JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Storage Elevates Phenolic Content and Antioxidant Activity but Suppresses Antiproliferative and Pro-apoptotic Properties of Colored-Flesh Potatoes against Human Colon Cancer Cell Lines

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ABSTRACT: Colored-flesh potatoes are an excellent source of health-benefiting dietary polyphenols, but are stored for up to 3−6 months before consumption. This study investigated the effect of simulated commercial storage conditions on antioxidant activity (DPPH, ABTS), phenolic content (FCR) and composition (UPLC-MS), and anticancer properties (early, HCT-116 and advanced stage, HT-29 human colon cancer cell lines) of potato bioactive compounds. Extracts from seven potato clones of differing flesh colors (white, yellow, and purple) before and after 90 days of storage were used in this study. The antioxidant activity of all clones increased with storage; however, an increase in total phenolic content was observed only in purple-fleshed clones. Advanced purple-fleshed selection CO97227-2P/PW had greater levels of total phenolics, monomeric anthocyanins, antioxidant activity and a diverse anthocyanin composition as compared with Purple Majesty. Purple-fleshed potatoes were more potent in suppressing proliferation and elevating apoptosis of colon cancer cells compared with white- and yellow-fleshed potatoes. The extracts from both fresh and stored potatoes (10−30 μg/mL) suppressed cancer cell proliferation and elevated apoptosis compared with the solvent control, but these anticancer effects were more pronounced with the fresh potatoes. Storage duration had a strong positive correlation with antioxidant activity and percentage of viable cancer cells and a negative correlation with apoptosis induction. These results suggest that although the antioxidant activity and phenolic content of potatoes were increased with storage, the antiproliferative and pro-apoptotic activities were suppressed. Thus, in the assessment of the effects of farm to fork operations on the health-benefiting properties of plant foods, it is critical to use quantitative analytical techniques in conjunction with in vitro and/or in vivo biological assays.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the fourth most important food crop worldwide and is the leading vegetable crop in the United States with per capita consumption of approximately 54 kg.[1] Potatoes are a good source of carbohydrates, minerals, and vitamins and are also rich in antioxidant polyphenols and carotenoids. Colored-flesh potatoes are gaining popularity among consumers due to greater levels of phenolic acids, anthocyanins, and carotenoids.[2] The total phenolic content of colored-flesh potatoes (90−400 mg GAE/100 gfw)[3] is generally comparable to that of common berries such as strawberries, blueberries, and cranberries (100−412 mg GAE/100 gfw).[4−6] However, potatoes are relatively inexpensive and can be consumed in larger quantities in one meal and can therefore contribute to maintaining a healthy population.

Chlorogenic acid, caffeic acid, and ferulic acid are among the prominent phenolic acids present in the potato, whereas *p*-coumaric acid, sinapic acid, and vanillic acid are present in minor quantities.[7] Purple-fleshed potatoes contain anthocyanins such as petunidin- and malvidin-3-rutinoside-5-glycosides acylated with *p*-coumaric and ferulic acid, whereas red-fleshed potatoes have pelargonidin- and peonidin-3-rutinoside-5-glycosides acylated with *p*-coumaric and ferulic acid.[8,9] Violaxanthin, antheraxanthin, lutein, and zeaxanthin are the major carotenoids found in potatoes.[10,11]

A number of studies have examined the antioxidant, antimutagenic, and anticancer effects of potato polyphenols. Pure soybean oil treated with freeze-dried extracts from peels of six potato cultivars showed a reduction in the peroxide value.[12] In our previous study, *Solanum jamesii* tuber extracts showed antiproliferative and cytotoxic effects against HT-29 human colon cancer and LNCaP human prostate cancer cell lines.[13] Chlorogenic acid and other polyphenols also exhibit strong antioxidant activity toward heart disease-related LDLs and thus may indirectly reduce the risk of heart disease.[14] Red- and purple-fleshed potato cultivars showed greater antioxidant potency, suggesting a role of anthocyanins as antioxidants.[15] We have previously reported that anthocyanin fractions from potato extracts were pro-apoptotic and induced apoptosis via both caspase-dependent and -independent pathways in LNCaP (androgen-dependent) and PC-3 (androgen-independent) prostate cancer cell lines.[16] The chemopreventive mechanisms of anthocyanins include scavenging free radicals, reducing cell proliferation, up-regulating/inducing apoptosis, and modulating mitogen-activated protein kinase (MAPK) activities.[17−19] A recent study using healthy

Received: March 16, 2011
Revised: June 8, 2011
Accepted: June 21, 2011
Published: July 08, 2011

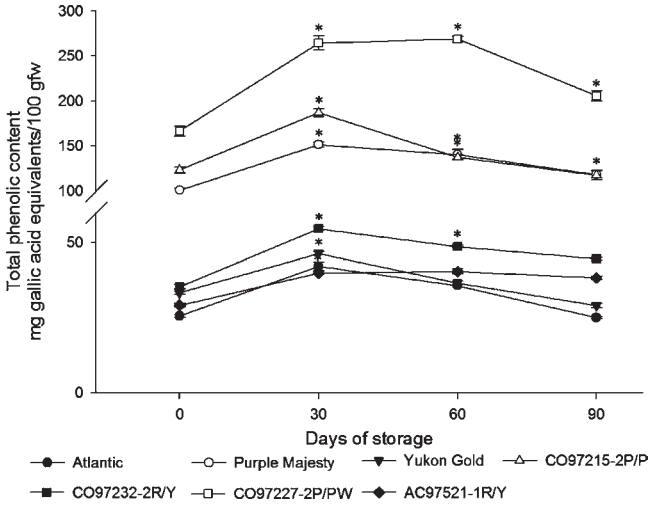

Figure 1. Initial storage-elevated total phenolic content in potato clones. Total phenolic content of the potato extracts was measured by Folin−Ciocalteu reagent assay as described under Materials and Methods and expressed as mg gallic acid equivalents/100 gfw. The letters (P/P, P/PW, and R/Y) after some of the advanced selections denote skin/flesh color: P, purple; PW, purple with white patches; R, red; Y, yellow. * indicates significant differences ($p < 0.05$) in the phenolic content compared with the initial time point. Results are presented as the mean ± SE of eight replicates for each time point.

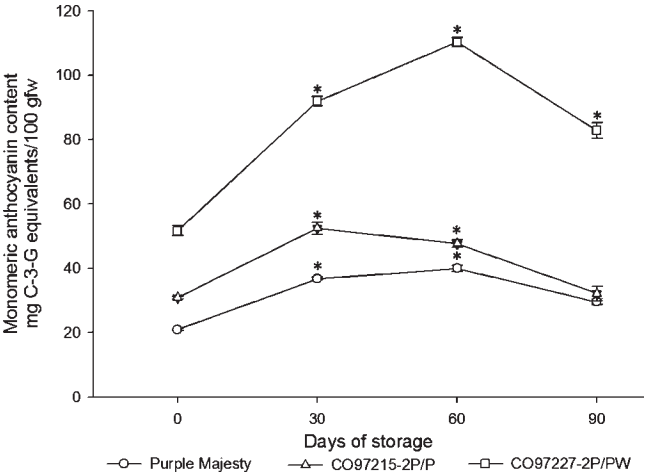

Figure 2. Initial storage caused de novo synthesis of anthocyanins in purple-fleshed clones. The trend is similar to the total phenolic content, suggesting that anthocyanins are major polyphenolic compounds present in purple-fleshed potatoes. Monomeric anthocyanin content was measured using the pH-differential method as described under Materials and Methods and expressed as mg C-3-G equiv/100 gfw. The letters (P/P and P/PW) after some of the advanced selections denote skin/flesh color: P, purple; PW, purple with white patches. * indicates significant differences ($p < 0.05$) in the anthocyanin content compared with the initial time point. Results are presented as the mean ± SE of eight replicates for each time point.

men showed that potato anthocyanins were anti-inflammatory and lowered plasma concentrations of C-reactive protein, 8-hydrodeoxyguanosine, and interleukin-6 in men consuming anthocyanin-rich purple potatoes compared with those consuming white potatoes.[20]

The literature suggests that cold storage (~5 °C) of potatoes either leads to an increase in the phenolic content or keeps it constant.[3,21−23] A similar observation has been reported for antioxidant activity.[24−26] Little information is available on the effect of storage on bioactivity and how analytical data (total phenolic and anthocyanin content, antioxidant activity) correlate with bioactivity (cell proliferation and apoptosis) before and after prolonged storage. In this study, we have investigated the effects of storage on the content, composition, and antioxidant activity of potatoes and on their anticancer properties using HCT-116 and HT-29 colon cancer cell lines.

The antioxidant activity of seven potato clones, white (Atlantic), yellow (Yukon Gold, CO97232-2R/Y, and AC97521-1R/Y), and purple (Purple Majesty, CO97215-2P/P, and CO97227-2P/PW), increased with storage. Phenolic content increased initially with storage followed by a decrease. However, after 90 days of storage, purple-fleshed potatoes showed significantly higher levels of phenolics compared with initial levels. Fresh purple-fleshed potato extracts showed more potent antiproliferative and pro-apoptotic properties in HCT-116 and HT-29 cell lines compared with extracts of white- and yellow-fleshed cultivars. Storage reduced the antiproliferative and pro-apoptotic properties of all clones tested. In summary, even though the content of phenolics increased, the anticancer activity decreased with storage. These findings suggest that it is critical to use analytical techniques in conjunction with in vitro and/or in vivo functional assays in assessing the effects of treatments on health-benefiting properties of plant foods.

MATERIALS AND METHODS

Chemicals. Solvents for the extractions were purchased from the Department of Central Receiving, Colorado State University (Fort Collins, CO). Reagents and chemicals for total phenolics, monomeric anthocyanins, and antioxidant activity assays and phenolic acid standards were procured from Sigma (St. Louis, MO). Gallic acid was purchased from Fisher Scientific (Pittsburgh, PA). Malvidin chloride, peonidin chloride, and pelargonidin chloride standards were procured from Indofine Chemicals (Hillsborough, NJ).

For the cell culture assays, McCoy's media, Dulbecco's modified Eagle's medium F-12, bovine serum albumin, and sodium bicarbonate were procured from Sigma. Fetal bovine serum, streptomycin/penicillin mix, and charcoal powder were obtained from Fisher Scientific (Pittsburgh, PA).

Potatoes. Seven potato clones, commercial cultivars (Atlantic, Purple Majesty, and Yukon Gold) and advanced selections (CO97232-2R/Y, AC97521-1R/Y, CO97215-2P/P, and CO97227-2P/PW), were grown at San Luis Valley Research Center, Colorado State University, Center, CO. The potatoes were grown in Dunul cobbly sandy loam soil for a growth period of 100−110 days, starting from mid-May until October. Vine killing was done approximately 3 weeks before harvesting using sulfuric acid. The potatoes were reconditioned for 3 weeks to allow sugar−starch conversion and then stored at 3 ± 1 °C. This was considered as "day 0". Potatoes were randomly placed in numbered bags and weighed at day 0 and then subsequently at monthly intervals before sampling for analysis to obtain moisture loss data. Tubers showed no signs of visual deterioration or significant loss of firmness even at day 90.

Preparation of Potato Extracts. Potato samples (10 g) were homogenized with 25 mL of 80% ethanol acidified with formic acid (0.1% v/v). The homogenized samples were poured into chloroform-resistant tubes and vortexed every 15 min for 1 h. Then 15 mL of chloroform was added to the tubes, and they were vortexed every 10 min for 0.5 h. The tubes were then centrifuged at 4000 rpm for 10 min and

stored overnight to allow layer separation. Supernatants (~15 mL) were collected and stored at −20 °C until further analyses. All potato samples were corrected for moisture loss during storage. To minimize intraclone variability for each clone, eight randomized tuber samples were taken at each time point and extracted separately for further analyses.

Quantification of Total Phenolics. Total phenolic content of the potato extracts was determined using a modified Folin−Ciocalteu colorimetric method.[27] In a 96-well microplate, 35 μL of extract was combined with 150 μL of 0.2 M Folin−Ciocalteu reagent and allowed to react for 5 min. Then 115 μL of sodium carbonate solution (7.5% w/v) was added, and the mixture was allowed to react for 30 min at 45 °C and cooled for 1 h. The absorbance was read at 765 nm using a microplate reader (Synergy-2, BioTek Instruments Inc., Winooski, VT) and expressed as milligrams of gallic acid equivalents per 100 g of fresh potato sample (mg GAE/100 gfw).

Quantification of Total Monomeric Anthocyanin Content. The total monomeric anthocyanin content was determined by pH differential method.[28] Two hundred and ninety microliters each of pH 1.0 and 4.5 buffers was separately added to 10 μL of the purple-fleshed potato extracts. After 15 min, absorbance was measured at 525 and 700 nm using a microplate reader (Synergy-2, BioTek Instruments Inc.). The difference in absorbance (A) at different pH values and wavelengths was obtained using the equation below:

$$A = (A_{525} - A_{700})_{pH1.0} - (A_{525} - A_{700})_{pH4.5}$$

Monomeric anthocyanin concentration (MAC) was calculated with an extinction coefficient (ε) of 26900 L/cm/mol, a molecular weight (MW) of 449.2 g/mol, a standard path length of 1 cm, and a dilution factor (DF) of 10 using the formula below:

$$\text{MAC (mg/L)} = (\text{A} \times \text{MW} \times \text{DF} \times 1000)/(\varepsilon \times 1)$$

Anthocyanin content was reported as milligrams of cyanidin-3-glucoside per 100 g of fresh potato sample (mg C-3-G equiv/100 gfw).

Antioxidant Activity Analysis. The antioxidant activity was measured using a modified 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay[29] and a modified 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay.[30,31] For the DPPH assay, freshly prepared 285 μL of diluted DPPH solution (240 μg/mL) was added to 15 μL of ethanol extracts in a 96-well microplate and allowed to react for 30 min. The absorbance was measured at 517 nm using a microplate reader (Synergy-2, BioTek Instruments Inc.) and compared with Trolox standards. The antioxidant activity was calculated as mg of Trolox equivalents per 100 g of fresh potato sample (mg TE/100 gfw).

For the ABTS assay, equal volumes of 3 mM ABTS radical and 8 mM potassium persulfate were allowed to react in the dark for at least 16 h at room temperature to form the mother solution. Then 5 mL of this mother solution was mixed with 145 mL of phosphate buffer (pH 7.4) to make the working solution. In a 96-well microplate, 290 μL of the ABTS working solution was mixed with 10 μL of ethanol extracts and allowed to react for 30 min. The absorbance was measured at 734 nm using a microplate reader (Synergy-2, BioTek Instruments Inc.). The antioxidant activity of the samples was expressed as milligrams of Trolox equivalents per 100 g of fresh potato sample (mg TE/100 gfw).

Ultraperformance Liquid Chromatography (UPLC) and Mass Spectrometry. Potato extracts (2 μL) were injected in a Waters Acquity UPLC system (Waters Corp., Milford, MA) using an HSS T3 column (1.8 μm, 1.0 × 100 mm) and a gradient from solvent A (100% water + 0.1% formic acid) to solvent B (95% methanol, 5% water, 0.1% formic acid). Injections were made in 100% A, which was held for 2 min, followed by a 13 min linear gradient to 100% B, followed by a 2 min hold at 100% B. The column was returned to starting conditions over 0.1 min and allowed to reequilibrate for 2.9 min. Flow rate was kept constant at 140 μL/min for the duration of the run. The column and the autosampler were held at 50 and 5 °C, respectively.

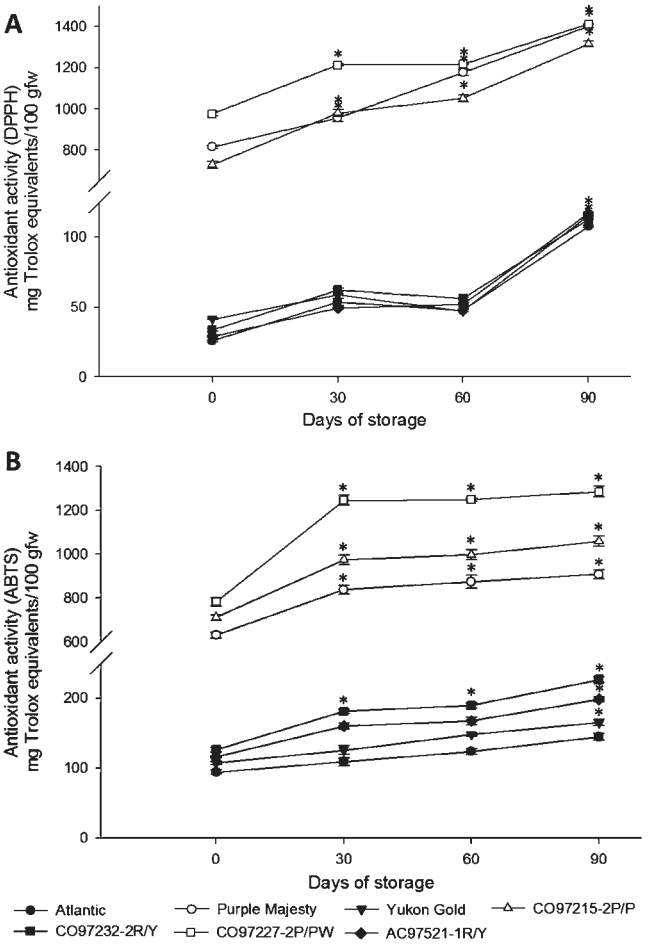

Figure 3. Antioxidant activity of potatoes as assessed by DPPH (A) and ABTS (B) assays, respectively. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. The letters (P/P, P/PW, and R/Y) after some of the advanced selections denote skin/flesh color: P, purple; PW, purple with white patches; R, red; Y, yellow. * indicates significant differences ($p < 0.05$) in the antioxidant activity compared with the initial time point. Results are presented as the mean ± SE of eight replicates for each time point.

Column eluent was infused into a Micromass Q-Tof Micro mass spectrometer (Waters Corp.) fitted with an electrospray source. Data were collected in positive ion full scan mode, scanning from m/z 50 to 1200 at a rate of 2 scans per second with an interscan delay of 0.1 s. Calibration was performed prior to sample analysis via infusion of sodium formate solution, with mass accuracy within 3 μL/L (ppm). The capillary voltage was held at 2200 V, the source temperature at 130 °C, and the desolvation temperature at 300 °C with a nitrogen desolvation gas flow rate of 300 L/h. The quadrupole was held at a collision energy of 7 V.

Peak detection was performed using MarkerLynx software (Waters MassLynx, v 4.1, Milford, MA). Peak areas were exported to SIMCA-P+ (Umetrics AB, v12.0, San Jose, CA) for principal component analysis. Data were scaled to unit variance and mean centered before principal component analysis.

Human Colon Cancer Cell Lines. HCT-116, p53+/+ cells were a generous gift from Dr. Bert Vogelstein, and HT-29 cells were purchased from ATCC (Manassas, VA). The cells were maintained at 37 °C in a humidified 5% CO_2 incubator in McCoy's medium supplemented with

Table 1. Phenolic Profile of Potato Extracts and the Effect of Storage[a]

			Atlantic		Yukon Gold		Purple Majesty		CO97227-2P/PW	
compound	molecular ion M^+ (m/z)	retention time (min)	day 0	day 90	day 0	day 90	day 0	day 90	day 0	day 90
chlorogenic acid	355	6.11	1.15 ± 0.02	2.13 ± 0.06	0.42 ± 0.02	1.94 ± 0.13	14.77 ± 0.13	23.77 ± 0.31	19.20 ± 0.65	30.09 ± 0.26
caffeic acid	181	6.15	0.87 ± 0.08	1.16 ± 0.09	0.58 ± 0.03	1.12 ± <0.01	5.73 ± 0.33	8.79 ± 0.45	5.98 ± 0.21	10.73 ± 1.13
ferulic acid	177	7.56		0.102 ± 0.01	0.043 ± <0.01	0.136 ± <0.01				
sinapic acid	207	7.76		0.041 ± <0.01		0.023 ± <0.01				
pet-3-rut-5-glc	787	5.79					215.4	449.7	297.1	761.8
mal-3-rut-5-glc	801	6.19					31.06	56.51	84.05	184.1
peo-3-coum-rut-5-glc isomer	917	7.7					0	0	128.9	297.2
pet-3-coum-rut-5-glc	933	7.92					6574	10720	11034	12026
pel-3-coum-rut-5-glc	887	8.11					0	0	1224	1514
peo-3-coum-rut-5-glc	917	8.21					306.8	416.2	7527	6225
mal-3-coum-rut-5-glc	947	8.31					668.7	834.2	1949	2516

[a] The phenolic acid values are expressed as mg/100 gfw potato. The anthocyanins have been reported as area under the curve. Values are presented as the mean ± SE of six replicates.

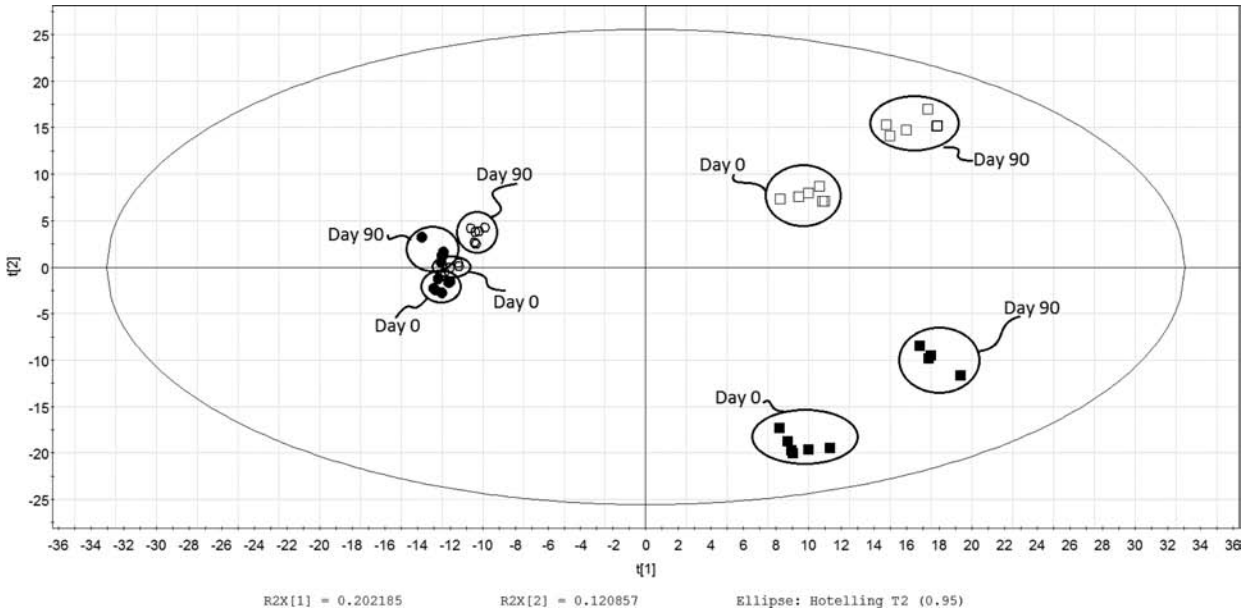

Figure 4. Principal component analysis revealed differences in phenolic profiles based on cultivar and storage. The purple-fleshed clones had different profiles from the white- and yellow-fleshed cultivars. The bubbles point out differences in phenolic profiles due to storage. Plot shows all of the individual data points. Data are represented for Atlantic (●), Purple Majesty (□), Yukon Gold (○), and CO97227-2P/PW (■) cultivars.

sodium bicarbonate (2.2 g/L), fetal bovine serum (50 mL/L), and streptomycin/penicillin mix (10 mL/L).

Cell Proliferation. Cell proliferation was assessed via 5-bromo-2′-deoxyuridine (BrdU) assay (Cell Signaling Technology, Beverly, MA) and cell counting using an automated cell counter (Nexcelom Bioscience, Lawrence, MA). Briefly, HCT-116 or HT-29 cells were grown in 96-well plates at 4000 cells per well in Dulbecco's modified Eagle's medium F-12 (DMEM). After 24 h, the cells were treated with potato extracts diluted in DMEM having final phenolic concentrations of 10, 20, and 30 μg GAE/mL. The treatments were added in triplicates at the volume of 1 mL per well and then allowed to incubate for 24 h. At the end of the incubation period, cell viability was assessed by quantifying the amount of BrdU incorporated into cellular DNA of proliferating cells using an anti-BrdU antibody. For cell counting, cells were plated at 50000 cells per well in a 12-well plate and treated as above and reported as percent reduction with respect to control.

Apoptosis. Apoptosis was measured using the Caspase-Glo 3/7 assay (Promega Corp., Madison, WI). After 24 h of incubation with the extracts, HCT-116 and HT-29 cells were counted, 15000 cells were added per well to a 96-well microplate, and the volume was made up to 200 μL using DMEM. Caspase-Glo 3/7 reagent (100 μL) was added to each well, and the plate was placed on a shaker at 300 rpm for 5 min. The plate was incubated in the dark at room temperature, and luminescence was measured after 30 min. Cells undergoing apoptosis have higher caspase-3 and caspase-7 activities, which result in a stronger luminescence signal.

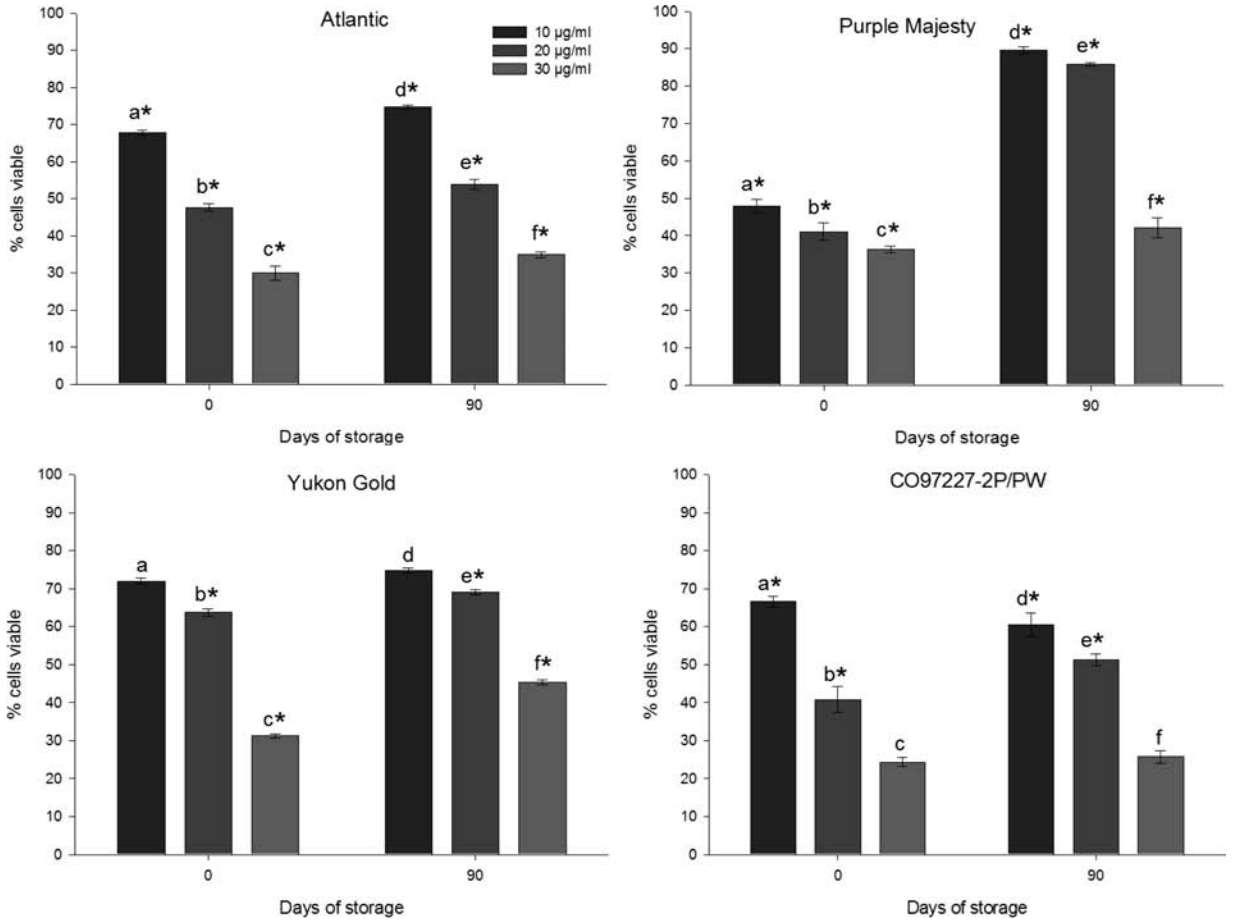

Figure 5. Potato extracts suppressed proliferation of HCT-116 cells in a dose-dependent manner. Cell number was measured using a cellometer as described under Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. * indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as the mean ± SE of four replicates for each time point.

Statistical Analysis. Fisher's protected *t* tests using the least squares means was used for comparing group differences, with $p < 0.05$ being considered as a statistically significant difference, and Pearson correlation coefficients were calculated using SAS Statistical Analysis System, v.9.2 (SAS Institute Inc., Cary, NC). All results have been expressed as the mean ± standard error (SE).

RESULTS AND DISCUSSION

Total Phenolic Content. Total phenolic content of seven clones (white-, yellow- or purple-fleshed) measured at 0, 30, 60, and 90 days of storage using the Folin−Ciocalteu reagent assay ranged from 25.6 ± 0.4 to 268.6 ± 3.3 mg GAE/100 gfw. This is in accordance with previous studies reporting that the total phenolic content of potato cultivars ranged from 90 to 400 mg GAE/100 gfw[3] and from 76 to 181 mg chlorogenic acid equivalents/100 gfw.[32] The phenolic content primarily depends upon the genotype, and slight variations within the genotype reported by different authors may be due to differences in the growing location, method of extraction, and sample preparation, as vigorous extraction methods can lead to an increase in the phenolic content.[33] The rank order for the phenolic content was purple-fleshed clones followed by yellow-fleshed clones and finally the white-fleshed cultivar. The phenolic content of purple-fleshed clones was approximately 6−8 times greater than that of Atlantic because of the presence of anthocyanins along with phenolic acids.

A significant increase ($p < 0.05$) in the phenolic content was observed after 30 days of storage for most clones (Figure 1), followed by a decline either to initial levels or to significantly above the initial levels after 90 days of storage depending upon the clone. CO97227-2P/PW had the greatest phenolic content among all clones tested. At day 0, its phenolic content was 166.2 ± 5.6 mg GAE/100 gfw, which increased to 268.6 ± 3.2 mg GAE/100 gfw at 60 days of storage and finally reduced to 205.4 ± 5.5 mg GAE/100 gfw at 90 days of storage. The lowest phenolic content was seen in the Atlantic cultivar (25.6 ± 0.4 mg GAE/100 gfw) at day 0.

In plants, environmental stresses such as low-temperature storage, strong light, wounding, or pathogen attacks have been shown to induce the generation of phenolic compounds via the phenylpropanoid pathway.[34] In potatoes, low-temperature storage,[35] light,[36] wounding,[37] and disease[38] can cause an increase in the phenolic content. Low temperature storage-induced activation of phenylalanine ammonia-lyase (PAL), a key regulatory enzyme in the biosynthesis of polyphenols including anthocyanins,[39] and de novo synthesis of secondary metabolites[21] may be responsible for an initial increase in the phenolic content

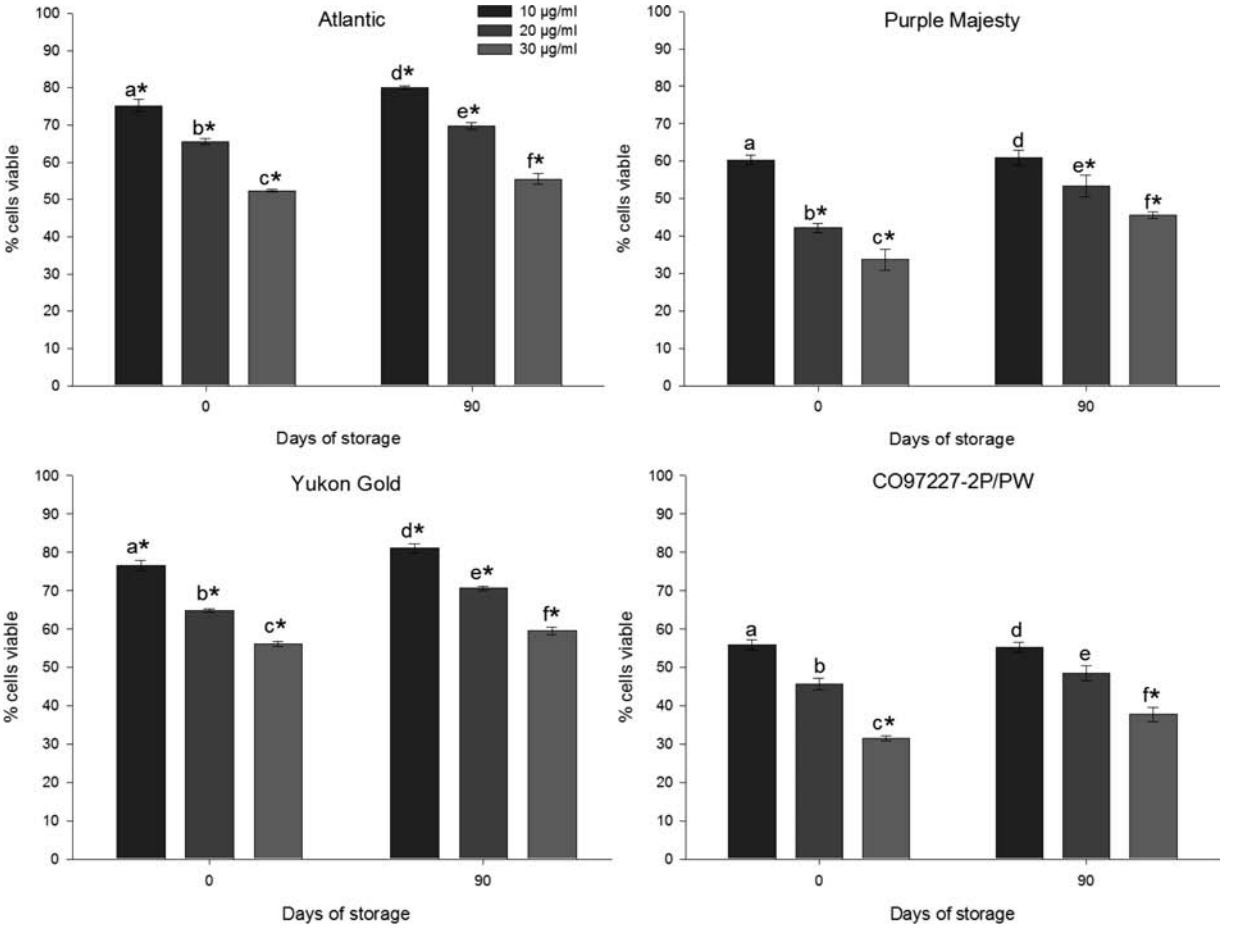

Figure 6. Potato extracts suppressed proliferation of HCT-116 cells in a dose-dependent manner. Cell viability was measured using the BrdU assay as described under Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. * indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as the mean ± SE of four replicates for each time point.

with storage. Between 30 and 90 days of storage, a decreasing trend in phenolic content may be due to degradation of the polyphenolic compounds, especially chlorogenic acid.[35]

Total Monomeric Anthocyanin Content. The anthocyanin content of purple-fleshed clones ranged from 20.9 ± 0.2 to 110.3 ± 1.4 mg C-3-G equiv/100 gfw (Figure 2). It has been documented that the anthocyanins in purple-fleshed cultivars can range from 11 to 174 mg C-3-G equiv/100 gfw.[32] This is in line with our current observation. CO97227-2P/PW had the highest anthocyanin content among all clones irrespective of the storage time. The initial anthocyanin content of CO92772-2P/PW was 51.7 ± 1.5 mg C-3-G equiv/100 gfw, which then increased to 110.3 ± 1.5 mg C-3-G equiv/100 gfw at day 60 and gradually decreased to 82.9 ± 2.4 mg C-3-G equiv/100 gfw at day 90. Purple Majesty, which showed the lowest anthocyanin content among the three clones tested, had 20.9 ± 0.2, 40 ± 1, and 29.6 ± 0.9 mg C-3-G equiv/100 gfw at 0, 60, and 90 days, respectively.

The anthocyanin content followed a trend similar to that of the phenolic content, suggesting that anthocyanins contribute to a major portion of polyphenols in purple-fleshed potatoes. Cold storage conditions are known to cause the conversion of starch to sugar,[40] which can up-regulate genes coding for dihydroflavonol reductase (DFR) and anthocyanidin synthase (ANS), which are involved in anthocyanin biosynthesis, and hence potentially cause an increase in the anthocyanin concentration.[41−44] Also, as suggested for the phenolic content, the initial increase might be due to enhanced PAL activity[39] and de novo synthesis of anthocyanins[21] during storage.

Antioxidant Activity. Antioxidant activity measured by the DPPH and ABTS assays showed an increase with storage (Figure 3). The antioxidant capacity measured by DPPH assay for fresh potatoes ranged from 25.8 ± 0.6 mg TE/100 gfw at day 0 to 107.8 ± 0.4 mg TE/100 gfw at day 90 for Atlantic and from 976.2 ± 11.46 mg TE/100 gfw at day 0 to 1412.2 ± 1.3 mg TE/100 gfw at day 90 for the advanced selection CO97227-2P/PW, which showed the highest antioxidant activity among the seven clones tested (Figure 3A). For the ABTS assay, the range was from 94 ± 3.2 mg TE/100 gfw at day 0 to 144.4 ± 4.9 mg TE/100 gfw at day 90 for Atlantic and from 782.3 ± 19.2 mg TE/100 gfw at day 0 to 1285.4 ± 25.1 mg TE/100 gfw at day 90 for CO97227-2P/PW (Figure 3B). The antioxidant values at day 90 were significantly higher compared with day 0 for all clones irrespective of the tuber flesh color.

The DPPH and ABTS methods measure the antioxidant activity as a result of their respective radical quenching ability. It has been reported that the antioxidant potential of pigmented cultivars can be 2−8 times higher than that of the

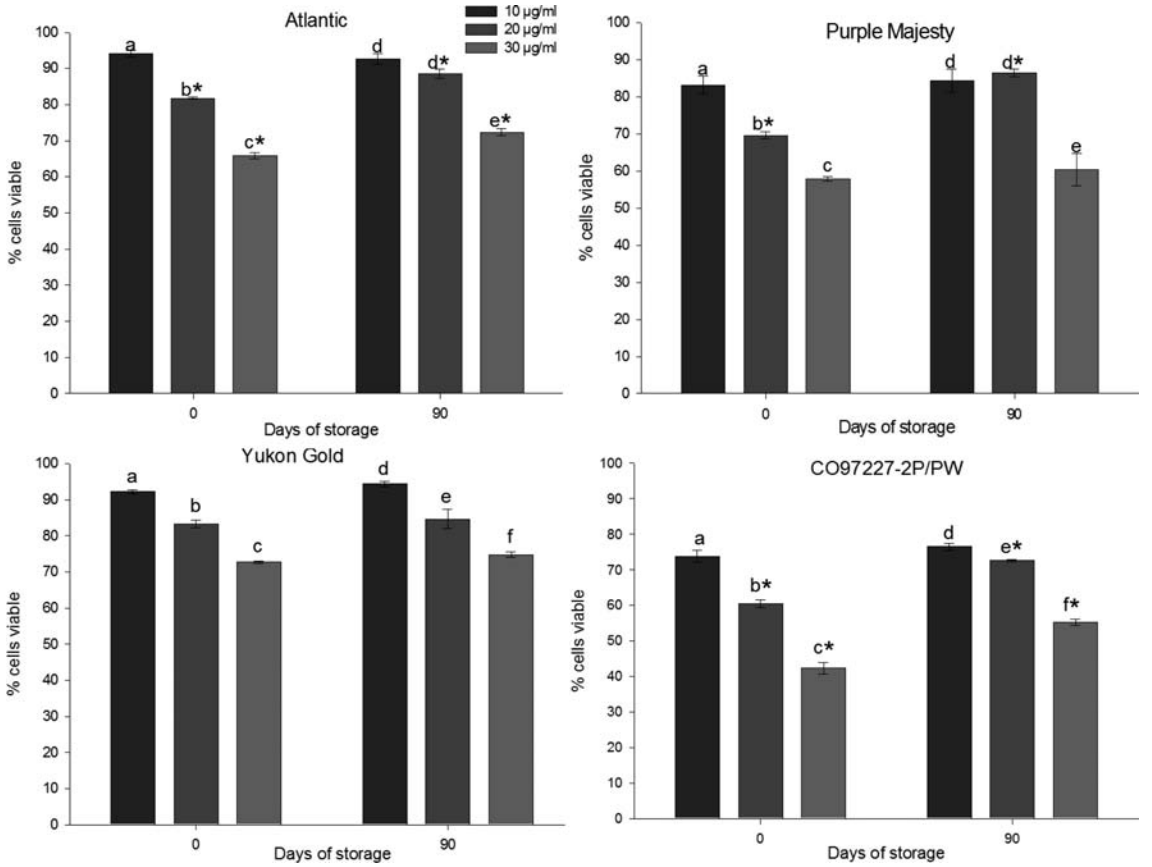

Figure 7. Potato extracts suppressed proliferation of HT-29 cells in a dose-dependent manner. Cell number was measured using a cellometer as described under Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. * indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as the mean ± SE of four replicates for each time point.

nonpigmented cultivars because of the presence of anthocyanins and/or carotenoids along with the phenolic acids.[3,45] In this study, CO97227-2P/PW had approximately a 10-fold greater antioxidant activity than the white cultivar. At 90 days of storage, the antioxidant activity had increased to its maximum for the duration of the study, although there was a trend toward reduction in the phenolic content between 30 and 90 days. These results indicate the contribution of some of the nonphenolic compounds such as vitamins and minerals to the antioxidant activity.[46,47]

UPLC-MS Profile of Phenolic Compound. On the basis of the data from total phenolic and anthocyanin contents and antioxidant activity assays, four clones were selected for phenolic profile screening. Of the four selected, three were commercially available cultivars representative of their color, Atlantic (white), Purple Majesty (purple), and Yukon Gold (yellow), and the fourth one was CO97227-2P/PW, a purple-fleshed advanced selection, which had the highest phenolic and anthocyanin content, and antioxidant activity. Chlorogenic acid was the most abundant phenolic acid in most clones (Table 1). It has been reported that chlorogenic acid may account for 90% of the total phenolic content in potatoes.[48] However, in this study only chlorogenic acid was measured but not its isomers. The chlorogenic acid content of Purple Majesty was approximately 35 times greater than that of Yukon Gold at day 0, which at day 90 reduced to approximately 12-fold. A previous study has reported approximately 10-fold difference in the chlorogenic acid concentration in pigmented cultivars such as Mountain Rose and Purple Majesty and in nonpigmented cultivars such as Yukon Gold.[3] Another study observed a 20-fold difference in the chlorogenic acid content.[49] In Atlantic and Yukon Gold cultivars, 90 days of storage increased the chlorogenic acid content approximately 2- and 4-fold, respectively. Among the purple-fleshed clones, a 1.5-fold increase was observed. Caffeic acid was the second most abundant phenolic acid. We have previously reported that caffeic acid content can range from 310 to 420 μg/100 gfw potato.[16,30] In the current study, caffeic acid ranged from 580 to 1160 μg/100 gfw potato in the white- and yellow-fleshed cultivars, irrespective of storage. The caffeic acid content in pigmented clones was greater than that in nonpigmented clones. The purple-fleshed clones contained caffeic acid ranging from 5.7 to 10.7 mg/100 gfw potato irrespective of storage. A previous study has reported a 100-fold difference in the caffeic acid contents of the yellow cultivar, Divina, and the purple cultivar Pollunta Chata.[49] Storage increased the caffeic acid content in Atlantic, Yukon Gold, Purple Majesty, and CO97227-2P/PW clones 1.3, 1.9, 1.5, and 1.8, times respectively. Thus, this increase in the phenolic acids could explain the observed increase in the total phenolic content with storage.

Purple-fleshed clones also contained anthocyanins in their glycosylated form; some of them were acylated with *p*-coumaric

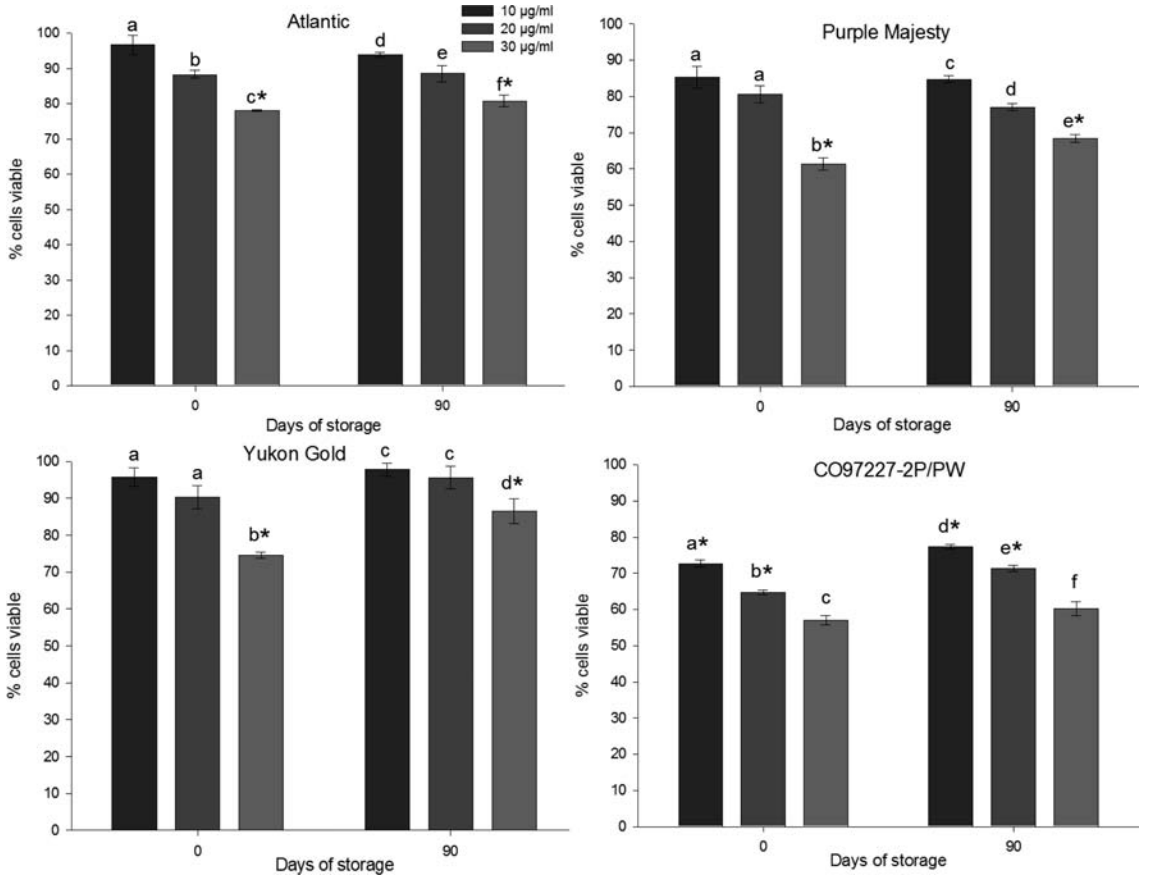

Figure 8. Potato extracts suppressed proliferation of HT-29 cells in a dose-dependent manner. Cell viability was measured using the BrdU assay as described under Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. * indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as the mean ± SE of four replicates for each time point.

acid. Pel-3-coum-rut-5-glc was observed only in CO97227-2P/PW. CO97227-2P/PW also had an approximately 3-fold higher amount of mal-3-coum-rut-5-glc as compared with Purple Majesty. Pet-3-coum-rut-5-glc was the most abundant anthocyanin in Purple Majesty, followed by mal-3-coum-rut-5-glc and then peo-3-coum-rut-5-glc. This agrees with another study that has reported Purple Majesty anthocyanins in the same order of abundance.[3] Storage increased the individual anthocyanins 1.2−2.5 times in both purple-fleshed clones with the exception of peo-3-coum-rut-5-glc in CO97227-2P/PW. This could explain the observed increase in the monomeric anthocyanin content from day 0 to day 90.

Principal component analysis indicated differences in the phenolic profiles among the clones and between the initial and final storage periods (Figure 4). The phenolic profiles of Purple Majesty (purple) were different not only from Atlantic (white) and Yukon Gold (yellow) but also from another purple clone, CO97227-2P/PW.

Cell Proliferation and Apoptosis Assays. The potential growth inhibitory effects of the extracts from the four clones (Atlantic, Purple Majesty, Yukon Gold, and CO97227-2P/PW) before and after storage were investigated using HCT-116 and HT-29 human colon cancer cell lines. Figures 5 and 6 illustrate the effects of different concentrations of the extracts (expressed as μg GAE/mL) on the proliferation of HCT-116 cells. The number of cells was quantified and reported as percentage reduction with respect to control treatment (only media). Potato extracts caused a dose-dependent reduction in the number of cells (Figures 5 and 6). The potato extracts could also suppress proliferation of HT-29 cells, which is an advanced human colon cancer cell line (Figures 7 and 8). However, the efficacy was lower as compared to the HCT-116 cell line.

The purple-fleshed clones showed more potent antiproliferative properties compared with the white- and yellow-fleshed cultivars. Significant reduction in the antiproliferative property was observed for all four clones with storage. CO97227-2P/PW was the most potent clone showing >70% reduction in cell proliferation at 30 μg GAE/mL.

Previous studies have shown reduction in cell proliferation of cancer cells treated with potato extracts. Purple Majesty extracts (2%) in the media have been reported to inhibit proliferation of MCF7 (estrogen-dependent) and MDAMB468 (estrogen-independent) breast cancer cells by approximately 70% after a 5-day incubation period.[3] Phenolic acids commonly identified in potatoes have been implicated in suppression of cancer cell proliferation in vitro.[50]

A similar trend was seen in the elevation of apoptosis (Figures 9 and 10). The potato extracts showed a dose-dependent increase in the induction of apoptosis with respect to the control (only media). Duration of storage negatively affected the pro-apoptotic activity of HCT-116 (Figure 9) and HT-29 (Figure 10) cancer cells. Purple-fleshed clones not only had higher

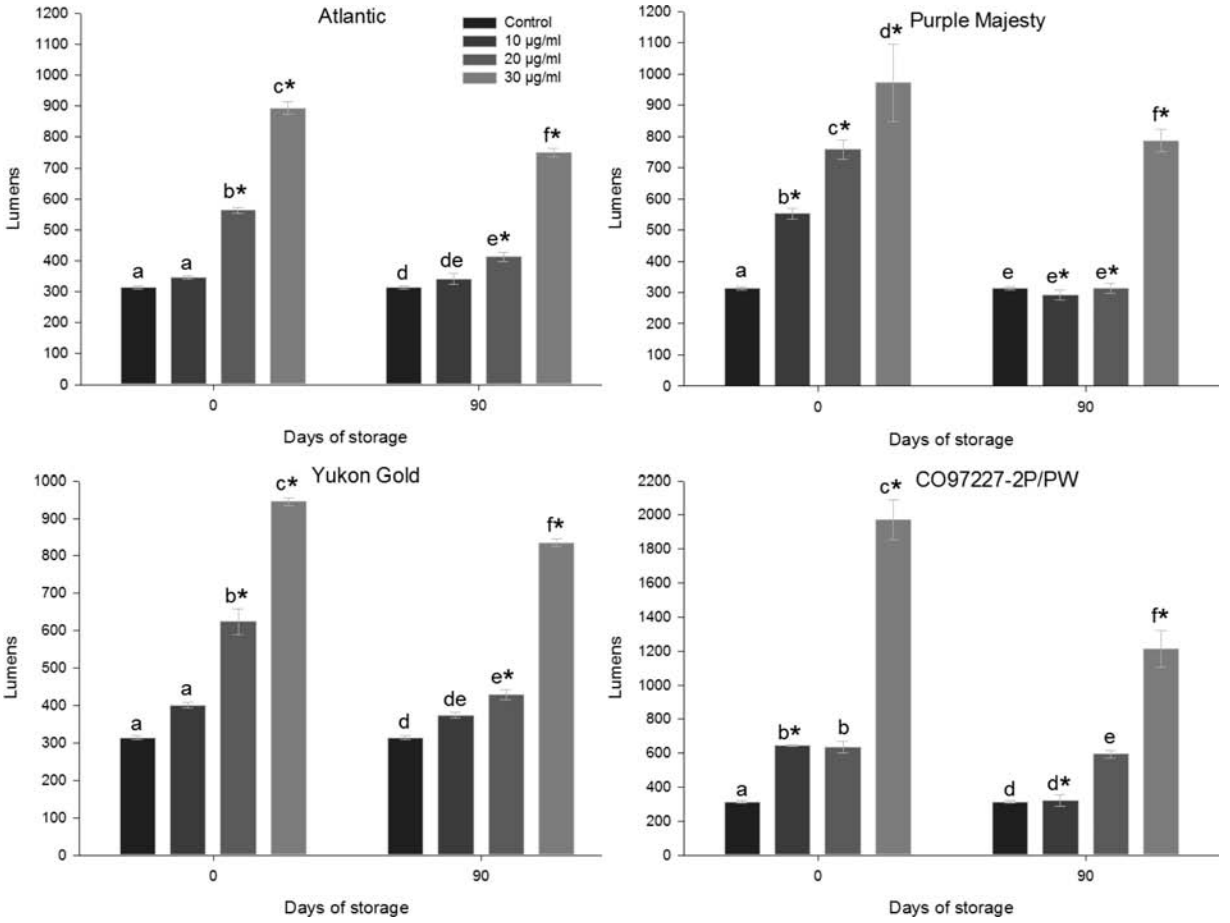

Figure 9. Potato extracts induced dose-dependent apoptosis in HCT-116 colon cancer cells. Apoptosis was measured using Caspase-Glo 3/7 assay as described under Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells at two different concentrations at a given time point. * indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given concentration. Results are presented as the mean ± SE of four replicates for each time point.

antiproliferative activity but also caused a greater increase in apoptosis as compared to the white- and yellow-fleshed cultivars. CO97227-2P/PW, with greater total phenolic content and antioxidant capacity, was the most potent clone, causing an almost 10-fold increase in the apoptotic cells as compared to the control. The diverse anthocyanin composition of CO97227-2P/PW as seen in the above section may explain the higher anticancer activity as compared with Purple Majesty.

Correlations. Significant positive correlations were observed for phenolic content and antioxidant activity ($R^2 = 0.90$, $p < 0.0001$) as well as anthocyanin content ($R^2 = 0.89$, $p < 0.0001$). This is in line with a strong positive correlation between the total phenolic and anthocyanin content ($R^2 = 0.91$) as reported by Reyes et al.[32] A significant positive correlation has been observed between the DPPH and ABTS assays for antioxidant activity measurement.[30,51,52] Similarly, correlation ($R^2 = 0.96$, $p < 0.0001$) was observed between the DPPH and ABTS assays.

Storage duration was positively correlated with antioxidant activity ($R^2 = 0.79$, $p < 0.02$) of all genotypes individually. Moderate to strong correlations were observed between storage duration and percentage of cancer cells viable for Atlantic ($R^2 = 0.74$, $p < 0.1$ for BrdU and $R^2 = 0.67$, $p < 0.1$ for cell counting) and Yukon Gold samples ($R^2 = 0.46$, $p < 0.36$ for BrdU and $R^2 = 0.99$, $p < 0.001$ for cell counting). However, apoptosis induction exhibited a strong negative correlation with storage duration for Atlantic ($R^2 = -0.93$, $p < 0.01$) and Yukon Gold ($R^2 = -0.95$, $p < 0.01$) samples, indicating a loss in the ability to induce apoptosis with storage duration. For the Purple Majesty samples, storage duration moderately correlated with percentage of viable cancer cells ($R^2 = 0.90$, $p < 0.01$ for BrdU and $R^2 = 0.66$, $p < 0.1$ for cell counting) and apoptosis ($R^2 = -0.58$, $p < 0.2$). For CO97227-2P/PW, the correlations were not significant, which could indicate that storage duration did not significantly suppress its anticancer properties.

Potatoes are receiving much attention lately for their antioxidant content. Potatoes are usually stored before consumption, so it is important to understand the effect of storage on the potato bioactive compounds. These results, for the first time, show that storage alters the phenolic compound profiles in potatoes and elevates total phenolic content but suppresses biological activity. Thus, it is important to optimize the storage conditions to retain the biological activity of potato bioactive compounds. It was also observed that colored-flesh potatoes, containing anthocyanins, had higher bioactivity as compared with the white- and yellow-fleshed ones. Hence, breeders can utilize these colored-flesh potato cultivars as parental material in the breeding programs to develop varieties with potent health benefits. Our results also warrant the use of in vitro and in vivo biological assays in conjunction with quantitative analytical techniques in assessing the genotype,

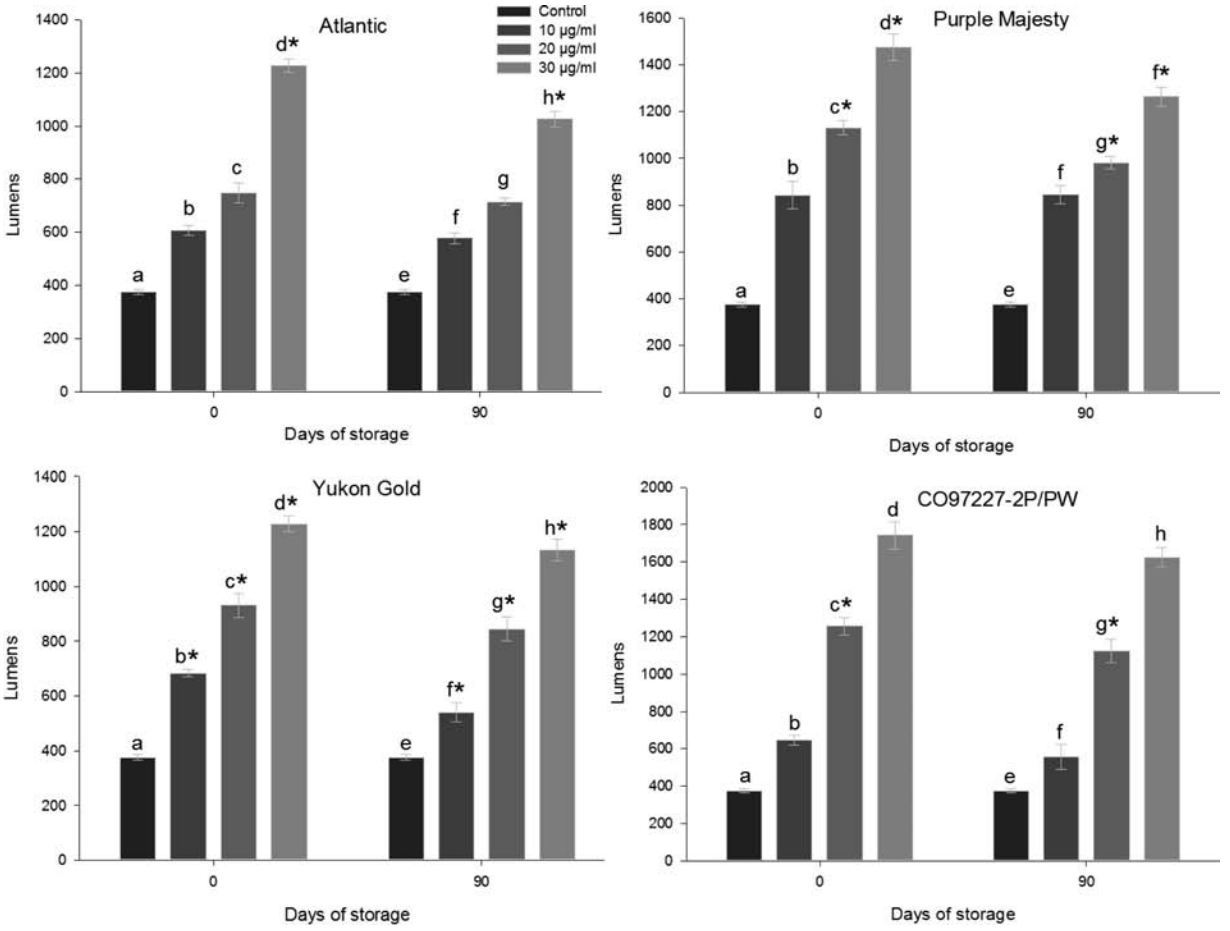

Figure 10. Potato extracts induced dose-dependent apoptosis in HT-29 colon cancer cells. Apoptosis was measured using Caspase-Glo 3/7 assay as described under Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells at two different concentrations at a given time point. * indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given concentration. Results are presented as the mean ± SE of four replicates for each time point.

storage, and processing effects on health benefits of fruits and vegetables. However, sensory attributes and consumer acceptance of these new cultivars should not be discounted.

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Funding Sources

This work was supported by National Research Initiative Grant 2009-55200-05197 from the USDA National Institute for Food and Agriculture (2009-2012).

ACKNOWLEDGMENT

We thank Sridhar Radhakrishnan, Fahrettin Goktepe, and Steven Keller for helping with the processing of potatoes and Corey Broeckling and Jim zumBrunnen for their help with UPLC-MS and statistical analysis, respectively.

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