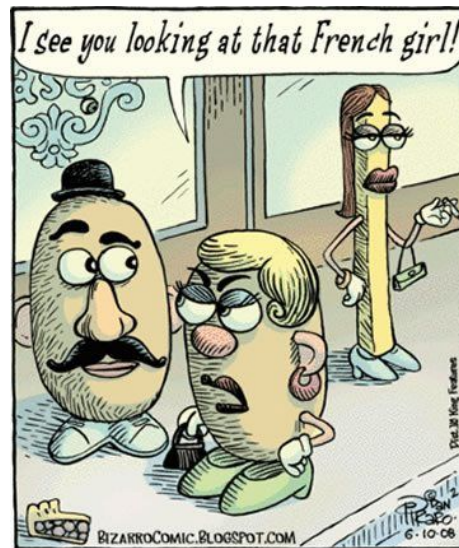


Centrifugation and Protein Determination

Lab Report 3

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Introduction:

In this experiment students will learn how to determine protein concentration using a spectrophotometer. Protein concentration is one of the most fundamental measurements made in physiology laboratories. Students will use bovine serum albumin (BSA) to make a standard curve of absorbance against protein concentration. Potatoes will be used to make a crude mixture of TES buffer and potato pieces to allow for the determination of concentration of protein. Students will use red and gold potatoes to see the difference of protein concentrations between different types.

Methods:

1. Primarily, approximately 5g of gold and red potato was sliced and homogenized for 30 seconds (Fig 1) then stored in an ice bath. The centrifugation step was overlooked and the samples were filtered and the supernatant was placed back in the ice bath.
2. A standard solution of concentration 1.000 mg/mL was synthesized using BSA and TES buffer solutions. Dilutions were made to obtain the following concentrations; 0.500 mg/mL, 0.100 mg/mL, 0.050 mg/mL, 0.010 mg/mL, 0.005 mg/mL.
3. Dye was made from a 4:1 ratio of solution of deionized water and Bio-Rad concentrate.
4. 0.100 mL of each of the standard solution concentrations were transferred into marked vials ranging from 0.000 mg/mL to 1.000 mg/mL, seven vials total of standard protein concentrate solutions (Fig 2).
5. 0.100 mL of prepared potato protein samples (red and gold) were also transferred into marked vials.
6. 5.000 mL of prepared dye reagent were added to all seven of the standard concentrate vials and the two potato sample vials (Fig 4).
7. All the vials were allowed a minimum of 10 minutes (a maximum of 1 hour) to incubate before inspection.
8. Absorbance was determined for each of the standards, to create a standard curve, as well as taken for each of the potato protein samples (Fig 6, Fig 7).

*NOTE: White potatoes were supposed to be used for this experiment, but Marcus said Wal-Mart was out of them. We exchanged the white potatoes with gold potatoes.

Materials:

- Varian Cary WinUV Spectrophotometer

- Tissue homogenizer
- Centrifuge
- Analytical balance
- 1 500 uL pipetman
- 12 100 mL beakers
- 2 30 mL centrifuge tubes
- 9 sample vials for protein determination
- Filter paper
- Bio-rad protein dye reagent
- 500 mL mM TES buffer (pH = 7.0)
- Bovine Serum Albumin (BSA)
- Red and gold potatoes

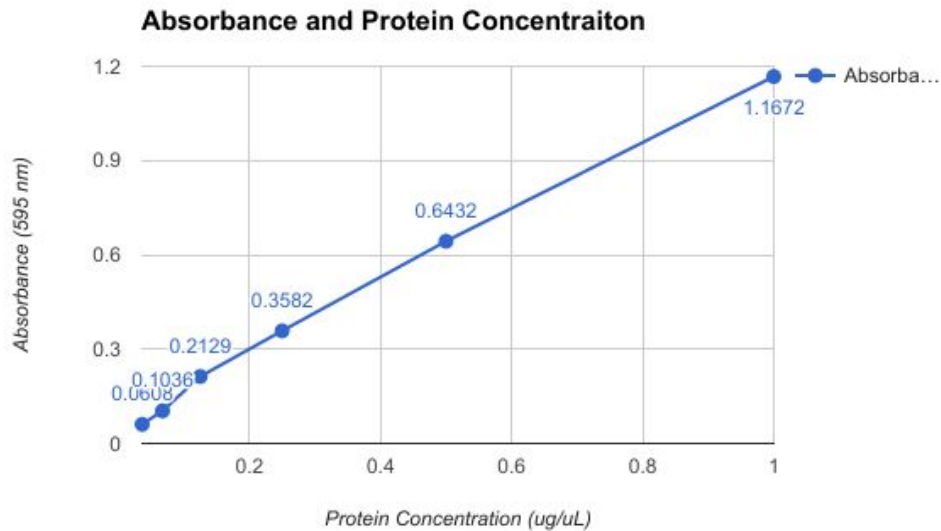
Results and Discussion:

The seven sample vials with protein concentrations of 0.0365, 0.0675, 0.1250, 0.2500, 0.5000, and 1.000 underwent color changes when Bio-rad dye was added (Figure 4). The vial sample solutions increased in deep blue color from least protein concentrated to most protein concentrated. This means that as protein concentration increases, the blue color of the dye increases, and the vials can act as a color code system for estimating the protein concentration of the two potatoes! And, the blue color system is just COOL to watch!

The protein standards, when plotted on a graph representing absorbance values measured at 595 nm and protein concentration values were expected to be a straight line (Graph 1). A nearly straight line was achieved, with a slight curve at the start of the line resulting from the first two absorbance values being slightly lower than expected, compared to the rest of the absorbance values that followed (Table 1).

Protein Concentration	Absorbance
0.0365	0.0608
0.0675	0.1036
0.125	0.2129
0.25	0.3582
0.5	0.6432
1	1.1672

Table 1: Protein concentration vs absorbance for the protein standard.



Graph 1. Absorbance vs Protein Concentration for protein standards.

Using the formula $m = \frac{\sum xy}{\sum x^2}$ the slope of the line (Graph 1) is 1.2100. The golden potato Varian Cary WinUV Spectrophotometer reading was 0.6431 and the reading for the red potato was 0.3779. Using the formula $x = y/m$, the protein concentrations for each potato can be calculated. The protein concentration for the golden potato was 0.7782 ug/uL and the protein concentration for the red potato was 0.4573 ug/uL. The golden potatoes have more protein than red potatoes.



Golden potato: "I am the mightiest!"

Obtaining samples of the red and gold potato protein concentrations from the other group, they reported values of 0.3039 $\mu\text{g}/\mu\text{L}$ and 0.4649 $\mu\text{g}/\mu\text{L}$ respectively. Using the formula, concentration (μ) = $\frac{\sum x_1 + x_2 + x_3 + \dots}{n}$ the average protein concentration of the red and gold potatoes are 0.3806 $\mu\text{g}/\mu\text{L}$ and 0.6216 $\mu\text{g}/\mu\text{L}$ respectively. If done

correctly, the standard error for the average protein values are 0.054 for the red potato and 0.11 for the golden potato. The standard error for the average protein values are found using the formula $SE = \sqrt{s^2/n}$ where $s^2 = \Sigma(x_i - \mu)^2/n - 1$. For the red potatoes average protein value the standard error was 0.0056 and for the white potatoes that standard error was 0.0353. From the registered values and calculations made, there is a significant difference in protein concentration between the two potatoes. The golden potato had a significantly lower absorbance ratio than the red.

At the end of the lab, there were potatoes left over. Kathryn took up Marcus on his generous offer of the potatoes, and took them home for her growing boys. This resulted in the boys learning about protein concentrations in the potatoes they were going to eat for dinner the next evening! This brought much excitement to Kathryn's house (Figure 8).



Figure 8: The remainder of the potatoes were taken home by Kathryn for her growing boys. They were very excited to learn about their potato protein sources and eat them!

Conclusion:

Through conducting this experiment we were able to conclude that gold potatoes had a significantly higher protein concentration than the red potatoes. By comparing the average concentrations of the protein from both potatoes we were able to successfully make our conclusion. The gold potato has significantly more protein due to the average being higher. Also, the range (by standard error) does not overlap with the average and range for the red potato. Group 3 had lower protein value in gold potato compared to the other groups which could be due to various reasons. Group 3 may have not cut up their gold potato in small enough pieces in order to obtain a better mixed potato/TES

solution. Also, difficulties filtrating the solution could lead to a decreased concentration for their gold potato.

Pictures:



Figure 1: In this photo you will see Morgan and Dr. Bidlack blending the potato into a solution that will have the buffer and potato liquid inside. We do this to allow a mixture of both. We did this with the machine in the picture called a Tissue Homogenizer.

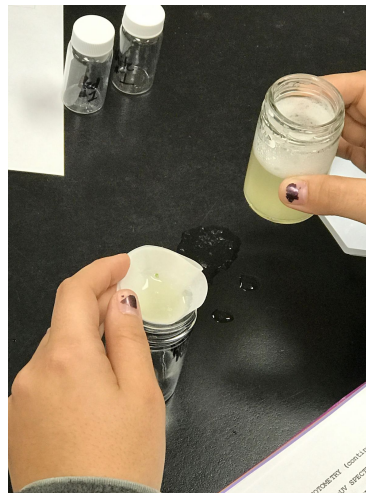


Figure 2: In this picture you will see Hannah filtering the solution to separate parts so we will have the ability to have 0.1 mL potato solution. We did this separate with filter paper which you can see in the picture.

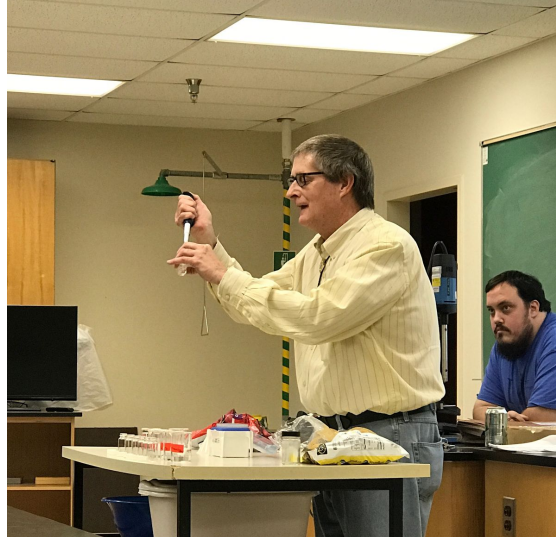


Figure 3: In this picture you can see Dr. Bidlack using the pipetman to transfer 0.1 mL of our potato buffer solution into a new “clean” vial. We are doing this to see the protein content into solution.

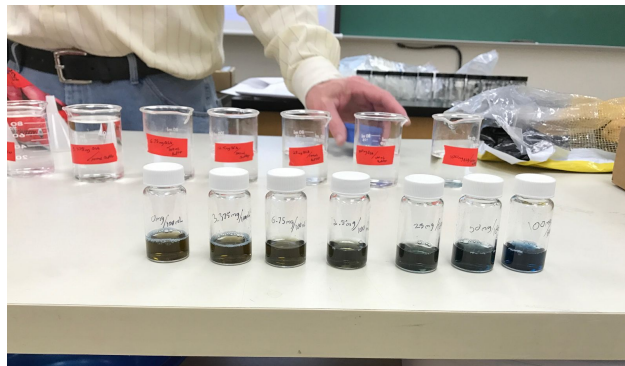


Figure 4: In this picture you can see the **BEFORE** solutions with an assortment of dilutions moving from left to right in a 0 to 100 mg per 100 mL.



Figure 5: In this picture you can see the **AFTER** solutions with an assortment of dilutions moving from 0 to 100 mg/mL, left to right. There is a distinct difference in color. You can also see that science is **FUN** based off of the students in the picture!



Figure 6: In this picture you can see the **GOAT** of the class performing an example of how to use the disposable spectrophotometer equipment to observe the absorbance of the protein in the potatoes and Bio-Rad protein dye reagent.

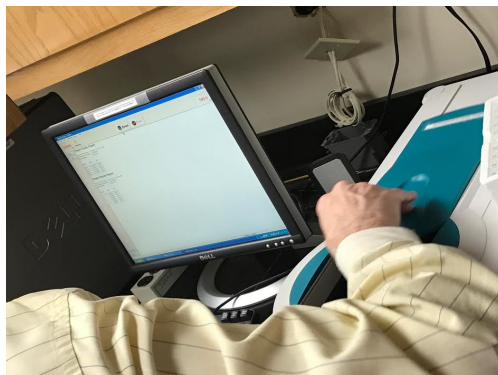


Figure 7: In this picture students can see how to use the spectrophotometer and what it is. In this you can see Dr. Bidlack showing off his skills to find the absorbance of the protein standards.