China: The “Greatest” Walk of My Life

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Dr. Norman Borlaug- although I never had the privilege to meet you in the flesh, I feel as if I know you very well through the stories and legacy that you have left. I will never be able to thank you enough for envisioning a brighter future and investing in us students to create a new world through science and dedication.

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Thank you to everyone who made this internship such a success.

Iowa? Where's Iowa? This was the common response I received when I told the Chinese students where I was from as they bombarded me with questions. Soon, I became even more proud to represent Iowa, the United States and most all of, the prestigious World Food Prize Foundation, as it was the reason I was even hearing this question posed from Chinese students in Beijing, China.

Standing at 6'2", I really stood out among the swarms of people. I got stares everywhere I walked, but it was alright with me. I knew that I had to persevere and make the very best of my experience here in Beijing. After seeing the Food Science Research building at the Chinese Academy of Agricultural Sciences, I knew that I was here for a purpose and I was so excited to dig into my research and try my best to make a difference in this world.

Never would I have thought that I would be living in the heart of China for two months, doing research with some incredible students and fully embracing Chinese culture. The things I have seen and done here will forever be with me and I can wholeheartedly say that my life has forever been changed for the better.

Background

I had some previous experience with the World Food Prize Foundation long before I was ever personally involved. My oldest brother was an intern eight years ago in Brazil and as a young student, I saw the impact it had on his life and the wonderful opportunity and experience the WFP Youth Institute had afforded him. I told myself, *I want to do that when I get old enough.* Eight years later, I received my chance and I am so glad that I was able to attend the 2009 World Food Prize Youth institute, as it marked the beginning of my long and fruitful journey.

My full name is Donovan Christian Richardson and I graduated from Creston High School in the spring of 2010. I am from Creston, Iowa, a small town of about 8,000 residents located in the southwest region of our great state. I live in a rural area with many fields and pasture acres nearby.

I have been an active member of both FFA and 4-H and have a strong grasp of basic agricultural education. I felt that I could contribute to the research done by WFP interns and that I could make a positive step in the direction of food security. The first step was to write my first research paper pertaining to the topic of "Food Insecurity" and attending the 2009 World Food Prize Youth Institute. I worked through the summer with my English teacher, Mrs. Qualseth, to write my paper which dealt with the country of Malawi and how a few simple steps could be taken to massively improve yields of crops. I recommended that some simple measures like subsidies being offered for fertilizer and hybrid seed could make a dramatic difference as had already been demonstrated. The "Malawi Miracle", as it had been dubbed, more than tripled the yields of the farms and even allowed the country to export to neighboring countries.

The Youth Institute was a wonderful experience and after talking to some of the other students, I knew there were many intelligent and bright individuals present. Round Table discussions became one of my favorite things to watch at the Symposium. They were so interesting and the people participating came from all walks of life with diverse areas of expertise in food. We packaged meals for families in third-world countries and the self-satisfaction I gained from that was phenomenal.

About the World Food Prize Foundation

The idea of the World Food Prize Foundation came from Dr. Norman Borlaug in 1970 when he was selected as a Nobel Peace Prize winner. Dr. Borlaug had dreams of forming his own prize for agriculture to recognize the accomplishments of agriculture laureates. In 1987, the first World Food Prize laureate was selected and the World Food Prize has continued to be awarded to deserving individuals who have made great strides in agriculture and providing enough food for the world. John Ruan then established the World Food Prize Foundation in 1990 and it has been thriving ever since. Borlaug-Ruan Internships began in 1994 and have grown each year to allow for high school students to travel to more countries. To date, over one hundred students have been awarded a Borlaug-Ruan internship which has allowed these students to travel to research centers and villages working on both scientific and social issues concerning agriculture.

Cultural Exchange

The culture I saw and experienced in China cannot be done justice with words. The beauty, rich history, heritage, and breathtaking views instilled such awe in me that sometimes my only reaction was to be quite literally speechless. I am so blessed that I was able to experience Beijing and the rich culture that China had to offer. I was able to visit the Forbidden City, I Dream of Red Chambers, Emperors' Palaces, and numerous other places. Climbing up to the top and walking along the Great Wall of China was an unforgettable moment. I felt like I was on top of the world!

Walking around the city of Beijing was simply indescribable. Everything felt surreal or perhaps like experiencing a very vivid dream. Just walking down the street, I had such a spring

in my step. Knowing that I had actually made it all this way after working so hard for nearly a year writing papers, attending events, interviewing and waiting for all of it to come to fruition. *I am here in Beijing,* I kept telling myself!

One of the things I enjoyed most while in Beijing was their customs and traditions. The fact that they honored a non-Chinese person with such traditions was even more humbling as a foreigner in China. Cheers were offered to me all the time during dinners like “welcome to Beijing” or “good luck to you with your work and best of times with your stay”. When they would explain the meaning of such things to me I felt it really was an honor to receive such hospitality. A common custom was to eat noodles because their length is a good sign of long life. We also ate dumplings when I left because it was a sign of good luck and they had to be eaten in pairs or even numbers. It was incredible to be a part of these traditions.

The history that exists in Beijing and China in general is utterly phenomenal. We have nothing like that here in America and although we learn about it in school, you cannot put it into perspective until you are actually on the Great Wall or walking through the Summer Palace. The way students took such pride in their country was contagious, I soon felt this sense of pride--of almost belonging in Beijing as I knew the same culture and had been at the site of such great emperors and events in Chinese history.

There were definitely a lot of differences between my hometown in Iowa and China. In all honesty, I was not too fond of some of them. The smog and general pollution was very bad in some places and the cleanliness of some parts of the city was very low. Some people were rude and I was expecting to hear the word “sorry” when someone accidentally got in the way or shoved past you. After being there for two or three weeks, I soon realized that this was part of

their culture. They were not doing anything wrong at all! It was I who was expecting too much and assuming that things would be similar to the United States. I soon had to embrace the culture and realize that this was completely normal in this country and that there was nothing wrong at all with what they did.

Research and Lab Work

My project began with some preliminary research and learning about the basis and purpose of the project. I was assigned a lab partner, Shihe Han, and she will be referred to many times throughout the remainder of this paper. I reviewed numerous articles and works from food science experts. I also spent a lot of time reacquainting myself with basic chemistry and biology as well as the structure of proteins as it would be essential to the continuation of my experiment.

BACKGROUND INFORMATION: I became aware of the large threat that food allergies pose against both adolescents and adults in not only America, but all over the world. In America, about 6 percent of children and 3.7 percent of adults suffer from a food allergy. Although there are many food products that may trigger a food allergy, there are only eight foods that make up 90 percent of all food-based allergies; peanuts being the third largest cause of food allergies. According to Health Day News, the number of peanut allergies in America has nearly tripled from 1997 to 2008. With peanut allergies on the rise all over the world, it creates a problem for supplying protein. Peanuts are an excellent, inexpensive source of protein and with the problem of allergies, parents are apprehensive in using or cooking with peanut products due to the large fear of anaphylaxis in their children, or themselves. Labeling problems, utensil contamination, and manufacturing errors are large problems all over the world as consumers who

are allergic to peanuts try to avoid them. This has put increased pressure on the Food Science sector to fully analyze and identify the allergenic proteins within peanuts.

PURPOSE: 1.) To extract and purify peanut proteins through various methods and then analyze proteins to detect all allergenic proteins from the peanut, 2.) testing how these allergenic proteins interact in our rabbit specimens and, 3.) study these allergenic proteins in-depth.

METHOD: To begin the experiment, starting with raw peanuts was essential. The shells were removed from the peanuts, then dried at 40°C until the moisture content was below 4 percent. Following the drying process, the red skins were removed from the peanuts and the peanuts were ground into a fine powder, similar to that of white household flour, using a kitchen grinder. Once the peanuts were in a fine powder, the addition of N-hexane was added in a 1:7 ratio of peanut powder to N-hexane respectively. The fat was removed, the mixture centrifuged, and dried at room temperature and then the process was repeated. Finally, the powder passed through a mesh strainer, number 60. The powder is now ready to be used for ammonium sulfate precipitation and isoelectric point precipitation.

Using a centrifuge was also something new to me. I learned basic protocols and instructions of using the machine and the various attachments for various sized test tubes. I had to be sure to weigh the test tubes with the solution so that they were equal. If I did not weigh the amounts and put them into the machine, it would become off-center and possibly damage the machine beyond use. I became proficient at this and would be able to come within approximately 0.05 gram of the desired amount.

Rough protein purification: The powder is blended into a solution of Phosphate Buffered Saline (PBS) in a 1:20 ratio meaning 1 gram of peanut powder to 20 mL of PBS buffer.

PBS buffer is a water based solution that is simplistic to combine the reagents. The peanut powder and PBS buffer are stirred together until the powder is dissolved into the PBS buffer. The solution is then transferred to an Ultrasonic Cell Crusher machine which sends ultrasonic waves down a rod that is sitting in the solution. Ultrasonic waves break down the cell walls of the peanut and they also aid and accelerate the protein purification process. The peanut powder and PBS solution had to be surrounded with ice water during the process as the ultrasonic waves elevate the temperature of the solution and therefore could have tainted our results if the proteins became denatured. The Ultrasonic Cell Crusher machine was allowed to run approximately twenty minutes. Following the use of this machine, the solution was centrifuged at 9,000 g for 10 minutes at 4°C (as to not denature the protein). After the centrifugation process, the solution is then divided into three different horizons: 1) lipids and oils on top, 2) the protein solution in the middle and 3) starches on the bottom. The fat that was possible to remove was discarded. Using a pipette, I had to be extremely careful and conscious to remove the protein layer without accidentally removing either of the other two layers. The starches and what other lipids escaped the previous removal, are then dissolved in an ethanol and water blend with a ratio of 20:80, respectively. We believe that there were possible proteins present in the starch so we also conducted protein purification with it. A blend of ethanol and water is used to extract some of the peanut protein. The buffers previously did not have a high enough alkalinity level to dissolve some of the proteins, such as Arah 2, which is dissolved in the ethanol blend. Once the protein is dissolved in a buffer solution, it can then be further studied through SDS-PAGE.

PBS buffer Protein Purification: Beginning with the PBS buffer protein solution, the purification process began. Ammonium sulfate precipitation simply requires a person to modulate the concentration of ammonium sulfate of a solution so that the solution in turn

precipitates the desired proteins. Using a concentration chart, I weighed out the required amount of ammonium sulfate to bring it to 40 percent concentration and gradually added it to the PBS protein solution until it was completely dissolved. The solution then sat for 30 minutes and then centrifuged at 9,000 g for five minutes at 4°C. After centrifugation, a stirring rod was utilized to remove the thin layer of lipids that remained from the rough protein purification process. Following this, the supernatant was transferred to a flask to be modulated to 50 percent ammonium sulfate concentration. The remaining precipitate in the test tube was our protein. 0.01mol PBS buffer was used to dissolve the protein and then transferred it to dialysis bags.

The dialysis bags had to be prepared before use. The bags are very stiff to begin with and must be soaked in pure ethanol for a few minutes and then soaked and stored in distilled water which causes a chemical reaction that makes the bags pliable and very easy to use. The bags are also reusable and had to be washed in a specific manner. We first washed them three times with tap water and soap, one time with distilled water, one time with ethanol and then three times with distilled water and then stored in distilled water again. When the dialysis bags are not in use, they should be stored in ethanol in a refrigerator so they do not become contaminated and skew results.

Precipitates that were dissolved in the PBS buffer from the test tubes were then transferred to the dialysis bags for a number of reasons. It is the final step of the protein purification process before samples can be extracted. Dialysis bags allow ethanol and distilled water to move out of the solution so that only the purified protein remains. The pores in the dialysis bags are small enough that we did not be worry about the protein moving out of the dialysis bag. We let the solutions sit in distilled water four hours, then added in PBS buffer to the

water so that the ammonium sulfate moves out of the dialysis bags. Additionally, the ammonium sulfate interferes with results when doing SDS-Page as it is a smaller molecule than protein.

Isoelectric Point Precipitation: The remaining half of the PBS buffer solution was precipitated using a process called Isoelectic Point Precipitation (IPP). It is similar to the ammonium sulfate precipitation in a number of the steps. Utilizing a pH meter and 0.1 M of citric acid, the pH of the PBS buffer protein solution was modulated to 6.6, and then sat for one hour. Research stated that the isoelectric point of peanut protein is between 6.4 pH and 4.0 pH. After the rest period, the solution was centrifuged at 9,000 g for 5 minutes. The samples were removed from the centrifuge and the supernatant was removed to a flask to have the pH further modulated. The precipitate was dissolved with PBS buffer and placed in a dialysis bag, following the same steps as previously discussed.

Continuing with IPP, the supernatant was titrated with citric acid after each centrifugation using the 0.1 M of citric acid and modulating in 0.2 pH increments. Using different amounts of the solution had to be tested and it was soon evident that 100 uL was a safe and time efficient starting point for modulating the pH..

Ethanol/water solution precipitations: As previously mentioned, the Rough Protein Purification divided the peanut powder in three parts: 1) lipids, which were removed, 2) protein solution, which was precipitated using two methods in a PBS solution and finally 3) starches, which will be precipitated using the same two methods. The starch was dissolved in a solution of ethanol and water in a 20:80 ratio, respectively. We used a 50 ml solution instead of a 100 mL solution with the PBS buffer as the starch layer was much smaller and the larger quantity is not needed.

The proteins that were present in the starch were precipitated and collected in the same manner as the protein solution from the original peanut powder. There were some differences in the ways that the different precipitates were handled. I experienced a large amount of difficulty in getting the ammonium sulfate to dissolve completely and this problem became evident even at 60 and 70 percent concentration. IPP also posed a problem sometimes as the pH changed very quickly even with a minute addition of citric acid. I had to adjust my titration technique by using the smallest measuring pipette the laboratory owned. This proved to be much more suitable.

Isolating, detecting and identification of proteins

Following the completion of the protein purification process, the isolation, detection and most importantly, identification of the proteins present in the peanut could begin. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis or more commonly referred to as SDS-PAGE, is a procedure used to isolate protein molecules and then examine them with computer software. A Sephadex G-100 Column is a purification procedure that also allows a look at molecules of proteins on a very small and exact scale. Both methods detect and identify proteins by their molecular mass as they precipitate through various gels. It was my responsibility to learn and master both of these procedures during my stay at CAAS.

Sephadex G-100 Column: Sephadex G-100 is a slow detection process, but it is quite accurate as computer software is used to pinpoint exactly where proteins are present. The Sephadex G-100 Column is best used after proteins have been isolated into one to three molecule strands. You may refer to **Figure 1-A** to see the general set up of the Sephadex G-100 column. My partner, Shihe Han had previously combined the reagents for the Sephadex gel and had prepared the station. The gel is added to the column and placed in a refrigerator that was kept at 10°C as to not denature or remove certain enzymes in the proteins and protect the UV detector.

Within the refrigerator, there is a pump that is hooked into the column that pumps PBS buffer into the tube so that it dissolves the protein. Other buffers are acceptable, but we decided it would give us the best test results if we utilized the same buffer as in our other tests.

Figure 1-A: UV detector and Sephadex Column

We set up the UV detector to cycle every ten minutes. If you refer to **Figure 1-A** once again, you will see the rack of test tubes present in the bottom of the cooler. These tubes collect the samples that are excreted from the Sephadex column. The UV detector measures the samples that pass through and then, if a protein is present, a spike in the graph occurs. The horizontal axis on the graph is measured in the time interval so we are able to trace back and find the test tube which contains the protein. They are measured in ten minute intervals allowing the user to trace the time back to the assigned test tube.

The Sephadex tube is emptied and put into each hole and the gel is washed. The gel is set up on a metal stand with a clamp that holds the tube in place. The wash procedure begins by placing a 500 mL beaker under the tube, and removing both end caps from the tube. Utilizing a rubber bulb, the gel is forced down with air pressure, it pushes the gel out the bottom and into the beaker. Using distilled water, the gel and distilled water are stirred together in the beaker and then allowed to sit until the solution separates. The supernatant is then poured out, leaving only

the gels. This process was repeated several times to ensure that there were no contaminants or foreign materials present in the gel. These foreign particles could affect the precipitation rate of peanut proteins within the gel and perhaps skew the results that the UV detector produces.

SDS-PAGE: This was another form of detecting proteins. The trials were shorter but the entire duration of the endeavor was longer. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is another method that detected proteins based on the molecular weight. It utilized an Electrophoresis machine. Personally, I found perfecting this process very hard as it required many precise measurements and a steady hand because some of the steps and procedures were very difficult. It also required a high amount of safety as many of the chemicals used in preparing the gels are toxic to humans.

This part of the experiment began by preparing the slides and setting up the electrophoresis machine. A rubber strip was put in between the slides to prevent the gel solution from leaking out as the solution set up into a gel. There was one gel on each side of the machine which was secured in place with a clip. After the gels were in place, the reagents for the isolation gel could be combined. The Isolation gel consisted of:

- 3.6 mL distilled water
- 5.2 mL acrylamide
- 3.0 mL tris pH-8.8
- 120 uL 10 percent concentrate SDS
- 120 uL ammonium persulfate
- 6.0 uL TEMED

These reagents then had to be stirred together very quickly and then distributed to each slide very quickly as the gel sets up rapidly. After the gel was distributed, the top of the gel was

covered with distilled water which needed to be added very slowly and deliberate as to not disturb the gel. The whole process had to be done quickly to create a good, solid gel for use in SDS-PAGE. This gel was allowed to set for ten minutes. Dump the excess distilled water out of the slides that was placed there to keep the gels moist and contained.

The Concentration gel consists of nearly the same reagents. The purpose of this gel is to concentrate the proteins into a straight line so that they precipitate in the same shape. Concentration gel consists of:

- 2.7 mL distilled water
- 0.67 mL acrylamide
- 0.5 mL TRIS
- 40 uL 20 percent concentrate SDS
- 40 uL ammonium persulfate
- 6.0 uL TEMED

This solution was combined in the same manner as the isolation gel. After the gel had been dispersed to the two slides, plastic combs were inserted into the slides to make wells for the proteins that would be added later. The combs were inserted slowly, and if there was any remaining concentration gel, it was added across the teeth of the comb to be sure to make a good, solid well. The gels then sat for approximately one hour to ensure they were completely set up. During the hour wait, the protein samples were prepared for the process.

The protein buffer solution was then prepared. It consisted of the following reagents:

- 0.24 mL of 0.5 M Tris-HCl, pH 6.8
- 1 mL glycerol
- 0.8 mL of 10% (w/v) SDS

- 0.2 ml of 2- mercaptoethanol
- 40mg bromophenol blue
- 7.76mL distilled water

The protein samples were first dissolved in PBS buffer in a 1:1 ratio in the sample tubes.

After the gel slides had set up, the plastic combs that formed the wells were removed and at this time, the rubber insert in between the slides was removed. Electrophoresis running buffer filled the gap between the two gel slides. Then, the protein samples were distributed into the wells using a 10.0 uL sample. A marker protein was also inserted into lane one of each gel so that a comparison can be made in the future. The top of the machine was then put into place and the electrodes were connected. The machine was set to eighty volts and is allowed to run until the samples formed a straight, horizontal line across where the concentration gel meets the isolation gel. The voltage was then increased to 120 volts and ran for approximately one to two hours until the samples had precipitated entirely to the bottom of the gel. After the completion of the process the machine was shut off and the running buffer solution was discarded. The slides were then removed from the machine and with great care and precision, the gels were removed into a glass petri dish. A Coomassie Brilliant blue staining solution was added to the dish so that it completely covered the slides. This allowed the proteins to be stained. The solution sat for about four hours and was then discarded. A destaining solution was then added for a two hour period and then changed to a fresh destaining solution. This was allowed to sit over night and then the following day, w. The solution and the gels needed to return to a near transparent color to allow a clear image of the proteins to be acquired from the machine.

With a clear gel acquired, a picture could then be taken of the gel. A specific type of photography machine was used as it brought the image up on a connected computer. Lighting

adjustments and other factors were manipulated to bring the gel into sight. The image was then saved onto a flash drive to be later transferred to a computer with Alphaerase software to analyze the image. I was instructed on how to use the software by my partner, Shihe Han. It took some work to become acquainted with it but it soon became relatively simple. A number was assigned to each of the heavy traces left in the gel, a mass that was known as it was the marker protein and had a sheet used to assign the numbers. We then assigned numbers to each of the blots on the remaining precipitates, paying special attention to the ones that were at similar levels. The software then calculated the mass of each compared to the marker. From there, the accuracy of our measurements were checked against known values of some of the identified peanut proteins.

As my time came to an end in Beijing, I was not able to complete all steps of this in-depth and tedious experiment. My lab partner and I continue to stay in touch and she is finishing the requirements of the experiment. She worked using the indirect ELISA procedure. The proteins were then treated by a process called enzymolysis.

RESULTS: On the following page, the analysis from the Alphaerase software can be found as well as the graphs produced from the UV detector in the Sephadex G-100 Column. We were able to get some great, clear results which were conclusive in studying the allergenic proteins within the peanuts.

Figure 1: Arah 1 allergenic protein

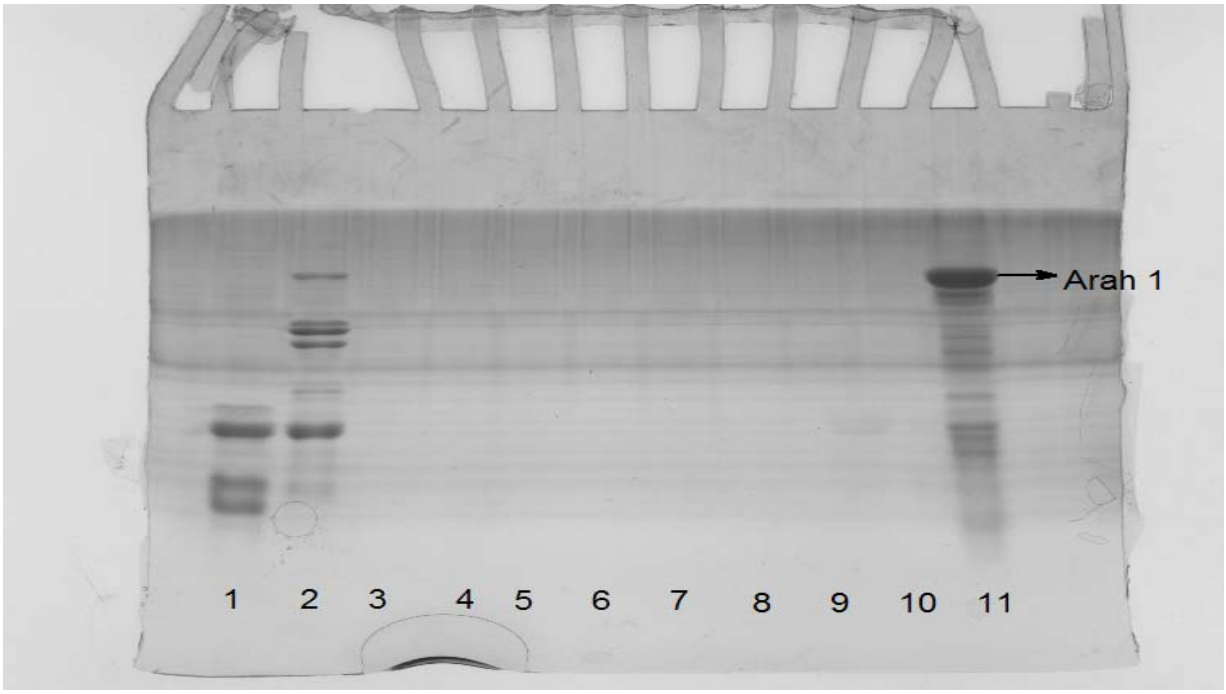

Figure 1: above clearly illustrates the allergenic peanut protein, Arah 1. In gel lane one, this is the ethanol mix, in gel lane is the PBS buffer and in lane eleven, is Arah 1.

Figure 2: Arah 2 allergenic protein

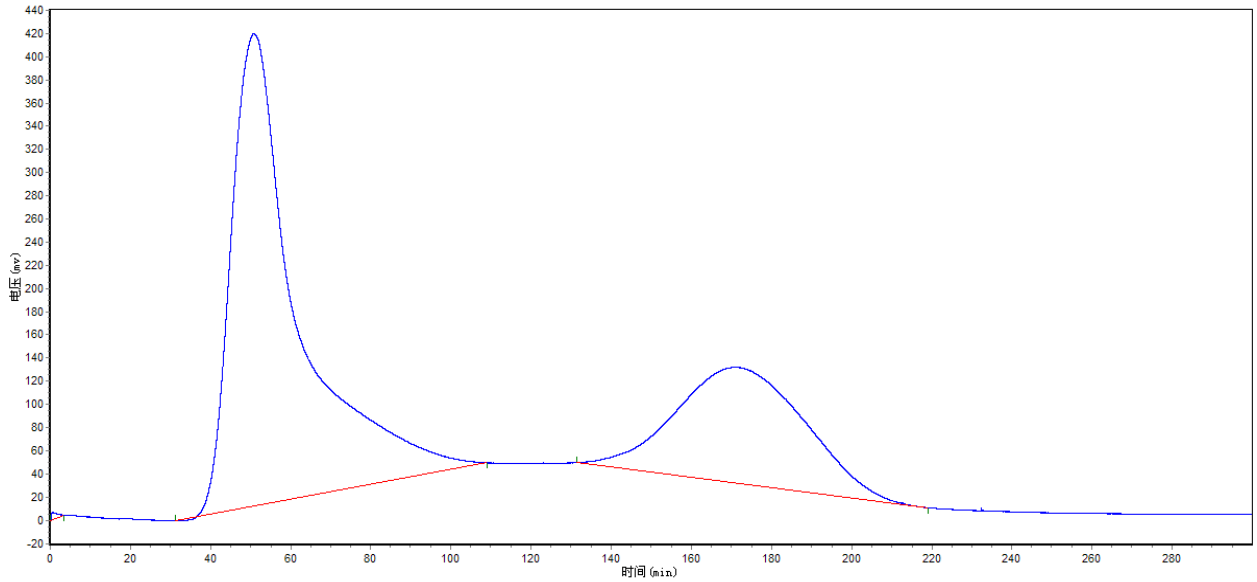

Figure 2: here shows a result from the Sephadex G-100 column and the UV detector. The large spike in the graph tells us there is a protein in that round. So, the sample is collected and then put through SDS-PAGE which produced the gel that was discussed earlier.

Figure 3: Arah 2 and Arah 3 allergenic proteins

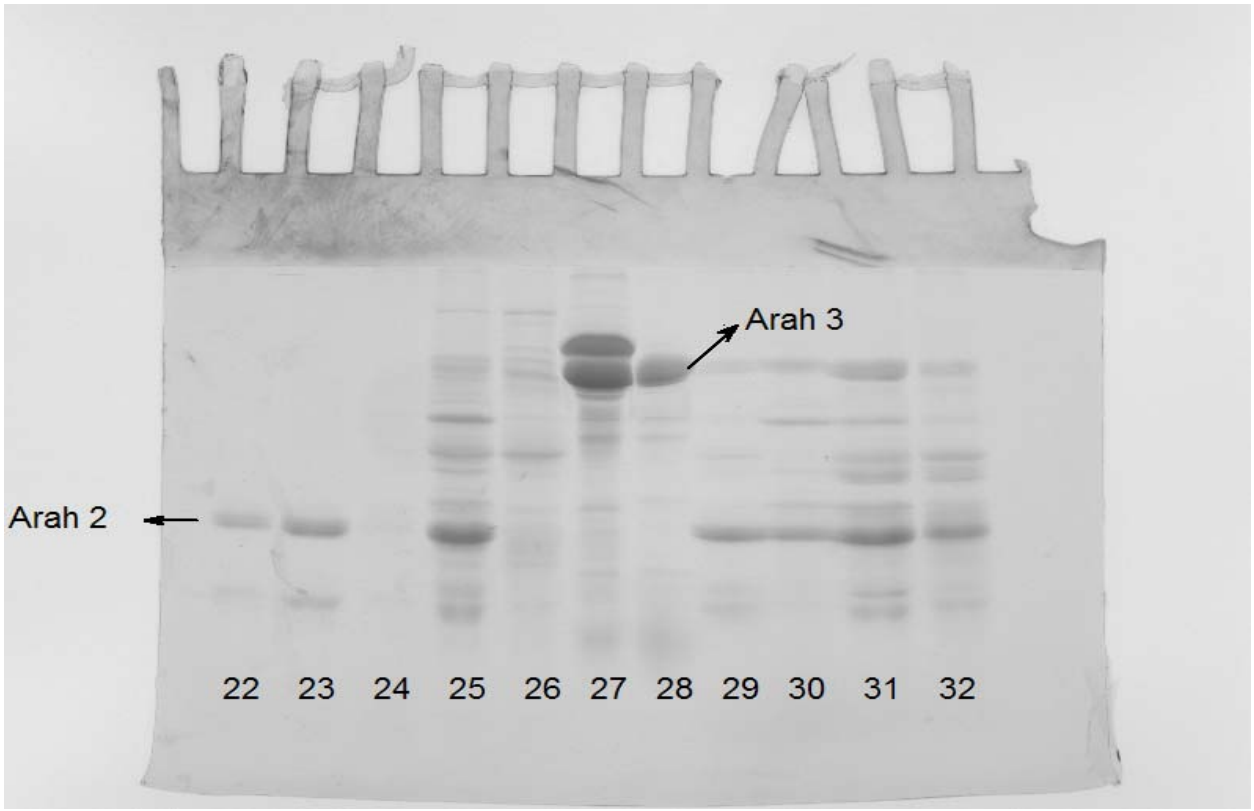

Figure 3: illustrates two known allergenic peanut proteins. The gels were facilitated through the Sephadex G-100 column yet again. The proteins Arah 2 and Arah 3 can distinctly be seen within this gel at sixty percent ammonium sulfate precipitation. The lanes of this gel are as follows: 22- ethanol, pH 6.4; 23- ethanol, pH 6.2; 24- PBS, 50% ammonium sulfate precipitation; 25- PBS, pH 5.6; 26- PBS, 60% ammonium sulfate precipitation; 27- PBS, 70% ammonium sulfate precipitation; 28-PBS, 80% ammonium sulfate precipitation; 29- PBS, 90% ammonium sulfate precipitation; 30- PBS, PH 6.4; 31- PBS, PH6.2; 32- PBS, PH 6.0.

CONCLUSIONS: From these results and the many trials that were completed in the lab, an accurate study of the allergenic proteins in peanuts was completed. In particular, we were able to cite Arah 1, 2, and 3 as commonly occurring allergenic proteins. The future of this experiment is deeply in-depth and labor intensive, but this small experiment and study has shown a positive light in that these proteins can be isolated and purified for additional study. The long term goal is to genetically engineer a peanut that is capable of providing a source of high quality protein as

well as not triggering peanut allergies. Peanuts are an inexpensive and easy source of protein to produce, something much sought after in today's world of food insecurity.

This experience that I was so incredibly fortunate to embark on was one that can be summed up in a simple word: unbelievable. The people that I met along my journey and the culture that I got to be a member of for eight weeks were absolutely phenomenal! When I entered into this internship and journey, I had plans to become a Food Scientist and after working at the Chinese Academy of Agricultural Sciences' Food Science Laboratory, those plans have been reaffirmed and strengthened. I cannot stress the importance of this internship enough and I cannot wait to see what the future brings. I hope to be a part of the fight against hunger and contribute from the Food Science and Technology sector.

As I walked the streets of Beijing, I looked around in amazement and just simply enjoyed how beautiful it was. The culture was fantastic to be enveloped in, and I truly enjoyed all aspects of my journey. All of the history, lab work, and sites blended together for this once-in-a-lifetime opportunity. As I reflected on this "great" walk in the journey towards my future, I remembered why I had traveled to China in the first place. I thought about the millions of people who starve and go hungry each and every night and I truly believe the research I did will hopefully benefit them someday in one form or another.

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