CHEM& 131 Name: _____

Protein Analysis

Background

Milk is composed of many biological molecules including proteins and fats. The major proteins in milk are caseins, which can be coagulated by several methods including agitation, acidification and heating. A number of food products including whipped cream, buttermilk and cheese are derived from coagulation of milk proteins.

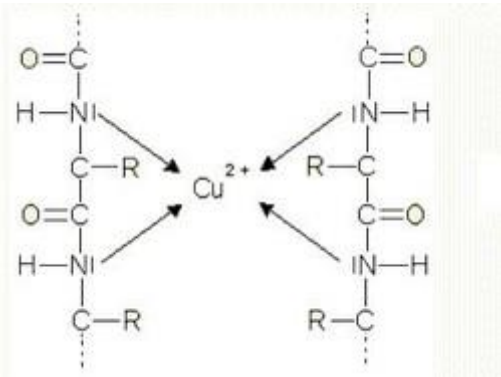
In this lab activity you will be coagulating milk by heating and analyzing the protein content by two protein measurement techniques, the Biuret test and the Xanthroproteic test.

Xanthroproteic test

The Xanthroproteic reagent uses nitric acid to nitrate (add a NO_2 group) to amino acids with aromatic side-chains. When protein is present this test results in a yellow color, the same color that appears when nitric acid is dropped onto human skin. Alternately, a strong base can be used to test for the presence of tryptophan or tyrosine. The addition of a strong base removes hydrogen from the hydroxyl group of tyrosine or the secondary amine of tryptophan. This shifts the absorbance frequencies of the aromatic ring leading to a new color of orange or rust.

Biuret test

The biuret reagent uses Cu^{2+} added to an alkaline (basic) protein solution. The Cu^{2+} ions form a complex with the amide nitrogens of the peptide backbone:



This complex involves 4 amide nitrogens chelated to a Cu^{2+} ion. This chelation shifts the absorbance characteristic of Cu^{2+} resulting in a color change from blue to purple or pink. A positive biuret test indicates the presence of molecules, like peptides, which contain two amide groups joined by a single carbon or nitrogen.

Waste disposal

Milk protein and other foods can go in the garbage. Wash the whey down the drain with plenty of water. Ethanol and diethyl ether should go into the organic waste container. Solutions containing HNO_3 and biuret reagent should go into the aqueous waste container.

Procedure

1. Place 30 g of milk in a 125 mL Erlenmeyer flask. Clamp the flask, containing your milk sample, to a ring stand and suspend the flask in a water bath set to a temp of about 40 °C. The water bath can be made by heating water in a 500 mL beaker. Heat sample for 10 minutes
2. After heating, remove flask from clamp and, while swirling the sample, add 8 drops of glacial acetic acid. Continue to add drops of acetic acid as long as casein continues to precipitate. Do not add too much acetic acid, be sure to stop after casein no longer precipitates with each drop. It can be hard to tell when the casein has stopped precipitating from solution, use your best judgment.
3. Filter the solid using a vacuum filtration apparatus. Place the filtrate into a new beaker labeled "whey" to test for protein later.
4. Place the solid in a 100 mL beaker and add 30 mL of a 1:1 mixture of ethanol-diethyl ether to dissolve the fat. Stir the mixture and filter the mixture through a new piece of filter paper on your vacuum filtration apparatus. Rinse the contents of the beaker onto the filter paper with small portions of the 1:1 ethanol-diethyl ether solvent.
5. Dry the protein by allowing suction to continue for several minutes.
6. Weigh a watch glass. Scrape the casein off of the filter paper, onto the watch glass and weigh it.
7. Test a small amount of the following foods using both the Xanthroproteic test and the Biuret test:
    a. Your prepared samples: Casein, whey
    b. Rice, butter beans, bread, sliced almond, swiss cheese and white meat.
    c. Marshmallow, radish and sugar.
8. Xanthroproteic test:
    a. Part I: Place 3-4 drops of concentration nitric acid on a **small** piece of food sample (1 grain of rice or 1 bean or a 3 mm x 3 mm piece of cheese) in a porcelain well plate. For the rice, bean and almond, you will need to break it down by grinding or slicing. The reaction should occur within 60 seconds. Record your observations.
    b. Part II: Add 3-4 drops of ammonium hydroxide to the spot. The reaction may take up to 10 minutes to notice an effect. Record your observations.
9. Biuret Test
    a. Add 3-4 drops of 6 M NaOH to a small piece of food sample on a porcelain well plate, then add 3-4 drops of 0.1% $CuSO_4$ solution. Record your observations.

Data/Calculations (show calculation for e and f)

1. CASEIN IN MILK
    a. Mass of milk sample: _____
    b. mass of watch glass: _____
    c. Mass of watch glass and casein: _____
    d. mass of casein: _____
    e. % casein in milk sample =
    f. % protein listed on the product label =

2. PROTEIN TESTS

Record observations and color changes for each protein test in the table below

	HNO_3	NH_4OH	$CuSO_4$ + NaOH	Analysis
Casein				
Whey				
White meat				
Radish				
Marshmallow				
Rice				
Beans				
Bread				
Sliced almond				
Cheese				
Sugar				

Post-lab questions

1. Based on your measurements, do you agree with the value for % protein listed on the label? Explain why or why not? How reliable/reproducible is your data? Describe steps you could take to make your data more reliable/reproducible. Attach more pages as needed.

2. Summarize the results of the Xanthroproteic and Biuret tests. Explain how specific pieces of data either confirmed or contradicted your expectations. Be sure to discuss all protein measurements, not just those for milk. Attach more pages as needed.