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Varietal Differences in Phenolic Content and Antioxidant and Antiproliferative Activities of Onions

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Epidemiological studies have indicated that the consumption of fruits and vegetables is associated with a reduced risk for the development of chronic diseases, such as cardiovascular disease and cancer. Phytochemicals, including phenolics and flavonoids, are suggested to be the major bioactive compounds contributing to the health benefits of fruits and vegetables. Onions are a major source of dietary flavonoids; however, there may exist varietal differences in composition, concentration, and beneficial activities. To characterize these differences, shallots and 10 onion (*Allium cepa* L.) varieties commonly available in the United States (Western Yellow, Northern Red, New York Bold, Western White, Peruvian Sweet, Empire Sweet, Mexico, Texas 1015, Imperial Valley Sweet, and Vidalia) were evaluated for total phenolic and flavonoid contents and antioxidant and antiproliferative activities. Shallots contained the highest total phenolic content (114.7 ± 10.0 mg/100 g of sample) among the varieties tested, with a 6-fold difference observed when compared to the variety with the lowest phenolic content (Vidalia, $p < 0.05$). Western Yellow onion variety exhibited the highest total flavonoid content (69.2 ± 3.7 mg/100 g of onion) of the varieties tested, with an 11-fold difference when compared to the variety with the lowest flavonoid content (Western White, $p < 0.05$). Shallots exhibited the highest total antioxidant activity (45.5 ± 2.1 μmol of vitamin C equiv/g of onion), followed by Western Yellow, New York Bold, Northern Red, Mexico, Empire Sweet, Western White, Peruvian Sweet, Texas 1015, Imperial Valley Sweet, and Vidalia. For all varieties, both total phenolic and flavonoid contents were strongly correlated with total antioxidant activity ($R^2 = 0.9668$, $p < 0.05$; and $R^2 = 0.7033$, $p < 0.05$, respectively). The proliferation of $HepG_2$ and Caco-2 cells was significantly inhibited in a dose-dependent fashion after exposure to the Western Yellow, shallots, New York Bold, and Northern Red extracts, with Western Yellow, shallots, and New York Bold exhibiting the highest antiproliferative activity against $HepG_2$ cells and New York Bold and Western Yellow exhibiting the highest antiproliferative activity against Caco-2 cells. However, the varieties of Western White, Peruvian Sweet, Empire Sweet, Mexico, Texas 1015, Imperial Valley Sweet, and Vidalia demonstrated weak antiproliferative activity against both $HepG_2$ and Caco-2 cells. These results may influence consumers toward purchasing onion varieties exhibiting greater potential health benefits and may significantly affect future breeding efforts to enhance onion nutritional qualities.

KEYWORDS: Onion; antioxidant; antiproliferative activity; phenolics; flavonoids; cancer; coronary heart disease (CHD); varietal differences

INTRODUCTION

Epidemiological studies have consistently shown that the consumption of fruits and vegetables is associated with a reduced risk for developing chronic diseases, such as coronary heart disease (CHD), cancer, diabetes, and Alzheimers's disease (*1*−*6*). Therefore, the National Cancer Institute recommends that Americans consume at least five servings of fruits and vegetables per day (*7*). It is estimated that one-third of cancers could be prevented by dietary modifications (*8*, *9*). Additionally, several cohort studies have shown a correlation between the consumption of fruits and vegetables and a decreased risk for CHD and cancer (*5*, *10*).

In organisms, most of the potentially harmful effects of oxygen are believed to be due to the formation and activity of reactive oxygen species (ROS). One of the defense mechanisms within the organism toward limiting the levels of ROS is

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antioxidants. The protective effect of fruits and vegetables against chronic diseases is attributed to their phytochemical content and corresponding antioxidant activity (*11*−*13*). Naturally occurring plant compounds such as phenolics, carotenoids, ascorbic acid, thiols, and tocopherols exhibit antioxidant activity. The actions of these natural antioxidants include scavenging free radical species and inhibiting the production of reactive species resulting from normal cell metabolism; antioxidants thereby prevent the damage to lipids, proteins, and nucleic acids and subsequent cellular damage and death (*1*). A reduction in oxidative DNA damage and possible prevention of the onset of carcinogenesis was shown in subjects consuming a diet rich in fruit (*14*).

Onions are one of the richest sources of flavonoids in the human diet. Onions possess a high level of antioxidant activity, which is attributed to the flavonoids quercetin, kaempferol, myricetin, and catechin (*15*−*17*). Flavonoid consumption has been associated with a reduced risk of cancer (*18*, *19*), heart disease (*20*−*22*), and diabetes (*23*). In addition, flavonoids have exhibited other biological effects such as the inhibition of plasma platelet aggregation and cyclooxygenase (COX) activity and the inhibition of histamine release and SRS-A biosynthesis in vitro; flavonoids have been shown in vitro to exhibit antibacterial, antiviral, anti-inflammatory, and antiallergenic effects (*24*). Anticarcinogenicity, antiaging, and antimutagenicity originate from these activities; thus, flavonoids potentially play a role in preventing a wide range of degenerative physiological processes (*16*). For these reasons, onions have been the focus of numerous investigations to determine and characterize their health benefits both in vivo and in vitro. Hertog et al. (*25*) found that the risk associated with CHD was inversely related to quercetin intake in the Zutphen Elderly Cohort Study. A high intake of flavonoids (∼30 mg/day) was associated with a ∼50% decrease in the mortality rate from CHD compared with individuals consuming a low level of flavonoids (∼19 mg/day). Fried onions have also been shown to reduce several biomarkers of oxidative stress in vivo, such as lymphocyte DNA damage and urinary malondialdehyde content (*26*). Raw onions have been shown to reduce platelet aggregation in dog blood vessels, indicating a possible preventative method for cardiovascular disorders (*27*). Oxidative damage caused by nicotine injections in rats was significantly reduced by onion oil; the concentration of thiobarbituric acid reactive substances, conjugated dienes, and hydroperoxides, all biomarkers of oxidative stress, were decreased by the consumption of onion oil (*28*).

The onion, the second most important horticultural crop in the world, is an extensively distributed and widely consumed vegetable in the United States (*29*). The U.S. onion industry contributes ∼2.4% of the world's production, with Peruvian Sweet and Mexico being two of the leading importers, in a national market where the retail value of the onion crop is approximately $3−4 billion. New York State is ranked seventh in total volume of onions produced in the United States, with the onion being a leading state commodity (*30*). Although milder and less pungent onions are becoming more popular in the United States, particularly for fresh consumption, stronger flavored varieties for cooking are still the most commonly sold (*31*). The pungency and flavor of onions are derived mainly from sulfur compounds and may be influenced by the bitter and astringent flavors imparted by flavonoid compounds (*32*). Great varietal diversity generally exists in both onion color and flavor, thereby influencing the composition and concentration of phenolic compounds. Although the edible bulb of red onions is generally higher in total flavonoids than the bulbs of white or sweet yellow onions due to the presence of anthocyanins (*33*), red onions do not generally contain higher levels of quercetin. Yellow onions have been found to contain higher levels of quercetin than red onions, with pink and white onions having the lowest concentrations (*15*). Growing location has also been found to influence quercetin content (*34*); this influence could be due to water and nutrient availability during the growing season and resulting dry matter accumulation in the edible bulb. Galmarini et al. (*35*) found both strong phenotypic and genotypic correlations between the soluble solids content, total dry matter, flavor, and nutritional benefits of onions. It is possible that varietal influences and growing conditions that result in onions with higher astringency, stronger flavor, greater pungency, and lower water content might also impart greater levels of flavonoids or specifically quercetin than milder varieties.

Consumer buying trends have increasingly been toward less pungent, milder onion varieties; U.S. consumers generally tend to prefer fruits and vegetables with low levels of bitterness and astringency (*36*). Consumers are also becoming increasingly concerned with maintaining good health, which will influence their purchasing patterns. Thus, it is of interest to further characterize intervarietal differences in phenolic composition and the associated health benefits of onions to enable consumers to make more informed choices as well as to facilitate breeding efforts toward those varieties with enhanced nutritional value.

The beneficial health-related effects of certain phenolics and flavonoids in onions are of importance both to consumers and to breeders. To our knowledge, there is limited literature on the study of the phenolic and flavonoid contents and associated antioxidant and antiproliferative activities of onions of different varieties from different growing locations. Our goal is to characterize the predominant onion varieties marketed in the United States. The objectives for this study were, for an array of onion varieties differing in color, pungency, and bitterness levels, to (1) determine the phenolic and flavonoid contents, (2) measure the total antioxidant activity, (3) determine the antiproliferative activity of onion extracts on human liver and colon cancer cells in vitro, and (4) determine correlations between antioxidant or antiproliferative activity and total phenolic or flavonoid content.

MATERIALS AND METHODS

Chemicals and Reagents. Sodium nitrite, (+)-catechin, Folin−Ciocalteu reagent (FCR), hydrochloric acid, glucagon, hydrocortisone, insulin, and α-keto-γ-methiolbutyric acid (KMBA) were purchased from Sigma Chemical Co. (St. Louis, MO). Aluminum chloride, sodium hydroxide, methanol, and acetone were purchased from Fisher Scientific (Pittsburgh, PA). Gallic acid was purchased from ICN Biomedical Inc. (Costa Mesa, CA). 2,2′-Azobis(amidinopropane) (ABAP) was purchased from Wako Chemicals (Richmond, VA). The HepG2 human liver cancer cells and Caco-2 human colon cancer cells were from the American Type Culture Collection (ATCC) (Rockville, MD).

Preparation of Onion Samples. Ten onion varieties and shallots were harvested at the ripe stage and stored at 4 °C in the laboratory. General descriptions of the onion varieties are given in **Table 1**. The edible onion bulb with stem and outer dry skin removed were selected for analysis; material was selected that was free from visible blemish or disease. All data collected for each onion variety were reported as means ± standard deviation (SD) for at least three replications.

Extraction of Onion Phenolic Compounds. Phenolics were extracted from fresh onions according to the method reported previously from our laboratory (*37*). Briefly, 100 g of onions was blended for 3 min in 200 g of 80% acetone using a Waring blender. The mixture was then homogenized in a Virtis High-Speed homogenizer for 3 min and filtered with a vacuum. Water in the filtrate was evaporated using

Table 1. Description of Shallot and Onion Varieties

variety	color	flavor	growing location
shallots	pink	strong, pungent	New York
Western Yellow	yellow	strong, pungent	Utah
New York Bold	yellow	strong, pungent	New York
Northern Red	red	strong, pungent	New York
Empire Sweet	yellow	mild	New York
Western White	white	strong, pungent	western U.S.
Peruvian Sweet	yellow	mild	Peru
Mexico	yellow	somewhat mild	Mexico
Texas 1015	yellow	mild	Mexico
Imperial Valley Sweet	yellow	mild	California
Vidalia	yellow	mild	Georgia

a rotary evaporator at 45 °C until the weight of the evaporated filtrate was <10% of the weight of the original filtrate. All extracts were stored at −40 °C until use. All extractions were performed in triplicate.

Measurement of Total Phenolic Content. The total phenolic content in onions was determined using the Folin−Ciocalteu colorimetric method (*38*), which was modified in our laboratory (*37*). Briefly, all sample extracts were diluted 1:5 with distilled water to obtain readings within the standard curve ranges of 0.0−600.0 μg of gallic acid/mL. The onion extracts were oxidized with FCR, and the reaction was neutralized with sodium carbonate. The absorbance was measured at 760 nm after 90 min at room temperature by an MRX II Dynex plate reader (Dynex Technologies, Inc., Chantilly, VA). The absorbance values were then compared with those of standards with known gallic acid concentrations. All values were stated as the mean (milligrams of gallic acid equivalents per 100 g of fresh sample) ± SD for three replications.

Measurement of Total Flavonoid Content. The total flavonoid content of the onion samples was determined using a modified colorimetric method (*37*, *39*). Briefly, 0.25 mL of diluted onion extracts was mixed with 1.25 mL of distilled water and, subsequently, with 0.075 mL of 5% sodium nitrite solution and allowed to react for 5 min. Then 0.15 mL of 10% aluminum chloride was added and allowed to further react for 6 min before 0.5 mL of 1 M sodium hydroxide was added. Distilled water was added to bring the final volume of the mixture to 3 mL. The absorbance of the mixture was immediately measured at 510 nm wavelength against a prepared blank using an MRX II Dynex spectrophotometer. The flavonoid content was determined by a catechin standard curve and expressed as mean (milligrams of catechin equivalents per 100 g of fresh sample) ± SD for the triplicate extracts.

Determination of Total Antioxidant Capacity. The total antioxidant capacity of onion extracts was measured using a total oxyradical scavenging capacity (TOSC) assay (*40*) as modified in our laboratory (*37*). Briefly, antioxidant activity was quantified after 15, 30, 45, and 60 min for four different onion extract concentrations and a control. The amount of ethylene generated by the reaction was expressed as peak area. The TOSC value corresponding to each extract concentration was calculated by integrating the area under the kinetic curve and assessed as the following equation: $TOSC = 100 - (\int SA/\int CA) \times 100$, where, $\int SA$ is the integrated area from the sample reaction and $\int CA$ is the integrated area from the control reaction. The median effective dose (EC_{50}) was determined for each onion variety from the dose−response curve of onion concentration versus TOSC value. The TOSC value is expressed as micromoles of vitamin C equivalents per gram of sample. All values were presented as the mean ± SD at least three replicates.

Measurement of Inhibition of $HepG_2$ and Caco-2 Cell Proliferation by Onion Extracts. The antiproliferative activity of different onion variety extracts was assessed by measurement of the inhibition of $HepG_2$ and Caco-2 human cancer cell proliferation. Antiproliferative activities were determined according to the colorimetric MTS assay (MTS-based cell titer 96 nonradioactivity cell proliferation assay) (Promega, Madison, WI) reported previously (*41*). $HepG_2$ cells were cultured in Williams' medium E (WME), containing 10 mM Hepes, 5 μg/mL insulin, 0.05 μg/mL hydrocortisone, 2 μg/mL glucagon, and 5% fetal bovine serum (Gibco, Life Technologies, Grand Island, NY), 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin. Caco-2 human colon cancer cells were maintained in DMEM, containing 10 mM Hepes, 5% FBS, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin. Both $HepG_2$ and Caco-2 cells were maintained in a 5% CO_2/37 °C incubator. A total of 2.5×10^4 $HepG_2$ or Caco-2 cells in growth media were placed in each well of a 96-well flat-bottom plate. Cell proliferation was measured by the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium (MTS) to formazan. After 4 h of incubation, the growth medium was removed and media containing various concentrations (10, 25, 50, 75, 100, 125, and 150 mg/mL) of onion extracts were added to the cells. Control cultures received the extraction solution minus the onion extracts, and blank wells contained 100 μL of growth medium without cells. Cell proliferation (percent) was determined at 96 h from the MTS absorbance (490 nm) reading for each concentration compared to the control, using at least three replications for each sample. The effective median dose (EC_{50}) was determined and expressed as milligrams of onion component per milliliter ± SD.

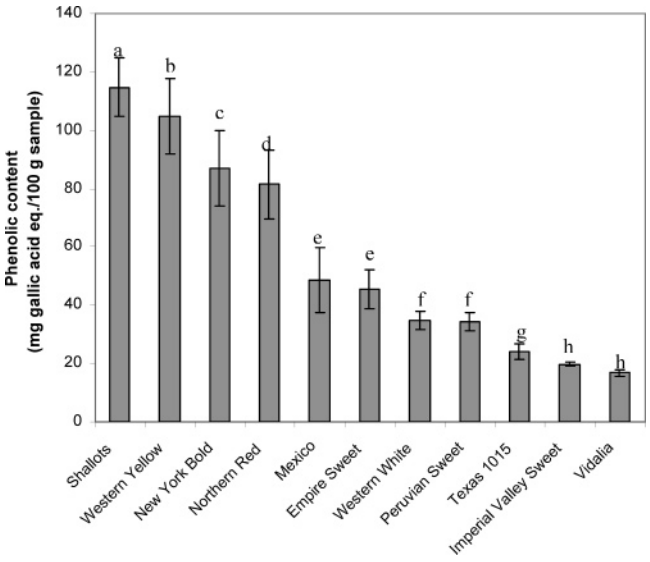

Figure 1. Total phenolic content of 10 onion varieties and shallots (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

Statistical Analysis. Statistical analysis was performed using Minitab Student release 12 (Minitab Inc., State College, PA) and SigmaStat version 8.0 (Jandel Corp., San Raphael, CA). Results were subjected to ANOVA, and differences between means were located using Tukey's multiple-comparison test. Correlations between various parameters were also investigated. Significance was determined at $p < 0.05$. All data were reported as the mean ± SD of three replications.

RESULTS

Phenolic Content of Onion Varieties. The total phenolic contents of the 10 onion varieties and shallots are shown in **Figure 1**. Shallots were found to have the highest phenolic content ($p < 0.05$) at 114.7 ± 10.0 mg of gallic acid equiv/100 g of sample, followed by Western Yellow (104.9 ± 13.0), New York Bold (86.9 ± 13.0), Northern Red (81.5 ± 11.8), Mexico (48.5 ± 11.2), Empire Sweet (45.5 ± 6.7), Western White (34.7 ± 3.2), Peruvian Sweet (34.3 ± 3.0), Texas 1015 (24.0 ± 2.7), Imperial Valley Sweet (19.8 ± 0.6), and Vidalia (16.8 ± 1.2). Significant differences were found in total phenolic content in comparisons between shallots, Western Yellow, New York Bold, Empire Sweet, Western White, and Texas 1015 varieties ($p < 0.05$); however, significant differences in total phenolic content were not found between Mexico and Empire Sweet, Western White and Peruvian Sweet, and Vidalia and Imperial Valley

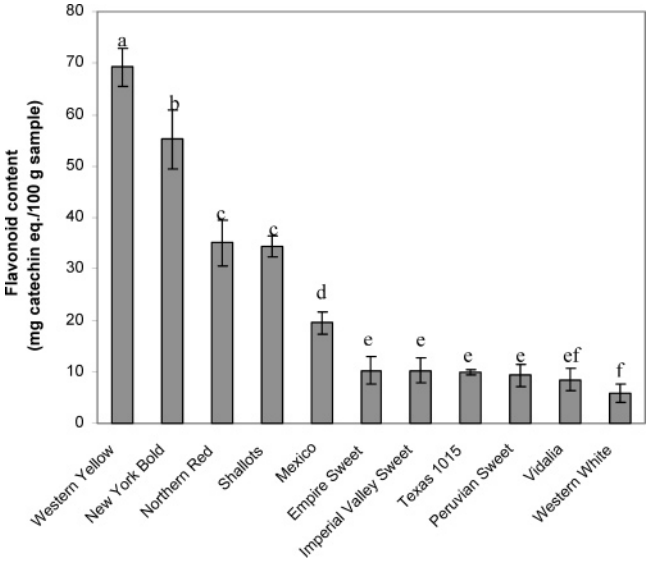

Figure 2. Total flavonoid content of 10 onion varieties and shallots (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

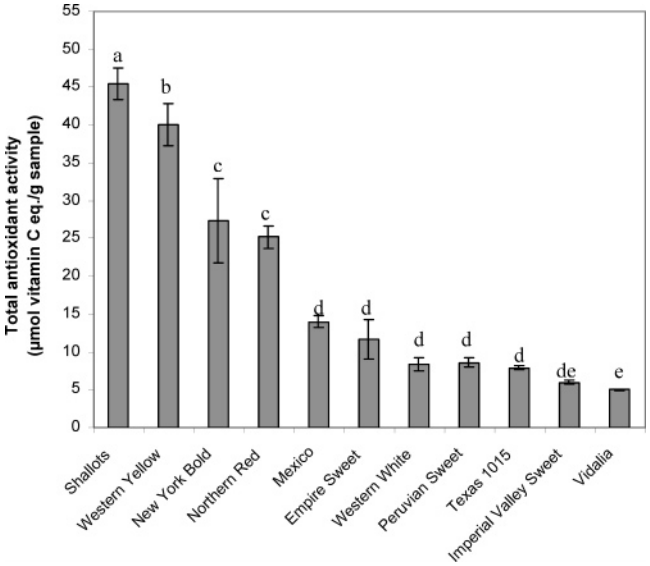

Figure 3. Total antioxidant activity of phytochemical extracts of 10 onion varieties and shallots (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

Sweet varieties ($p > 0.05$). There was a 6-fold difference in phenolic content between the highest and lowest ranked varieties, shallots and Vidalia ($p < 0.05$).

The total flavonoid contents of the 10 onion varieties and shallots are shown in **Figure 2**. Western Yellow variety had the highest flavonoid contents (69.2 ± 3.7 mg of catechin equiv/100 g of sample, $p < 0.05$), followed by New York Bold (55.2 ± 5.8), Northern Red (35.1 ± 4.5), shallots (34.4 ± 2.1), Mexico (19.5 ± 2.1), Empire Sweet (10.3 ± 2.6), Imperial Valley Sweet (10.3 ± 2.4), Texas 1015 (10.0 ± 0.5), Peruvian Sweet (9.3 ± 2.1), Vidalia (8.5 ± 2.1), and Western White (5.8 ± 1.8). The flavonoid contents of Western Yellow, New York Bold, Northern Red, Mexico, Texas 1015, Empire Sweet, and Peruvian Sweet were significantly different from each other ($p < 0.05$). However, significant differences in the total flavonoid content were not found in comparisons between Northern Red and shallots or among Texas 1015, Imperial Valley Sweet, Empire Sweet, Vidalia, and Peruvian Sweet varieties ($p > 0.05$). An 11-fold difference in flavonoid content was found between the highest and lowest ranked varieties, Western Yellow and Western White ($p < 0.05$), respectively.

Total Antioxidant Activity. The total antioxidant activities of the 10 onion varieties and shallots, expressed as micromoles of vitamin C equivalents per gram of sample, are summarized in **Figure 3**. Shallots had the greatest antioxidant activity (45.5 ± 2.1 μmol/g, $p < 0.05$), followed by Western Yellow (40.1 ± 2.8), New York Bold (27.3 ± 5.6), Northern Red (25.2 ± 1.5), Mexico (14.0 ± 0.8), Empire Sweet (11.7 ± 2.6), Western White (8.4 ± 0.9), Peruvian Sweet (8.6 ± 0.6), Texas 1015 (7.9 ± 0.3), Imperial Valley Sweet (6.0 ± 0.2), and Vidalia (5.0 ± 0.1). A statistically significant difference ($p < 0.05$) was found between shallots, Western Yellow, and New York Bold, between Western Yellow and Mexico, and between Mexico and Vidalia. No significant difference ($p > 0.05$) in antioxidant activity was found between New York Bold and Northern Red. Similarly, no significant difference ($p > 0.05$) was found among Western White, Peruvian Sweet, Texas 1015, Imperial Valley Sweet, and Vidalia.

Inhibition of Human Cancer Cell Proliferation. The antiproliferative activities of 10 onion varieties and shallots toward the growth of $HepG_2$ human liver and Caco-2 human colon cancer cells in vitro are shown, respectively, in **Figures 4** and **5**. Among the 10 onion varieties and shallots, shallots, Northern Red, Western Yellow, and New York Bold showed relatively high antiproliferative activities toward both $HepG_2$ and Caco-2 cells in a dose-dependent manner. The antiproliferative activities of onion varieties are expressed as the median effective dose (EC_{50}), with a lower EC_{50} value signifying a higher antiproliferative activity (**Figure 6**). The phytochemical extracts of Western Yellow (61.9 ± 2.1 mg/mL), shallots (62.2 ± 1.0), and New York Bold (63.8 ± 1.5) had the highest antiproliferative activities toward $HepG_2$ cells with the lowest EC_{50} values ($p < 0.05$), followed by Northern Red (74.5 ± 2.9) and Mexico (113.8 ± 4.6). The phytochemical extracts of Peruvian Sweet and Western White exhibited a weak antipro-

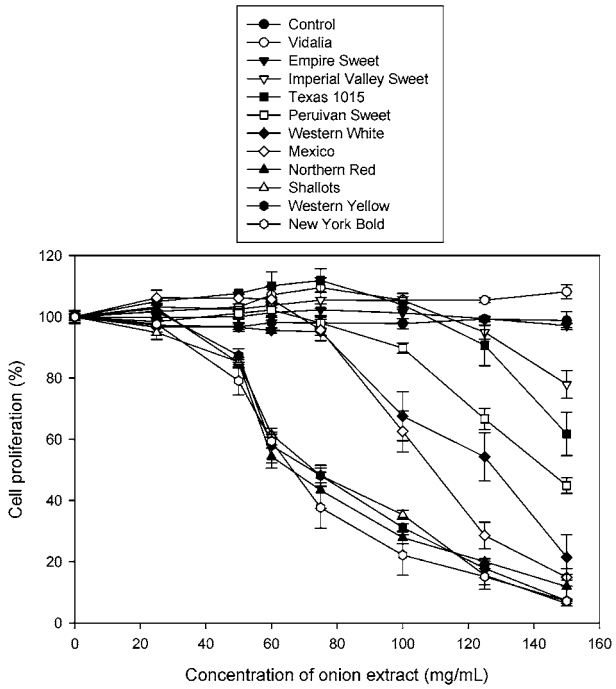

Figure 4. Percent inhibition of $HepG_2$ cell proliferation by extracts of 10 onion varieties and shallots (mean ± SD, $n = 3$).

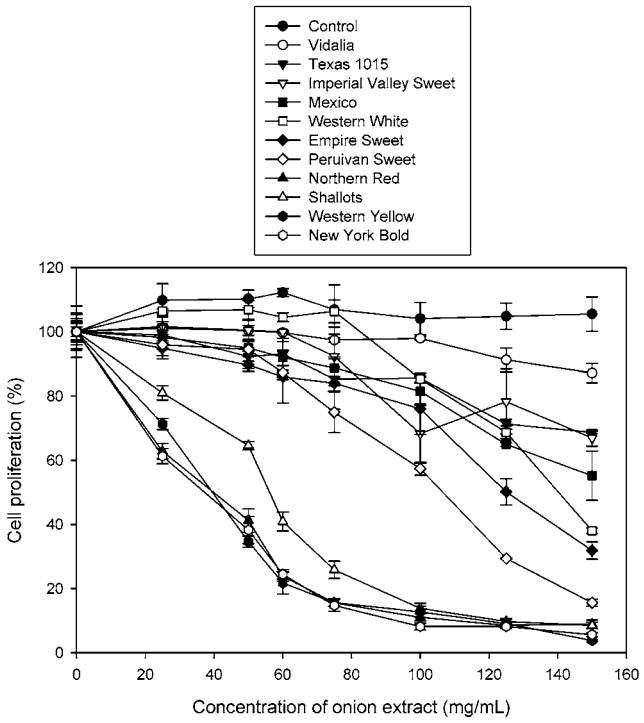

Figure 5. Percent inhibition of Caco-2 cell proliferation by the extracts of 10 onion varieties and shallots (mean ± SD, $n = 3$).

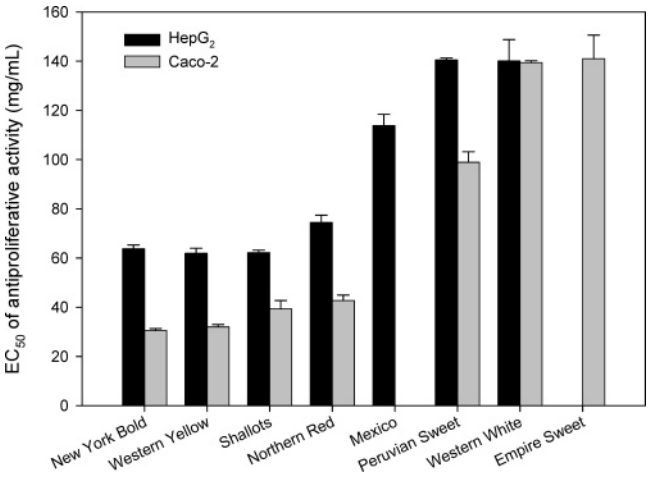

Figure 6. EC_{50} values of antiproliferative activity of phytochemical extracts of seven onion varieties and shallots (mean ± SD, $n = 3$).

liferative activity toward $HepG_2$ cells at higher doses with EC_{50} values of 140.5 ± 0.8 and 140.1 ± 8.7 mg/mL, respectively. The EC_{50} values toward $HepG_2$ cells of the Texas 1015, Imperial Valley Sweet, Empire Sweet, and Vidalia varieties could not be determined at the doses of onion extracts used in this experiment.

The antiproliferative activities of onion extracts toward Caco-2 human colon cancer cells were slightly different from those toward $HepG_2$ cells. The phytochemical extracts of New York Bold and Western Yellow exhibited the highest antiproliferative effect ($p < 0.05$) toward Caco-2 cells with the lowest EC_{50} values (30.5 ± 0.8 and 32.0 ± 1.0 mg/mL, respectively), followed by shallots (39.3 ± 3.4 mg/mL) and Northern Red (42.7 ± 2.2 mg/mL). The phytochemical extracts of Peruvian Sweet, Western White, and Empire Sweet showed weak antiproliferative activities at higher doses with EC_{50} values of 98.9 ± 4.3, 139.4 ± 0.8, and 141.0 ± 9.6 mg/mL, respectively (**Figure 6**). However, the EC_{50} values of the Mexico, Texas 1015, Imperial Valley Sweet, and Vidalia varieties could not be determined at the doses of onion extracts used in this experiment.

Table 2. Correlation Analysis of Phytochemical Content, Total Antioxidant Activity, and Antiproliferative Activity

	total antioxidant act.[a]	antiproliferative act.	
		$HepG_2$ cells	Caco-2 cells
phenolics	0.9668*[b]	0.9255*	0.7873*
flavonoids	0.7033*	0.7818*	0.7650*
total antioxidant act.		0.8301*	0.7059*

[a] Correlation coefficient R^2. [b] *, Significantly different, $p < 0.05$.

Relationship among Phytochemical Content, Total Antioxidant Activity, and Antiproliferative Activity. The correlations between phytochemical content and total antioxidant and antiproliferative activities are summarized in **Table 2**. A significant linear relationship was found between total phenolic content and total antioxidant activity ($R^2 = 0.9668$, $p < 0.05$) and between flavonoid content and total antioxidant activity ($R^2 = 0.7033$, $p < 0.05$) in the phytochemical extracts from different onion varieties. The positive correlation indicates that the higher total phenolic/flavonoid contents resulted in a higher total antioxidant activity. The relationship between total phenolic/flavonoid contents and median effective dose (EC_{50}) toward the inhibition of $HepG_2$ and Caco-2 cell proliferation was examined. There was a strong correlation between inhibition of $HepG_2$ cell proliferation and phenolic content ($R^2 = 0.9255$, $p < 0.05$) and between inhibition of $HepG_2$ cell proliferation and total antioxidant activity ($R^2 = 0.8301$, $p < 0.05$). Weak correlations were observed between the inhibition of Caco-2 cell proliferation and phenolic content ($R^2 = 0.7837$, $p < 0.05$), flavonoid content ($R^2 = 0.7650$, $p < 0.05$), and total antioxidant activity ($R^2 = 0.7059$, $p < 0.05$). Similarly, a weak correlation existed between the inhibition of $HepG_2$ cell proliferation and flavonoid content ($R^2 = 0.7818$, $p < 0.05$).

DISCUSSION

Our work has clearly shown that the phytochemicals in onions have potent antioxidant and antiproliferative activities and that antioxidant activity in onions is positively correlated with total phenolic and flavonoid contents. In assessing 10 onion varieties and shallots for the total phenolic and flavonoid contents, it was found that broad variability exists. A 6-fold difference was found in phenolic content between shallots and Vidalia varieties, and an 11-fold difference in flavonoid content was measured between Western Yellow and Western White varieties. Those differences could be due to genetic differencs and/or to growing location, climate, maturity, and harvest season variation.

It is well-known that both genetic and agronomic or environmental factors play important roles in the phenolic composition and thus nutritional quality of crops (*42*). Three of four varieties exhibiting the greatest total phenolic content and total flavonoid content were grown in New York (New York Bold, Northern Red, shallots); Western Yellow was grown in Utah. Shallots, the two strongest flavored yellow varieties (Western Yellow and New York Bold), and the red variety (Northern Red) were all found to be highest in total flavonoid and total phenolic contents. These four varieties also exhibited the greatest antioxidative and antiproliferative activities. Although we would expect differences in flavonoid composition to distinctly differ among pink, red, and yellow varieties, the total levels were similar; specific phenolic or flavonoid composition most likely

is significantly different among these varieties. Additionally, it is possible that other phenolic compounds or phytochemicals characteristic of the color or flavor of each variety were present, which may affect health-benefiting properties.

A positive correlation between phenolics and total antioxidant activity has been previously demonstrated for a variety of fruits and vegetables both in our laboratory and by others (*41*, *43*−*48*). A study by our group has shown that vitamin C contributes only 2.6% to the total antioxidant activity in onions; phytochemicals such as phenolic compounds were largely responsible for the remaining activity (*44*). This experiment suggests that phenolics and flavonoids in onions comprise a generous portion of the total antioxidant activity ($R^2 = 0.9668$, $p < 0.05$; and $R^2 = 0.7033$, $p < 0.05$, respectively).

The phytochemicals present in the 10 onion varieties and shallots also contributed to their antiproliferative activity. The inhibition of cell proliferation was observed in a dose-dependent manner after exposure to the extracts of Western Yellow, shallots, New York Bold, and Northern Red varieties; these varieties demonstrated greater antiproliferative activity than the Western White, Empire Sweet, Peruvian Sweet, Mexico, Texas 1015, Imperial Valley Sweet, and Vidalia varieties. The antiproliferative activities of onion varieties differed for the $HepG_2$ and Caco-2 cell lines. Overall, the 10 onion varieties and shallots possessed a greater ability to inhibit Caco-2 colon cancer cell proliferation than $HepG_2$ liver cancer cell proliferation. We may hypothesize that onion phytochemicals target different molecules at the various stages in the progression of cell mutagenicity and carcinogenicity in distinct cell types; differences in phytochemical composition, other than what was characterized in our study, may also have an impact on antiproliferative effects between cell types. This discrepancy in vitro suggests that onions generally may have a greater ability to prevent tumor growth in the colon than in the liver in vivo. However, there are many additional important factors, such as bioavailability, that will influence antiproliferative activity in vivo. Quercetin glucosides comprise the majority of the phytochemicals in onions; these compounds may not reach the colon in amounts necessary to exhibit antiproliferative activity because 52% of the quercetin glucosides in onions was found to be absorbed by the small intestine (*49*).

Total phenolic and flavonoid contents and antioxidant and antiproliferative activities exhibited significant variation among the 10 onion varieties and shallots in this experiment. Western Yellow, shallots, New York Bold, and Northern Red consistently had high levels of antioxidant and antiproliferative activities. Previous studies have shown that pungent yellow and red onions naturally may have higher levels of antioxidant activity than milder onions, such as Empire Sweet, Peruvian Sweet, and Vidalia, as well as Western White varieties studied in this experiment (*15*). The genetic makeup and color of the onion varieties need to be factored in when differences in antioxidant activity are considered. Our study also shows that Northern Red and New York Bold onion varieties, which have high sugar contents and strong, pungent, and bitter flavors, exhibited higher antioxidant and antiproliferative activities. Vidalia, perhaps the mildest and lowest solids content variety, was found to exhibit the lowest antioxidant and antiproliferative activities and the lowest levels of flavonoids and total phenolics. It would thus be of interest to further study the relationship between antioxidant and antiproliferative activities and the sensory properties of different onion varieties, including more varieties commonly distributed in different parts of the United States. Information regarding the varying health properties and flavor profiles of different onion varieties is valuable in such a profitable retail market because it could affect consumer spending and commercial use of onions. Our work can provide consumers with increased awareness of the variability in the health benefits of different available onion varieties.

Knowledge of specific differences in the phenolic and flavonoid profiles among onion varieties may be additionally of potential value to breeders in selecting for onions with specific anticipated health benefits. Further work is needed to specially characterize synergistic and individual effects of phytochemical compounds.

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