) and around 30−33 mN/m

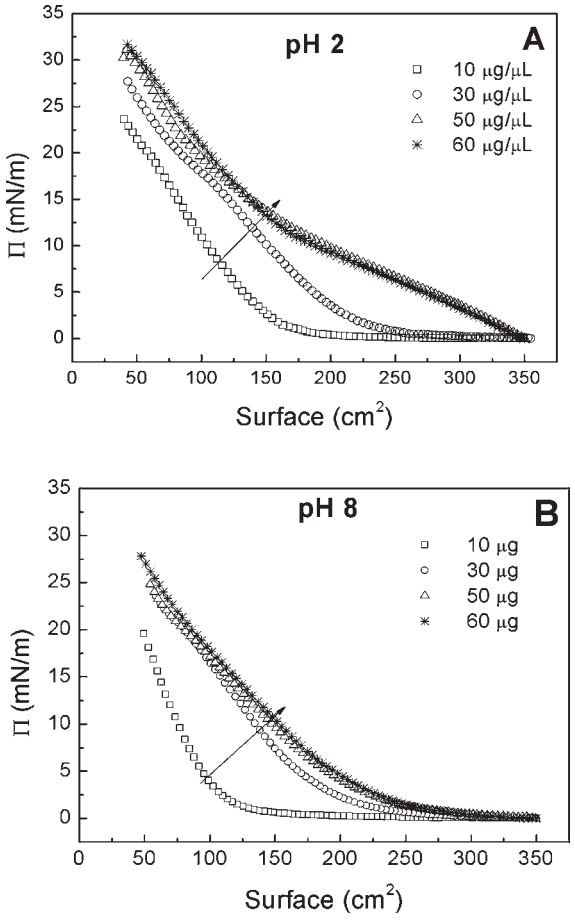

Figure 3. Surface pressure isotherms (compression curves) at the air−water interface by compression−expansion cycle at 20 °C for different potato protein concentrations (10, 30, 50, and 60 μg) at (A) pH 2 and (B) pH 8. At least three isotherms were performed for each sample.

for 50−60 μg. Small differences are noticed between the two pH values studied (see below). The values of collapse are similar to those found for β-casein by Rodriguez Patino et al.[36] and for crayfish and rice proteins under the same conditions (unpublished results). Isotherms were displaced toward larger pressure values as the amount of protein was raised (see arrow). This evolution tends to be logarithmic for either of the two pH values studied, as we observe an important gap between the lowest and the two biggest concentrations.

Figure 4 shows a double cycle for 30 μg of potato protein solution at pH 8, where no differences between the first and the second cycle were noticed. This reproducibility suggests a complete recovery of the protein film at the interface. The same control was performed at pH 2 with the same conclusions (not shown).

Figure 5 shows the pressure (corresponding to collapse pressure) obtained at the minimum area (maximum compression) for PPI at pH 2 and 8 as a function of interfacial concentration. All of the systems exhibit an initial growth in Π with interfacial concentration followed by an evolution toward a maximum value that corresponds to the saturation of the interface (Π^{sat}). We can notice that for the same concentration protein films at pH 2 exhibit a higher collapse pressure than those made at pH 8.

The higher values of collapse pressure and the better general spreading efficiency exhibited at pH 2 as compared to pH 8 can be related to the lower level of repulsive interactions between

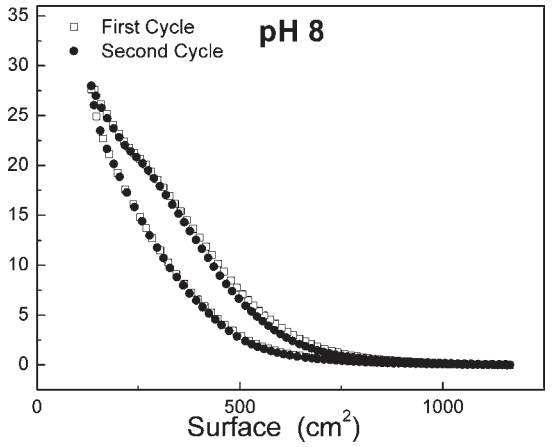

Figure 4. Surface pressure isotherms (compression−expansion curves) at the air−water interface at pH 8 and at 20 °C by a double compression−expansion cycle with 30 μg of potato protein spread. At least three isotherms were performed for each sample.

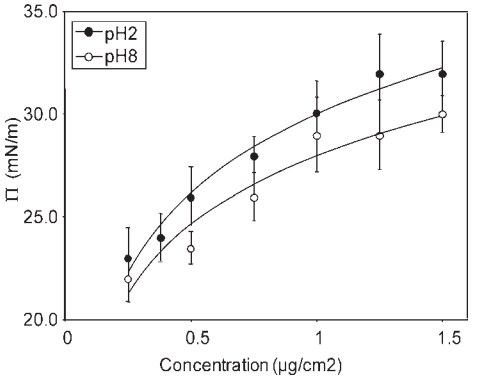

Figure 5. Surface pressure at the air−water interface at maximum compression as a function of potato protein concentration at pH 2 and 8, at 20 °C. At least three experiments were performed for each sample.

protein residues at the air−water interface. After determination, the zeta potential at pH 2 appeared to be as low as 0.9 ± 0.5 mV, whereas pH 8 leads to a zeta potential of −11.4 ± 0.5 mV (results not shown). The poor electrostatic repulsions between proteins deduced from the low zeta potential at pH 2 can explain a better interfacial packing of proteins at this pH, particularly at saturation conditions or at high concentrations.

In addition, the general better spreading efficiency observed at pH 2 as compared to pH 8, even at low concentrations, could be related to intrinsic capacities of spreading, and we will have to check in a future study the molecular structure of the PPI at these two pH values.

Oil−Water Interface. The transient interfacial tension versus time, corresponding to adsorption kinetics of potato proteins at the oil−water interface, is shown in Figure 6A and B as a function of protein concentration at pH 2 and 8, respectively.

The adsorption kinetics present two phases. The first one is characterized by a rapid decrease of interfacial tension and corresponds to the phase of protein adsorption. The second one, from 200 s, is characterized by a slow evolution of interfacial tension with time and corresponds to the phase of conformational rearrangements of proteins at the oil−water interface. Each kinetic tends to an equilibrium tension value (σ_{eq}) obtained

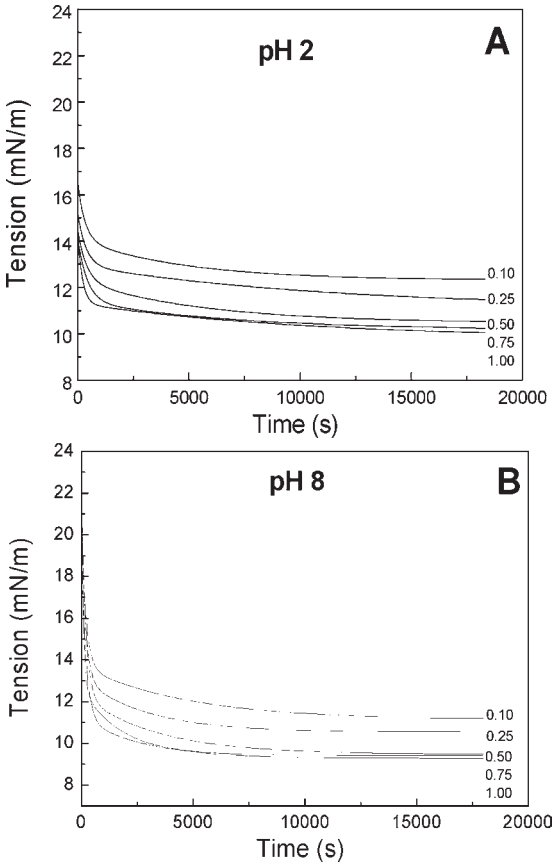

Figure 6. Interfacial tension kinetics of potato protein solutions as a function of protein concentration at sunflower oil−water interface at different pH values: (A) pH 2 and (B) pH 8, at 20 °C. Time is indicated in seconds. At least three experiments were performed for each sample.

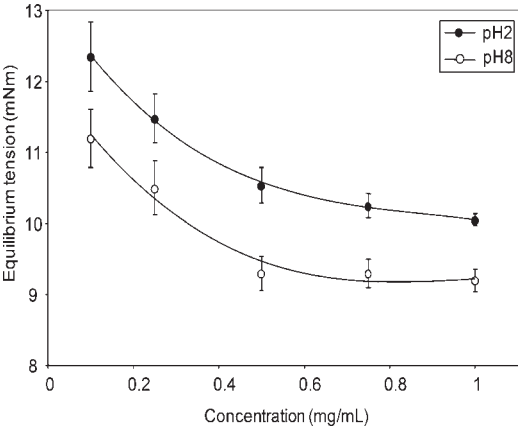

Figure 7. Equilibrium interfacial tension at sunflower oil−water interface as a function of potato protein concentration at pH 2 and 8, at 20 °C. At least three experiments were performed for each sample.

at long time (5 h), even if the interfacial tension is still evolving extremely slowly.

Figure 7 shows the evolution of σ_{eq} as a function of potato protein concentration in the solution. As may be observed, a decrease in equilibrium tension takes place as protein concentration increases, showing a tendency to a plateau value after which σ_{eq} does not change with protein concentration. This value is

dx.doi.org/10.1021/jf2019853 |*J. Agric. Food Chem.* 2011, 59, 9466–9474

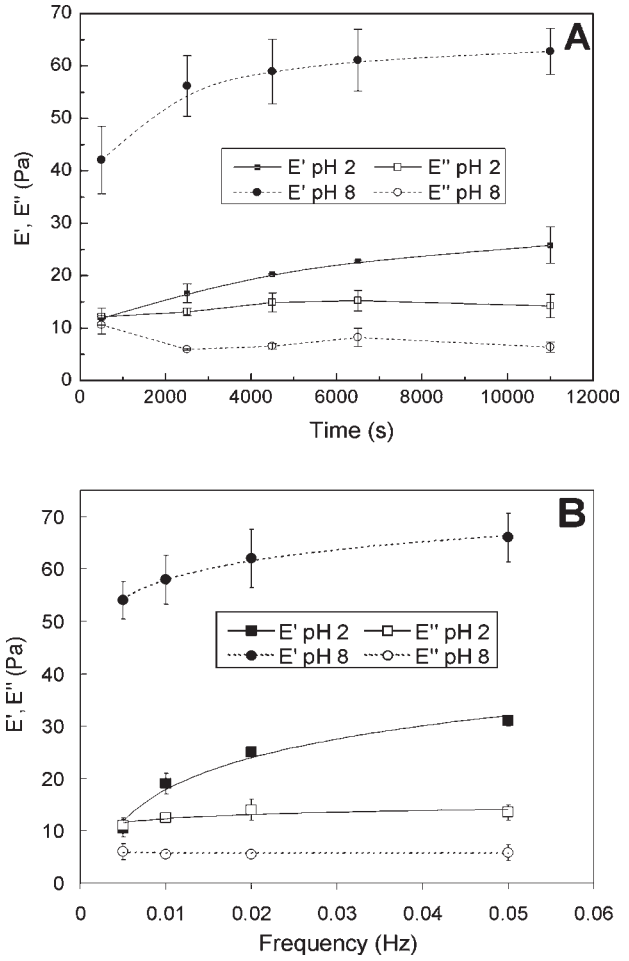

Figure 8. Evolution of dilatational (E') and loss (E'') modulus at sunflower oil−water interface films of potato proteins (1 g/L) at pH 2 and 8, at 20 °C as a function of (A) time in s (frequency 0.02 Hz), and (B) frequency in Hz (time 11 000 s). At least three experiments were performed for each sample.

known as the equilibrium tension at saturation ($\sigma^{sat}{}_{eq}$). According to this evolution, the saturation of the interface is reached at about 0.75 mg/mL for the two pH values, showing values of ca. 10 and 9.2 mN/m for $\sigma^{sat}{}_{eq}$. Therefore, both results put forward that protein adsorption at the interface was very similar whatever the pH value, even if pH 8 allows a lower equilibrium interfacial tension.

As stated by Benjamins and Lucassen-Reynders,[5] it is important to have information not only on the interfacial tension value, related to adsorption, but also on the interfacial viscoelastic behavior, to evaluate the resistance of interface to mechanical constraints, particularly with the aim of understanding the role of stabilization of oil droplets.

Interfacial rhelogical properties were evaluted at 1 mg/mL potato protein, ensuring the interface is satured at either of the two pH values studied. Figure 8 shows dilatational properties of interfacial films submitted to dilatational fluctuations of area at constant strain amplitude. These measurements were performed either during the protein adsorption at a constant frequency (Figure 8A) or at the equilibrium, when the protein was completely adsorbed at the interface, as a function of frequency (Figure 8B). Figure 8A puts forward the higher values obtained for the elastic modulus (E') over the loss modulus (E''). This behavior (high difference between elastic and loss moduli) is much more prononounced at pH 8 than at pH 2. This is confirmed by the value of the tan of the phase angle (tan δ), which is lower for the film at pH 8 as compared to pH 2 (Table 2). Furthermore, whatever the time, the value of elastic modulus (E') is much more important at pH 8 than at pH 2. All of these indicators point out very clearly the more pronounced elastic behavior of films of PPI at pH 8 as compared to pH 2. In Figure 8A, we can observe an increase of elastic modulus (whatever the pH) with time. This time dependency is to be put in relation with the interface protein concentration in the way E' depends on the interface coverage, which increases with time. A plateau is reached at pH 8 at about 4500 s, and this value is largely in the zone of saturation of the oil−water interface.

Table 2. Interfacial Air−Water and Oil−Water Characteristics for Potato Protein Solution at pH 2 and 8[a]

pH	Π^{sat} a−w (mN/m)	σ_{eq} o−w (mN/m)	E^*_{eq} o−w (mN/m)	tan δ_{eq} o−w
2	32.1 ± 2.1a	10.0 ± 0.1a	29.6 ± 2.0a	0.64 ± 0.23a
8	30.1 ± 1.9a	9.2 ± 0.1b	63.1 ± 4.6b	0.10 ± 0.01b

[a] Saturation pressure (Π^{sat}) is measured at maximum surface compression, equilibrium interfacial tension δ_{eq} is obtained after 11 000 s, and dilatational modulus (E^*) and delta tangent are measured at 0.02 Hz. At least three experiments were performed for each sample. Means within a column with different subscripts are significantly different ($p < 0.05$).

Figure 8B shows a low frequency dependence for viscoelastic moduli with an obvious predominant elastic response, particularly at pH 8. Typically the dependency of elastic modulus with frequency is rather poor with films constituted with proteins.[37−40] Nevertheless, whatever the frequency, the E' value of the film is much more pronounced at pH 8 than at pH 2. It is then worth mentioning that the potato protein stabilized interface at pH 8, at which the contribution of electrostatic interactions is relevant (−11 mV), shows remarkable higher elastic-like characteristics values (with higher values for the elastic modulus and much lower for the loss tangent) than the pH 2 system from the early stages of film formation (Figure 8A) and until it reaches the equilibrium (Figure 8B). It can be deduced that important electrostatic repulsions inside the interfacial films help in maintaining a rigid and organized structure. This will have to be imaged in our next study using an AFM device. On the other hand, it is well-known that protein films formed at minimum electrostatic repulsion conditions lead to poor viscoelastic modulus.[41] At pH 2, the interface seems to be close to this case.

Comparison between Air−Water and Oil−Water Interfacial Properties. Table 2 shows the values of some characteristics obtained from air−water or oil−water interfacial properties for the protein systems studied at pH 2 and 8. Surface pressure at maximum compression (air−water) and equilibrium interfacial tension (oil−water) are similar between acidic and alkaline conditions. In the case of equilibrium surface tension, the means are significantly different between pH 2 and 8, but the values are too near to represent distinct behaviors. On the other hand, pH yields a remarkable and significant influence for the dilatational rheology characteristics at the oil−water interface (E^*_{eq} and tan δ_{eq}). These results reveal an important elastic reinforcement of the oil−water interface at pH 8 as compared to that at pH 2.

As described by Benjamins et al.,[35] rheology of interfacial film (related to the interactions between adsorbed molecules) and interfacial tension (related to amphiphilic character and flexibility of proteins) can be put in connection (Figure 9). This figure plots the dilatational complex modulus as a function of interfacial

dx.doi.org/10.1021/jf2019853 |J. Agric. Food Chem. 2011, 59, 9466–9474

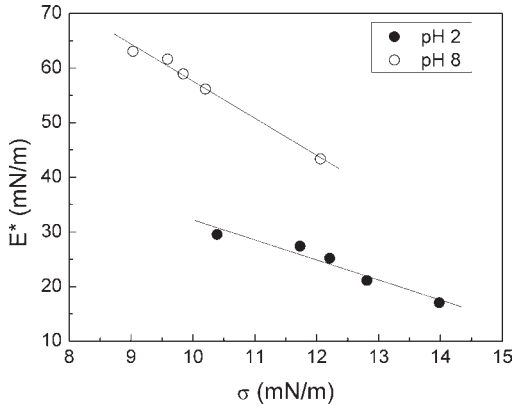

Figure 9. Complex dilatational modulus at 0.02 Hz as a function of interfacial tension at sunflower oil−water interface films of potato proteins at 1.0 g/L (pH 2 and 8). At least three experiments were performed for each sample.

tension at the oil−water interface for both pH values. We can observe that the slope is much more pronounced at pH 8 than at pH 2, and it can be related to a higher interfacial strengthening ability for a similar reduction in the interfacial tension level. In fact, Benjamins et al.[35] demonstrated that the different slope obtained for different proteins can be attributed to the structural characteristics of each protein film. A higher slope was found for compact proteins as compared to the more flexible one that exhibited a less pronounced slope. As a consequence, the different slope obtained at the two pH values studied highlights differences in the internal structure of adsored proteins due to pH changes, and the mean value of elastic modulus reflects differences in intermolecular interactions inside the film.

In summary, we can observe that surface pressure (air−water interface) or interfacial tension (oil−water interface) characteristics behave simarly whatever the pH studied, but that rheological parameters studied at oil−water interfaces reveal important differences. We would like now to assess if these differences played a role in emulsion formation and stabilization with potato protein.

Emulsions. *Droplet Size Distribution and Interfacial Concentration.* Figure 10 shows the droplet size distribution profiles obtained for 50% wt/vol stabilized by 3 wt % PPI as a function of aging time at pH 2 and 8. All of the droplet size distribution curves display a wide polydispersity, as may be deduced from the values obtained for the uniformity ratio shown in Table 3. It is clear that pH 8 provides the formation of finer emulsions than does pH 2: $d_{4,3}$ = 0.58 μm at pH 8 versus 0.84 μm at pH 2, the uniformity of the droplet population being quite similar between the two pH values. We can also observe Table 3 that the percentage of adsorbed proteins at the oil−water interface is significantly higher at pH 8 than at pH 2. This confirms the better ability we have observed in this Article to decrease interfacial tension at the oil−water interface.

Furthermore, an evolution along time toward higher size and a decrease of uniformity of droplets, related to coalescence phenomena, can be noticed in Figure 8 and Table 3. It is clear that the increase in $d_{4,3}$ observed after 20 days is much more important at pH 2 (51.5% of increase) than at pH 8 (15.5%). These results can be linked with the higher viscoelastic properties observed at pH 8 at the oil−water interface, as compared to those at pH 2. These results are in agreement with those found for sweet potato

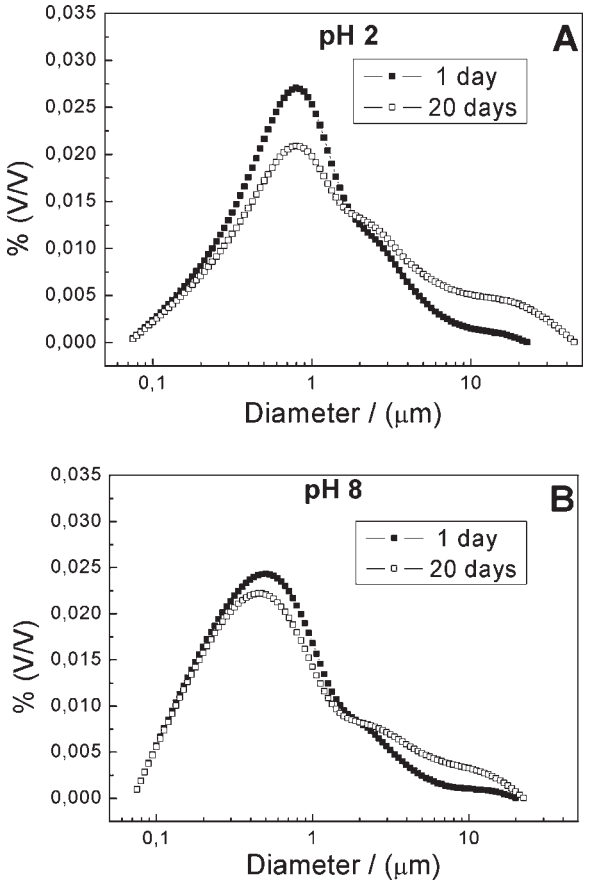

Figure 10. Droplet size distribution profiles for emulsions containing 50 wt % oil and 3 wt % potato protein as a function of storage time (1 day and 20 days) at (A) pH 2 and (B) pH 8. All samples were diluted in deflocculation agent (SDS). At least three experiments were performed for each sample.

protein isolate by Mu et al.,[8] who reported an enhancement in the emulsifying activity and stability indexes by increasing pH.

Values of interface protein concentration (Γ) were very similar to those obtained by van Koningveld et al.[15] for emulsions of 10% w/v of oil with 7.5 mg/mL of potato protein isolate, determined as the surface excess from the total and bulk concentration. Furthermore, according to Bos and Van Vliet,[42] the adsorption or surface excess concentration of most proteins is found to be approximately 2−3 mg m^{-2}, depending on the pH and ionic strength of the solution. Whatever the pH, the percentage of adsorbed proteins (AP) is around 60% and the interfacial concentration is around 2 mg/m^2, without significant differences in the interfacial concentration.

Concluding Remarks. PPI exhibits low solubility in the whole range of pH values, being always lower than 40 wt %, with the presence of submicrometer aggregates. Applying soluble supernatants with the same composition, we have demonstrated that the pH value of the aqueous phase exerts a small effect on interfacial tension (oil−water interface) or surface pressure (air−water interface), but displays very important differences in viscoelastic properties of the interfacial films formed between oil and water. Clearly, pH 8 provides a major elastic response at oil−water interfaces as compared to pH 2. This behavior could be linked to important electrostatic repulsions between adsorbed proteins that may allow the constitution of a cohesive film in these conditions.

Table 3. Characteristics of Emulsions Containing 50 wt % Oil and Stabilized by 3 wt % Potato Proteins at pH 2 and 8[a]

			1 day		20 days	
pH	AP (%)	Γ (mg/m^2)	$d_{4,3}$ (μm)	U (−)	$d_{4,3}$ (μm)	U (−)
2	56.2 ± 0.9a	2.1 ± 0.4a	0.84 ± 0.01a	1.20 ± 0.04a	1.27 ± 0.08a	2.23 ± 0.21a
8	65.1 ± 1.7b	1.8 ± 0.1a	0.58 ± 0.03b	1.38 ± 0.27a	0.67 ± 0.05b	2.22 ± 0.10a

[a] Volumetric mean diameter, $d_{4,3}$; index of uniformity, U; % of adsorbed proteins, AP. At least three experiments were performed for each sample. Means within a column with different subscripts are significantly different ($p < 0.05$).

In continuation, our results reveal that pH 8 displays a much higher ability to produce fine and stable emulsions than pH 2. As a consequence, it can be concluded that there is an apparent relationship between the rheological properties of the oil−water interfacial films and the macroscopic emulsion behavior. First, important electric charges appearing at pH 8 can cause efficient electrostatic repulsion between droplets limiting risks of flocculation and coalescence. Second, the strong elastic character of the interfacial films at pH 8 leads to a better resistance to mechanical constraints and then to a better resistance against coalescence. Moreover, the link between the adsorption ability and the emulsion formation and stabilization seems, in this case and whatever the type of interface (air−water or oil−water), less essential.

We have to determine in the future the mechanism explaining the differences of film viscoelastic properties between pH values with a particular focus (i) on the zeta potential and the nature and intensity of interactions between proteins inside the interfacial film, (ii) on the properties of submicrometer aggregates that could be reformed at the interface, and (iii) on the molecular structure of PPI in relation to their spreading capacities modulated by pH. Furthermore, AFM imaging of such interfacial films at different pH values and in dynamic conditions (pH variations) has to be assessed to confirm the structure differences of films hypothesized here.

Consequently, the pH-dependence of potato protein interfacial films in relation with their ability to form and stabilize oil-in-water emulsions could be used to enhance emulsions made with plant proteins and/or to intend tunable oil−water interfaces. Some other investigation concerning foaming properties of potato proteins should also be performed to assess their capacities to form and stabilize food foams.

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Funding Sources

We acknowledge financial support from the MEC (FPU grant). This work was also part of research project no. AGL2007-65709 supported by the Spanish MCYT and FEDER.

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