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**Title: Improving Value-Added Health Attributes of Colorado Potatoes**

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**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 are the positive nutritional attributes of potato as a food source. Potatoes are a good source of vitamin C, estimated to provide up to 30% of daily intake (Finlay et al., 2003). Dietary intake of antioxidants, especially from natural foods compared to supplementary pills, is widely accepted by consumers as a positive contribution to a healthy lifestyle. From our research data during the past three years, and from Wu et al., (2004) there is now emerging evidence that potatoes are a good source of antioxidants derived from phenolic compounds. While the health impacts from consumption by mammals require much more study, we now know that cultivars and advanced selections in breeding programs can provide an important source of biodiversity in content and radical scavenging capacity. While growing-season environmental conditions, post harvest conditions, and cooking methods also contribute to antioxidant status; the best opportunity to maximize antioxidant health attributes appears to be through genetics and plant breeding.

It is likely that marketing of potato cultivars might take new directions to meet nutrition conscious consumer demands. Special attributes of new cultivars such as high antioxidant and vitamin C content may provide unique marketing opportunities. For example, new selections with colored flesh possess 3 to 5xs antioxidant activity, and selections vary considerably in vitamin C content and retention following storage and cooking.

During the past three years we focused on analytical characterization of cultivars and advanced selections from the breeding program. This work has demonstrated that valuable diversity exists and provides a basis for the next steps. For example, from some very recent preliminary work, we have learned that aqueous extracts from baked and freeze-dried tuber tissue of a new introduction, 'Rio Grande' inhibited cell division of two breast cancer cultures more effectively than five other cultivars that we tested. While cell culture inhibition studies must be followed with animal and human feeding trials, among other studies, these data suggest exciting possibilities. Accordingly, in this proposal we outline a two- pronged research strategy. The research scope will encompass continuation of analytical screening on a reduced scale in number of entries tested. We feel this is appropriate since most of the advanced selections have now been tested for two and three years. At the same time we propose to add research that will focus on testing inhibition potential of cancer cell cultures with selected cultivars and advanced selections, within budgetary limitations. This can be accomplished by collaborating with the Cancer Prevention Laboratory that is experienced in conducting such research. Our two laboratories are well equipped to conduct analytical antioxidant research to screen promising selections for the breeding and marketing program, and to initiate culture

inhibition studies with cancer cell lines. We therefore propose to continue research on the following objectives:

- (1) Characterize **genetic diversity** of antioxidant content, radical scavenging capacity, and vitamin C for 30 to 40 advanced selections and new cultivars. This will include uncooked and cooked samples.
- (2) Employ breast **cancer cell culture** screening to examine potential inhibitory effects of selected cultivars and advanced breeding lines based upon data from antioxidant analyses.
- (3) A longer-term objective is to conduct a **rat feeding trial** to examine breast cancer development response to diets containing six potato cultivars.
- (4) **Publish** results from previous work, including breast cancer cell culture inhibition study.
- (5) Prepare grant proposals to seek funds to study basic mechanisms underlying breast cancer cell inhibition properties of potato extracts.

#### **Methods, procedures, and facilities:**

##### **(a) Work in progress and plans for 2005**

- (1) Tubers from 88 **cultivars and selections** grown at San Luis Valley Research Center in 2003 were collected, lyophilized, and assayed for total phenolics (Table 1), ABTS antioxidant radical scavenging capacity (Table 2) and vitamin C (Table 3). Seventy-four cultivars and selections have been collected and lyophilized from the 2004 crop. Analysis of the 2004 crop is presently underway and will continue during 2005.
- (2) Tubers of eight cultivars and selections (R. Burbank [control], R. Norkotah, R. Nugget, Chipeta, Yukon Gold, CO 94165 [Purple Majesty], CO 94183-R/R) obtained from five climatically different Colorado **production environments** in 2002 (San Luis Valley, Weld county, Delta, Arkansas Valley, and Powder Horn). Three locations supplied tubers from the 2003-growing season. These samples have been analyzed and a graduate student dissertation is in preparation. Temperature means, extremes and growing degree-day heat units plus precipitation and elevation will be used to interpret climatic effects on antioxidant status.
- (3) **Cooking stability** of endogenous antioxidants was studied in selected cultivars of the 2003 and 2004 harvest by comparing uncooked samples to those boiled for 30 minutes, micro waved at full power for 5 minutes/tuber, and baked for one hour at 350 F. Selected Colorado cultivars will be examined again in 2005.
- (4) **Vitamin C** content as influenced by genotype, cooking method, and storage interval has been analyzed from selections and cultivars grown in 2003, and will be completed for those grown in 2004.

(5) The Cancer Prevention Laboratory has generously lyophilized 150 lbs. of tubers from each of six cultivars (Rio Grande, CO94183-1R/R, Russet Burbank, Russet Nugget, Yukon Gold and Purple Majesty). These freeze dried powders are intended for use in a **rat feeding trial** to test their possible impact on altering antioxidant status and inhibiting breast cancer, in vivo.

In a preliminary in vitro cell culture experiment, extracts from these samples were tested for their capacity to inhibit cell cultures of two breast cancer lines. Aqueous extracts from Rio Grande inhibited cancer cell growth more effectively than extracts from the other entries.

Tubers for the proposed studies in 2005 will again be obtained from San Luis Valley Research Center test plots. Samples will be stored in coolers in the Shepardson building, Fort Collins. Lyophilization, spectrophotometric, GC, and HPLC analyses will be conducted in C. Stushnoff's laboratory. Cell culture experiments will be conducted in the Cancer Prevention Laboratory. All necessary analytical equipment is available, but funds are 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 potato industry in Colorado.** The work proposed is intended to provide data to assist selection of new cultivars with high antioxidant and vitamin properties 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 a "carbo-phobic" diet fad 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, ABTS radical scavenging capacity and vitamin C assays will be completed for 74 entries grown in 2004. A new set of entries (30-40) will be collected and analyzed from the 2005 harvest. New selections and cultivars will be added annually as an evaluation component of the breeding program.
- A study to examine the impact of environment on antioxidant content and capacity has been completed. The student intends to graduate in May 2005, and papers will shortly be submitted for publication.
- Total phenolics and ABTS analyses will also be completed on eight entries in the 2003/2004-storage/reconditioning-chip study.
- Cooking heat stability effects on antioxidant and vitamin C status will be examined for ten cultivars and as many of the 74 genotypes as time and resources permit.
- Confirm preliminary data on breast cancer cell culture inhibition studies.
- Screen 20-30 advanced selections for breast cancer cell culture inhibition.
- Assist marketing agencies attempting to promote potatoes for health benefits.

*Longer term (3-5 year) expectations*

- Add new advanced selections and cultivars for genotype analyses and storage studies from the breeding program. Provide data to aid marketing of new cultivars based upon nutritional properties.
- Examine screening protocols for breast, colon, kidney and/or liver cancer cell culture inhibition by potato extracts from promising advanced selections and cultivars.
- Evaluate the impact of the planned rat feeding trial.
- Initiate an analytical biochemical study designed to identify and characterize the mode of action responsible for inhibition of cancer cultures by potato extracts.
- Assist marketing agencies attempting to promote potatoes for health benefits.

## Budget

1. Personnel: Analytical technical support with training to conduct micro plate spectrophotometric antioxidant and HPLC analyses. This could be a postdoctoral associate or hourly research technician, depending on experience and training. It is anticipated that  $\frac{3}{4}$  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
  
2. Materials and Supplies: reagents, micro plates, tips, and gases cell culture supplies. 4,000
3. Travel: local and part expenses for one national research meeting 1,500
4. Equipment (none)
5. Services: Service contract for micro plate reader 2,800
6. Total: \$30,688

## Justification of estimates for personnel costs:

1. Genotype/cultivar assays: 40 entries x triplicate assays x 2 (freeze dried and micro waved) = 280 assays. Maximum of 20 total phenolics and 10 ABTS assays per day including lyophilization, sample preparation, data entry and analyses. Approximately 42, 8-hour days.
2. Vitamin C: HPLC 40 entries x 3 =120 assays, 10 assays/day. = Approx. 12 days.
3. Cell culture: 30 entries x 3 replicates; 3 days/5 entries/ assay = 54 days
4. Cooking heat stability of advanced selections: 10 entries x 4 treatments x 3 =120 assays. Approx 16 days.

The assays are listed in order of priority for available resources.

## Literature Cited

Finlay, M., Dale, B., Griffiths D.W. and D.T. Todd. 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.

Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E. and R.L. Prior. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J. Agric. Food Chem. 52:4026-4037.

Table 1. Evaluation data for 2003 crop based on total phenolics. The highest and lowest groups represent genotypes that fall into 20% of the total population of 88 tested from the 2003 harvest. The remaining 60% fall into the middle of the population. Some of these may possess other outstanding characteristics that make them desirable for additional antioxidant testing. Based upon the past three years experience, the very lowest can be discarded after one year, but two years testing would be desirable for promising selections. The entries are listed in descending rank for total phenolics based on uncooked and cooked samples. Russet Burbank is listed first as the control standard for comparison. Data are means of three replicate tubers.

Highest Sources			Lowest Sources		
Identity	Phenolics uncooked & cooked samples (mg/gdw)	Phenolics cooked samples only (mg/gdw)	Identity	Phenolics uncooked & cooked samples (mg/gdw)	Phenolics cooked samples only (mg/gdw)
			R. Burbank	2.24	2.44
CO97227-2P/P	26.6	19.2	BC0894-2W	1.8	1.5
CO97226-2R/R	19.6	16.5	CO95070-7W	2.5	2.5
CO97216-3P/P	16.2	13.1	VC1015-7R/Y	3.0	2.7
CO97216-1P/P	16.2	11.2	CO95051-7W	2.9	2.7
PAC99929-1R/R	15.6	12.1	CO97274-2W/Y	2.5	2.8
CO97306-1R/R	14.5	11.7	CO96293-4W	3.1	2.9
CO972077-2RU	12.5	11.0	VC1002-3W/Y	3.7	2.9
CO97215-2P/P	12.5	7.7	CO94035-15RU	3.3	3.1
CO97222-1P/R	11.8	13.3	CHIPETA	2.8	3.1
CO94183-1R/R	11.7	9.5	AC99329-7W/Y	2.4	3.2
CO94165-3P/9 Purple Majesty	11.0	8.0	CO94084-12RU	3.3	3.3
CO94065-2R/R	10.3	10	CO96141-4W	3.7	3.3
Rio Grande R.	9.9	8.6	AC94296-5W	3.8	3.6
All Blue	8.4	6.1	ATC98509-1R/Y	3.9	3.6
RNK#S3	8.4	7.9	AC9405-2RU	3.8	4.0
CO97219-1R/R	8.2	6.1	VC0967-5R/Y	5.8	5.0
CO99364-3R/R	8.2	6.0			
Durango Red	8.0	7.7			
<b>Other cultivars</b>					
Centennial R.	3.0	2.5			
Cherry Red	5.7	5.5			
Freemont R.	5.5	5.6			
Keystone R.	6.3	6.3			
R. Nugget	5.9	4.6			
RNK#S8	8.2	7.2			
Sangre#S10	7.5	6.7			
Silverton R.	6.5	6.1			
Yukon Gold	na	4.1			

Table 2. Evaluation data for 2003 crop based on ABTS/Trolox equivalent antioxidant capacity (TEAC). The highest and lowest groups represent genotypes that fall into 20% of the total population of 88 that was tested from the 2003 harvest. The remaining 60% fall into the middle of the population. The entries are listed in descending rank for ABTS/TEAC based on uncooked and cooked samples. Russet Burbank is listed first as the control standard for comparison. Data are means of three replicate tubers.

Highest Sources			Lowest Sources		
Identity	ABTS/ TEAC (uM/gdw) Cooked & uncooked	ABTS/ TEAC (uM/gdw) Cooked only	Identity	ABTS/ TEAC (uM/gdw) Cooked & uncooked	ABTS/ TEAC (uM/gdw) Cooked only
R. Burbank Standard	0.24 ± 0.02	0.23± 0.02	R. Burbank Standard	0.24 ± 0.02	0.23± 0.02
CO97227-2P/P	7.1	2.7	BC0894-2W	0.3	0.3
CO97226-2R/R	2.6	1.9	CO95070-7W	0.2	0.2
CO97216-3P/P	3.0	1.5	VC1015-7R/Y	0.2	0.2
CO97216-1P/P	4.2	1.3	CO95051-7W	na	na
PAC99929- 1R/R	2.5	1.7	CO97274-2W/Y	0.5	0.4
CO97306-1R/R	2.2	1.3	CO96293-4W	na	na
CO972077-2RU	2.2	0.6	VC1002-3W/Y	0.4	0.2
CO97215-2P/P	3.7	0.7	CO94035-15RU	0.4	0.3
CO97222-1P/R	na	1.2	CHIPETA	0.2	0.3
CO94183-1R/R	1.7	0.8	AC99329-7W/Y	0.4	0.4
CO94165-3P/9 Purple Majesty	2.6	0.8	CO94084-12RU	na	na
CO94065-2R/R	0.7	0.7	CO96141-4W	na	na
Rio Grande R.	0.5	0.4	AC94296-5W	0.3	0.3
All Blue	2.3	0.7	ATC98509-1R/Y	na	na
RNK#S3	0.5	0.4	AC9405-2RU	na	na
CO97219-1R/R	1.6	0.7	VC0967-5R/Y	0.4	0.3
CO99364-3R/R	1.4	0.6			
Durango Red	0.2	0.3			
<b>Other cultivars</b>					
Centennial R.	0.3	0.3			
Cherry Red	0.2	0.2			
Freemont R.	0.3	0.3			
Keystone R.	0.3	0.3			
Russet Nugget	0.4	0.3			
RNK#S8	0.4	0.4			
Sangre#S10	0.3	0.3			
Silverton R.	0.3	0.3			
Yukon Gold	0.3	0.3			

Table 3. Evaluation data for 2003 crop based on vitamin C content and stability following 7 months storage (36-38F, 90% RH). The highest group represents genotypes that fall into 18% of the total population of 88 that was tested from the 2003 harvest. Russet Burbank is listed with cultivars as the control standard for comparison. Data are means of three replicate tubers.

Identity	Vitamin C at harvest (mg/100gFW)	Vitamin C after 7 months storage (mg/100gFW)	Vitamin C retained after 7 months storage (%)	Vitamin C retained after cooking (%)
<b>Advanced selections (top 16)</b>				
AC99330-1P/Y	38.8	14.2	37	na
CO99135-2RU/Y	31.8	10.6	33	na
VC1115-1RU	30.7	14.0	46	na
CO95007-1RU	29.8	10.1	34	na
CO97219-1R/R	29.1	8.2	28	na
ATC98513-1W/Y	28.8	15.9	55	na
CO99338-3RU/Y	28.6	12.9	45	na
AC99329-7RW/Y	27.0	12.2	45	na
CO96109-7RU	25.9	12.1	46	na
CO99364-3R/R	25.8	11.5	45	na
AC95405-2RU	25.7	15.8	62	na
AC94296-5W	24.5	15.9	65	na
VC1123-2W/Y	22.9	11.4	51	na
CO95070-7W	22.6	11.3	51	na
CO96141-4W	22.6	11.1	49	na
CO97274-2W/Y	23.4	9.4	40	na
<b>Cultivars</b>				
Yukon Gold	23.4	11.8	50	71
All Blue	22.7	7.1	31	69
Russet Nugget	20.2	14.4	72	94
Cherry Red	20.2	10.0	49	51
Purple Majesty	19.6	7.4	38	na
RNK-S8	18.5	12.0	65	88
Silverton R.	18.4	12.0	65	85
Rio Grande	18.1	12.9	72	94
Centennial R.	17.7	10.2	57	89
Sangre-S10	17.8	11.3	63	82
Freemont R.	17.2	10.2	59	88
RNK-S3	15.5	12.2	79	77
Keystone R.	14.4	13.5	94	94
R. Burbank	14.3	12.8	89	74