

1 **Nutritional composition of mungbean and soybean sprouts compared to their adult**
2 **growth stage**

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12

13 **Short version of title**

14 Nutritional composition of mungbean and soybean sprouts (. . .)

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16

17 **ABSTRACT**

18 This study determined the level of phytonutrients in mungbean and soybean sprouts
19 compared to mature mungbean grain and vegetable soybean. The comparison included
20 landraces and improved mungbean and soybean varieties to assess the effect of breeding
21 on the phytonutrient content of both crops. Sprouting mungbean enhanced vitamin C
22 content 2.7-fold compared to mature mungbean grain. Relatively old mungbean accessions
23 were superior in protein, calcium (Ca), iron (Fe), zinc (Zn), carotenoid and vitamin C content
24 compared to improved mungbean lines at the fully mature stage. With regards to nutritional
25 value, the vegetable soybean stage was superior to soybean sprouts in terms of content of
26 protein (14% increase), Zn (45%), Ca (72%), and Fe (151%). Isoflavones, reported to have
27 beneficial effects on human health, are found in high concentrations in soybean sprouts and
28 could easily provide the recommended anticarcinogenic dose range from 1.5 to 2.0 mg/kg of
29 body weight per day.

30

31 **Keywords:** Mungbean, Soybean, Sprouts, Vegetable soybean, Mungbean grain,
32 Phytonutrients

33

34 **Chemical compounds studied in this article**

35 Caffeic acid (PubChem CID: 689043; chlorogenic acid (PubChem CID: 1794427; kaempferol
36 (PubChem CID: 5280863); luteolin (PubChem CID: 5280445); lutein (PubChem CID:
37 5281243); violaxanthin (PubChem CID: 448438); neoxanthin (PubChem CID: 5281247); α -
38 carotene (PubChem CID: 4369188); β -carotene (PubChem CID: 5280489)

39

40 1. Introduction

41 The regular intake of nutritious food such as non-starchy vegetables, fruit, legumes,
42 nuts, fish, etc. reduces the risk of diet-related diseases like obesity, diabetes, cardiovascular
43 disease, hypertension, stroke, and cancers that are escalating worldwide due to increased
44 consumption of fast food with high trans fat content and high caloric density, and
45 sweetened beverages (Hurt, Kulisek, Buchanan, & McClave, 2010; Mozaffarian, 2016). Apart
46 from these behavioral changes in food consumption, climate change, especially rising
47 carbon dioxide (CO₂) levels, is threatening human nutrition. Global climate models predict a
48 gradual increase in atmospheric CO₂ concentration from the current level of ~400 ppm to as
49 much as 700 ppm by 2100 (IPCC, 2014). At global CO₂ levels of 550 ppm, expected to be
50 reached in the next 40-60 years, many of the food crops for human nutrition will have
51 decreased nutritional value when compared with the same plants grown under present CO₂
52 levels (Myers et al., 2014). According to Meyers and coworkers (2014), elevated CO₂
53 resulted in significant decreases of iron (Fe) and zinc (Zn) in grains and legumes that follow
54 the C₃ photosynthetic pathway. These crops represent major dietary sources of these
55 elements for a large portion of the population in developing countries.

56 There is also evidence that past breeding efforts, usually geared towards enhancing
57 yield and appearance of produce, led to a decline in nutritional value of modern cultivars.
58 This is supported by a review study conducted on 48 garden crops based on USDA food
59 composition data, which revealed a statistically significant decline of six nutrients—protein,
60 calcium (Ca), phosphorus (P), Fe, riboflavin and ascorbic acid—between 1950 and 1999
61 (Davis, Epp, & Riordan, 2004). It is likely that these changes were caused by the replacement
62 of older, more nutritious cultivars with modern ones. Similar trends have been observed in
63 wheat (Fan et al., 2008) and potato tubers (White et al., 2009). These observations inspired

64 Unilever to assemble a scientific consortium to identify ‘pre-domesticated’ varieties of crops
65 (mainly fruit and vegetables) that have not been touched by breeders and could be used to
66 develop the next generation of nutrient-rich food and beverages (Unilever, 2012). Vegetable
67 genebanks like the one maintained by the World Vegetable Center (WorldVeg) harbor many
68 landraces, farmer selections and crop wild relatives, which have hardly undergone any
69 breeding and could be explored for the generation of nutrient-rich food.

70 Phytonutrient levels in plants vary according to the growth stage. There is some
71 decrease in phytonutrients from the seedling (sprout/microgreen) to the fully grown stage
72 as shown in lettuce (Pinto, Almeida, Aguiar, & Ferreira, 2015), brassicas (Sun et al., 2013),
73 broccoli (Guo, Yang, Wang, Guo, & Gu, 2014), a range of different beans (Nakamura,
74 Kaihara, Yoshii, Tsumura, Ishimitsu, & Tonogai, 2001), and amaranth (Ebert, Wu, & Yang,
75 2015). Germination of seeds has been reported to enhance the nutritive value of legumes
76 by activating enzymes that reduce or eliminate anti-nutritional factors (Bau, Villaume,
77 Nicolas, & Mejean, 1997) and has been found to be quite effective in reducing phytic acid,
78 stachyose and raffinose in mungbean, and, on the other hand, helping to better retain many
79 minerals compared to other processes such as dehulling, boiling, autoclaving and microwave
80 cooking (Mubarak, 2005).

81 Sprouts and microgreens are fresh, functional and nutraceutical foods that are increasingly
82 becoming popular for healthy eating (Ebert, 2015; Kyriacou et al., 2016). As more people
83 worldwide are moving to urban areas, city administrators need solutions to provide healthy
84 diets for urban dwellers at affordable prices (Cohen & Garrett, 2010). Sprouts and
85 microgreens have the potential to contribute to food and nutrition security in cities as they
86 can be easily grown in urban and peri-urban settings where land is often a limiting factor,

87 either by specialized producers or the consumers themselves, independent of seasonal
88 growth cycles, inside, or around, residential areas (Ebert, 2015).

89 Legumes are second only to cereals in their importance as human food crops (Nair et
90 al., 2013), and grain legumes contribute 33% of the dietary protein nitrogen (N) needs of
91 humans (Vance, Graham & Allan, 2000). Under subsistence agriculture in the tropics and
92 subtropics, the proportion of legume protein N in the human diet can reach twice this
93 figure. About 90% of global mungbean production occurs in South, East and Southeast Asia
94 (Nair et al., 2013). In South Asia, mungbean is consumed mainly as *dhal* (porridge), while in
95 the rest of Asia it is consumed as sprouts, or as noodles. Mungbean and soybean sprouts
96 have long been an essential, year-round component of Asian and vegetarian diets (Ebert,
97 2015; Ghani et al., 2016) and the former are now finding their way into supermarket chains
98 in the Americas, Europe and East Africa. In the U.S., producers of Chinese foods perform
99 canning of sprouts alone, in water or mix them with other vegetables, such as water
100 chestnuts. Mungbean sprouts are also formulated and offered as dietary supplement in
101 healthcare (Kovacs, 1996).

102 However, there is scarce information pertaining to the nutritional properties of this
103 produce, which is now becoming more mainstream. The objective of this study was to
104 determine the level of phytonutrients in mungbean and soybean sprouts compared to
105 mature mungbean grain and vegetable soybean. The comparison included landraces from
106 the WorldVeg genebank and mungbean and soybean varieties improved by WorldVeg
107 breeders to assess the effect of breeding on the phytonutrient content of both crops.

108

109 **2. Materials and Methods**

110 *2.1 Plant material*

111 Accessions of each crop with contrasting geographical origin and breeding status (landrace
112 versus improved cultivar) were chosen from the WorldVeg genebank to verify potential
113 genotypic differences.

114 **Mungbean.** The experimental setup consisted of two relatively old landraces and two
115 improved mungbean lines of the WorldVeg genebank as follows:

- 116 • VI000197 A-G (landrace): 100-seed weight: 2.89 g; shiny seeds with green seed coat
117 color; year of acquisition: 1972; origin: Afghanistan
- 118 • VI000323 B-G (landrace): 100-seed weight: 4.93 g; dull seeds with green seed coat
119 color; year of acquisition: 1972; origin: Taiwan
- 120 • VI060081 B-G (improved mungbean line): 100-seed weight: 6.62g; dull seeds with
121 green seed coat color; year of acquisition: 2010; developed by WorldVeg breeders in
122 Thailand
- 123 • VI060110 A-G (improved mungbean line): 100-seed weight: 6.69 g; shiny seeds with
124 green seed coat color; year of acquisition: 2010; developed by WorldVeg breeders in
125 Thailand.

126 **Soybean.** For soybean sprouts and vegetable soybean, the following WorldVeg genebank
127 landraces and improved soybean lines were used:

- 128 • VI015437 (landrace): medium seed size; 100-seed weight: 19.2 g; yellow seed coat
129 color; year of acquisition: 1973; origin: China; used for sprouts only
- 130 • VI015726 (landrace): medium seed size; 100-seed weight: 20 g; yellow seed coat
131 color; year of acquisition: 1973; origin: North Korea; used for sprouts and vegetable
132 soybean
- 133 • VI016706 (landrace): small seed size; 100-seed weight: 7 g; light green/yellow seed
134 coat color; year of acquisition: 1973; origin: Indonesia; used for sprouts only

- 135 • VI023379 (landrace): small seed size; 100-seed weight: 7.6 g; black seed coat color;
136 year of acquisition: 1975; origin: India; used for sprouts only
- 137 • VI022144 (landrace): large seed size; 100-seed weight: 31.1 g; light green seed coat
138 color; year of acquisition: 1979; origin: Japan; used for vegetable soybean only
- 139 • AVSB8001 (improved soybean line): medium seed size; 100-seed weight: 13-17 g;
140 yellow seed coat color; year of release: 1985 in Taiwan, 1991 in Vietnam; used for
141 sprouts and vegetable soybean
- 142 • AVSB9301 (improved soybean line): medium seed size; 100-seed weight: 20-23 g;
143 yellow seed coat color; year of release: 2004 in Vietnam; used for sprouts and
144 vegetable soybean.

145 Small-seeded soybean varieties are commonly used for sprouting, resulting in the highest
146 sprout yield per 100 seeds. Medium-seeded soybean varieties are used for processing to
147 produce tofu, soybean milk, soybean oil, etc. Both small- and medium-seeded soybean
148 varieties are harvested at yellow or R8 stage; while large-seeded soybean varieties with >30
149 g per 100 seeds are used for vegetable soybean, which are harvested and consumed at
150 green or R6 stage.

151

152 *2.2 Production of mungbean and soybean sprouts, vegetable soybean and mungbean grain*

153 For the production of mungbean and soybean sprouts, seeds were soaked for eight
154 and four hours respectively, in distilled water, followed by rinsing. Seeds (60 g per replicate)
155 were then placed in a single layer on a sheet of paper towel inside perforated plastic trays,
156 which were enclosed in solid plastic boxes for drainage of excess water and to maintain high
157 moisture content for the sprouting process. The experiment was conducted in two batches,
158 with three replicates each, for nutritional analysis. Boxes were arranged in a randomized

159 complete block design (RCBD) and kept in a laboratory room at 26±2 °C. The seedlings were
160 carefully watered three times daily. Mungbean and soybean sprouts were harvested at the
161 end of the third day of germination. The sprouts were washed, seed coats removed and
162 samples were used for nutritional analysis.

163 For the production of vegetable soybean and mungbean grain, seeds were sown
164 towards the end of summer (soybean in mid-September; mungbean at the beginning of
165 October) in WorldVeg experimental fields in southern Taiwan with three replicates arranged
166 in RCBD. The mungbean field was previously grown to pepper and the soybean field to
167 tomato. Soybean samples were harvested at 69-73 days after sowing (depending on
168 maturity stage of each accession) and mungbean samples at 97 days after sowing, for
169 nutritional analysis.

170

171 *2.3 Chemicals used*

172 The chemicals 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Trolox, β-
173 carotene, lutein, quercetin and kaempferol were purchased from Sigma-Aldrich Chemical
174 Co. (St. Louis, MO, USA), while α-carotene and caffeic acid were obtained from Fluka Co.
175 (Neu-Ulm, Germany). Genistein, daidzein, glycitein, genistin, daidzin and glycitin were
176 purchased from Nacalai tesque INC. (Kyoto, Japan). Violaxanthin and neoxanthin were
177 purchased from ChromaDex Co. (Irvine, CA, USA.), while luteolin was obtained from
178 Extrasynthese Co. (Genay, France). Chlorogenic acid was purchased from MP Biomedicals,
179 LLC. (Santa Ana, CA, USA), 6''-O-malonyl genistin, 6''-O-malonyldaidzin and 6''-O-malonyl
180 glycitin from LC laboratories INC. (Woburn, MA, USA), and 2,4-dinitrophenylhydrazine
181 (DNPH) was obtained from Panreac Co. (Barcelona, Spain). Ascorbic acid and 2,6-

182 dichlorophenolindophenol (DCPIP) was obtained from Merck Chemical Co. (Darmstadt,
183 Germany).

184

185 *2.4 Nutritional analysis*

186 About 250 g to 300 g of sprouts and vegetable soybean seeds per replicate were
187 collected. Seventy (70) g were weighed and stored at -70 °C for vitamin C analysis within a
188 week; the remaining portion was weighed, freeze-dried, ground into fine powder and stored
189 at -20 °C for subsequent analysis. Vitamin C is more sensitive than other phytonutrients to
190 heat, light and drying processes, including freeze drying, thus the sampling and preparation
191 method for vitamin C analysis differed from the other phytonutrients. For mungbean grain,
192 no drying process was required and the grain was directly ground to fine powder and stored
193 at -20 °C for nutritional analysis.

194 Micro-Kjeldahl digestion followed by distillation (AOAC, 1990a) was used to
195 determine protein content of samples. The determination of Ca, Fe, and Zn content was
196 performed by ashing procedure, strong acid washing, followed by detection with Atomic
197 Absorption Spectroscopy (AOAC method 975.03, p. 42; AOAC, 1990b).

198 Total ascorbic acid content (vitamin C) was determined on the basis of coupling 2,4-
199 dinitrophenylhydrazine (DNPH) with the ketonic groups of dehydroascorbic acid through the
200 oxidation of ascorbic acid by 2,6-dichlorophenolindophenol (DCPIP) to form a yellow-orange
201 color in acidic conditions as described by Hanson et al. (2004). Antioxidant activity was
202 measured using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical (Re et
203 al., 1999) and expressed as trolox equivalents (TE). Details are given by Yang et al. (2006).

204 **Carotenoid determination.** Two hundred (200) mg of freeze dried fine powder was
205 briefly rinsed in 0.8 ml of distilled water, followed by 9.2 ml of acetone. The extract was

206 evaporated under a nitrogen air flow and re-dissolved in 2 ml of 1 % tetrahydrofuran (THF)
207 in methanol. Separation and identification of carotenoids was performed using a high
208 performance liquid chromatography (HPLC) system (Waters 2695, Milford, MA, USA)
209 equipped with an auto-sampler, a photodiode array detector (Waters 996) monitoring 210 –
210 700 nm, Millennium software, and a C₃₀ Column (YMC™, 3.0 μm, 4.6 mm x 150 mm). The
211 running conditions were set at 30 °C using a gradient at 1.3 ml/min from 0 to 1% THF in
212 methanol at 0 –15 min, 1 to 25% THF in methanol at 15 – 25 min, 25 to 70% THF in
213 methanol at 25 – 50 min, and the final 100% THF at 50 – 60 min. Identification of
214 carotenoids (α-carotene, β-carotene, violaxanthin, neoxanthin, and lutein) in samples was
215 performed by comparing retention time and light absorption spectra (350 nm – 700 nm) of
216 known standards and co-elution of spiked known standards to samples. The peak areas
217 were calibrated against known amounts of standards. The content of carotenoid standards
218 was measured according to their optical density reading and specific extinction coefficient
219 ($E^{1\%}_{1\text{cm}}$) in the respective solvent.

220 **Flavonoid determination.** Nine flavonoid aglycones were determined in the frozen
221 samples after hydrochloric hydrolysis of the flavonoid derivatives. A 100 mg frozen sample
222 placed in a 20 mL tube containing 10 mg ascorbic acid dissolved in 5 mL of acidified
223 methanol (1.2 M HCl) was flushed with nitrogen air for 30 sec and then refluxed at 80 °C for
224 2 h. After cooling down to room temperature, the sample was sonicated for 10 min and
225 centrifuged at 4000 g for 10 min. The supernatant, approximately 2 mL, was taken and
226 filtered through a 0.2 μm syringe filter (Millipore, Bedford, MA). The filtrate was kept at 10
227 °C for HPLC analyses within 12 h. Flavonoid aglycons were separated using the HPLC system
228 equipped with a Waters 2695 separation module and an Agilent Zorbax ODS column (3.5μm,
229 4.6 x 150 mm) at 35 °C using a gradient from 0 – 15 min, 1 to 25% acetonitrile (ACN) in 1%

230 aqueous formic acid (FA); and 15 – 50 min, 25% – 40% ACN in 1% aqueous FA at a flow rate
231 of 0.7 mL/min. The column elute was monitored using a Waters 996 photo diode array
232 detector (250 –700 nm). Identification and quantification of individual flavonoids was
233 carried out using commercial standards.

234 **Isoflavones** were separated using the HPLC system equipped with a Waters 996
235 separation module and an Lichrospher R 100 RP-18 column (5µm, 4 x 250 mm) at 30 °C
236 using a gradient from 0 – 30 min, 90 to 50% of solvent A (0.1% acetic acid in water) and 10
237 to 50% of solvent B (acetonitrile); 31 – 34 min, 50% of solvent A and 50% of solvent B; 35 –
238 39 min, 50 to 0 % of solvent A and 50 – 100 % of solvent B at a flow rate of 1.0 mL/min. The
239 column elute was monitored using a Waters 2996 photo diode array detector (210 –400
240 nm). Identification and quantification of individual isoflavones (genistein, daidzein, glycitein,
241 genistin, daidzin, glycitin, 6''-O-malonyl genistin, 6''-O-malonyldaidzin, 6''-O-malonyl glycitin)
242 was carried out using commercial standards.

243

244 *2.5 Statistical analysis*

245 Data was statistically analyzed by two-way analysis of variance (ANOVA) using
246 Statistical Analysis Software (SAS) 9.4. Duncan's multiple range test (DMRT) was used to
247 determine significant differences at the $p < 0.05$ level.

248

249 **3. Results and discussion**

250 *3.1 Nutritional analysis of mungbean sprouts and mungbean grain*

251 At the sprouting stage, the improved mungbean lines (VI060081 and VI060110)
252 showed the highest dry matter and protein content, while Ca, Fe and Zn content did not
253 differ among the four genotypes tested (Table 1). At the fully grown stage, mungbean grain

254 of the two relatively old genebank accessions, acquired at the start of the operation of the
255 WorldVeg genebank (VI000197 and VI000323), were superior in protein, Ca, Fe and Zn
256 content compared to the improved mungbean lines. Protein content of all four genotypes
257 was higher than the values reviewed and reported by Nair et al. (2013). The observed lower
258 content of protein and some essential micronutrients of improved mungbean lines
259 compared to relatively old landraces confirms earlier observations with garden crops in the
260 United States (Davis et al., 2004), that breeding for size and yield may have a detrimental
261 effect on the nutrient value of the produce.

262 The lower values of protein and minerals observed in mungbean sprouts compared
263 to mature grains (Table 1) are primarily a result of water uptake and ensuing biochemical
264 processes during sprouting (Tang, Dong, Ren, Li, & He, 2014) and are in line with
265 observations made by Fordham, Wells, & Chen (1975).

266 Mungbean sprouts showed very low levels of carotenoids; the content of
267 violaxanthin and neoxanthin was below the detection threshold (Table 1). However, mean
268 vitamin C content was 2.7-fold higher at the sprouting stage compared with mature
269 mungbean grain. Sprouts of improved mungbean line VI060081 had the highest vitamin C
270 content at this early growth stage. At the fully mature stage, mungbean grain of the
271 relatively old genebank accession VI000323 from Taiwan presented the highest carotenoid
272 and vitamin C content among all four genotypes (Table 1). While the content of carotenoids
273 was enhanced at the grain stage compared to mungbean sprouts, vitamin C content was
274 considerably lower at the fully mature stage.

275 The vitamin C content of mungbean sprouts was 1.7-fold higher than that of soybean
276 sprouts (Tables 1 & 2) and 2.5-fold higher than that of amaranth sprouts (Ebert, Wu, & Yang,
277 2015). Our results are in agreement with Fordham et al. (1975) who observed a 7.9-fold

278 increase of total ascorbic acid content in mungbean sprouts compared to mungbean grain.
279 With regard to vitamin C content, our findings are also in line with reports by Chavan &
280 Kadam (1989) who concluded that sprouting of cereal grain generally improves their vitamin
281 value.

282 Little variation in antioxidant activity (AOA) was measured among mungbean
283 genotypes at the sprouting stage, with the exception of VI000197 having inferior content
284 (Table 1). Antioxidant activity of mungbean sprouts reached only 48% of the value observed
285 in soybean sprouts (Table 2). However, AOA levels of mungbean grain were 6.7- to 11.3-fold
286 higher than at the sprouting stage (Table 1), clearly exceeding AOA levels measured in
287 soybean sprouts, vegetable soybean (Table 2), amaranth sprouts and microgreens (Ebert et
288 al., 2015).

289 In mungbean sprouts, only caffeic acid and kaempferol were found in detectable
290 quantities, while chlorogenic acid and luteolin were below the detection level (Table 1). In
291 mungbean grain, caffeic acid and kaempferol reached a 13.8-fold and 7.8-fold higher level,
292 respectively. The genebank accession VI000323 from Taiwan showed the highest level of
293 these phytochemicals at both development stages. Phenolic compounds such as phenolic
294 acids and flavonoids are an important source of antioxidants and are relevant for stability,
295 color, flavor and nutritional value of food (Chen & Ho, 1997).

296 Our results have shown that mungbean sprouts and mungbean grain are of high
297 nutritional value. They can be considered as functional foods that may lower the risk of
298 various diet-related diseases through their antioxidant, antimicrobial, anti-inflammatory,
299 antidiabetic, antihypertensive, and antitumor effects as reviewed by (Tang et al., 2014).

300

301 *3.2 Nutritional analysis of soybean sprouts and vegetable soybean*

302 At the sprouting stage, dry matter and protein content was highest in VI015437, a
303 relatively old genebank accession from China, while the highest contents of Ca, Fe and Zn
304 were determined in the improved soybean line AVSB9301, released in 2004 in Vietnam
305 (Table 2). While Ca levels in our experiments were similar to those reported in Korean and
306 Chinese soybean sprouts (Lee, 2015), mean protein, Fe and Zn levels were about 2-fold
307 higher in the current studies.

308 At the vegetable soybean stage, there were only minor differences among genotypes
309 regarding protein, Ca and Zn contents, while Fe content did not differ significantly (Table 2).
310 Compared to the sprouting stage, there was a slight increase in the mean value of protein
311 (14%) content and a more pronounced increase in Zn (45%), Ca (72%), and Fe (151%)
312 content at the vegetable soybean stage.

313 When comparing the mean protein and mineral content of mungbean and soybean
314 sprouts (Tables 1 & 2), it is noted that the values of soybean are much higher for protein
315 (2.71-fold), Ca (7.95-fold), Fe (2.96-fold), and Zn (2.18-fold). This is in agreement with earlier
316 reports made by Abdullah and Baldwin (1984) who observed comparable or higher contents
317 of several minerals in soybean seeds and sprouts compared to mungbean, on a dry weight
318 basis.

319 The content of carotenoids in soybean sprouts was rather low (Table 2). The highest
320 content of α -carotene, β -carotene and vitamin C was obtained with VI016706, a relatively
321 old genebank accession from Indonesia, while the improved cultivar AVSB9301, released
322 2004 in Vietnam had the highest violaxanthin, neoxanthin, and lutein contents. The AOA
323 content of soybean sprouts was more than 2-fold higher than that of mungbean sprouts
324 (Tables 1 & 2), with AVSB8001, released in 1985 in Taiwan, reaching 3049 $\mu\text{m TE}$.

325 Vegetable soybean showed an enhanced carotenoid content compared with
326 soybean sprouts; this was especially the case with neoxanthin—a 8.9-fold increase—and α -
327 carotene—a 4.4-fold increase (Table 2). The highest levels were observed with the improved
328 soybean line AVSB9301. Vitamin C content more than doubled at the vegetable soybean
329 stage, while AOA was only slightly enhanced compared with soybean sprouts.

330 With a mean content of 23.82 mg vitamin C, the consumption of 100 g vegetable
331 soybean would have made a 40% contribution to the previously established recommended
332 dietary allowance (RDA) of 60 mg/d, out of which 46 mg/d are necessary to prevent the
333 vitamin C deficiency disease, scurvy (Carr & Frei, 1999). These authors successfully argued
334 for a reevaluation of the established RDA value for vitamin C to reduce the risk of other
335 chronic diseases such as cancer, cardiovascular disease, and cataract, which are—possibly
336 through antioxidant mechanisms—associated with vitamin C intake. The RDA values for
337 vitamin C recently were adjusted to 90 mg/d for male and 75 mg/d for female non-smoking
338 adults (National Institutes of Health, 2016). Smokers require an additional intake of 35 mg
339 vitamin C per day.

340 In soybean sprouts, the highest levels of isoflavones, a main class of phytoestrogens,
341 were obtained with the improved breeding line AVSB8001, followed by the relatively old
342 genebank accession VI015437 from China (Table 3). The highest individual isoflavone share
343 was registered with the malonylglucosides 6''-O malonyl genistin, followed by 6''-O malonyl
344 daidzin and 6''-O malonyl glycitin. The isoflavonoids genistein, daidzein and glycitein were
345 only present in low concentrations and were completely absent in vegetable soybean.

346 The mean isoflavone contents of vegetable soybean reached only 43.2% of the
347 contents found in soybean sprouts, on average (Table 3). The landrace VI022144 presented
348 the highest isoflavone content among vegetable soybean genotypes.

349 The observed isoflavone contents of vegetable soybeans are very similar to those
350 reported in the USDA database (Bhagwat, Haytowitz & Holden, 2008), while the isoflavone
351 contents of soybean sprouts are 3.2-fold higher in our experiments compared to the values
352 found in the USDA database. The isoflavone concentrations of vegetable soybean and
353 soybean sprouts measured in our experiments are within the range reported by Wang &
354 Murphy (1994) for commercial soybean foods. Our results confirm the observations made
355 by Kim et al. (2006) that the isoflavone content in soybean sprouts is higher than in the
356 seed. Large variation in isoflavone content has been observed among soybean sprout
357 genotypes (Ghani et al., 2016) making genetic selection for high isoflavonole content an
358 interesting breeding goal.

359 Isoflavones, found in particularly high concentrations in soybeans and flax seeds, are
360 reported to have beneficial effects on human health, particularly on hormone-dependent
361 conditions, such as cancer, menopausal symptoms, cardiovascular disease and osteoporosis
362 (Setchell & Cassidy, 1999). Vegetable soybean and especially soybean sprouts could easily
363 provide the anticarcinogenic dose range from 1.5 to 2.0 mg/kg of body weight per day
364 recommended by (Hendrich, Lee, Xu, Wang, & Murphy, 1994). The present study has shown
365 that both soybean sprouts and vegetable soybean are of high nutritional value containing a
366 wide range of health-promoting phytochemicals, thus justifying the promotion of these food
367 items for healthy diets.

368

369 4. Conclusions

370 **Mungbean.** At full maturity, mungbean landraces were superior in protein, Ca, Fe,
371 Zn, carotenoid and vitamin C contents compared to improved varieties. Mean vitamin C
372 content was 2.7-fold higher at the sprouting stage compared to mature mungbean grain. In

373 contrast, the content of the phenolic compounds caffeic acid and kaempferol and
374 antioxidant activity was much higher in mungbean grain than in sprouts. A landrace from
375 Taiwan (VI000323) showed the highest levels of caffeic acid and kaempferol at both
376 developmental stages.

377 **Soybean.** There was no clear pattern of higher nutritional value of either
378 unimproved or improved soybean lines. Vegetable soybean was superior to soybean sprouts
379 in terms of content of protein (14% increase), Zn (45%), Ca (72%), and Fe (151%). Vitamin C
380 content more than doubled at the vegetable soybean stage, neoxanthin and α -carotene
381 contents showed a 9-fold and 4-fold increase, respectively. Isoflavones were found in high
382 concentrations in soybean sprouts and could easily provide the recommended
383 anticarcinogenic dose range from 1.5 to 2.0 mg/kg of body weight per day.

384 Soybean sprouts were superior to mungbean sprouts in the content of protein (2.71-
385 fold), Ca (7.95-fold), Fe (2.96-fold), and Zn (2.18-fold). The antioxidant activity of soybean
386 sprouts was more than 2-fold higher than that of mungbean sprouts.

387

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395

396 **Author Contributions**

397 A. Ebert designed the study, interpreted the results and drafted the manuscript. J.
398 Chang and M.-R. Yan supervised the implementation of the mungbean and soybean trials
399 and collected and analyzed the data, respectively. R.-Y. Yang supervised the nutritional
400 analyses and contributed to the manuscript.

401

402 **Conflict of interest statement**

403 The authors declare that there are no conflicts of interest.

404

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529

530 **Tables**

531

532 **Table 1**533 Content¹ of dry matter, protein, minerals, carotenoids, vitamin C, antioxidant activity (AOA), phenolic acids (chlorogenic acid, caffeic acid), and
534 flavonoids (luteolin, kaempferol) of mungbean sprouts and mungbean grain per 100 g edible portion of fresh weight.

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Accession /cultivar	Dry matter (g)	Protein (g)	Minerals			Carotenoids			
			Ca (mg)	Fe (mg)	Zn (mg)	Violaxanthin (mg)	Neoxanthin (mg)	Lutein (mg)	α-Carotene (mg)
Mungbean sprouts									
VI000197 A-G	9.72 ± 0.42 ^b	3.38 ± 0.16 ^b	7.13 ± 0.15 ^a	0.36 ± 0.07 ^a	0.49 ± 0.02 ^a	n.d. ²	n.d.	0.18 ± 0.02 ^a	0.01 ± 0.00
VI000323 B-G	11.42 ± 0.95 ^b	3.80 ± 0.25 ^b	7.88 ± 1.09 ^a	0.81 ± 0.14 ^a	0.59 ± 0.11 ^a	n.d.	n.d.	0.18 ± 0.03 ^a	0.01 ± 0.00
VI060081 B-G	14.37 ± 1.03 ^a	4.72 ± 0.26 ^a	8.53 ± 1.23 ^a	0.77 ± 0.51 ^a	0.67 ± 0.32 ^a	n.d.	n.d.	0.15 ± 0.04 ^a	0.01 ± 0.00
VI060110 A-G	14.01 ± 0.87 ^a	5.19 ± 0.18 ^a	7.74 ± 0.37 ^a	0.57 ± 0.11 ^a	0.63 ± 0.05 ^a	n.d.	n.d.	0.19 ± 0.02 ^a	0.01 ± 0.00
Mean ± SD ³	12.38 ± 2.12	4.27 ± 0.77	7.82 ± 0.89	0.63 ± 0.30	0.60 ± 0.16	n.d.	n.d.	0.18 ± 0.03	0.01 ± 0.00
Mungbean grain									
VI000197 A-G	90.78 ± 0.09 ^a	27.54 ± 0.73 ^{ab}	109.17 ± 6.11 ^a	7.17 ± 0.68 ^{ab}	4.56 ± 0.11 ^a	0.08 ± 0.00 ^a	0.10 ± 0.01 ^{ab}	0.87 ± 0.05 ^{bc}	n.d.
VI000323 B-G	90.35 ± 0.14 ^a	28.65 ± 1.99 ^a	101.85 ± 4.65 ^a	7.83 ± 0.88 ^a	4.48 ± 0.31 ^a	0.07 ± 0.01 ^a	0.11 ± 0.02 ^a	0.98 ± 0.03 ^a	n.d.
VI060081 B-G	90.37 ± 0.49 ^a	25.33 ± 0.38 ^b	92.12 ± 3.28 ^b	6.26 ± 1.27 ^b	2.45 ± 0.54 ^b	0.07 ± 0.03 ^a	0.10 ± 0.02 ^{ab}	0.92 ± 0.04 ^{ab}	n.d.
VI060110 A-G	90.13 ± 0.73 ^a	26.00 ± 0.22 ^b	83.09 ± 1.13 ^c	6.28 ± 0.68 ^b	2.97 ± 0.07 ^b	0.09 ± 0.02 ^a	0.07 ± 0.02 ^b	0.83 ± 0.04 ^c	n.d.
Mean ± SD	90.41 ± 0.45	26.88 ± 1.64	96.56 ± 10.90	6.88 ± 1.04	3.61 ± 1.00	0.08 ± 0.02	0.10 ± 0.02	0.90 ± 0.07	n.d.

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537 Table 1 continued...

Accession /cultivar	β-Carotene (mg)	Vitamin C (mg)	AOA (μmol TE)	Chlorogenic acid (μmol)	Caffeic acid (μmol)	Luteolin (μmol)	Kaempferol (μmol)
Mungbean sprouts							
VI000197 A-G	0.053 ± 0.01 ^b	17.61 ± 0.86 ^b	962 ± 55 ^b	n.d.	9.8 ± 0.8 ^b	n.d.	0.32 ± 0.10 ^a
VI000323 B-G	0.067 ± 0.01 ^{ab}	18.44 ± 0.41 ^b	1255 ± 52 ^a	n.d.	11.3 ± 0.7 ^a	n.d.	0.37 ± 0.10 ^a
VI060081 B-G	0.067 ± 0.02 ^{ab}	20.33 ± 1.05 ^a	1214 ± 119 ^a	n.d.	8.8 ± 0.5 ^{bc}	n.d.	0.35 ± 0.21 ^a
VI060110 A-G	0.073 ± 0.01 ^a	19.05 ± 1.16 ^{ab}	1348 ± 61 ^a	n.d.	8.1 ± 0.5 ^c	n.d.	0.40 ± 0.10 ^a
Mean ± SD	0.065 ± 0.01	18.86 ± 1.30	1195 ± 163	n.d.	9.5 ± 1.4	n.d.	0.36 ± 0.12
Mungbean grain							
VI000197 A-G	0.16 ± 0.01 ^b	4.72 ± 0.28 ^b	10888 ± 305 ^b	0.08 ± 0.00 ^a	127.6 ± 4.6 ^{bc}	1.21 ± 0.06 ^{bc}	3.10 ± 0.90 ^a
VI000323 B-G	0.21 ± 0.03 ^a	9.82 ± 1.24 ^a	8399 ± 426 ^c	0.07 ± 0.01 ^a	142.7 ± 2.0 ^a	1.37 ± 0.03 ^a	3.08 ± 0.93 ^a
VI060081 B-G	0.16 ± 0.02 ^b	7.58 ± 2.04 ^{ab}	9616 ± 1171 ^{bc}	0.07 ± 0.03 ^a	133.2 ± 3.6 ^{ab}	1.25 ± 0.09 ^{ab}	2.39 ± 1.46 ^a

VI060110 A-G	0.13 ± 0.02 ^b	6.03 ± 2.08 ^{ab}	12597 ± 106 ^a	0.09 ± 0.02 ^a	120.6 ± 9.7 ^c	1.12 ± 0.08 ^c	2.73 ± 0.88 ^a
Mean ± SD	0.17 ± 0.04	7.04 ± 2.40	10375 ± 1715	0.08 ± 0.02	131.0 ± 9.8	1.24 ± 0.11	2.82 ± 0.96

538 ¹Values are means ± standard deviation. Means within a column with different upper case letters are significantly different at $P < 0.05$. An absence of letters indicates no
539 significant difference was observed. ² not detected; ³ standard deviation.
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Table 2

Content¹ of dry matter, protein, minerals, carotenoids, vitamin C, and antioxidant activity (AOA) of soybean sprouts and vegetable soybean per 100 g edible portion of fresh weight.

Accession/ cultivar	Dry matter (g)	Protein (g)	Minerals		
			Ca (mg)	Fe (mg)	Zn (mg)
Soybean sprouts					
VI015437	28.15 ± 1.03 ^a	13.65 ± 0.91 ^a	65.3 ± 2.55 ^{bc}	1.99 ± 0.20 ^{abc}	1.32 ± 0.04 ^{bc}
VI015726	24.70 ± 1.24 ^b	12.73 ± 0.83 ^b	54.7 ± 5.35 ^{cd}	2.04 ± 0.53 ^{ab}	1.34 ± 0.25 ^{abc}
VI016706	17.93 ± 0.79 ^c	9.03 ± 0.18 ^c	46.0 ± 4.76 ^d	1.43 ± 0.29 ^c	1.04 ± 0.06 ^d
VI023379	16.37 ± 0.95 ^d	9.17 ± 0.42 ^c	62.0 ± 2.08 ^{bc}	1.56 ± 0.21 ^{bc}	1.14 ± 0.04 ^{cd}
AVSB8001	25.53 ± 0.80 ^b	12.03 ± 0.46 ^b	68.7 ± 7.74 ^{ab}	1.98 ± 0.42 ^{abc}	1.41 ± 0.10 ^{ab}
AVSB9301	25.87 ± 0.85 ^b	12.89 ± 0.38 ^{ab}	76.7 ± 7.64 ^a	2.22 ± 0.23 ^a	1.58 ± 0.09 ^a
Mean ± SD ²	23.09 ± 4.56	11.58 ± 1.94	62.2 ± 11.18	1.87 ± 0.40	1.31 ± 0.21
Vegetable soybean					
VI015726	34.03 ± 0.15 ^{ab}	13.51 ± 0.08 ^a	117.3 ± 14.8 ^a	4.52 ± 0.37 ^a	1.89 ± 0.01 ^{ab}
VI022144	32.71 ± 0.18 ^b	12.23 ± 0.25 ^b	94.4 ± 4.8 ^b	4.64 ± 0.04 ^a	1.70 ± 0.01 ^b
AVSB8001	35.29 ± 0.92 ^a	13.32 ± 0.23 ^a	106.0 ± 17.0 ^{ab}	4.86 ± 0.18 ^a	1.97 ± 0.06 ^a
AVSB9301	34.46 ± 1.01 ^a	13.70 ± 0.16 ^a	109.0 ± 16.1 ^a	4.77 ± 0.70 ^a	2.04 ± 0.20 ^a
Mean ± SD	34.12 ± 1.14	13.19 ± 0.62	106.7 ± 14.7	4.70 ± 0.37	1.90 ± 0.16

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Table 2 continued...

Accession/ cultivar	Carotenoids					Vitamin C (mg)	AOA (μmol TE)
	Violaxanthin (mg)	Neoxanthin (mg)	Lutein (mg)	α-Carotene (mg)	β-Carotene (mg)		
Soybean sprouts							
VI015437	0.050 ± 0.002 ^b	0.0051 ± 0.0004 ^b	0.15 ± 0.02 ^c	0.0012 ± 0.0005 ^b	0.030 ± 0.005 ^b	13.00 ± 0.86 ^b	2534 ± 136 ^{bc}
VI015726	0.057 ± 0.009 ^b	0.0060 ± 0.0015 ^b	0.18 ± 0.04 ^c	0.0030 ± 0.0018 ^{ab}	0.033 ± 0.004 ^b	8.67 ± 0.93 ^e	2704 ± 371 ^{ab}
VI016706	0.053 ± 0.012 ^b	0.0066 ± 0.0006 ^b	0.25 ± 0.02 ^b	0.0132 ± 0.0122 ^a	0.093 ± 0.040 ^a	14.67 ± 0.56 ^a	2156 ± 131 ^{cd}
VI023379	0.040 ± 0.009 ^b	0.0047 ± 0.0004 ^b	0.18 ± 0.02 ^c	0.0015 ± 0.0008 ^b	0.043 ± 0.004 ^b	10.67 ± 0.28 ^{cd}	1795 ± 282 ^d
AVSB8001	0.050 ± 0.015 ^b	0.0068 ± 0.0024 ^b	0.26 ± 0.05 ^{ab}	0.0038 ± 0.0052 ^{ab}	0.050 ± 0.000 ^b	9.67 ± 1.47 ^{de}	3049 ± 291 ^a
AVSB9301	0.087 ± 0.013 ^a	0.0093 ± 0.0005 ^a	0.31 ± 0.00 ^a	0.0043 ± 0.0017 ^{ab}	0.053 ± 0.016 ^b	11.30 ± 0.63 ^c	2761 ± 515 ^{ab}
Mean ± SD	0.056 ± 0.017	0.0064 ± 0.0019	0.22 ± 0.06	0.0045 ± 0.0062	0.050 ± 0.026	11.33 ± 2.23	2500 ± 502
Vegetable soybean							

VI015726	0.053 ± 0.003 ^a	0.050 ± 0.001 ^{bc}	0.25 ± 0.01 ^{ab}	0.023 ± 0.007 ^{ab}	0.043 ± 0.015 ^a	25.79 ± 5.09 ^a	2853 ± 255 ^a
VI022144	0.070 ± 0.011 ^a	0.043 ± 0.005 ^c	0.19 ± 0.02 ^b	0.013 ± 0.005 ^b	0.043 ± 0.015 ^a	21.38 ± 5.66 ^a	2651 ± 284 ^a
AVSB8001	0.063 ± 0.022 ^a	0.057 ± 0.010 ^b	0.28 ± 0.06 ^{ab}	0.013 ± 0.005 ^b	0.047 ± 0.021 ^a	25.22 ± 1.66 ^a	3061 ± 509 ^a
AVSB9301	0.083 ± 0.021 ^a	0.077 ± 0.009 ^a	0.32 ± 0.06 ^a	0.030 ± 0.009 ^a	0.050 ± 0.011 ^a	22.89 ± 1.90 ^a	3042 ± 333 ^a
Mean ± SD	0.067 ± 0.018	0.057 ± 0.014	0.26 ± 0.06	0.020 ± 0.009	0.046 ± 0.014	23.82 ± 3.89	2902 ± 352

548 ¹ Values are means ± standard deviation. Means within a column with different upper case letters are significantly different at $P < 0.05$. ² standard
549 deviation.

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Table 3

Content¹ of isoflavones in soybean sprouts and vegetable soybean (mg per 100 g edible portion of fresh weight).

Accession / cultivar	Genistein	Daidzein	Glycitein	Genistin	Daidzin
Soybean sprouts					
VI015437	0.71 ± 0.03 ^b	0.24 ± 0.02 ^b	0.007 ± 0.002 ^b	3.27 ± 0.14 ^c	6.43 ± 0.45 ^a
VI015726	0.52 ± 0.10 ^b	0.15 ± 0.03 ^c	n.d. ²	2.15 ± 0.44 ^d	3.41 ± 0.62 ^c
VI016706	0.58 ± 0.16 ^b	0.32 ± 0.02 ^a	0.017 ± 0.001 ^b	3.74 ± 0.11 ^b	4.55 ± 0.08 ^b
VI023379	0.24 ± 0.02 ^c	0.12 ± 0.01 ^c	0.027 ± 0.001 ^a	1.55 ± 0.03 ^e	1.74 ± 0.11 ^d
AVSB8001	1.04 ± 0.22 ^a	0.33 ± 0.01 ^a	0.013 ± 0.005 ^b	5.04 ± 0.32 ^a	6.86 ± 0.29 ^a
AVSB9301	0.64 ± 0.03 ^b	0.22 ± 0.01 ^b	n.d.	3.93 ± 0.14 ^b	4.58 ± 0.11 ^b
Mean ± SD ³	0.62 ± 0.27	0.23 ± 0.08	0.011 ± 0.008	3.28 ± 1.21	4.59 ± 1.81
Vegetable soybean					
VI015726	n.d.	n.d.	n.d.	0.63 ± 0.06 ^b	0.74 ± 0.25 ^b
VI022144	n.d.	n.d.	n.d.	2.25 ± 0.23 ^a	1.66 ± 0.06 ^a
AVSB8001	n.d.	n.d.	n.d.	0.79 ± 0.22 ^b	0.63 ± 0.21 ^b
AVSB9301	n.d.	n.d.	n.d.	0.59 ± 0.16 ^b	0.51 ± 0.07 ^b
Mean ± SD	n.d.	n.d.	n.d.	1.07 ± 0.73	0.89 ± 0.50

Table 3 continued....

Accession / cultivar	Glycitin	6''-O-Malonyl genistin	6''-O-Malonyl daidzin	6''-O-Malonyl glycitin	Total isoflavones
Soybean sprouts					
VI015437	1.91 ± 0.03 ^a	61.06 ± 1.98 ^b	59.20 ± 1.52 ^a	9.81 ± 0.18 ^a	142.61 ± 2.30 ^b
VI015726	0.48 ± 0.06 ^e	51.62 ± 10.19 ^c	33.64 ± 5.95 ^c	3.73 ± 0.28 ^e	95.70 ± 17.19 ^d
VI016706	0.80 ± 0.04 ^c	49.94 ± 2.40 ^c	33.99 ± 1.29 ^c	6.38 ± 0.16 ^c	100.48 ± 3.67 ^d
VI023379	1.17 ± 0.04 ^b	25.14 ± 1.00 ^d	15.16 ± 0.90 ^d	9.72 ± 0.14 ^a	54.87 ± 2.12 ^e
AVSB8001	1.26 ± 0.09 ^b	81.98 ± 2.96 ^a	53.12 ± 2.64 ^b	8.33 ± 0.44 ^b	157.97 ± 6.51 ^a
AVSB9301	0.70 ± 0.03 ^d	67.62 ± 4.53 ^b	34.15 ± 2.69 ^c	4.36 ± 0.07 ^d	116.21 ± 7.44 ^c
Mean ± SD	1.05 ± 0.48	56.23 ± 18.50	38.21 ± 15.06	7.05 ± 2.50	111.28 ± 35.17
Vegetable soybean					
VI015726	0.83 ± 0.17 ^b	15.80 ± 0.93 ^b	13.12 ± 0.81 ^b	5.89 ± 0.34 ^a	37.02 ± 2.53 ^b
VI022144	1.27 ± 0.09 ^{ab}	35.91 ± 2.56 ^a	21.09 ± 1.45 ^a	6.52 ± 0.80 ^a	68.71 ± 4.56 ^a
AVSB8001	1.97 ± 0.56 ^a	23.56 ± 7.01 ^b	11.66 ± 2.78 ^b	10.91 ± 3.27 ^a	49.52 ± 14.00 ^{ab}
AVSB9301	1.45 ± 0.58 ^{ab}	17.85 ± 6.04 ^b	8.77 ± 2.56 ^b	7.91 ± 3.97 ^a	37.08 ± 13.38 ^b
Mean ± SD	1.38 ± 0.55	23.28 ± 9.15	13.66 ± 5.09	7.81 ± 3.00	48.08 ± 16.00

¹ Values are means ± standard deviation. Means within a column with different upper case letters are significantly different at $P < 0.05$. An absence of letters indicates no significant difference was observed. ² not detected; ³ standard deviation.