Stushnoff

2003

Resource Needs

The project primarily requires an M.S. student interested in economic market modelling. Data will be collected from secondary sources including the USDA National Agricultural Statistics Service and the Colorado Agricultural Statistics Service. Minimal travel will be needed and the majority of work can be conducted on campus. Depending on availability some data may need to be purchased and addition software may be to be acquired to develop and test the time series forecasting models.

This project is part of the authors interest in developing tools for explaining and forecasting economic conditions for the different minor commodities produced by Colorado agriculture. Resources from this project will be used to demonstrate the process and outcomes. Funding will be sought from other Colorado growers associations and the Colorado Department of Agriculture. This project is not an isolated effort. Similar work may be initiated on Colorado fruit tree crops, vegetables, sugar beets, and floriculture industries.

Outcomes Time Line

The research should be completed one year after an appropriate M.S. student is identified. The student will be employed on a half-time basis. Two months will be needed to review previous research and identify relevant institutions in the potato market. Four months will be needed to collect secondary data and develop preliminary economic models. Four months will be needed to complete the modelling process. This involves model specification, testing, and developing the forecasts. Two months will be needed to complete a final report. Preliminary results can be presented from the effort and we will discuss alternative questions answerable from the models.

Budget

Item	Dollars
M.S. Student (12 months – projecting 2003 FY rates)	
Salary and Fringe	$15,000
Tuition Waiver	$3,400
Travel	$500
Software and Data	$500
Total	$19,400

Title: Effects of Genotype, Production Environment, Post Harvest Storage, and Heat Stability on Antioxidant Content and Radical Scavenging Capacity of Potato Breeding Germplasm

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

Nature, scope and objectives of the proposed research: Potato tubers (*Solanum tuberosum L.*) provide an important source of high-quality protein and energy to millions world-wide, especially in cool-temperate climates. Less appreciated is the importance of potato as a source of vitamin C, estimated to provide up to 30% of daily intake from fruits and vegetables (Finlay et al., 2003). Potatoes also contain other important antioxidants, primarily derived from phenolic compounds that have not been well studied from a genetic, production, storage, or cooking heat-stability standpoint. Our research during the past two years has shown that colored flesh genotypes possess 3 to 5Xs antioxidant activity of common cultivars, and that storage and cooking temperature can also impact content and radical scavenging activity. Our preliminary antioxidant results plus recent work at Texas A&M on anthocyanin content of colored tubers, strongly suggest that cool temperatures may enhance antioxidant status. Thus, we have initiated a student project that will compare eight cultivars grown at five environmentally diverse production sites in Colorado.

Even though most food experts contend the recent concern over acrylamide in cooked potato products is not a reason for major concern, we think it might be prudent to address this issue to be sure a new advanced selection is not among those cultivars most prone to a potential problem. We may also identify unique selections that are much less prone to generate acrylamide during cooking. Acrylamide is classified as a probable human carcinogen, and has been detected in cooked food that contains glucose and asparagine (Mottram et al., 2002). Potato products are of concern because asparagine may comprise 64.2% of total amino acids in processed potato products (Becalski et al., 2003).We are especially interested in examining this issue because we have already completed research to quantify glucose by gas chromatography in a storage study for several cultivars (McSay et al., 2002), and because of the potential interaction with antioxidants. Interestingly, cooking potato slices in oil with rosemary, a herb that contains phenolic antioxidant activity, has been found to reduce the level of acrylamide (Becalski et al., 2003). This suggests that an investigation of high and low phenolic antioxidant potatoes relative to acrylamide production might prove to be very interesting. Because we have already shown that low temperature storage of tubers induces different levels of glucose in different cultivars, the production of asparagine, the predominant amino acid in potato and a requirement for acrylamide production should also be assayed in potato cultivars and selections. We therefore propose the following objectives:

(1) Characterize **genetic diversity** of antioxidant content and radical scavenging capacity for advanced selections and new cultivars.

(2) Determine the impact of **production environment** on antioxidant status.

(3) Evaluate the effects of **storage conditions** on antioxidant status.

(4) Evaluate **antioxidant heat stability** for different cooking methods and selected cultivars.

(5) Quantitate **asparagine, acrylamide and glucose** content in advanced selections and new potato cultivars.

Methods, procedures and facilities.

(1) Tubers from 72 **cultivars and selections** grown at San Luis Valley Research Center in 2002 were collected and lyophilized for antioxidant analyses in C. Stushnoff's laboratory, Fort Collins, CO.

(2) Tubers of eight cultivars and selections (R. Burbank [control], R. Norkotah, R. Nugget, Chipeta, Yukon Gold, CO 94165 [purple], CO 94183 [red]) were obtained from five climatically different Colorado **production environments** (San Luis Valley, Weld county, Delta, Arkansas Valley, and Powder Horn). Temperature means, extremes and growing degree-day heat units plus precipitation and elevation will be used to interpret climatic effects on antioxidant status.

(3) Antioxidant status will also be determined for tubers from 10 cultivars **stored** at 1.1 C (34 F), 2.2 C (36 F), 3.3 C (38 F), 4.4 C (40 C) sampled monthly from November thru June, and for eight cultivars stored for four months at 2.2 C (36 F) following four weeks reconditioning at 50 and 60 F.

(4) **Heat stability** of antioxidant status will be determined for selected cultivars by comparing uncooked samples to those boiled for 30 minutes, microwaved at full power for 5 minutes/tuber, and baked for one hour at 350 F.

(5) **Asparagine, acrylamide** and water soluble vitamins will be examined by HPLC with a 'Discovery' RP C-16 column eluted with methanol (30 to 55%) and acetonitrile, phosphate buffer mobile phase decreasing from pH 5.5 to 3.0. The same column using a different mobile phase can be used to quantify ascorbic acid and six other water soluble vitamins. Ascorbic acid is an important antioxidant and needs to be quantified to interpret tuber antioxidant interactions. We will examine new releases, and genetic lines that are extreme for antioxidant activity to determine how antioxidant status, asparagine and glucose interact as determinants of acrylamide production in cooked potato products.

Tubers for these studies will be obtained mostly from San Luis Valley research test plots and other sites as indicated. Samples will be stored in newly renovated coolers in the Shepardson building, Fort Collins. Lyophilization, spectrophotometric, GC, and HPLC analyses will be conducted in C. Stushnoff's laboratory. All necessary analytical equipment is available, but funds are required for technical labor, service contracts, maintenance, columns, solid phase purification, reagents, gases and micropipette tips.

Relationship of proposed research to the potato industry in Colorado. The work proposed comprises our role as part of a team that is working to assure introduction of high quality, well adapted new cultivars for Colorado producers. Our specific role is to seek value-added attributes that focus on human health through dietary intervention. The

role of plant-derived antioxidants is receiving increasing attention. While potatoes provide a major source of starch in diets, changing lifestyles and other factors threaten growth in consumption. Research data on positive health attributes can assist marketing and consumer confidence. This work provides an opportunity to enhance potentially beneficial attributes of potatoes through plant breeding, and to determine environmental and storage conditions that can maximize antioxidant and health components of new introductions.

Potential to leverage research funding. We anticipate that opportunities to seek funding in collaboration with the newly established 'Cancer Prevention Laboratory' will be enhanced with data from this work. Results from this work should also enhance opportunities to seek funds from value-added and health related funding programs.

Timeline and expected milestones.

Short term (1 year) expectations.

- Total phenolics and ABTS radical scavenging capacity assays have been completed for 38 cultivars and selections from the 2001 growing season. Seventy two genotypes grown in 2002 have been lyophilized and uncooked samples will be analyzed in 2003 for genotype characterization, objective 1. New selections and cultivars will be added annually as an evaluation component of the breeding program.

- Tubers from five distinct environmental production sites for eight cultivars have been prepared for total phenolics and ABTS radical scavenging analyses in 2003. A second year's data will be collected again in 2003 as part of a PhD project.

- Total phenolics and ABTS analyses will also be run on eight entries in the storage study.

- Cooking heat stability effects on antioxidant status will be examined for ten cultivars and as many of the 72 genotypes as time and resources permit. We have microwaved, baked and boiled all 72 entries, and will analyze the most important based upon data from the uncooked samples.

- Analytical protocols for asparagine and acrylamide quantitation by HPLC will be developed for a few selected cultivars in year one. A protocol for glucose has been used in our lab for several years.

Longer term (3-5 year) expectations

- Add new advanced selections and cultivars for genotype analyses and storage studies as they arise from the breeding program.

- Complete determination of environmental effects and cooking heat stability on antioxidant status in year 2 or 3.

- Characterize tendencies to produce asparagine, acrylamide and glucose for advanced selections and new cultivars.

- Develop a screening protocol for water soluble vitamins and apply to advanced selections.

Budget

1. Personnel: Analytical technician with training to conduct microplate spectrophotometric antioxidant, HPLC and GC analyses. This could be a postdoctoral associate or hourly research technician, depending on experience and training. It is anticipated that ¾ time equivalent of a postdoc position would be required to conduct all of the proposed work.

Salary (3/4 time)	18,750
Fringe benefits (19.4%)	3,638
Total	22,388/ year

Graduate assistant:

Half of a (1/4 time position)	3,900
Fringe (3.5%)	137
Total	4,037 (for 2003 only)

2. Materials and Supplies: Reagents, microplates, tips, gases. 1,000
3. Travel: Local and part expenses for one national research meeting 1,500
4. Equipment (none)
5. Services: Half of service contract for microplate reader 1,400
6. Total: 30,325

Justification of estimates for personnel costs:
Genotype assays: 72 entries x 3 replicates x triplicate assays = 648 assays. Maximum of 30 total phenolics and 10 ABTS assays per day including lyophilization, sample preparation, data entry and analyses. Approximately 87, 8-hour days.

Environmental study: 5 sites x eight cultivars x 3 replicates x triplicate assays = 360 assays. Approx. 48 days.

Storage study: 10 entries x 4 temps x 3 x 3 =360 assays. Approx. 48 days.

Heat stability: 4 entries x 4 temps x 3 =144 assays. Approx 20 days.

Acrylamide: HPLC development done primarily by C. Stushnoff. Samples require 30 to 45 minutes each plus preparation. Estimated technical support time required = 30 days in year one and proportionately more as routines are developed in year two and beyond. Undergraduate work-study students will be assigned to conduct routine analyses.

References Cited

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Finlay, M., Dale, B., Griffiths, D.W., Todd, D.T. 2003. Effects of genotype, environment, and postharvest storage on the total ascorbate content of potato (*Solanum tuberosum*) tubers. J. Agric. Food Chem. 51: 244-248

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Mottram, D.S., B.L., Wedzicha, Dodson, A.T. 2002. Acrylamide is formed in the maillard reaction. Nature 419:448.