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Effect of Enzymatic Treatment of Extracted Sunflower Proteins on Solubility, Amino Acid Composition, and Surface Activity

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Industrial proteins from agriculture of either animal or vegetable origin, including their peptide derivatives, are of great importance, from the qualitative and quantitative point of view, in food formulations (emulsions and foams). A fundamental understanding of the physical, chemical, and functional properties of these proteins is essential if the performance of proteins in foods is to be improved and if underutilized proteins, such as plant proteins (and their hydrolysates and peptides derivatives), are to be increasingly used in traditional and new processed food products (safe, high-quality, health foods with good nutritional value). In this contribution we have determined the main physicochemical characteristics (solubility, composition, and analysis of amino acids) of a sunflower protein isolate (SPI) and its hydrolysates with low (5.62%), medium (23.5%), and high (46.3%) degrees of hydrolysis. The hydrolysates were obtained by enzymatic treatment with Alcalase 2.4L for DH 5.62 and 23.5% and with Alcalase 2.4L and Flavorzyme 1000 MG sequentially for DH 46.3%. The protein concentration dependence on surface pressure (surface pressure isotherm), a measure of the surface activity of the products (SPI and its hydrolysates), was obtained by tensiometry. We have observed that the degree of hydrolysis has an effect on solubility, composition, and content of the amino acids of the SPI and its hydrolysates. The superficial activity and the adsorption efficiency were also affected by the degree of hydrolysis.

KEYWORDS: Sunflower protein isolate; protein hydrolysis; food emulsifier; food dispersions; air−water interface; surface activity at equilibrium

INTRODUCTION

Plant proteins are increasingly being used as an alternative to proteins from animal sources in human nutrition. Among plants, legume seeds (soybean, chickpea, lupine, lentil, etc.) and oilseeds (sunflower, rapeseed) represent a rich source of proteins (*1*). Fat extraction from plant seeds yields a large amount of defatted meal which, among other nutritional and undesirable components, contains a significant amount of protein and fiber which could be used as food ingredients. These byproducts were traditionally used as a source for animal food, but the high protein content can increase the value of the byproducts if these proteins are directed to human nutrition. Another problem derived from plant proteins is the poor solubility at pH around the isoelectric point and at high temperature (*2*), which is detrimental for the main functional properties of proteins (including foaming and emulsifying capacity). The undesirable compounds for an enzymatic treatment (fiber, phenols) can be eliminated if defatted meal is processed into a protein concentrate and subsequently into a protein isolate (*3*−*5*). However, a more interesting alternative is the use of protein isolate as the starting material for the preparation of protein hydrolysates with food-grade proteases (*2*, *5*, *6*). Some protein hydrolysates display high solubility across a wide range of pH and temperatures, in contrast to native proteins. Most of these proteins are used as emulsifiers (*2*, *6*) in the production of food dispersions (emulsions and foams). Thus, the interfacial characteristics of these protein isolates and their hydrolysate derivatives by themselves or in combination with other emulsifiers (lipids, phospholipids, etc.) are key factors to define the functionality of these ingredients in food formulations (*7*−*14*) and/or for the production of "tailor-made hydrolysates".

Enzymatic hydrolysis is frequently used to improve functional and nutritional properties of food proteins. Protein hydrolysates can be classified into three major groups depending on the degree of hydrolysis, which determines their applications (*15*):

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hydrolysates with a low degree of hydrolysis (1−10%) with improved functional properties (mainly foaming and emulsifying capacity) (*16*−*23*), hydrolysates with a variable degree of hydrolysis that are used as flavorings, and extensive hydrolysates (>10%) that are used as nutritional supplements (*16*−*26*) and in special medical diets, like hypoallergenic hydrolysates (*27*), correction of metabolic errors (*28*), and hepatic diseases (*29*−*32*), for deficient or reduced intestinal activity (*32*), and others (*33*−*36*). However, the hydrolysis typically has a negative impact on long-term emulsion/foam stability, and on heat-set gelation behavior. Furthermore, many peptides produced by hydrolysis commonly have a bitter off-taste which makes them undesirable as food ingredients. This bitter off-taste can be avoided if the protein hydrolysates are produced by a sequential combination of endo- and exoproteases, as those used in this work (Alacalase and Flavorzyme, respectively). An additional negative aspect is the production of large amounts of protein hydrolysates with specific characteristics (for improvement of specific functional properties, production of bioactive peptides, etc.). Sunflower constitutes an interesting raw material for the preparation of protein hydrolysates. It is one of the more important oilseed crops cultivated in the world. Defatted sunflower meal contains about 30% protein, which could be used as a food ingredient (*36*) either as a high-quality sunflower protein isolate or for the preparation of protein hydrolysates with the food-grade proteases Alcalase (endopeptidase), Flavorzyme (exopeptidase), and with Alcalase and Flavorzyme sequentially (*5*, *37*).

In this contribution we initiate a systematic study centered on the production of a sunflower protein isolate (SPI) and SP hydrolysates with different degrees of hydrolysis and their influence on the most important functional properties of spread and adsorbed films at fluid interfaces (such as adsorption and structural, topographical, and dynamic characteristics), as a function of the protein concentration in the bulk phase and at the interface, including their foaming and emulsifying characteristics. In this paper we have analyzed the production of SPI and SP hydrolysates, their chemical and amino acid compositions, solubility, and the surface activity at equilibrium of adsorbed films at the air−water interface. The surface activity, that is the reduction of surface or interfacial tension due to the adsorption of proteins at fluid interfaces, is an important factor for the formation, stability, and optimization of the input of energy involved in the emulsification or foaming process (*8*, *38*). In addition, the surface pressure isotherm is also of utility to describe relaxation phenomena and interactions or compatibility between emulsifiers at fluid interfaces (*9*, *12*−*14*).

MATERIALS AND METHODS

Materials. Sunflower (*Helianthus annuus L.*) protein hydrolysates were prepared from defatted sunflower meal provided by MIGASA (Seville, Spain). A protein isolate prepared as previously describe (*5*, *37*) was used as the substrate for the hydrolysis. Bromophenol blue, disodium EDTA, dithiothreitol (DTT), sodium dodecyl sulfate (SDS), 2,4,6-trinitrobenzene-sulfonic acid (TNBS), and D-L-α-aminobutyric acid were purchased from Sigma Chemical Co. (St. Louis, MO). Acrylamide, *N*,*N*′-methylenebisacrylamide, and Coomassie Brilian blue G-250 were purchased from Serva (Heidelberg, Germany). Standards for electrophoresis and gel filtration were supplied by Amersham Pharmacia Biotechnology (Uppsala, Sweden). Diethyl ethoxymethylenemalonate was obtained from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade.

The proteases used were Alcalase 2.4L and Flavorzyme 1000 MG (Novo Nordisk, Bagsvaerd, Denmark). These are two enzymes with the specifications recommended by FAO/OMS for food applications. Alcalase 2.4L is an endopeptidase from *Bacillus licheniformis*, with Subtilisin Carlsberg as the major enzymatic component, having a specific activity of 2.4 Anson units (AU) per gram. One AU is the amount of enzyme that under standard conditions digests hemoglobin at an initial rate that produces an amount of tricloroacetic acid-soluble product which gives the same color with the Folin reagent as 1 mequiv of tyrosine released per minute. Flavorzyme 1000 MG is an exopeptidase and endoprotease complex with an activity of 1.0 leucine aminopeptidase unit (LAPU) per gram. One LAPU is the amount of enzyme that hydrolyzes 1 mmol of leucine-*p*-nitroanilide per min, using the method AF 298/1 (Novo Nordisk).

Samples for interfacial characteristics of protein isolate and hydrolysate films were prepared using Milli-Q ultrapure water and were buffered at pH 7. Trizma-HCl [$(CH_2OH)_3CNH_2$/$(CH_2OH)_3CNH_3Cl$] for buffered solutions at pH 7 was used as supplied by Sigma (>95%) without further purification.

Preparation of Protein Isolate. An initial concentrate of defatted sunflower meal was obtained by sedimentation−flotation according to Villanueva et al. (*5*). Afterward, this concentrate was suspended (1:10 w/v) in a solution of 0.25% (w/v) Na_2SO_3 at pH 10.5 by stirring for 15 min at room temperature (*19*). After centrifugation at 7500*g* for 15 min at room temperature the supernatants were pooled. To recover the soluble proteins two additional extractions were carried out at the isoelectric point (pI 4.35). The precipitate was washed with distilled water adjusted to pH 4.35 and atomized.

Enzymatic Hydrolysis. The protein isolate was hydrolyzed batchwise by treatment with Alcalase and/or Flavorzyme in a pH-stat. The hydrolysis was conducted in a reaction vessel equipped with stirrer, thermometer, and pH electrode. A constant concentration of substrate was used in all hydrolysis (10%, w/v). Three different degrees of hydrolysis (DG) were obtained. A 5.62% DG hydrolysate was obtained by Alcalase 2.4L treatment for 15 min, with an enzyme/substrate (E/S) ratio of 0.0025 AU/g of protein, at 20 °C and pH 8.0. A 23.5% DG hydrolysate was obtained by Alcalase 2.4L treatment for 45 min, with an E/S = 0.3 AU/g of protein, at 50 °C and pH 8.0. Finally, a 46.3% DG hydrolysate was obtained by sequential Alcalase 2.4L/Flavorzime 1000 MG treatment. The sequential treatment was carried out with an initial hydrolysis (45 min, E/S = 0.3 AU/g of protein, and at pH 8.0) using Alcalase and a second one (30 min, E/S = 50 LAPU/g of protein, at 50 °C and at pH 7.0) using Flavorzyme. Hydrolysis was stopped by dropping the pH to 5. Hydrolysates were clarified by centrifugation at 4000*g* for 30 min at 16 °C to remove insoluble substrate fragments, and the supernatants were lyophilized and freeze-dried for later use. The degree of hydrolysis, defined as the percentage of peptide bonds cleaved, was calculated by the determination of the free amino groups by reaction with TNBS according to Adler-Nissen (*39*). The total number of amino groups was determined in a sample that had been 100% hydrolyzed at 110 °C for 24 h in 6 N HCl (10 mg of sample in 4 mL of HCl).

Gel Filtration Chromatography. The molecular masses of the hydrolysates were determined by FPLC. Samples were passed through a PD-10 column (Amersham Pharmacia) to remove nonprotein components. Gel filtration was carried out in a fast protein liquid chromatography system (FPLC) equipped with a Superose 12 HR 10/30 column (Armsham Pharmacia). The injection volume was 200 μL. The eluent was 20 mM phosphate buffer, 0.5 M sodium chloride buffer, pH 8.3, at a flow rate of 0.4 mL/min. Elution was monitored at 214 nm to detect small peptides lacking aromatic residues. The molecular masses were determined with a calibration curve made with blue dextran (2000 kDa), catalase (240 kDa), β-amylase (200 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), ribonueclease (13.8 kDa), citochrome C (12.5 kDa), and bacitracine (1.45 kDa) as molecular weight standards. All these reagents were obtained from Sigma.

Amino Acid Analysis. Amino acids were determined by high-performance liquid chromatography (HPLC), according to the method by Alaiz et al. (*40*). The HPLC system consisted of a model 600E multisystem with a 484 UV−vis detector (Waters, Milford, MA). The content of tryptophan was determined according to the method by Yust et al. (*41*). The experimental error for this analysis was never higher than 5%.

Table 1. Physicochemical Properties of Sunflower Protein Isolate

component	composition (%)
moisture	5.10 ± 0.83
ash	3.00 ± 0.08
protein content	82.81 ± 0.45
soluble sugars	0.22 ± 0.02
polyphenols	0.45 ± 0.08
others	8.60

Table 2. Chemical Composition of Sunflower Protein Isolate and Protein Hydrolysates (HP) from Sunflower Protein Isolate at Different Degrees of Hydrolysis

degree of hydrolysis (%)	protein ($N \times 25$) (%, w/w)	modified protein
sunflower meal	33.3	
isolate	82.1	
HP 5.62	93.9	60.5
HP 23.3	80.8	83.9
HP 46.3	74.5	94.7

Solubility Curve Determination. The protein isolate and hydrolysates (10 g) were extracted twice with 200 mL of 1 N NaOH stirring for 2 h at room temperature. Aliquots were taken for precipitation of the proteins at different pH values adjusted with HCl. The samples were centrifuged at 7500*g* for 15 min at 16 °C, and the nitrogen content was determined in the supernatant. With these values the percent solubility was calculated as the percent distribution of protein in the soluble and insoluble fractions.

Surface Tension Measurements. Surface tension measurements were used to determine protein adsorption at the air−water interface. The surface activity was expressed by the surface pressure, $\pi = \sigma_o - \sigma$, where σ_o and σ are the aqueous subphase surface tension and the surface tension of the aqueous solutions of protein, respectively. Measurements were performed with a Sigma 701 digital tensiometer (KSV, Finland), based on the Wilhelmy method, with a roughened platinum plate, as described elsewhere (*42*). True equilibrium adsorption does not seem to be possible with proteins, even after 2 or 3 days (*42*). Therefore, we considered the surface pressure measured after 24 h as the pseudoequilibrium value. Surface tension measurements were reproducible within ± 0.5 mN/m.

RESULTS AND DISCUSSION

Chemical Properties of Sunflower Protein Isolate and Hydrolysates. The purification of proteins by extraction in a basic medium and further precipitation at its pI is the habitual way to obtain protein isolate (*43*, *44*). Proteins represent the main component of sunflower protein isolate (**Table 1**). A high protein content is essential in order to use this byproduct for fortification of foods (*4*, *45*, *46*) or for the production of protein hydrolysates (*15*). Soluble sugars represent a minor component. Reduced sugar content is an important goal during the production of protein products because sugars can have adverse effects on the functional properties of proteins, on the bioavailability of some amino acids (*47*), and can cause flatulence (although is not a case of sunflower protein isolate) during the ingestion of some foods (*48*). Soluble sugars also have a significant effect on the interfacial properties of protein-adsorbed films at fluid interfaces (*9*, *49*, *50*). Other minor components are polyphenols, which may be beneficial because they are strong antioxidants (*51*).

In **Table 2** we can see that protein concentration in the isolate and in protein hydrolysates is higher than in the original sunflower meal. However, the protein concentration decreases as the degree of hydrolysis increases. Thus, the protein concentration is lower in the extensive protein hydrolysates than in the original SPI. The decrease of the protein concentration in the SP hydrolysates with a higher degree of hydrolysis was due to, on the one hand, salt content increasing during the hydrolysis with Alcalase for the addition of NaOH to maintain the optimum pH of this enzyme and subsequent addition of HCl in the final step of the hydrolytic process for the inactivation of the proteases at acid pH and, on the other hand, the ash content (>80%) showed in the commercial crude extract used during the second hydrolysis with Flavorzyme.

The modified protein, expressed as the differences observed in percent of solubility between the sunflower protein hydrolysates and the protein isolate in the isoelectric point, also increases with the degree of hydrolysis due to the action of the enzyme on native sunflower proteins.

Amino Acid Composition. The products of the different hydrolyses performed present different molecular size and amino acid composition (**Figure 1**, **Table 3**). Similar results were also reported by Parrado et al. (*52*) and Villanueva et al. (*37*). At higher degrees of hydrolysis (**Figure 1**, parts **C** and **D**), the protein fraction with lower molecular mass increases in relation to the molecular masses of the original protein isolate (**Figure 1A**). However, minor differences are observed in the amino acid composition between the protein isolate and protein hydrolysates derived from the treatment with Alcalase and/Flavorzyme (**Table 3**), which is a characteristic of the hydrolytic treatment used in this work, which maintains intact the quality of the original proteins.

The amino acid composition of SPI and SP hydrolysates satisfied the FAO requirements for the essential amino acids (*53*) except for lysine (**Table 3**). The low level of lysine is typical for SP hydrolysates (*37*, *52*). Although this amino acid is considered as an indication of the nutritional quality of a protein, the consideration of its concentration alone is insufficient to evaluate the nutritional value of a protein. A low level of methionine is also observed in SPI and SP hydrolysate with a 5.62% degree of hydrolysis. But, the level of this amino acid increases for high degrees of hydrolysis (at 23.5 and 46.3%). The hydrolysis also reduces slightly the level of phenylalanine of the original SPI.

Solubility. Solubility is one of the most important characteristics of proteins, not only by itself, but also for its influence on other functional properties (especially those related with foaming and emulsifying capacity). The solubility of sunflower protein isolate is high at basic pH, and gradually decreases with decreasing pH values up to very low solubility at pH lower than 6 (**Figure 2**). However, solubility was unusually low in the range of pH below 3. A possible explanation could be that the SPI was obtained in a plant pilot where the extraction and dried conditions of the product could involve a higher denaturation, and consequently insolubilization, of sunflower proteins at acid pH. Although protein is the main component in the protein isolate, the heterogeneous nature of the isolate (**Table 1**) may facilitate interactions between proteins and other components that can modify the net charge and hydrophobicity of proteins affecting protein solubility (*17*). The strong pH dependence of the solubility and the associated functional properties (*2*) mean that the optimum functionality of sunflower protein isolate occurs at pH > 8, which limits their application as food ingredients. Thus, in this respect the hydrolysis of the SPI would improve the solubility and the use of sunflower proteins in food formulations.

The solubility increases with the degree of hydrolysis (**Figure 2**). In this sense the solubility is maximum (solubility ≈ 90%)

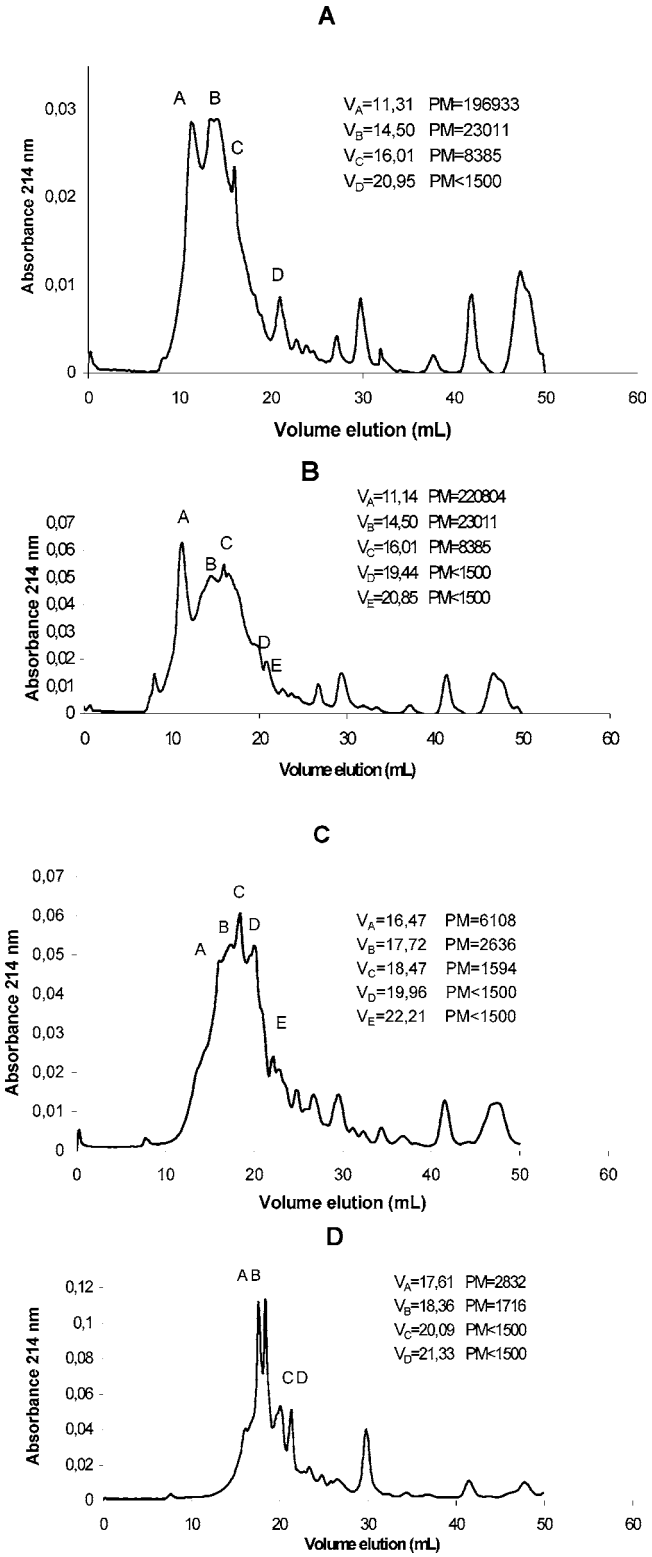

Figure 1. Gel filtration chromatographs of sunflower protein isolate (**A**) and protein hydrolysates at 5.62 (**B**), 23.2 (**C**), and 46.4 (**D**) degrees of hydrolysis.

for degrees of hydrolysis higher than 23.5%, over the overall range of pH studied. However, at the lower degree of hydrolysis (at DH = 5.62%) the solubility is pH dependent, with the maximum solubility at pH > 8, but this solubility decreases at lower pH and tends to a plateau at pH < 5 with a solubility of about 40%. As expected (*37*), the greatest increase in solubility was observed around the isoelectric point of the original SPI and at the higher degrees of hydrolysis. From these results we

Table 3. Amino Acid Composition of Sunflower Protein Isolate and Protein Hydrolysates from Sunflower Protein Isolate at Different Degrees of Hydrolysis[a]

amino acid	protein isolate	hydrolysate 5.62%	hydrolysate 23.5%	hydrolysate 46.3%	FAO (1985)
aspartic acid [b]	10.3	13.8	11.9	12.1	
glutamic acid[c]	26.3	29.1	21.6	24.2	
serine	6.1	5.7	5.3	5.7	
histidine	3.2	2.7	2.6	2.8	1.9
glycine	7.3	6.6	7.2	6.6	
threonine	5.0	4.2	4.7	4.6	3.4
arginine	11.4	11.2	11.5	11.4	
alanine	5.6	5.2	5.3	5.2	
proline	6.7	6.1	7.0	5.9	
tyrosine	2.5	2.2	2.4	2.5	
valine	5.9	5.3	5.7	6.2	3.5
methionine	0.6	1.1	6.6	4.7	2.5[d]
cysteine	0.6	0.3	0.8	0.2	
isoleucine	5.5	4.5	4.5	5.0	
tryptophan	0.5	1.4	2.0	1.9	
leucine	8.6	7.6	8.0	7.9	6.6
phenylalanine	6.7	5.9	5.9	6.0	6.3[e]
lysine	3.7	3.0	3.3	3.3	5.8

[a] Data (g of amino acid/100 g of protein) are the average of three determinations. [b] Aspartic acid + aspargine. [c] Glutamic acid + glutamine. [d] Methionine + cysteine. [e] Phenylalanine + tyrosine.

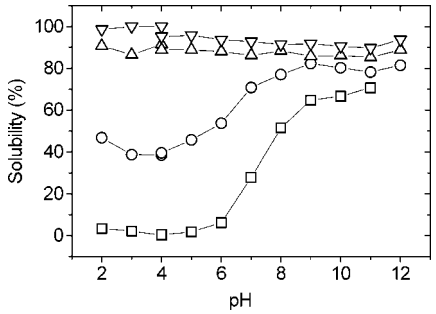

Figure 2. Solubility at different pH values of sunflower protein isolate (□) and protein hydrolysates at 5.62 (O), 23.2 (Δ), and 46.4 (▽) degrees of hydrolysis.

can deduce that the solubility increases (**Figure 2**) as the protein fraction with lower molecular mass increases (**Figure 1**) at higher degrees of hydrolysis. These two phenomena may have different effects on the formation and stability of food dispersions (emulsions and foams). In fact, the reduction of molecular masses in protein hydrolysates might promote foam and emulsion formation due to the faster diffusion of molecules to fluid interfaces (air−water and oil−water, respectively) (*9*, *12*, *49*, *50*). On the other hand, peptides formed during hydrolysis may be too small, especially at higher degrees of hydrolysis (**Figure 1**), to stabilize fluid interfaces, which is essential for the stability of the dispersed system (*2*, *8*, *38*). Clearly, it is necessary to counterbalance the positive effect of the degree of hydrolysis on the solubility and protein fractions with lower molecular mass and the negative effect of lower protein−protein interactions in protein fractions with lower molecular masses necessary for the stabilization of the interfacial film. Thus, knowledge of the effect of hydrolysis on the main physicochemical properties of the interfacial film (adsorption, structure, stability, dynamic phenomena, mechanical properties, etc.) and their relation to the formation and stability of food dispersions (emulsions and foams) is essential for the use of these proteins in traditional or new (functional foods) food formulations.

Adsorption of Sunflower Proteins at the Air−Water Interface at Equilibrium. The equilibrium properties of

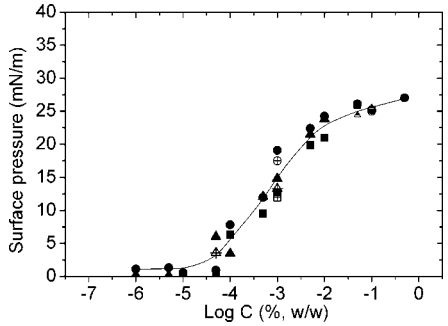

Figure 3. Concentration dependence on surface pressure (surface pressure isotherm) for sunflower protein isolate adsorbed films on the air–water interface. C is the protein concentration in the sunflower protein isolate in the bulk phase. Temperature, 20 °C; pH 7. The different symbols are for different measurements with new protein solutions.

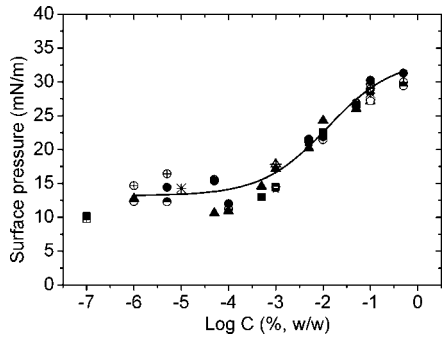

Figure 4. Concentration dependence on surface pressure (surface pressure isotherm) for protein hydrolysates from sunflower protein isolate adsorbed films on the air–water interface. Degree of hydrolysis, 5.62%. C is the protein concentration in the hydrolysate from SPI at DH = 5.62% in the bulk phase. Temperature, 20 °C; pH 7. The different symbols are for different measurements with new protein solutions.

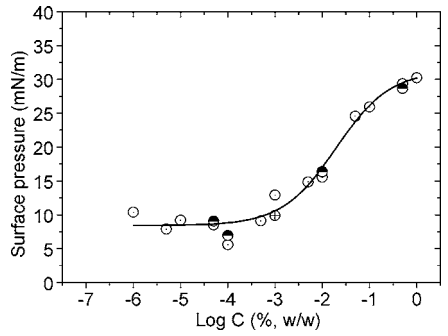

Figure 5. Concentration dependence on surface pressure (surface pressure isotherm) for protein hydrolysates from sunflower protein isolate adsorbed films on the air–water interface. Degree of hydrolysis, 23.3%. C is the protein concentration in the hydrolysate from SPI at DH = 23.3% in the bulk phase. Temperature, 20 °C; pH 7. Different symbols are for different measurements with new protein solutions.

adsorbed sunflower protein fractions (protein isolate and hydrolysates) are of utility for the interpretation of structural and dynamic properties (*9*, *12*−*14*) of the studied proteins at fluid interfaces.

The protein concentration dependence on surface pressure (surface pressure isotherm) for sunflower protein isolate (**Figure 3**) and hydrolysates at 5.62% (**Figure 4**), 23.3% (**Figure 5**), and 46.3% (**Figure 6**) degrees of hydrolysis on water at 20 °C and at pH 7 showed a sigmoidal behavior. The same protein concentration dependence on surface pressure was observed for milk (*42*) and soy (*54*) proteins. For sunflower protein isolate

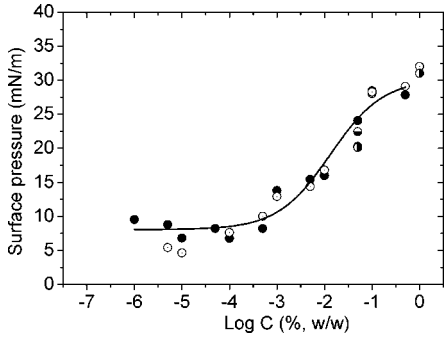

Figure 6. Concentration dependence on surface pressure (surface pressure isotherm) for protein hydrolysates from sunflower protein isolate adsorbed films on the air–water interface. Degree of hydrolysis, 46.4%. C is the protein concentration in the hydrolysate from SPI at DH = 46.4% in the bulk phase. Temperature, 20 °C; pH 7. The different symbols are for different measurements with new protein solutions.

(**Figure 3**) at low protein concentrations, the initial solutions caused only a small increase in the surface pressure. The surface pressure increased with protein concentration and tended to a plateau at the higher protein concentrations. The behavior of adsorbed protein films (**Figure 3**) can be interpreted in terms of monolayer coverage. At the lower protein concentrations, as the surface pressure is close to zero, the adsorbed protein residues may be considered as a two-dimensional ideal gas. Proteins at higher concentrations, but lower than that of the plateau, form a monolayer of irreversibly adsorbed molecules. As the plateau is attained, the monolayer is saturated by protein that is irreversibly adsorbed (*55*).

However, the results for the SP hydrolysates are unexpected but reproducible because at the lower protein concentrations in solution the surface pressure is higher than zero (π > 10 mN/m). This behavior may be associated with the formation of small peptides with an amphiphilic character during the hydrolysis of SPI (**Table 2**). In fact, the surface pressure isotherm for SPI is typical of milk and soy proteins (*12*, *56*, *57*) with a surface pressure close to zero at a protein concentration in solution in the range of 1×10^{-6} to 1×10^{-5} %, p/v. In this context, SP hydrolysates adsorbed at the air−water interface behave as low molecular weight emulsifiers (LMWE) giving measurable surface pressures at low LMWE concentrations in aqueous solutions (*58*). Although the SPI have fractions with low molecular masses such as the hydrolysates (**Figure 1**), the absence of this phenomenon in SPI may be due to the different hydrophilic character of the amino acids in the native SPI and those generated during the hydrolysis process. Interestingly, the major differences between amino acid composition of SPI and the 5.6% hydrolysate, with a very similar polypeptide composition, correspond to hydrophobic amino acids (Met and Trp). Thus, the system may be analyzed as competitive adsorption between different polypeptides, modulated by the bulk concentration of each one, that changes with the degree of hydrolysis. Clearly, the isolation and analysis of the amino acid composition of peptide derivatives generated from the hydrolysis of a native protein (**Figure 1**) could give an insight at a molecular level on the surface activity of protein hydrolysates as a function of the degree of hydrolysis. That is, the production of "tailor-made hydrolysates" with specific functional properties requires further structure−function relationships at a molecular level. This work is underway at present.

The highest protein concentration at which the plateau is attained is the adsorption efficiency (AE). At higher protein concentrations, the protein molecules may form multilayers

Table 4. Adsorption Efficiency (AE) and Superficial Activity (SA) of Sunflower Protein Isolate and Protein Hydrolysates from Sunflower Protein Isolate at Different Degrees of Hydrolysis[a]

	AE (% wt/wt)	SA (mN/m)
sunflower protein isolate	>0.5	>26.9
hydrolysate, DH = 5.62	0.1	30.5
hydrolysate, DH = 23.5	1.0	30.5
hydrolysate, DH = 46.3	0.5	28.5
β-casein	0.15	23.6
WPI	2.5	26.6
7S	0.1	26.4
11S	0.1	21.0

[a] Milk and soy globulins are included as reference (*58*). Temperature, 20 °C; pH 7; I = 0.05 M.

beneath the primary monolayer, but these structures do not contribute significantly to surface pressure (*55*). The maximum surface pressure at the plateau is the superficial activity (SA).

The results in **Table 4** show that the superficial activity (SA) is lower for SPI than for the hydrolysates, and decreases at the highest degree of hydrolysis. The adsorption efficiency (AE) is also affected by the degree of hydrolysis, but in a complicated manner. The maximum value of AE was observed for the hydrolysate with a DH = 23.5%, but the opposite was observed for a DG = 5.62%. For comparison purposes, in **Table 4** we have included the results of the adsorption efficiency and superficial activity for some selected milk and soy globulins (*57*). Some differences exist between milk and soy proteins at neutral pH. The superficial activity (SA) is almost identical for WPI, and 7S, but lower for β-casein and 11S soy globulin. At this pH value the surface activity of SPI and its hydrolysates is higher than that for milk and soy proteins.

The relationship between the equilibrium properties of protein films at fluid interfaces and the stability of the food dispersion (emulsion or foam) has been recently established for sodium caseinate (*59*). However, different mechanisms are involved in the dispersion formation and stability, where the equilibrium properties of the adsorbed film play a complicated role (*18*, *60*−*63*). Clearly, for emulsion and foam formation the dynamic properties (rate of protein adsorption and surface rheological properties) of adsorbed protein layers play a larger role than the equilibrium properties. This work is underway at present for sunflower protein isolate and its hydrolysates.

Conclusions. In this paper we have analyzed the production of SPI and SP hydrolysates and their chemical and amino acid compositions, solubility, and the surface activity at equilibrium of adsorbed films of SPI and SP hydrolysates at the air−water interface. The degree of hydrolysis has an effect on solubility, composition, and content of the amino acids of the hydrolysate. The problem of low solubility of SPI in acidic aqueous solutions is overcome for its hydrolysates. The solubility of SP hydrolysates increases with the degree of hydrolysis. The SPI present a high protein content which is important from a nutritional point of view (for fortification of foods) and as a raw material for the production of protein hydrolysates. The SPI and SP hydrolysates have a low content of soluble sugars, which is important for food formulations. The amino acid composition of SPI and SP hydrolysates satisfies the FAO requirements for the essential amino acids except for lysine. The adsorption isotherms present the typical sigmoidal shape, giving a saturated monolayer at the higher protein concentration in the bulk phase, and at this protein concentration the surface pressure tended to a plateau. The superficial activity (the maximum surface pressure at the plateau) is lower for the protein isolate than for the hydrolysates and decreases at the highest degree of hydrolysis. The adsorption efficiency (the protein concentration at which the plateau is attained) is also affected by the degree of hydrolysis. In summary, enzymatic hydrolysis can be used to improve functional and nutritional properties of underutilized SPI in food formulations.

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