PROGRESS REPORT

PROJECT TITLE: Purification of procyanidins for nitrous oxide reduction in corn fields
PROJECT NUMBER: 6107-23DD
REPORTING PERIOD: July 1 to September 30, 2023
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1.) PROJECT ACTIVITIES COMPLETED DURING THE REPORTING PERIOD. (*Describe project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

This project aims to explore cost-effective strategies for procyanidins production and to assess the potential of the resultant products in mitigating N_2O emissions. During the second reporting period, postdoctoral scientist CheJen Hsiao worked closely with Dr. Chen at the Department of Food Science and Nutrition, University of Minnesota, to explore two concurrent approaches for achieving high-purity procyanidins cost-effectively: 1) Synthesizing procyanidins through the catalysis of catechin or epicatechin in a potassium phosphate buffer using horseradish peroxidase (HRP; EC 1.11.1.7) and hydrogen peroxide (H_2O_2). Catechin and epicatechin are the monomeric units of procyanidin; 2) Purifying grape seed extracts by eliminating contaminants, notably tartaric and gallic acids, to enrich the procyanidins content.

1. The procyanidins synthesis approach:

We assessed the influence of five distinct soil amendments on soil denitrification enzyme activities (DEA): water, grapeseed extract (GSE), a mixture of catechin, horseradish peroxidase, and H_2O_2 (CHH2O2), catechin combined with horseradish peroxidase and water (CHW), and catechin mixed with H_2O_2 and water (CWH2O2) (Fig. 1). Our results indicated that both GSE and CHH2O2 led to a significant 30% reduction in DEA as compared to the water control. On the other hand, CHW and CWH2O2 induced a 10-20% increase in DEA, probably because the microbes may use catechin as an extra carbon substrate for growth. These findings reveal that a mixture of catechin, HRP, and H_2O_2 effectively reduced the soil's denitrification potential by 30%, a result comparable to that achieved with 1 mg g^{-1} of GSE.

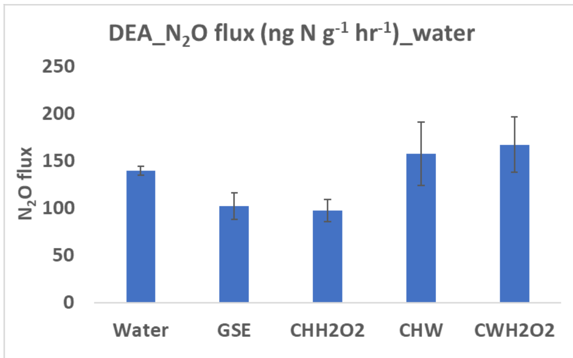

Figure 1. Denitrification enzyme activity (DEA) of soil amended with various amendments after 7 days of soil incubation. The amendments included water as control; GSE, procyanidins from USP (USP Grape seeds oligomeric proanthocyanidins; 1 mg g^{-1}); CHH2O2, catechin + horseradish peroxidase + H_2O_2; CHW, catechin + horseradish peroxidase + water; CWH2O2, catechin + H_2O_2 + water. Results are given as means ± standard errors of the mean (n = 3).

We then evaluated the denitrification inhibition efficiency using a mixture of epicatechin, horseradish peroxidase, and H_2O_2 (EHH2O2) (Fig. 2). The results showed that EHH2O2 did not significantly affect soil DEA.

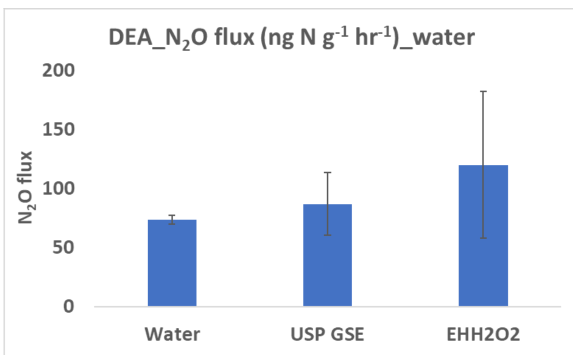

Figure 2. Effects of soil amendments on DEA after 7 days of soil incubation. The amendments included water as control; USP Grape seeds oligomeric proanthocyanidins; 1 mg g^{-1} (USP GSE); and a mixture of epicatechin, horseradish peroxidase, and H_2O_2 (EHH2O2). Results are given as means ± standard errors of the mean (n = 3).

2. The impurities removal approach

The strategies for this approach encompassed several steps: 1) Evaluating the denitrification inhibition efficiency of grape seed extracts sourced from multiple vendors, each with varied procyanidin compositions; 2) Chemically characterizing these grape seed extracts; 3) Applying individual components to microcosm tests to identify impurities; 4) Removing the detected impurities and applying the refined products to microcosms for an assessment of their denitrification inhibition efficiency.

We evaluated the soil DEA response after applying 1 mg g^{-1} of five distinct commercially available grape seed extracts: Morel pure grape seed powder (Morel, $1.44/oz); Cenalga organic grape seed extract powder (Cenalga, $6.66/oz); BulkSupplements.com grape seed extract (Bulk Supply, $3.97/oz); and NuSci grape seed extract powder standardized 95% proanthocyanidins OPC (NuSci, $4.82/oz). Water was used as a negative control, while USP GSE ($14,000/oz) was used as a positive control. Our results indicated that USP GSE outperformed the other brands (Fig. 3). Soil N_2O emissions increased following application of Morel, Cenalga, Bulk Supply, and NuSci brands of GSE. This unsatisfactory N_2O inhibition corresponded with observations of significant impurities and subpar solubility in the GSE solutions from Morel, Cenalga, Bulk Supply, and NuSci. The hues of the GSE solutions from Morel, Cenalga, Bulk Supply, and NuSci diverged from the expected beige tone characteristic of USP GSE, either appearing excessively reddish or lacking the anticipated coloration altogether. Interestingly, the DEA of soils treated with USP GSE wasn't reduced. This anomaly might be attributed to the age of the USP GSE as it came from a bottle opened 2.5 months ago.

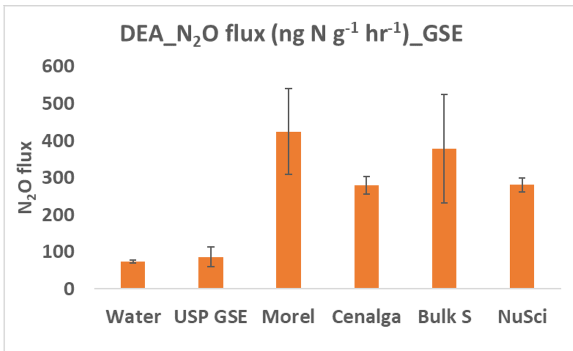

Figure 3. Effects of various GSE candidates on DEA after 7 days of soil incubation. The GSE candidates included USP Grape seeds oligomeric proanthocyanidins (USP GSE); Morel pure grape seed powder (Morel); Cenalga organic grape seed extract powder (Cenalga); BulkSupplements.com grape seed extract (Bulk S); NuSci grape seed extract powder standardized 95% proanthocyanidins OPC (NuSci). Water was used as negative control. Results are given as means ± standard errors of the mean (n = 3).

In the next phases of our research, we have outlined the following objectives:

1. **Optimization of HRP oxidative conditions**: We aim to identify the most favorable and economically viable conditions for HRP-catalyzed reactions that yield the highest procyanidin concentrations and maximal N_2O inhibition potential.

2. **Cost estimation**: As we scale up to mesocosm-level experimentation (with dimensions of 1.5 x 1.5 x 1.35 m each unit), it is critical to determine the costs associated with procyanidin production. Considering a soil bulk density of 1.3 Mg m^{-3} and assuming that procyanidins mainly influence the top 20 cm of soil, our projections indicate a requirement of approximately 150 L of CHH2O2 solution for each mesocosm. Preliminary estimates under the current setup are $180 for catechin, $350 for HRP, $750 for potassium phosphate buffer, and $120 for H2O2, resulting in a total of $1,400. We believe there is potential for further cost reduction.

3. **Mesocosm testing**: Our plan is to apply procyanidins to the mesocosms and assess their impact on N_2O inhibition as well as corn growth and productivity. If additional funding is possible, we will also explore the effects on soil microbial communities, focusing on denitrification-related microbes (i.e., *narG*, *napA*, *nirS*, *nirK*, and *norB* functional genes related to soil denitrifiers).

4. **Efficiency of denitrification inhibition**: We intend to evaluate the efficiency of denitrification inhibition and the associated costs of removing impurities like tartaric acid and gallic acid from grape seed extracts.

By focusing on these objectives, we aim to further our understanding and practical application of procyanidins in mitigating N_2O emissions and promoting soil health in corn fields.

2.) IDENTIFY ANY SIGNIFICANT FINDINGS AND RESULTS OF THE PROJECT TO DATE.

1. So far, the "Procyanidins synthesis" approach is more advantageous both in efficacy and cost-efficiency compared to the "Impurities removal" methodology.
2. The combination of catechin, HRP enzyme, and H_2O_2 is the most promising for denitrification inhibition.
3. Based on current estimations, the utilization of catechin in combination with HRP enzyme and H2O2 for the production of procyanidins—capable of reducing soil denitrification by 30% for a 1.5 m x 1.5 m patch of soil—has a budget ceiling of approximately $1,400.

3.) CHALLENGES ENCOUNTERED. (*Describe any challenges that you encountered related to project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

Dr. CheJen Hsiao may be transitioning to a different professional role by the end of October. However, to ensure project continuity, Dr. Hsiao will stay on in a part-time research associate capacity for several more months until we can onboard and transition responsibilities to a suitable successor for the procyanidins project.

4.) FINANCIAL INFORMATION (*Describe any budget challenges and provide specific reasons for deviations from the projected project spending.*)

NA

5.) EDUCATION AND OUTREACH ACTIVITES. *(Describe any conferences, workshops, field days, etc attended, number of contacts at each event, and/or publications developed to disseminate project results.)*

Dr. CheJen Hsiao participated in the Minnesota Corn podcast (https://www.mncorn.org/minnesota-corn-podcast/) and talked about our latest work on procyanidins that is showing considerable promise in reducing denitrification and nitrous oxide emissions. The podcast will be released on October 14.

Dr. Hsiao keeps collaborating with Food Scientists to explore strategies for enhancing the efficiency of procyanidin production while ensuring economic viability.