2010

Proposal Submission to CCPGA Royalties for Potato Research

Title: Colorado's Nutritionally Enhanced Potatoes

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Nature, scope and objectives of the proposed research

Background Nutritional attributes of fruit and vegetable produce continue to interest health conscious consumers and all indications are that this trend will continue. Evidence is mounting that certain potato cultivars are a good source of antioxidants (Brown et al., 2003; Freidman, M. 1997; Stushnoff et al., 2007, 2008, 2010; Wu et al., 2004) minerals and fiber. A recently published handbook by The US National Potato Board, 2010, "Potatoes Goodness Unearthed" (Anon, 2010), describes health virtues of potatoes as part of its comprehensive campaign to inform consumers and encourage consumption. Antioxidant research at Colorado State University, along with work of several other prominent research universities has been recognized in this handbook. An invited symposium presentation and a peer reviewed publication in the Amer. Journal of Potato Research, as well as a recent publication in the highly respected European Journal of Experimental Botany have also provided visibility for the Colorado program. Some cultivars and advanced selections developed by the Colorado breeding program are very high in antioxidants.

Potato is an important and popular staple food, but we must continue to work on improving its' image as a nutrition-rich food source. While previous research has established that selections and cultivars from the Colorado breeding program possess desirable vitamin C, total phenolics content, and radical scavenging capacity, nutritional profiles can be improved by adding data on mineral content and sensory properties. Potatoes are especially rich in potassium, a very important essential element that is also implicated in regulation of blood pressure among other benefits, but differences among cultivars are not well documented.

While growing-season environmental conditions, postharvest conditions, and cooking methods may alter antioxidant and nutrient status, the simplest and least costly option that is readily available to producers who wish to market potatoes with health attributes is to identify cultivars with the highest content of desirable nutrients, vitamins and antioxidants. Additional research data is required to do this.

To complete the picture, we propose to submit samples for analysis of N, P, K, S, Ca, Mg, Zn, Fe, Mn and Cu, to Ward Laboratories, Kearney, NB. Data from this analysis will then be combined with antioxidant and vitamin content data that we have accumulated over several years along with high quality photos and information on sensory properties, and pertinent available information on cancer cell culture bioactivity for potato cultivars grown in Colorado. This will enable compilation of a high-quality comprehensive publication that can be made available to seed and fresh market producers, and to consumers. While the National Potato Board handbook is an important step in this direction, specific cultivar properties that benefit producers directly still need

to be documented. This is an essential step to build on positive attributes of Colorado grown potatoes.

In this proposed research project we plan to consolidate several years of antioxidant data in a comprehensive compendium with additional nutritional information. Each cultivar and advanced selection will be documented with pertinent nutritional data, high quality photos, sensory attributes, and other distinguishing special features. It is anticipated that a high quality compendium can be published either by CPAC, CAES, or a commercial publisher (to be determined) that will primarily raise national and international visibility, as well as chronical and archive Colorado cultivars to benefit producers and create positive awareness among consumers.

Accomplishments in 2009. In addition to antioxidant analyses of stored and cooked samples of selected cultivars and twenty some advanced selections the following have been accomplished.

(1) A chlorogenic acid HPLC analytical protocol was developed for this important antioxidant (Feng, et. al. 2005). Data (Fig. 1, 2, Table 1) revealed that cultivars with purple and red tissues were 3 to 5 fold higher than non-pigmented cultivars. While chlorogenic acid was degraded by cooking in some taxa, its' stability varied by cultivar and selection. Chlorogenic acid dropped during storage in some cultivars, but levels increased after 6 months storage in others, especially in the purple and red fleshed types. This suggests high chlorogenic acid cultivars that are stable after cooking and storage can be developed by breeders.

(2) A protocol to test colon cancer cell culture inhibition by potato extracts has been developed. The test utilizes a tetrazolium based colorimeteric assay that measures respiration in cultured colon cancer cell mitochondria, providing a direct measure of either apoptotic programmed cell death of cancer cell cultures, or inhibition of proliferation as a result of introducing buffered potato extracts to the cultures. This was made possible with a cell culture incubation protocol and developmental work by Tatiana Zuber a PhD student who was funded in 2007 on a 2-year USDA Crops for Health fellowship.

(3) A collaborative project with Mark Taylor of the Scottish Crop Research Institute, (SCRI) Dundee, Scotland, facilitated by this project and a research award from the Royal Edinburgh Society, Scotland, enabled utilizing genetic information from the recently sequenced potato genomic data base at SCRI. This resulted in a genomic/metabolomic study that identified 27 genes and 16 pigment and antioxidant metabolites that are responsible for differences between purple and white colored flesh in potato tubers (Fig. 3). This study also identified a new transcription factor gene that is responsible for pigment deposition. Two previously unknown structural genes were discovered, and genes associated with glycoalkaloid deposition and down regulation were also evident (Stushnoff et. al., 2010).

In this proposed project we plan to grow at least five biological replicates of cultivars and selections at SLV in 2010 and 2011 and to conduct the following work:

(1) prepare and send potato tuber extracts for analysis of major and minor mineral elements to Ward Laboratories, Kearney, NB to determine endogenous levels of: N, P, K, S, Ca, Mg, Zn, Fe, MN, and Cu;

(2) prepare and analyze samples from the same replicates in (1) for vitamin C, and antioxidant properties at Fort Collins;

(3) screen a limited number of the most promising taxa based on 2009/2010 data for inhibition of colon cancer in our Fort Collins lab;

(4) Prepare a compendium based upon at least two years data for antioxidant and vitamin C, sensory data, photos, mineral nutrient content, impact of cooking (microwave, and baking) and storage on antioxidant properties, and relationship to mineral contents.

Methods, procedures, and facilities

Work plans for 2010
Ten disease-free cultivars and 10 advanced selections grown at San Luis Valley Research Center will be harvested and lyophilized for analysis. Samples will be assayed for total phenolics, ABTS, and DPPH antioxidant radical scavenging capacity, vitamin C and mineral content.

Harvested tubers will be stored in refrigerated coolers in the Shepardson building, Fort Collins, prior to freeze drying, spectrophotometric, and HPLC analyses in C. Stushnoff's laboratory. All necessary analytical equipment is available. Funds are requested for student hourly assistance (Michaela Kaiser) and partial support for a graduate student (Tatiana Zuber), plus hourly labor for sensory analyses supervised by Food Science personnel. Funds are also required for analysis of mineral nutrients, a service contract, maintenance and repairs, chromatography supplies, solid phase filtration and purification, travel to SLV, reagents, and supplies for microplate spectrophotometric analyses.

Relationship of proposed research to the Colorado potato industry. The work proposed is intended to: (1) raise awareness of the positive health attributes of Colorado cultivars and (2) to provide data to assist selection of new cultivars with high antioxidant and bioactivity properties for Colorado producers. The primary goal is to add information on nutrient content of Colorado cultivars and to compile a cultivar compendium. The role of plant-derived antioxidants and nutrient content is receiving increasing attention and is consistent with goals of the College Crops for Health Program. While potatoes have long been recognized as a dietary staple, their image is sometimes more akin to contributing to obesity than as a healthy food, and has thus not kept pace with other vegetable crops. Research data is required to support putative health claims. This work provides an opportunity to characterize and document potentially beneficial attributes of potatoes.

Potential to leverage research funding Results from this research project have been instrumental in receiving a CSU/AEP grant, an invitation to present a talk at a major

symposium at the North American Potato Association Annual Meeting, Idaho Falls, ID, August 2007, as well as an invitation to collaborate on molecular dissection research in Scotland. It is anticipated the increased exposure from publishing a comprehensive compendium will directly leverage sales of Colorado seed cultivars and market potatoes. National exposure of cultivar attributes should assist future funding of research projects from various sources.

Timeline and expected milestones *Short term (1-2 year) expectations.*
We plan to complete analyses for total phenolics, radical scavenging capacity, vitamin C and mineral contents within one year of harvest. Two years field data should be collected to strengthen confidence in testing genotype X environment interactions. The compendium will be prepared in a manner that will permit addition of data from subsequent years of testing, but plans are to complete a document with at least 2 years data for elemental nutrient composition, and more for antioxidant properties.
Long term (3-5 year expectations)
Publications on health attributes provide visibility and may increase consumption of Colorado potatoes.

The genomic and metabolite profile data for pigment antioxidant production and the transcription factor with the sectorial model system (Stushnoff et. al., 2010) will enable more efficient breeding for several secondary metabolite genes. Linking genes with metabolites will also enable better understanding of mechanisms controlling health attributes.

A PhD graduate student is testing potato cultivars and selections for colon cancer inhibitory properties. While some genotypes have shown inhibitory activity the work is not far enough along to predict how significant it may be. These data coupled with the work on gene dissection at SCRI should enable narrowing of putative metabolites that may be involved.

Budget for 2010	**2010**
1. Personnel: Michaela Kaiser ($12.00/h X 12 hrs/week x 36)	
Salary plus fringe benefits (4.3%)	5,184
Tatiana Zuber ($14.00/h X 5 hrs/week x 26) plus (5.0% fringe)	1,911
Student hourly assistant ($10/h X 12 hrs/week X 26)	3,120
2. Materials and Supplies:	1,500
3. Travel: Fort Collins to SLV	300
a. Service contract for micro plate reader	2,400
b. Expenses for mineral analyses (20 cultivars x 3 replicates) x $19.80/sample.	1,188
Total:	15,603

Justification of estimates for personnel costs

1. Michaela Kaiser has prepared samples in our lab for several years and has expressed an interest in this project. We also request part time support for student hourly support to assist with sample preparation and sensory analysis. Cultivar/advanced selection assays will require freeze drying, extraction, data entry and analyses of ~20 entries x triplicate biological assays.
2. Funds are also requested to cover travel to SLV and lab fees for mineral analysis. Ward laboratories have conducted very satisfactory plant sample nutrient analyses for us in the past.

Literature Cited

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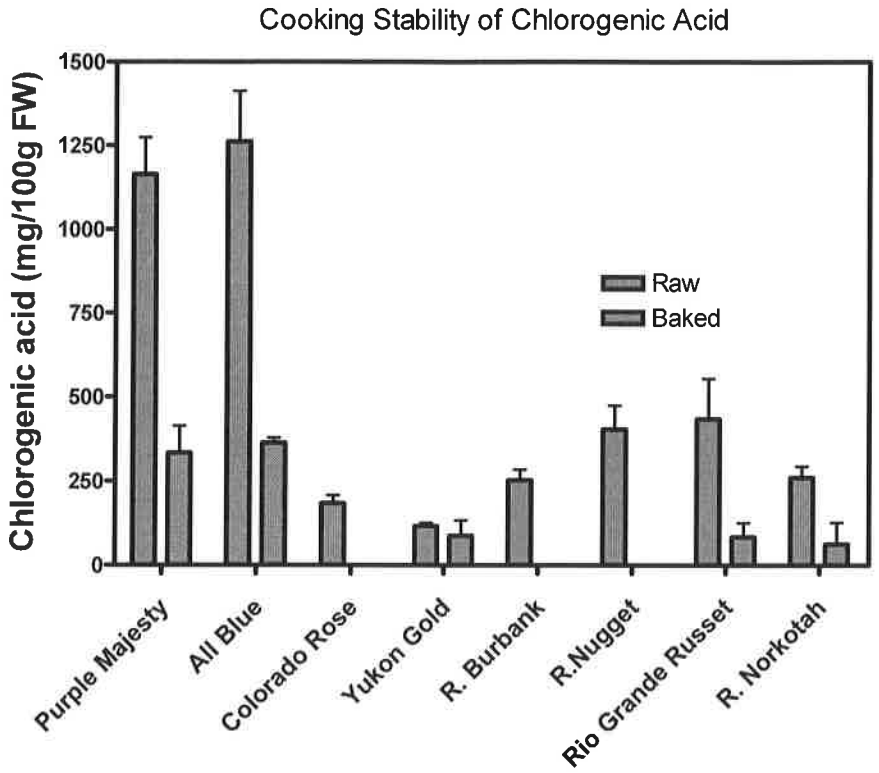

Figure 1. Chlorogenic acid content in eight cultivars following microwave cooking at harvest in 2008.

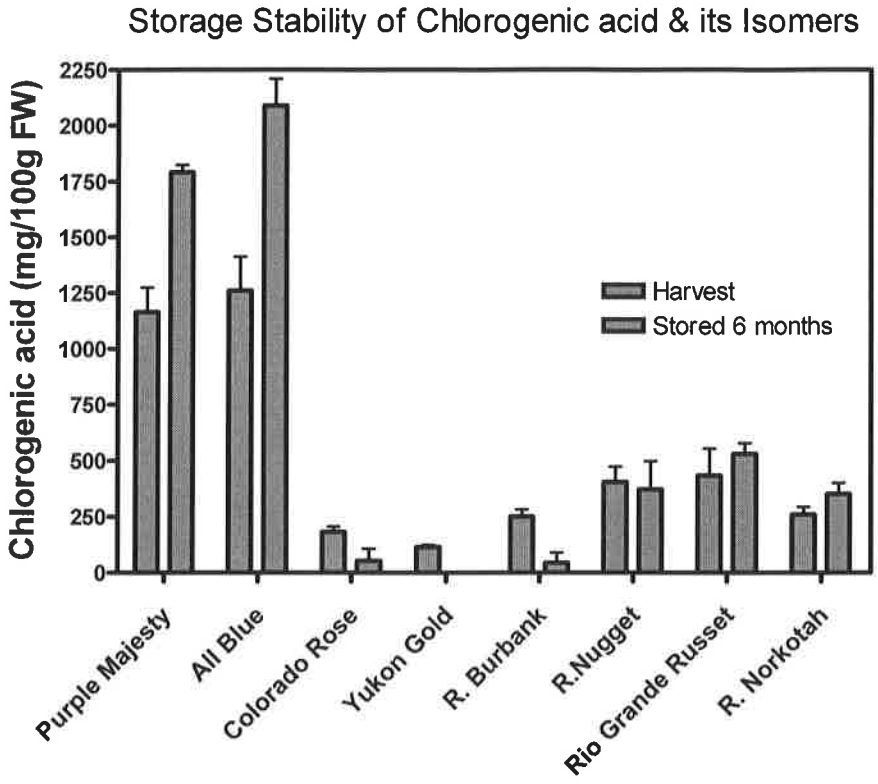

Figure 2. Chlorogenic acid content after 6 months storage at 5C in eight cultivars grown in 2008

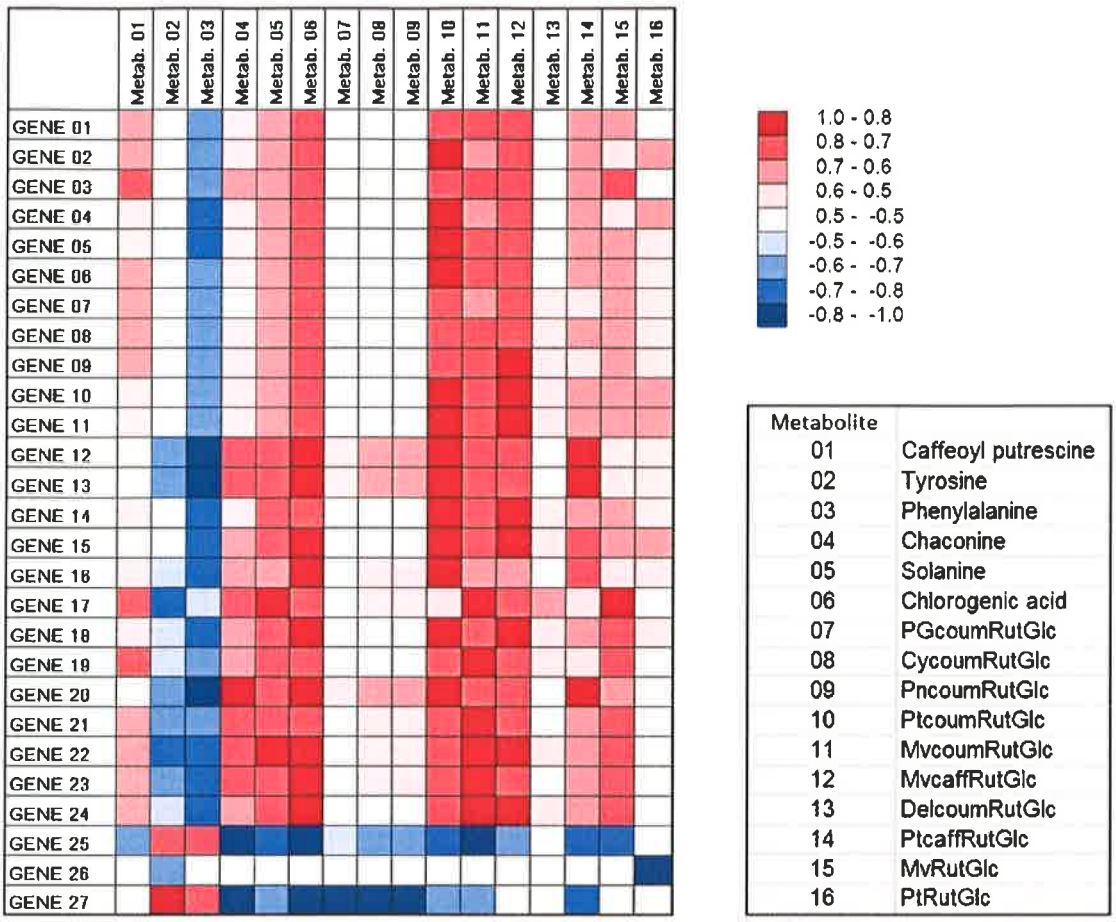

Figure 3. Gene heat unit map showing 27 genes that increase or decrease 16 secondary metabolites with pigment and antioxidant properties in purple fleshed tubers.

Table 1. Stability of chlorogenic acid in microwave cooked and stored tubers. Data are HPLC absorbance units.

	Harvest samples			Stored 6 months	
	Raw	Microwave	% Retained	Raw	% Change from harvest
Adirondack Red	2425	883	36	3542	146
All Blue	1262	394	31	2089	165
Colorado Rose	184	0	0	54	29
Purple Majesty	1165	334	29	1793	154
Rio Grande R.	434	84	19	531	122
R. Burbank	253	0	0	46	18
R. Norkotah	261	63	24	352	135
R. Nugget	404	0	0	373	92
Yukon Gold	117	88	75	0	0
CO95086-8RU	180	78	43	531	295
CO95172-3RU	447	0	0	596	133
CO97215-2P/P	979	0	0	596	133
CO97216-3P/PW	2386	219	9	1955	82
CO97232-2R/Y	3171	0	0	268	8
CO99364-3R/R	1540	364	24	2519	163