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Foaming behaviour of enzymatically modified sunflower protein in proximity to *pI*

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ABSTRACT

Improving the foaming properties of sunflower protein could substantially expand its use in a variety of food products. The enzymatic hydrolysis of this protein would promote the increment of surface tension properties in a probed way as we previously studied. The objective of the work was to study the effect of enzymatic treated sunflower protein isolate (SP) on the foaming properties at different pH near the *pI*. Foaming properties were determined in a traditional way by whipping methods and also measured with a rheological approach, to correlate both manner of parameters obtained, as a secondary objective. We studied previously at pH7, that a limited enzymatic treatment substantially enhanced foaming properties of SP. In this study we investigated the effect of hydrolysis of SP on the foaming properties at different pHs surrounding the *pI* of the protein, as a potential use for others food applications. The results showed that solubility was increased as higher were the degree of hydrolysis. This, relates with a higher overrun or drainage rate at all pH studied in a different way, as a result of different protein content. The stability against collapse follows a slightly similar performance by compared with drainage stability due to another mechanism to carry out each phenomenon. The very good correlation between whipping and rheological methods, to study the foaming properties, results in a useful tool to analyze these parameters.

Keywords: *Protein, Hydrolysates, Foam, Foam stability, Rheology*.

1. INTRODUCTION

Between vegetable proteins, most of them have been extensively studied; however, there is a limited application of sunflower protein isolate. The little use has been attributed to phenolic compounds presence that makes food colored and undesirable. Nevertheless, extending the pHs applicability, their use would expand and could be hide in many others formulations in the industrial processing.

The functional properties of proteins surrounding the *pI* depend of the protein structure; in example, the gliadin portion from wheat gluten is poorly soluble near neutral and acidic pH, showing little foamability. Thus, their use in a broad range of food systems can be limited [1,2,3]. Regarding β-lactoglobulin, it was observed a foam capacity increase at high pH. It was attributed to velocity adsorption protein changes at liquid interface with pH increment [4]. It was made heating denaturing treatments on egg proteins [5,6] through pH modifications [7] or organic acids addition [8]. It was observed that pH denaturing effect would promote an increase of foam functionality and rheology properties [9,10].

In this study we investigated the effect of enzymatic hydrolysis of sunflower protein on the foaming properties at different pHs surrounding the *pI* of the protein, as a potential use for food application, expanding its employ in a variety of products. At same time we compared whipping with rheological methods to analyze their foam stability properties.

2. EXPERIMENTAL SECTION

2.1. Materials. Same sunflower protein isolate (SP) as previously described [11], was hydrolysate as before it was declared [12]. The isoelectric point of protein was 4.5. and the pH was 6.9. The degree of hydrolysis (DH), defined as the percentage of peptide bonds cleaved, was calculated from identical earlier procedure according to Adler-Nissen [13]. Protein hydrolysates with degree of hydrolysis (DH) of 1.5%; 7% and 9.8% were obtained. The pI of hydrolysates resulted almost unchanged respect to SP. Only a pI reduction of 0.5 units was observed in the 9.8% DH. As a results pI of samples was 4-4.5. The chemical composition and molecular weight of SP and their hydrolysates can be observed in the Table 1.

Table 1. Molecular Weigh and Chemical Composition of sunflower protein isolate and their hydrolysates.

Sample (Molecular Weigh (kDa))	SP (200,60,45,28,20)	1.5% DH, (60, <6.5)	7%DH, (50,<6.5)	9.8%DH, (45,28,<6.5)
Protein content (%)(± 0.5)	83.8	80.43	81.72	88.24
Soluble sugars (%)(± 0.02)	0.25	0.22	0.22	0.2
Polyphenols, (%)(± 0.1)	0.6	0.4	0.4	0.6
Moisture , (%)(± 0.9)	5.5	5.0	4.95	5.7
(%)Ash (±0.1)	3	5	6	5
(%)Others	6.85	8.95	6.7	0.26

2.2. Electrophoresis. Soy and hydrolyzed proteins were analyzed by PAGE-electrophoresis as was describe elsewhere [11] according to the procedure of Laemmli [14].

2.3. Preparation of solutions. The sunflower protein isolate and the hydrolysates were prepared in distilled water and pH was adjusted to 3, 5 or 6 with 0.1 HCl. To avoid bacterial growth, 0.2g/l sodium azide was added to the solutions.

Protein hydrolysates had different solubility at these pHs. Protein solutions at 3% wt/total wt were prepared and the soluble fraction after centrifugation (4000 x g for 30 min) was used. In the Table 2 can be observed the approximate protein quantity of each sample.

Table 2. Soluble fraction of protein used to make the foams for each sample.

Soluble protein (%) (± 0.9)	SP	1.5%DH	7%DH	9.8%DH
pH 3	19.27	40.21	63.74	67.06
pH 5	20.00	38.61	80.08	86.47
pH 6	20.95	60.32	65.37	72.36

2.4. Solubility. The procedure of Martínez [15] was used. Samples of hydrolysate proteins prepared at 2% w/w were centrifugated at 12,857xg for 30 min at room temperature. The supernatant containing the total soluble fraction was lyophilized for 48 h in a Stokes freeze-dryer (Barber-Colman,Philadelphia PA 19120, USA), operating at a condenser plate temperature of 40° C and a chamber pressure of less than 100 mm Hg. Then, samples were weighted and solubility was expressed as:

S% = (total soluble solids (g) / total solids (g)) x 100 (1)

2.5. Foam: formation, drainage and collapse. 30 mL of solutions were foamed at similar conditions as were used in the cited previous publication (25 ºC, 3 min, 2500 rpm with Griffin & George stirrer). Where Foam Overrun (FO) was calculated as:

FO (%) = [(final foam vol. – initial solution vol.) / initial solution vol.] 100 (2)

Foam stability was also measured as previous work [11] through the volume of liquid drained to the bottom of the graduated tubes and foam height as collapse recorded over time. Drainage velocity was calculated as previously:

$$k_{dr} = n / V c^{1/} \quad (3)$$

where *V is* the maximum drained volume; *n* is a constant related to the sigmoid shape of the curves; and *c* a constant related to drainage half time by $c^{1/n}$.

Foam collapse was described by two parameters: t_c, the time when the collapse started and the rate of volume decay after that lag time, that was fitted with the following linear model:

$$V = K_c t + b \quad (4)$$

Where K_c is the collapse rate.

All data reported, FO, drainage and collapse measurements are means of at least two replicates. The relative error was about 10%.

2.6. Rheological method for foam stability. Drainage and disproportionation kinetics were determined by a rheological method as was described elsewhere [16]. It was used a Brookfield DV-LVT viscometer with a T-spindle (C). The following mathematical model was applied to describe foam apparent viscosity as a function of time [17]:

$$\mu_{app}(t) = a\,[\exp(-K_2 t^{0.5}) - \exp(-K_1 t)] \quad (5)$$

where μapp is the apparent viscosity of foam at time t, a is a constant related to maximum viscosity, and K_1 and K_2 are rate constants for drainage and disproportionation, respectively.

2.7. Statistical analysis. All the experiments were performed in duplicate or triplicate. The model goodness-of-fit was evaluated by the coefficient of determination (R^2) and the analysis of variance (ANOVA), using Statgraphics Plus 3.0. software.

3. RESULTS SECTION

3.1. Solubility. In the Figure 1 it can be seen the solubility as a function of pH for SP and their hydrolysates of 3%wt/ total wt for each sample.

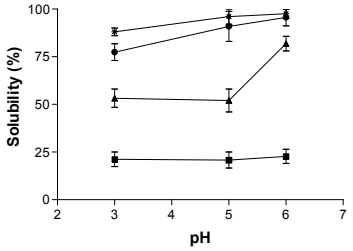

Figure 1. Solubility (%) as a function of pH for SP ■; 1.5% ▲; 7% *; 9.8% ●.

SP had the minimum solubility at all pH values, with a slightly increment at 6. This low solubility could be attributed to protein aggregates formed during the industrial processing of the starting sunflower meal. However, when the protein was hydrolyzed, the solubility increment was notable. At 1.5%DH it was observed a minimum of solubility near the isoelectric point, largely increased when the pH was 6. Surrounding the *pI* normally, the proteins have a minimum of solubility due to scarce of molecular charges which led to minimum water interactions and protein aggregation. On the other hand, when the hydrolysis degree were higher (7 or 9.8%DH), the increment of solubility was important at all pH studied, with an inverse behavior observed near the pI at these level of hydrolysis.

Some authors also found an increase of solubility at the isoelectic point of sunflower hydrolysates [18]. They stated that the presence of salt could strongly influence the protein solubility, attributable to modification of charges, modifying the repulsion among protein molecules [19].

3.2. Foam overrun. The Figure 2 shows the foam overrun (FO) for SP and their hydrolysates at pH 3, 5 and 6.

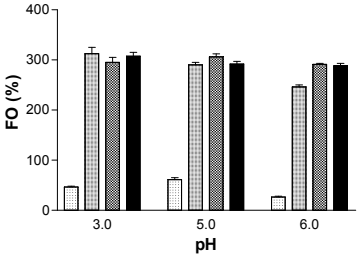

Figure 2. Foam overrun (%) for SP; 1.5%; 7% and 9.8% from left to right as a function of the pH. Conditions: temperature 25°C, ionic strength aproxim. 0.01 M.

Foam overrun was increased by protein hydrolysis, which is in accordance with results reported for limited hydrolysis of other proteins such as sunflower [11] soybean [20], wheat [21] and rapeseed [22]. The decreased molecular size, flexibility and the

exposure of hydrophobic areas resulting from hydrolysis [23]; the increased solubility (Figure 1), which reveals a higher protein quantity in the foams, increases the affinity and faster adsorption to the interface and hence leads to the observed higher overrun. It is important to emphasize that more significant fractions for each sample resulted: 200 and 60 kDa for SP; 60 kDa for 1.5%DH; 50 kDa for 7%DH and 6.5 kDa for 9.8%DH.

Regardless the correlation between SP and hydrolysates through the two parameters, it can be seen that FO also depend of the molecular size. This can be seen at 1.5%DH at pH 6, where a highest solubility were observed for this hydrolysate, (indicating an relative higher protein quantity in the foam) however, it was not shown an increment of FO, being one of the lowest at this pH.

3.3. Rate of liquid drainage. Depending on the protein and enzyme used, limited protein hydrolysis may improve foam stability of proteins. Nevertheless, these properties decrease when DH was high and the smaller peptide size does not allow an appropriate interfacial structure for foam stabilization [20] or alteration in the peptides aggregation to the liquid interface [17]. These results were obtained in the previous work at pH 7 [11].However, as is shown in the Figure 3 at pH 3, 5 and 6, it was observed a clear tendency; as more hydrolyzed were the SP, better drainage stability were obtained, with an exception of 7%DH at pH 5.

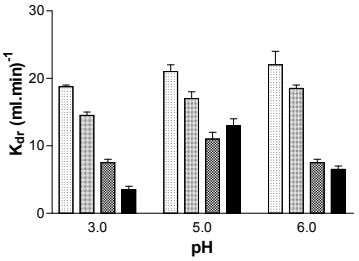

Figure 3. Kdren for SP; 1.5%; 7% and 9.8% from left to right as a function of the pH. Conditions: temperature 25°C, ionic strength aproxim. 0.01 M.

This, correlates with the high solubility (in 7 and 9.8% DH at all pH) (Figure 1) showing that more protein quantity were necessary to improve this property. It could be said that the quantity of peptides at solution is, as well as the size, determinant. On the other hand, the apparent higher drainage stability for SP, could be due to a lower foam formed that lacks of gravity phenomenon for drainage occurrence [16].

3.4. Foam collapse. Figure 4 a-b shows the time when the collapse of foams started (tc) (a), and the rate of decrease of the foam high, called Kc (b).

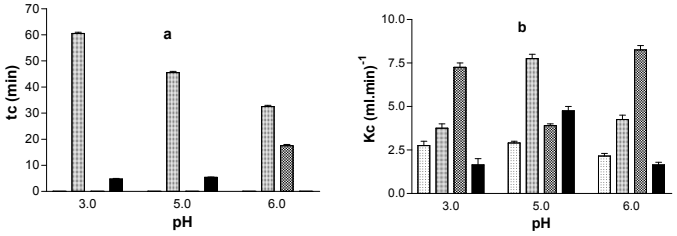

Figure 4. Time to collapse started, tc (a) and Collapse rate, Kc (b) for SP; 1.5%; 7% and 9.8% from left to right as a function of the pH. Conditions: temperature 25°C, ionic strength aproxim. 0.01 M.

Firstly, it can be seen that some tc values resulted zero. It corresponds to those cases where began to collapse at the end of whipping process, immediately.

It was observed that, the time when the collapse of foams started (a) was decreased up to 100 % by SP hydrolysis at all pH studied. The tc for foam collapse is influenced by the time prior to drainage conducts to very thin films that break at foam top. Therefore, when drainage rates are lower, it is suppose that higher the lag times for collapse should be result.

From the analysis it can be concluded that 1.5%DH was the best in the increment stability against collapse of foams at all studied conditions. At second place would be 7%DH at pH 6.

The rates at which foams collapses after the tc were decreased depending on the pH studied (Figure 4b). The hydrolysis not always decreased the rates of collapse for the intact SP as was expected. Again, the more hydrolyzed proteins showed the best performance in general as was seen for the k_{dren}. Thus, it can be observed that the stability against liquid drainage and collapse of foam are led for many factors; whereas a high quantity of protein for drainage is necessary, a limited hydrolysis (1.5%DH) shows to be a good option to obtain high stability against collapse.

Whereas, the capacity of a protein to successfully produce a foam is essential, its stability subsequent to the formation is the main criterion for its usefulness in whipping applications.

As a result, depend the application, it would be the hydrolyzed protein selected and conditions used at industrial processing.

3.5. Rheological method for foam stability determination. Characteristic viscosity of foams as a function of time for SP and hydrolyzed protein foams are shown in Figure 5 as a model curves.

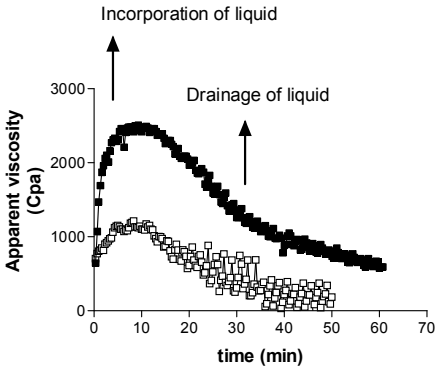

Figure 5. Apparent viscosity as a function of time for SP ■; 1.5% and 9.8% DH. Conditions: temperature 25°C, ionic strength aproxim. 0.01 M.

Apparent viscosity at first increased to a maximum due to the liquid drainage. After, disproportionation produced a decrease [16].

Both parts of the apparent viscosity and maximum point, are influenced by the protein state and structure (not shown).

It can be seen in the Figure 5 a curve as a model of apparent viscosity behavior.

The Figure 6 a-b shows K_1 and K_2 obtained from the rheological method for foam stability (expression 5) as a function of pHs for SP and their hydrolysates.

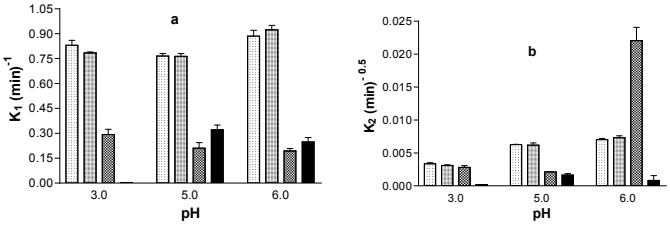

Figure 6. Rate constant for drainage, K_1 (a) and rate constant for disproportionation, K_2 (b) for SP; 1.5%; 7% and 9.8% from left to right as a function of the pH. Conditions: temperature 25°C, ionic strength aproxim. 0.01 M.

It can be observed that both K_1 (Figure 6 a) and K_{dr} drainage rate (Figure 3) were correlated for each foam (R^2=0.950–0.986). A similar behavior was observed for the K_2 (Figure 6 b) of the rheological method with the Kc (R^2=0.920–0.897) (Figure 4 b) of the bubbling method. The correspondence between foaming properties and viscosity analysis would promote a very useful technique to measure the performance of hydrolyzed sunflower protein foams at different pHs through diverse methods. Apart from stability parameters obtained against drainage and collapse of foams, the apparent viscosity could be obtained to deepen and characterize the foaming properties through flow properties during the foaming study.

4. CONCLUSIONS

Thus, it is essential to know the future application to select the appropriate hydrolysate of sunflower protein and the required pH.

It is important to keep in mind the little use given to this protein, and using it at different pHs would expand the possibility of application in the food industry.

Enzymatic treatment sunflower protein was used to start the study with foaming properties analysis and the effect of pHs. It is currently studying the same hydrolysates on other functional properties applied to food systems, such as gels and emulsions.

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