Enhancing the foamability of beverages proteins by ultrasound

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Abstract—The objective was to study the foamability of two proteins after ultrasound application. Soy protein and whey protein isolate were used as starting material at pH used in the food processing. Ultrasound was used to analyze the foamability effect on the solutions relating with the bubble size change.

The samples were sonicated at same conditions using an ultrasonic processor. Foam formation was measured by conductimetric and optical methods. Moreover, the evolution of the bubble size change was registered. Whey protein isolate solution was found a good choice to use as foaming agent at beverages preparations improved by ultrasound application.

Keywords—foamability, soy protein isolate, ultrasound, whey proteins isolate.

I. INTRODUCTION

Soybean proteins are widely used in many foods as functional and nutritional ingredients [1]. These proteins are used in a wide range of food applications, including processed meat, nutritional beverages, infant formulas, and dairy product replacement. Glycinin and β-conglycinin, the major components of soybean protein, account for approximately 70% of the proteins in soybeans [2]. Most studies were done using native soy protein isolate, glycinin and β-conglycinin, which are of limited value for the understanding of commercially available soy isolates.

Whey protein concentrates and isolates are important food ingredients because of their desirable functional properties, such as gelation, foaming and emulsification. Whey proteins are a significant source of functional protein ingredients for many traditional and novel food and beverages products [3]. The main proteins in whey are β-lactoglobulin (β-lg), α-lactalbumin (α-lac) and bovine serum albumin (BSA) and they account for 70% of total whey proteins [4]. These proteins are responsible for the functional properties of whey proteins, such as solubility in water, viscosity, gelation, emulsification, foaming, colour, flavor and texture enhancement and offer numerous nutritional advantages to formulated products [5].

The effect of ultrasound is related to cavitation, heating, dynamic agitation, shear stresses, and turbulence [6]. It may cause physical changes producing aggregates through non-covalent bonds by cyclic generation and collapse of cavities depending of structural or aggregation protein state.

In the present work, effects of ultrasound of high intensity on the foamability of two different proteins at food pH were analyzed. Bubbling method is the unique system to form the foam that gives the precise liquid and gas used to form them, having thus, the exact density of foams obtained.

Soy protein and whey protein isolates were used as starting material. The foaming formation together with the bubble size change was analyzed.

II. MATERIALS AND METHODS

2.1 Protein samples preparation

Soy protein isolate (SPI) was provided by Instituto de la Grasa, Seville, Spain and the complete description was published elsewhere [7].

Soluble SPI (SSPI) at pH 3 was used as starting material for the current work. Protein solution, at 4% w/w, was centrifuged for 1 hour at room temperature at 10,000 g. The protein content was determined in the soluble fraction by the Kjeldhal method (N x 6.25), resulting in 0.46.

Whey protein isolate (WPI) was provided by Milkaut, Argentina. The protein was used at 2 % wt/wt and adjusted further to pH3.

These final solutions were treated by high intensity ultrasound (HIUS).

2.2 Foam formation

The foams were made using a Foamscan instrument (Teclis-It Concept, Logessaigne, France). The foam is generated by blowing nitrogen gas at a flow of 45 mL/min through a porous glass filter of 0.2 μm at the button of a glass tube where 20 ml of the foaming
aqueous solutions (25 ± 1°C) is placed. In all experiments, the foam was allowed to reach a volume of 120 ml. The bubbling was then stopped and the evolution of the foam was analyzed by means of conductimetric and optical measurements. The Final Time of Foaming (FTF), the Total Gas Volume (TGV) and the Final liquid volume (FLV) were taken from the table results after each experiment. The generated foam rises along a thermostated square prism glass column, where the volume is followed by image analysis using a CCD camera. The evolution of the bubble size change in the foam was also determined by a second CCD camera set with a macro objective which allows to capture the variation of the air bubble size every 5 s. Thus, the images of initial obtained foam could be obtained to characterize them.

The following parameters were determined: Foam Expansion (FE), as the inverse of the Foam Maximum Density (MD) determined by (1), is a measure of the liquid retention in the foam; the Overall Foaming Capacity (OFC, mL/s) was determined from the slope of the foam volume curve up to the end of the bubbling. The Foam Capacity (FC), a measure of gas retention in the foam, was determined by (2)

\[
FE = \frac{V_{\text{foam}}}{V_{\text{liquid}}} \\
FC = \frac{V_{\text{i}}}{V_{\text{gas(f)}}}
\]

where \(V_{\text{liq}(i)}\) and \(V_{\text{liq}(f)}\) are the initial and the final liquid volumes; \(V_{\text{foam}(f)}\) is the final foam volume and \(V_{\text{gas}(f)}\) is the final gas volume injected.

2.3 High-intensity ultrasound (HIUS) treatment
Protein solutions were sonicated for 20 min using an ultrasonic processor Vibra Cell Sonics, model VCX at a frequency of 20 kHz and an amplitude of 20%, which were constant. A 13 mm high grade titanium alloy probe threaded to a 3 mm tapered microtip was used to sonicate 10 ml of the solutions. Samples contained into glass test tubes were immersed into a glycerine-jacketed at 0.5°C to dissipate most of the heat produced during sonication treatments (Polystat, Cole-Parmer).

Statistical analysis
All the experiments were performed in duplicate or triplicate. The model goodness-of-fit was evaluated by the coefficient of determination (R²) and the analysis of variance (ANOVA), using Statgraphics Plus 3.0. software.

III. RESULTS AND DISCUSSION
3.1 Efficiency in foam formation
Since the foaming protocol determines that the nitrogen flow stops once the foam height reaches 120 ml, the foaming time, the gas required during the bubbling and the remaining liquid will depend on the ease of the solution to form the foam, which would relate to the overall foaming capacity in each case. In the Table 1 it can be seen the Final Time of Foaming (FTF), the Total Gas Volume (TGV) incorporated into the foam and the Final liquid volume (FLV), corresponding to the remaining liquid after foam formation; for untreated (SSPI and WPI) and HIUS treated proteins. Thus, we can assume a better efficiency in foam formation when the Final Time of Foaming is low, also the Total Gas Volume incorporated and high the liquid incorporated, that it means, a low remaining FLV [7].

| Protein   | FTF(±0.5 s) | TGV(±5 cm³) | FLV(±3 cm³) |
|-----------|-------------|-------------|-------------|
| SSPI      | 241.5       | 177         | 10.25       |
| SSPI HIUS | 180         | 131         | 8.45        |
| WPI       | 207         | 151.5       | 18          |
| WPI HIUS  | 160         | 116         | 14.05       |

The Table 1 shows for SSPI that ultrasound provoked a decreasing effect of FTF, indicating that the formation of foam is facilitated by the treatment. However, at the same time it is observed that at the end of the formation, less amount of gas and similar liquid were incorporated by the TGV increase and FLV unchanged parameter. This means that the better formation of the foam, by the HIUS effect, does not allow to include a greater quantity of protein at the final formed foam, which, could be very influencing factor for the later stability as same way.

For WPI HIUS provoked a decreased of the FTF, it indicates a greater velocity in the formation of the foam as same way, less gas amount incorporated by comparing with the untreated WPI, and more liquid quantity. This means that the greater speed in reaching the set foam height, is accompanied also by greater incorporation of liquid, thus, more protein to the foam formed was present after bubbling. In a previous submitted work, we investigated the ultrasound effect on these proteins at pH7. In that work, WPI accelerated also the formation foam velocity, however, less amount of liquid (and protein) was possible to add to the final foam. Therefore, this pH, which is the food habitually used, would be very favorable to obtain foams with higher velocity and higher amount of protein at same time. As the concentration were the same in both analysis (pH 7 and pH 3), a possible explanation would be the solubility difference cause by the pH condition. WPI, has an isoelectric point around 6, thus, more protein effective molecules at pH 3.
could be present and diffuse faster to air-water interface of the dispersed system. [8]

In the other hand, when SSPI was compared, in the present work almost no differences were obtained (foaming time and final foam density), however, at pH 7, we observed that more time took to form the foam, but more quantity of protein were incorporated. In this case, an evident more soluble protein quantity was present and would be possible to diffuse to the air-water interface. Obviously, if different pHs was compared, no equal protein quantity could be present in both samples, however, it is important to have into account the aggregation effect by the ultrasound at different protein [9]. It is said in general that HIUS effects promote foaming changes attributed to partial denaturation of proteins and then, the ability to form the foam [10].

3.2 Effects of HIUS on other parameters for formation of foams

In Figure 1 (a-d) it can be seen the HIUS effect for SSPI and WPI on FE, FC, OFC and MD

![Graphs showing FE%, FC, OFC, and MD for soy soluble protein isolate (SSPI) and whey protein isolate (WPI) at similar concentrations, pH 3.]

It can be seen, in general that there were no changes in FE, FC, OFC and MD, for SSPI, as a consequence of HIUS treatment. Although faster foam was produced, it was not reflected in the formation parameters on this protein. In the Table 2, it can be seen the Initial Aspect of the foam obtained for each protein.

| Protein | Initial Foam Aspect |
|---------|---------------------|
| SSPI    | ![Image of SSPI foam] |
| HIUS    | ![Image of HIUS foam] |
| WPI     | ![Image of WPI foam]  |
| HIUS    | ![Image of HIUS foam] |

Table 2: Initial Foam Aspect for untreated proteins (SSPI and WPI) and HIUS treated solutions.
The foam for SSPI presented similar to bigger bubbles compared to the foam obtained without the treatment. Thus, neither better effect was obtained on soy protein after HIUS treatment in the present work conditions. In the other hand for WPI solution, the Figure 1 shows an increment of almost all parameter after HIUS. On this particular protein, it could be seen not only an improvement of FC and OFC but also a higher MD, indicating a more dense foam. It is also relates very well to the data obtained previously (Table 1). It was possible to see that the time of foam formation was decreased and more liquid was incorporated, giving more efficient foams after treatments. The corresponding images in the Initial Aspect (Table 2), showed foams with smaller size bubbles, by comparing with untreated WPI solution. This confirms that HIUS causes greater incorporation of liquid on this protein.

IV. CONCLUSION

Two different source of protein were used to study the effect of ultrasound of high intensity on foam production. Soluble soy protein (SSPI) and whey protein isolates (WPI) were prepare for food and beverages applications, at pH3 at similar conditions. When HIUS was applied, for SSPI practically no changes were observed, whereas, a decrease of foaming time and higher foam density at same time was found for WPI. Thus, an extraordinary HIUS effect was observed for the last protein. Foaming parameters were improved and at same time, more efficient liquid incorporation was obtained. The essential difference between proteins would be the solubility at this pH. Isoelectric point of soy protein is around 4, close to the employed pH at the present study. However, whey protein has an isoelectric point of 6, some near to the used here. While proximity to the isoelectric point causes a null charge on the proteins which promotes an easy diffusion to the air-liquid interface during the whipping, the low solubility causes a lack of protein available under such circumstances. In addition, the recorded bubbles images of the corresponding obtained foams with the second camera showed bigger bubbles for HIUS treated SSPI, and smaller ones for WPI, confirming the above attained results. Therefore, whey protein isolate solution was found a good choice to use as foaming agent at food and beverages processing improved by ultrasound application.

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