Improving Value-Added Health Attributes of Colorado Potatoes

Report of Progress and Results for Activity in 2003
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Cecil Stushnoff and Ann McSay
Department of Horticulture & Landscape Architecture,
Colorado State University, Fort Collins, CO

David Holm
San Luis Valley Research Center, Center, CO

SUMMARY OF RESULTS FROM POTATO RESEARCH ACTIVITY IN 2003

Project justification: Last year we proposed a series of investigations focused on the potential to increase value of potato cultivars based upon health benefits in human diets. We proposed to emphasize screening and characterization of cultivars and advanced selections for antioxidant properties and water soluble vitamins. We also proposed characterizing cultivar tendencies to produce acrylamide in cooked products. Following suggestions from Chris Voigt and the CAPC screening committee it seemed most prudent to focus our resources on antioxidant and vitamin screening and to drop the acrylamide investigations.

Although not a stated goal of this project, but at the encouragement of Farm Fresh Direct, we also conducted some preliminary research to develop an in vitro technique to explore the possibility of characterizing potato cultivars for glycemic properties. A brief summary of that work will also be included in this report.

Significant accomplishments and potential impacts:

I. Genetic Diversity

1. Average total phenolic content of microwaved, boiled, and baked cultivars and selections was used to characterize each of 67 entries we processed from the 2002 crop (Table 1, 3). Because potatoes are not consumed uncooked, raw sample phenolic content was not included in these average scores.
    - Compared to the standard Russet Burbank: 12 selections and 2 cultivars were significantly higher, 21 selections and 8 cultivars were similar, while 22 selections and 2 cultivars contained significantly less total phenolics, $P< 0.05$.
    - Seven selections in the highest group had purple or red flesh, three had red skin and yellow flesh and two had russet skin with white flesh. The highest selection contained 91.3 mg/gdw gallic acid equivalent which is 5 times the level found in Russet Burbank.

2. Radical scavenging capacity, (expressed as mM Trolox , a water soluble form of vitamin E) was determined for microwaved, boiled, and baked samples from 12 cultivars and 29 of the selections that had the highest total phenolic content (Table 2,3). Radical scavenging capacity was also determined for uncooked and microwaved samples from all 67 entries (Appendix).

    - With very few exceptions, radical scavenging was also highest in those selections with colored flesh and very high phenolic content (Appendix A). CO97226-2R/R was 23xs more active than Russet Burbank, and 13 selections with yellow or white flesh had higher radical scavenging capacity than Russet Burbank. This suggests that new cultivars with high antioxidant properties need not be only those with colored flesh.

- All but three of the cultivars grown at SLV had higher antioxidant properties than Russet Burbank. All blue was the highest followed by Durango Red, Keystone Russet, Centennial Russet and Yukon Gold.

3. A protocol was developed to screen cultivars and selections for vitamin C content in uncooked tubers using reverse phase HPLC. Freeze dried samples of 72 entries (stored less than 60 days at 38F) were finely ground and subjected to ammonium sulfate to remove proteins, then extracted in a phosphate buffered saline solution with dithiothreotol as a reducing agent to stabilize the extract against loss of activity during analysis.
    - Vitamin C content ranged from 8 to 68 mg/100 grams fresh tuber weight. The highest content was found in CO95172-3RU with 3.5xs the level in Russet Burbank.
    - Vitamin C content was normally distributed (Fig. 1) with a few selections containing much higher levels than average. This suggests breeding and selection can produce new cultivars with substantially higher vitamin C.
    - The USDA Potato Nutrient Database for standard reference lists potato containing 19.6 grams vitamin C per 100 grams fresh tuber weight. This compares favorably with our data for most cultivars including Russet Burbank (Table 4). However, data from Medallion Labs provided by Chris Voigt is considerably lower. This may reflect the analytical method used or a storage effect. Vitamin C has been reported to drop by 50% or more following 4-6 months storage.

II Production Environment

Total phenolic content and ABTS radical scavenging capacity assays have been completed for the 2002 crop year. Russet Burbank, Russet Norkotah, Russet Nugget, Chipeta, Yukon Gold, CO94165 P/P, and CO94183 R/R were grown at San Luis Valley, Powder Horn, Delta, Arkansas Valley and Weld County. The same study was repeated in 2003 at three locations and is presently being analyzed to provide two years data.Both years provided extreme heat stress at the Arkansas Valley. Chipeta and Yukon Gold appeared to tolerate the heat stress better than other entries in 2002. This project is in its final year.

III Storage/Reconditioning

ABTS radical scavenging capacity of chips processed from 40F, 50F and for those reconditioned 40/60 versus 50/60 differed substantially among entries, but there was no consistent pattern for the 2002 crop that could be associated with a specific temperature treatment for all entries. Chips from CO94183 R/R, CO94165 P/P, and from AC98069 W/Y were highest in antioxidant radical scavenging capacity from 2002 tubers (Fig. 2). Chips were also processed from some of the same cultivars for the 2003 crop. These are presently being analyzed and will be reported next year.

IV Cooking Heat Stability

There were substantial differences in stability among entries for different cooking methods (Appendix A). Values generally decreased, but in some cases (20 entries) antioxidant properties increased in cooked samples, for example AC89536-5RU. Even though the colored fleshed types lost antioxidant properties during cooking the cooked levels still generally were much higher than in most of the white-fleshed types (Fig. 3,4).

V Glycemic Index

Preliminary research was conducted to adapt an in vitro assay to estimate the glycemic index of Colorado potatoes. An in vitro assay might be used in place of the much more expensive in vivo assays that use human subjects with blood tests to screen cultivars and selections. The in vitro assay requires a complex series of enzymatic digestions designed to estimate rapid release of glucose from starch plus HPLC to quantify glucose. Glucose released within 20 minutes in humans stimulates insulin activity and is considered hyperglycemic compared to slowly available glucose from more complex and less digestible starch molecules.

- We were able to demonstrate some cultivar differences (Table 5), although not many showed major differences, and replication in these initial trials was limited.
- The glycemic index is based upon comparison with glucose and/or white bread. Glucose is assigned a score of 100 and our results show that all the glucose in the standard indeed appeared within 20 minutes, as did white bread and wheat flour. The lowest glycemic cultivar in this preliminary work was Florisant (51.2) and the highest was CO94183R/R (85.5).
- In our opinion this assay could be adapted as a screen, but more developmental work is required to increase through put and develop the necessary statistical confidence for routine analyses.

Table 1. Total phenolic content of Colorado potato cultivars and breeding lines compared to Russet Burbank grown in 2002 at SLV. Ranks are based on means of three cooking methods (bake, boil, microwave), expressed as Gallic acid equivalents mg/gdw. Values for entries are significantly different (p= .05) between the three columns, but not within columns.

Equal to R. Burbank (16.9 ± 4.7 mg/gdw)	**Greater than R.Burbank** (16.9 ± 4.7 mg/gdw)	**Less than R. Burbank** (16.9 ± 4.7 mg/gdw)
AC87340-2W (14.3)	AC87084-3RU (23.9)	AC93047-1RU
AC92009-4RU (18.9)	AC89536-5RU (24.8)	AC93026-9RU
ATC98515-1R/Y (16.0)	CO94183-1 R/R (29.46)	AC95405-2RU
ATC98495-1W/Y (15.7)	CO94165-3 P/P (29.2)	AC94296-5W
	CO97219-1R/R (22.8)	AC97521-1R/Y
CO97216-1P/P (16.4)	CO97215-2P/P (22.8)	ATC98444-1R/Y
CO89097-2R (16.1)	CO97216-3P/P (31.6)	ATC98509-1R/Y
CO97232-2R/Y (16.1)	CO97227-2P/P (91.3)	BC0894-2W
CO92077 (22.1)	CO97306-1R/R (69.8)	CO94157-2W/Y
CO93001-11RU (18.1)	CO97232-1R/Y (23.0)	C094035-15RU
CO95172-3RU (19.7)	CO97233-3R/Y (24.5)	CO94084-12RU
CO94019-1R (18.8)	VC0967-5R/Y (23.8)	CO94222-6RU/Y
CO94065 (19.9)		CO97274-2W/Y
		CO93016-3RU
NDC6184-3R (14.8)		CO95086-8RU
NDC5372 (19.4)		CO95070-7W
NDC5281-2R (17.5)		CO95051-7W
PAC99P29-1R/R (14.9)		NDC6084C-2W
TC1675-1RU (17.2)		VC1075-1R
VC1106-1RU/Y (18.4)		VC1015-7R/Y
VC1009-1W/Y (19.9)		VC1123-2W/Y
VC1015-1R/Y (19.5)		VC1002-3W/Y
VC0967-2R/Y (18.0)		
Cherry Red (16.8)	All Blue (39.8)	Chipeta (12.8)
Centennial R. (20.6)	Durango Red (24.0)	Yukon Gold (12.2)
Freemont R. (14.8)		
Keystone (20.1)		
RNK#3 (21.2)		
RNK#8 (19.4)		
Sangre –S10 (21.6)		
Silverton R. (14.9)		

Table 2. Radical scavenging capacity of Colorado cultivars and breeding lines that had the highest total phenolics content. Data are expressed as Trolox (water soluble vitamin E) equivalents mM/gdw compared to the control cultivar Russet Burbank = 0.17 mM TEAC/gdw. Values for entries are significantly different between the two columns, but not within columns (P=.05).

Equal to R. Burbank (0.17 ± 0.04 mM/gdw)	**Greater than R.Burbank** (0.17 ± 0.04 mM/gdw)	**Greater than R.Burbank** (0.17 ± 0.04 mM/gdw)
CO95070-7W (0.14)	AC97521-1R/Y (0.34)	All Blue (3.65)
Cherry Red (0.21)	AC89536-5RU (1.91)	Centennial Russet (1.14)
Sangre (0.20)	AC93047-1RU (0.40)	Chipeta (0.27)
Silverton Russet (0.21)	AC94296-5W (0.22)	Durango Red (2.24)
	ATC98495-1W/Y (0.23)	Freemont (0.26)
	ATC98509-1R/Y (0.22)	
	ATC98515-R/Y (0.23)	Keystone Russet (1.31)
	CO95007-1RU (0.28)	Russet Norkotah #3 (0.37)
	CO94165-3P/P (0.81)	Russet Norkotah#8 (0.43)
	CO95086-8RU (0.26)	Yukon Gold (1.03)
	CO95172-3RU (0.35)	
	CO97215-2P/P (1.72)	
	CO97216-1P/P (1.73)	
	CO97216-3P/P (1.16)	
	CO97219-1R/R (0.53)	
	CO97226-2R/R (4.01)	
	CO97227-2P/P (2.580	
	CO97306-1R/R (1.76)	
	CO97232-1R/Y (0.22)	
	CO97232-2R/Y (0.33)	
	CO97233-3R/Y (0.32)	
	NDC5372-1-RU (1.31)	
	PAC99P29-1R/R (1.61)	
	VC1009-1W/Y (1.06)	
	VC1015-1R/Y (0.64)	
	VC1015-7R/Y (0.24)	
	VC1075-1R (0.27)	
	VC0967-5R/Y (0.33)	

Table 3. Potato selections and cultivars proposed for tests in 2003/04 based on total phenolics, ABTS radical scavenging and vitamin C. Entries are the top ten of those tested from the 2002 harvest.					
Entry	**Total raw Phenolics** (Rank)	**Total micro Phenolics** (Rank)	**ABTS Raw** (Rank)	**ABTS Micro** (Rank)	**Vit. C Raw** (Rank)
CO97227P/P	163 (1)	102 (1)	6.03 (1)	4.09 (1)	.049
CO97306R/R	118 (2)	73 (2)	3.87 (4)	1.77 (7)	.054
CO97216P/P	95 (3)	37 (3)	5.37 (3)	2.09 (4)	.064
All Blue	81 (4)		3.38 (5)		.09
CO94165P/P	75 (5)	34 (4)	2.66 (7)		.07
CO97215P/P	54 (6)	26 (8)			.03
CO974183R/R	48 (7)	31 (5)			.07
CO97219R/R	48 (8)	23			.08
CO97232R/Y	41 (9)	24 (10)			.166 (3)
VC1015R/Y	39 (10)	11			.154 (6)
CO86218R	33	29 (6)	2.96 (6)	2.95 (3)	.07
AC87084RU	27	28 (7)			.05
VC0967 RY	25	25 (9)			.07
CO97226R/R	36		6.02 (2)	3.81 (2)	.098
PAC99P29R/R	23	15	2.62 (8)	1.44 (8)	.048
AC89536 RU	33	23	2.48 (9)	1.80 (5)	.097
NDC5372RU	22	22	2.21 (10)	1.78 (6)	.07
Keystone R	19	17		1.5 0 (7)	
CO92077 RU	34	23		1.17 (8)	
VC1081W/Y	33	21		1.02 (9)	
Cent. Russet RU	21	23		0.91 (10)	
CO95007RU	21	10			.185 (1)
CO95172RU	26	20			.170 (2)
VC1009W/Y	33	21			.161 (4)
CO95086RU	18	8			.159 (5)
CO97233R/Y	32	20			.154 (7)
AC93047RU	18	13			.140 (8)
CO94157W/Y	14	7			.133 (9)
CO95070W	11	4			.128 (10
Standards?					
Russet Burbank					.08
Russet NorkotahS3	22	18			.05

Table 4. Vitamin C content of Colorado cultivars from our lab compared to data from Medallion Labs and the USDA Nutrient Database Standard Reference (1998) for Potato. Our data are from tubers grown at SLV, freeze dried in 60 days from harvest.

Cultivar	USDA Mg/100gfw	Medallion Mg/100gfw	Our data Mg/100gfw
USDA cultivar not specified	19.6		
All Blue		7.13	36.0
Centennial Russet		<1.0	32.0
Cherry Red		5.7	15.2
Chipeta			28.0
Durango Red		6.81	27.6
Freemont Russet			25.2
Keystone Russet			20.0
Russet Burbank			32.0
Russet Norkotah # 3			20.0
Russet Norkotah #8			24.0
Russet Norkotah		9.79	
Russet Nugget		8.55	
Banana Fingerling		6.67	
CO94183 R/R		5.84	26.4
CO94165 P/P			29.6
Yukon Gold (stored 6 months)		11.1	8.0

Table 5. Summary of glycemic index preliminary data

	Free sugar glucose	Rapidly available glucose	Glycemic Index Rag as % glucose	Total Glucose	RAG as % total glucose
ACHILLE	0.008109	0.011823	77.7	0.01146	100.0
CO94183R/R	0.007234	0.013016	85.5	0.0119	100.0
CO94183R/R (COOL)	0.006619	0.010245	67.3	0.011165	91.8
FLORISANT	0.004331	0.007794	51.2	0.01187	65.7
CO94165P/P	0.004547	0.009897	65.0	0.00926	100.0
ATLANTIC	0.00578	0.008846	58.1	0.01016	87.1
RED GOLD	0.005659	0.009948	65.3	0.00893	100.0
R.BURBANK	0.004602	0.009242	60.7	0.009123	100.0
CHELLAH	0.00538	0.009942	65.3	0.010923	91.0
RNK3 (COOL)	0.005281	0.009437	62.0	0.010906	86.5
RNK3	0.005954	0.0095	62.4	0.010209	93.1
POT STARCH (SIGMA)	0.005043	0.009255	60.8	0.01377	67.2
WHEAT FLOUR	0.005365	0.01757	115.4	0.013006	100.0
WHITE BREAD	0.004942	0.01596	104.8	0.012137	100.0
RICE	0.004882	0.010309	67.7	0.011296	91.3
SANGRE	0.006055	0.009787	64.3	0.010921	89.6
DURANGO	0.005855	0.010156	66.7	0.010459	97.1
VALISA	0.004775	0.010507	69.0	0.010665	98.5
C093001-11RU	0.009588	0.009683	63.6	0.005062	100.0
Glucose	0.01576	0.015223	100.0	0.015799	96.4

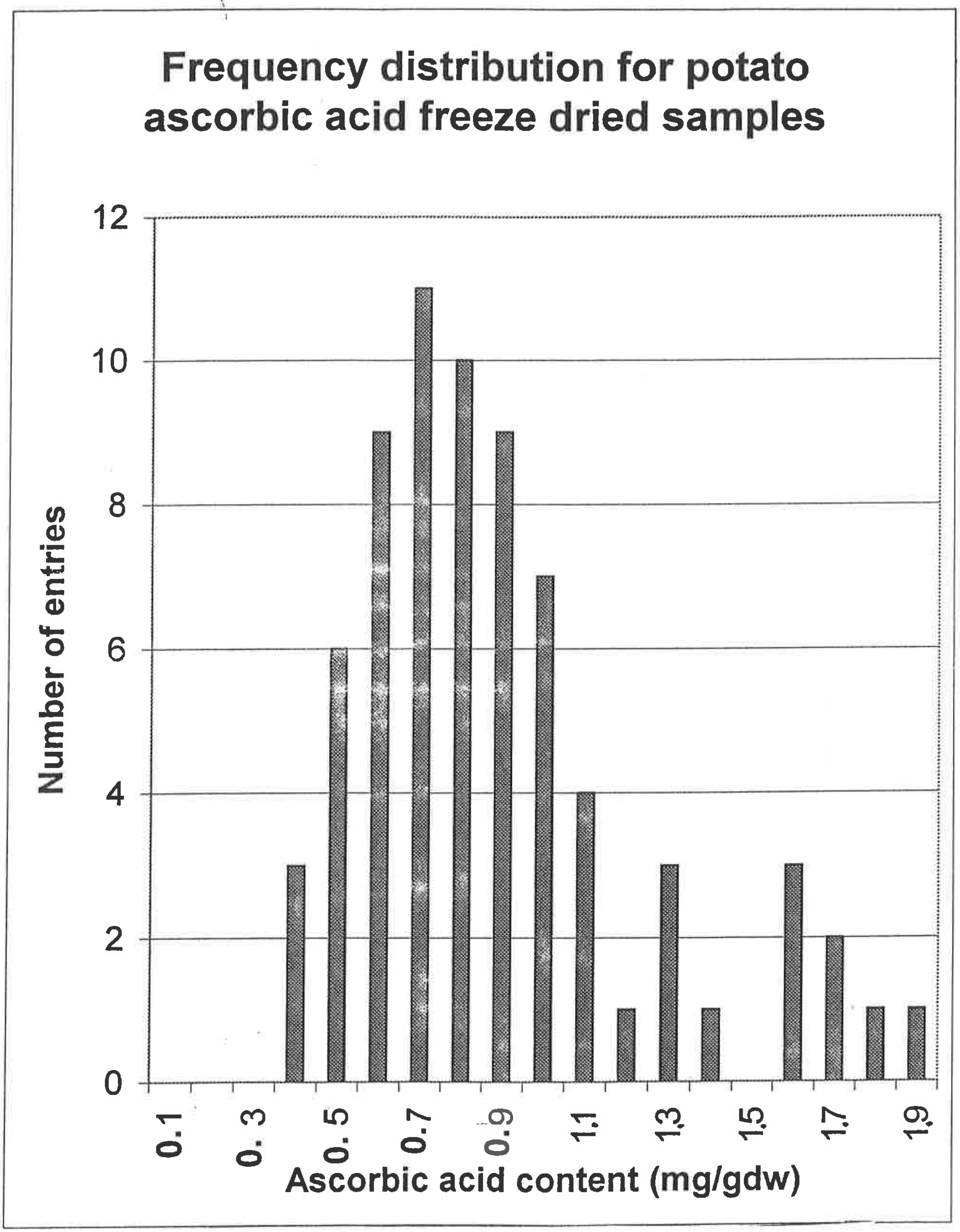

Figure 1. Frequency distribution for the number of cultivars and selections that fall into 19 classes of vitamin C in freeze dried uncooked potato samples. Classes are mg/gdw x 10 vitamin C.

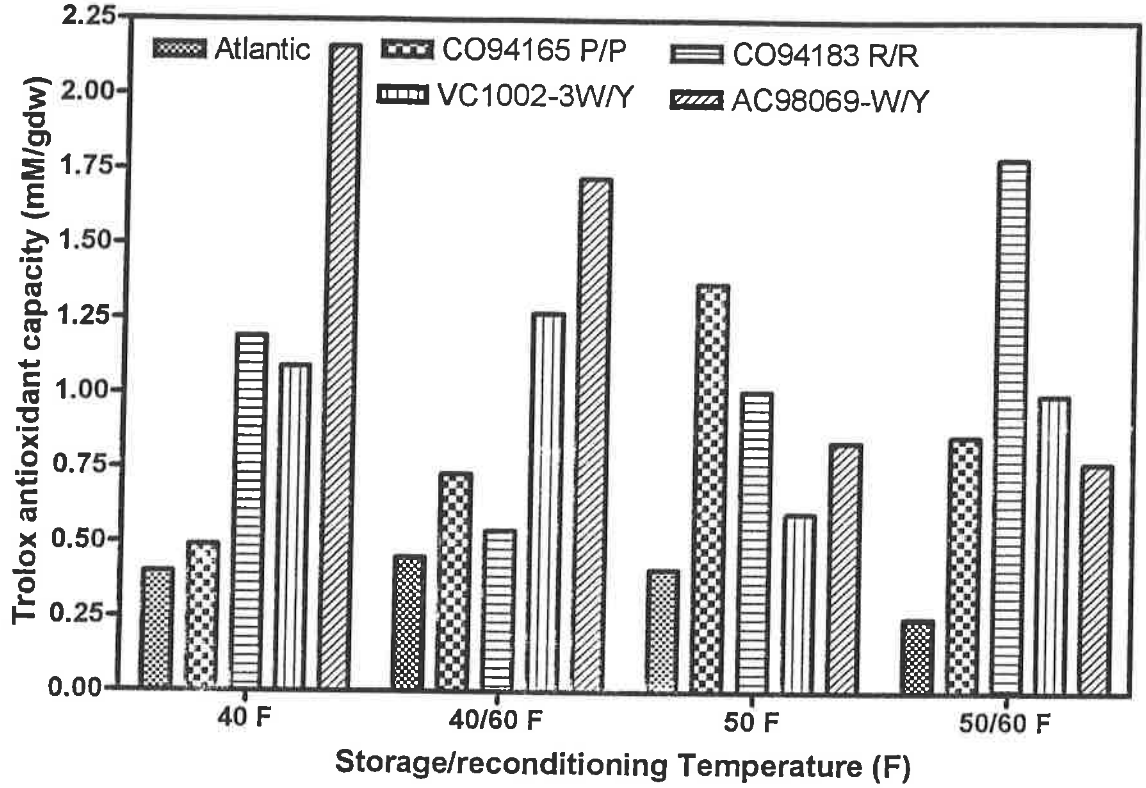

Figure 2. Radical scavenging capacity in chips prepared after storage or reconditioning at temperatures indicated.

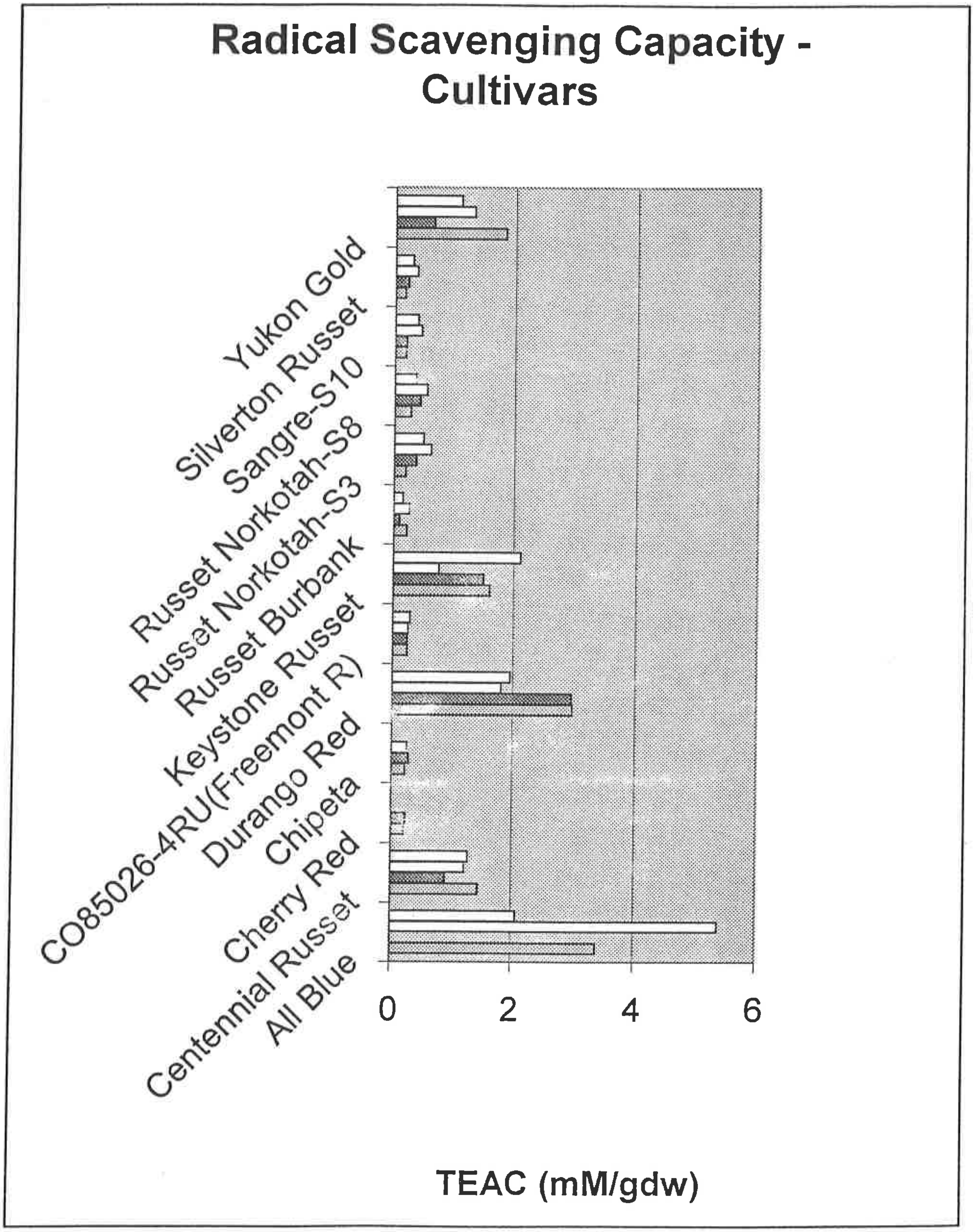

Figure 3. Radical scavenging capacity expressed as Trolox (water soluble vitamin E) equivalent antioxidant capacity (mM/gdw) for 13 cultivars comparing uncooked freeze dried samples to microwaved, boiled and baked samples.

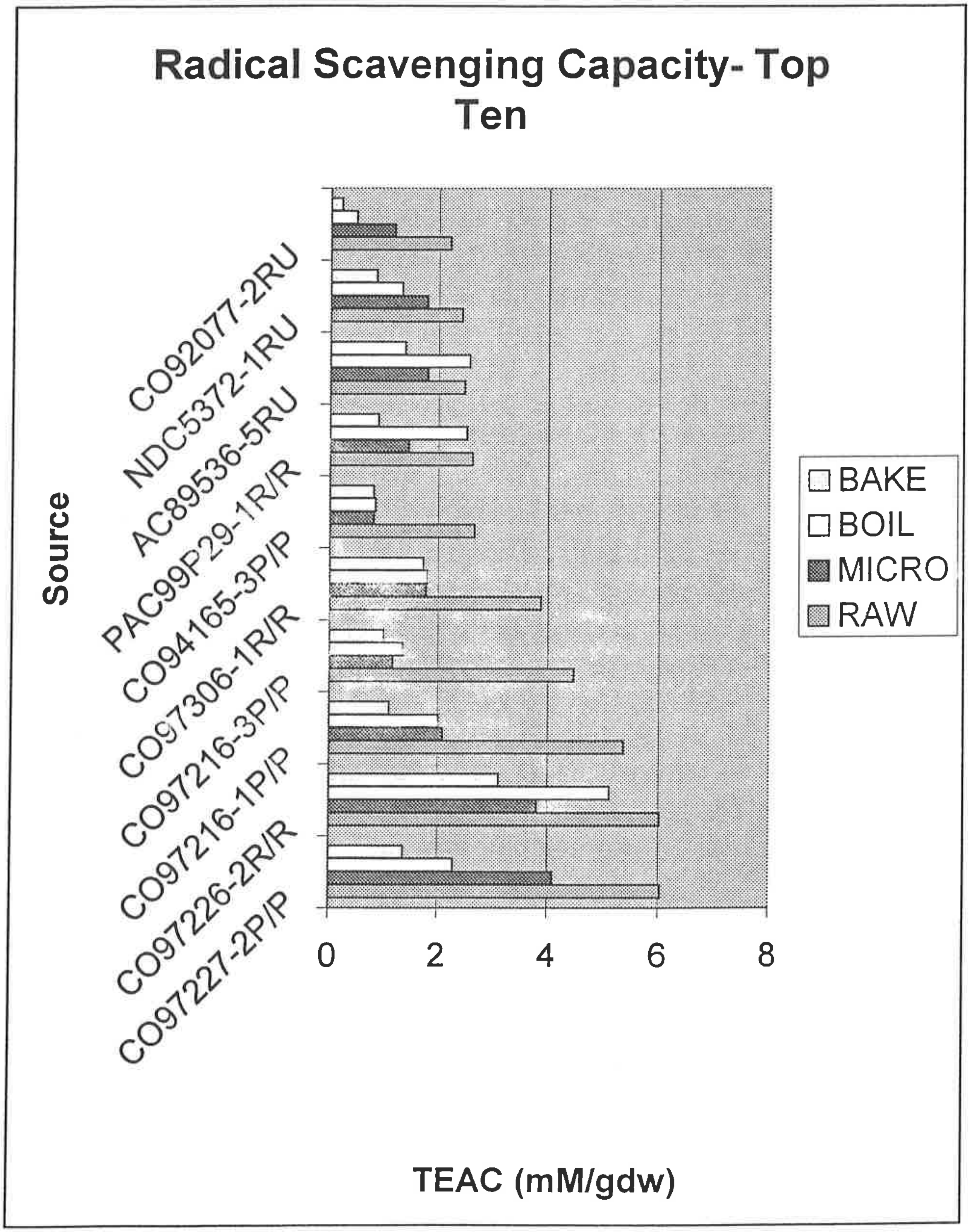

Figure 4. Radical scavenging capacity expressed as Trolox (water soluble vitamin E) equivalent antioxidant capacity (mM/gdw) for the 10 highest selections comparing uncooked freeze dried samples to microwaved, boiled and baked samples.

Appendix A

Total phenolics content as gallic acid equivalents, radical scavenging capacity as Trolox (water soluble vitamin E equivalents) and vitamin C content for all cultivars and selections grown in 2002 and evaluated in 2002 and 2003.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Potato phytochemical summary for 2002 crop													
2			ABTS Radical Scavenging Capacity					Total Phenolics as gallic acid equivalents						
3	Lab.		ABTS (TEAC) Trolox Vit. E equivalents uM/gdw					Phenolics (mg/gdw)					Vitamin C	
4	Code	Selection/Cultivar	RAW	MICRO	BOIL	BAKE		RAW	BAKE	BOIL	MICRO		Raw	Z
													mg/ml	mg/100gfw
5	1	PAC99P29-1R/R	2.62	1.44	2.52	0.88		23.25	5.91	22.82	15.9	PAC99P29-1R/R	0.048	19.2
6	2	CO97215-2P/P	1.58	0.81	2.76	1.58		54.06	22.14	20.21	26	CO97215-2P/P	0.031	12.4
7	3	CO97216-1P/P	5.37	2.09	2.01	1.09		29.66	24.18	11.88	13.13	CO97216-1P/P	0.086	34.4
8	4	CO97216-3P/P	4.47	1.15	1.34	0.98		94.84	24.79	33.35	36.55	CO97216-3P/P	0.064	25.6
9	5	CO97219-1R/R	1.22	0.58	0.38	0.64		47.54	28.13	17.14	23.19	CO97219-1R/R	0.078	31.2
10	6	CO97222-1R/R										CO97222-1R/R	0.119	47.6
11	7	CO97227-2P/P	6.03	4.09	2.29	1.36		163	62.45	109.17	102.4	CO97227-2P/P	0.049	19.6
12	8	CO97274-2W/Y	0.15					14.73	7.644	11.18	11	CO97274-2W/Y	0.061	24.4
13	9	CO97306-1R/R	3.87	1.77	1.79	1.72		117.78	65.73	71.13	72.51	CO97306-1R/R	0.054	21.6
14	10	ATC98444-1R/Y	0.27	0.1				16.74	13.03	8.75	8.665	ATC98444-1R/Y	0.081	32.4
15	11	ATC98495-1W/Y	0.34	0.18	0.29	0.21		24.25	12.99	16.31	17.96	ATC98495-1W/Y	0.127	50.8
16	12	ATC98509-1R/Y	0.21	0.12	0.27	0.26		13.22	6.17	9.91	8.68	ATC98509-1R/Y	0.099	39.6
17	13	ATC98515-1R/Y	0.21	0.24	0.28	0.17		14.61	13.29	14.38	20.49	ATC98515-1R/Y	0.07	28
18	14	AC97521-1R/Y	0.21	0.18	0.44	0.4		16.18	19.3	10.6	11.63	AC97521-1R/Y	0.101	40.4
19	15	CO97226-2R/R	6.02	3.81	5.12	3.11		35.5				CO97226-2R/R	0.098	39.2
20	16	CO97232-1R/Y	0.2	0.17	0.25	0.23		24.11	23.24	21.99	23.99	CO97232-1R/Y	0.122	48.8
21	17	CO97232-2R/Y	0.38	0.23	0.44	0.31		41.39	13.17	21.9	13.1	CO97232-2R/Y	0.166	66.4
22	18	CO97233-3R/Y		0.22	0.41	0.34		13.17	30.35	23.3	19.73	CO97233-3R/Y	0.154	61.6
23	19	CO94157-2W/Y						13.72	10.45	9.73	6.66	CO94157-2W/Y	0.133	53.2
24	20	VC1106-1RU/Y	0.39	0.31				21.13	20.14	15.44	19.65	VC1106-1RU/Y	0.086	34.4
25	21	VC1123-2W/Y	0.07	0.14				20.67	13.64		2.72	VC1123-2W/Y	0.053	21.2
26	22	AC94296-5W	0.16	0.26	0.16	0.25		14.41	10.16	9.21	10.01	AC94296-5W	0.115	46
27	23	AC95405-2RU	0.29	0.24				13.43	12.85	10.4	8.04	AC95405-2RU	0.061	24.4
28	24	CO95007-1RU	0.29	0.13	0.44	0.26		20.88	14.26	19.23	9.75	CO95007-1RU	0.185	74
29	25	CO95051-7W	0.23	0.13				17.79	8.12	14.83	8.35	CO95051-7W	0.077	30.8
30	26	CO95070-7W	0.24	0.11	0.16	0.15		10.59	4.78	7.49	3.99	CO95070-7W	0.128	51.2
31	27	CO95086-8RU	0.26	0.2	0.4	0.18		17.78	11.38	18.15	13.62	CO95086-8RU	0.159	63.6
32	28	CO95172-3RU	0.33	0.28	0.39	0.38		25.75	23.67	19.84	19.51	CO95172-3RU	0.17	68
33	29	VC1009-1W/Y	1.75	1.02	1.38	0.79		32.76	12.09	26.4	21.33	VC1009-1W/Y	0.161	64.4
34	30	VC1015-1R/Y	1.18	0.37	0.91	0.63		38.74	16.7	31.39	10.53	VC1015-1R/Y	0.154	61.6
35	31	VC1015-7R/Y	0.3	0.24	0.23	0.25		6.4	7.26	13.09	7.13	VC1015-7R/Y	0.1	40
36	32	VC1075-1R	0.25	0.24	0.23	0.34		19.67	12.92	17.01	14.15	VC1075-1R	0.092	36.8
37	33	CO94019-1R	0.34	0.39				30.31	21.61	17.44	17.91	CO94019-1R	0.049	19.6
38	34	CO94035-15RU	0.22	0.14				13.08	13.61	10.54	12.36	CO94035-15RU	0.073	29.2
39	35	CO94065-2R	0.74	0.71	0.73	0.72		22.2	21.8	18.9	19.14	CO94065-2R	0.058	23.2
40	36	CO94084-12RU	0.23	0.13				15.72	11.61	8.49	8.54	CO94084-12RU	0.069	27.6
41	37	CO94165-3P/P	2.66	0.8	0.83	0.8		74.56	27.31	26.5	33.85	CO94165-3P/P	0.074	29.6
42	38	CO94183-1R/R	1.16	0.66	0.48	0.68		47.68	35.31	22.18	30.88	CO94183-1R/R	0.066	26.4
43	39	CO94222-6RU/Y	0.26	0.2				11.18	6.47	10.51	5.35	CO94222-6RU/Y	0.06	24
44	40	NDC6084C-2W	0.14	0.11				8.73	8.86	8.43	8.5	NDC6084C-2W	0.065	26
45	41	NDC6184-3R	0.35	0.25				19.56	18.41	11.82	14.33	NDC6184-3R	0.087	34.8
46	42	VC0967-2R/Y	0.18	0.2				19.17	23.59	13.42	17.01	VC0967-2R/Y	0.034	13.6

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Potato phytochemical summary for 2002 crop													
2			ABTS Radical Scavenging Capacity					Total Phenolics as gallic acid equivalents					Vitamin C	
3	Lab.		ABTS (TEAC) Trolox Vit. E equivalents uM/gdw					Phenolics (mg/gdw)					Raw	Z
4	Code	Selection/Cultivar	RAW	MICRO	BOIL	BAKE		RAW	BAKE	BOIL	MICRO		mg/ml	mg/100gfw
47	43	VC0967-5R/Y	0.26	0.25	0.32	0.43		25.41	23.32	20.96	25.25	VC0967-5R/Y	0.073	29.2
48	44	VC1002-3W/Y	0.24	0.17				11.74	7.83	6.36	9.11	VC1002-3W/Y	0.059	23.6
49	45	AC93026-9RU	0.25	0.17				24.96	13.01	22.92	8.65	AC93026-9RU	0.06	24
50	46	AC93047-1RU	0.39	0.29	0.47	0.45		17.8	18.95	12.09	12.8	AC93047-1RU	0.14	56
51	47	CO93001-11RU	0.12	0.24				20.22	22.36	17.99	13.83	CO93001-11RU	0.09	36
52	48	CO93016-3RU	0.31	0.23				19.73	13.71	10.57	18.3	CO93016-3RU	0.08	32
53	49	CO93037-6R	0.28	0.24						24.55	22.42	CO93037-6R	0.07	28
54	50	AC92009-4RU	0.28	0.29				19.2	26.25	14.86	15.69	AC92009-4RU	0.08	32
55	51	CO92077-2RU	2.21	1.17	0.47	0.2		34.01	16.42	26.68	23.27	CO92077-2RU	0.11	44
56	52	NDC5281-2R	0.28	0.23				23.39	16.34	19.63	16.41	NDC5281-2R	0.056	22.4
57	53	NDC5372-1RU	2.43	1.78	1.31	0.84		21.47	20.96	15.54	21.73	NDC5372-1RU	0.077	30.8
58	54	TC1675-1RU	0.32	0.33				18.76	13.27	18.24	20	TC1675-1RU	0.086	34.4
59	55	CO85026-4RU(Freemont R)	0.24	0.24	0.26	0.29		12.61	15.93	14.11	14.31	CO85026-4RU	0.063	25.2
60	56	AC87084-3RU	0.4	0.46				27.5	17.85	25.84	28.07	AC87084-3RU	0.052	20.8
61	57	AC89536-5RU	2.48	1.8	2.57	1.37		33.53	18.8	31.76	23.84	AC89536-5RU	0.097	38.8
62	58	CO86218-2R (Durango Red)	2.96	2.95	1.81	1.95		32.46	19.64	23.21	29.29	CO86218-2R	0.069	27.6
63	59	DT6063-1R(Cherry Red)	0.21	0.24				25.12	17.16	14.74	15.79	DT6063-1R	0.038	15.2
64	60	CO89097-2R	0.2	0.25	0.28			14.46		14.9	19.4	CO89097-2R	0.045	18
65	61	BC0894-2W	0.18	0.15	0.16	0.2		9.19	7.16	6.02	7.2	BC0894-2W	0.041	16.4
66	62	AC87340-2W	0.15	0.15				16.83	18.23	11.46	13.07	AC87340-2W	0.045	18
67	63	All Blue	3.38		5.38	2.07		80.97	37.79	41.7		All Blue	0.09	36
68	64	Centennial Russet	1.45	0.91	1.23	1.28		21.43	19.14	19.93	22.98	Centennial Russet	0.08	32
69	65	Chipeta	0.23	0.29	0.26			19.01	16.08	10.41	12.07	Chipeta	0.07	28
70	66	Keystone Russet (AC83064-	1.6	1.5	0.77	2.11		18.75	29.91	12.9	17.55	Keystone Russet (AC8	0.05	20
71	67	Russet Burbank	0.22	0.1	0.26	0.15		28.42	14.93	22.24	13.47	Russet Burbank	0.08	32
72	68	Russet Norkotah-S3	0.2	0.37	0.62	0.49		22.23	25.87	31.27	18.38	Russet Norkotah-S3	0.05	20
73	69	Russet Norkotah-S8	0.27	0.43	0.55	0.36		25.69	14.94	22.85	20.59	Russet Norkotah-S8	0.06	24
74	70													0
75	71	Sangre-S10	0.18	0.2	0.45	0.39		26.38	23.75	19.42	21.56	Sangre-S10	0.07	28
76	72	Silverton Russet (AC83064-6	0.17	0.22	0.37	0.3		21.61	13.07	18.48	13.32	Silverton Russet (AC83	0.09	36
77	73	Yukon Gold	1.84	0.65	1.32	1.11		19.68	13.63	14.64	8.64	Yukon Gold	0.02	8
78										Z=http://nutrition.aboutcom/library/foodfind/blpotatoes.htm				
79										list raw potato as having 24.043 mg/122 g fresh tuber				

Appendix B

Copy of proposal submitted to USDA by Cecil Stushnoff and David Holm (not funded)

High Vitamin C, High Antioxidant and Low Glycemic Potato Cultivars Can Improve Health Attributes and Enhance Marketing

TITLE: **High Vitamin C, High Antioxidant and Low Glycemic Potato Cultivars Can Improve Health Attributes and Enhance Marketing**

INVESTIGATORS: Cecil Stushnoff and David Holm, Department of Horticulture & L.A., Colorado State University, Fort Collins, and San Luis Valley Research Center, Center, CO
COOPERATORS: Chris Voigt, Executive Director, Colorado Potato Administrative Committee Area II, Center, CO 81125
Charlie Higgins, PhD, Farm Fresh Direct, Hooper, CO 81136
GRANT REQUEST PERIOD: July 1, 2004 to June 30, 2005

SUMMARY OF PROBLEM: Potatoes (*Solanum tuberosum L.*) provide high-quality protein and energy to millions world-wide, but recent negative publicity over obesity related health issues too often implicates consumption of potato products. Recent research, including our preliminary data, suggests considerable genetic diversity exists for vitamin C and beneficial antioxidant properties in breeding lines. These potentially positive attributes are not yet well recognized by the consuming public. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommend carbohydrates as a better dietary energy alternative than high fat foods. Recently shippers of fresh market potatoes have received consumer inquiries regarding the effect of potato consumption on blood sugar. This has given rise to questions regarding potential differences in glycemic indicies among potato cultivars. Characterization and public awareness of beneficial phytochemicals, as well as identification of low glycemic index (GI) cultivars can provide valuable features to boost public confidence in potato as a healthy vegetable, and to improve market opportunities. Marketing agencies such as 'Fresh Market Direct' require research data on positive attributes to assist their activities.

JUSTIFICATION: ***Phytochemicals*** While potato is a major carbohydrate staple, especially in cool-temperate climates, less appreciated is its importance as a source of vitamin C, estimated to provide up to 30% of daily intake from fruits and vegetables in some European diets. Vitamin C content, determined recently in 87 cultivars and selections in the Colorado potato breeding program, ranges from 0.3 to 1.85mg/gfw, suggesting considerable potential for enhancement.

Potatoes also contain other important antioxidants, primarily derived from phenolic compounds that have not been well studied from genetic, production environment, storage, or cooking heat-stability perspectives. Our recent assays show that some genotypes possess 3 to 5X..s the antioxidant content and activity of common cultivars, and that storage and cooking temperature can also impact content and radical scavenging activity. 'All Blue' a purple-fleshed cultivar grown in the San Luis Valley, CO contains substantially higher levels of cyanidin 3-glucoside anthocyanin and chlorogenic acid antioxidant compared to tubers grown in two warmer Texas sites (Reyes Cisneros-Zevallos, 2003) suggesting a production environment response for this potentially important phytonutrient effect.

Glycemic index (GI) FAO and the WHO have endorsed the use of GI to classify carbohydrate rich foods, especially in view of recommendations that carbohydrates are a better energy alternative than high fat foods. Some dietetic agencies in Australia, Canada, and Europe now recommend use of low GI food as a dietary intervention strategy. Significant effects of blood sugar on several physiological functions including brain function, satiety, stress resistance, the immune system, and chronic diseases have been detected (Kimura, 2003). It thus appears that the concept of food GI will receive increasing attention as a decision factor in the nutrition/health field, especially as concerns over an obesity epidemic increase. This emerging concern will be an issue for potato consumers and producers, but it may also present an opportunity to expand consumption if we can discover and document low GI cultivars and potato products.

Starch has been categorized by digestibility for nutritional purposes as: (1) rapidly digestible, (2) slowly digestible, and (3) resistant. An in vitro assay can be used to determine rapidly available glucose (RAG) by HPLC (Englyst et al., 1999). Generally potato tuber and legume starch granules are more resistant to hydrolysis by pancreatic amylases in the raw state, however, when cooked the granules are fully gelatinized and readily digestible. Upon cooling, re-crystallization or retrogradation can occur to form resistant polymers, especially for linear amylose forms found in potato (Champ et al., 1999; Englyst et al., 1992). Published in vivo data on GI for boiled potato relative to glucose (100) varies among cultivars: Desiree (101), Nardine (70), Ontario White (58), Pontiac (56) and Sebago (87) (Foster-Powell et al., 2002). While troublesome to interpret, because these reports arose from several different production regions, these data suggest that cultivar differences do exist. Good research data on cultivars that differ in specific gravity, skin, and tuber tissue features (white, russet, red, yellow, purple), grown and prepared under standard conditions are very much needed.

OBJECTIVES: (1) Characterize cultivars and advanced selections for water-soluble vitamins, especially vitamin C; (2) characterize antioxidant content and radical scavenging capacity; (3) evaluate cooking heat stability and impact of storage duration on (1) and (2);(4) adapt the in vitro assay of Englyst et al., (1999) to screen for glycemic index based on rapidly available glucose (RAG); (5) publish results and make data available to consumers, producers and marketing agencies such as 'Fresh Market Direct'.

PLAN OF RESEARCH: Ten selected cultivars and 60 advanced selections will be grown in replicated plots and stored under standard industry conditions at the Colorado State University, San Luis Valley Research Center. Laboratory assays will be conducted in a fully equipped physiology/biochemistry lab, Shepardson Bldg., Fort Collins, CO. Instrumentation includes: HPLC, GC, microplate spectroscopy, freeze drier, centrifuges, vacufuge, and all other necessary lab equipment and computers. Vitamin assays and specific phenolic compound quantitation will be run with a HP1050, HPLC. Total phenolics and ABTS radical scavenging assays will be conducted with a 'Spectromax 640' microplate spectrophotometer using well-established protocols (see attached descriptions, Appendix A). Sugars will be assayed either by derivatization and gas chromatography, or by Dionex amperometric detection HPLC. Our laboratory has conducted these assays on apple and potato for the past two years.

Because the RAG in vitro assay correlated well, ($r = 0.981$, $p = <.0001$, $n = 8$), with in vivo data using the same food carbohydrate sources, albeit from a trial with a limited number of human subjects, we plan to adapt this assay as a screen and evaluate its effectiveness. We propose to initially evaluate RAG and to determine if this published technique is sensitive enough to sort the RAG cultivars from the slowly available glucose (SAG) cultivars. In **phase one** we will adapt a method in our lab based upon any necessary modifications of Englyst's(1999) published protocol. In **phase two** we will test tubers of Colorado cultivars grown in one location (SLV Research Center), using best cultural practices and standardized postharvest storage, handling, cooking and analytical practices. Specific gravity, soluble sugar and starch content of raw tubers of approximately the same size will be determined prior to conducting the in vitro assay for GI. These data should demonstrate if the proposed assay can distinguish differences in GI, and they should also enable us to determine if some cultivars have special attributes that can add market value.

Literature Cited

Champ M, Martin L, Noah L, Gratas M. (A) In vivo techniques to quantify resistant starch. (B) Analytical methods for resistant starch. In Cho S, Prosky L, Dreher M Eds. *Complex Carbohydrates in Foods: Definition, Functionality, and Analysis.* New York: Marcel Dekker.

Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH. 1999. Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. Am. J. Clin. Nutr. 69:448-454.

Englyst HN, Kingman SM, Cummings JH. 1992. Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Res. 46:33-50.

Foster-Powell K, Holt SHA, Brand-Miller C. 2002. International table of glycemic index and glycemic load values. 2002.Am. J. Clin. Nutr. 76:5-56.

Kimura S. 2003. Glycemic carbohydrate and health: background and synopsis of the symposium. Nutrition Reviews 61:1-4.

Nantel G. 2003. Glycemic carbohydrate: An international perspective. Nutrition Reviews 61:34-39.

Reyes LF and L, Cisneros-Zevallos. 2003. Wounding stress increases the phenolics content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum L.)*J. Agric. Food Chem. 51:5296-5300.

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

OMB Approved 0518-0025

GRANT AND ASSISTANCE TYPE COOPERATIVE AGREEMENT BUDGET

				DURATION PROPOSED	ARS USE ONLY
Recipient Name: **Agreement No.**				Months:	Months: ______
PRINCIPAL INVESTIGATOR(S) PROJECT DIRECTOR(S)				FUNDS REQUESTED BY PROPOSER	FUNDS APPROVED BY ARS (If different)
A. Salaries and Wages	ARS FUNDED WORK MONTHS				
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)	6			12,500	
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorate					
b. ___ Other Professionals					
c. ___ Graduate Students					
d. ___ Pre-Baccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop, and Other				$2,000.00	
Total Salaries and Wages →				$14,500.00	
B. Fringe Benefits (If charged as Direct Costs) a. @19.4%				$2,425.00	
C. Total Salaries, Wages, and Fringe Benefits *(A plus B)* →				$16,925.00	
D. Nonexpendable Equipment *(Attach supporting data. List items and dollar amounts for each item.)*					
E. Materials and Supplies				$2,000.00	
F. Travel 1. Domestic (Including Canada)				$1,500.00	
2. Foreign (List destination and amount for each trip)					
G. Publication Costs/Page Charges				$1,000.00	
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amount. Details of subcontracts, including work statements and budget should be explained in full in proposal (subcontract to consultant)				$1,900.00	
J. Total Direct Costs (C through I) →				$23,325.00	
K. Indirect Costs (Specify rate(s) and base(s) for on off campus activity.) (Where both are involved, identify itemized costs included in on off campus bases.)				on campus @45% $10,496.00	
L. Total Direct and Indirect Costs (J plus K) →				$33,821.00	
M. Less Residual Funds (If applicable) →					
N. TOTAL AMOUNT of this REQUEST (L minus M) →				$33,821.00	
O. COST SHARING →					

COMMENTS

Please fill out Column E only for the budget portion of your proposal.

Form ARS-455 (4/93) USDA-ARS

BIOGRAPHICAL SKETCH

Cecil Stushnoff, Professor, Department of Horticulture & Landscape Architecture, Colorado State University, Fort Collins, CO 80523, 970-491-7110, stushnof@lamar.colostate.edu

Educational Training

INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
Rutgers University, New Brunswick, NJ	Ph.D	1967	Horticulture, Botany
University of Saskatchewan Saskatoon, SK, Canada	M.S.	1964	Horticulture
	B.S.	1963	Agriculture

Academic Positions

1975-80	Professor; 1971-75, Associate Professor; 1967-70, Assistant Professor; Department of Horticultural Science and Landscape Architecture, Univ. of Minnesota, St. Paul,
1981-89	Professor and Head, Dept. of Horticulture Science, Univ. of Sask., Saskatoon, Canada
1989-90	Senior Research Scientist, Department of Biochemistry, Colorado State University
1990-present	Professor, Dept. Horticulture & Landscape Architecture, Colorado State University

Professional Experience

1976	Guest researcher, University of Tromso, Inst. of Biology & Geology, Tromso, Norway
1979	Invited Research Administrator, USDA/CSREES, Washington, D.C.
1983-88	Associate Editor, Canadian Journal of Plant Science,
1985-88	Expert Ctte. Horticulture and Plant Gene Resources
1986-88	Visiting Professor, Dept. of Biochemistry
1990-00	Affiliate Administrator, USDA, CSREES, Washington, DC
1994-02	Director, Plant Biotechnology Program, Colorado State University
2000-01	Sabbatical, Crop and Food Research Institute, Food and Human Nutrition and Lincoln University, New Zealand

Honors

1963-64	Canadian National Research Council Graduate Scholarship
1967	Sigma Xi, New Jersey Chapter
1967	Phi Kappa Phi
1971&79	Paul Howe Shepard research paper award, Amer. Pomological. Soc.
1973	Joseph Harvey Gourley research paper award, Amer. Soc. for Hort. Science
1974	NSF/ASHS Plenary Address at XIX th. ISHS Congress, Warsaw, Poland
1980	George M. Darrow research paper award, Amer. Soc. Hort Science
1984-1986	President and Vice president, Canadian Society for Horticulture Science,
1988&91	C. J. Bishop research paper award, Can. J. Plant Science
1993	Fellow, American Society for Horticultural Science
1993	Colorado State University Chapter of Gamma Sigma Delta Research Award of Merit
1995	ESCOP Leadership Development Program

Professional Societies

American Chemical Society
American Society of Plant Physiologists
American Society for Horticultural Science
North American Pomological Society

GRADUATE STUDENTS and POST-DOCTORAL FELLOWS

Postdoctoral fellows (4)
PhD students (12 completed, 3 present)
MS students (28 completed)

Experience relevant to proposed research.

In 2001, I spent a six-month sabbatical in a Food Nutrition Research Laboratory, Crop Research Institute, Lincoln, NZ, learning analytical technologies and developing antioxidant assays with potato and apple. Upon return to Fort Collins, CO, our laboratory has established several antioxidant assays using microplate spectroscopy and HPLC. We have initiated and applied assays to examine genetic diversity, environmental, and cooking heat stability impact on antioxidant status of potato cultivars and breeding lines. We have also completed a two-year, USDA funded project that examined genetic diversity in a core collection of apple germplasm in collaboration with the National Apple Germplasm Unit at Geneva, NY.

PUBLICATIONS. (Recent publications selected from a total of 130 refereed papers, books, chapters and patents)

Cox, S.E. and C. Stushnoff. 2003. Relationship of fruit color and light exposure to lycopene content and antioxidant properties of tomato. Can. J. Plant Science (In press)

Javanmardi, J., C. Stushnoff, E. Locke and J.M. Vivanco. 2003. Antioxidant activity and total phenolic contant of Iranian *Ocimum* accessions. Food Chemistry 83:547-550.

McSay, A.E., C. Stushnoff , D. Holm and R. Davidson. 2003. Storage Characteristics of new potato introductions. Acta Horticultura (In press)

Pennycooke, J.C., M.L. Jones and C. Stushnoff. 2003. Down-regulating alpha-galactosidase enhances freezing tolerance in transgenic petunia. Plant Physiology 133:1-9.

Stushnoff, C. A. E. McSay, P.L. Forsline and J.J. Luby. 2003. Diversity of phenolic antioxidant content and radical scavenging capacity in the apple germplasm core collection. Acta Horticultura (In press)

Cox, S.E., and C. Stushnoff. 2001. Temperature-related shifts in soluble carbohydrate content during dormancy and cold acclimation in *Populus tremuloides*. Can. J. For. Res. 31:1-8.

Ali, A., D.L. Johnson and C. Stushnoff. 1999. Screening lentil, (*Lens culinaris*) for cold hardiness under controlled conditions. J. Agric. Sci. Cambridge, 133:313-319.

CURRICULUM VITAE

NAME: David G. Holm

CURRENT POSITION: Professor of Horticulture (Potato Breeding)
San Luis Valley Research Center
Colorado State University
0249 East Road 9 North
Center, CO 81125
Phone: 719-754-3594; Fax: 719-754-2619
e-mail: spudmkr@lamar.colostate.edu

EDUCATION: **Ph.D.** [Major: Horticultural Science, Minor: Supporting (Plant Physiology and Plant Breeding), Collateral Field: Statistics], University of Minnesota, 1977.
M.S. (Plant Science), University of Idaho, 1974.
B.S. (Plant Science), University of Idaho, 1972.

PROFESSIONAL EXPERIENCE:

- **Professor**, Colorado State University, Department of Horticulture and Landscape Architecture, San Luis Valley Research Center, 1996-present.
- **Superintendent**, Colorado State University, San Luis Valley Research Center, 1983-97.
- **Potato Breeding and Selection Project Leader** (COLO0712 - "Development of New Potato Cultivars for Colorado via Germplasm Enhancement and Evaluation), Colorado State University, San Luis Valley Research Center, 1982-present.
- **Associate Professor**, Colorado State University, Department of Horticulture, San Luis Valley Research Center, 1992-96.
- **Acting Superintendent**, Colorado State University, San Luis Valley Research Center, 1982-83.
- **Assistant Professor**, Colorado State University, Department of Horticulture, San Luis Valley Research Center, 1978-92.
- **Junior Scientist**, University of Minnesota, Department of Horticultural Science, 1977-78. Assisted in managing the "Cultural and Physiological Studies on Vegetable Crops and Potatoes" project.
- **Research Assistant**, University of Minnesota, Department of Horticultural Science, 1974-77; "Cultural and Physiological Studies on Vegetable Crops and Potatoes" project.
- **Graduate Assistant**, University of Idaho, Department of Plant and Soil Science, 1972-74; "Development of Improved Disease Resistant Potato Varieties" project.

MEMBERSHIP IN PROFESSIONAL SOCIETIES AND COMMITTEES:

- **Potato Association of America**
- **European Association for Potato Research**
- **American Society for Horticultural Science**
- **WCC-27 (Western Regional Coordinating Committee for Potato Variety Development)**
- **Southwest Regional Potato Cultivar Development Working Group**

HONORS AND AWARDS:

Alpha Zeta (1970), Agronomy Student Award (1971), Phi Sigma Society (1971), Sigma Xi (1978), Gamma Sigma Delta (1978), Gamma Sigma Delta Faculty Research Award of Merit (2000).

MAJOR RESEARCH ACCOMPLISHMENTS:

- Six potato cultivars have been released. They are Sangre(1982), Ute Russet (1986), Russet Nugget (1988), Chipeta (1993), Keystone Russet (2000), and Silverton Russet (2000). Advanced selections/recent releases undergoing commercialization include Cherry Red (DT6063-1R), Fremont Russet (CO85026-4), and Durango Red (CO86218-2) and BC0894-2. Plant Variety Protection applied for Keystone Russet and Silverton Russet.
- Cooperated with other agencies in the release of Gemchip (1989), Frontier Russet (1990), Ranger Russet (1991), Century Russet (1995), and Russet Legend (1999). Russet Legend was selected from a cross of Century Russet and WNC672-2 made by the Colorado Potato Breeding and Selection Program in 1983.
- Clonal selections released by CSU include Sangre-Selection 10, 11, and 14 and Russet Norkotah-Selection 3 and Selection 8.

- Cultivars and clonal selections developed by Colorado State University accounted for 57% of the 2002 fall potato acreage planted in Colorado. Approximately 48% of the Colorado certified seed acreage accepted for certification, was represented by cultivars and line selections developed by CSU or in cooperation with other agencies. Advanced selections accounted for another 6% of the seed acreage.
- Russet Nugget, released by Colorado in 1988, accounted for 13% of the acreage making it second in area planted in the San Luis Valley and the seventh most popular russet cultivar in the United States.
- Of the Russet Norkotah fall potato acreage in Colorado, 66% was planted to Colorado Russet Norkotah Selections 3 and 8.
- Silverton Russet, named in 2001, is currently among the top five russet cultivars being grown in six fall potato production states. For russet cultivars, Silverton Russet ranked fourth overall in acreage planted in the United States.
- Conservatives estimates indicate that new potato cultivars and clonal selections increase the value of the Colorado fall potato crop by $11-$12 million annually due to improved yield and quality.

PUBLICATIONS: Total 151: 2 theses, 16 refereed journal articles, 77 technical reports, 26 abstracts/proceedings, 24 extension articles, and 6 popular/trade articles.

SELECTED PUBLICATIONS:

Holm, D. G., Miller, J. C., Jr., and Smallwood, D. G.. 1992. Russet Nugget: A fresh market and processing potato cultivar with resistance to common scab. Am. Potato J. 69:331-336.

Holm, D. G. 1996. Russet Norkotah clonal selection studies: a three year summary (Abstr.). Am. Potato J. 73:363.

Maga, J. A. and Holm, D. G.. 1992. Subjective and objective comparison of baked potato aroma as influenced by variety/clone. Pages 537-541 in G. Charalambous, ed. Food Science and Human Nutrition. Developments in Food Science 29. Elsevier, Amsterdam.

Maga, J. A. and Holm, D. G.. 1993. Mineral composition of the skins and interiors of raw potatoes grown at different locations. Pages 247-251 in G. Charalambous, ed. Food Flavors, Ingredients and Composition. Elsevier, Amsterdam.

McSay, A. E., Stushnoff, C., Holm, D., and Davidson, R. 2002. Storage characteristics of new potato introductions. XXVth International Horticultural Congress & Exhibition. Toronto, Canada. p. 133. (Abstr.).

Mosley, A. R., James, S. R., Rykbost, K. A., Love, S. L., Stanger, C. E., Shock, C. C., Pavek, J. J., Corsini, D. L., Miller, J. C., Jr., Love, S. L., Thornton, R. E., Holm, D. G., and Voss, R. E.. 2000. Century Russet: A high-yielding fresh market cultivar with *Verticillium* resistance. Am. J. Potato Res. 77:161-165.

Mosley, A. R., James, S. R., Shock, C. C., Love, S. L., Rykbost, K. A., Charlton, B. A., Holm, D. G., Love, S. L., Corsini, D. L., Pavek, J. J., and Thornton, R. E. 2000. Russet Legend: A full season long russet for processing and fresh market use. Am. J. Potato Res. 77:77-81.

Park, Don Keun, Maga, J. A., and Holm, D. G.. 1998. Time color changes among raw and baked potato varieties. J. Food Proc. Pres. 22:333-344.

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Appendix A. Antioxidant Assays

ABTS Assay for Antioxidant Activity. This assay is based upon oxidation of 2,2' azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (ABTS) to the activated ABTS+· radical using MnO_2 based upon (Miller and Rice Evans, 1997). Activity in extracts is measured spectrophotometrically as the blue-green colored ABTS+· radical, with an absorption max at 734 nm. ABTS+· is decolorized by antioxidants under constant time and temperature. A water-soluble analog of vitamin E, Trolox, (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is used as a standard to compare aqueous acetone extracts of fruit, vegetables, juice, wine or supplements.

Miller NJ and CA Rice-Evans. 1997. Factors influencing the antioxidant activity determined by the ABTS+· radical cation assay. Free Rad. Res.26:195-199.

Total Peroxyl Radical-trapping Potential (TRAP) Assay. This assay is based upon the potential of antioxidants in extracts to scavenge peroxyl radicals generated by thermal decomposition of 2,2' diazobis (2-amidinopropane) dihydrochloride (AAPH) (Valkonen and Kuusi (1997) modified by Wilson (2001) for human or rat plasma or serum. Detection of the oxidation product is based upon colorimetric absorption of the oxidation product, dichlorofluorescein (DCFH to DCF) at 504 nm. The extent to which sample extracts or plasma can scavenge AAPH peroxyl radicals and prevent oxidation is measured as a lag phase and the TRAP value is expressed µM peroxyl radicals trapped per L of plasma or extract based upon a known concentration of Trolox as an internal standard.

Valkonen M and T Kuusi. 1997. Spectrophotometric assay for total peroxyl radical-trapping potential in human serum. J. Lipid Res. 38:828-833.

Wilson P. 2001. TRAP assay for human or rat plasma (or serum). Personal communication. Phytochemicals & Health Group, Nutrition & Health Team, NZ Institute for Crop & Food Research, 10p.

FOX3 (Lipid Peroxidation) Assay. Lipid hydroperoxides are detected by their oxidation of Fe (II), in the presence of xylenol orange, to a Fe (III)-xylenol orange complex. Free radical-mediated chain oxidation is initiated in micelles of lipid (Intralipid ®) by irradiation with ultraviolet light. Color change is measured at 570 nm against H2O2 standards and the data are expressed as the amount of extract that inhibits lipid peroxidation by 50% (IC 50).

Hermes-Lima M, Willmore WG and K.B. Storey. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. Free Radical Biology & Medicine 19:271-280.

Total Phenolics. Total phenolics in an extract are measured spectrophotometrically at 765nm based upon a color reaction of phenolic compounds with Folin-Ciocalteu, a phosphomolybdio-phosphotungstic reagent (Spanos and Wrolstad, 1990). Total phenolics are expressed as gallic acid equivalents.

Spanos GA and RE Wrolstad. 1990. Influence of processing and storage on the phenolics composition of Thompson seedless grape juice. J. Agric. Food Chem. 38:1565-1571.

All assays are run with a temperature regulated Spectromax microplate and cuvette reader using Max Pro and Excel software. Samples can be analyzed directly or after lyophilization and storage at -80 C.

Appendix B. Summary of the glycemic index in vitro assay.

1. **Prepare samples for analysis**

i. Boil for 30 minutes and maintain at 60-70 °C during preparation for enzymatic digestion.

ii. Boil as in (i) then refrigerate to produce retrograded (resistant) starch.

iii. Microwave at full power (700 watts, rotating turntable) for (x = to be determined) minutes per gram fresh weight, *ie*. the same standardized conditions for each cultivar.

2. **Measure in vitro free sugar content (fructose and glucose).** Weigh cooked potato samples into 50 mL polypropylene centrifuge tubes with internal standard (arabinose or glucopyranoside). Vortex vigorously, boil 30 minutes, vortex and cool to 37 °C, add invertase, shake at 37 °C for 30 minutes. Add sample to absolute ETOH, vortex. Measure free sugars in NaOH buffer with Dionex ,PAD HPLC (Englyst et al., 1999).

3. **Measure in vitro RAG, SAG, total glucose and starch**. Weigh cooked potato samples into 50 mL polypropylene centrifuge tubes with internal standard (arabinose or glucopyranoside), and freshly prepared pepsin-guar gum solution. Vortex and incubate in a water bath at 37 °C for 30 minutes to hydrolize proteins by pepsin. Add sodium acetate buffer, pH 5.2 with glass balls. To one sample add pancreatic enzyme mixture plus amyloglucosidase and invertase, incubate at 37 °C for exactly 20 minutes, stop digestion of RAG by adding solution to absolute ETOH. Stop a second sample for SAG in a similar manner after an additional 100 minutes digestion. Measure free sugars in NaOH buffer with Dionex ,PAD HPLC (Englyst et al., 1999). Total glucose and starch are determined after digestion with amyloglucosidase and referenced to pure analytical grade potato starch.

Appendix C. Production, storage, and cooking.

- Tubers from 70 cultivars and selections grown at San Luis Valley Research Center in 2004 will be collected and lyophilized for antioxidant analyses.

- Tubers of eight cultivars and selections (R. Burbank [control], R. Norkotah, R. Nugget, Chipeta, Yukon Gold, CO 94165 [purple], CO 94183 [red]) will be 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.

- Antioxidant status will also be determined for tubers from 5 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.

- Heat stability of antioxidant status will be determined for selected cultivars by comparing uncooked samples to those boiled for 30 minutes or microwaved at full power for 5 minutes/tuber.

- 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

Colorado Potato Administrative Committee, Area II
1305 Park Ave./P.O. Box 348
Monte Vista, Colorado 81144
Phone (719) 852-3322 or Fax (719) 852-4684
cvcpac@fone.net

October 13, 2003

ARS/USDA
Martha Hollenbeck, Program Assistant
Office of the Deputy Administrator
National Program Staff
5601 Sunnyside Ave.
GWCC 4-2140A
Beltsville, MD 20705

Dear Ms. Hollenbeck,

I am writing to support the grant request from Dr. Cecil Stushnoff and Dr. David Holm, entitled "High Vitamin C, High Antioxidant and Low Glycemic Potato Cultivars Can Improve Health Attributes and Enhance Marketing". This is an important project for the entire potato industry and plays a key role in the local marketing of Colorado potatoes.

The potato is the most popular and versatile vegetable in the United States. The research project that Dr. Stushnoff and Dr. Holm are conducting will greatly enhance the marketability of the potato crop. Currently, despite wonderful nutritional features, the potato is being perceived as an unhealthy food item. High protein/low carbohydrate and low glycemic index diets are creating less demand for potatoes. This research is needed to "re-invent the potato". Our organization's efforts are concentrated on marketing a better tasting, more nutritious, and safer potato. This research will help us achieve our marketing goals. It will give us a product that has greater consumer appeal and hopefully grow the consumption of potatoes.

We have worked with Dr. Stushnoff and Dr. Holm for many years and have found their research to be of the best quality. I sincerely hope that you give strong consideration for the funding of this valuable project. We appreciate the partnership and interest that the ARS has taken in providing research funding for projects that benefit the potato industry, the environment, and the consumers of our products.

Sincerely,

Chris Voigt
Executive Director

David Holm, Ph.D.
Colorado State University
San Luis Valley Research Center
Center, Colorado 81125

Dr. Holm:

The devastating decrease in the fresh potato market from misinformed consumer fears of excess carbohydrates makes it necessary for all potato growers and marketers to obtain the best data to support the nutritional value of potatoes. We request that Colorado State University conduct tests of the impact of potato consumption upon the glycemic index to help us educate our customers. We need to know what varieties are most healthy for our customers.

Some previous research has indicated Colorado's San Luis Valley can produce a healthier potato as far as antioxidant and vitatmin C levels are concerned. Higgins Farms, Inc., Farm Fresh Direct, L.L.C., and all other potato growers in the San Luis Valley ask for your assistance in searching for research money and conducting research that will help us grow and market healthier potatoes.

Sincerely

Charles Higgins, President phone 719 378 2388, 719 588 2388
Higgins Farms, Inc.
60819 Highway 112
Hooper, Colorado 81136