Lab 4: Protein Functionality: Solubility and Foam Formation

I. PURPOSE:
The purpose of this experiment was to prepare a standard curve of dilutions of a protein solution of unknown concentrations by using spectrophotometer and to find the pH adjustment of Whey and Casein solutions.

II. INTRODUCTION:
Functional properties of food proteins are categorized into various ways depending upon their attributes. Some general properties include binding, hydration, sensory, structural and matrix formation. They all have different functional properties related to their structures. Properties are completely depends upon proteins structure and changes in structures vary in the change in properties as well. Basic structure of protein contains amino acid composition and sequence that include secondary and tertiary structures, shape, molecular size etc.

Since food proteins are a mixed group of different protein, solubility tests are performed to find their sequence, protein conformation, pH, concentration and temperature of the solution. Protein solubility in water must have a polar surface to the water. It has hydrophilic and hydrophobic ends on both sides that occur via hydrogen bonding.

Foam formation refers to the ability of a solution to form foam. Measurements, such as pH, temperature viscosity, are taken to find the exact volume of foams in the solution. The foam in bread is stabilizes after baking making it a staple food throughout the world. The major protein component found in bread is gluten. It is poorly soluble in water due to their low numbers of ionizable amino acids.

In this experiment, we looked closely at two broad classes of milk proteins: the caseins and the whey. Casein protein has clusters of hydrophobic peptide chains with a pH of 4.6 at precipitation while the pH of whey protein is 4.6 at the state of
solubility. Both of the proteins possess unique functional properties that reflect their structures. Whey proteins make up 20% of the protein in milk and Caseins make up 80% of the protein in milk. They are considered two broad classes of milk proteins and are very important in food industry. Whey is used for food additives in cheese making via new methods of processing.

III. PROCEDURE:
The procedure followed for the experiment is found in “FS&T 101A-Food Composition Laboratory” (2012) pages 42 through 45 titled “Lab 4: Protein Functionality: Solubility and Foam Formation”. There were not any modifications made during this experiment. The procedure in the lab manual was followed as it is written.

IV. DATA/GRAPHS:
Table 1: Concentration and Absorbance data for standard curve made with BSA

<table>
<thead>
<tr>
<th>Tube (13*100 mm)</th>
<th>Absorbance @ 595nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.197</td>
</tr>
<tr>
<td>3</td>
<td>0.339</td>
</tr>
<tr>
<td>4</td>
<td>0.588</td>
</tr>
<tr>
<td>5</td>
<td>0.900</td>
</tr>
</tbody>
</table>

Table 2: Absorbance data reflecting solubility of heated and non-heated whey and casein proteins in the designated pH environments.

<table>
<thead>
<tr>
<th>Whey pH</th>
<th>Absorbance @ 595nm heated</th>
<th>Absorbance @ 595nm unheated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.53</td>
<td>0.71</td>
<td>0.708</td>
</tr>
<tr>
<td>3.5</td>
<td>0.456</td>
<td>0.756</td>
</tr>
<tr>
<td>4.17</td>
<td>0.702</td>
<td>0.748</td>
</tr>
<tr>
<td>5.5</td>
<td>0.64</td>
<td>0.78</td>
</tr>
<tr>
<td>6.8</td>
<td>0.86</td>
<td>0.92</td>
</tr>
<tr>
<td>7.65</td>
<td>0.86</td>
<td>0.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Casein pH</th>
<th>Absorbance @ 595nm heated</th>
<th>Absorbance @ 595 unheated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.704</td>
<td>0.454</td>
</tr>
<tr>
<td>3.5</td>
<td>0.043</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 3: Calculated data showing % solubility of the two proteins, heated or non-heated as a function of pH.

<table>
<thead>
<tr>
<th>Whey pH</th>
<th>% solubility heated</th>
<th>% solubility non-heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.53</td>
<td>57.80</td>
<td>57.60</td>
</tr>
<tr>
<td>3.5</td>
<td>34.60</td>
<td>62.0</td>
</tr>
<tr>
<td>4.17</td>
<td>57.10</td>
<td>61.30</td>
</tr>
<tr>
<td>5.5</td>
<td>51.40</td>
<td>64.0</td>
</tr>
<tr>
<td>6.8</td>
<td>10.20</td>
<td>76.90</td>
</tr>
<tr>
<td>7.65</td>
<td>10.20</td>
<td>78.80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Casein</th>
<th>% solubility heated</th>
<th>% solubility non-heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>57.20</td>
<td>34.40</td>
</tr>
<tr>
<td>3.5</td>
<td>3.000</td>
<td>3.000</td>
</tr>
<tr>
<td>4.5</td>
<td>39.90</td>
<td>34.80</td>
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<tr>
<td>5.5</td>
<td>58.30</td>
<td>34.00</td>
</tr>
<tr>
<td>6.5</td>
<td>43.30</td>
<td>38.20</td>
</tr>
<tr>
<td>7.5</td>
<td>37.00</td>
<td>34.40</td>
</tr>
<tr>
<td>8.5</td>
<td>41.90</td>
<td>38.60</td>
</tr>
</tbody>
</table>
Graph 1: concentration and standard data for standard curve made with BSA

\[ y = 1.092x + 0.0774 \]
\[ R^2 = 0.9699 \]

Graph 2: % Solubility of Unheated and Heated Casein Protein as a function of pH
V. CALCULATIONS:

- Final whey concentration at pH 3.5 = 0.456 (original concentration = 0.1g/100ml)
  \[ y = 1.092 + 0.0774 \times 0.456 \]
  \[ X = 0.03467 \text{g/100ml} \]

- % Solubility whey = \( \frac{0.03467}{0.1} \times 100 = 34.67\% \text{ solubility} \)

- Final casein concentration at pH 4.5 = 0.514 (original concentration = 0.05g/100ml)
  \[ y = 1.092 + 0.0774 \times 0.514 \]
  \[ X = 0.03998 \text{g/100ml} \]

- % solubility casein = \( \frac{0.03998}{0.05} \times 100 = 39.98\% \text{ solubility} \)

VI. DISCUSSION:

In this experiment, we used a standard curve to find the concentrations and % solubility of whey and casein proteins. The trendline equation from graph 1 was used to find the concentrations and the % solubility of both proteins. To find the protein concentration, we used standard curve absorbance at 595nm at calculated pH. In my calculations for final whey concentration, I used pH 3.5 value at absorbance of 0.456 and ended up with an answer of 34.67% solubility which is close to the value in the table 2.
Errors might include the incorrect way of plotting the trend line or mistakes while doing the actual experiment.

Electrostatic charge determines the solubility of a protein. Isoelectric point is also known as the pH value of a protein and it is the pH at which the protein is least soluble. Looking at the graph 1, we can see that the Isoelectric point for Whey is approximately 4.1. Compared to the actual isoelectric point for whey (4.6), this value is similar to the calculated one giving the experiment a correct value. Looking at graph 2, we see that the isoelectric point for casein is approximately 5.5. Compared to the actual isoelectric point for casein (4.6), the calculated value is quite higher due to an unexpected error in the experiment.

The % solubility calculations for whey and casein tell us the functional application of both proteins in their interactions with water. The pH affects the net charge on whey and casein which directly affects the ionic interactions of both. This results in hydration. Heat causes denaturation of whey protein which leads to a mixture of whey protein and casein protein.

VII. CONCLUSION:

In this experiment, we prepared a standard curve by using BSA and functional properties of whey and casein proteins in heated and unheated environments. I learned about the different properties of both proteins and the solubility points. Protein solubility is very important than other methods such as foaming, gelation because for all these methods, the initial solubility of a protein is used. The methods we used were very straightforward and easy to follow and no modifications were made during this experiment. My group did not had any problems in following the directions and getting good results. The only problem we had was the time limit which was shorter than the time it took us to finish the entire experiment.

VIII. QUESTIONS:

1. Using your data, identify the pH at which whey and casein proteins are least soluble.

   Describe the condition of the protein at this pH which causes this point of minimum solubility.

   Whey is least soluble at the pH of 4.1 which is also the isoelectric point of this protein. Casein is least soluble at the pH of 3.5. Whey is precipitated at pH 4.6 and is soluble at
this pH and is the protein which remains after casein is removed by precipitation. Casein is least soluble at the pH of 5.5. At the least solubility point, the water binding and the net charge is zero.

2. At their respective points of minimum solubility, contrast the degree of solubility between whey and casein proteins for both heated and non-heated protein solutions. Discuss how molecular structure influences the degree of solubility for each type of protein.

Looking at both heated and non-heated protein solutions, the degree of solubility in the whey protein of heated solution is less than the unheated solutions. The degree of solubility in the casein protein of heated solution is also less than the unheated solutions.