

Nebraska Soybean Board
FINAL Research/Extension Education Report Form

Note: Submit this report no later than 60 days after the NSB-funded project officially terminates.

This post-project 60-day time-frame will allow the Lead PI/Extension Educator time to complete any final data analysis and a final technical report, plus the drafting of any articles for submission to scientific journals.

This completed report will be provided to the National Soybean Checkoff Research Database: soybeanresearchdata.com.

Project # and Title:

Identification of Anti-Inflammatory Bioactive Compounds in Soybean Sprouts

PI / Extension Educator:

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Co-PI's / Co-Extension Educator's:

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Project Date (Including No-Cost Extension): 10/1/21 to 9/30/22

Total Budget for Project: \$ 48,050

1. Briefly State the Rationale for the Research.

Chronic inflammation in the gastrointestinal (GI) tract often leads to various metabolic disorders including obesity and type II diabetes. Daily dietary intake of anti-inflammatory compounds from natural foods draws significant attention to prevent chronic inflammation. The research team elucidated in the FY19 research project funded by NSB that the soybean sprout germinated for four days can exhibit anti-inflammatory activity in cultured human intestinal cells after simulated gastrointestinal digestion. The in vivo anti-inflammatory effects of the sprouted soybean were tested using mouse model in the FY20 project. In this FY22 project, a chemical fractionation approach is used to identify the anti-inflammatory bioactive compounds in soybean sprouts, which can be used as biochemical markers for generating soybean genotypes with enhanced anti-inflammatory activity in a future project.

2. Research Objectives: (copy from original proposal, but keep in a brief bullet format).

Objective 1: Fractionation of phytochemicals in digested soybean sprouts

Objective 2: Identification of bioactive compounds in the bioactive fractions

3. General Approach Used and (if applicable) the Nebraska Test Location.

Objective 1: Fractionation of phytochemicals in digested soybean sprouts

Food grade soybeans were sprouted under lab conditions at room temperature for four days. Five hundred grams of the sprouted soybean was digested by mimicking human gastrointestinal (GI) digestion using digestive enzymes. The chemical compounds in the digest were fractionated into six fractions by cation exchange chromatography to roughly separate compounds by chemical classes as per their ionic strength. The fractions were tested for the biological activity to identify the most potent fraction.

Objective 2: Identification of bioactive compounds in the bioactive fractions

The chemical compounds in the fractions were determined by gas chromatography-mass spectrometry (GC-MS) analysis (primary metabolites), liquid chromatography (LC)-MS/MS analysis (secondary metabolites), and LC-MS/MS analysis (oligopeptides). The chemicals specifically accumulated in the most potent fraction are the candidates of bioactive compounds.

4. Describe Deliverables & Significance Attained for Each Research Objective.

Objective 1: Fractionation of phytochemicals in digested soybean sprouts-

Digestion and quality check: The primary aim under this objective is to fractionate the chemical compounds in the gastrointestinal (GI) digest of soybean sprouts. To perform this analysis, we first perform the GI digestion of the sprouted soybeans. The quality check by the peptide content analysis of the first digestion showed poor digestion efficiency. The peptide content in the new batch is much lower (~380 µg/mg) than the previous attempts, indicating some anomalies during digestion. The investigation identified that the enzyme activity was an issue, and thus we ordered a new batch of enzymes to perform the GI digestion. The GI digestion with the new enzymes substantially improved the peptide content (~450 µg/mg). We selected the new batch for the fractionation analysis.

Fractionation: To fractionate the GI digest we have optimized the fractionation process. In the extensive tests of the combination of columns and buffers, a Superdex 30 Increase 10/300 GL column with 10 mM sodium phosphate and 140 mM sodium chloride as a running buffer showed the best separation of chemicals. We further optimized the chromatographic parameters, and the best separation was achieved with the following procedure; equilibration for 2 column volume (CV; 48mL) at a rate of 0.8mL/min, with elution for 2CV at a rate of 0.1/mL/min. We repeated the chemical fractionation multiple times and collected the fractions to obtain sufficient amounts of chemicals for further analyses. The anti-inflammatory activity assay for each fraction is currently under way.

4. Describe Deliverables & Significance Attained for Each Research Objective. (continued)

Challenges: The challenges faced in this project substantially reduced the progress of the project. The primary obstacle that was faced after fractionation was in determining the biological activity. The buffers used for the fractionation contain salts and the salts remain in the fractions. Thus, it is critical to remove the salt before using it in the cell culture study, as these salts can be toxic to the cells in the anti-inflammatory activity assay. Despite a salt cleanup method used to remove the salt, the fractions remained toxic to the cells. The further salt separation significantly reduce the yield of the fractionated material which make it difficult for re-assessment. Thus, we needed to repeat the entire process in order to fractionate and identify the compounds responsible for bio-activity without causing cytotoxicity.

Objective 2: Identification of bioactive compounds in the bioactive fractions- We are currently analyzing the contents of chemical compounds in the fractionated soybean sprout digests. As fractionation reduce the complexity of the samples making the chemical determination simpler, we expect it allows us for more precise detection of previously known bioactive compounds in the soybean sprout digests. Once the bioactivity assay identify the fraction with the most potent anti-inflammatory activity, we will analyze the compounds particularly in the fraction as the candidates of anti-inflammatory compounds.

5. List where the Project Research Results/Findings were Publicized.

The manuscript reporting the anti-inflammatory activity in the digested soybean sprout was submitted for the publication in a peer-reviewed scientific journal last year, based on the critical comments received from the reviewer's we are now revising the manuscript and the revised version will be submitted soon. The initial publication will lay the foundation of the work and after completion of the identification of the bio-active compounds (pending from this project) will be then published to establish a co-relation between the active compounds, the changes occurs during sprouting, and their respective bio-activity.

Note: The Final Report comprised of the above listed items must be kept to THREE PAGES.

A Technical Report of no more than TEN PAGES (preferably fewer) can be appended to this report.

Submit the form with the following file name format: #XXX_FINAL_Project Title_LastName

Please submit this completed form with attached files to the Agriculture Research Division, jmcmahon10@unl.edu, based on the reporting schedule given to you.

If you have any questions, please call Jen McMahon at the Agricultural Research Division (402) 472-7082.

Please click to attach technical reports, etc. Please check your information before submitting the form.

Please note: Attach files button may not work in some versions of Acrobat Reader. You may need to save a copy of this form and then attach files to the copy.