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A biochemical mini-research project: Can yeast fermentation decrease the amount of phytic acid in soybeans?

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Abstract: This mini-research project studied the effect of yeast fermentation on the nutritional value of soybeans, specifically the concentration of phytic acid, protein, and lipids. With prior experience in protein and lipids analysis, the students (n = 12) in this laboratory applied their critical thinking and problem-solving skills to design and conduct their own assays for phytic acid extraction and quantification. All students decided to use ion exchange chromatography to extract phytic acid and UV/vis spectroscopy with Wade's reagent for phytic acid quantification. These methods were evaluated by the students as simple and effective in analyzing phytic acid from soybeans. This project allowed students to use several of the methods used in other parts of the semester in addition to adding new methods to evaluate a research question.

Keywords: *Mini research project, Phytate, Phytic acid, Soybeans, Yeast fermentation.*

1. Introduction

Phytic acid (show in Figure 1), also known as hexavalent phytate anion, is a naturally occurring compound abundantly present in cereal grains and legumes where it serves as a major storage form of phosphorous as the $Ca^{2+}$and Mg^{2+} salts [1]. Although being an important component of plant seeds, phytic acid is indigestible for non-ruminant animals (e.g. swine, poultry, and humans) because these animals lack the enzyme phytase required to hydrolyze the phosphoester bond [2, 3, 4]. Phytate also has a strong binding affinity for mineral cations (calcium, iron, magnesium, manganese, zinc, etc.), rendering them unavailable for intestinal absorption [5]. Several methods of lowering the level of phytic acid have been reported, including cooking, soaking, germination, and bacterial fermentation. This biochemistry laboratory course mini-research project, done over the last three weeks of the course, tested the effect of yeast fermentation on the concentration of phytic acid of soybeans. This project was designed to give the students more critical thinking skills as they worked to evaluate, experimentally, an interesting problem in the animal nutritional field. The soybeans were presoaked overnight in water prior to fermentation. The students were responsible for doing a literature search for analytical methods of phytic acid quantitation and submitting their lists of needed materials to the instructor and teaching assistant. Along with phytic acid, protein and lipid levels were also required to be measured. The instructions given to students (the assignment and grading assessment) are shown in appendix A.

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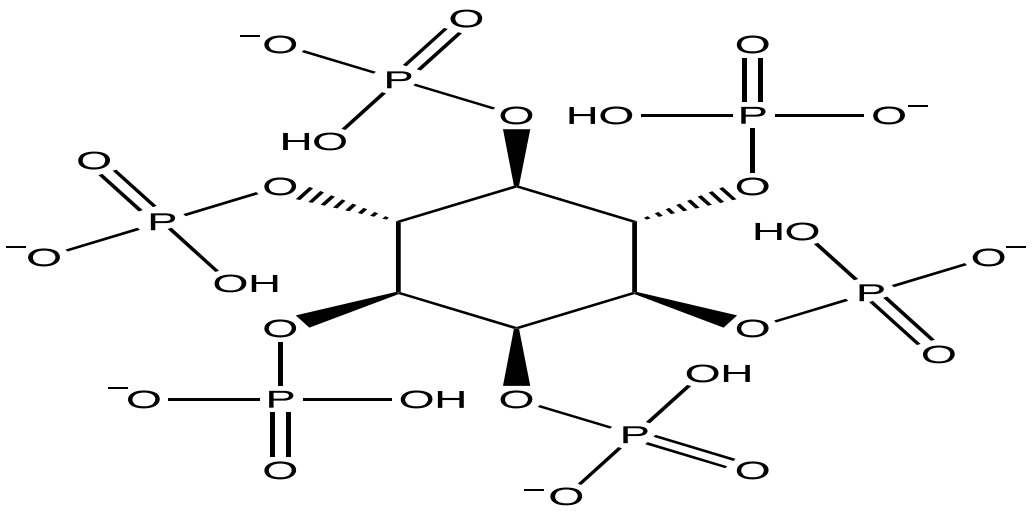

Figure 1.
Structure of phytate (Inositol hexakisphosphate).

2. Methodology

2.1. Pre-Soaking The Soybeans for the Entire Class

Dry soybeans were soaked overnight in deionized water (300 g beans/ 800 mL water) and without draining the water, were blended in a Waring Blender the next day.

2.2. Fermenting the Soybeans

Each student received about 40 grams of blended soybeans, which they divided into two zip locked bags. One bag underwent fermentation with 10 mL of yeast culture in sterile Yeast Extract, Peptone, Dextrose medium (YPD: 10 g yeast extract, 20 g peptone; 20 g glucose per L water) and the other bag received 10 mL of sterile YPD as a control. Note: dried Baker's yeast for this experiment can be obtained from a grocery store. A variation in this yeast preparation could be to use a measured weight of dry yeast to each bag of soybean blend. Both bags were incubated overnight at room temperature and then spread on paper plates (tared) to dry until the next week's lab. Total dry weight of soybean was then determined. Figure 2 shows various steps in the protocol.

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Figure 2.
Soybeans at different stages in the fermentation process. Top left: Soybeans as soaking began. Top right: Soybeans after 24 hours of soaking. Bottom left: Bean homogenate in fermentation bag. Bottom right: Post-fermentation plating to allow materials to dry.

2.3. Phytic Acid Analysis

Phytate was isolated from soybeans and semi-purified via ion exchange chromatography using DEAE-cellulose resin (batch method) in 50 mM HEPES buffer, pH 7.4. A phytate standard curve was constructed, using a standard phytate solution of the dipotassium salt of phytic acid (Sigma Chemical Company; St. Louis, MO) at 1 mg/mL H_2O and Wade's reagent [6] to determine the concentration of phytate in the samples. Wade's reagent is a solution containing the complex of $FeCl_3$ and sulfosalicylic acid which exhibits an absorption maximum around 500 nm. Phytate reacts with iron (III) to form a more stable phytate complex, which results in a decreased absorbance of initial sulfosalicylic acid complex. Figure 3 shows a typical phytic acid standard curve using the Wade's reagent. This procedure was modified from the method of Latta and Eskin [7].

Note: Some students did a HCl extraction method from the literature, rather than the ion exchange resin method, but found it unsatisfactory and then followed the method of their peers.

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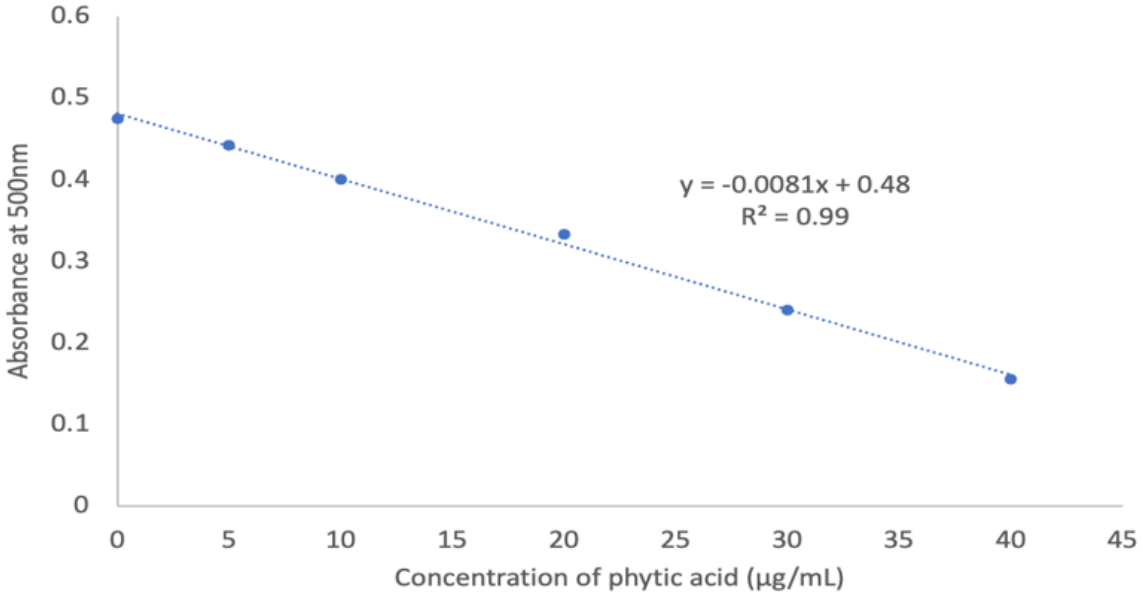

Figure 3.
Phytic acid standard curve using Wade's reagent.

2.4. Protein Analysis

The protein concentrations in soybeans were determined by the Murphy-Kies assay [8]. The students had used this method several times during the semester to quantitate protein.

2.5. Lipid Analysis

Total lipids were isolated from soybeans using the method of Bligh and Dyer [9] and analyzed by thin layer chromatography (TLC) using silica gel G as the stationary phase and chloroform/methanol/water (65/25/4, v/v/v) as the mobile phase. Earlier in the semester, the students had done this same method of lipid extraction and analysis.

3. Discussion: Student Perceptions of the Project and Safety Issues

An anonymous survey (summarized in Table 1) was completed by the students (n = 12) at the end of the project. About half of the students liked both procedures because they were easy to follow and doable. Because everyone had carried out UV/vis spectroscopy for protein quantification previously in the semester, all students were comfortable at doing colorimetric assay with the Wade's reagent. Although all students agreed that ion exchange chromatography was a good technique for extracting phytic acid, it took them a lot of time to isolate and quantitate the phytic acid and they could not complete all steps within the limited time frame of the lab. This is also the main reason why the students did not prefer or had ambiguous feelings about the extraction method. Students' reflection on the hardest part of the lab showed that they were not used to having the freedom to choose what to do. Most students wanted more instructions and time to do the project.

Safety issues: In all labs, students wore safety glasses and disposable gloves
There are several safety issues associated with the chemicals used in this project:

- The Wade's reagent used in phytic acid quantification contains sulfosalicylic acid.
- Chloroform and methanol were used in the lipid extraction and analysis.
- Sodium hydroxide (5 mM) was used in protein assay.
- Hydrochloric acid (used by some students for phytic acid extraction)

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Table 1.
Student survey about the project.

Survey questions	Did you like the methods for isolating and characterizing phytic acid from soybeans?	Did your data come close to the literature value?	Did the previous lab experiments prepare you for this project?	Did you like the challenge of doing a research project?	What was the hardest part of this mini research project?
Number of students who said "Yes"	7	3	12	11	- Doing research on how to isolate, purify, and quantitate phytic acid and deciding which were the best methods. - Balancing all the tasks within the limited lab time. - Trying out new procedures without knowing whether it works
Number of students who said "No"	1	4	0	1	
Number of students who said "Somewhat/ Not sure"	4	5	0	0	

4. Conclusion

Although almost all students valued the research experience of this project, they wished they had received more time and instructions. This was not surprising because all students were carrying out phytic acid extraction for the first time. There are various procedures for the extraction process, most of which mentioned soaking dry soybeans in hydrochloric acid for more than an hour. The soybeans used in this project were presoaked in water, making phytic acid readily available for isolation and using acid was not necessary. Many students had to redo the extraction step after getting unsatisfying result when using hydrochloric acid. This could have been the reason for why extra time was needed. This mini-research project clearly challenged the students to use techniques and equipment that they had used previously in the semester to 'tackle' a new problem. They also selected, following a literature search, some new techniques to use to test their hypothesis. This three-week project was an interesting and challenging way to end the semester for this biochemistry course and challenged students to apply critical thinking skills.

Acknowledgements:

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Appendix A: Instructions for Mini-Research Project

Biochemistry 343 Name:

Mini Research Project Biochemistry Lab 343 (Done as individuals)

This project will address the problem of phytic (phytate) in soybeans especially for nonruminant animals (such as pigs, chickens, and turkeys).

Major Question to Address:
Will fermentation of the soybean (with yeast) change the amount of phytate (as well as protein and lipid) in soybean? You will thus want to measure the following with and without yeast fermentation:
a. mass of phytic acid and estimate of purity (from your isolation method)
b. mass of protein
c. mass of lipid and types (via TLC)

Timeline:
Wed. April 7: soak beans overnight in di water (target 20g of beans per student)

Thurs. April 8 homogenize (Chris will do this at beginning of class) and give each student 2 aliquots (one for yeast fermentation and one as non yeast control); incubations will be at room temperature in zip lock baggies; note: jones will prepare yeast for each student to add to their fermentation and have yeast culture medium for your control baggie

This will also be a good time to do literature search for how to isolate and quantitate phytate (since you already know how to measure protein and lipid masses) as well as literature on phytate and its use by animals, plants, and microorganisms.
Fri. April 9: spread beans to dry
Wed. April 14: Weights of dried bean preparations; indicate your materials/reagent needs
Thurs. April 15: start analyses
Wed. April 21: pre-lab chat/discussion
Thurs. April 22: finish analyses
Wed. April 28: pre-lab chat/discussion

Prepare for Zoom meeting and your formal lab report

MINI RESEARCH PROJECT BIOCHEMISTRY LAB 343 (done as individuals)

Name:_____

This is worth 120 points (20 points for presentation and 100 points for report)

FORMAL REPORT: _____/100 POINTS due Wed May 5 before 10 am; no late reports will be accepted. Please send this as a Word Document via email to both Chris (cfapuzz@ilstu.edu) and myself (majone3@ilstu.edu) [NOTE: this is the only way I will accept your report]

In your typed, formal report, report, be sure to address the following:

Introduction: what you wanted to do and why
Format statement of hypothesis
Show flow diagram
Show calculations and use appropriate sig figs

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Show controls and blanks for all analytical methods; also be sure to briefly explain how you isolated, evaluated, and quantitated your phytate.
Bibliography and use correct citation format in test (see Jones Rules for Writing)
Label Figures, Tables, Graphs correctly; give title; label x and y axes appropriately; do line trend as appropriate; remove grid lines in graphs
Results: show data and have text explaining these data
Discussion: explain what the data mean and put into context with other literature (how do your data compare to literature values)
Conclusion(s): What did you conclude (learn)
Future work: what should be done next
For your Presentation: _____/20 points Name:
Present 4 slides (in PowerPoint as the only acceptable way to present) and have the following:
1). What you did and why do it
2). How do it
3). Results and conclusions
4). References
Each student will have 5 ½ min for your presentation; Chris and I will ask questions at the end of each presentation
Please send your PowerPoint to both your TA and I by Tuesday May 4 before 4pm and no late material will be accepted

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