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Section A07, Thu 9-11:50 am

Partner: Sh. G

Lab 4 (effect of pH and heat on whey & casein proteins)

Purpose/ objective:

The purpose of this lab is to understand how different structure of protein can affect the function of them. Functional properties of proteins are the physical and chemical properties of them that can affect their behavior in food processing. In this lab the functionality of proteins were evaluated.

Introduction:

Properties of proteins are the physical and chemical properties of them that can affect their behavior in food processing. Consumers don't really understand these behavior but food technologists are interested in knowing more about these properties so they can manipulate proteins in order to get more consistent product. Functional properties of food proteins can be:

1-Protein must interact with other components of the food system (binding)
2-Hydration properties (Water absorption/retention; Wettability; Swelling;
Adhesion, Dispersability, Solubility, Viscosity)
3-Protein-Protein Interactions (Gelation, Dough/fiber formation)
4-Surface properties (Emulsification, Foaming film formation)
5-sensory (color, flavor, odor)

The structure of protein affects the function of them and any change in their structure will change their functions as well. For example for the formation of foam the white egg needs to be unfolded from their native conformation. It is important that we understand the molecular properties of proteins. Adding a polar group to a protein will increase its net charge and alter solubility and foaming and emulsification capacity.

Usually proteins are mixed in food and not only one single kind of protein and they could interact with each other or respond differently to their surrounding conditions. Functionality might be difficult to

measure. Functionality can be influenced by whether they are denatured or native and environmental conditions like pH or salt. One of the important functions of protein is their interaction with water. Protein hydration is important in functions such as solubility, viscosity and etc.

Factors influencing hydration can be:

1- Protein concentration (ratio of polar to apolar amino acids)

2-pH

3-Ionic strength and concentration

4-Surface charge

5-Temperature of solution

In general solubility will increase with polarity of the protein surface and decrease as molecular weight decrease.

Procedure:

The procedure followed for the experiment is found in "Principles of Food Composition Laboratory Manual" (2013) Experiment 4, protein functionality: solubility and foam formation, pages 37-48. The only change in this lab was at part 2 in the table where the BSA and water addition was different in 5 tubes.

Data/result:

Table 1 concentration and absorbance data for standard curve made with BSA:

Data from our group:

Tube	BAS	Concentration (mg/mL)
1	0.000	0.0
2	0.163	0.1
3	0.355	0.2
4	0.610	0.4
5	1.080	0.8

Data from group 12:

Tube	BAS	Concentration (mg/mL)
1	0.000	0.0
2	0.107	0.1
3	0.176	0.2
4	0.363	0.4
5	0.730	0.8

Table 2: absorbance data reflecting solubility of heated and non heated whey and casein proteins in the designated pH environment.

Data from our group:

pH	Absorbance Heated	Absorbance non heated
2.69	0.544	0.476
3.31	0.524	0.486
4.50	0.504	0.532
5.53	0.865	0.726
6.58	0.458	0.520
7.50	0.304	0.478
8.37	0.706	0.766

Data from group 12:

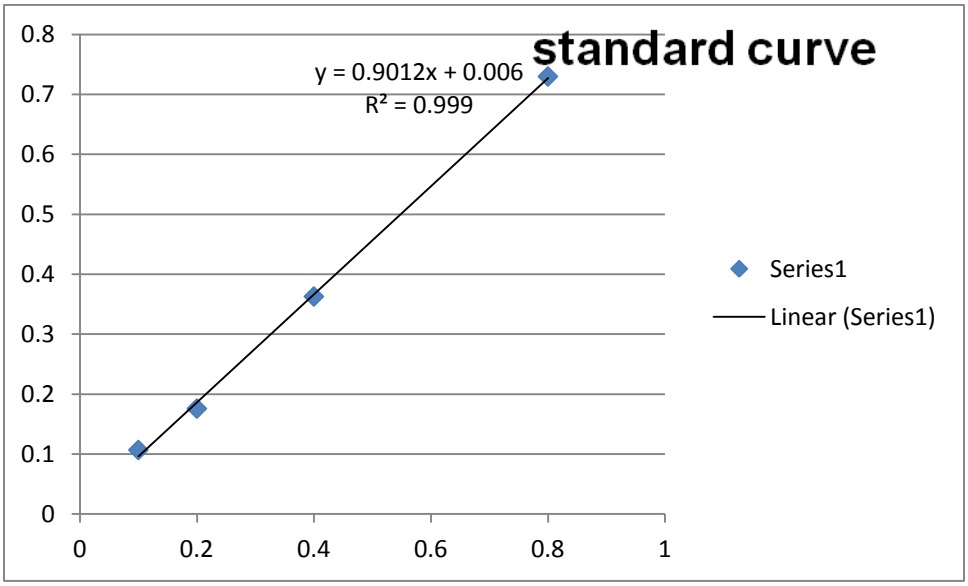
pH	Absorbance Heated	Absorbance non heated
2.60	0.333	0.283
3.42	0.376	0.323
4.57	0.530	0.286
5.38	0.534	0.267
6.55	0.486	0.422
7.52	0.522	0.430
8.32	0.504	0.418

Table 3: % solubility of the 2 proteins:

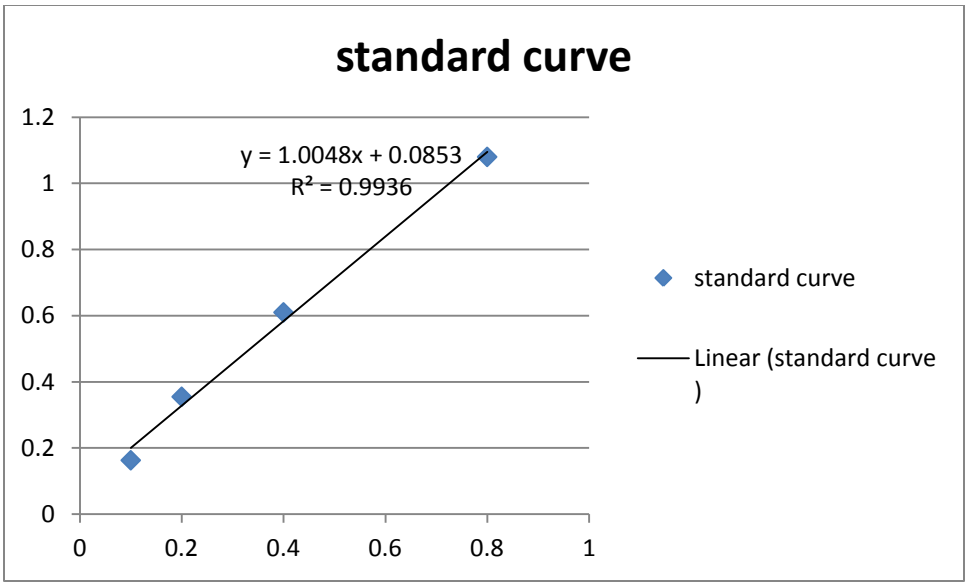
PH	Whey(heated)	Whey (non-heated)	PH	Casein (heated)	Casein (non-heated)
2.69	91.30%	77.76%	2.60	32.63%	27.63%
2.31	87.32%	79.76%	3.42	36.93%	31.63%
4.50	83.34%	88.90%	4.57	52.33%	27.93%
5.53	155.18%	127.52%	5.38	52.73%	26.03%
6.58	74.18%	86.52%	6.55	47.93%	41.53%
7.50	43.52%	78.54%	7.52	51.53%	42.33%
8.37	123.54%	135.48%	8.32	49.73%	41.13%

Graph 1: standard curve

Data from group 12:

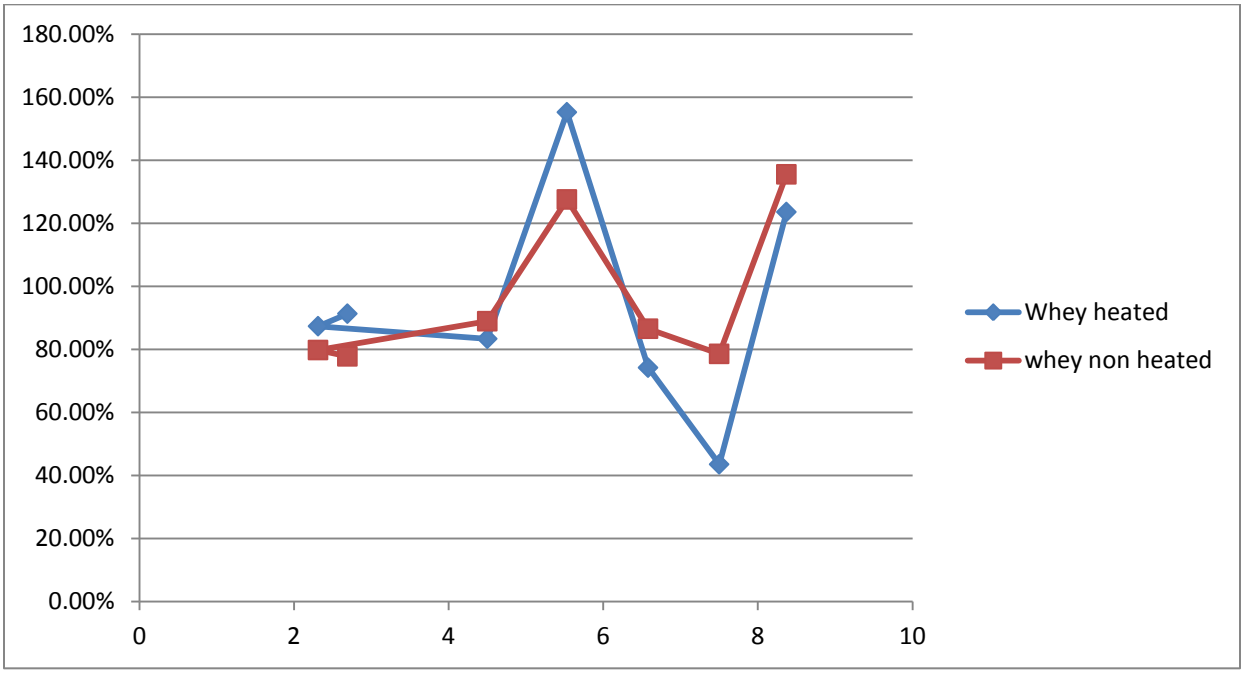


Data from our group:

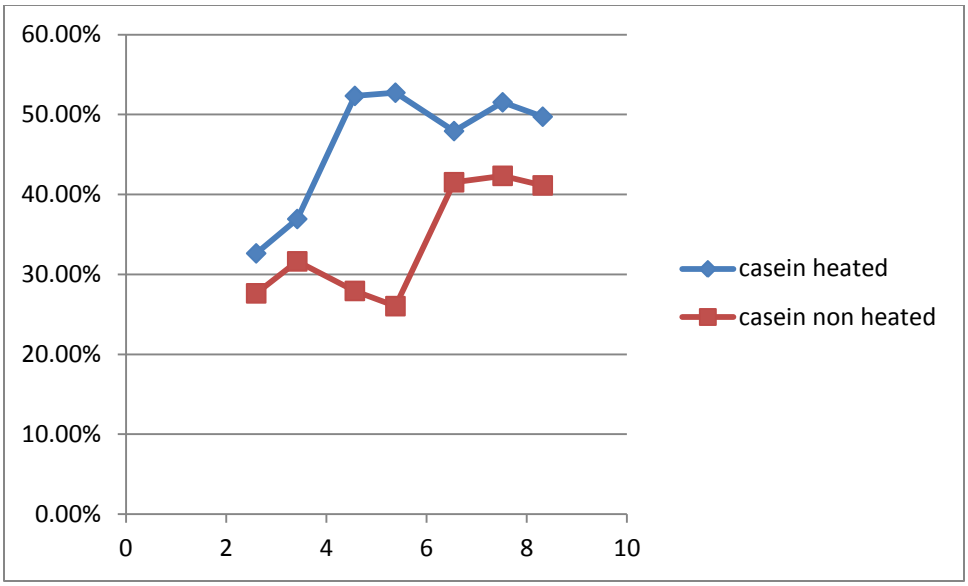


Graph 2:

% solubility of Whey as a function of pH:



% solubility of casein as a function of pH:



Calculation:

% Solubility whey= final protein concentration* 100/original protein concenteration=-→ 0.4565*100/0.5=91.3%

% Solubility casein= final protein concentration* 100/original protein concenteration=-→0.3263*100/1=32.63%

Finding final whey concentration:

Y = 1.0048x + 0.0853

Where Y is Absorbance and X is the Concentration of protein

0.544=1.0048X + 0.0853

X= 0.544-(0.0853) / 1.0048

X=0. 4565 (mg/mL)

Final Concentration of Casein:

Y= 0.9012X+0.006

Where Y is Absorbance and X is the Concentration of protein

0.333= 0.9012X+0.006

X= 0.333-(0.006)/ 0.9012

X= 3263 (mg/mL)

Discussion:

Standard curve was used to get the concentration of the proteins. This formula ($y = 0.9012x + 0.006$, $R^2 = 0.999$) was used for casein protein and this ($y = 1.0048x + 0.0853$, $R^2 = 0.9936$) was used for whey protein. Change of Heat and pH of milk causes denaturation of whey proteins, leading to a complex mixture of whey protein aggregates and casein micelles. Heat and pH change the structure of proteins and that result in the change of their function. Whey is more sensitive to heat and it can be denature by adding heat to it. It would unfold and it can get insoluble by denaturing it because of structural change and disulfide bridges. The caseins protein precipitate at pH 4.6 and the whey remain after the casein have been removed by precipitation and are soluble at pH 4.6; as we saw in our data that Casein has the lowest solubility at pH approximately 5.38 and whey protein has the least solubility at pH approximately 7.5. Whey is more compact so it is more resistance to pH. Casein has the lowest solubility at pH approximately 5.38 and whey protein has the least solubility at pH approximately 7.5. At these points the pH of solution is at isoelectric point so the net charge is zero (it is neutral) and water binding and solubility is at minimum point. In other word based on the change in pH the solubility of the proteins change because of the change in their structures.

Conclusion:

In this lab we learned that function of proteins depends on their structure. In general solubility increases with polarity of the protein surface and decreases as molecular weight increases. Proteins provide the body critical amino acids. The major proteins in milk are casein and whey. These two milk proteins are both excellent sources of all the essential amino acids, but they differ in one important aspect, whey is a fast digesting protein and casein is a slow digesting protein. The caseins are phosphoproteins which can be precipitated at pH 4.6 and the whey is soluble at pH 4.5 and remains after caseins precipitated are removed. Whey makes approximately 20% of the protein of milk and caseins makes 80%. Caseins exist in colloidal aggregates (micelles) but whey proteins are dissimilar of globular and heat labile. The whey proteins are much more divers and sensitive to heat than caseins. The globular protein unfold easily with the addition of heat and becomes denature and insoluble due to structural change. At some point

the pH of the solution is at isoelectric point so the net charge would be zero and water binding and solubility would be at minimum point which for the whey protein was at 7.5 and for casein was at 5.38. Having a compact and more globular structure of whey protein makes it more divers. The higher level of cystine in this protein results in more disulfide bonds which stabilizes the shape.

Questions:

1- Casein has the lowest solubility at pH approximately 5.38 and whey protein has the least solubility at pH approximately 7.5. At these points pH the solution is at isoelectric point so the net charge is zero and water binding and solubility is at minimum point.

2- In general solubility increases with polarity of the protein surface and decrease as molecular weight increases. The caseins exist as colloidal containing several thousands of individual protein molecules, whey proteins are dissimilar group of protein and they are mostly globular and heat labile. Whey proteins are more divers, they tend to be more hydrophobic and hydrophilic amino acid along their peptide chains and possess higher level of cystine. This is the reason that whey protein is globular and compacted. The whey protein is more sensitive to heat. Casein protein is more negatively charged because it is more hydrophobic. Whey protein is more compact so it is more stable. (According to lab manual). Whey is more sensitive to heat and it can be denature by adding heat to it. It would unfold and it can get insoluble by denaturing it because of structural change and disulfide bridges.