



SURFACE PROPERTIES OF MAIZE, FISH AND BOVINE SERUM PROTEIN HYDROLYSATES

PROPIEDADES SUPERFICIALES DE HIDROLIZADOS PROTEÍNICOS DE MAÍZ, PESCADO Y SUERO DE BOVINO

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Abstract

The surface properties of commercial protein hydrolysates from fish (FPH), bovine serum (BSPH), maize by acid hydrolysis (MPHA) and maize by enzymatic hydrolysis (MPHE) were evaluated. The emulsifying activity (EA), stability (ES), and capacity (EC); foaming capacity (FC) and stability (FS); fat holding capacity (FHC); and solubility (S) were determined. Electric conductivity was used for evaluating the emulsifying properties. Average molecular weights were determined by SDS-PAGE. FC was determined by measuring the percentage increase in volume of the hydrolysates solutions upon stirring, whilst FS was determined by measuring the remaining foam volume after a given period of time. MPHA displayed the best EA ($255 \mu\text{S}$) ($p < 0.05$); MPHE showed the best ES (49.14 min) ($p < 0.05$); MPHA and MPHE exhibited the highest FHC values (6.7 mL/g); and MPHE had the highest FC (62.5%) ($p < 0.05$). FPH displayed the highest EC (340 g oil/g protein) ($p < 0.05$). Highest FS was shown by MPHA. In general, the best overall properties were displayed by the maize hydrolysates.

Keywords: protein hydrolysates, hydrolysis degree, emulsifying activity, foaming activity, fat retention capability.

Resumen

Se evaluaron las propiedades de superficie de hidrolizados proteínicos comerciales de pescado (FPH), suero de bovino (BSPH), maíz por hidrólisis ácida (MPHA) y maíz por hidrólisis enzimática (MPHE). Las propiedades determinadas fueron: actividad (EA), estabilidad (ES) y capacidad emulsificante (EC); capacidad (FC) y estabilidad (FS) espumante; capacidad de retención de grasa (FHC); y solubilidad (S). Las propiedades emulsificantes se evaluaron por conductividad eléctrica. Los pesos moleculares promedio fueron determinados por electroforesis SDS-PAGE. La FC se calculó midiendo el porcentaje de incremento en el volumen al agitar las soluciones con los hidrolizados. La FS, por medio del volumen remanente de la espuma en el tiempo. MPHA desarrolló la mejor EA ($255 \mu\text{S}$) ($p < 0.05$); MPHE tuvo la mejor ES (49.14 min) ($p < 0.05$); MPHA y MPHE mostraron los mayores valores de FHC (ambos con 6.7 mL/g) y el MPHE la mayor FC (62.5%) ($p < 0.05$). FPH tuvo la mayor EC (340 g aceite/g proteína) ($p < 0.05$). La mayor FS fue para MPHA. En general puede decirse que las mejores propiedades fueron exhibidas por los hidrolizados de maíz.

Palabras clave: hidrolizados proteínicos, grado de hidrólisis, actividad emulsificante, actividad espumante, retención de grasa.

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1 Introduction

Protein hydrolysates have an upmost importance in the food industry as functional ingredients (Martínez *et al.*, 2009), and they represent an option as source of nitrogen in the preparation of diets for enteral products, hypoallergenic formulas and dietetic beverages (Adler-Nissen, 1986; Mahmoud and Cordle, 2000; Chabanon *et al.*, 2007).

Protein hydrolysis and the control in the degree of hydrolysis are carried out because of several reasons, including the improvement of nutritional properties and texture of foods, increasing or decreasing the protein solubility, achieving better emulsifying and foaming properties, reducing or eliminating disagreeable off-flavours and odours, removing toxic ingredients or anti-nutritional factors and for contributing to texture build-up in manufactured foods (Lahl and Braun, 1994; Pedersen, 1994; Jamdar *et al.*, 2010). Criteria for adequately selecting protein hydrolysates include their nutritional value, cost, flavour, solubility and functionality.

Most protein hydrolysates possess the ability to reduce the interfacial tension between phases, and thus, may be able to form and stabilize emulsions and foams (McClements, 1999; Miñones and Rodríguez-Patiño, 2007). The proteins, at the same time as they decrease the interfacial tension, can form a continuous film at the interface through intermolecular interactions that provide structural rigidity (Wilde, 2000).

It is known that low molecular weight emulsifiers achieve a better interface covering than the intact proteins, because they can diffuse more rapidly to the interface, providing more stability (Dickinson, 2001; Wilde, 2000). Thus, the surface properties of protein hydrolysates could be improved as their molecular weight diminishes due to higher diffusion rate to and increased rate of adsorption at the interface than intact proteins (Caessens *et al.*, 1999).

Protein hydrolysates of vegetable origin have been increasingly used as an alternative for protein hydrolysates of animal origin in food products, so that it is of the utmost importance to acquire information regarding the functional and physicochemical properties of these compounds, to be able to use them effectively in the design of new food products and for improving existing ones (Miñones and Rodríguez-Patiño, 2007).

Protein hydrolysates can be obtained by acidic or enzymatic methods, and can be classified in two groups, depending on their degree of hydrolysis (Pedroche *et al.*, 2004): (i) hydrolysates with low degree of hydrolysis (between 1 and 10%), which are characterized for having improved functional properties (mainly emulsifying and foaming properties), and (ii) hydrolysates undergoing extensive hydrolysis (> 10%), that are usually used as nutritional supplements and for developing enteral formulas (Pedroche *et al.*, 2004). The functionality of hydrolysates is tied to the nature and composition of peptides generated during hydrolysis (Chabanon *et al.*, 2007).

Given that knowledge of their functional properties is fundamental for their efficient application in food products, the aim of this work was to study and compare the surface properties of four commercial protein hydrolysates from: bovine serum (BSPH), fish (FPH), maize by acid hydrolysis (MPHA), and maize by enzymatic hydrolysis (MPHE).

2 Materials and methods

2.1 Materials

Commercial food grade protein hydrolysates of fish (Saria Industries Morbihan, Cedes, France, protein content = 79.05 %); spray dried beef plasma (Proliant B6302, Ingredientes Funcionales de México, S.A. de C.V., Mexico, protein content = 69.50 %), and of maize by acid and enzymatic hydrolysis (Complementos Alimenticios S. A. de C.V., Mexico, protein content of 40.58% and 34.93 %, respectively) were purchased.

2.2 Degree of hydrolysis

Degree of hydrolysis (DH) is defined as the percentage of free amino groups cleaved from protein, which was calculated from ratio of α -amino nitrogen (AN) and total nitrogen (TN) ratio. The AN was determined by a modified formol titration method (Ninsang *et al.*, 2005). Ten mg of sample was added with an equal amount of distilled water. The mixture was adjusted to pH 7.0 using 0.1N NaOH. Then 10 mL of 38% (v/v) formaldehyde solution was added into the mixture and titration was continued to the end point at pH 9.5 with 0.2N standard NaOH solution. TN was determined by Kjeldahl method (AOAC, 2000).

2.3 Electrophoresis

Average molecular weight of the protein hydrolysates was determined as reported by Laemmli (1970), with the modifications introduced by Petruccelli and Añón (1994). Sodium dodecyl sulphate-polyacrylamide gels electrophoresis (SDS-PAGE) was used and runs were done in gel mini-slabs (Mini Protean II Model, Bio-Rad Laboratories, Hercules, CA, USA). Samples were prepared by re-suspending the trichloroacetic acid (TCA) precipitates of each isolate in sample buffer. The soluble fraction obtained at pH 4.5 was precipitated with TCA (volume ratio 18.8% TCA solution:aliquot 1:0.5, final concentration of TCA 12.5 %) and analysed by SDS-PAGE with β -mercaptoethanol. The molecular weight of polypeptides was calculated using the MW-Precision Plus Protein Standard 10-250 kDa (BIO-RAD Laboratories Inc., USA).

2.4 Hydrolysates solubility

Protein hydrolysates solubility was determined as indicated by Morr *et al.* (1985), with slight modifications. About 500 mg of dry protein hydrolysate were accurately weighed into separate 150 mL standard beakers and several aliquots of 0.1 M NaCl were added with stirring to form a smooth paste. Additional, 0.1 M NaCl solution was then added to bring the total volume of the dispersion to about 40 mL. The beaker contents were stirred with a magnetic stirrer at a rate that just failed to form a vortex. The pH of the dispersion was immediately determined and adjusted to pH 3.0 or 7.0 using 0.1N HCl or NaOH solution. The dispersion was stirred for a total of 1 h under these conditions and the pH was intermittently monitored and maintained at the prescribed value throughout the stirring period. The dispersion was then transferred into a 50 mL volumetric flask, diluted to the mark with additional 0.1 M NaCl solution and mixed by inverting and swirling. An aliquot of the dispersion was centrifuged 30 min at 20,000 \times g and the resulting supernatant fraction was filtered through Whatman No. 1 filter paper. The protein content of the filtrate was determined using the biuret reaction (Gornall, 1949). The solubility of

the protein hydrolysates was calculated as:

$$\text{Protein hydrolysate solubility (\%)} =$$

$$\left(\frac{(\text{Supernatant protein concentration}) \left(\frac{\text{mg}}{\text{mL}} \right) (50)}{(\text{Sample weight (mg)}) \left(\frac{\text{Sample protein content (\%)}}{100} \right)} \right) (100) \quad (1)$$

Analysis was performed by triplicate.

2.5 Emulsifying activity and stability

The specific electric conductance method of Kato *et al.* (1985) was followed for determining the emulsifying activity (EA) and stability (ES). Protein hydrolysates solutions (90 mL at 0.1 % w/v) and 30 mL of sunflower oil were poured into a 250 mL beaker. The mixture was homogenized with a high shear homogenizer (Poly-Tron PT MR 2100, Kinematica, Switzerland) at 12,000 rpm during 2 min at 20°C. Temperature was controlled by means of a Brookfield TC 500 recirculation water bath (Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA). The conductivity of the emulsion was measured with a conductivity meter (YSI, model 33, Simpson Electric Co., USA) for a period of 10 min. The pH of the protein hydrolysate solution was adjusted previously to 7.0 with 0.1N NaOH.

The EA was determined by measuring the conductivity difference between that of the protein hydrolysate solution and the minimum electric conductivity displayed by the emulsion during one min. The emulsifying stability was determined from the conductivity curves initial gradient after emulsion formation. The ES is defined by the following equation:

$$ES = (C_s - C_e) \left(\frac{\Delta t}{\Delta C} \right) \quad (2)$$

where C_s is the electric conductivity of the protein hydrolysate solution, C_e is the minimum conductivity of the emulsion, and $\frac{\Delta t}{\Delta C}$ is the reciprocal of the initial slope of the conductivity curves (Kato *et al.*, 1985). All the experiments were performed in triplicate.

2.6 Emulsifying capacity

The method of Linder *et al.* (1996) was used for determining the emulsifying capacity (EC). The conductivity of the emulsion was monitored

continuously during the homogenization of a 0.05% (w/v) protein hydrolysate solution to which 16.5g/mL of sunflower oil was poured at a constant rate. The samples were maintained in an ice bath at $20\pm2^\circ\text{C}$, stopping the sunflower oil addition at the moment that phase inversion occurred, confirmed by a sharp decrease in conductivity. Results were expressed as g of emulsified sunflower oil per g of protein hydrolysate. Experiments were done in triplicate.

2.7 Fat holding capacity

The fat holding capacity (FHC) was determined using the method of Hordur *et al.* (2000) with some modifications. A sample of protein hydrolysate (500 mg) was put into a centrifuge tube and added 10 mL of sunflower oil. The mixture was blended with a spatula every 10 min during 30 min at room temperature. The mixture was then centrifuged (IEC 20a centrifuge, Damon7 EC Division, USA) during 25 min at 3800 rpm. Fat absorption was determined by decanting the supernatant oil and reporting the remaining oil by weight difference. The analysis was done in triplicate and the result was reported as mL of absorbed fat per g of protein hydrolysate.

2.8 Foaming capacity and stability

Foaming capacity (FC) and stability (FS) were determined by the method reported by Liceaga-Gesualdo and Li-Chan (1999) with slight modifications. A sample of each protein hydrolysate (3 g) was dispersed in 100 mL of distilled water, and whipped in a Waring Variable

Speed Laboratory Blender (model LB10S, Waring Products, Inc., New Hartford, CT, USA) operated at 3000 rpm for 1 min. The mixture was transferred to a 250 mL graduated cylinder and the volume occupied by the foam was registered 0, 1, 5, 10, 20, 30, 40 and 60 min. The FC was expressed as the % volume increase and the FS as the remaining foam volume after a given time.

2.9 Statistical analysis

Treatments were arranged in a completely randomized design. Data were analysed by analysis of variance and significant differences between treatments determined by Tukey's test at $p = 0.05$ using the NCSS version 5 statistical software (Wireframe Graphics, Kaysville, UT). All experiments were done in triplicate.

3 Results and discussion

3.1 Degree of hydrolysis, average molecular weight, and solubility

The degree of hydrolysis (DH), average molecular weight and the solubility of the different commercial protein hydrolysates are given in Table 1. Protein hydrolysates are made up by a mixture of polypeptides, so that it is convenient to report their average molecular weight (AMW). The highest AMW was displayed by BSPH (143.831 kDa), followed in descending order by MPHA (128.761 kDa), MPHE (121.691 kDa), and FPH (75.190 kDa) (electrophoretic patterns not shown).

Table 1. Protein content, hydrolysis degree, average molecular weight, and solubility of the hydrolysates.

Protein hydrolysate code	Hydrolysis degree (%)	Average molecular weight (kDa)	Solubility (%) pH 3.0	Solubility (%) pH 7.0
BSPH	4.50 ± 0.5^a	143.830 ± 0.4^d	10.24 ± 0.07^c	25.80 ± 0.5^c
FPH	9.89 ± 0.7^b	75.190 ± 0.9^a	5.28 ± 0.03^b	7.13 ± 0.03^b
MPHA	47.65 ± 0.3^d	121.691 ± 0.8^b	0.82 ± 0.18^a	0.87 ± 0.05^a
MPHE	41.39 ± 0.7^c	128.761 ± 0.5^c	0.63 ± 0.16^a	0.82 ± 0.01^a

The results are significant with a significance level of 95% ($p < 0.05$). Values reported are mean values of at least three replicates \pm standard deviations. Mean values follow by same superscript letter are not significantly different from each other.

BSPH = bovine serum protein hydrolysate; FPH = fish protein hydrolysate; MPHA = maize protein hydrolysate obtained by acid hydrolysis; MPHE = maize protein hydrolysate obtained by enzymatic hydrolysis.

The hydrolysates of protein animal origin had low DH (4.50% for BSPH and 9.89% for FPH) compared to that of vegetable origin (41.39% for MPHA and 47.65% for MPHE) which suffered a high DH. The lower the DH of the protein hydrolysates, higher was the protein content. In general, the higher the protein content of the protein hydrolysates, the lower was their solubility. Additionally, solubility of all of the protein hydrolysates increased as pH was increased from 3.0 to 7.0. This phenomenon is in agreement with findings reported by Mahmoud (1994), who found that as pH tended to the isoelectric point, proteins exhibited a lower capacity to solubilise in water, because their net charge is close to zero, and protein-protein interactions are favoured over protein-water interactions. As pH moved farther away from the isoelectric point, more protein functional groups are ionized, and protein-water interactions are favoured over protein-protein interactions. This phenomenon is more clearly noticed in BSPH and FPH. Kristinsson and Rasco (2000) reported that hydrolysis of fish proteins increased their solubility, but that more important than the DH, was the balance existing between hydrophilic and hydrophobic forces. The relatively low solubility shown by the vegetable protein hydrolysates might be a consequence of the extended DH that they suffered. It has been reported that the solubility of whey protein decreased with an increase of DH. This effect was noticeable even when the degree of hydrolysis was increased by only 1 % (Forstrom *et al.*, 2004). However, this trend cannot be generalized, because it depends on the type of protein being studied. For example, the solubility of soybean protein hydrolysates increased with increasing in DH (Martínez *et al.*, 2009).

3.2 Emulsifying activity and stability

An oil-in-water emulsion (O/W) is an oil, water and emulsifier system in which oil droplets are suspended in the water. The emulsifier adsorbs (i.e. protein hydrolysates) at the oil-water interface to reduce the interfacial tension (hence to prolong the life time of this metastable system). Surface charge arises from the dissociation of the protein hydrolysates ionic groups, and strong lateral interactions between the proteins amino acid residues may surge that result in the formation of a structured interfacial layer, whose

mechanical properties will greatly determine the emulsion stability against droplet coalescence (Dickinson and McClements, 1996). The electrical conductivity in O/W emulsions depends on the degree of dispersion. O/W emulsions having fine droplet size will exhibit low resistance, i.e. high electrical conductivity, but if resistance increases, it is a sign of droplet aggregation and instability (PDFTop, 2010). Table 2 shows the emulsifying activity and stability data for the O/W emulsions formed with the different protein hydrolysates. The highest emulsifying activity was shown by MPHA (255.0 μ S) followed in descending order by MPHE (180.0 μ S) > FPH (98.0 μ S) > BSPH (97.5 μ S). Thus, it is evident that the protein hydrolysates of vegetable origin (MPHA and MPHE) had a greater emulsifying activity than their animal origin counterparts (FPH and BSPH). MPHA formed the finer emulsion, while BSPH and FPH formed the coarsest emulsions.

Regarding the emulsifying stability, the bigger the conductivity difference the lower the stability of the emulsion. A relative stability index can be obtained for an easy comparison of emulsion stabilities having different initial conductivities, by dividing the emulsifying activity by the conductivity gradient (Garti *et al.*, 1981). The greatest emulsifying stability was displayed by the emulsion stabilized with MPHE (49.14 min), followed by MPHA (21.16 min) and FPH (19.50 min), and the less stable emulsion was formed by BSPH (9.75 min). These results indicate that neither the DH nor the AMW of the protein hydrolysates affected emulsion stability, and that it was the nature of the protein hydrolysates that influences this parameter. These findings are in contrast with those of Linder *et al.* (1996) who stated that smaller peptides and amino acid fractions diffused more rapidly to the interface and covered more efficiently the oil droplets surfaces.

Cheftel *et al.* (1989) suggested that protein hydrophobicity contributed to achieve a higher protein concentration at the oil-water interface, lower interfacial tension, and thus to the stability of the emulsion. Our solubility data (Table 1) are in agreement with this point of view, as emulsifying stability was inversely proportional to the protein hydrolysates solubility.

An in depth discussion regarding the effect of the protein hydrolysates on the surface properties so far considered, and those to be considered below, are beyond the scope of this study, as pro-

Table 2. Emulsifying activity, stability and capacity, and fat retention capacity of the different protein hydrolysates.

Protein hydrolysate code	Conductivity of protein hydrolysates solutions (μS)	Initial conductivity of emulsions (μS)	Conductivity gradient ($\mu\text{S}/\text{min}$)	Emulsifying activity (μS)	Emulsifying stability (min)	Emulsifying capacity (g emulsified oil/g protein)	Fat holding capacity (mL/g protein)
BSPH	170.0 ± 3.6 ^a	267.5 ± 0.02 ^a	10.00 ± 0.06 ^c	97.5 ± 1.5 ^a	9.75 ± 0.02 ^a	272.0 ± 2.3 ^b	5.5 ± 0.8 ^a
FPH	202.0 ± 1.4 ^b	300.0 ± 0.08 ^b	5.02 ± 0.04 ^b	98.0 ± 1.1 ^a	19.50 ± 2.08 ^b	340.0 ± 1.2 ^d	4.7 ± 0.5 ^a
MPHA	495.0 ± 4.8 ^c	750.0 ± 0.02 ^d	12.04 ± 2.02 ^c	255.0 ± 6.5 ^c	21.16 ± 1.08 ^b	293.5 ± 1.5 ^c	6.7 ± 0.6 ^b
MPHE	200.0 ± 2.3 ^b	380.0 ± 0.02 ^c	3.66 ± 0.05 ^a	180.0 ± 4.3 ^b	49.14 ± 0.06 ^c	210.0 ± 4.4 ^a	6.7 ± 0.5 ^b

The result are significant with a significance level of 95% ($p < 0.05$). Values reported are mean values of at least three replicates ± standard deviations.

Mean values follow by same superscript letter are not significantly different from each other.

BSPH = bovine serum protein hydrolysate; FPH = fish protein hydrolysate; MPHA = maize protein hydrolysate obtained by acid hydrolysis; MPHE = maize protein hydrolysate obtained by enzymatic hydrolysis.

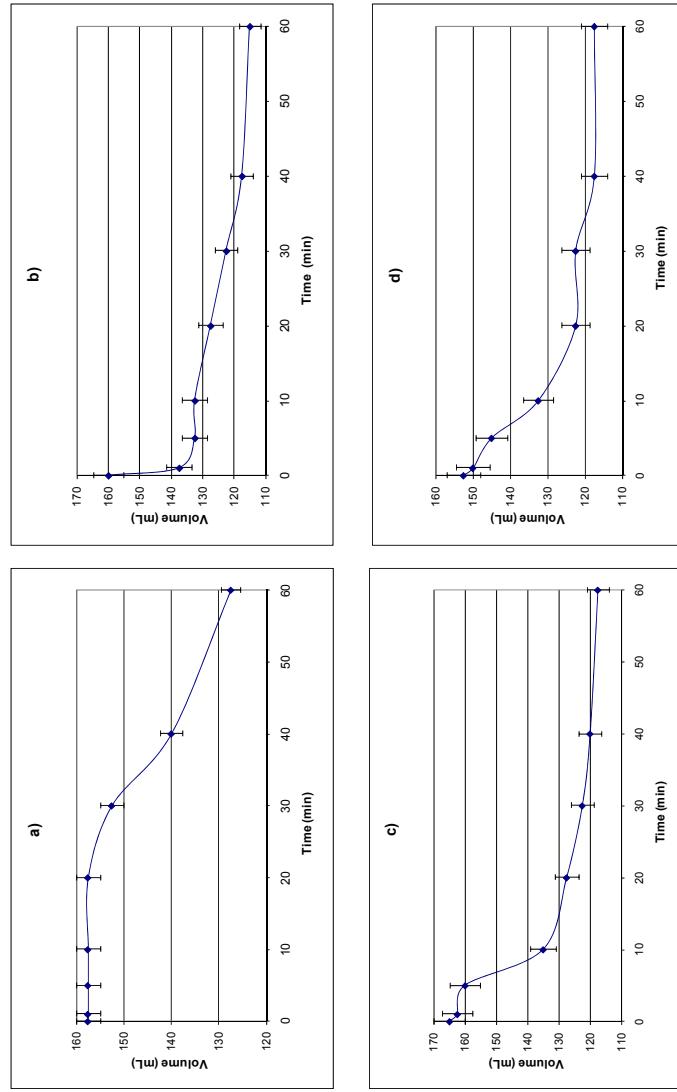


Fig. 1. Remaining foam volume-time curves for foams formed with: a) bovine serum protein hydrolysate (BSPH); b) fish protein hydrolysate (FPH); c) maize protein hydrolysate obtained by enzymatic hydrolysis (MPHE); d) maize protein hydrolysate obtained by acid hydrolysis (MPHA).

teins are polyelectrolytes that occur in a bewildering variety of composition, structure, and properties that physicochemical polymer theory is of limited use for understanding them. The properties ultimately depend on the primary structure of a protein, i.e., what amino acid residues occur and in what sequence. Their most important properties may be their charge, which determines the charge of the protein as a function of pH; and the hydrophobicity, which is of prime importance for conformation and solubility. The conformation is the total three-dimensional folding of the peptide chain, where some levels of structure can be distinguished, i.e., the secondary structure that involves fairly regularly orderings of amino acid residues strongly bonded, and the tertiary structure involves the further folding of the peptide chain, including secondary structure elements (Walstra, 2003).

3.3 Emulsifying capacity

The emulsifying capacity determines the maximum amount of oil that can be emulsified in a given volume of emulsifier solution of known concentration (Swift and Sulzbacher, 1963; Cheftel *et al.*, 1989). The emulsifying capacity of the different protein hydrolysates is given in Table 2, and the values of this parameter from higher to lower were: FPH > MPHA > BSPH > MPHE. The difference in the EC of the protein hydrolysates may be attributed to their rate and extent of unfolding at the oil-water interface, which depends on the flexibility of the protein molecule, i.e., on the strength of the forces maintaining the secondary and tertiary structure. Globular proteins tend to unfold more slowly and less extensively because they have fairly compact structures which are stabilized by disulfide bonds and ordered secondary structure. A large portion of the secondary structure of globular proteins is maintained after adsorption to the interface (Dickinson and McClements, 1996). Thus, we might assume that FPH and MPHA suffered to a larger degree the loss of secondary structure upon adsorption at the oil-water interface, possessing a relative greater ability to cover a larger oil droplet superficial area, than BSPH and MPHE, and hence, their higher EC. This explanation is likely in view of the marked differences in EC found between MPHA and MPHE which were obtained from the same protein source. The enzymatic

modification of proteins has advantages over traditional chemical techniques because milder reaction conditions can be used and proteins can be modified at selected sites (Dickinson and McClements, 1996).

3.4 Fat holding capacity

The ability of protein hydrolysates to absorb fat is an important functionality that influences the taste of the product that is required in various food industries (Kelfala *et al.*, 2010). For instance, the fat holding capacity of proteins is of most importance in the manufacture of meat and milk products. In solid and semi-solid food products where fat is dispersed in a continuous matrix, fat holding properties are influenced by more factors than the interfacial film and the whole structure must be taken into account, as proteins tend to form part of the structure (Mitchell and Ledward, 1986). Suffice here, that proteins that display better fat holding capacity on their own, are likely to contribute to a better fat holding capacity in the structure of foods into which they are incorporated.

The fat holding capacity of the protein hydrolysates is shown in Table 2. The FHC was significantly higher for MPHE and MPHA than for BSPH and FPH. As in the case of EA, neither the DH nor the AMW of the protein hydrolysates affected FHC.

3.5 Foaming capacity and stability

Foams are not simply gas-in-water suspensions. Foams are almost made by beating or whipping, rather than bubbling, and an excess of the disperse phase (the gas) rather than a fixed amount, generally occurs. The prolonged and severe mechanical stresses ensure that some coalescence occurs, with the final product containing a very large volume of gas bubbles distorted into polyhedral shapes. Further differences from an emulsion are that the thin liquid lamellae between these bubbles may contain particulate material and the gas, unlike oil, is soluble in the aqueous phase (Mitchell and Ledward, 1986).

All of the different protein hydrolysates produced considerable volume increases after whipping. However, the foaming stability-time curves (Fig. 1) indicated that they affected differently FS. Fig. 1a shows that BSPH produced

the most stable foam, retaining its volume for about 20 min, followed by a gradual decrease in volume of around 19% in the following 40 min. This result is better than that reported by Liu *et al.* (2010) for protein plasma hydrolysate (DH= 6.2%) which exhibited a foam stability of around 22% after 3 min. The least stable foam was formed by FPH (Fig. 1b) whose volume sharply decreased by about 16 % in the first 2 min, and broke-down almost completely after 1 h of formation. The FS for the commercial FPH was within the range of the results reported by Pacheco-Aguilar *et al.* (2008) for a fish protein hydrolysate (DH= 10%) from Pacific whiting (*Merluccius productus*). MPHE (Fig. 1c) and MPHA (Fig. 1d) also formed unstable foams, whose volume decrease was more gradual than that of the FPH foam, but nevertheless, foam volume maintenance was short lived, and remaining foam volume at the end of 1 h was negligible.

The effect of the different protein hydrolysates on foam capacity and stability cannot be explained on terms of their DH, AMW or solubility (hydrophobicity). Nonetheless, Dickinson and McClements (1996) stated that experiments with globular proteins show that their interiors are densely packed and highly incompressible, almost like solids. Packing constraints sometimes lead to the presence of cavities in proteins interiors, and these are believed to increase the flexibility of protein molecules. Proteins with cavities have smaller effective compressibility and larger effective volumes. The foaming capacity of proteins has been found to increase as their compressibility increases, which are probably because the molecules are more flexible and can unfold more easily at the air-water interface. This may be the case of FPH.

Conclusions

This work provides knowledge regarding the surface properties of commercial protein hydrolysates obtained from bovine serum protein, fish protein and maize protein (by acid and enzymatic hydrolysis). A higher degree of hydrolysis did not resulted in greater solubility. The maize hydrolysates with DH higher than 40% exhibited lower solubility (MPHE: 0.63-0.82 % and MPHA: 0.82-0.87 %) than BSPH with a

DH of 4.50 which displayed a solubility of 10.24-25.80 %. Likewise, solubility was independent of the average molecular weight of the hydrolysates. MPHA showed the highest EA (255 μ S), while MPHE exhibited the highest ES (49.14 min). Both, MPHE and MPHA displayed the highest FHC (6.7 mL of oil/ g protein), and also the highest FC (61.0 - 65.5 %, $p > 0.05$). With this knowledge, improvement of existing food products and the design of new food product with specific desired functional properties may be achieved. None of the protein hydrolysates evaluated was capable of providing the best results in all the surface properties studied, so that selection of the most adequate protein hydrolysate must be done on basis of the specific property wanted to be enhanced in a food product.

References

- Adler-Nissen, J. (1986). *Enzymatic hydrolysis of food proteins*. Elsevier Applied Science Publishers, London, UK.
- AOAC, (2000). "Official Methods of Analysis" 17Th ed., Association of Official Analytical Chemists, Washington, DC.
- Caessens, P., Visser, S., Gruppen, H., and Voragen, A.G.J. (1999). β -lactoglobulin hydrolysis. I. Peptide composition and functional properties of hydrolysates obtained by the action of plasmin, trypsin, and *Staphylococcus aureus* V8 protease. *Journal of Agricultural and Food Chemistry* 47, 2973-2979.
- Chabanon, G., Chevalot, I., Framboisier, X., Chenu, S. and Marc, I. (2007). Hydrolysis of rapeseed protein isolates: Kinetics, characterization and functional properties of hydrolysates. *Process Biochemistry* 42, 1419-1428.
- Cheftel, J.C., Cuq, J.L. and Lorient, D. (1989). *Proteínas alimentarias*, Pp. 115-120. Editorial Acribia, España.
- Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloid & Surfaces B* 20, 197-210.

- Dickinson, E. and McClements, J. (1996). *Advances in food colloids*. Blackie Academic & Professional. London.
- Forstrom, C. K., Vegerud, G., Langsrud, T., Risberg, E. M., and Egeland, B. (2004). Hydrolyzed whey proteins as emulsifiers and stabilizers in high-pressure processed dressing. *Food Hydrocolloids* 18, 757-767.
- Garti, N., Magdasi, S., and Rubinstein, A. (1981). A new method for stability determination of semi-solid emulsions, using conductivity measurements. *Colloids & Surfaces* 3 (3), 221-231.
- Gornall, A.G., Bardawill, C.J., and David, M.M. (1949). Determining serum proteins by means of the biuret reaction. *Journal of Biological Chemistry* 177, 751-766.
- Jamdar, S.N., Rajalakshmi, V., Pednekar, M.D., Juan, F., Yardi, V., and Sharma, A. (2010). Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. *Food Chemistry* 121, 178-184.
- Hordur, G., Kristinsson, B. and Rasco, A. (2000). Fish protein hydrolysates: Production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition* 40 (1), 43-81.
- Kato, A., Fujishige, T., Matsudommi, N. and Kobayashi, K. (1985). Determination of emulsifying properties of some proteins by conductivity measurements. *Journal of Food Science* 50, 56-59.
- Kelfala, M.B., Amadou, I., Foh, B.M., Kamara, M.T., and Xia, W. (2010). Functionality and antioxidant properties of tilapia (*Oreochromis niloticus*) as influenced by the degree of hydrolysis. *International Journal of Molecular Science* 11, 1851-1869.
- Kristinsson, H., and Rasco, B. (2000). Biochemical and functional properties of Atlantic salmon (*Salmo salar*) muscle proteins hydrolyzed with various alkaline proteases. *Journal of Agricultural and Food Chemistry* 48, 657-666.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685
- Lahl, W.J., and Braun, S.D. (1994). Enzymatic production of protein hydrolysates for food use. *Food Technology* 48(10), 68-71.
- Liceaga-Gesualdo, A.M. and Li-Chan, E.C.Y. (1999). Functional properties of fish protein hydrolysate from herring (*Clupea harengus*). *Journal of Food Science* 64, 1000-1004.
- Linder, M., Fanni, J., and Parmentier, M. (1996). Functional properties of veal bone hydrolysates. *Journal of Food Science* 61, 712-720.
- Liu, Q., Kong, B., Xiong, Y.L., and Xia, X. (2010). Antioxidant activity and functional of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. *Food Chemistry* 118, 403-410.
- Mahmoud, M.I. (1994). Physicochemical and functional properties of protein hydrolysates in nutritional products. *Food Technology* 48(10), 89-94.
- Mahmoud, M.I., and Cordle, C.T. (2000). Protein hydrolysates as special nutritional ingredients. In: *Novel macromolecules in food systems* (G. Doxastakis and V. Kiosseoglou, eds.), Pp. 181-215. Elsevier, Amsterdam.
- Martínez, K.D., Carrera, S.C., Rodríguez-Patiño, J.M. and Pilosof, A.M.R. (2009). Interfacial and foaming properties of soy protein and their hydrolysates. *Food Hydrocolloids* 23, 2149-2157.
- McClements, D.J. (1999). *Food emulsions: Principles, practice, and techniques*. CRC Press, Boca Raton, FL.
- Miñones, C.J., and Rodríguez-Patiño, J.M. (2007). Phospholipids and hydrolysates from a sunflower protein isolate adsorbed at the air-water interface. *Food Hydrocolloids* 21, 212-220.
- Mitchell, J.R., and Ledward, D.A. (1986). *Functional properties of food macromolecules*. Elsevier Applied Science Publishers. London.

- Morr, C.V., German, B., Kinsella, J. E., Regenstein, J.M., Van Buren, J.P., Kilara, A., Lewis, B.A, and Mangino, M.E. (1985). A collaborative study to develop a standardized food protein solubility procedure. *Journal of Food Science* 50, 1715-1718.
- Ninsang, S., Lertsiri, S., Suphantharika, M., and Assavanig, A. (2005). Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. *Journal of Food Engineering* 70, 571-578.
- Pacheco-Aguilar, R., Mazorra-Manzano, M.A., and Ramírez-Suárez, J.C. (2008). Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease. *Food Chemistry* 109, 782-789.
- PDFTop (2010). Emulsions.http://www.pdftop.com/ebook/emulsions/perc.ufl.edu/courses/intephen01/_files/06_emulsion.ppt. Accessed August 4, 2010.
- Pedersen, B. (1994). Removing bitterness from protein hydrolysates. *Food Technology* 48(10), 96-99.
- Pedroche, J., Yust, M.M., Lqari, H., Girón-Calle, J., Alaniz, M., and Vioque J. (2004). *Brassica carinata* protein isolates: Chemical composition protein characterization and improvement of functional properties by protein hydrolysis. *Food Chemistry* 88, 337-346.
- Petruccelli, S., and Añón, M.C. (1994). Relationship between the method of obtention and structural and functional properties of soy protein isolates. 1. Structural and hydration properties. *Journal of Agricultural and Food Chemistry* 42, 2161-2169.
- Swift, C. E., and Sulzbacher, W. L. (1963). Factors affecting meat proteins as emulsion stabilizers. *Food Technology* 15, 224-226.
- Walstra, P. (2003). *Physical chemistry of foods*. Marcel Dekker, Inc. New York.
- Wilde, P. (2000). Interfaces: Their role in foam and emulsion behavior. *Current Opinion in Colloid and Interface Science* 5, 176-181.