

# Radiant Energy and the Determination of Protein and Carbohydrate Content of Foods

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## 1. Abstract

The purpose of our research was to investigate and suggest a method of protein detection that leverages spectrophotometry to determine the protein or carbohydrate concentration of a given sample. The method involves utilizing a standard curve created by data collected through spectrophotometers, which function by reading levels of radiant energy. After a couple of extensive labs, it was found that comparisons could be made between the standards and samples. Even though the research group was unable to fully justify this method, the data received from the labs helped justify that this method was an improvement on current methods.

## 2. Introduction

In China there was a concern about the poor production of food and usage of hazardous chemicals. This issue also included the recent pet food scare, which resulted in the illness or death of many animals in the United States as well, and may eventually endanger human lives.

Food is a necessary part of life, and in the current expansion of the human population and their pets, it has become increasingly difficult and complex to

provide the entire population with nutrition. For this reason, food manufacturing and transportation has become a science in itself and therefore must be monitored. Also, as the population grows, so must the food industries. Unfortunately, that allows for more frequent errors and corruptions, which was the case in China.

In light of the recent pet food scandal, there have been serious health issues concerning the use of tainted protein in feed, which resulted in the death or illness of numerous animals. As a result, there have been questions concerning the methods of protein detection and use of hazardous chemicals. However, spectrophotometry, which is the process of measuring light in order to calculate the concentration values, seems to provide a better method of detecting protein and carbohydrate concentrations.

Another problem is the transportation of agricultural goods, which are susceptible to perishing. Therefore, it has become necessary to measure the level of production, consumption and distance from market must be factored in to maximize efficiency. This especially holds true for tropical plants that are highly susceptible

to rotting and over-ripening. For this reason, certain methods of preserving the product can be used until they have reached the shelves. At this point though, it is important to assure high quality, which can be done through analysis using spectrophotometers. We investigated the method of spectrophotometry in order to avoid cases similar to the pet food scare and to solve the produce issue.

### **3. Background Information**

#### **A. Overview**

In our day and age, food has become a major commercial issue as the myriads of peoples no longer produce their own food. Instead, they rely on mass production to obtain this basic necessity. As a result, monitoring and maintaining sanitation and economical efficiency is becoming increasingly difficult for the food industry. For this reason, researching new methods of testing food products to ensure quality and safety is of utmost importance. However, there naturally are a multitude of obstacles and problems to overcome. Of the many, two that will be discussed and addressed concern the recent pet food scare and the degradation of fresh products.

#### **B. Pet Food Scare**

One issue involves the recent scare resulting from the poor method of protein measurement of several pet food companies. These companies used wheat gluten protein in their pet food, but the company that supplied this protein used a chemical toxin, melamine as to boost the nitrogen content of their product in place of actual protein because it contains approximately sixty percent nitrogen and would lead the tests to measure nitrogen rather than protein,

they showed higher nitrogen levels than the actual levels of protein. Unfortunately, as a result of the use of this poor practices and testing method, numerous pets were poisoned by melamine, resulting in damage to the reproductive system and bladder cancer. Moreover, it has been discovered that the method of using melamine as a substitute was also implemented in livestock feed and subsequently has become a possible agent for biomagnifications, the accumulation of toxins in the food chain, which could potentially reach the human population.

Surprisingly, the practice of using melamine as a protein substitute is common in China. Therefore, it was necessary to replace the poor method with a more appropriate method of protein detection, one that would measure the amount of actual protein rather than nitrogen. Before a new test could be suggested, the subject being tested and means for testing must be thoroughly understood.

#### **C. Protein Background**

A protein is a group of large, complex organic compounds composed of amino acids. The sequence of these amino acids determines the overall structure and function of the protein. Also, proteins are found in all living cells and are essential parts of organisms, as they create the actual structure of every physical part of the organism. Proteins also play a crucial role in metabolism and functioning of living matter. Each protein molecule mainly contains one of twenty residues or “R-groups”, and the main chain, which comprises a series of elemental atoms of nitrogen, carbon, and oxygen. There are various chemical bonds between the residues and also the bonds

between the main chains, which are known as peptide bonds, are formed as the result of a dehydration reaction, which is the junction of compounds by removing a water molecule. These reactions and forces then shape the protein into its correct shape with the aide of other proteins and enzymes in four levels of organization, the primary, secondary, tertiary, and quaternary. These levels are different steps of organization for the protein. The amino acid sequences are coded according to information transferred from genomic sequences, which creates a correlation between an organism's DNA and physical form.

Proteins are the "building blocks" of organisms, and are important in food, and therefore are preferred in high quantities. Using Melamine in food products is very common in China for the purpose of increasing the apparent quantities of protein content of food. This is why companies added Melamine to the pet food, as they hoped to increase sales, despite the illegal and hazardous results. Therefore, it is strongly believed and suggested that a more appropriate method of protein detection would be to measure the amount of actual protein rather than the amount of nitrogen. For this reason, it is sensible to use protein assays, which require spectrophotometers, as it is a possible method of detecting protein, rather than the nitrogen that the protein is composed of, as it has proven to never always be the case.

#### **D. Transportation, Storage, and Packaging**

The other major issue the food processing and production industry has recently been confronted with involves the transportation and preservation of

produce from the farm to the place of consumption. Produce and perishable foods are common in the diets of those living in developed nations. However, many perishable goods cannot be produced in certain areas due to climatic, agricultural, structural, and economic obstacles. As a result, many of these goods must be shipped long distances to reach their consumers. In the course of this journey there is a high risk of these products degrading to the point of decay.

#### **E. Carbohydrate Background**

Carbohydrates are molecules that are mainly composed of carbon, hydrogen, and oxygen. The general formula for Carbohydrates is  $C_nH_{2n}O_n$ . Carbohydrates can be categorized into those used for energy, those used for structure, and those used for chemical intercellular communication, thus making Carbohydrates a necessity for organisms. These Carbohydrates are essential for life because they are the main source of easily obtainable energy for living organisms, which is imperative to perform all metabolic processes through the synthesis of ATP during glycolysis. Although it is possible to derive ATP from other organic compounds, such as amino and nucleic acids, these other compounds allow for the production of less energy than Carbohydrates and are generally needed for other tasks in the organism.

Carbohydrates can be separated into groups depending on the number of individual Carbohydrate units, or saccharides, in the entire molecule. If there is only one Carbohydrate unit, it would be called a monosaccharide. Carbohydrate molecules with two units are called disaccharides. Molecules with three units are called trisaccharides and

so on. Carbohydrate molecules with many units are called polysaccharides.

Carbohydrates used for energy are essential for life because they are a main source of easily obtainable energy for living organisms, which is imperative to perform all metabolic processes through the synthesis of ATP in glycolysis. Although it is possible to derive ATP from other organic compounds, such as amino and nucleic acids, these other compounds produce less energy than Carbohydrates and are generally needed for other tasks in the organism. This category of Carbohydrates contains simple and complex Carbohydrates. Simple Carbohydrates are easier to break down to its basic components than complex ones. This is because complex Carbohydrates contain many more bonds than simple Carbohydrates, therefore requiring more reactions in order to break the molecule into its simpler constituents. Since simple Carbohydrates are easier to convert into energy, it acts as a more immediate source of energy, while complex Carbohydrates can serve as storage for energy. The process of breaking complex Carbohydrates into its simpler parts is known as hydrolysis, which is removal of water molecules in order to cleave the Carbohydrate.

Carbohydrates also play an important role in the structure of certain organisms. These Carbohydrates are generally more difficult to break down and digest. For example, the cellulose in plant cells is made up of Carbohydrates which allow the plant to maintain its shape and structure. However, the plant does not use the cellulose as an energy source; it uses starch as energy instead. Another example of a structural carbohydrate is chitin, which makes up the exoskeleton of arthropods and is

usually the substance making the cell wall of fungal cells.

The amount of time elapsed in plant ripening can be determined by the concentration of Carbohydrates in the produce.<sup>1</sup> In the lab, the specific case study of this problem was a comparison of time versus concentration of Carbohydrates in bananas. Bananas serve as an excellent example of this issue because they are only sustainable in tropical areas, but are still in high demand in milder locations. Furthermore, a banana, like all fruits, undergoes a process known as ethylating, commonly known as ripening. However, in the banana this process can be very acutely observed by the drastic color changes occurring in the banana peel. This ethylating process proceeds due to the plant hormone ethylene, which is also responsible for converting cellulose into simple-sugar Carbohydrates.

In both cases the concentration of the substances is a key aspect of the problem. The change in concentration of carbohydrate can be calculated using radiant energy and spectrophotometers, and the amount of time elapsed generalized by the various stages of banana ripening from green, to yellow to brown. The comparison of these three concentration values can then be used to optimize the timing of fruit transportation. Similarly, the concentration of protein (rather than nitrogen) can be calculated using this same technology. But this then begs the question, how does radiant energy technology work and how can this be applied to this problem?

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<sup>1</sup> According to Doctor Rong Di and Professor Raul Machado

## F. Spectrophotometers

Spectrophotometers work by reading and analyzing levels of radiant energy. More specifically in this lab, their function is to read the amount of change in radiant energy. Radiant energy is simply energy of electromagnetic waves. Its wavelengths range from radio waves with wavelengths as long as thousands of meters to gamma rays with wavelengths as short as a million-millionth of a meter. It is also the quantity of radiation emitted by a light source and propagates in the form of electromagnetic waves, which may be viewed as streams of photons. In fact, every object emits radiant energy in the form of electromagnetic waves, which is absorbed by an object and converted to heat.

As electromagnetic waves are important to radiant energy, it is important to understand the physical qualities of electromagnetic waves and electromagnetic radiation. Electromagnetic radiation is defined as self-propagating waves that can be classified by their wavelength and frequency. The more energy a wave has, the higher its frequency will be and the shorter its wavelength will be. Wavelength is measured in meters, and the typical range is  $10^{-16}$  to  $10^8$  m, the latter being lowest energy. Frequency is measured in Hertz, ranging from  $10^{24}$  to 1 Hz, in decreasing energy. Some high-energy waves include x-rays, gamma rays, and ultraviolet light, while some low energy rays include microwaves, radio waves, and infrared radiation. In the middle is visible light, whose wavelength spectrum ranges from 380 nm to 740 nm. At these frequencies, the cone cells in the retina of a human's eye can detect and interpret changes in the frequencies that we call color. These

changes occur when photons of visible light with varying frequencies, which is essentially the different colors, are emitted from an object and are either absorbed or reflected by that object. The reflected photons can then be detected by our eyes. Thus, the color that we perceive an object to be is the frequency of light that it reflects.

The different colors occur at different frequencies between the 380 nm to 740 nm thresholds. The visible light spectrum, which are the different values of frequencies representing each color, goes from red, orange, yellow, green, blue, indigo, to violet in order of increasing frequency. The intensity of this color depends on the concentration of the pigment in the object that is absorbing or reflecting the light. Due to this, scientists have been able to use devices known as colorimeters and spectrophotometers, which is the subject of study, to measure the absorbance of a specific wavelength of light of different solutes to derive the concentration of given solute based on the intensity of color.

A colorimeter is a very basic instrument that shines a light source through a colored filter. This filtered light then travels through a cuvette, which contains the pigmented solution, and the remaining unabsorbed light is measured using a detector, usually a photo resistor. The colorimeter is usually attached to a computer, which processes the results. Spectrophotometers perform a very similar process, but replace the cuvette with a 96 well-plate, which allows 96 solutions to be read at once.

Most of the time spectrophotometers and colorimeters are used to identify the concentration of a solution with an unknown concentration. These identifications can be made using

the Beer-Lambert Law or by making a standard curve using known concentrations of the solution and plotting this versus their measured absorbance. The Beer-Lambert law gives a mathematical relationship between absorbance ( $A$ ), molar absorptivity ( $\alpha$ ), distance the light travels through solution ( $l$ ), and concentration of the solution ( $c$ ). this relationship is expressed mathematically as:  $A = \alpha l c$ . This relationship mathematically proves that there is a direct relationship between the absorbance of light of a pigmented solution and the concentration of the pigment in that same solution.

#### **G. Alternative Methods**

For the spectrophotometry method of determining protein concentration in the lab, the Bradford assay was used. The Bradford assay works by measuring the shift of light from 465 nm to 595 nm. The pigment is created by adding the dye Coomassie<sup>®</sup> Brilliant Blue, which works by binding stoichiometrically, or chemically in specific proportions, to the protein molecules. The Coomassie<sup>®</sup> Brilliant Blue alone will be read at 465 nm, while Coomassie<sup>®</sup> Brilliant Blue bound to protein will be read at 595 nm. Therefore, the radiant energy value of the 595 nm light of several samples of known concentrations can be inputted into a standard curve graph, allowing for any concentration value to be found, as explained earlier. There are several other types of assays, including Biuret protein assay Lowry protein assay Bicinchoninic acid protein assay Amido black protein assay. Although each has their own advantages and disadvantages, the Bradford assay is the easiest and fastest method and can be performed in room temperature without the use of special

equipment. For these reasons, the Bradford assay is the ideal choice as it is the most likely to keep pace with the mass production methods of companies.

The method of determining the protein concentration through finding the nitrogen concentration can be done through the Kjeldahl method, which involves the breakdown and boiling of organic substances containing nitrogen. This method was discovered by Kjeldahl in 1883, in which it was found that by boiling the organic nitrogen, it would release ammonium sulfate, which could then be measured. It is also important to note that this is a relatively simple process, and therefore can be implemented at a low cost, even lower than that of spectrophotometry. Unfortunately, this method is meant only to find the nitrogen content, as explained earlier before, it will not find the true value of the protein content. Therefore, when using this method, companies may and have been able to bypass the system and make it appear that there is more protein in the product by increasing the amount of nitrogen, but not necessarily the amount of protein. There are other methods of nitrogen determination, including the Dumas and Combustion methods, but unfortunately, they create the same issue as the Kjeldahl, and therefore should be avoided despite their variability in standard deviation and expense. In any case, whatever the method used, it is imperative to access a new method to possible future food processing problems.

## 4. Experimental Design

### A. Overview

The purpose of this experiment is to identify the value of the unknown concentration of a sample solution. In our experiments, these solutions were five different protein solutions and solutions of glucose derived from three bananas at different stages of ripeness.

To do this the proteins and Carbohydrates had to first be isolated. In the case of the protein solutions, this had already been done for us. In the case of the banana carbohydrate solutions, we took a small specific amount of fruit, mixed it with water using a vortex machine and then centrifuged this sample so we could separate the cellulose from the glucose.

In order to pigment the solutions so the spectrophotometer could differentiate between concentrations we mixed the solutions with specific dyes. We then raised the temperature of the solutions to activate the dye.

After being dyed, the absorbency of the sample was analyzed using the spectrophotometer. By plotting the known concentration of the standard solutions against the measured absorbency, a standard curve was made. This created a linear equation from which values for concentration could be calculated by comparing the measured values of absorbency.

### B. Protein Assay

The first lab performed was the protein assay, which utilized the BioRad's Bradford kit in order to measure the concentration of protein in given samples. The samples had to be mixed with a dye and stock base from before their absorbency could be measured. The stock base, BSA, was made through the combination of 3  $\mu$ L

of stock BSA (50  $\mu$ g/mL) with 147  $\mu$ L of dH<sub>2</sub>O, and then put into the vortex until well mixed. For the measurement of these very minute volumes, we used micro-pipettes.



(above: micropipettes)



(above: tubes and tray used)

We multiplied 2 by 6 to determine the amount of dye plus six (the number of standards) yielding the total number of tubes. This number was doubled by 2 mL, which is the amount of the solution needed for each test sample. Thus, the amount needed was 36 mL of solution. However, to promote ease of measurement, the total volume of the solution had to be a multiple of 5. Therefore, 40 mL of dye was made. Thus 32 mL dH<sub>2</sub>O and 8 mL of dye were mixed using a mini vortexer.



(above: mini vortex machine)

Each of the 18 tubes then had 2 mL of dye added along with a given amount of the stock BSA. The amounts for each solution are found on the Table 1.1. 3  $\mu$ L of each sample being tested was then added to its respective tube in duplicate, was placed in a centrifuge at 1400 rpm, and was allowed to sit for 5 minutes.



(above: centrifuge)

**Table 1.1**

BSA ( $1\mu\text{g/mL}$ )	Dye (mL)
0	2
2.5	2
5.0	2
7.5	2
10.0	2
12.5	2

The standards and samples were then distributed into a 96-well plate placing 200  $\mu$ L per well.

The tray was then read at 595 OD using the protocol “bjlBRAD.prt”. This program will read the change of pigments in the solutions to the 595 nm value, which then will provide how many  $\mu\text{g}$  of protein is present.

The results of this measurement were then inputted into Microsoft Excel and a standard curve was derived. The standard curve, by plotting the known concentration of the standard solutions against the measured absorbency, allowed us to derive the concentration of the samples.

### C. Carbohydrate Assay

The second lab performed was the carbohydrate assay, which compared the concentration of glucose in unripe, ripe, and overripe bananas based on a standard curve made using known concentrations of several standards. Half of a gram from an unripe, a ripe, and an overripe banana were obtained and each were weighed and placed in a weight boat on top of a top-loading balance. The banana samples were then placed in a mortar along with 6 mL of distilled water and grounded using a pestle. The samples were then poured from the mortar into a 15 mL conical

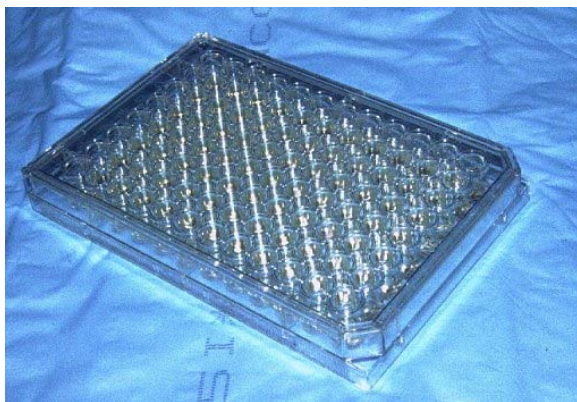
tube. The mortars were then rinsed with 6 mL of fresh distilled water and this was also poured into the conical tubes. This resulted in a banana to water ratio of approximately 1:25.

The conical tubes were then centrifuged for 10 minutes at 1500 rpm at room temperature.

During the 10 minutes, 30  $\mu\text{L}$  of eight different standards at the values of 0, 50, 100, 200, 400, 600, 800, 1000  $\mu\text{M}$  glucose were added in duplicated to a series of labeled centrifuge tubes. 100  $\mu\text{L}$  of an unknown substance (Solution A) and 100  $\mu\text{L}$  of another unknown substance (Solution B) were added to each tube and mixed gently.

At this point the 3 samples could also be put into smaller tubes with solutions A and B. All of the tubes were then capped and placed in boiling water at 85 to 90 degrees Celsius for 10 minutes. The tubes were removed, were allowed to cool, and were gently mixed. They were then centrifuged for a few seconds to integrate any condensation.

200  $\mu\text{L}$  from each tube was then transferred into a 96-well assay plate.



(above: 96 well plate)

The absorbency of each well was then analyzed at 450 nm using a spectrophotometer.

A standard curve was then plotted from the glucose concentrations

and absorbency readings of the standards. The slope and intercept of the curve was then determined. The concentrations of the banana extracts were then compared to that of the standards to determine the glucose concentrations of each banana sample.



(above: spectrophotometer)

## 5. Results

For the protein assay, the results of the standards, as found of appendix A, were used to make a standard curve. That way, the concentration of the unknowns could be calculated through comparison with those of the standards. Next, the data of the standards was edited so that the extreme outliers in the group would not adversely affect the final standard curve. For example, the six concentrations of the first group of standards were 0.57, 0.58, 0.34, 0.34, 0.26, and 0.33. However, the concentrations of 0.57, 0.58, and 0.26 do not fit the correlation of their standard curve. This data was removed from the group of standards to prevent the standard curve from being inaccurate. An inaccurate standard curve would have very adversely affected the overall experiment because we derive the value of the concentrations of the unknown directly using this standard curve, and any noticeable inaccuracy would cause

*For the entire Protein Assay results, see Appendix A.*

an inaccuracy in the calculated concentration of the unknown.

When the values were inputted, it was found that the results showed a trend in which higher concentrations read at higher spectrophotometric values. The increase was also increasing as a constant, therefore making the relationship between the two values linear.

The data of the standards was then inputted on a Microsoft Excel spreadsheet and graphed as a standard curve. From this standard curve we could use the data values for the absorbance of the unknowns received by using the spectrophotometer to derive the concentration. All this required was finding the absorbance value for the unknown on the x-axis and finding the corresponding value for concentration on the y-axis by means of the standard curve. From this comparison, the concentrations of the protein solutions with unknown concentrations were determined to be: 0.942, 1.494, 3.346, and 1.808 molal.

For the carbohydrate assay, the same basic method was used, and the unknown concentrations of the green, yellow, and brown bananas were determined to be: 24919.889, 27049.042, and 30855.566  $\mu$ molal respectively. This shows a growing trend in carbohydrate concentration as the banana ripens.

## 6. Laboratory Errors

There were noticeable extreme outliers in the data, which are most likely the result of human error, exacerbated by multiple students participating in the same experiment.

Ideally, each student would perform the experiment individually, rather than all four students participating

in one. However, this would be very expensive.

The reason these circumstances are not ideal is that the pressure each individual exerts on the micro-pipette changes drastically from person to person when operating with such small volumes. As a result, the values received from multiple people vary significantly, and makes the derived data unreliable and difficult to analyze. When one person works on an entire experiment, the pressure exerted on the micro-pipette is far more similar, and even if the data is not accurate in relation to overall accepted values, it is accurate in relation to itself. Since the unknown concentration is determined based on the standard curve that the same experimenter determined, accuracy in relation to accepted values is not very important, but accuracy in relation to itself is of utmost importance.

For this reason, it is important to perform the removal of extreme outlying data from the unknowns. For example, one group of samples with the same unknown concentration of protein received absorbance values of:

1.16	0.76	0.65
0.61	0.58	1.04

All of the above absorbance values should have been the same, or very similar since they all used the same concentration of protein solution. However, the above highlighted values were significantly different from the other four absorbance values. Because they varied tremendously from the other four, we removed the highlighted value and assumed the actual concentration of

*For the entire Carbohydrate Assay results, see Appendix B.*

the solution was of the solutions with the absorbance of around 0.6.

Keeping in mind the circumstances, the data we received was relatively acceptable. The correlation coefficient ( $r^2$ ) for our standard curve came out to be 0.918, where the value closest to 1 is ideal.

## 7. Conclusions

As seen from the various experiments performed, the research group was able to conclude that spectrophotometry could be viable methods for detecting protein directly, bypassing the issue currently seen with current nitrogen tests. Therefore, it was concluded that instead of the method in which some pet food companies used to detect protein content through nitrogen concentration, the methods involving spectrophotometry and colorimetry should be used to detect protein content fairly and correctly. Even though the proposed methods of spectrophotometry and colorimetry would probably be more costly than the current method used by the pet food companies, the use of spectrophotometry would be able to halt the use of Melamine and other nitrogen rich toxins from being used in pet food, or in fact, any food product. It would then prevent food related illness for pets and people alike.

Likewise, it is imperative to find methods of determining carbohydrate content in order to increase produce transportation efficiency. By using spectrophotometers, the amounts of Carbohydrates can be determined in imported produce, which can then be analyzed in order to assure the plants will ripen at the correct time. Else, the product would go to waste, thus creating an economic deficit. This is also important to detect possible bacteria

agents, which can ruin the food, and cause illness of death if consumed.

It could not be concluded though, that the method is entirely correct as there was no definite evidence as true values to calculate the stand deviation of the spectrophotometry test were absent, and will be further investigated as future work.

## 8. Future Work

Theoretically, the method researched can be a solution to the current issues, but due to a lack of information, the method's accuracy will be assessed in the future as well with other alternative methods of protein and carbohydrate determination.

A large cause of the recent pet food scare was cost. If the spectrophotometric test earlier described had been used in place of the test that only measured nitrogen content, the toxic chemical used to supplement the nitrogen content of the pet food would not have been used. However, since this test is far more costly and less efficient, the dangerously unspecific alternate test was used. The cost and limited extent to which spectrophotometry could be advanced to the point of being cheap and efficient enough to be a viable alternative expose the necessity for an alternate test that would prove equally efficient as that already in use and arguably as accurate and specific as spectrophotometry. We have recognized this necessity and devised a plausible alternate method.

This method would make use of a protein-dependent bacteria and microscope. The bacterium may be one already in existence, or a genetically modified strain that has had the gene coding for the proteins enabling the metabolism of Carbohydrates replaced

by one that codes for the metabolism of proteins. Before the test could be performed, like in spectrophotometry, a standard curve of sorts would have to be made, relating the amount of protein to the dependent growth of the bacteria. When this relationship has been established, a known volume of a given sample of food would be added to a known quantity of bacteria in the form of a culture. By monitoring the growth of the bacteria over time (time would also have to be factored into the standards established), one would be able to determine the protein content of the sample using no more than a microscope, measurement device, and bacterial culture. As opposed to the spectrophotometric method, where the protein sample must be isolated, then processed, then dyed, mixed, measured, tested, analyzed and the data derived, this method would require fewer steps, much lower cost, and far less expensive lab equipment. Also, it is likely the lab-employees performing the test could be far less experienced without receiving any less accurate data.

To make this testing process successful, the bacterium with aforementioned qualities would have to be isolated and mass produced. Also, an accurate means of tagging the bacteria would need to be identified, as well as the standard relationship between bacterial growth and amount protein over time. Furthermore, it would be necessary to make sure that the bacterium would consume all types of protein, and only protein. However, if these tasks were to be successfully completed, a new viable, reliable, accurate, and efficient means of measuring protein content would ensure the integrity of all processed food.

Naturally, spectrophotometers can have a promising future in other studies and objectives. This is due to the fact that radiant energy can be applied to analyze any desired substance or material in a solution, there is a wide range in which this field can expand. Likewise, radiant energy can provide a more correct method of concentration and substance detection, as the infrared system is capable of not only detecting objects, but also their sizes, shapes, and lengths without being disturbed by outside forces or obstacles. Already, there is a method of detecting early stages of cancer by analyzing a patient's diluted cell plasma's, which have been treated with an aluminum oxide adsorbent, with spectrophotometers. Although no future works are currently being pursued by the project group in the field, spectrophotometer analysis has potential for solving various situations.

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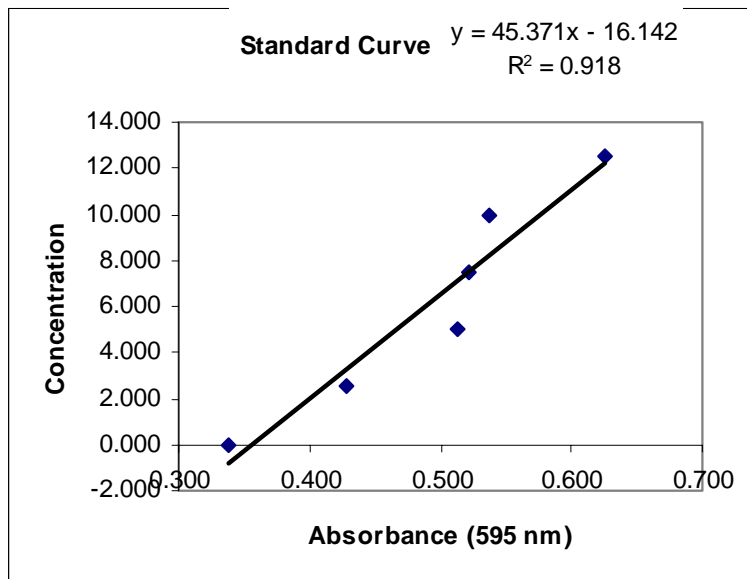
## Appendix A: Data of Protein Assay

Sample	Absorbance			Mean	Std. Dev.	ug/4 ul	Conc. (ug/ul)
A	0.505	0.525	0.525	0.439	0.088	3.768	0.942
	0.351	0.367	0.360				
B	0.513	0.572	0.583	0.488	0.079	5.976	1.494
	0.410	0.423	0.424				
C		0.763	0.650	0.651	0.080	13.383	3.346
		0.612	0.578				
D	0.414	0.500	0.464	0.515	0.099	7.232	1.808
	0.506	0.502	0.705				
E	0.697	0.574	1.121	1.227	0.780	39.506	9.876
	2.241	0.570	2.156				

*Sample of Unknown Concentration*

Standard	Absorbance			Mean	Std. Dev.	Conc. (ug/ml)
1			0.343	0.338	0.005	0.000
		0.338	0.333			
2		0.436	0.436	0.428	0.009	2.500
		0.429	0.419			
3		0.504	0.521	0.512	0.010	5.000
		0.517	0.524			
4			0.516	0.522	0.009	7.500
			0.514			
5			0.526	0.536	0.010	10.000
		0.530	0.535			
6			0.547	0.625	0.052	12.500
		0.546	0.524			
			0.622			
		0.568	0.585			
			0.650			

*Standard Input of Values*



## Appendix B: Data of Carbohydrate Assay

Standard	Absorbance			Mean	Std. Dev.	Conc. (uM)
1	0.357	0.445		0.401	0.062	0
2	0.466	0.500		0.483	0.024	50
3	0.619	0.725		0.672	0.075	100
4	0.910	0.920		0.915	0.007	200
5	1.364	1.350		1.357	0.010	400
6	1.275	1.422		1.349	0.104	600
7	1.462	1.585		1.524	0.087	800
8	1.721	1.568		1.645	0.108	1000

*Standard Input of Values*

Sample	Absorbance			Mean	Std. Dev.	Conc./25	Conc. (uM)	
1	1.835	1.838		1.837	0.002	967.676	24191.889	
2	2.030	1.959		1.995	0.050	1081.962	27049.042	
3	2.205			2.205	#DIV/0!	1234.223	30855.566	

*Sample of Unknown Concentration*

