Title: Improving Value-Added Health Attributes of Colorado Potatoes - (2006 revised version with reduced budget)

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Summary of revisions as of April 27, 2006

Briefly, the following changes are proposed. The budget has been reduced by 50%. Research to be done will be adjusted accordingly, primarily to complete previous work and to pursue the most promising goals. All vitamin C analyses and cooking studies will be dropped. Screening for antioxidant properties will be reduced from 69 advanced selections and cultivars in 2005/06 to 20. We propose to follow up on unique contributions to antioxidant properties based on pigment composition and chlorogenic acid content will continue. Work will be done in these areas to the extent resources permit.

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. For example, potatoes are a good source of vitamins C and B6, potassium, magnesium, zinc and fiber. Dietary intake of antioxidants from natural foods is also 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 vitamin C and phenolic antioxidants than conventional cultivars. Growing-season environmental conditions, post harvest conditions, and cooking methods may alter antioxidant status, but the best opportunity to maximize antioxidant health attributes appears to be by identifying and selecting antioxidant-rich cultivars through genetics and plant breeding.

Marketing potato cultivars with enhanced health attributes such as high antioxidant and vitamin C content may provide a new marketing opportunity to meet demands of nutrition conscious consumers. 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 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 and provides a basis for the next steps. We have also learned 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. While cell culture inhibition studies cannot be used to predict if consumption of this product will prevent breast cancer in vivo, we are encouraged to follow up with additional research. Accordingly, more in-depth chemical analyses using LC/MS with collaborators in New Zealand was conducted to search for cultivar differences and compounds that may be

involved in inhibition of human breast cancer cell cultures. The results of these analyses are presented in this report.

We propose to continue high through-put microplate based chemical analytical screening of Colorado cultivars and selections for antioxidant properties for the following objectives.

(1) Characterize **genetic diversity** of antioxidant content, radical scavenging capacity, and vitamin C for 20 advanced selections and new cultivars for the breeding program.

(2) Based upon promising data obtained from tests with Rio Grande Russet in 2004/05 we will attempt to develop a HPLC protocol to quantify **chlorogenic acid,** as resources permit.

(3) As needed, we may conduct antioxidant assays to support preparation of grant proposals in collaboration with agricultural economists who may seek funds to enhance marketing of Colorado potato cultivars with enhanced health attributes.

Methods, procedures, and facilities

(a) Work in progress and plans for 2006

(1) Tubers from 74 **cultivars and selections** grown at San Luis Valley Research Center in 2004 were collected, lyophilized, and assayed for total phenolics (Table 1), ABTS antioxidant radical scavenging capacity (Table 2) and vitamin C (Table 3).

(2) Sixty nine cultivars and selections from the breeding program have been collected and lyophilized from the 2005 crop. An additional 105 samples were provided for analyses as part of a nutrient study in Collaboration with Jorge Delgado, USDA and David Holm, SLV. Antioxidant properties and vitamin C have been analyzed and will be interpreted with reference to tuber elemental nutrient levels.

(3) **Cooking stability** of endogenous antioxidants and vitamin C are being studied in selected cultivars of the 2003, 2004, and 2005 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.

(4) **Vitamin C** content as influenced by genotype, cooking method, and storage interval has been analyzed from selections and cultivars grown in 2003, 2004 and will be completed for those grown in 2005.

Tubers for the proposed studies in 2006 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. All necessary analytical equipment is available. 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 Colorado potato industry 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 are a dietary staple, potatoes can benefit from boosting consumer confidence and creating enhanced awareness of positive nutritive benefits. Research data is required to support putative health claims. 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 Results from this work should enhance opportunities to seek funds from value-added marketing and health related funding programs.

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

- Total phenolics, ABTS radical scavenging capacity and vitamin C assays have been completed for 69 selections and cultivars (plus 105 entries for Delgado & Holm) grown in 2005. A new set of entries (20) will be collected and analyzed from the 2006 harvest.
- Assist marketing studies if requested, to promote potatoes for health.

Objective 1. Antioxidant properties: Summary of results

Total phenolic content expressed as gallic acid equivalents (Table 1) for cultivars grown in Colorado and for the top selections demonstrate that the highest values were obtained from entries with purple and red pigmented skin and tuber flesh tissues. Of the 75 to 90 selections tested each year since 2002, the highest selections consistently arose from pigmented selections. The obvious assumption that the anthocyanin pigments in these selections were responsible for these high values is correct, but in addition we were surprised to discover that in two of these that we tested, chlorogenic acid, a very important antioxidant was also very high. More detail on this will be presented later. Only Purple Majesty and Mountain Rose were evaluated for phenolic constituents this past year, and these data suggest other promising pigmented selections should be tested for chlorogenic acid as well.

Table 1. **Summary of total phenolic content (gallic acid equivalents/100g FW) for the highest content selections.**

Selection	**2002**	**2003**	**2004**	**2005**	**Average**
CO97227-2P/P	41.6	48.9	19.0	14.5	31.0
CO97226-2R/R	28.4	28.8	20.2	13.7	22.8
CO97222-1R/R	7.5	6.0	13.6	13.8	10.2
CO97215-2P/P	27.0	25.0	16.8	11.0	19.9
CO97216-3P/P		25.5	4.9	10.6	13.7
CO97216-1P/P	28.8	33	46.5	10.0	29.7
CO97306-1R/R	58.9	23.3	15.8	9.6	26.9
CO99364-3R/R		15.2	11.2	9.4	11.9
CO01357-4R/R				9.2	9.2
Cultivars	**2002**	**2003**	**2004**	**2005**	**Average**
All Blue	40.5	15.2	22.9	7.6	21.6
Centennial Russet	10.7	4.4	7.8	3.9	6.7
Cherry Rd	12.6	6.4	7.4		8.8
Colorado Rose	7.2	4.8	5.0	3.7	5.2
Mountain Rose	23.8	18.6	14.9	7.7	16.3
Purple Majesty	37.3	20.4	16.2	8.6	20.6
Rio Grande Russet	16.8	13.6	5.0	4.1	9.9
Russet Burbank	14.2	1.6	9.9	4.0	7.4
Russet Norkotah S3	11.1	9.8	8.3	5.7	8.7
Russet Nugget	10.6	9.8	5.4	3.9	7.4
Yukon gold	9.8		4.3	3.3	5.8

Radical scavenging capacity expressed as Trolox equivalents, a water soluble vitamin E analogue, closely follow data for total phenolic contents (Table 2). Once again the highest radical scavenging capacity was detected in the pigmented cultivars, with the exception of Rio Grande Russet. Rio Grande Russet was also high in chlorogenic acid, and although well within established limits for glycoalkaloids, it contains higher levels than other cultivars tested.

Table 2. Radical scavenging capacity of high and low entries (ABTS/TEAC uM/g DW) 2001 to 2004.

Highest Entries

2001		2002		2003		2004	
Purple Majesty*	34.7	CO97227-2P/P*	60.3	CO97227-2P/P*	70.9	CO97216-1P/P*	59.7
Mt. Rose*	23.9	CO97226-2R/R*	60.2	CO97216-1P/P*	42.0	CO97226-2R/R*	53.9
TC1675-1RU	17.5	CO97216-3P/P*	44.7	CO97215-2P/P*	36.6	CO97227-2P/P*	40.9
		CO97306-1R/R*	38.7	CO97216-3P/P	30.2	Purple Majesty*	39.1
		All Blue*	33.8	CO97226-2R/R*	26.5	CO97215-2P/P*	33.3
		CO86218-2R	29.6	Purple Majesty*	26.0	All Blue*	31.4
		Purple Majesty*	26.6	PAC99P29-1R/R*	24.8	Mt. Rose*	29.8
		PAC99P29-1R/R*	26.2	All Blue*	22.9	CO99364-3R/R	23.6
		Rio Grande	24.8	CO97306-1R/R*	21.9	CO97219-1R/R	21.4
		CO92077-2RU	22.1	Mt. Rose*	16.6	CO97306-1R/R*	16.8

* = Among the highest two out of four years

Lowest entries

2001		2002		2003		2004	
CO92027	5.6	AC87084-3RU	4.0	CO97232-1R/Y	3.1	CO00279-2R/Y	9.8
Russet Nugget	3.9	Sangre-S10	1.8	AC99323-1W	2.5	CO99045-1W/Y	9.4
AC87084-3RU	3.5	BC0894-2W	1.8	DT6063-1R cherry r.	2.5	CO97138-7RU	8.9
CO3016-3RU	2.8	AC94296-5W	1.6	AC96010-3RU	1.9	CO00275-1R/Y	8.9
Yukon Gold	2.5	AC87340-2W	1.5	Durango Red	1.8	R. Norkotah#S3	6.8
2044sl21	2	CO97274-2W/Y	1.5	CO96141-4W	1.7	CO93037-6R	6.3
		NDC6084C-2W	1.4	Freemont Russet	1.6	R. Norkotaht#S8	6.3
		CO93001-11RU	1.2	ATC98509-1R/Y	1.4	R. Nugget	5.5
		VC1123-2W/Y	0.7	CO95051-7W	1.3	R. Burbank	5.4
				AC92009-4RU	1.2	TC1675-1RU	5.3

Data in Table 3 demonstrate that several selections, as well as the new cultivars Rio Grande Russet contain considerably more vitamin C than the lowest selections and several older cultivars. Potatoes are an important source of Vitamin C, an important health nutrient. This fact is not well known by the public and can perhaps be used to enhance public appreciation of potato as a healthy food.

Table 3. Highest and lowest entries for vitamin C content (mg/100 g FW) uncooked samples
Highest Entries

2002		2003		2004	
CO95007-1RU*	73.7	AC99330-1P/Y*	38.8	Yukon Gold	33.5
CO95172-3RU*	67.7	CO99135-2RU/Y	31.8	CO95086-8RU*	32.9
CO97232-2R/Y*	66.1	CO95007-1RU*	29.8	AC99330-1P/Y*	31.6
CO95086-8RU*	63.3	CO97219-1R/R*	29.1	Rio Grande R*.	29.7
CO97233-3R/Y	61.3	ATC98513-1W/Y	28.8	CO95172-3RU*	29.3
AC93047-1RU	55.8	Rio Grande Russet*	28.6	C097232-2R/Y*	29.2
CO94157-2W/Y*	53.0	AC99329-7RW/Y	27.0	AC96052-1RU*	28.2
AC98495-1W/Y	50.6	CO96109-7RU*	25.9	C094157-2W/Y*	28.1
CO97222-1R/R	47.4	AC95405-2RU	25.7	CO96109-7RU*	26.4
AC97521-1R/Y	45.8	AC94296-5W	24.2	CO97219-1R/R*	24.9
CO92077-2RU	43.8	Yukon Gold	23.4	Yukon Gold	24.4
AC97521-1R/Y	40.2	C094157-2W/Y*	22.3	CO93037-6R	20.4
Rio Grande R*.	38.6	AC99323-1W	21.6		
		CO95172-3RU*	21.3		

* = Appears in at least two of three years

Cultivars

2002		2003	2004	*2005*
All Blue	35.8	22.7	21.1	***21.0***
Centennial Russet	31.8	17.7	16.8	***19.5***
Cherry Red	15.1	20.2	17.1	-
Colorado Rose	17.9	22.3	18.1	***20.5***
Mountain Rose	26.3	17.1	19.2	***19.0***
Purple Majesty	29.5	19.6	25.4	***24.5***
Rio Grande Russet	38.6	28.6	29.7	***30.5***
Russet Burbank	31.9	14.3	16.7	***18.2***
Russet Norkotah S3	19.9	15.5	21.9	***21.5***
Russet Nugget	-	20.2	19.6	***18.7***
Yukon Gold	23.9	23.4	24.4	***33.5***

Objective 2. 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 4 and 5, from three repetitions 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.

Table 4. 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 5. 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).			
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

Objectives 1 & 2. LC/MS 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. LC/MS instrumentation, 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.

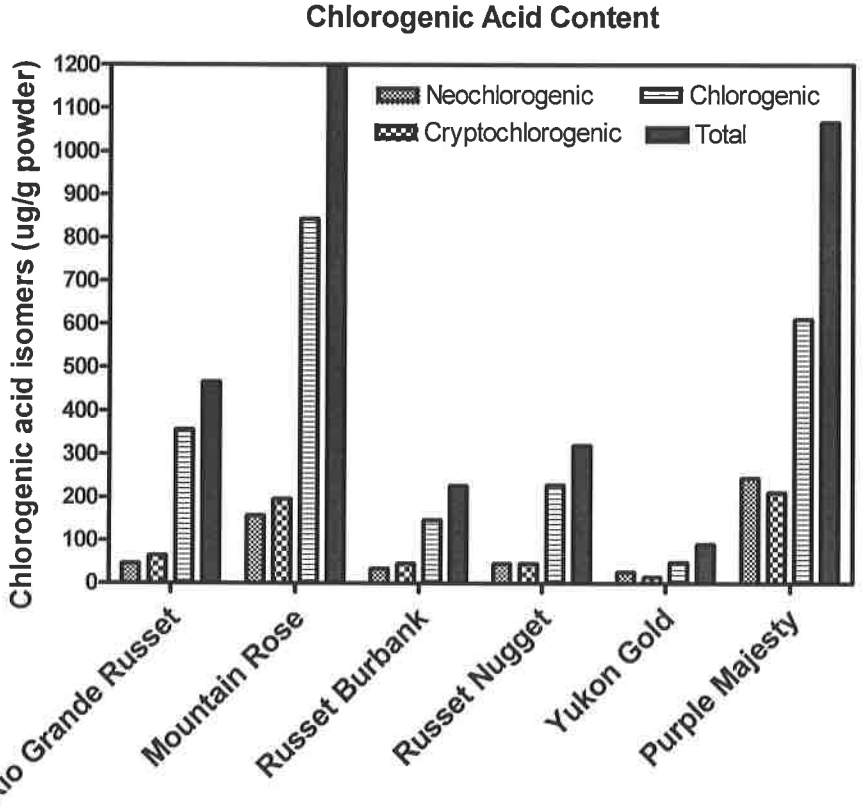

Figure 1. Chlorogenic acid isomers detected in extracts from potato cultivars baked for one hour at 170 °C, detected by LC/MS.

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 (alpha-solanine + alpha 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.

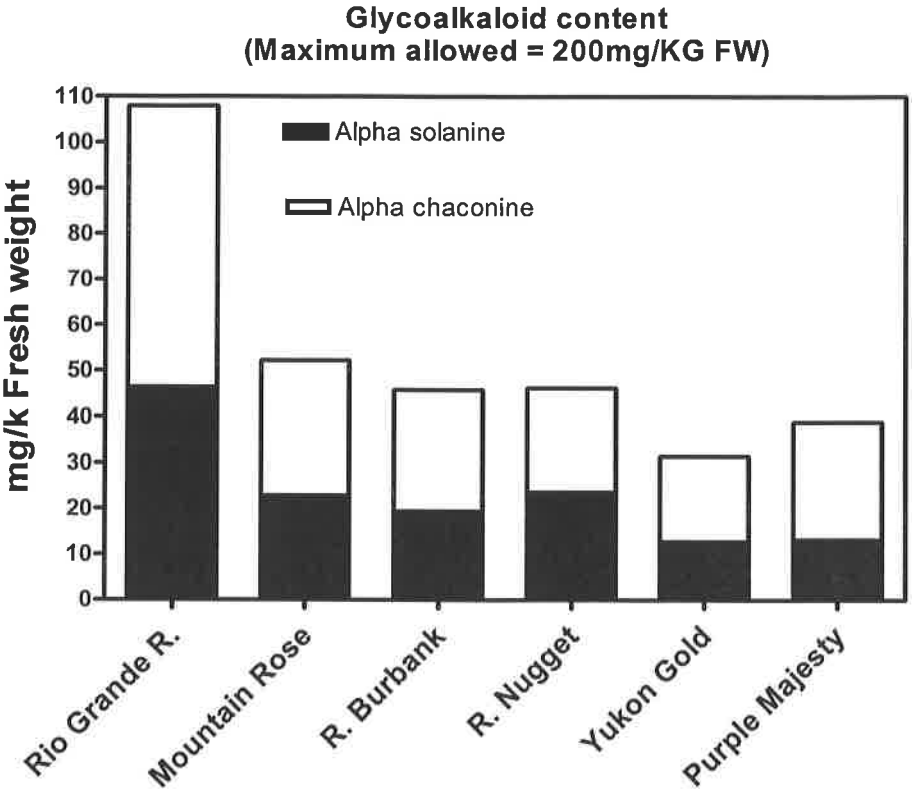

Figure 2. Glycolalkaloid content in tubers baked one hour at 170 C.

Amended Budget for 2006/2007

1. Personnel: The major component of our budget is for part time analytical technical or student hourly support to run the assays. If this revised proposal is funded we will attempt to maintain only key elements at a reduced level, (1/2 time will be reduced to ¼ time).

Salary (1/4 time, 65 days @$12/hour)	6,250
Fringe benefits (19.4%)	1,212

2. Materials and Supplies: reagents, micro plates, tips, supplies.	1,000
3. Travel: local	500
4. Equipment (none)	
5. Services: Partial service contract for micro plate reader	1,400
6. Total:	$10,362

Justification of estimates for personnel costs

1. Genotype/cultivar 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.

30 days

2. Develop HPLC assay for chlorogenic acid and screen the most promising russet selections (approximately 30-40 days)

Total 60-70 days

Literature Cited

Feng, R.T., Lu, Y., Bowman, L.L., Qian, Y., Castronova, V., Ding, M. 2005. Inhibition of AP-1, NF-kB and MAPKs and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. 21 June. http://www.jbc.org/cgi/reprint/M503347200v1.pdf.

Freidman, M. 1997. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. J. Agric. Food Chem. 45: 1523-1540.

Lee, K.R., Kozukue, N., Han, J.S., Park, J.H., Chang, E.Y., Baek, E.J., Chang, J.S., Freidman, M. 2004. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. J. Agric. Food Chem. 52:1832-2839.

Mensinga, T.T., Sips, A., Rompelberg, C.J.M., van Twillert, K., Meulenbelt, J., van den Top, H.J., van Egmond, H.P. 2005. Potato glycoalkaloids and adverse effects in humans: an ascending dose study. Regulatory Toxicology and Pharmacology 41:66-72.

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.

Zhang, Y.J., Vareed, S.D., Nair, M.G. 2005. Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. Life Sciences 76:1465-1472.