JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Changes of Folate and Other Potential Health-Promoting Phytochemicals in Legume Seeds As Affected by Germination

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ABSTRACT: Folate deficiency associated with low dietary intake is a well-documented public health problem, resulting in serious health and socioeconomic burdens. Therefore, optimization of the germination process of different cultivars of legume seeds in relation to the content and composition of folate, vitamin C, and total phenolics and total antioxidant capacity was carried out to maximize the health-promoting properties. The content and composition of folate, vitamin C, and total phenolic and total antioxidant capacities varied between species, among cultivars, and with germination time. During germination, total folate content was maximum at 815.2 μg/100 g fresh weight in soybean sprout and at 675.4 μg/100 g fresh weight in mungbean sprout on the fourth day, which were equivalent to, respectively, 3.5- and 3.9-fold increases in the seed's content, and total folate content strongly decreased thereafter. 5-CH_3-H_4folate was the most abundant folate species in legume sprouts and reached a maximum on the fourth day. Vitamin C was not detected in raw seeds, and its content increased sharply in soybean and mungbean sprouts and reached a maximum at the fourth day of germination (29 and 27.7 mg/100 g fresh weight, respectively). Germination of soybean and mungbean for 4 days provided the largest amount of total folate as well as the more stable species 5-CH_3-H_4folate and also brought about large amounts of vitamin C and total phenolics and substantial antioxidant capacities.

KEYWORDS: *folate, phytochemicals, sprout, soybean, mungbean*

INTRODUCTION

Folate deficiency is a well-documented public health problem in the developing world, resulting in severe health and socioeconomic burdens.[1,2] Folate deficiency decreases DNA biosynthesis and thus affects cellular functions, growth, and development. There are a variety of disorders associated with folate deficiency, including neural tube defects (NTDs), such as spina bafida and anencephaly, megaloblastic anemia, occlusive vascular disease, colon cancer, Down's syndrome, and Alzheimer's disease.[3,4] Folate deficiency causes approximately 300 000 NTDs per year[5] and is responsible for 10% of adult deaths from heart disease.[6] Furthermore, folate deficiency is the main cause of anemia in at least 10 million pregnant women in the developing world.[7] Humans and animals cannot synthesize folates de novo and, therefore, depend completely on their dietary sources.[2] Thus, plant foods are the main source of folates in the human diet. Food folate levels vary among different crops species; the staples wheat, maize, and, especially, rice contain low amounts of folates to meet the recommended dietary allowances (RDAs) of 400 μg/day for adults and 600 μg/day for pregnant women.[8] Enrichment of food with folate is, therefore, an important global challenge and high priority in research.

Defeating folate deficiency in humans is a tremendous challenge. Several strategies have been proposed to fight folate deficiency. Folate biofortification, the enhancement of folates in crop through plant breeding or biotechnology, offers the most sustainable solution;[1] it is, however, a long-term process. Supplementation and food fortification strategies, however, are less feasible in developing countries due to economic or social reasons.[9] Moreover, concerns have arisen about human life-threatening diseases due to chronic exposure to synthetic folic acid from the fortified foods.[4] In contrast to synthetic folic acid fortification, a natural way to increase folate levels is germination of edible plant seeds as has been reported in recent years.[10,11] During germination, rapid cell division increases the demand for carbon one units for cell metabolism and for nucleotide biosynthesis; as a result, folate synthesis is accelerated in developing seedlings.[12,13] In this way, contents of folates have been reported to increase 1.7−4.3 times compared with ungerminated seeds.[10,11] A complement or alternative to mandatory folic acid fortification could be to increase the natural folate content by sprouting.

It has been widely reported that sprouts provide higher nutritive value than raw seeds, and their production is simple and inexpensive. Legume species sprouts are becoming a functional food that has been recommended for the human diet because they have the advantages of germinated seeds. In comparison to other legume seeds surveyed, soybean and mungbean seeds appear to be superior in folate content[14] and, therefore, offer the possibility of decisively increasing folate intake by sprouting. However, folate deficiency in several Asian countries such as China is partly attributed to the off-season limited supply of fresh vegetables.[1,15] Legume sprouts can be produced within a short time in households; hence, they could be an alternative and vital source of folate during the off-season of vegetable supply. For that purpose, we focused on the optimization in key legume sprouts bioactives (folate, vitamin

Received: June 2, 2012
Revised: August 20, 2012
Accepted: August 20, 2012
Published: August 20, 2012

dx.doi.org/10.1021/jf302403t | J. Agric. Food Chem. 2012, 60, 9137−9143

C, and phenolic compounds) and the antioxidant capacity in different cultivars as a source of genotypic variability.

The aims of this study were as follows: (i) to evaluate the effect of germination of legume seeds on folate content and composition and other potential health-promoting phytochemicals; and (ii) to select suitable legume cultivars and to optimize germination times in relation to concentrations of these compounds.

MATERIALS AND METHODS

Safety. General guidelines for work with organic solvents and acids were considered. Dithiothreitol and 2,3-dimercapto-1-propanol (BAL) have been indicated to be toxic by European Union regulatory information.

Chemicals and Reagents. Pteroyl-γ-L-glutamic acid (PteGlu) and the reduced forms of monoglutamyl folates, (6,*S*)-5,6,7,8-tetrahydrofolate sodium salt (H_4folate), (6,*S*)-5-formyl-5,6,7,8-tetrahydrofolate sodium salt (5-HCO-H_4folate) and (6,*S*)-5-methyl-5,6,7,8-tetrahydrofolate sodium salt (5-CH_3-H_4folate) were a kind gift from Merck & Cie (Schaffhausen, Switzerland). Pteroyltri-γ-L-glutamic acid ($PteGlu_3$) and 10-formylfolic acid, sodium salt (10-CHO-folic acid), were purchased from Schirck's Laboratories (Jona, Switzerland). Folates standard were stored under argon atmosphere at −80 °C until use. Liquid chromatography (LC)-grade acetonitrile, and methanol, L(+)-ascorbic acid (99%, crystalline), 2,3-dimercapto-1-propanol (BAL), rat serum (EC no 3−4−22−12), α- amylase (Type I-A, from porcine pancreas, 23.5 units/μL, EC No. 3−2−1−1), Protease (Type XIV, from *Streptomyces griseus*, ≥ 3.5 units/mg EC no 232−909−5), Folin−Ciocalteu's phenol reagent, 4,6-tripryridyl-*s*-triazine (TPTZ) were purchased from Sigma-Aldrich (St Louis, OM). LC grade phosphoric acid (85%) was purchased from Tedia Company, Inc. (Fairfield, OH). Monobasic potassium phosphate (≥99%, purity), dibasic potassium phosphate (≥99%, purity), sodium acetate (≥99%, purity) and sodium chloride (≥99%, purity) were from Merck (Darmstadt, Germany). Meta-phosphoric acid (MPA) and gallic acid were from Acros Organics (Geel, Belgium). Dithiothreitol (DTT) was from Bio-Rad Laboratories, Inc. (Hercules, CA). Chicken pancreas (EC no 3−4−22−12) was purchased from Shanghai Wingch Chemical Technology Co. Ltd., (Shanghai, China). Powdered certified reference material (BCR-485) were purchased via Sigma-Aldrich (St Louis, OM) from the Institute for Reference Material and Measurements (Geel, Belgium) and stored as vacuum-packed subsamples (2 g) at −80 °C until analysis. All other chemicals were of analytical grade commercially obtained from local chemical suppliers. Water was purified (≤0.1 μS cm^{-1}) using a Milli-Q system (Millipore, Billerica, MA).

Plant Materials and Experimental Conditions. *Seed Collection.* Four legume cultivars from two different origins, two soybean (*Glycine max* cv. HeiNong48 and Bangladesh soybean-4) and two mungbean (*Vigna radiata* cv. Sulv3 and BARI mung-4), were used in this study. HeiNong48 and Sulv3 were native to China and collected from Dong bei, China. Bangladesh soybean-4 and BARI mung-4 were native to Bangladesh and collected from the Bangladesh Agricultural Research Institute (BARI), Bangladesh.

Seed Germination. Seeds of each cultivar (250 g) was rinsed in deionized water, surface sterilized by 0.07% sodium hypochlorite (NaOCl) for 30 min, and drained. They were then placed in aerated deionized water (23 ± 3 °C) and soaked overnight. After pouring off soaking water, the seeds were placed over moist filter paper in black plastic germination trays (27 × 37 × 5 cm). Each cultivar had three replications.

Germination Condition. The trays were randomly placed in a controlled environment chamber (made by http://www.zjuee.cn) with a 16 h photoperiod and air temperatures of 25 ± 0.5 and 20 ± 0.5 °C day/night. The relative humidity was maintained at 60% (day) and 80% (night). Photosynthetically active radiation of 300 μmol m^{-2} s^{-1} was provided by a combination of fluorescent tubes (Philips TLD 36W/83, NY and Sylvania F36W/GRO, Munich, Germany) and metal halide lamps (Osram HGI.T 400W, Munich, Germany).

Sampling Procedure. Sprout samples from each tray were collected at 2, 4, 6, 8, and 10 days after sowing. For each day, two subsamples from each replication were rapidly and gently collected for fresh weight (FW), dry weight (DW), folate, vitamin C, total phenolic compounds, and total antioxidant capacity analysis. Germination was stopped by pouring liquid nitrogen into the sprout, which was then immediately stored at −80 °C, if needed. The seed and freeze-dried samples were thoroughly homogenized by using a ball mill (Retsch, MM-301, Germany).

Folate Analysis. *Enzyme Preparation.* Folate conjugase preparation, storage, and activity checking were performed as described in our previous study.[16] Chicken pancreas solution was prepared by dissolving 5 mg of chicken pancreas in phosphate buffer (30 mL, 0.1 M, pH 7.0) containing 1% ascorbic acid. Protease was dissolve in distilled water (5 mg/mL), and α-amylase was used directly without any pretreatment. Protease was kept at −20 °C and α-amylase at 4 °C for a maximum of 3 months.

Folate Extraction. The seed/sprout samples (5 g FW) were immediately ground to a fine powder in liquid nitrogen, and 15 mL of extraction buffer (0.1 M of phosphate buffer containing 1.0% of L-(+)-ascorbic acid (w/v) and 0.1% BAL (v/v), pH 6.1, freshly prepared) was added. Subsequently, 60 μL of α-amylase was added to it. After 10 min at room temperature, 0.8 mL of protease was added, and the mixture was flushed with argon gas, capped, and incubated for 1 h at 37 °C. The capped tube was then placed on a water bath at 100 °C for 10 min and then rapidly cooled on ice. Tubes were then centrifuged at 27000*g* for 20 min at 4 °C. The supernatants were filled to an exact volume in 25 mL volumetric flasks with extraction buffer. For deconjugation of polyglutamylated folates, 175 μL of rat serum and 2 mL of chicken pancreas solution were added to the 5 mL of extraction solution and flushed with argon gas before capping, which was then incubated on a shaking water bath at 37 °C for 2 h. An additional treatment of 5 min at 100 °C was carried out to inactivate enzyme, again followed by cooling on ice. The samples were then centrifuged again at 27000*g* for 20 min at 4 °C. The final solution in the centrifuge tube was then filtered through a 0.45 μm pore size, 25 mm ⌀ nylon disposable syringe filter. The filtrate was then purified using solid-phase extraction (SPE) on strong anion exchange (SAX) Isolute cartridges (3 mL/500 mg of quaternary amine N^+, counter ion Cl^-, Supelco, Bellefonte, PA, USA) using a visiprep SPE vacuum manifold (Supelco) as described in our previous study.[16] Folate extraction and sample purification were carried out under yellow fluorescent light.

Folate Quantification. A LC system (Agilent 1200 series, Agilent Technologies, Germany) was used, consisting of a gradient quaternary pump, a thermostated autosampler, a thermostated column compartment, an ultraviolet detector (UV), a fluorescence detector (FLD), and a computer running Agilent Chemstation software (rev B. 04.01 SP1). The separation of folates was performed on a Zorbax SB, C_{18}, column 250 × 4.6 mm, 5 μm (Agilent Technologies, Santa Clara, CA, USA), at 23 °C. The flow rate was 0.4 mL/min; the injection volume was 20 μL with a total running time 42 min; the temperature in the thermostated autosampler was 8 °C. For the detection and quantification of folates a fluorescence detector (ex/em 290/360 nm for reduced folates and 360/460 nm for 10-HCO-folic acid) and a UV detector were used (290 nm). Peak purity and identity were confirmed by comparison of relative peak areas in both detectors. The mobile phase and chromatographic elution program were the same as described in our previous study.[16] Preparation and storage of the standard stock solutions (200 μg/mL) and calibration solutions were according to our previously described method.[16] The actual concentration of folates standard was assured spectrometrically as detailed in our previous study.[16] Quantification was based on fluorescence detection, except for PteGlu, using a multilevel (n = 8) external calibration curve with a linear range over 0.5−100 ng/mL for H_4folate, 0.3−100 ng/mL for 5-CH_3-H_4folate, and 10−600 ng/mL for 5-HCO-H_4folate, 10-HCO-folic acid, and PteGlu. The calibration curves had a correlation coefficient >0.9996 for all folate forms. Endogenous 5-CH_3-H_4folate found in each batch of rat serum was corrected by running blank samples and the concentration subtracted

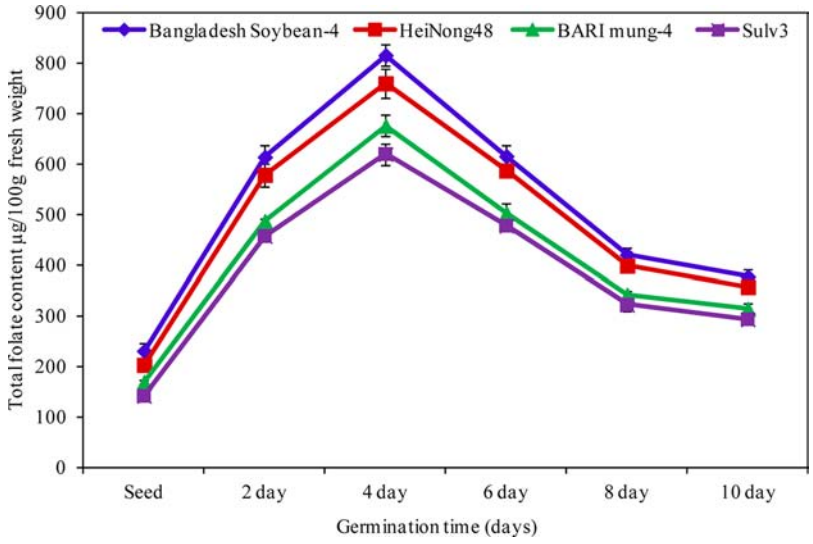

Figure 1. Total folate content in different cultivars of soybean and mungbean seeds during germination. Duplicate determinations were made on folate extractions from each replication. Data are means of all determinations. Error bars indicate the standard deviation.

from the real sample extracts. The sum of different folate forms in seed/sprout samples was expressed in micrograms of folic acid per 100 g of fresh weight after conversion using the molecular weight as detailed in our previous study.[16]

Check of Accuracy and Quality Control. Certified reference material mixed vegetables (BCR-485) were analyzed in each batch of samples to check the accuracy and for quality control. The certified value of total folate content in BCR-485 was 315 ± 28 by microbiological assay. In BCR-485, only H_4folate and 5-CH_3-H_4folate were quantified, and the sum of folates, expressed as folic acid, was 236.3 ± 1.5 μg/100 g (n = 10). However, 5-HCO-H_4folate was masked and could not be quantified, and 10-HCO-folic acid was not detected, which were well in line with results previously reported by Kariluoto[11] and Jastrebova et al.[17] Sensitivity was assessed by evaluating the LOD (S/N > 3) and LOQ (S/N > 10) values. Intra- and interday precisions in BCR-485 were <8.2% relative standard deviation (RSD) obtained on one day and on different days at three levels for both folate forms. Recovery tests were performed in duplicate by the addition of folate standards (50 and 100% of initial content) before extraction to BCR-485, and the mean recovery percentages were 97 ± 1 for H_4folate and 98 ± 2 for 5-CH_3-H_4folate.

Vitamin C Analysis. Vitamin C contents, ascorbic acid, and dehydroascorbic acid were determined by LC method after reduction of dehydroascorbic acid to ascorbic acid.[18] All steps in the analysis were performed under yellow fluorescence light. The seed/sprout samples (1 g FW) were ground in liquid nitrogen and extracted with 20 mL of 4.5% ice-cold MPA. For reduction of dehydroascorbic acid, an aliquot of the extract was added to 1% DTT solution and allowed to react for 30 min at pH 7.0; the pH was adjusted with K_2HPO_4, prior to LC analysis.

Samples were analyzed on an Agilent 1200 series (Agilent Technologies, Germany) LC, using an isocratic method with a flow rate of 1.0 mL/min. The mobile phase consisted of 25% $NH_4H_2PO_4$ (15 mM) and 75% acetonitrile, with the pH adjusted to 3.9. A 20 μL portion was injected from each sample. A Zorbax SB, C_{18} column, 250 × 4.6 mm, with a particle size of 5 μm (Agilent Technologies, Santa Clara, CA, USA), was used. Absorbance was measured in a UV detector at 254 nm. Samples were quantified using a multilevel (n = 7) external ascorbic acid standard calibration curve with a linear range over 10−600 μg/mL. Intra- and interday precisions were <3.3% RSD obtained on one day and on different days at three levels.

Determination of Total Phenolic Compounds. Total phenolic content was analyzed using Folin−Ciocalteu's phenol reagent as described,[19] with slight modifications. The seed/sprout samples (1 g for FW and 0.5 g for DW) were macerated and extracted in 6 mL of 80% (v/v) acetone and thoroughly shaken at room temperature for 1 h, followed by centrifugation at 3500 rpm for 10 min. An aliquot of 1/10 diluted supernatant was oxidized with Folin−Ciocalteu's phenol reagent, and the reaction was neutralized with 7.5% (w/v) sodium carbonate. The mixture was allowed to stand at room temperature for 90 min before the absorbance at 765 nm was read (Lambda 35, UV−vis spectrophotometer, PerkinElmer Ltd., Shelton, CT, USA). A blank was prepared using 50 mL of 80% (v/v) acetone. A gallic acid standard curve (n = 8) with a linear range over 0−60 μg/mL was prepared from a freshly made 1 mg/mL gallic acid [in 80% (v/v) acetone] stock solution. Intra- and interday precisions were <3.6% RSD obtained on one day and on different days at three levels.

Determination of Total Antioxidant Capacity. Extraction and analysis of total hydrophilic antioxidant capacity were performed as described[20] with minor modification. Seed/sprout samples (1 g FW) were homogenized in liquid nitrogen and extracted in 10 mL of water, followed by centrifugation at 12000g for 10 min. An aliquot of 5 μL of supernatant was added to 2 mL of ferric reducing antioxidant power (FRAP) reagent and incubated at 37 °C for 30 min. Absorbance at 593 nm (Lambda 35, UV−vis spectrophotometer, PerkinElmer Ltd.) was determined relative to a reagent blank also incubated at 37 °C. The total antioxidant capacity of samples was determined against a standard of known FRAP value, ferrous sulfate (1000 μM). Intra- and interday precisions were <3% RSD obtained on one day and on different days at three levels.

Statistical Analysis. The results were subjected to analyses of variance (ANOVA) by using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). The results shown are mean values (mean ± standard deviation), and the differences between cultivars and samplings were compared by using a multiple-range test (least significance difference) at p < 0.05 probability level (Duncan's test).

RESULTS

Folate Content and Composition in Legume Seed and Sprouts. Legume seeds showed significant (p < 0.05) differences in total folate content among the species and cultivars (Figure 1). Total folate content in ungerminated soybean seeds ranged from 202.9 to 230.5 μg/100 g FW. Bangladesh soybean-4 presented a higher content of total folate (230.5 μg/100 g FW) than HeiNong48 (202.9 μg/100 g FW). H_4folate was the predominant vitamer in soybean seed and accounted for 33.6 and 35.2% of total folate content in Bangladesh soybean-4 (Figure 2A) and HeiNong48 (Figure 2B), respectively. 5-CH_3-H_4folate was the second highest vitamer in soybean seeds and accounted for 25.3 and 25.2% of

dx.doi.org/10.1021/jf302403t | J. Agric. Food Chem. 2012, 60, 9137−9143

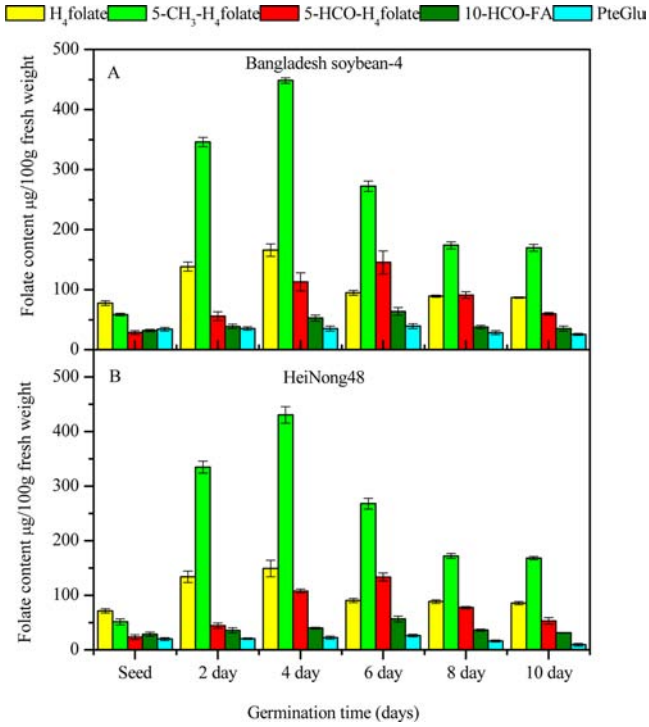

Figure 2. Folate speciation in different cultivars of soybean seeds (A, Bangladesh soybean-4; B, HeiNong48) during germination. Duplicate determinations were made on folate extractions from each replication. Data are means of all determinations. Error bars indicate the standard deviation.

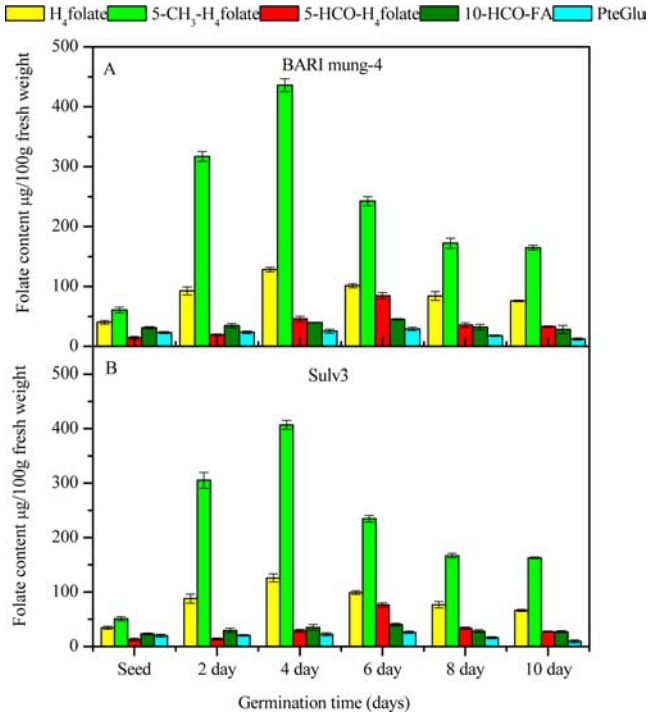

Figure 3. Folate speciation in different cultivars of mungbean seeds (A, BARI mung-4; B, Sulv3) during germination. Duplicate determinations were made on folate extractions from each replication. Data are means of all determinations. Error bars indicate the standard deviation.

the total folate content in Bangladesh soybean-4 and HeiNong48, respectively. The percentage of formylated folates on average accounted for 26.2 and 25.5% of total folate content in Bangladesh soybean-4 and HeiNong48, respectively. Furthermore, the ungerminated seeds of the two soybean cultivars presented considerable amounts of PteGlu (34.4 μg/100 g FW) in Bangladesh soybean-4 and (28.4 μg/100 g FW) in HeiNong48.

The total folate content in ungerminated mungbean seeds ranged from 141.1 to 168.9 μg/100 g FW (Figure 1). BARI mung-4 presented a higher content of total folate (168.9 μg/100 g FW) than Sulv3 (141.1 μg/100 g FW). In contrast to soybean seeds, 5-CH_3-H_4folate was the predominant vitamer in mungbean seeds and accounted for 35.8 and 36.1% of total folate content in BARI mung-4 (Figure 3A) and Sulv3 (Figure 3B), respectively. H_4folate was the second highest vitamer in mungbean seeds and accounted for 23.9 and 24.2% of the total folate content in BARI mung-4 and Sulv3, respectively. The percentage of formylated folates on average accounted for 26.8 and 25.5% of total folate content in BARI mung-4 and Sulv3, respectively. Furthermore, PteGlu contributed a considerable amount to the total folate content (13.5 and 14% for BARI mung-4 and Sulv3, respectively).

Total folate content was strongly influenced by germination in both species, as germination time increased folate concentrations significantly ($p < 0.05$). In soybean sprouts, germination brought about a sharp increase in total folate content (Figure 1) and individual folate species (Figure 2). Germination led to maximum 3.5- and 3.7-fold increases in total folate content in comparison to seed on the fourth day, accounting for 815.2 and 759.5 μg/100 g FW in Bangladesh soybean-4 and HeiNong48, respectively, and, total folate content strongly declined thereafter. In contrast to raw seeds, 5-CH_3-H_4folate was the most abundant, and H_4folate was the second highest vitamer in sprouts of both soybean cultivars. 5-CH_3-H_4folate and H_4folate became more abundant at the second and fourth days, being maximum on the fourth day, and decreased thereafter. Interestingly, 5-HCO-H_4folate and 10-HCO-FA became more abundant at the fourth and sixth days, being maximum on the sixth day, and decreased thereafter. On the other hand, PteGlu content was increased slightly during germination, became maximum on the sixth day, and decreased thereafter.

In mungbean sprouts, germination brought about a sharp increase in total folate content (Figure 1) and individual folate species (Figure 3). Germination led to maximum 3.9- and 4.3-fold increases in total folate content in comparison to seed content on the fourth day, accounting for 690.89 and 633.9 μg/100 g FW in BARI mung-4 and Sulv3, respectively, and, thereafter strongly declined. 5-CH_3-H_4folate was the most abundant and H_4folate the second highest vitamer in sprouts of both mungbean cultivars. 5-CH_3-H_4folate and H_4folate became more abundant on the second and fourth days, being maximum on the fourth day, and decreased thereafter. In a trend similar to that of the soybean sprouts, 5-HCO-H_4folate and 10-HCO-FA became more abundant on the fourth and sixth days, being maximum on the sixth day, and decreased thereafter. PteGlu content increased slightly during germination, becoming maximum on the sixth day, and decreased thereafter.

Vitamin C Content of Legume Seed and Sprouts. Vitamin C was not detected in unsprouted soybean and mungbean seeds (Figure 4A). Germination brought about sharp and gradual increases of the content of this natural antioxidant, and the highest value was reached at the fourth day of the sprouting period in all studied cultivars and thereafter

dx.doi.org/10.1021/jf302403t | J. Agric. Food Chem. 2012, 60, 9137−9143

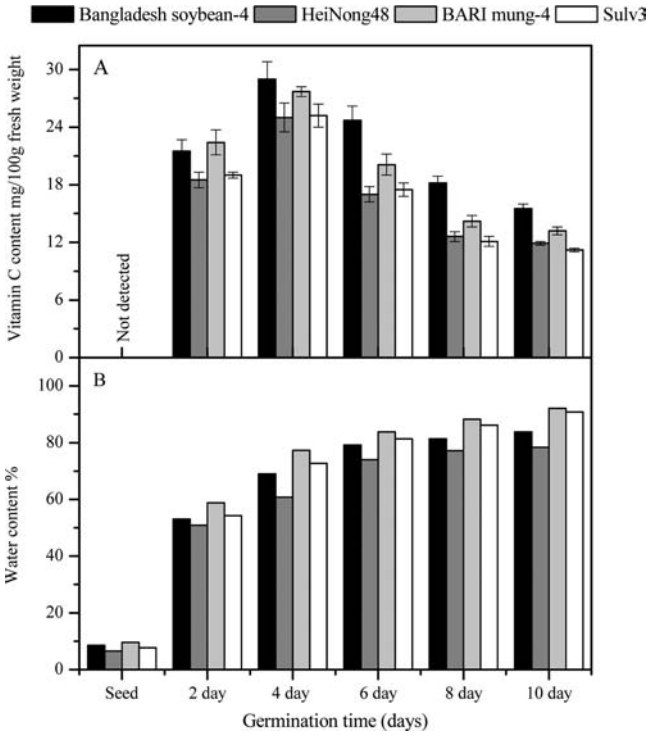

Figure 4. Vitamin C levels (A) and water content (B) measured in different cultivars of soybean and mungbean seeds during germination. Duplicate determinations were made on vitamin C extractions from each replication. Data are means of all determinations. Error bars indicate the standard deviation.

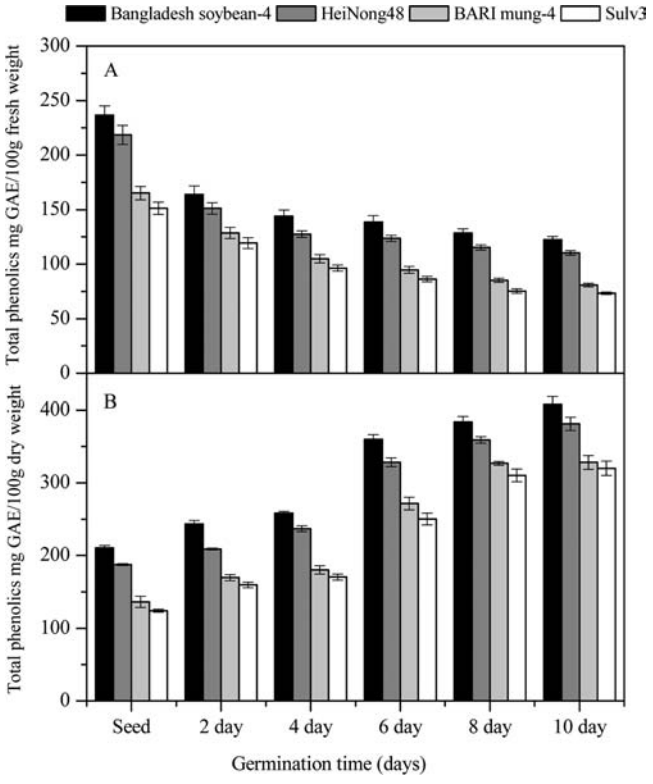

Figure 5. Total phenolic content measured in different cultivars of soybean and mungbean seeds during germination (A, fresh weight basis; B, dry weight basis). Duplicate determinations were made on phenol extractions from each replication. Data are means of all determinations. Error bars indicate the standard deviation.

decreased. Although the evolution of vitamin C content over time was analogous in the different sprouts, significant ($p < 0.05$) differences in concentrations were observed among the species and cultivars. Levels of 29 and 25 mg/100 g FW were reached in Bangladesh soybean-4 and HeiNong48, respectively, after 4 days of germination, whereas sprouts of BARI mung-4 and Sulv3 mungbean at 4 days contained 27.7 and 25.2 mg/100 g FW, respectively.

Total Phenolic Compounds in Legume Seed and Sprouts. The analyzed legume seeds showed significant ($p < 0.05$) differences for total phenolics contents (Figure 5). The total phenolic content in raw seeds was significantly higher ($p < 0.05$) in Bangladesh soybean-4 (236.6 and 210.6 mg GAE/100 g, FW and DW, respectively) than in HeiNong48 (218.5 and 187.5 mg GAE/100 g, FW and DW, respectively). Total phenolics recorded in BARI mung-4 (165.1 and 136.1 mg GAE/100 g, FW and DW, respectively) were also higher than in Sulv3 (151.1 and 124.1 mg GAE/100 g, FW and DW, respectively) over the monitored period. Germination led to marked decreases in total phenolic content when results were expressed on a fresh weight basis (Figure 5A), opposite the trends found when results were expressed on a dry weight basis (Figure 5B).

Total Antioxidant Capacity in Legume Seed and Sprouts. Germination brought about a reduction in total antioxidant capacity (Figure 6). For the FRAP assay, the values ranged from 1.32 to 0.85 mmol Fe^{2+}/100 g FW in seeds and from 0.35 to 0.91 mmol Fe^{2+}/100 g FW in the sprouts, with statistically significant ($p < 0.05$) variations among the species and cultivars.

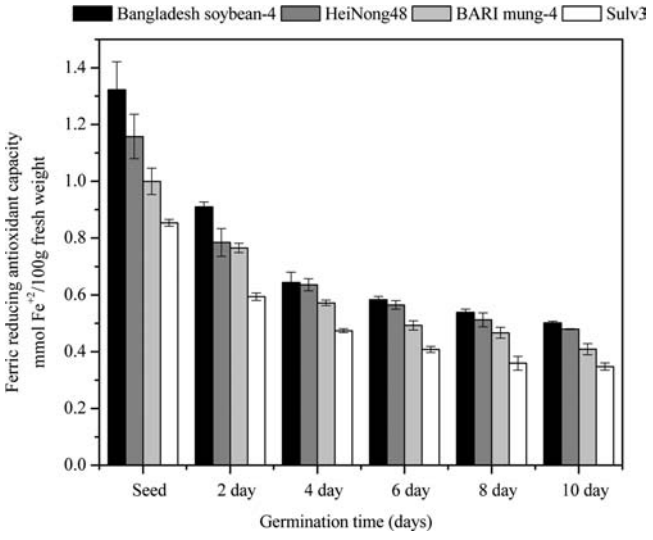

Figure 6. Ferric reducing antioxidant potential (FRAP) measured in different cultivars of soybean and mungbean seeds during germination. Duplicate determinations were made on FRAP assay from each replication. Data are means of all determinations. Error bars indicate the standard deviation.

DISCUSSION

Results on total folate content in legume seeds (Figure 1) were comparable to existing data, except for a known discrepancy that can occur between microbiological versus chromatographic analysis. The majority of folate data are based on micro-

dx.doi.org/10.1021/jf302403t | J. Agric. Food Chem. 2012, 60, 9137−9143

biological assays, and total folate content varied from 189 to 375 μg/100 g FW[21,22] and from 273 to 625 μg/100 g FW[21,23] for soybean and mungbean seeds, respectively. To our knowledge, this is the first report of folate content and composition in sprouted soybean and mungbean obtained by LC-UV/FLD. Total folate content studied is in accordance with stable isotope dilution assay (318 and 277 μg/100 g FW in soybean and mungbean seeds, respectively),[14] except for folate derivation during sample preparation. In the case of legumes, total folate contents showed a naturally high variation, due to their varying dry matter fraction and climate as well as genetic influences. In addition, the chemical stability of folate during processing could also contribute to the differences in total folates.[2] In general, folate content in foods determined by LC was 20−52% lower than reported using the microbiological assays, which can be explained by interconversions of different folate forms, yield variable in microbiological assay, and lack of all folate standards for chromatographic separation.[24,25] Besides total folate content, analyze the folate species distribution is important, as they are differ in stability.[2] With regard to the folate species, H_4folate was the predominant form and 5-CH_3-H_4folate was the second highest form in soybean seeds (Figure 2). In contrast to soybean seeds, 5-CH_3-H_4folate was found to be the dominant and H_4folate the second highest folate species in the mungbean seeds (Figure 3), in agreement with results reported by Rychlik et al.[14]

The effect of germination on total folate content and species distribution in the soybean and mungbean cultivars indicated that the differences found seem to be due to both genetic factors and germination time. To the best of our knowledge, no literature data on total folate content and species distribution in soybean and mungbean sprouts are yet available. Therefore, our data can best be compared to findings on other sprouts. The only study available, which dealt with broccoli and cereal sprouts, found folate increased 1.7−4.3-fold relative to ungerminated seeds,[10,11] which is well in line with our data. Analogously to other plant matrices, 5-CH-H_4folate was the predominant and H_4folate also was the second highest folate species in legume sprouts, increasing up to the fourth day and decreasing thereafter. Usually, H_4folate is the first intermediate in folate biosynthesis and a central point in folate metabolism. However, during legume seed germination, this folate species appears to play only a minor role. By contrast and in accordance with other authors, 5-CH_3-H_4folate has been observed to be most abundant[26] as it provides the methyl group for methionine biosynthesis[27] and methylation via *S*-adenosylmethionine.[28] Interestingly, 5-HCO-H_4folate and 10-HCO-FA became more abundant at the fourth and sixth days, but decreased thereafter. This finding can be viewed in the context of previous results, which found the folate distribution strongly dependent on the physiological condition of the plants and its homeostasis within the plant compartments.[29] Furthermore, formylated folates are mainly involved in purine biosynthesis,[30] which appears to occur during the fourth and sixth days according to our findings. As folate species decreased again after 6 days of germination, it can be concluded that high folate concentrations are necessary only in the early stages of germination.

Results on the behavior of vitamin C content in legume seeds and sprouts were comparable to existing data. Vitamin C was absent in soybean and mungbean seeds (Figure 4A), in agreement with other studies in which vitamin C was not found in seeds and significantly increased during germination.[31,32] Vitamin C concentration after germination varies considerably between species. For example, in soybean, vitamin C ranged from 2.6 mg/100 g FW on the first day after sowing to 21.8 mg/100 g FW on the fourth day after sowing. With an increase in germination time, the vitamin C content also increased, reaching a maximum value after a few days of growth. In soybean, after that moment, the vitamin C content decreased until reaching original values.[32] Vitamin C has been directly involved in the modulation of plant growth, including the early stage of germination of embryos.[33] The increment of vitamin C content during germination is attributed to the increase of the activity of L-galactono-γ-lactone dehydrogenase (EC 1.3.2.3), a key enzyme in vitamin C biosynthesis, which catalyzes the oxidation of L-galactono-1,4-lactone to ascorbic acid in soybean sprouts.[32]

The results of total phenolics content in legume seeds and sprouts provided by this work are in agreement with published data by different authors expressed on both fresh weight and dry weight bases.[19,31,34] When results are expressed on a fresh weight basis (Figure 5A), germination caused a decrease of total phenolic compounds in soybean and mungbean, suggesting a dilution effect of phenolics after imbibition and growth, and increased water absorption (Figure 4B) caused a reduction of total phenolic compounds compared with seed contents. On the contrary, when results are expressed on a dry weight basis (Figure 5A), the moisture content is eliminated and the total phenolics content increased with germination time, well in line with previous results.[31]

Differences in FRAP found in soybean and mungbean seeds and sprouts were probably due to the presence of polyphenolic compounds. The increased hydrophilic antioxidant capacity in foods has been correlated to the rise in the presence of biologically active biomolecules with pronounced antioxidant activity such as polyphenols, vitamins C, vitamin E, and carotenoids.[31] Our own investigations resulted in strong correlations between contents of total phenolics and FRAP (R^2 = 0.99−0.97) in relation to cultivars and germination time. Nevertheless, our results agree with a previously published study indicating that hydrophilic antioxidant capacity in broccoli cultivars decreased with germination time.[35]

Sprouted legume has been promoted as a functional food. It has the beneficial effect of reducing antinutritional factors and increasing the bioavailability of both macro- and micronutrients, such as protein, minerals, and vitamins. With regard to folates, a usual serving of 1 cup of 4-day-old legume sprouts can be assumed to weigh 50 g fresh mass, corresponding to an approximately equivalent RDA of folate, 400 μg/day. According to the current study, the intake of only 137 g of 4-day-old soybean sprouts or 181 g of 4-day-old mungbean sprouts covers the vitamin C RDA for an adult (40 mg/day). Therefore, legume sprouts should be held more highly valued for folate- and other micronutrient-deficient people all over the world.

In conclusion, our data confirm that 4-day-old legume sprouts are an important source for dietary folate as well as most stable species 5-CH_3-H_4folate content and also contained large amounts of vitamin C and total phenolics and showed substantial antioxidant capacity. From the results of the current study, soybean species, especially Bangladesh soybean-4, were identified as the most promising to obtain the highest amount of folate, vitamin C, and antioxidant capacity during sprouting. These results provide valuable information about the production of legume sprouts as a functional food with optimized health-promoting properties.

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Funding

This work was financially supported by a Ph.D. grant from the Chinese Government, the HarvestPlus-China Program (No. 8234), a Program from the Ministry of Science and Technology, China (No. 2009AA06Z316), and Fundamental Research Funds for the Central Universities, China (No. 2012FZA6008).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Folate standards were a kind gift from Merck & Cie (Schaffhausen, Switzerland). For this purpose, we thank Courdula Mouser. We acknowledge Dr. Jean-Paul Schwitzguébel for helping us to obtain folate standards from Schirck's Laboratories (Jona, Switzerland). We also acknowledge the Bangladesh Agriculture Research Institute (BARI) for providing the soybean and mungbean seeds. For this purpose, we thank Imrul Mosaddek Ahmed.

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