2007 #18,000

Title: Improving Value-Added Health Attributes of Colorado Potatoes

Investigators: Cecil Stushnoff, Department of Horticulture & Landscape Architecture,
David Holm and Sastry Jayanty, San Luis Valley Research Center
Colorado State University

Nature, scope and objectives of the proposed research

Background Potato (*Solanum tuberosum L.*) is an essential staple food source providing high-quality protein and energy to millions world-wide. Less appreciated are several other positive nutritional attributes of potato as a food source, including a rich source of vitamins C and B6, phenolic antioxidants, potassium, magnesium, zinc and fiber. Dietary intake of antioxidants from natural foods is fast becoming recognized as a positive contribution to a healthy lifestyle. From our recent research data and from Wu et al., (2004) evidence is mounting that potatoes are a good source of several specific phenolic based antioxidants. While the health impacts from human consumption require more study, we have identified cultivars and advanced selections that are much higher in antioxidants than conventional cultivars. While growing-season environmental conditions, post harvest conditions, and cooking methods may alter antioxidant status, the best opportunity to maximize antioxidant health attributes appears to be by identifying and selecting antioxidant-rich cultivars.

A recent consumer marketing survey, conducted under our USDA/CSREES/NRI project included Purple Majesty in the questionnaire. Health conscious consumers identified with higher antioxidant properties and were willing to pay a premium when aware of potential benefits. A recent article in "Spudman" (Stushnoff and Higgins, 2007) surfaced several positive comments from various sources around the country about enhanced health attributes in potato.

During the past four years we have focused on analytical characterization of cultivars and advanced selections from the breeding program. This work has demonstrated that significant diversity exists in potato germplasm. Preliminary data suggests that aqueous extracts from baked and freeze-dried tuber tissues of Rio Grande Russet inhibited proliferation of two breast cancer cultures more effectively than five other cultivars that we tested. This work needs to be repeated. While cell culture inhibition studies cannot be used to predict if consumption of this product will prevent breast cancer in vivo in humans, we were encouraged to follow up with additional research. Accordingly, more in-depth chemical analyses using LC/MS with collaborators in New Zealand was conducted that revealed interesting and important findings on potato antioxidants.

In this proposal we plan to continue high through-put microplate based chemical analytical screening of Colorado cultivars and selections for antioxidant properties. The following specific goals will be pursued.

(1) Characterize antioxidant content, radical scavenging capacity, and vitamin C for 20 advanced selections and new cultivars from the breeding program using existing techniques and equipment.

(2) Based upon promising data obtained from LC/MS and upon tests with Rio Grande Russet in 2004/05 we will attempt to develop a HPLC protocol to quantify chlorogenic acid.

(3) Develop application of cell culture assays to screen for inhibition of colon cancer. This component was included in a previous submission that involved The Cancer Prevention Laboratory (CPL). Because of funding obligations on other projects at CPL and lack of resources on our part we were unable to complete this work. We still feel this would provide important information on possible inhibitory compounds in other potato germplasm that was not tested in the initial work with Rio Grande Russet. While we would expect that (CPL) will wish to assist us and continue to collaborate, such screening more directly serves the needs of the breeding program and the potato industry than it does CPL. Thus, we would work towards refurbishing an existing incubator in Shepardson that belongs to CPL, so appropriate techniques to conduct the screening can be established and shift the burden of these tests to our lab from CPL.

(4) Develop an antioxidant index that incorporates different assays so potato cultivars can be compared directly for antioxidant properties.

Methods, procedures, and facilities

(a) Work plans for 2007

(1) Twenty lbs. of tubers from 20 pigmented and white fleshed cultivars and advanced selections grown at San Luis Valley Research Center will be collected and lyophilized.
(2) Samples from (1) will be assayed for total phenolics, ABTS and DPPH antioxidant radical scavenging capacity.
(3) Samples from (1) will be used to quantify chlorogenic acid content from raw, micro waved (approximately 100 C) and baked (approximately 160 C) samples to explore its temperature stability.
(4) Use of cell culture assays for colon cancer will be explored with new cultivars and selections including Rio Grande Russet.
(5) Develop an antioxidant index for potatoes.

Samples will be stored in coolers in the Shepardson building, Fort Collins. Lyophilization, spectrophotometric, and HPLC analyses will be conducted in C. Stushnoff's laboratory. All necessary analytical equipment is available. Repairs are required to refurbish a 37 °C incubation chamber to conduct cell culture assays. Funds are also required for technical labor, service contracts, maintenance, columns, solid phase purification, reagents, gases 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 profile of Colorado cultivars and (2) to provide data to assist selection of new cultivars with high antioxidant properties for Colorado producers. Our specific role is to better understand phytochemical attributes of Colorado cultivars that may be related to human health. The role of plant-derived antioxidants is receiving increasing attention. Chlorogenic acid has been implicated in several anticancer processes including inhibition of cell proliferation and tumor blood vessel development. While potatoes have long been recognized as a dietary staple, their image as healthy food has suffered. Research data is required to support putative health claims. This work provides an opportunity to identify and characterize potentially beneficial attributes of potatoes.

Potential to leverage research funding Results from this work should enhance opportunities to seek funds from value-added marketing and health related funding programs. The Cancer Prevention Laboratory has included potato as a target for future studies. Data generated from this proposal may aid their work and is consistent with the College of Agriculture's new Crops for Health initiative.

Timeline and expected milestones *Short term (1 year) expectations.*

- We expect to develop a HPLC protocol for chlorogenic acid in 2007/2008, and to test 20 cultivars and advanced selections.
- We expect to complete analyses for total phenolics and radical scavenging capacity.
- We expect to develop a reliable colon cancer cell culture screening protocol.

Cell culture summary of results Preliminary tests with six cultivars revealed that Rio Grande Russet was most active in inhibiting human breast cancer cell cultures. Purple Majesty had some inhibition. Mountain Rose and Yukon Gold had no inhibitory effect. Data in Tables 1 and 2, from three replications demonstrate both dose and time dependency in two different breast cancer cell culture lines. These data should not be taken to suggest similar inhibition will occur following human consumption of Rio Grande Russet potato products. The results have, however, prompted us to consider colon cancer cell cultures for possible inhibitory effects. Unlike breast cancer potato products when consumed are directly exposed to colon cells. We hypothesize that if inhibitory effects are present action is more likely to be from direct contact as opposed to breast cancer that would involve more convoluted, complex metabolic pathways.

Table 1. Dose-dependent effect of extracts from samples of baked Rio Grande Russet potato on the growth of *in* vitro human breast cancer cell cultures (% of control, average of three repetitions).

Concentration of extract (%)	MCF on day 5 (estrogen dependent)	MDAMB-468 on day 2(estrogen independent)
0.00	100	100

0.28	76	72
0.55	55	49
1.10	43	38

Table 2. Time-dependent effect of 0.55% concentration extracts from samples of baked Rio Grande Russet potato on the growth of *in vitro* human breast cancer cell cultures (% of control, average of three repetitions). Note these are not rat cell cultures.

MCF-7 (estrogen dependent)		MDAMB-468 (estrogen independent)	
Day 1	91	Day 1	63
Day 3	75	Day 2	49
Day 5	55	Day 3	37

Characterization of phenolic composition and glycoalkaloid content.
Data collected from total phenolics, radical scavenging and vitamin C assays suggested more information on differences in bioactive compounds among cultivars may provide valuable insight into further understanding health benefits of potato cultivars. This was achieved through a collaborative arrangement with Paula Wilson of the New Zealand Crop & Food Research Institute. A LC/MS instrument and especially the protocol for running the tests with potato are not available locally or elsewhere in the USA that we know about.

Summary of Findings

I. Phenolics

The major phenolic peaks were identified as three chlorogenic acid isomers, neochlorogenic acid, cryptochlorogenic acid, and chlorogenic acids (3-, 4-, and 5-, caffeoylquinic acids). The pigmented cultivars, Purple Majesty and Mountain Rose contained considerably higher amounts than the pigmented cultivars. There is approximately a ten fold difference between the highest (Purple majesty) and the lowest (Yukon Gold) cultivars (Fig. 1). They also contain a variety of anthocyanin pigments, but at lower concentrations than the phenolic acids. Freidman (1997) reported that chlorogenic acid was destroyed upon baking. However, we found unexpectedly high levels from our baked potato extracts. This discrepancy may be due to the fact that the cultivars used in Freidman's 1997 review were low before baking compared to the very high levels we found in the pigmented cultivars, or the pigmented cultivars are more stable upon baking. We have not yet analyzed the uncooked samples for content prior to baking.

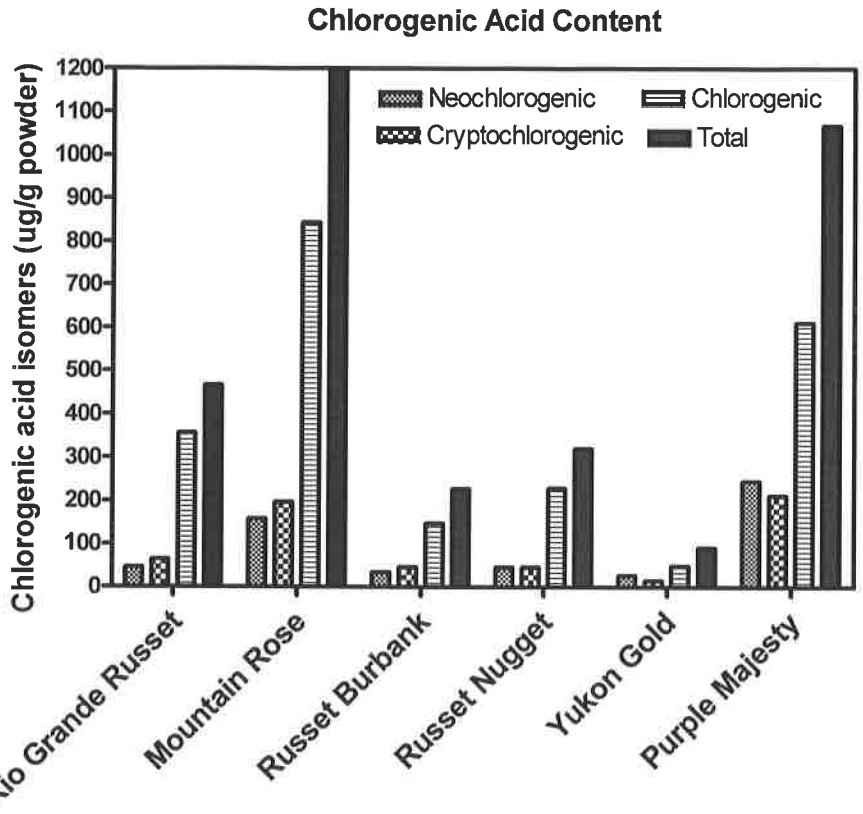

Figure 1. Chlorogenic acid isomers detected in extracts from potato cultivars baked for one hour at 170 ˚C, detected by LC/MS.

II Anthocyanins

Five pelargonidin glycosides and one peonidin glycoside were identified in Mountain Rose (Table 6). Five petunidin glycosides, one delphinidin, one peonidin, and one malvidin glycoside were identified in Purple Majesty (Table 7). None were as abundant as the chlorogenic acid isomers. Several of these pigments have been identified by others in dark highly pigmented fruits and eggplant, and some have been shown to possess cancer cell culture antiproliferation properties (Zhang et al. 2005). A recent report demonstrated that chlorogenic acid inhibited cell proliferation, blood vessel development (angiogenesis), triggered cancer cell death (apoptosis) and activated phase II enzymes know to disrupt carcinogenesis (Feng et al. 2005).

Table 6. **Anthocyanin concentrations in Mountain Rose baked one hour at 170 C**

Cultivar	Anthocyanin	Ug/g	Total
Mountain Rose	Pelargonidin-3-rutinoside+coumaric acid	1040	1638
	Pelargonidin-3-rutinoside-5-glucoside	315	
	Pelargonidin-3-rutinoside	159	
	Pelargonidin-3-rutinoside-5-glucoside+ferulic acid	124	
	Peonidin-3-rutinoside-5-glucoside+coumaric acid	trace	

Table 7. Anthocyanin concentrations in Purple Majesty (ug/g) baked one hour at 170 C.

Anthocyanin	Ug/g	Total	Activity
Petunidin-3-rutinoside-5-glucoside+coumaric	1579	1913	Apoptosis
Malvidin-3-rutinoside-5-glucoside+coumaric	119		Antiproliferation
Petunidin-3-rutinoside-5-glucoside	73		Apoptosis
Petunidin-3-rutinoside-5-glucoside+ferulic acid	61		Apoptosis
Delphinidin-3-rutinoside-5-glucoside+coumaric	43		Antiangiogenic
Peonidin-3-rutinoside-5-glucoside+coumaric	38		None found

III Glycoalkaloids

Total glycoalkaloid content (α-solanine + α chaconine) of Rio Grande Russet was approximately two to three times higher than that of the other cultivars, but well below levels considered undesirable. (Fig. 2) Glycoalkaloids are relatively heat stable and are not destroyed by baking (Mensinga et al, 2005). Lee et al. (2004) reported that α-solanine and α-chaconine are potent inhibitors of human colon (HT29) and liver (HepG2) cancer cells.

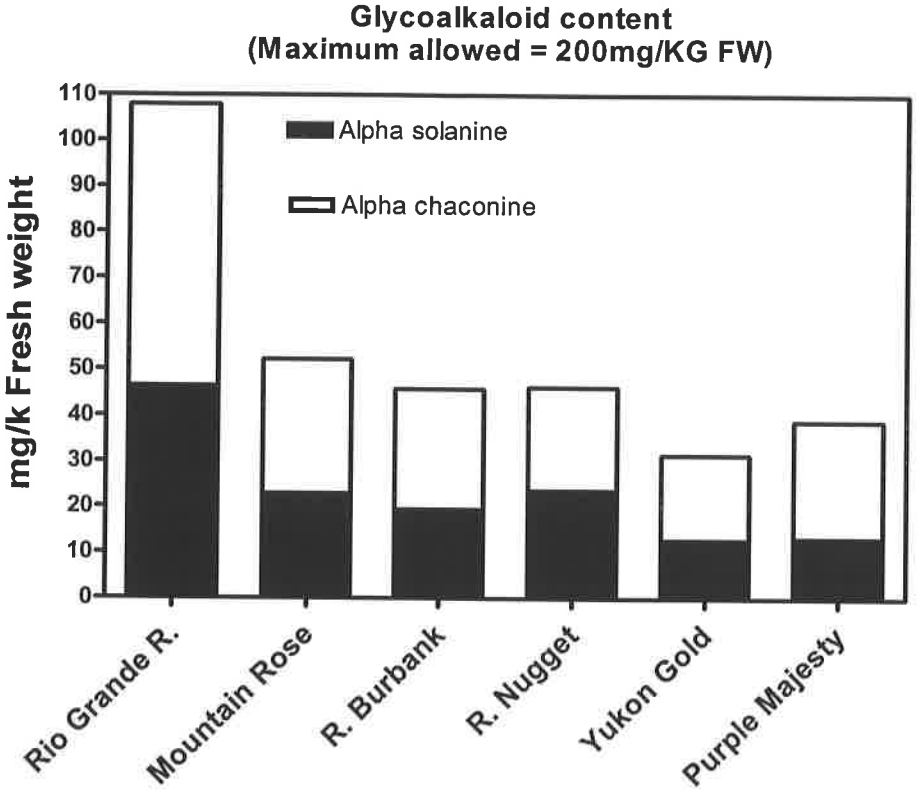

Figure 2. Glycolalkaloid content in tubers baked one hour at 170 C.

Budget for 2007

1.	Personnel: Part time analytical technical or student hourly support to run the assays.	
2.	Salary (1/2 time, 65 days @$12/hour)	12,500
	Fringe benefits (19.4%)	2,424
3.	Materials and Supplies: reagents, micro plates, tips, supplies.	1,000
4.	Travel: local	500
5.	Equipment (repair of 37° C incubator)	1,000
6.	Services: Partial service contract for micro plate reader	1,400
7.	Total:	$18,824

Justification of estimates for personnel costs

1. Cultivar/advanced selection assays for top selections: Preparation, freeze drying, extraction, data entry and analyses of 20 entries x triplicate assays. Approximately 30 (8-hour) days for total phenolics and radical scavenging assays.

2. Develop HPLC assay for chlorogenic acid and screen the most promising russet selections (approximately 30-40 days)
3. While a suitable incubator exists that is required to run the cell culture screening, it requires repair to become functional.

Literature Cited

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