

2015 GRANT APPLICATION FOR POTATO RESEARCH

METABOLOMICS TO EVALUATE NUTRITION AND HEALTH TRAITS IN COLORADO POTATO

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RELEVANT FUNDING SOURCE: CCPGA royalties

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EXECUTIVE SUMMARY

Potato nutrition and health traits are important targets for modern breeding. Health traits have the potential to add value to the potato industry by increasing consumer demand. Further, potato is an important source of calories world-wide, and therefore efforts to better understand its nutritional quality are important to global food safety and security.

Drs. Heuberger and Prenni (PI and co-PI, Colorado State University, CSU) use a biochemical profiling technique referred to as "metabolomics." This method can evaluate total biodiversity in nutrition and health traits in plant foods for thousands of compounds ("metabolites") in a single experiment. Previous studies have demonstrated the genetic basis of nutrition traits in cooked food [17], supporting that food crops can be bred for many compounds related to health in a single effort. This is in contrast to traditional breeding with a focus

on a single nutrient (e.g. high-iron or high-zinc), or a single class of compounds (e.g. "antioxidants"). Rather, metabolomics provides the opportunity to monitor many health-related compounds in a single experiment. A metabolomics approach addresses major goals of the potato industry by (i) enabling the development of rapid, high-throughput profiling methods to evaluate nutrition and health traits and (ii) documenting which health traits exist in Colorado potato, and which traits are areas for improvement through breeding. While the breadth of health-related metabolites in potato has been well-documented, variation in quality (type of nutrient) and quantity (amount per gram of cooked potato) in Colorado potatoes is largely unknown. In the proposed study, we seek to characterize the health-promoting quality of a diverse set of potato in the Colorado Potato Breeding program.

The overall objectives of this research are to:

1. Characterize the type and quantity of health-related compounds among and within market classes
2. Develop a metabolite profiling workflow to characterize health-related metabolites in potato

The developed method will utilize multiple metabolite extraction and detection methods to quantify health-promoting compounds in potato. A total of 60 cooked and raw potatoes from the Colorado Potato Breeding and Selection Program will be evaluated and includes several market classes (e.g. russet, yellow, specialties). **This study directly addresses the priority outcome: "improved economic sustainability through potato varieties" by developing "potato varieties with enhanced nutritional/health attributes." This work also supports the CCPGA goal to provide "new marketing strategies for seed sales."** Future efforts that use metabolomics technologies in potato research can include:

- rapid screening of potato varieties for nutritional and health-promoting compounds
- identifying genetic markers to facilitate the development of new varieties with enhanced nutrition
- understanding how management practices affect tuber biochemistry

We anticipate no significant obstacles with the completion of the proposed work and these goals can be met within the 2-year funding timeframe. Our laboratory is experienced in extracting and detecting metabolites in food and plant products and developing new chemical detection methods.

NATURE, SCOPE, AND OBJECTIVES OF THE PROJECT

Nutrients and bioactive compounds are novel targets for potato breeding. Potato has been well-characterized for an abundance of nutrients and other health-promoting compounds. Nutrients are defined as molecules necessary for the maintenance of basic human metabolic and physiological functions. Nutrients are broadly classified as macronutrients (fats, carbohydrates, proteins), micronutrients (vitamins, minerals), and fiber (complex carbohydrates, or oligosaccharides). Cooked potato contains many of the essential macro and micronutrients. Potato also contains anti-nutrients, or compounds that inhibit the absorption or activity of nutrients such as phytic acid. In addition, potato contains a class of molecules referred to as "bioactive compounds" (BACs). BACs are thought to provide no known nutritional value (*i.e.* not necessary for basic human functions), however still affect human health through the prevention of chronic diseases. BACs are commonly referred to as "phytochemicals" or "antioxidants", however potato contains a specific suite of BACs that warrant individual investigations. BACs reported in potato include phenolics, amines, sterols, carotenoids, alkaloids, and many others (Table 1). Individually, each BAC has been demonstrated to affect diverse set processes related to human health, and consuming them together in the diet an important facet of the health-promoting properties of consuming whole potato.

| Metabolite class | Sub-class/name | Reported bioactivity | Reference |
|-------------------------|--------------------------------------|---------------------------------------|------------------|
| phenolics | chlorogenic acid | ↓ inflammation; ↓ carcinogenesis | [1,2] |
| | anthocyanins | ↓ hypertension; ↓ carcinogenesis | [3] |
| amines/polyamines | kukoamines | ↓ hypertension; ↑ neuroprotection | [4,5] |
| | putrescine | ↑ immune development | [6] |
| sterols | sitosterol, stigmasterol | ↓ cholesterol absorption | [7] |
| | oxy-sterols (<i>anti-nutrient</i>) | ↑ atherosclerosis | [8,9] |
| alkaloids | glycoalkaloids | ↑ or ↓ carcinogenesis; effect unclear | [10-12] |
| carotenoids | xanthophylls | ↑ eye health | [13] |

Mass spectrometry-based metabolomics is a high-throughput method to profile nutrients and BACs for potato. Mass spectrometry (MS) is a chemical detection method commonly used for metabolomics. MS-metabolomics platforms can quantify thousands of compounds in a short time (~10-30 minutes per sample). MS is therefore a high-throughput method, whereby hundreds to thousands of samples can be screened for nutrients and BACs in a single experiment. The following MS-platforms are available for use at Colorado State University: UPLC-MS (lipids, alkaloids), GC-MS (amino acids, carbohydrates), ICP-MS (minerals), and UPLC-MS/MS, described in the “Methods and Facilities” section below.

A major limitation to utilizing MS-profiling for nutrient and BAC quantitation is the initial method development procedures. Initial experiments are required to (i) determine if metabolites are detected under the chosen extraction and detection conditions/platforms and (ii) the development of secondary methods to provide quantitation of many compounds. Typically, samples are first run on UPLC-MS and GC-MS platforms to characterize the extent of biochemical diversity in a system. The nutrients and BACs that are detected on those platforms are then calibrated on a UPLC-MS/MS system. The UPLC-MS/MS system is more sensitive, faster, and cheaper than the UPLC-MS and GC-MS counterparts. The UPLC-MS/MS system provides the ability to screen more potato varieties for less cost and is more amenable to incorporation as a technology to facilitate breeding for nutrients and BACs.

Rationale. Nutrients and BACs are novel targets for potato breeding. To breed for healthier potato, there is a critical need to evaluate how much these compounds differ among varieties and market classes, and also a need to develop rapid and low-cost methods for analysis. *We hypothesize that Colorado potato contains variation in nutrients and BACs due to variety and market class.* A better understanding of the genetic contribution to nutrient and BAC composition of potato can provide the opportunity to breed for nutrition as with traditional targets such as yield and disease resistance. Thus, the major objectives of this project are to:

1. Characterize the type and quantity of health-related compounds among and within market classes
2. Develop a metabolite profiling workflow to characterize health-related metabolites in potato

The proposed work (detailed in the “Methods and Facilities” section of this proposal) seeks to utilize MS-profiling to evaluate biochemical diversity in the tuber. The long-term goal of our study is to provide the framework to develop Colorado potato varieties with enhanced nutritional and health-promoting traits. This includes characterizing the nutrient and BAC content of Colorado potatoes and developing methods for high-throughput screening of additional material.

METHODS AND FACILITIES

Overview. The proposed research will characterize the types and quantity of nutrients and BACs in 60 Colorado potato lines. The experiment will first evaluate different extraction and detection methods using UPLC-MS and GC-MS, and later develop methods for ICP-MS and UPLC-MS/MS (Table 2). All MS platforms are available at Colorado State University core facilities.

Table 2. Mass spectrometry (MS) platforms for profiling nutrients and BACs in potato.

| Platform | Metabolites detected | Run-time (approximate) | Objective/Year |
|------------|---|------------------------|----------------|
| UPLC-MS | phenolics, sterols, anthocyanins | 20 min/sample | Obj. 1, Year 1 |
| GC-MS | amino acids, sugars, fatty acids | 30 min/sample | Obj. 1, Year 1 |
| ICP-MS | minerals (iron, zinc, calcium) | 5 min/sample | Obj. 1, Year 2 |
| UPLC-MS/MS | amino acids, phenolics, sterols, anthocyanins | 10 min/sample | Obj. 2, Year 2 |

Objective 1: Characterize the type and quantity of health-related compounds among and within market classes. A total of 60 potato varieties were selected from the Colorado Potato Breeding and Selection Program that span multiple market classes. These include Russets (14 lines), Reds (11), Chippers (12), and Specialty (23, e.g. yellows, purple/yellow, red/red). The lines within each market class were chosen based on breeding distance; that is, the lines were derived from a diverse set of parents. We hypothesize that a larger genetic distance among lines within a market class will result in variable quantities of nutrients and BACs.

Potatoes from each line will be evaluated for biochemical profiles in both cooked and raw tuber. By analyzing cooked and raw tubers, we will be able to determine if raw material is sufficient to inform on biochemical profiles for future studies. Cooked potatoes will be microwaved using procedures as previously reported [14], where the cooking time is adjusted based on the weight of the tuber. Cooked and raw tubers will be lyophilized to remove water content and ground to a fine powder. Metabolites will be extracted by adding 1 mL of 80% methanol to 100 mg of ground potato powder and incubated for 2 hr on vortex mixer. The supernatant, referred to as the "metabolite extract", will be collected for further analysis.

Metabolites will be detected and quantified using three MS platforms: ultra performance liquid chromatography (UPLC-), gas chromatography (GC-), and inductively coupled plasma (ICP-) coupled to MS. For UPLC-MS, 1 μ L of the metabolite extract will be sampled and the data will be analyzed as previously described [15]. For GC-MS, 500 μ L of the extract will be prepared, analyzed, and the data will be analyzed as previously described [16]. For ICP-MS, 5 g of powder subjected to microwave-assisted acid digestion and 1 mL of sample will be analyzed. Objective 1 will utilize univariate (ANOVA) and multivariate statistics (PCA, PLS methods) to characterize variation in the metabolome (all detected chemical signals) among and within market classes. Correlations will be performed to evaluate relationships between cooked and raw potato metabolites and among metabolites. Additionally, several known potato nutrients and BACs will be searched for in the MS-data, such as compounds described in Table 1.

Objective 2: Develop a metabolite profiling workflow to characterize health-related metabolites in potato. The methods used to quantify nutrient and BACs from Objective 1 will be translated to a UPLC-MS/MS platform. UPLC-MS/MS requires first running authentic standards (e.g. purchased chlorogenic acid) and then

re-calibrating the mass spectrometer. The re-calibration includes: (i) speeding up the run-time of the machine and (ii) focusing the mass spectrometer to only look for the compounds of interest. The final results is a faster and more sensitive method to quantify nutrients and BACs in potato. For this Objective, we will develop a method to quantify a mixture of ~50 potato nutrients and BACs in a single run. It is estimated to require 10 days of UPLC-MS/MS run-time to calibrate the instrument. However, the 10 days of run-time are expected to be spread out of the course of 1-2 months, as calibration requires running a standard (~10 min), analyzing the information obtained from the instrument, and then re-running the standard alongside potato extract to determine the efficacy of the calibration. Further, compounds mixtures (e.g. chlorogenic acid + glycoalkaloids) will be assessed to determine that there are no overlapping signals in the instrument.

OUTCOMES AND ENHANCING COMPETITIVENESS OF CO POTATO GROWERS

Expected outcomes. The major outcomes from Objectives 1 and 2 are expected to include:

- Characterization of nutrients and BACs in cooked potato from Colorado breeding lines and varieties
- Determination if nutrient and BAC quantities in raw tuber are correlated to cooked tuber
- Evaluation of specific nutrients and BACs as breeding targets, within and among market classes
- Development of a high-throughput and low-cost assay to screen for many nutrients and BACs in a single experiment

These outcomes can increase the competitiveness of Colorado potato growers by documenting the presence of health traits. The overall health-benefit of potato is unclear, cooked tuber perceived to contain excessive carbohydrate content and products are often fried prior to consumption. However, potato contains many nutrients and BACs that consumers search for in more obscure plant-foods or medicines (e.g. kukoamines). Further, developing a high-throughput and low-cost assay to measure many nutrients and BACs at a single time can provide the ability to ensure that potato varieties released by the CSU Potato Breeding and Selection program are documented to contain health-promoting compounds.

EXTENSION AND OUTREACH PLAN

Drs. Dave Holm (co-PI) and Heuberger will facilitate the following extension and outreach activities for this project:

- Field days/open house events
- Annual Advanced Selection and Evaluation Meeting
- Professional meeting presentations (Potato Association of American annual symposia; Southern Rocky Mountain Agricultural Conferences)
- Articles for SpudItems (when appropriate)
- Colorado Potato Breeding and Selection website

TARGETS FOR ADDITIONAL OUTSIDE FUNDING

The data generated in the proposed study is critical to secure Federal funding for future research. The USDA-AFRI-NIFA Foundational program section *Food Safety, Nutrition, and Health* priority *Improving Food Quality* supports research to improve our knowledge and understanding of the chemical properties of foods. We believe that the biochemistry learned from Colorado potato can translate to other crops of national importance. Further, BACs identified in our research will allow for studies on the AFRI-NIFA priority area *Function and Efficacy of Nutrients* to further our understanding of how potato metabolites affect human health and disease.

TIMELINE

| | Spring 2015 | Summer 2015 | Fall 2015 | Winter 2015 | Spring 2016 | Summer 2016 | Fall 2016 | Winter 2016 |
|---|-------------|-------------|-----------|-------------|-------------|-------------|-----------|-------------|
| Obj. 1: potato sample preparation | ■ | | | | | | | |
| Obj. 1: UPLC-MS and GC-MS profiling | | ■ | | | | | | |
| Obj. 1: UPLC-MS and GC-MS data analysis | | | ■ | ■ | | | | |
| Obj. 1: ICP-MS profiling | | | | | ■ | | | |
| Obj. 1: ICP-MS data analysis | | | | | | ■ | | |
| Obj. 1: manuscript preparation | | | | | | | ■ | |
| Obj. 2: UPLC-MS/MS method development | | | | | ■ | ■ | | |
| Obj. 2: manuscript preparation | | | | | | | ■ | ■ |

BUDGET INFORMATION AND JUSTIFICATION

Year 1: Characterize nutrient and BAC variation in potato

*Design: 60 varieties * 2 processes (cooked/raw) * 3 replicates = 360 potato samples*

UPLC-MS profiling: 360 * \$25/sample = \$9,000

GC-MS profiling: 360 * \$22.5/sample = \$8,100

Materials and reagents for metabolite extraction and metabolomics preparation: \$640

Data analysis: 15% salary for post-doctoral scientist: \$7,907

Travel to attend Southern Rocky Mountain Agricultural Conference: \$750

Total year 1: \$26,396

Year 2 (estimate): Discovery of mineral variation in potato and development of an accelerated method

*Design: 60 varieties * 2 processes (cooked/raw) * 3 replicates = 360 potato samples*

ICP-MS profiling: \$0

Design: calibration of ~50 nutrients and BACs on UPLC-MS/MS

UPLC-MS/MS method development (est. 10 days of run-time): \$8,740

authentic standards: \$2,500

Data analysis: 25% salary for post-doctoral scientist: \$13,705

Travel to attend Southern Rocky Mountain Agricultural Conference: \$750

Total year 2 (estimate): \$24,945

The proposed study requires significant efforts to interpret the biochemical data derived from the mass spectrometer. Thus, 15% of a post-doctoral scientist salary is requested for this project. Jacqueline Chaparro, a current graduate student in the Department of Horticulture and Landscape Architecture at CSU has begun her post-graduate training in the laboratories of Drs. Heuberger and Prenni. Chaparro will defend her Ph.D. thesis in March 2015, after which Drs. Heuberger and Prenni will co-advise as a Postdoctoral Scientist in Spring 2015. For Objective 2, 25% of salary for a postdoctoral scientist is requested to support the additional time required for Chaparro to develop UPLC-MS/MS method and write two manuscripts to report the data. Authentic standards (commercial and pure nutrients and BACs) are required to develop UPLC-MS/MS assays. Travel is requested to attend the Southern Rocky Mountain Agricultural Conference to present findings. Work to grow and transport potatoes and the ICP-MS analysis will be provided at no cost by the PIs of this proposal.

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