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High intensity ultrasound application on rheological properties effects of native soy protein isolate

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ABSTRACT

Industrial protein modification methods are mainly based on the use of heat, enzymes or reducing agents of achieving various functional properties that make them suitable for use in various foods. The use of high intensity ultrasound (US) has been applied in various operations in food processing. Soy protein was isolated. The solubility; the flow behavior by viscometer, the size distribution and zeta potential particle were studied. The gel point was analyzed by dynamic rheometry and frequency sweeps characterizing the viscoelasticity of the gel formed. Samples at a frequency of 20 kHz and amplitude of 20%, were treated for 20 minutes. The solubility showed a large increase. However, the viscosity showed no changes after HIUS; matching the results obtained in the study with particle size distribution. The zeta potential increased significantly with the unaltered capacity of gelation would be related to a structural modification imparted by the HIUS.

Keywords: *Soy protein, high intensity ultrasound, nanotechnology.*

1. INTRODUCTION

The native soy proteins, although possessing very good solubility, may not have an adequate behavior to form colloidal structures in foods. Therefore, industrially they can be made different processes of modifications of their structure and functionality to achieve specific characteristics. Modification methods at the industrial level are based on the use of heat, enzymes or reducing agents.

In this way, various functional properties are achieved which make them suitable for use in various foods.

The so-called emerging technologies, based on non-thermal physical processes, are proving promising to achieve the inactivation of contaminating and pathogenic microorganisms, reducing undesirable changes in food. Among these technologies are pulsating electric fields, high hydrostatic pressure and high intensity ultrasound.

Gordon et al., used high intensity ultrasound to control particle size and morphology in combination with different treatment times, whey protein concentration and concentration (WPI) found a reduction in particle size Of WPI 7.5% w / w with the time of US application, saw that this decrease was more marked in the first two minutes of treatment [1]. In the study of the combined treatment of ultrasound and temperature found that at a temperature of 85-93 ° C, the lowest concentration (7.5% w / w) the particle size was reduced; When the concentration was 9% w / w no changes were observed and when the most concentrated sample (12% w / w) was treated, a strong increase in particle size was observed, as compared to the untreated sample [1].

Thus, the result obtained for the WPI case 7.5% w / w suggested that the effect of ultrasound was predominant over that of the temperature aggregation that prevailed in the case of the sample at 12% w / w [1]. For all of these cases polidispersed size distributions were obtained with a very wide range for the case of WPI 12% w / w, which is corroborated by confocal microscopy where a great polydispersity is observed [1].

The objective of this work was to characterize the effect of ultrasound technology of native soy protein at room temperature, in order to promote structural alterations that would lead to the improvement some rheological properties.

2. EXPERIMENTAL SECTION

2.1. Soy protein isolates characterization and sample preparation. Native soy protein isolate (SPI) was obtained from soybean defatted flour (Sanbra S.A., Brazil). It was characterized by differential scanning calorimetry (DSC) for verification of its structural state. The sample presented an endothermic peak, which indicates qualitatively the presence of a native fraction, so it is concluded that the extraction process was successful.

2.2. High-intensity ultrasound treatment. Samples were sonicated using a Vibra Cell Sonics ultrasonic processor, model VCX 750 (Sonics & Materials INC., Newtown, USA) at a frequency of 20 Khz and a 20% amplitude, for 20 continuous minutes.

2.3. Study of solubility. The 2% w / w protein solution in distilled water was centrifuged for 30 minutes at room temperature. The supernatant with the soluble fraction was lyophilized in a Stokes apparatus for 48 hrs, weighed and the solubility calculated according to equation (1):

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

2.4. Determination of the viscosity of the solutions. The flow behavior of 2 % solutions was studied with a Brookfield LTV viscometer with cone and dish at room temperature (25 °C), in the speed range of 90 - 150 RPM. The shear force as an average of the ascent and descent values at each deformation speed. All measurements were made in duplicate for each protein solution.

2.5. Particle size distribution: Dynamic light scattering. The dynamic light scattering assays were performed on a dynamic light scattering apparatus (Zetasizer Nano-Zs, Malvern instruments, Worcestershire, England) provided with a He-Ne laser (633 nm) and a digital correlator, Model ZEN • 3600, in the range of 0.6 nm to 6 µm. The samples were measured after filtering with 0.45 and 0.22 µm filter and unfiltered. Measurements for SPI solutions were performed at 25°C. Samples were placed in disposable 1-cm polystyrene buckets arranged in the equipment. Particle size measurements are reported as the mean and standard deviation of at least five measurements.

2.6. Dynamic Rheometry.

2.6.1. Dynamic viscoelasticity. The sample was placed in the measuring system, which was thermostated at the initial heating temperature (25° C). Heating was made at a rate of 10°C / min to the desired temperature of 90° C. This was maintained for 15 minutes and then the temperature was lowered with the same speed ramp to 25°C. The elastic, viscous, complex and tangent modulus of the phase angle were recorded using a deformation of 0.01% and a frequency of 1 Hz. These conditions were previously evaluated as the linear viscoelasticity zone of the systems.

The determinations were performed in duplicate with differences less than 10%. **2.6.2. Frequency sweep.** The G 'and G' modules were recorded as a function of the oscillation frequency, f, is 0-5 Hz, with 0.01% deformation, under response conditions of the linear viscoelastic range. The results were analyzed in duplicate or triplicate, concluding effects studied using Anova, Statgraphycs, 3.0.

3. RESULTS SECTION

3.1. Solubility of SPI. It was performed in 2%. The effect of storage time on the solubility of SPI was previously analyzed. The results showed that the SPI at room temperature increased its solubility from 16% to 19% at 24 hours storage. From these results, all subsequent treatments were carried out with the samples dissolved during one week of storage. Thus, an increase in solubility by HIUS effect was determined from 19 ± 0.47 to 56 ± 5.66 and there was a considerable increase in the parameter due to the effect of the treatment.

3.2. Determination of the viscosity of the solutions. The behavior of the 2% solution flow curves and their respective treatments, in the velocity range of 90 - 150 RPM allowed to determine the Newtonian behavior in all the samples studied, given their linear relationship.

Thus, the values obtained for its analysis correspond to the slope of the Shear Stress curve (D/Cm2) vs Shear Rate (1/sec), its Viscosity (Cp).

It was not observed a viscosity change after treatment. The results showed 1.5 ±0.3 for untreated simple and 1.5±0.2 for HIUS treated sample.

The effect of the treatment on viscosity was not directly related to the solubility found in the ultrasound effect. This could be due to the fact that the aggregates formed in the samples at 2% were not completely significant to appreciate differences in the study of viscosity by their concentration. However, it can be concluded that the effect of US does not induce changes in flow behavior, although there is a slight increase in solubility.

Changes in viscosity during a treatment; are often related to the change in the molecular size of the sample. In the present case, the treatment can give rise to a different type of joints, which would consequently favor to alter the functional properties.

3.3. Zeta Potential (ζ). The surface charge of a protein is due to the partial ionization of several amino acid residues [2]. Typically the Zeta potential of a protein is positive if there are more positively charged amino acids present than the negatively charged amino acids [3]. Table 1 shows the values of the potential Zeta (ζ) of the SPI.

Table 1. Zeta Potential (ζ) for SPI

Treatment	2%
Without HIUS	-17,1 ± 8,2 [a]
HIUS	-23,9 ± 5,8 [ab]

We can see that the samples tested showed negative values, indicating that the samples contain more negatively charged amino acids than positively charged amino acids. This is correct because of their natural pH, since they are above their isoelectric point.

An increase in modulus was observed compared to untreated SPI.

The increase in Zeta potential in modulus is due to the formation of aggregates, that is to say, that when applied to HIUS, it could increase the negative surface charge of the protein, strengthening the electrostatic repulsions between the particles. In cases where no changes in average particle size are observed (as will be seen in the next section), it can be concluded that the HIUS causes surface changes in the previous aggregates, inducing the exposure of negatively charged residues and increasing in consequently their surface charge, this leads to the improvement of the stability of the protein dispersions.

3.4. Particle Size Distribution. The SPI particle size distribution was performed by dynamic light scattering.

Based on the results provided by the software of the team, we proceeded to analyze the %volume. This is a quantitative indicator of the population so it will describe the representative percentage in each case and, although other sizes are found, the majority share is the one that will essentially determine the functional properties. For this reason, we decided to analyze the

effects of the treatment by describing the peak/s majority/s in Vol.%

In Figure 1 we can observe the average of 18 measurements of the particle diameter (nm) size distributions in volume% of the SPI for each sample.

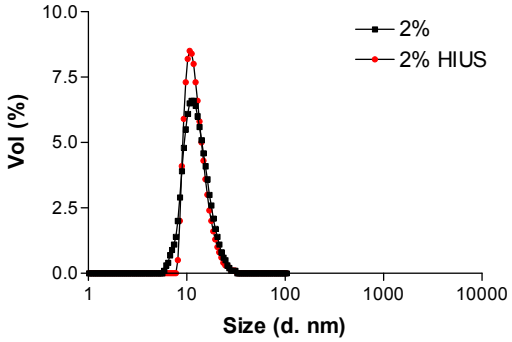

Figure 1. Size distribution particle for untreated and HIUS treated SPI.

The results show a major peak around 10 nm in the mean diameter of the particle size distribution for untreated SPI. These results are consistent with previous studies by our research group on denatured soy protein [4].

Previous studies have shown that the effect of HIUS is related to the decrease in particle size; While the temperature tends to add it, increasing the size of said majority peak. However, in the case of HI-treated SPI no difference was observed ie no effect of HIUS was found at room temperature relative to particle size. However, the increase in solubility (19 to 56%) and zeta potential (Table 1), shown above, indicates an obvious structural modification due to the effect of HIUS, which does not directly translate into its size distribution. Similarly, the viscosity study also showed no changes after treatment and being more directly related to particle size, one could confirm the null effect of the parameter on SPI when treated with HIUS.

3.5.1. Dynamic rheometry: Viscoelasticity and gelation. These experiments were carried out with a temperature program ranging from 25 ° C to 90 ° C, with a speed of 10 ° / min, maintaining this temperature for 15 min and then descending at the same speed up to 25 ° C. Dynamic rheology studies of SPI were performed, with deformation of 0.01% and at a fixed frequency of 1 Hz; the evolution of the storage modulus (G '), loss (G' ') and phase angle (tan δ)

Figure 2 shows the evolution of the components as a function of time for SPI as an example. The "gel point" is indicated in this case.

In this case as an example at higher concencetration (4% w/w), a "Tgel" gelation temperature is observed in 3.5 min of approximately 60°C and a subsequent evolution of G '> G ", evidencing the gel structure from that moment.

Table 2 shows the gelling times and temperatures, next to the value of G ' for SPI at 2%.

Table 2. Gelling point (time, temperature) and corresponding value of G 'of SPI at 2%. Gel time ± 0.4 min; Temp.gel ± 5 ° C; G "± 5 Pa.

Treatment	SPI (%)	Time Gel (min)	Gel Temp. (ºC)	G´ (Pa)
Without HIUS	2	---	---	---
HIUS	2	---	---	---

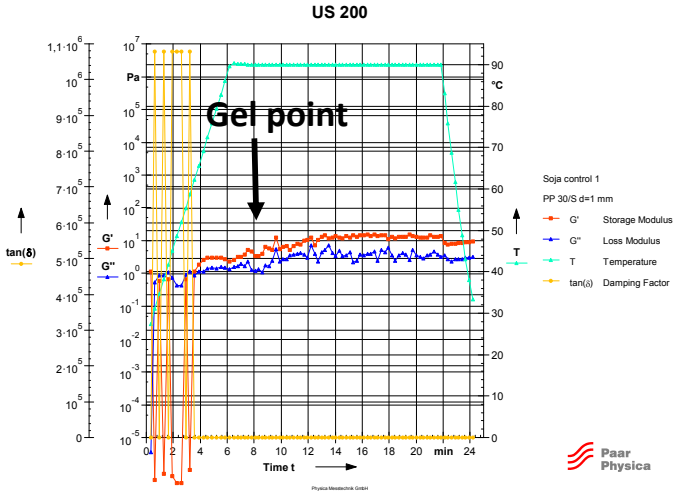

Figure 2. Evolution of storage or elastic components (G '), loss or viscous (G "), tan and temperature ramp, for SPI.

In Table 2 it can be seen that the 2% SPI did not reach the "gel point", showing an erratic evolution of the components, with no defined crossing point, which determines the non-gelling in the measured conditions. It is difficult to find the gel point due to the low protein concentration, as we found in previous works with the same soy protein but denatured [5]. Non effect of HIUS for gelation can be seen. Although an increase in solubility was observed with the treatment, HIUS appears to generate molecular changes in the native proteins, increasing the affinity for the water and at the same time unchanging the afinity by itself.

3.5.2. Frequency sweep. The variation of the G' and G" components in the frequency range of 0.05 - 5 Hz at 25 ° C with a deformation of 0.01% of the SPI samples was studied. Figure 3 shows the evolution of the components as a function of frequency for a sample as an example.

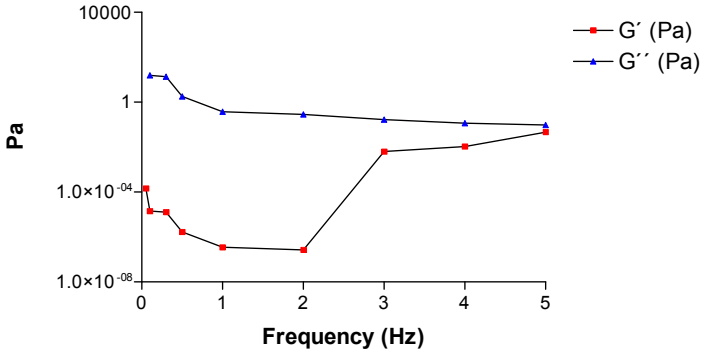

Figure 3. Evolution of solid components (G ') and viscose (G"), with frequency for SPI.

The dependence of the components with the frequency and the relationship between them will indicate the existence and type of a gelled network or solution.

In the present work it was observed that in all cases where the G' and G" junction existed, the viscous component (G") was greater than the solid component (G') in all of the range of frequency studied. This situation could correspond to an entanglement networks system or also called "pseudogeles" [6]. They are systems formed by simple interactions between polymer chains, rather than true junctions between them. It is described that at low frequencies, the flow is similar to that of high viscosity liquids.

4. CONCLUSIONS

The isolated protein structure (SPI) conserved native fractions after fractionation.

The solubility of SPI at 2% w/w was increased with storage time and application of HIUS at room temperature.

The viscosity was related to the mean particle size of the SPI, which did not show significant changes with the treatment.

The surface potential of the aggregates, evidenced a modification of the treatment, generating structures of similar average size but of greater exposure of negative charge protein residues.

The application of HIUS unchanged the gelling capacity of the SPI samples, presenting a pseudogel formation.

The molecular modifications caused by HIUS could alter some functional properties in a very directed way due to the subtle change generated, without probably provoking greater variations in the basic properties of the native soybean proteins (hydration properties, eg interfacial foaming and emulsification). To investigate the possible applications, these studies should be studied in greater detail and in different conditions of temperature, pH, ionic strength, etc.

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