JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effect of Germination on Phytochemical Profiles and Antioxidant Activity of Mung Bean Sprouts (*Vigna radiata*)

Xinbo Guo,[†,§] Tong Li,[†] Kexuan Tang,[§] and Rui Hai Liu*[,†]

[†]Department of Food Science and Institute of Comparative and Environmental Toxicology, Stocking Hall, Cornell University, Ithaca, New York 14853, United States

[§]State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan-SJTU-Nottingham Plant Biotechnology R & D Center, Morgan-Tan-International Center for Life Sciences, Fudan University, Shanghai 200433, China

ABSTRACT: Epidemiological studies have shown that regular consumption of fruits and vegetables is associated with reduced risk of chronic diseases. It is recommended to increase consumption of fruits and vegetables to prevent chronic diseases related to free radical-induced oxidative stress. Different varieties of fruits and vegetables provide different vitamins, phenolics, flavonoids, minerals, and dietary fibers for optimal health benefits. Mung bean sprouts are one of the major vegetables in human diet. However, the profiles of phytochemicals and effect of germination on phytochemical content and antioxidant activity of mung bean sprouts have not been studied. The objective of this study was to determine the effect of germination on phytochemical profiles and antioxidant activity of mung bean sprouts. Germination of mung beans dramatically increased vitamin C content in mung bean sprouts in a time-dependent manner and reached the peak on day 8 of germination up to 285 mg/100 g DW, almost 24 times higher than the initial concentration in mung bean seeds ($p < 0.05$). On fresh weight basis, one serving of mung bean sprouts (about 104 g) provides 21.6 mg of vitamin C, which could meet 36% of Daily Value (DV). In addition, the germination dramatically increased total phenolic compounds and total flavonoids in mung bean sprouts in a time-dependent manner, up to 4.5 and 6.8 times higher than the original concentration of mung bean seeds, respectively. Quercetin-3-*O*-glucoside content was significantly increased in mung bean sprouts after germination. The total antioxidant activity of mung bean sprouts was increased by 6 times higher than that of mung bean seeds. Therefore, the germination of mung bean sprouts significantly increased phytochemical content, vitamin C content, and antioxidant activity.

KEYWORDS: *mung bean sprouts, phenolics, flavonoids, quercetin-3-O-glucoside, vitamin C, antioxidant activity, vegetables*

INTRODUCTION

Regular consumption of fruits and vegetables has been strongly associated with reduced risk of developing chronic diseases, such as cardiovascular disease (CVD), cancer, diabetes, Alzheimer disease, cataract, and age-related functional decline.[1] Phytochemicals, the secondary metabolites of plants, have been proposed to be responsible for the health benefits of fruits and vegetables.[2] They exhibit a wide range of biological effects beyond antioxidant activity.[2] Phytochemicals have been shown to have beneficial effects in inhibiting LDL oxidation, regulating lipid profiles and blood pressure, and lowering C-reaction protein.[3] All these effects are responsible for the CVD prevention of fruits and vegetables. Phenolics and flavonoids have been demonstrated to have potent anticancer activity through the different mechanisms of action including antioxidant activity, antiproliferative activity, regulation of tumor suppressor gene/oncogene expression through the signal transduction pathways, inhibition of NF-κB gene expression regulating related COX-2 activity and PGE2 synthesis, induction of apoptosis, and antiangiogenesis.[4−6] Vitamin C is known for its health benefits in regulation of collagen synthesis and antioxidant activity.[7] Fruits and vegetables are considered as good sources of vitamin C.

The 2010 Dietary Guidelines for Americans[8] recommend most people should eat at least nine servings of fruits and vegetables a day, four servings of fruits and five servings of vegetables, based on a 2000 kcal diet. Plant-based foods, such as fruits, vegetables, whole grains, and legumes, which contain significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition to reduce the risk of developing chronic diseases.[2]

Mung bean (*Vigna radiata*) sprouts are one of the common vegetables consumed in the world. Previous research suggested that beneficial phytochemicals (mainly glucosinolate of sulforaphane) of broccoli sprouts and cauliflower sprouts were significantly increased after the germination,[9] which provided potent anticancer activity. However, there is no report on phytochemical profiles and antioxidant activity on germination of mung beans. Our hypothesis was that the germination of mung bean might significantly increase contents of vitamin C and phenolics, and total antioxidant activity in mung bean sprouts. Therefore, the objective of this study was to determine the effects of germination on the phytochemical profiles and antioxidant activity of mung bean sprouts.

MATERIALS AND METHODS

Chemicals and Materials. Ascorbic acid (ASA), 2,6-dichloroindophenol sodium salt hydrate, potassium hydroxide (KOH), sodium borohydride ($NaBH_4$), aluminum chloride, chloranil, tetrahydrofuran (THF), quercetin dihydrate, catechin hydrate, vanillin, Folin-

Received: June 10, 2012
Accepted: October 22, 2012
Published: October 22, 2012

Ciocalteu reagent, metaphosphoric acid, and dichlorofluorescin diacetate (DCFH-DA) were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4), methanol, alcohol, acetone, acetic acid, hydrochloric acid (HCl), and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from J.T. Baker (Phillipsburg, NJ). Sodium bicarbonate ($NaHCO_3$) was 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). All reagents used were of analytical grade. Mung bean seeds (*V. radiata*) were obtained from Johnnys Seed Company (Winslow, ME).

Germination Condition. Mung bean seeds were cleaned with running water, and then were soaked in water for 1 h at room temperature (20 °C). After removed excess water, seeds were placed into a sprouter (60 × 150 mm), 20 g/cup, and kept in the dark at room temperature for germination. The sprouts were rinsed with running water every 12 h and the germination period was 9 days.

Moisture Content of Mung Bean Sprouts. The moisture content was determined by using the oven-dry method as described previously in our lab.[10] Briefly, 2 g of mung bean sprouts was dried in an oven at 102 °C to a constant weight. The measurements were expressed as percent of dry weight in triplicate. Data were reported as mean ± SD.

Determination of Vitamin C. Vitamin C of mung bean sprouts was measured using 2,6-dichloroindophenol (DIP) titrimetric method, as reported previously and modified in our laboratory.[11] Briefly, 2 g of mung bean sprouts was blended in a Waring blender using 30 mL of chilled 2% (w/v) metaphosphoric acid buffer for 5 min. Samples were then homogenized with a Polytron homogenizer for 3 min. The homogenates were centrifuged at 1400*g* for 5 min. The extract solutions were filtered (Whatman no. 1) for titration. A total of 1 mL of ascorbic acid standard solution (1 mg/mL) was added to 50 mL conical beaker, and then mixed with 10 mL of 2% (w/v) metaphosphoric acid buffer. Ascorbic acid was titrated with 2, 6-dichloroindophenol solution until the color turned pink for 15 s. The measurement was expressed as milligram of vitamin C per 100 grams of sample. Data were reported as mean ± SD for at least three replicates.

Phytochemical Extractions of Mung Bean Sprouts. Phytochemicals of mung bean sprouts were extracted using the method as reported previously by our lab.[12,13] Briefly, for the extraction of soluble-free phytochemical, 2 g of fresh samples were blended in a Waring blender using 30 mL of chilled 80% acetone (1:2, w/v) for 5 min. Samples were then homogenized with a Polytron homogenizer for 3 min. The homogenates were then centrifuged at 1400*g* for 5 min. Supernatants were collected, and then another 20 mL of chilled 80% acetone was added to the residues, and the extraction was repeated three times. All the supernatants were pooled and evaporated using a rotary evaporator under vacuum at 45 °C until 10% of the supernatants had been retained. The free phytochemical extracts were brought to 10 mL in water and were kept at −40 °C until analysis. Bound phytochemicals of vegetables and fruits were extracted using the method reported previously and modified in our laboratory.[13] For the extractions of bound phytochemical, the residues from above soluble free extraction were flushed with nitrogen gas and hydrolyzed directly with 20 mL of 4 N NaOH at room temperature for 1 h with shaking. The mixture was acidified to pH 2 with concentrated hydrochloric acid, centrifuged at 1400*g* for 5 min, and extracted six times with ethyl acetate. The ethyl acetate fractions were evaporated at 45 °C under vacuum to dryness, and were reconstituted in 10 mL of water and stored at −40 °C until analysis.

Determination of Total Phenolics. The total phenolic contents of mung bean sprouts were determined by the Folin-Ciocalteu colorimetric method,[14] and modified in our laboratory.[15] All extracts were diluted with Milli-Q water in order to obtain readings that fall within the standard curve concentration range of 0.0−600.0 μg gallic acid/mL. For each analysis, 100 μL of the standard gallic acid solution or extracts was added to 0.4 mL of Milli-Q water in a test tube. Folin-Ciocalteu reagent (0.1 mL) was added to the solution and allowed to react for 6 min to ensure that the Folin-Ciocalteu reagent reacted completely with the oxidizable phenolates in the sample. Then, 1 mL of 7% sodium carbonate solution was added to raise the pH, and 0.8 mL of deionized water was added into the test tubes to adjust the final volume to 2.4 mL. The samples were mixed and allowed to stand for 90 min at room temperature. After the color was developed, 200 μL of each sample was added into a 96-well plate in duplicate and the absorbance was read at 760 nm using a MRX II Dynex spectrophotometer (Dynex Technologies, Inc., Chantilly, VA). Results were calculated based on the standard curve of gallic acid concentrations and expressed as milligram per 100 grams of dry weight for triplicate. Data were reported as mean ± SD.

Determination of Flavonoids. The total flavonoid contents were determined using the sodium borohydride/chloranil protocol (SBC) developed in our laboratory.[16,17] Briefly, 1 mL of phytochemical extracts of tested samples was added into test tubes (15 × 150 mm), placed under nitrogen gas to dryness, and reconstituted in 1 mL of terahydrofuran/ethanol (THF/EtOH, 1:1, v/v). Catechin hydrate standard (0.3−10.0 mM) was prepared fresh in 1 mL of THF/EtOH (1:1, v/v). Each test tube with sample or standard was added with 0.5 mL of 50 mM $NaBH_4$ solution and 0.5 mL of 74.6 mM $AlCl_3$ solution. Then, the test tubes were shaken in an orbital shaker at room temperature for 30 min. An additional 0.5 mL of 50.0 mM $NaBH_4$ solution was added into each test tube with shaking continued for another 30 min at room temperature. Chilled 2.0 mL of 0.8 M acetic acid solution was added into each test tube and kept in the dark for 15 min after being thoroughly mixed. Then, 1 mL 20.0 mM chloranil was added in each tube and heated at 95 °C with shaking for 60 min. The reaction solutions were cooled using tap water, and the volume was brought to 4 mL using methanol. Then, 1 mL of 16% (w/v) vanillin was added into each tube and mixed. Then, 2 mL of 12 M HCl was added into each tube and kept in the dark for 15 min after a thorough mix. The reaction solutions were centrifuged at 1400*g* for 5 min, 200 μL of each solution was added into a 96-well plate in duplicate, and the absorbance was measured at 490 nm using a MRX Microplate Reader with Revelation workstation (Dybex Technologies, Inc., Chantilly, VA). Results were calculated by using the standard curve of catechin hydrate concentration. Total flavonoid content was expressed as milligram per 100 grams of dry weight of sample. Data were reported as mean ± SD for at least triplicates.

Analyses of Quercetin, Myricetin, and Quercetin-3-*O*-glucoside by HPLC-UV. Quercetin, myricetin, and quercetin-3-*O*-glucoside were determined using the method of HPLC-UV reported previously by our laboratory.[18] Briefly, quercetin, myricetin, and quercetin-3-*O*-glucoside were determined by the RP-HPLC procedure with a Supelcosil LC-18-DB column (150 mm × 4.6 mm, and 3 μm pore size). A Waters 515 HPLC pump (Waters Corp., Milford, MA) and one Waters 2487 dual-wavelength absorbance detector set at 370 nm were used for all HPLC analyses. For analyses of quercetin, myricetin, quercetin-3-*O*-glucoside in mung bean sprouts extracts, the solvent system used was 40% (v/v) methanol in acidified water (pH 2; triflouroacetic acid). Injections (100 μL) were made for each sample. The recovery of quercetin-3-*O*-glucoside was 96.7 ± 1.5% from the phytochemical extracts. Quercetin and myricetin were not detected in mung bean sprouts extracts. Quercetin-3-*O*-glucoside contents were expressed as milligram per 100 grams of dry weight according to the standard curve of quercetin-3-*O*-glucoside concentration. Results obtained for sample extracts were expressed as mean ± SD for triplicates.

Determination of Total Antioxidant Activity. Total antioxidant activity was measured using the hydrophilic peroxyl radical scavenging capacity (Hydro-PSC) assay as reported previously.[19] Ascorbic acid and phytochemical extracts were diluted in appropriate concentration by 75 mM phosphate buffer (pH 7.4). Ascorbic acid was made fresh and diluted to 6.3, 4.8, 3.2, 2.4, and 1.0 μg/mL. Gallic acid was made fresh and diluted to 5.5, 3.5, 2.7, 1.4, and 0.9 μg/mL. The reaction mix contained 75 mM phosphate buffer at pH 7.4, 40 mM ABAP, 13.26 μM DCFH dye, and the appropriate concentrations of the pure antioxidant compound or sample extracts. The dye was prehydrolyzed with 1 mM KOH to remove the diacetate moiety just prior to use in

the reaction, and the reaction was carried out at 37 °C, in a total volume of 250 μL using a 96-well plate. Fluorescence generation was monitored (excitation at 485 nm and emission at 538 nm) with a Fluoroskan Ascent fluorescent spectrophotometer (Thermo Lab systems, Franklin, MA). Data were acquired with the Ascent Software, version 2.6 (Thermo Lab systems, Franklin, MA) running on a PC. The areas under the fluorescence reaction time kinetic curve (AUC) for both control and samples were integrated and used as the basis for calculating peroxyl radical scavenging capacity (PSC) using the equation PSC (value) = 1 − (SA/CA), where SA is AUC for the sample or standard dilution and CA is AUC for the control reaction. Compounds or extracts inhibiting the oxidation of DCFH produced smaller SA and higher PSC values. The parameter EC_{50} was defined as the dose required to cause a 50% inhibition (PSC unit = 0.5) for each pure compound or sample extract, and was used as the basis for comparing the antioxidant activities of different compounds or samples. Results obtained for antioxidant activities of sample extracts were expressed as micromoles of vitamin C equivalents per 100 g of sample ± SD for triplicate analyses.

Statistical Analysis. Statistical analyses were performed using Sigmaplot software 11.0 (Systat Software, Inc., Chicago, IL) and dose−effect analysis was performed using Calcusyn software version 2.0 (Biosoft, Cambridge, U.K.). The differences among the mung bean sprout samples were determined by two tailed *t* test with a *p*-value of 0.05. Significance of relationships was calculated by multivariate method. Results were subjected to ANOVA calculated using SPSS software 13.0 (LEAD Technologies, Inc.). All data were reported as mean ± SD for triplicate analyses.

RESULTS

Changes in Moisture Content during the Germination of Mung Beans. The moisture content of dry mung beans was 9.25 ± 0.09% (Figure 1). With the germination, the moisture

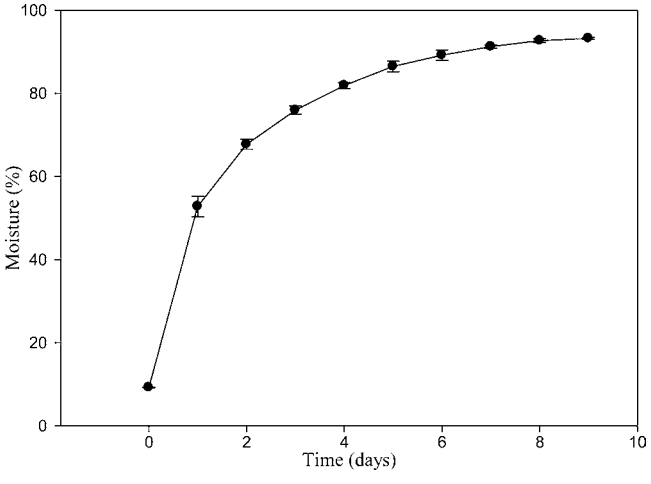

Figure 1. Changes in moisture content during the germination of mung bean sprouts (mean ± SD, n = 3).

content of mug bean sprouts was dramatically increased, up to 52.77 ± 2.47% after 24 h of germination. Then, the moisture content was continuously increased and reached the plateau on day 6 (89.22 ± 1.27%) and kept the similar levels from day 6 to day 9 (93.24 ± 0.23%; Figure 1).

Changes of Vitamin C Content in Mung Bean Sprouts. Vitamin C content of mung bean seeds was 11.69 ± 0.38 mg/100 g DW (Figure 2). The germination led to a significant increase (p < 0.05) in vitamin C content after 2 days of germination (Figure 2). The vitamin C contents were increased in a time-dependent manner and reached the peak on day 8 at the concentration of 285 ± 25.7 mg/100 g DW, almost 24

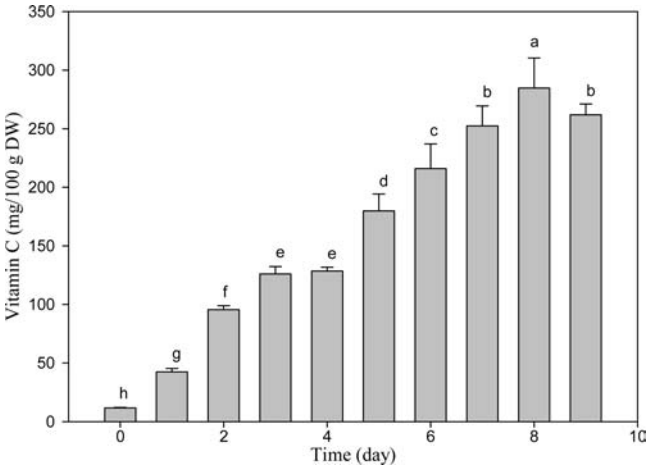

Figure 2. Time kinetics of vitamin C content expressed as dry weight during the germination of mung beans (mean ± SD, n = 3). Bars with no letters in common are significantly different (P < 0.05).

times higher than the initial concentration in mung bean seeds (Figure 2).

Phenolic Content in Mung Bean Sprouts. Total phenolic contents of mung bean seeds and mung bean sprouts are presented in Figure 3. Total phenolic content of mung bean

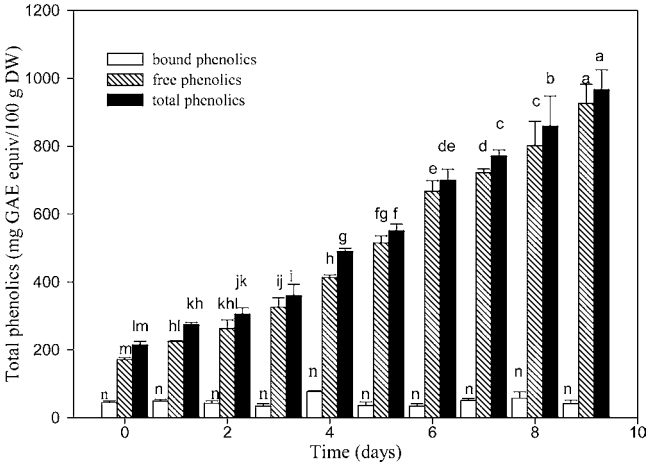

Figure 3. Phenolic content of mung bean and mung bean sprouts (mean ± SD, n = 3). Bars with no letters in common are significantly different (P < 0.05).

seeds was 214.7 ± 10.7 mg GA equiv/100 g DW. The free phenolic content of mung bean seeds was 170.8 ± 5.7 mg GA equiv/100 g DW, up to 80% of total phenolics, while the bound phenolic content was 43.91 ± 5.23 mg GA equiv/100 g DW, accounted for 20% of total phenolics (Figure 3). Total phenolic contents were significantly (p < 0.05) increased in a time-dependent manner after the germination and were up to 966.4 ± 58.3 mg GA equiv/100 g DW on day 9, which were 4.5 times higher than the original concentration of mung bean seeds. Free phenolic contents of mung bean were dramatically increased (p < 0.05) with the germination and were 925.4 ± 56.7 mg GA equiv/100 g DW on day 9 after the germination, which were 5.4 times higher than the initial concentration of free phenolics in mung bean seeds. Free phenolics accounted for 96% of total phenolics on day 9 of the germination (Figure 3). There was no significantly changes (p > 0.05) in bound

phenolic contents of mung bean sprouts. The bound phenolics on day 1 and day 9 were 48.26 ± 5.58 and 40.96 ± 10.7 mg GA equiv/100 g DW, respectively (Figure 3).

Flavonoid Content in Mung Bean Sprouts. The total flavonoid content of mung bean sprouts determined using the SBC assay is shown in Figure 4. Total flavonoid content of mung bean seeds was 195.3 ± 21.8 mg catechin equiv/100 g DW (Figure 4). Total flavonoid contents of mung bean sprouts were significantly increased ($p < 0.05$) with the germination in a time-dependent manner (Figure 4), and reached the peak on day 7 of the germination. The change patterns of total flavonoids during the germination are similar to these of total phenolics in mung bean sprouts. The flavonoid content reached the maximum concentrations on day 9, approximately 1319 ± 143 mg catechin equiv/100 g DW, which was 6.8 times higher than the initial concentration in mung bean seeds.

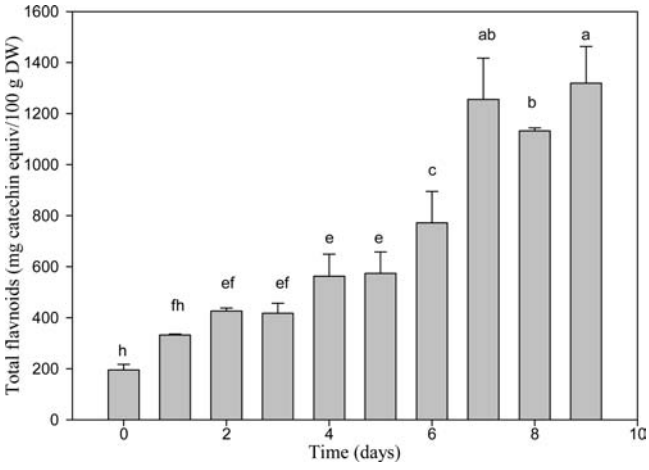

Figure 4. Total flavonoid content of mung bean and mung bean sprouts (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($P < 0.05$).

Quercetin-3-*O*-glucoside Content in Mung Bean Sprouts. The concentrations of represented flavonoids, quercetin, quercetin-3-*O*-glucoside, and myricetin, were analyzed by HPLC/UV and the results are shown in Figure 5. Quercetin-3-*O*-glucoside was detected in all samples of mung bean seeds and sprouts, but quercetin and myricetin were not detected. Quercetin-3-*O*-glucoside concentration in mung bean seeds was 0.49 ± 0.02 mg/100 g DW (Figure 5). There were no changes in quercetin-3-*O*-glucoside concentrations in the first 3 days of germination. Quercetin-3-*O*-glucoside concentrations were significantly increased in mung bean sprouts after 4 days of germination (on day 4: 5.95 ± 1.34 mg/100 g DW), and reached the peak on day 5 (10.98 ± 1.24 mg/100 g DW) and then kept stable from day 5 to day 9 (Figure 5).

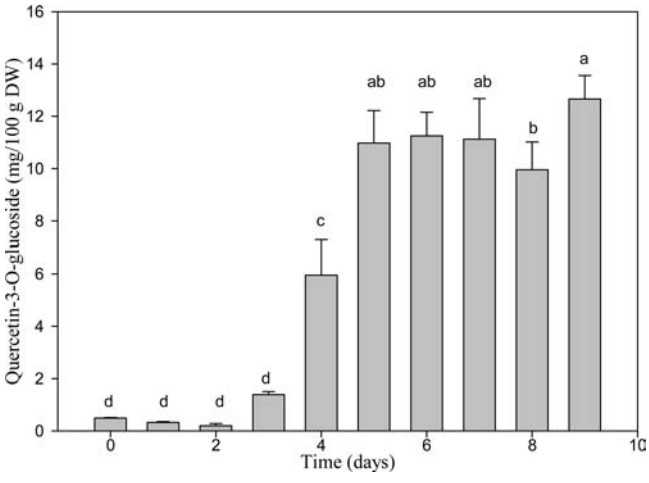

Figure 5. Quercetin-3-*O*-glucoside content of mung bean sprouts (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($P < 0.05$).

Total Antioxidant Activity in Mung Bean Sprouts Extracts. Total antioxidant activity measured by the PSC assay for mung bean seeds and mung bean sprouts are presented in Figure 6 with the PSC values expressed as micromoles of vitamin C equivalents per 100 grams of DW. Total antioxidant activity of mung bean seeds was 451.9 ± 66.7 μmol of vitamin C equiv/100 g DW. The total antioxidant activities of mung bean sprouts were significantly increased ($p < 0.05$) after the 2 days of germination. On day 4, PSC values were increased up to 2168 ± 228 μmol of vitamin C equiv/100 g DW, which was 5 times higher than the initial antioxidant activity of mung bean seeds (Figure 6), and kept at the similar level of antioxidant activity from day 4 to day 7. On day 8, total antioxidant activity was increased to reach the peak at 2657 ± 156 μmol of vitamin C equiv/100 g DW, which was 6 times higher than the initial antioxidant activity of mung bean seeds (Figure 6).

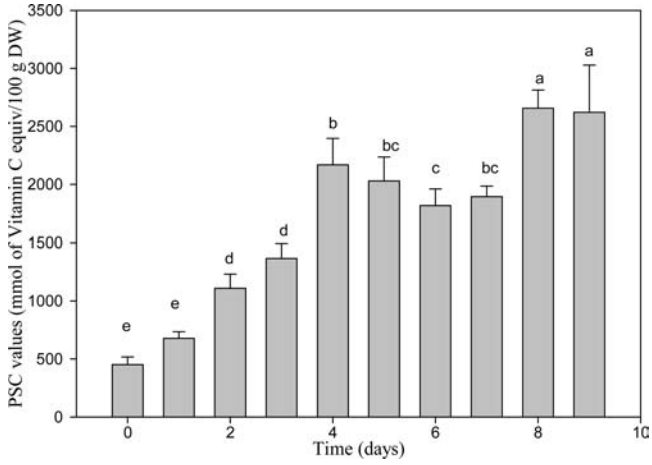

Figure 6. Total antioxidant activity of mung bean sprouts (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($P < 0.05$).

DISCUSSION

Epidemiological studies have been consistently demonstrated that a diet high in fruits and vegetables is associated with a decreased risk of developing chronic diseases, such as CVD, cancer, diabetes, obesity, Alzheimer disease, cataract, and age-related functional decline.[1] The 2010 Dietary Guidelines for Americans[8] recommend most people should consume 9−13 servings of fruits and vegetables per day for the optimal nutrition in the prevention of chronic diseases. Most Americans do not meet the recommendation of 2010 Dietary Guidelines. Therefore, the key is to increase servings and varieties of fruits and vegetables daily to obtain different bioactive compounds for the maximum health benefits. Mung bean sprouts are one of the common consumed vegetables and are rich in vitamins,

minerals, and phytochemicals, which may provide complementary nutrients and bioactive compounds together with other fruits and vegetables. Here we demonstrated the germination of mung bean sprouts significantly increased concentrations of vitamin C, phenolics, and flavonoids, and total antioxidant activity.

Vitamin C is an essential nutrient for collagen synthesis in human. Deficiency of vitamin C leads to scurvy disease. In addition, vitamin C is an excellent antioxidant, especially in a food system to help maintain the active state for many bioactive compounds, such as vitamin E, flavonoids and some phenolics. Vitamin C has been routinely used as a marker of nutritional quality, especially in fruits and vegetables, and their processed products. Fruits and vegetables are good sources for vitamin C. Here we reported the germination of mung bean sprouts significant increased vitamin C content. Vitamin C content of mung bean seeds was 11.69 mg/100 g DW, and the germination of mung bean sprouts significantly increased vitamin C content in a time-dependent manner and reached the peak on day 8 at the concentration of 285 mg/100 g DW, almost 24 times higher than the initial concentration in mung bean seeds. On fresh weight basis, one serving of mung bean sprouts (about 104 g) provides 21.6 mg of vitamin C, which could meet 36% of Daily Value (DV). This is very important for people to obtain sufficient amount of vitamin C in many areas of the world. Therefore, mung bean sprouts are excellent source of vitamin C in human diet.

Phenolics are the products of secondary metabolism in plants, which provide essential functions in the growth and reproduction of the plants.[20] Epidemiological and experimental studies demonstrated that phenolic compounds in human diet may provide health benefits associated with reduced risk of chronic diseases.[21] The beneficial effects associated with plant-based food consumption are in part due to the existence of phenolics. Phenolics are present in free and bound (cell wall-associated) forms in plants. Mung bean seeds contained 80% free phenolics and 20% bound phenolics (Figure 3). The germination changed the ratio of free and bound phenolics; 95% free phenolics and 5% bound phenolics, indicating phenolics more bioavailable in small intestine. In addition, the germination dramatically increased total phenolic compounds in mung bean seeds in a time-dependent manner, up to 966 mg GA equiv/100 g DW on day 9, which was 4.5 times higher than the original concentration of mung bean seeds. On fresh weight basis, one serving of mung bean sprouts (about 104 g) provides 68 mg of total phenolics. Compared with the data published previously on phenolic contents of common vegetables,[20,21] the concentration of total phenolics in mung bean sprouts after 8 days germination was similar to those of broccoli, brussels sprouts and spinach, which contained higher levels of phenolics among the 27 vegetables commonly consumed in the United States.

Flavonoids are a major group in the family of phenolic compounds with antioxidant and biological activity that have been identified in fruits, vegetables, and other plant foods. Flavonoids have been linked to reducing the risk of major chronic diseases. This study was the first to determine total flavonoid content in mung bean sprouts using a new method developed recently.[16] Determination of total flavonoids in foods is challenging. The most common methods for determining the content of total flavonoids include aluminum chloride ($AlCl_3$) colorimetric assay[5] and high performance liquid chromatography (HPLC). However, these two methods each have certain limitations. $AlCl_3$ colorimetric assay only measures partial flavonoids and therefore cannot be used accurately to determine total flavonoids.[16] HPLC is an excellent method to determine individual flavonoids, but cannot be used to determine the total flavonoids because the method is limited by the availability of flavonoid standards and many unidentified flavonoids are present in foods.[2] The SBC assay developed by our lab can detect all types of flavonoids, including flavones, flavonols, flavanones, flavanonols, isoflavonoids, flavanols, and anthocyanidins.[16] The total flavonoid content in mung bean seeds, measured by SBC assay, was 195 mg catechin equiv/100 g DW. The total flavonoid content mung bean sprouts on Day 9 was 1319 mg catechin equiv/100 g DW, which was 6.8 times higher than the initial concentration in mung bean seeds ($p < 0.05$). In dormant and geminating mung bean seeds, daidzein, genistein, and their 7-*O*-glucodides, daidzin and genistin, were reported using HPLC-immunoanalysis.[22]

Quercetin-3-*O*-glucoside is one of the common flavonoids in many fruits and vegetables related to human health benefits. Quercetin-3-*O*-glucoside was detected in all samples of mung bean seeds and sprouts. Quercetin-3-*O*-glucoside concentration in mung bean seeds was 0.49 mg/100 g DW, and was significantly increased in mung bean sprouts after 4 days of germination, and reached the peak on day 5 (10.98 mg/100 g DW), which was 22 times higher than that of mung bean seeds, and then kept stable from day 5 to day 9 (Figure 5). Therefore, the germination significantly increased quercetin-3-*O*-glucoside synthesis in mung bean sprouts and improved nutritional quality of mung beans.

Total antioxidant activity was determined using PSC assay and expressed as PSC values (μmol of vitamin C equiv/100 g DW; Figure 6). Total antioxidant activity of mung bean seeds was 452 μmol of vitamin C equiv/100 g DW. The germination significantly increased PSC values after the two days of germination. The PSC values was increased to 2168 on day 4 of germination, which was 5 times higher than the PSC values of mung bean seeds ($p < 0.05$). On day 8, the PSC values were increased to the maximum at 2657 μmol of vitamin C equiv/100 g DW, which was 6 times higher than the initial antioxidant activity of mung bean seeds.

Correlations among total phenolics, total flavonoids, vitamin C, quercetin-3-*O*-glucoside, and antioxidant activity were analyzed. Total phenolics of mung bean sprouts was highly correlated to the vitamin C contents ($R^2 = 0.967$, $p < 0.05$), total flavonoid contents ($R^2 = 0.961$, $p < 0.05$), antioxidant activities ($R^2 = 0.896$, $p < 0.05$), and quercetin-3-*O*-glucoside contents ($R^2 = 0.923$, $p < 0.05$). This is consistent with the results previous reported in the literature.[13] Total flavonoid contents of mung bean sprouts were also highly correlated to the contents of vitamin C ($R^2 = 0.937$, $p < 0.05$) and quercetin-3-*O*-glucoside contents ($R^2 = 0.84$, $p < 0.05$). The antioxidant activity of mung bean sprouts was highly correlated to the concentrations of vitamin C ($R^2 = 0.9$, $p < 0.05$), total flavonoids ($R^2 = 0.817$, $p < 0.05$), and quercetin-3-*O*-glucoside ($R^2 = 0.849$, $p < 0.05$).

In summary, the germination of mung bean sprouts dramatically increased vitamin C content, total phenolics, total flavonoids, and total antioxidant activity, when compared to those of mung bean seeds. Mung bean sprouts are excellent source of bioactive compounds with high antioxidant activity. Consumers are encouraged to increase consumption of fruits and vegetables in their diets for the optimum nutrition in the prevention of chronic diseases.

AUTHOR INFORMATION

Corresponding Author

*Address: Department of Food Science, Stocking Hall, Cornell University, Ithaca, NY 14853-7201. Telephone: (607) 255-6235; fax: (607) 254-4868; e-mail: RL23@cornell.edu].

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Liu, R. H. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.* **2003**, *78*, 517S−520S.

(2) Liu, R. H. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J. Nutr.* **2004**, *134*, 3479s−3485s.

(3) Liu, R. H.; Finley, J. Potential cell culture models for antioxidant research. *J. Agric. Food Chem.* **2005**, *53*, 4311−4314.

(4) Liu, R. H.; Liu, J. R.; Chen, B. Q. Apples prevent mammary tumors in rats. *J. Agric. Food Chem.* **2005**, *53*, 2341−2343.

(5) Sun, J.; Liu, R. H. Cranberry phytochemical extracts induce cell cycle arrest and apoptosis in human MCF-7 breast cancer cells. *Cancer Lett.* **2006**, *241*, 124−134.

(6) Yoon, H.; Liu, R. H. Effect of selected phytochemicals and apple extracts on NF-kappa B activation in human breast cancer MCF-7 cells. *J. Agric. Food Chem.* **2007**, *55*, 3167−3173.

(7) Carr, A. C.; Frei, B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am. J. Clin. Nutr.* **1999**, *69*, 1086−1087.

(8) USDA, Dietary Guidelines for Americans, 2010; www.dietaryguidelines.gov (2011).

(9) Fahey, J. W.; Zhang, Y.; Talalay, P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10367−10372.

(10) Wolfe, K. L.; Liu, R. H. Apple peels as a value-added food ingredient. *J. Agric. Food Chem.* **2003**, *51*, 1676−1683.

(11) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010−3014.

(12) Jiratanan, T.; Liu, R. H. Antioxidant activity of processed table beets (*Beta vulgaris* var. conditiva) and green beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **2004**, *52*, 2659−2670.

(13) Sun, J.; Chu, Y. F.; Wu, X.; Liu, R. H. Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem.* **2002**, *50*, 7449−7454.

(14) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152−178.

(15) Liu, R. H.; Sun, J. Antiproliferative activity of apples is not due to phenolic-induced hydrogen peroxide formation. *J. Agric. Food Chem.* **2003**, *51*, 1718−1723.

(16) He, X. J.; Liu, D.; Liu, R. H. Sodium borohydride/chloranil-based assay for quantifying total flavonoids. *J. Agric. Food Chem.* **2008**, *56*, 9337−9344.

(17) Zhang, M. W.; Zhang, R. F.; Zhang, F. X.; Liu, R. H. Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *J. Agric. Food Chem.* **2010**, *58*, 7580−7587.

(18) Boyer, J.; Brown, D.; Liu, R. H. Uptake of quercetin and quercetin 3-glucoside from whole onion and apple peel extracts by Caco-2 cell monolayers. *J. Agric. Food Chem.* **2004**, *52*, 7172−7179.

(19) Adom, K. K.; Liu, R. H. Rapid peroxyl radical scavenging capacity (PSC) assay for assessing both hydrophilic and lipophilic antioxidants. *J. Agric. Food Chem.* **2005**, *53*, 6572−6580.

(20) Song, W.; Derito, C. M.; Liu, M. K.; He, X. J.; Dong, M.; Liu, R. H. Cellular antioxidant activity of common vegetables. *J. Agric. Food Chem.* **2010**, *58*, 6621−6629.

(21) Boivin, D.; Lamy, S.; Lord-Dufour, S.; Jackson, J.; Beaulieu, E.; Cote, M.; Moghrabi, A.; Barrette, S.; Gingras, D.; Beliveau, R. Antiproliferative and antioxidant activities of common vegetables: a comparative study. *Food Chem.* **2009**, *112*, 374−380.

(22) Lapcik, O.; Hill, M.; Cerny, I.; Lachman, J.; Al-Maharik, N.; Adlercreutz, H.; Hampl, R. Immunoanalysis of isoflavonoids in *Pisum sativum* and *Vigna radiata*. *Plant Sci.* **1999**, *148*, 111−119.