CSU - Cultivar Royalty Funds
Proposals – 2010

Sastry Jayanty
Potato Postharvest Physiology
San Luis Valley Research Center Department of Horticulture & LA

Title: *Improving the food source to prevent future health risks and concerns*

Most relevant funding source: *CCPGA royalties*

Nature, scope, objectives of proposed research

Diet and exercise are the two most widely prescribed words to reduce obesity and related health issues. But despite the fact that availability of low calorie food choices and the importance of healthy life style there is a significant population affected by chronic diseases associated with obesity. A new and emerging approach is to design foods that controls appetite and energy intake. This is achieved through promoting gastric relaxation that delays gastric emptying, and finally limiting meal size; in order to promote satiety-enhancing neuroendocrine feedback responses.

Improvements in nutrition potentially add value to the crop and impact consumers and growers on a global food production scale. Potato is the most significant vegetable and staple food consumed worldwide. Potatoes are popular in various segments of the population and there are reports that there is a perceived linkage between potato consumption and obesity. Potatoes are rich in resistant starch, proteinase inhibitors, phenolic acids, and carotenoids. All these compounds have a role in controlling obesity, glycemic load and Type 2 Diabetes and related cardiovascular diseases. Understanding the role of these compounds derived from potatoes will help in selection and breeding strategies for new cultivars. Improving the food source is like treating and preventing future health risks and concerns.

Obesity is generally accompanied by insulin resistance and impaired glucose utilization. Over 60% of the adult US population is overweight or obese. This is a potentially important market segment to which value-added potatoes can be offered for improved health benefits. Potato starches are one of the main sources of dietary carbohydrates. One of the long term goals of this project is to identify potato cultivars that when consumed as a cooked product release glucose slowly from the intestinal tract and/or promote glucose uptake from the blood stream via peripheral tissues by improving insulin sensitivity. Resistant starches are non-digestible oligosaccharides that contribute to the growth of potentially beneficial bacteria in the colon. Potential health benefits include inhibition of intestinal infection reduction in cancer risk and modification of the glycemic response.

Huge losses are incurred by the potato industry due to disease spread in potato storages. These diseases are caused by both bacterial and fungal pathogens. PIs are wound induced and they are systemically expressed in potato leaves, suggesting that the proteinase inhibitors are involved in the plant defense response against invading predators such as insects or fungi. We would like to test whether PIs in potato tubers offer resistance to diseases during storage.

The outcome of this proposal will be identification of cultivars that have value-added traits, such as elevated PI2 for use as a human satiety supplement; and resistant starches, phenolics (chlorogenic acid and caffiec acid) useful in modulating the glycemic index; and carotenoids for obvious health benefits. This project will ultimately benefit both the consumer through improved healthy functional food options and potato growers in diversifying their end markets.

Back Ground and Significance

There is growing recognition of the importance of whole food-based approaches for chronic disease prevention (World Health Organization 2003 & 2007). Satiety is important in regulating food intake and has important public health significance in the control of obesity. A survey of the literature reveals that concerns regarding potato consumption have focused on fried potatoes (French fries or potato chips) as a source of excessive caloric intake (Receveur et al.,2008) and on potential problems associated with potatoes' seemingly high glycemic index (Halton et al., 2006), despite the fact that the glycemic index can be markedly reduced by the method of food preparation (Fernandes et al., 2005, Englyst et al., 1992). These issues generally address concerns related to the occurrence and consequences of obesity, heart disease, and type-2 diabetes. Attention has recently been given to the role of dietary glyemic index (GI) for management and prevention of chronic diseases.

Our team has validated a screening procedure for the glycemic index in potato cultivars using an appropriate animal model. As shown in the Figure 1, a strong dose response exists between dose of glucose administered and glucose uptake. However, when six potato cultivars that had measurable differences in resistant starch were evaluated, and the area under curve (AUC) data was regressed on RS content, variance was large, reflecting the importance of screening in an animal model. Whether differences are due to carbohydrate characteristics or the effects of potato phytochemicals on insulin metabolism and glucose utilization remains to be determined. Overall, among the potato cultivars screened, there was a 2.5 fold range in glucose uptake. **Chlorogenic acid** is a family of naturally occurring antioxidants, also slows the release of glucose into the bloodstream after a meal (Johnston et al., 2003). It is also an antioxidant and an inhibitor of the tumor promoting activity of phorbol esters. Chlorogenic acid and caffeic acid are antioxidants in vitro and might therefore contribute to the prevention of Type 2 Diabetes Mellitus (Paynter et al., 2006) and cardiovascular disease (Lincoln et al., 2000). Chlorogenic acid has been proven in animal studies in vitro to inhibit the hydrolysis of the enzyme glucose-6-phosphatase in an irreversible fashion. This mechanism allows chlorogenic acid to reduce hepatic glycogenolysis and to reduce the absorption of new glucose
Caratenoids are indispensable to plants and play a critical role in human nutrition and health. A wide variety of health benefits of carotenoids have been reported. Some carotenoids have provitamin A activity but all are antioxidants (Palozza and Krinksy, 1992), and may reduce the risks of cancer (Khachik et al., 1999), cardiovascular disease (Gey, 1995). The carotenoid content of white-fleshed potato varieties has been reported to be low (< 100 µg per 100 g fresh weight (FW)), whereas, the carotenoid content of yellow-fleshed varieties may be as high as 560 µg per 100 g FW (Nesterenko and Sink, 2003).

Resistant starch (RS): Starches are one of the main forms of dietary carbohydrates. Starch consists of two types of molecules, amylose (normally 20-30%) and amylopectin (normally 70-80%). Amylopectin is a compact molecule with a higher molecular weight than amylose. Amylose has an extended shape and hydrogen bonds between these extended chains make it resistant to amylases. The amount of resistant starch (RS) in a given cultivar is dependent on the amount of amylose. Dr. Jayanty's postharvest lab at the San Luis Valley Research Center, has been working on estimating the RS and amylose content in potato cultivars developed in Colorado. Our initial results indicate that there are significant differences among cultivars. Some of the cultivars having high RS in raw tubers but lose significant amounts of RS when they are baked.

Proteinase inhibitors: In order to take advantage of these opportunities for the improvement of potato cultivars for chronic disease prevention, a greater understanding of the macronutrient composition and small molecules in potato is required. Breeding and selection of potato cultivars for human health benefits are expected to heavily depend on high throughput profiling of cultivars and selection for desirable traits.

Specific objectives and deliverables

We hypothesize that obese mice consuming RS will have suppressed colonic and systemic oxidative stress compared to a chow diet. Whether differences are due to carbohydrate characteristics (RS) or the effects of potato phytochemicals such as chlorogenic acid, carotenoids, or proteinase inhibitors on insulin metabolism and glucose utilization remains to be determined.

Objective 1. Screening for resistant starch levels, chlorogenic acid and carotenoids in different potato cultivars and germplasm accessions. (Jayanty, Holm)

Nugent (2005) and Cummings et al (1996) have identified four types of RS, RS1-RS4, in foods. Potatoes are said to be high in RS2, which is resistant to digestion by pancreatic enzymes due to the structure of the starch granules. RS2 is found in abundance in raw potatoes; however, this form of starch in potatoes becomes easily digested after cooking.

Quantifying RS in potato cultivars:

Resistant starch analysis will be conducted primarily at the San Luis Valley Research Center, Colorado State University. Popular potato cultivars grown in different regions of the US will be collected from the collaborators. Initial screening will be determining amylose and amylopectin levels in all cultivars. Interesting cultivars with high or low amylose and phosphorous content will be further analyzed for the resistant starch. Tubers will be stored at different temperature regimes for different time periods. Cooking conditions (baking versus microwave cooking) will be tested. Samples will be cooked and cooled to room temperature and then freeze dried. Resistant starch will be estimated in freeze dried samples. Representative wild potato species will be collected from NRSP (Sturgeon Bay, WI) will be analyzed. Amylose and amylopectin amounts will be estimated in different potato cultivars by the Hovenkamp-Hermelink method. RS will be estimated using the AOAC method 2002.02 using a kit from Megazyme International Ltd., Ireland.

Objective 2. Screening cultivars for expression of proteinase inhibitor genes at different developmental stages and studying the relationship with glycemic index. (Jayanty, Holm)

Identification of PI genes from potato genome sequence: Twenty eight PI genes (cDNA sequences) from *S. tuberosum*, *S. phureja*, *S. nigrum*, *S. americanum* were used to search the shotgun genome sequence of doubled monoploid potato *S. phureja* DM1-3 516R44 (Potato Genome Sequencing Consortium, www.potatogenome.net). This preliminary analysis identified 16 unique PI genes in *S. phureja* genome (Table 2). Similar analysis was performed with the partial BAC sequence data available for diploid *S. tuberosum* variety RH89-039-16. Only seven unique PI genes were identified from this analysis due to partial genome coverage in this data

Expression analysis of PI genes: Based on the available data, primers unique to each of the PI genes will be designed for quantitative real-time reverse transcription (RT)-PCR. Real-time RT-PCR amplification will be performed with cDNA synthesized from DNase-treated total RNA from three developmental stages of tubers of different breeding lines. These studies will identify potato breeding lines with high expression levels of different PI genes and differential expression levels.

Postharvest disease incidence and PI gene expression: Potato lines with high and low PI gene gene expression will be used to test disease susceptibility during potato storage. Freshly harvested potatoes with or without fungicide treatments along with controls will be sprayed or inoculated with the *Fusarium sambucinum, Phytophthora infestans, P. erythroseptica, and, Erwinia carotovara,* to evaluate resistance and disease development. Sprayed and inoculated potatoes will be kept in containers supplied with constant humid compressed air, stored at different temperatures. Potatoes will be scored based on the severity of symptoms during 3, 6 and 12 weeks storage. These tubers will be stored in a disease-favorable environment (1 to 2 weeks at 60°F and low airflow). Disease incidence and PI gene expression will be correlated for role of PI genes in improved postharvest storage of potato.

Objective: 3. Estimating glycemic load for different potato cultivars using animal models (Jayanty, and Thompson)

Glycemic index will be Determining the using pre-clinical animal model GI is a measure of the effects of carbohydrates on blood glucose levels. Carbohydrates that break down rapidly during digestion, releasing glucose rapidly into the bloodstream have a high GI; carbohydrates that break down slowly, releasing glucose gradually into the bloodstream, have a low GI. For most people, foods with a low GI have significant health benefits. The concept was developed by Dr. David J. Jenkins and colleagues in 1980–1981 at the University of Toronto (Jenkins et al., 2002).

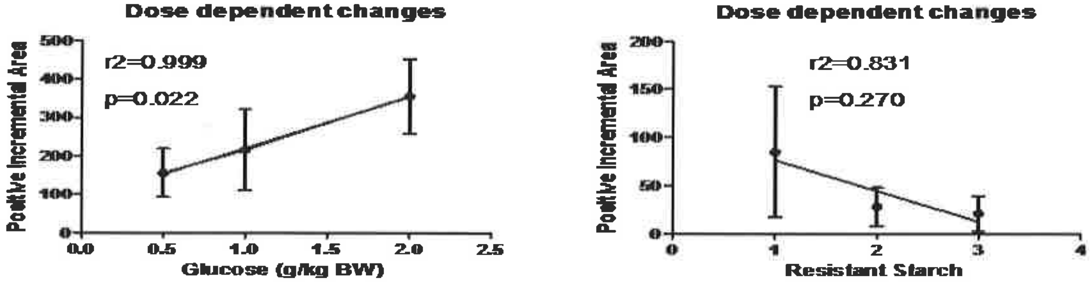

Figure-2
Dose dependent studies with glucose and resistant starch using animal models.

We propose potatoes with a low GI, when combined with other dietary constitutents, will reduce risk for the development of diabetes, heart diesease, obesity and cancer.
As shown in the Figure 2, a strong dose response exists between dose of glucose administered and glucose uptake measured as area under the curve. However, when six potato cultivars were evaluated that had measured differences in RS, when the AUC data was regressed on resistant starch content, variance was large and values fell off the regression line reflecting the importance of screening in the animal model.

Potential for results to leverage additional outside funding:
Preliminary results obtained by this study will be used in developing multi disciplinary proposals targeting Specialty Crops Research Initiative and other federal grants.

Timeline and expected short term (1 yr) and longer term (3-5 yrs) outcomes

Objectives and PD and colloboratorsent	Outcomes		
	Year -1	Year -2	Year -3
1. Screening for resistant starch levels (Jayanty and Thompson)	Screening cultivars for resistant starch Estimating glycemic load	Screening exotic germplasm for resistant starch Estimating glycemic load	Establishing relationship between resistant starch and glycemic index Identifying cultivars with high amounts of resistant starch
2. Screening cultivars for protease inhibitors (Jayanty, Wusirika, Holm)	Identification of PI genes from potato genome sequence Estimating glycemic load	Expression analysis of PI genes	Analysis of glycemic index Postharvest disease incidence
3. Screening cultivars for Chlorogenic acid and Carotenoids (Jayanty, Holm)	Identification of PI genes from potato genome sequence Estimating glycemic load	Expression analysis of PI genes	Analysis of glycemic index Postharvest disease incidence

Detailed annual budget (personnel, materials and supplies, travel, equipment, services) and a budget justification.

Budget: (Current year)

Requested funding for 2010:	**$24, 000.00**
Research Associate (50%):	$10,000.00
Animal Studies:	$10,000.0
Chemicals, Supplies and Services:	$3,000.00
Travel:	$1000.00