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Original Article

Functional food characteristics of potato cultivars (*Solanum tuberosum* L.): Phytochemical composition and inhibition of 1-methyl-1-nitrosourea induced breast cancer in rats

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ABSTRACT

Effects of freeze-dried potato powder, prepared from baked potato with skin and incorporated into a purified diet, on the post-initiation phase of chemically induced breast carcinogenesis in rats, were evaluated for both dose dependence and variation in anticancer activity among cultivars. Associations among anticancer activity, select phytochemicals, and antioxidant capacity were investigated. No adverse effects were observed in rats fed diets containing between 5% and 50% (w/w) freeze-dried potato powder. While Russet Burbank potato (RB) (5%, w/w) had marginal effects on the carcinogenic response, feeding a range of dietary concentrations (12.5%, 25%, and 50%, w/w), of a red pigmented cultivar, cv. Mountain Rose (MR), with higher content of chlorogenic acid derivatives and anthocyanin content than RB, showed greater inhibition of carcinogenesis. Overall, MR-fed rats had a 23% reduction in cancer incidence ($p = 0.009$) and a 49% reduction in cancer multiplicity (2.1 vs. 4.0 cancers per rat, $p = 0.004$) with evidence of a dose dependent effect on cancer multiplicity. Evaluation of additional cultivars showed significant variation for anticancer activity that is likely to be sufficient to build upon for crop breeding and improvement.

1. Introduction

Potatoes (*Solanum tuberosum* L.) are an important staple food providing from 5% to 15% of dietary calories for various populations around the world (World Cancer Research Fund/American Institute for Cancer Research, 2007). Potato ranks fourth behind rice, wheat, and maize in terms of world food production as an energy source. Whereas potatoes have long been a dietary staple in parts of South and Central America, Europe and North America, they are now emerging as an important food crop in other areas, particularly China (Food & Agriculture Organization, 2008). Consequently, improvement of potatoes for health benefit would be of great value and likely impact the emerging global crisis resulting from the epidemic increase in chronic diseases (World Health Organization, 2003, 2007).

Potatoes are tubers, which are the tips of underground stems that swell with starch accumulation and water uptake. While potatoes are often categorized as vegetables, for example by USDA, other classification systems have combined tubers with roots and plantains so as to divide vegetables into starchy and non-starchy subgroups (Riboli & Norat, 2003; van Gils et al., 2005; World Cancer Research Fund/American Institute for Cancer Research, 2007). This reflects the fact, not unique to the potato, that staple crops are predominantly viewed as a source of dietary starch. However, relative to human health, potato has begun to receive increasing attention as a source of nutrients, antioxidants, and other bioactive phytochemicals (Brown, Wrolstad, Durst, Yang, & Clevidence, 2003; Im et al., 2008; Stushnoff et al., 2008). These efforts to develop an understanding of phytochemicals in potato that may benefit human health have met with success, but seemingly greater attention has been paid to toxicological issues

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Abbreviations: MNU, 1-methyl-1-nitrosourea; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalents; DPC, days post-carcinogen.

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related to food processing-induced acrylamide formation and cancer risk (Felton & Knize, 2006; Gorman, 2002; Pelucchi et al., 2003) and the biological activities of potato glycoalkaloid constituents (Friedman, 2006; Mensinga et al., 2005).

There is growing recognition of the importance of food-based approaches for chronic disease prevention (World Health Organization, 2003, 2007). 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, Morou, Gray-Donald, & Macaulay, 2008) and on potential problems associated with potatoes' seemingly high glycemic index (Halton et al., 2006), despite the fact that glycemic index can be markedly reduced by the method of food preparation (Englyst, Kingman, & Cummings, 1992; Fernandes, Velangi, & Wolever, 2005). These issues generally address concerns related to the occurrence and consequences of obesity, heart disease, and type-2 diabetes. However, with the growing recognition that cancer shares common etiological factors with these chronic diseases, such as altered glucose homeostasis, chronic inflammation, and cellular oxidation (Marshall, 2006), the investigation of potato consumption on cancer risk is also merited. In 2007, a review by the World Cancer Prevention Fund/American Institute for Cancer Research, regarding food consumption patterns and cancer prevalence, concluded that there was insufficient evidence to make any dietary recommendations for tubers, roots, and plantains (World Cancer Research Fund/American Institute for Cancer Research, 2007).

Based on reports that: (1) potato cultivars have been shown to vary significantly in phytochemical composition (Stushnoff et al., 2008); (2) a number of phytochemicals identified in potato have been reported to inhibit the growth of human breast cancer cells in monolayer culture (Denton, Koszewski, & Notides, 1992; Hakimuddin, Paliyath, & Meckling, 2004; Lee & Zhu, 2006; Neto, 2007; Xuan, Endo, & Fujimoto, 2002); and (3) potato consumption has been associated with a reduced risk for breast cancer, although the study cohort was small and other factors may have accounted for the observed response (Hirose et al., 1995), the experiments reported herein were designed to determine the effect of potato consumption on the occurrence of experimentally induced breast cancer and to investigate the association between the carcinogenic response and a select number of potato cultivars that varied in phytochemical content and antioxidant capacity.

2. Materials and methods

2.1. Chemicals

Acetone, Trolox, fluorescein sodium salt, and 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All reagents used were ACS grade or higher.

2.2. Potatoes

Approximately 75 kg (fresh weight) of each potato cultivar was obtained from the San Luis Valley Research Center of the Colorado Agricultural Experiment Station. Potatoes were scrubbed, rinsed, and dried, then sent overnight to VanDrunen Farms (Momence, IL, USA) where each cultivar was separately baked at 170 °C for 60 min and then immediately freeze dried and milled into powder which was sent to Colorado State University for storage at −20 °C until incorporation into the AIN-93G based diet. Powders included both flesh and skin of the potatoes since this is representative of the phytochemical and nutrient potential of the food and there is no consensus that potato skins should not be eaten. The decision was made to use baked potato because potatoes prepared in this manner can be eaten unadulterated by additive ingredients and baking does not require special technology, unavailable in certain regions of the world. The potatoes were chosen to represent production trends, color, and total phenolic content (Stushnoff et al., 2008).

2.3. Extraction of 80% acetone-soluble phytochemicals

Potato phytochemicals were extracted in 80% aqueous acetone, similar to other extraction methods (Wu et al., 2004). Extraction was carried out in 15 ml conical tubes with 500 mg of dried potato powder and 10 ml of 80% (v/v) acetone. Samples were rotated at a velocity sufficient to maintain insoluble material in suspension and the extraction lasted for 2 h in the dark at 4 °C. Tubes were centrifuged at 2000 × *g* for 15 min before extract supernatant was removed and diluted for assay.

2.4. Determination of antioxidant capacity by ORAC

Antioxidant capacity of the potato extracts was determined by the oxygen radical absorbance capacity (ORAC) method (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). Briefly, 25 μl of dilute extract (1:8) was mixed with 150 μl of 127 nM fluorescein solution and reacted with 25 μl of 37 mM AAPH in solution (10 mM phosphate-buffered saline (PBS), pH 7.4, 37 °C). (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), a water-soluble vitamin E analog, was used to generate a standard curve. A Gemini XS microplate reader (Molecular Devices, Sunnyvale, CA, USA) with excitation at 485 nm and emission at 520 nm was used to quantify the decrease in fluorescent signal over time using an area under the curve (AUC) approach. All values are expressed as mean ± SEM (μmoles of Trolox equivalents per gram potato powder). Each potato powder was extracted in triplicate and run in triplicate in the assay.

2.5. Extraction of phytochemicals for LC/MS

Potato powder was extracted with 10 mL of 50 mM PBS, pH 7.13, for 20 h at 4 °C in the dark. After centrifugation at 3220 × *g*, for 10 min at 4 °C, the supernatant was filtered through a 0.22 μm syringe filter and stored immediately at −20 °C until analyzed by LC/MS. Extracts were diluted six-fold with 15% acetic acid in methanol for LC/MS injection. The analyses were conducted on the potato powders to determine the chemical composition of the powders used in the animal feeding studies and may not be representative of random biological samples for cultivars. Variation among technical replicates was less than 10%.

2.6. Analysis for glycoalkaloids

Previously described LC/MS glycoalkaloid methodology was adapted for this work (Stobiecki, Matysiak-Kata, Franski, Skala, & Szopa, 2003). Briefly, extract components were separated with a Synergi Fusion RP80, 4 μm, 150 mm × 2.1 mm column with 4 mm × 2 mm guard cartridge from Phenomenex Inc. (Torrance, CA, USA) and a water/acetonitrile gradient elution containing 1% formic acid at a column temperature of 35 °C. A Thermo Finnigan LTQ Ion Trap in electro-spray ionization (ESI) positive ion mode was used for mass determination.

2.7. Analysis for flavonols and phenolic acids

LC/MS methodology was based on the work of others (calde-Eon, Saavedra, de Pascual-Teresa, & Rivas-Gonzalo, 2004; Fang, Yu, & Prior, 2002; Wu & Prior, 2005) and was similar to that used in glycoalkaloid analysis with the additional use of positive ion mode