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ARTICLE

Comprehensive Profiling of Isoflavones, Phytosterols, Tocopherols, Minerals, Crude Protein, Lipid, and Sugar during Soybean (*Glycine max*) Germination

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Isoflavone, phytosterol, tocopherol, mineral, protein, lipid, and sugar contents of soybeans were analyzed during 7-day germination with or without exposure to light. The levels of phytosterols and tocopherols increased significantly during the 3 day germination. Although malonyl glycosides were the predominant forms of isoflavones in soybean seeds, 77% of malonyl daidzin and 30% of malonyl genistin were converted to corresponding daidzin, daidzein, genistin, and genistein during the germination period. Slight decreases in malonal glycidin and malonyl glycidin concentrations were also observed while the total molar concentration of isoflavones remained constant. An increase of approximately 4% in the protein level was accompanied by a 5−6% reduction in the carbohydrate and lipid contents after the 7-day germination. Mineral (Ca, Cr, Fe, Zn Cu, K, Mg, Mn) levels did not vary much during germination, and the presence of light during germination had only a little, if any, effect on the levels of the micro- and macronutrients in soybeans.

KEYWORDS: Soybean germination; isoflavones; phytosterols; tocopherols; mineral; macronutrient

INTRODUCTION

Soybeans are one of the major sources of nutrients in the diets of animals and humans. Many health benefits, such as reducing cholesterol, improving HDL/LDL ratio, increasing bone density, relieving menopause symptoms for women (*1*), and decreasing cancer risk (*2*, *3*) and heart disease risk (*4*−*7*), are attributed to soybean food intake.

Soybean sprouts have been consumed as vegetables for centuries in oriental countries. Sprouting or germination is the fastest growing period of plant life and is known to increase essential nutrient levels, bioavailability, and palatability of soybeans (*8*, *9*). It also decreases antinutritional factors and "beanny" flavors such as trypsin inhibitor, lipoxygenase, and hemagglutinins. Therefore, greater utilization of germinated soybeans in preparing food for humans and animal feed would be very beneficial for both.

Although many beneficial effects of soybean germination have been suggested, comprehensive assessments of important nutrient profile changes during the germination process are still lacking. A more systematic and comprehensive nutrient profile assessment during soybean germination should greatly improve the preparation of healthy food/feed by maximizing the availability of nutrients.

Isoflavone is one of the families of phytoestrogens that are recognized for their well-established positive effects on health, including antioxidative activity, cancer prevention, menopause symptom release, cholesterol lowering, and bone density improvement. Soybeans are considered to be the richest source of isoflavones. Three major types of isoflavones in soybeans can exist in four chemical forms: aglycon (genistein, daidzein, glycitein), glucoside (genistin, daidzin, glycitin), malonyl glucoside (malonyl genistin, malonyl daidzin, malonyl glycitin) and acetyl glucoside (acetyl genistin, acetyl daidzin, acetyl glycitin). Apparently, the aglycon form is the biologically most active isoflavone for mammal metabolism. The effects of water soaking and germination on the isoflavone levels in soybeans have been reported with conflicting results from different researchers (*10*−*12*). This premise deserves more comprehensive investigation to confirm the validity of the reported effects.

Phytosterols are essential for stabilization of plant cell membranes. These molecules also serve as precursors of plant hormones. The presence of phytosterols in the diet is known to reduce serum cholesterol levels because of their structural similarity to cholesterol (*13*). Phytosterols also exhibit antipyretic, anti-inflammatory, antineoplastic, and immunomodulating activity (*14*). Soybeans are rich in phytosterols, which have been reported to increase during soybean germination (*9*), although confirmation is currently lacking.

Soybeans are also rich in tocopherol (vitamin E), a well-known vitamin that is essential for human health (*15*). One report showed that the α-tocopherol content in cotyledons remained the same when soybean seedlings were grown in the dark; however, the presence of light during germination resulted in an increase in the α-tocopherol level, whereas the γ- and δ-tocopherol contents decreased rapidly (*16*). Another study showed that tocopherol

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increased during soybean germination (*17*). Relatively little is known about the impact that soybean germination has on phytosterols and tocopherols, and it deserves more comprehensive investigation.

Light exposure during germination has resulted in increased levels of isoflavones (*18*, *19*). However, this effect of light on isoflavones could not be confirmed by another study which concluded that light does not have a significant effect on the content of isoflavone during germination (*10*). It has also been reported that light has varying effects on the concentrations of some minerals in soybean sprouts (*6*, *20*). Additional information about the effects of light on other nutrients, such as tocopherols, phytosterols, and some of the macronutrients during germination, is absent. Light effects on the nutrient profile changes during soybean germination deserve further study.

Comprehensive assessments of changes in the nutrient profile during the germination process are necessary for the optimization of nutrients in the preparation of soybean food and animal feed. In this study, the micro- and macronutrients in soybeans during the germination process were assessed. The key micronutrients: isoflavones, tocopherols, and phytosterols, were analyzed by high performance liquid chromatography (HPLC) and gas chromatography−mass spectrometry (GC−MS) methods. The contents of major macronutrients soybean proteins, digestible carbohydrates, and lipids were monitored using the AOAC standard method and other published methods. The major mineral elements, Cu, Cr, Mg, Mn, Zn, Ca, Fe, and K, were detected by inductively coupled plasma mass spectrometry (ICP-MS) and flame atomic absorption spectrometry (FAA) methods. This nutrient profile data should greatly benefit future soybean food research and development.

MATERIALS AND METHODS

Chemicals and Reagents. The soybeans used in this study were newly harvested local food-grade soybeans (Missouri Gold soybean). Isoflavones (malonyl genistin (MGN), malonyl daidzin (MDN), malonyl glycitin (MGL), daidzin (DN), genistin (GN), glycitin (GL), daidzein (DEN), genistein (GEN), and glycitein (GLE)), tocopherols (α-, γ-, δ-tocoperol), phytosterols (campesterol, stigmasterol, β-sitosterol), 5α-cholestane, apigenin (APN), and cholesterol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) at the highest purity (>97%) available. A standard stock solution containing a mixture of Cu, Cr, Mg, Mn, Zn, Ca, Fe, and K was purchased from Perkin-Elmer (Norwalk, CT). DMSO, acetonitrile, acetic acid, ascorbic acid, methanol, hexanes, anhydrous sodium sulfate, trace-metal-grade nitric acid, $FeCl_3 \cdot 6H_2O$, sulfosalicylic acid, and all other chemicals and solvents were purchased from Fisher Scientific (Pittsburgh, PA).

Soybean Germination. The soybeans were first washed and soaked in drinking tap water for 8 h, prior to being placed in a sprouter to start germination. The germination temperature was 25 °C. To determine whether light exposure would have any impact on the nutrient levels of soybeans during germination, a 100 W incandescent light bulb was placed at a distance of 1 ft above the sproutor. The germination test in the dark was conducted in a dark chamber without any light during the entire time. The germinating soybeans were rinsed with fresh water twice daily. A small portion of the bean sprouts was removed each day before rinsing for micronutrient and macronutrient analysis. The soybeans were germinated for up to day 7. All the soybean samples were pat-dried with a paper towel to remove extra water on the sprouts and weighed prior to lyophilizing with a Labconco freeze-dryer. The dried samples were weighed to determine the moisture content and were then finely ground. The ground dry samples were stored in sealed bottles at −20 °C in a manual-defrost freezer in the dark until the nutrient analysis.

Isoflavone Analysis. Isoflavones were extracted by following a published procedure (*21*). Briefly, a 0.50 g ground, dry soybean sample was weighed into a vial and 0.2 mL of apigenin (APN) internal standard (2 mg/mL in DMSO) was added. After mixing, isoflavones were extracted with acetonitrile/water (5 mL/3 mL) mixture in a vial capped with a Teflon linear cap by shaking for 2 h on a shaker. An additional 1.8 mL of water was then added to make the total solvent volume 10 mL. The sample was centrifuged, and the supernatant was filtered with a 0.45 μm nylon membrane filter before HPLC analysis. The isoflavone analysis was performed with a Perkin-Elmer ISS 200 HPLC system (Norwalk, CT) which included a binary pump, an autosampler, and a photodiode array detector. Separation was accomplished using a Waters C18 column (5 μm particle size, 150 × 3.9 mm); the mobile phase solvent A was 0.1% acetic acid in water, and solvent B was 0.1% acetic acid in acetonitrile; the flow rate of the mobile phase was 1.5 mL/min. The linear gradient was from 10% to 30% B in 30 min, then 90% B for 5 min. The column was equilibrated with 10% B for 7 min between runs. UV absorbance at 260 nm was used for the quantitation of all isoflavones. The calibration curves were prepared in linear concentration ranges of from 2 mg/L to 100 mg/L.

Tocopherol and Phytosterol Analysis. For tocopherol and phytosterol extraction, a published method (*22*) was used with some modification. The new method using gas chromatography−mass spectrometry allowed simultaneous and rapid determination of tocopherols and phytosterols. One gram of the sample was accurately weighed into a 50 mL screw capped glass vial. A 5α-cholestane surrogate was added, along with 0.1 g of ascorbic acid, as an antioxidant for prevention of tocopherol oxidation during the extraction procedure. After adding 10 mL of 2 N KOH in methanol, the vial was capped tightly and subjected to ultrasonication for 15 min at 65 °C. The unsaponifiables were extracted three times with 10 mL of hexane each time. The pooled hexane extract was washed with 2.5% NaCl aqueous solution to remove any saponifiable residue. The sample was concentrated to 2 mL by rotary-evaporation, followed by nitrogen blow-down. Cholesterol was added as an internal standard prior to GC−MS analysis.

For the GC−MS analysis, a Hewlett-Packard 5890 series-II GC interfaced with a HP5971 series mass selective detector was used. The column was a 5 m length fused silica capillary column with 0.25 mm i.d. and 0.25 μm film of 100% polysiloxane (J&W scientific, Folsom, CA). Helium was used as a carrier gas at a constant flow rate of 1.54 mL/min. The GC oven was programmed from 200 to 270 °C at a rate of 20 °C/min. GC injection port temperature was held at 270 °C, and the transfer line was at 280 °C. An extracted ion chromatogram was used for quantification. The data was collected and processed with HP ChemStation software.

Mineral Analysis. The mineral concentrations of germinated soybeans were determined by ICP-MS and FAA methods after acid digestion. The conventional acid digestion method was used. Each 0.5 g subportion of freeze-dried soybean sample was digested with 15 mL of trace-metal-grade nitric acid. After complete digestion, the nitric acid was heat evaporated to a volume of about 1 mL, and the sample was then diluted to 50 mL with ultrapure water. Cu, Cr, Mg, Mn, and Zn were analyzed by ICP-MS, after appropriate dilution, while Ca, Fe, and K were detected by FAA. An Elan 9000 ICP-MS system (Perkin-Elmer SCIEX, Norwalk, CT) equipped with a cross-flow type nebulizer and nickel cones was used. The plasma rf power was 1000 W. Argon flow rates for the plasma and auxiliary gas were 15 L/min and 0.95 L/min, respectively. Samples were delivered at 1 mL/min by a peristaltic pump. The initial instrument calibration was carried out in a linear concentration range of 0.1−500 μg/L. The FAA used was a model 3100 flame atomic absorption spectrometer (Perkin-Elmer SCIEX, Norwalk, CT). The digested sample solution was directly aspirated into an air−acetylene oxidizing flame after appropriate dilution and the absorbance was recorded for quantification. Fe, Ca, and K single element hollow cathode lamps were used to detect the corresponding element. The concentrations were obtained with an external standard calibration method in a linear range of the calibration curves.

Total Sugar Analysis. Total sugar level in the germinated soybeans was determined following a published rapid procedure (*23*). Each subportion of 150 mg of ground, dry germinated soybean was mixed with 10 mL of D.I. water and 1 mL of concentrated HCl. After the autoclave for 20 min at 120 °C, the mixture was cooled and neutralized with 275 μL of 40% NaOH. The final sample was diluted with water and the total sugar content was determined by using the phenol-sulfuric acid method. Glucose was used as a calibration standard at a linear concentration range of 10 to 120 mg/L.

Crude Protein Analysis. Crude protein was detected by following the AOAC standard method using a FP-428 nitrogen/protein analyzer

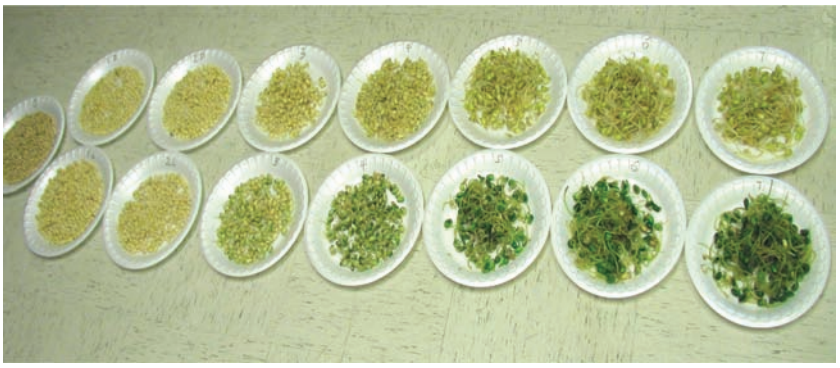

Figure 1. Soybeans germinated with (bottom row) or without (top row) exposure to light. Daily samples from 7-day germination period are arranged from left to right.

Table 1. Moisture Content of Soybeans during Germination

sample ID	description	moisture (%)
seed	soybean seeds before soaking	6.17
1D	8 h soaked soybeans in the dark (day 1)	57.81
1L	8 h soaked soybeans under light (day 1)	57.37
2D	day 2 germinated in the dark	60.58
2L	day 2 germinated under light	59.98
3D	day 3 germinated in the dark	65.87
3L	day 3 germinated under light	65.69
4D	day 4 germinated in the dark	70.44
4L	day 4 germinated under light	69.72
5D	day 5 germinated in the dark	76.13
5L	day 5 germinated under light	74.95
6D	day 6 germinated in the dark	79.81
6L	day 6 germinated under light	79.00
7D	day 7 germinated in the dark	82.52
7L	day 7 germinated under light	81.21

(LECO, St. Joseph, MI). A subportion of 60 to 100 mg of the freeze-dried, ground soybean sample was used for the detection of each replicate.

Lipid Analysis. The total lipid content of the soybean sample was determined using a method similar to that used by Kim et al. (*24*). A 1 g aliquot of the freeze-dried, ground soybean sample was extracted with 10 mL of hexane with shaking for 1 h. A 5 mL portion of the supernatant, after centrifugation, was transferred to a preweighed vial. The hexane was evaporated using a ~60 °C heating block until a constant weight was reached. The weight of the extracted oil was calculated.

Quality Control and Statistical Analysis. The analytical methods to be used were validated before the samples were analyzed. Blank, spike recovery, reproducibility, instrument calibration and linear range were all tested and continuously monitored during sample analysis. All of the samples were analyzed in triplicate and the data were carefully reviewed for statistical validity. The results were given as a mean of the triplicate data, and the relative standard deviation (RSD) was calculated for each sample. Differences between variables were tested for significance by using Student's *t* test.

RESULTS AND DISCUSSION

Physical Appearance of Soybean Germinated with/without Light Exposure. We used incandescent light to simulate natural sunlight, which is different from methods reported by others that used certain wavelengths of light during germination study (*18*, *19*, *25*). **Figure 1** shows the appearance of soybeans at different times during the 7-day germination period, with and without exposure to light. The soybeans germinated without light exposure maintained a yellow color, while those exposed to light gradually changed to a green color due to the photosynthesis of chlorophyll. The green-colored soybean sprouts looked like those reported by Kim et al. (*18*) who used different light conditions.

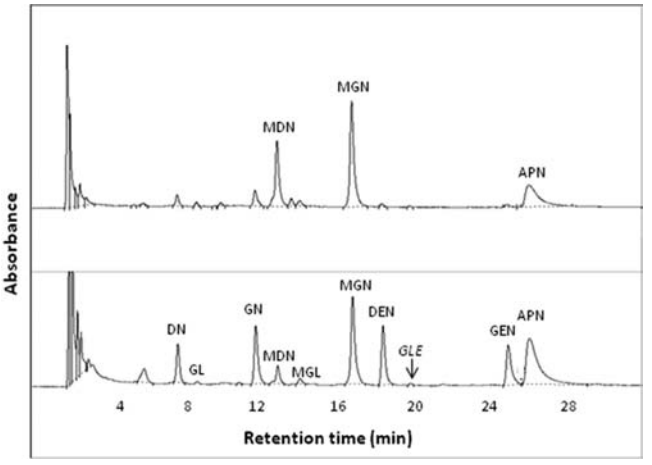

Figure 2. HPLC separation of isoflavones extracted from germinated soybeans: top, day 1 germinated soybean in the dark; bottom, day 6 germinated soybean in the dark.

Moisture Content of Soybeans during Germination. The moisture content of the soybean sprouts was monitored daily during germination. **Table 1** shows the moisture content of germinated soybeans, with and without light exposure, during germination. The moisture content of the soybeans increased from 6% to 57% during the 8 h of initial soaking in water. This result agreed with the reported moisture increase in soaked soybeans with a moisture content of 55.95 to 57.87% (*11*). The moisture content continued to increase gradually from 57 to 82% within the 7-day germination duration. Light exposure only slightly (1.5%) lowered the moisture content of germinated soybeans.

Isoflavone Profile of Soybeans during Germination. The soybeans' isoflavone profile changed dramatically during the germination process. Representative chromatograms in **Figure 2** show the isoflavone changes. The top panel of the figure is a chromatogram of a soaked soybean sample (soaked for 8 h in the dark) at the beginning of germination; the bottom panel is a chromatogram of a soybean sample germinated to day 6. MGN and MDN were the predominant isoflavones found in soybeans before germination. During germination, MGN and MDN decreased, with corresponding increases of DN, GN, DEN, and GEN, presumably by the loss of malonyl moiety, and then further loss of glycoside (*26*, *27*). Data documenting changes in the isoflavone profile during the 7-day germination (with/without light) are tabulated in **Table 2**. In the table, the total isoflavone for each sample was the sum of all of the malonyl glycone forms, glycone forms, and aglycon forms. Acetyl glycoside forms of isoflavone acetyl daidzin, acetyl genistin, and acetyl glycitin were not

analyzed since they are generally present only in fairly low concentrations in soybeans and soybean sprouts. The aglycon form of isoflavones, DEN and GEN, increased the most during the 7-day germination period. DEN increased 4.9 times (7D/1D ratio) in the dark and increased 6.6 times (7 L/1 L ratio) under light; GEN increased a little more slowly, 2.3 times in the dark and 4.0 times under light; the glycoside isoflavones (DN and GN) increased approximately 3.3 and 2.5 times respectively, while the level of corresponding malonyl glycoside isoflavones decreased. The glycitin isoflavone group behaved differently from the other two groups of isoflavones during germination; both MGL and GL decreased while GLE was not detectable in all of the samples. Although individual isoflavone profiles changed drastically during germination, the total molar concentrations of isoflavones remained almost constant. This indicated that isoflavones convert to different forms, but do not convert to any significant extent to other nonisoflavone compounds during this germination period.

Table 2. Isoflavone Content of Soybeans during Germination with/without Light[a]

	isoflavone concns (μM/g)								
sample ID	DEN	DN	MDN	GEN	GN	MGN	GL	MGL	total
RD	<MDL	0.269	2.408	0.095	0.424	1.913	0.157	0.236	5.502
1D	0.219	0.322	1.929	0.225	0.381	2.233	0.153	0.224	5.685
1L	0.204	0.332	2.096	0.229	0.380	2.460	0.164	0.222	6.087
2D	0.225	0.322	2.037	0.220	0.350	2.463	0.158	0.227	6.002
2L	0.202	0.313	1.936	0.201	0.345	2.270	0.148	0.238	5.653
3D	0.226	0.329	1.901	0.216	0.347	2.381	0.102	0.239	5.741
3L	0.195	0.317	1.902	0.189	0.297	2.388	0.134	0.248	5.671
4D	0.231	0.367	1.682	0.181	0.350	2.159	0.124	0.226	5.321
5D	1.039	0.615	0.563	0.699	0.666	1.488	0.141	0.177	4.349
5L	1.056	0.601	0.600	0.776	0.629	1.546	0.098	0.173	5.478
6D	1.074	0.605	0.419	0.826	0.703	1.238	0.087	0.190	4.068
6L	1.141	0.680	0.374	0.907	0.735	1.118	0.087	0.177	5.219
7D	1.080	1.066	0.448	0.515	0.955	1.561	<MDL	0.186	5.811
7L	1.333	0.744	0.308	0.906	0.774	0.954	<MDL	0.170	5.230
7D/1D ratios	4.938	3.313	0.232	2.286	2.503	0.699		0.832	1.022
7L/1 L ratios	6.547	2.239	0.147	3.950	2.037	0.388		0.765	0.859

[a] Concentration unit is μmol/gram dry sample. Mean values from triplicate samples are reported. MDL = method detection limit.

GEN and DEN are biologically more active forms of isoflavones. An important health benefit offered by germination is the production of more biologically available forms of isoflavones (*28*). Our results were not consistent with a previously reported study (*10*) in that all isoflavones increased remarkably after 5 days of germination.

There are conflicting results in the literature on the impact of isoflavone levels obtained by soaking soybeans in water (*10*, *11*). Comparing GEN and DEN in soybean seeds soaked for 8 h (**Table 2**) in our study, DEN increased from being nondetectable to 0.219 μmol/g, and GEN increased from 0.269 to 0.322 μmol/g. These results agree with those reported by Lee et al. (*10*), but are different from other results showing that the DEN content does not increase after 5 and 10 h soaking (*11*). Most recently, Goes-Favoni et al. (*12*) also reported the increases of aglycons, GEN and DEN, to 0.5 and 0.8 μmol/g, respectively, when soybeans were soaked in water. In addition, we detected a very low aglycon isoflavone content that agreed well, but was different with the results from Lee et al. (*10*) in which DEN and GEN concentrations were much higher in soybean seeds that had not been soaked. Our result that showed very low or nondetectable DEN and GEN concentrations and high levels of malonyl glycoside forms in nonsoaked soybean seeds may represent the true isoflavone forms in newly harvested soybeans. The soybeans used in our study were freshly harvested and stored for a very short period.

Light exposure had only a slight impact on the isoflavone levels in this study. No significant effect from light exposure on the isoflavone levels of soybeans during the early germination stage was observed. Aglycons, DEN and GEN, increased more during the later stage (after day 5) of germination, when light was present. The same phenomenon was not seen with other types of isoflavones. Although these results were in general agreement with results reported by Lee et al. (*10*), another study showed that varying light conditions cause different effects on the isoflavones in soybean seedlings, while the soybean genotypes play an extra role (*25*).

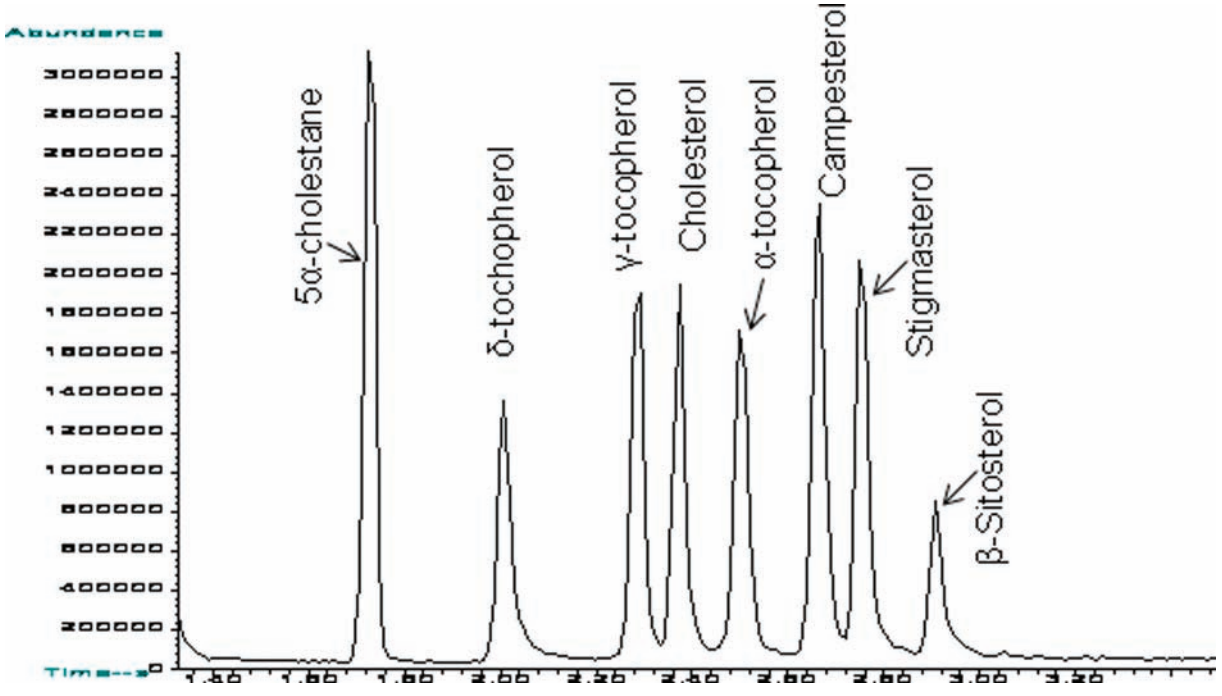

Figure 3. Rapid gas chromatographic separation of phytosterols and tocopherols.

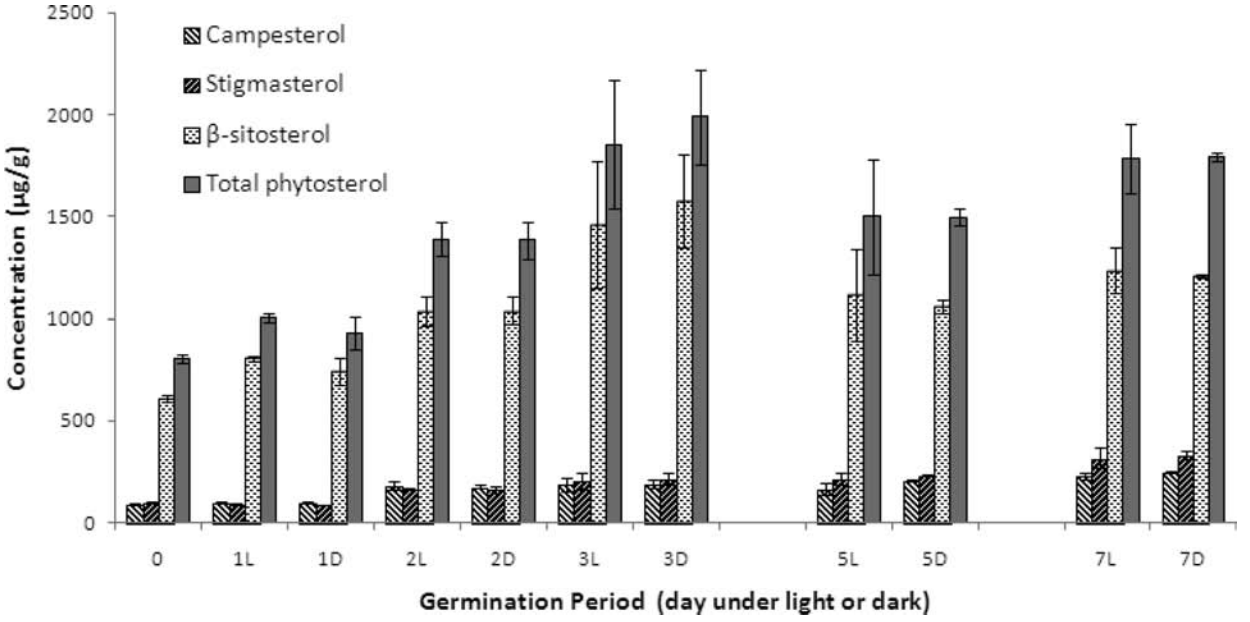

Figure 4. Phytosterol profile of soybeans during germination (L) with or (D) without light.

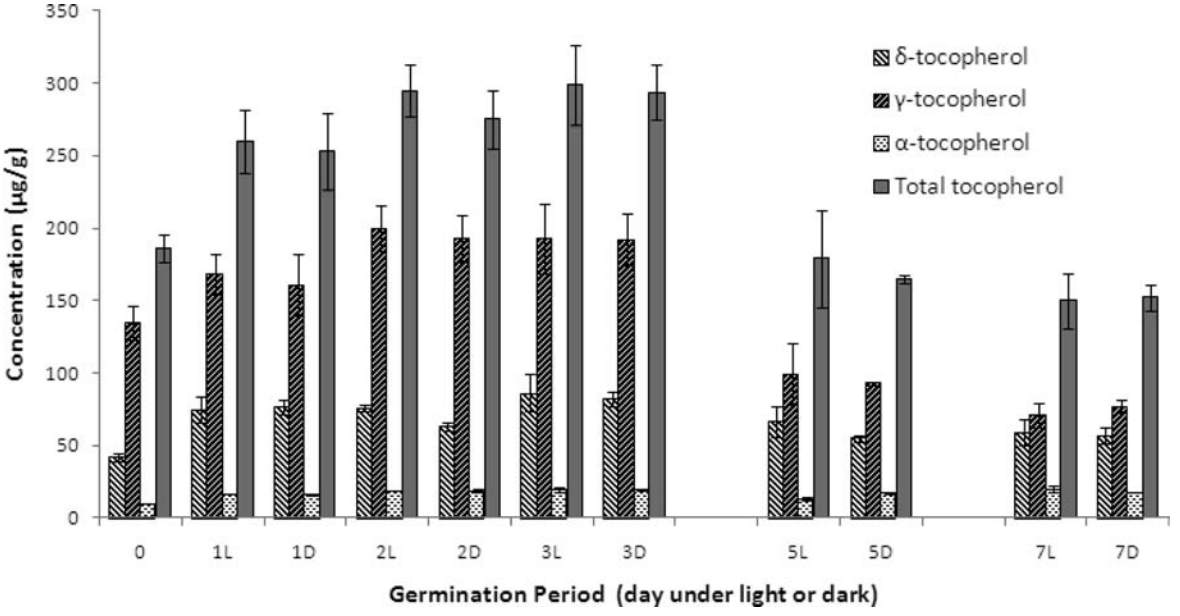

Figure 5. Tocopherol profile of soybeans during germination (L) with or (D) without light.

Tocopherol and Phytosterol Profile of Soybeans during Germination. The GC/MS separation of the phytosterol and tocopherol standard mixture is shown in **Figure 3**. Using the new rapid GC−MS method, all of the major tocopherols and phytosterols were separated and detected simultaneously in 3 min. This new method allowed greater efficiency, when compared to conventional methods (*13*). The total phytosterol level varied from 1004 to 1987 μg/g during the 7-day germination, with the β-sitosterol as the predominant form. The profile changes of phytosterols are shown in **Figure 4**. The phytosterol concentrations increased from day 1 to day 3, almost doubled, and then remained at an almost constant level. Soybeans are rich in phytosterols. The concentrations of β-sitosterol, campesterol, and stigmasterol were reported as147.1 mg/100 g, 82.8 mg/100 g, and 81.4 mg/100 g, respectively (*9*). Very little information about the effects of germination on the phytosterols is available, and confirmation is lacking although increased phytosterol levels (β-sitosterol 222.7 mg/100 g, campesterol 94.9 mg/100 g, stigmasterol 99.9 mg/100 g) were reported during germination (*9*).

Tocopherol, a vital antioxidant present in many foods, reduces the risk of cardiovascular diseases and cancer. It has also been reported to be able to slow the aging process. Soybeans are a good dietary source of this key antioxidant. The concentration of tocopherol in germinating soybean was found to range 150−299 μg/g, with γ-tocopherol as the major form, followed by δ-tocopherol, with α-tocopherol as the lowest. The profile changes of tocopherols are shown in **Figure 5**. γ- and δ-tocopherol levels increased from day 1 to day 3, and then decreased gradually; α-tocopherol concentrations were low and remained constant during the germination process. To date, not much research has been conducted to determine the effect of soybean germination on tocopherol concentration. In contrast, Feldheim et al. (*29*) reported that a 45-day growth of soybeans increased α-tocopherol and β-tocopherol (from approximately

Table 3. Mineral Concentrations in Germinated Soybeans[a]

	mineral concn							
	(mg/g)			(μg/g)				
sample ID	K	Mg	Ca	Fe	Zn	Mn	Cu	Cr
seed	20.4	3.23	0.786	73.0	55.2	33.2	16.4	2.00
1D	18.4	3.29	0.760	80.8	46.0	30.4	16.8	2.21
1L	18.6	3.21	0.735	85.7	49.4	33.4	16.1	1.97
2D	18.5	3.06	0.753	76.3	49.1	33.8	16.3	1.57
2L	19.3	3.19	0.806	83.9	48.8	32.5	15.7	2.06
3D	18.9	3.29	0.728	77.6	50.4	32.5	17.0	1.65
3L	18.9	3.29	0.681	74.5	47.1	32.5	16.4	2.15
4D	18.7	3.33	0.824	79.1	48.6	31.9	16.6	1.65
4L	19.8	3.17	0.724	79.2	50.5	33.5	17.2	1.96
5D	19.3	3.18	0.778	80.6	50.2	31.6	15.0	1.16
5L	19.4	3.50	0.740	76.9	52.9	33.5	16.3	1.61
6D	18.7	3.27	0.836	77.9	50.9	32.3	16.4	1.50
6L	20.3	3.44	0.814	82.6	56.0	33.2	16.4	2.15
7D	19.1	3.33	0.731	55.0	52.8	32.2	16.6	1.36
7L	19.3	3.53	0.747	66.0	59.0	32.4	15.0	1.28

[a] Mean values from triplicate samples are reported.

2 to 8 mg/100 g), whereas δ-tocopherol decreased from 7−8 to 5−6 mg/100 g; γ-tocopherol tended to decrease. Due to the large differences in germination duration and conditions employed, direct comparisons of results from two different studies are not appropriate.

Light exposure had no significant effect for either phytosterol or tocopherol levels during the germination phase.

Mineral Content of Soybeans during Germination. The mineral concentrations of Cu, Cr, Mg, Mn, Zn, Ca, Fe, and K in germinated soybeans are shown in **Table 3**. The reproducibility of replicated samples yielded relative standard deviations of less than 10%, except for a few samples with up to 18.3% for Cr. The highest mineral concentration in the germinated soybean was K (~19 mg/g), followed by Mg (~3.3 mg/g) and Ca (~0.8 mg/g). Zhou et al. (*30*) reported that soybean meal contained 16.15 g/kg of K, 2.93 g/kg of Mg, and 2.65 g/kg of Ca. The lowest concentration was Cr, at around 1 to 2 μg/g. The concentrations of K, Mg, Ca, Mn, and Cu did not change significantly during germination. Zn concentration increased slightly in the late stage of germination, while the Fe concentration fluctuated more during germination. Although Cr concentrations varied for different samples, its concentration was close to the method detection limit (MDL), so the variations were probably due to analytical inaccuracy, not to concentrations in the samples. Our results for Ca and Mg did not agree with the reported increase of Ca (11.4%) and Mg (21.47%) in the 5-day germinated soybeans (*8*). On the other hand, Lee et al. (*20*) reported decreasing K, Ca, Fe, and Mg levels in soybeans during germination and attributed the losses to leaching into the soaking water.

The effect of light on mineral concentrations has not been reported. Our study showed that light exposure during germination had no significant effect on the level of minerals in soybeans.

Protein, Lipid, Sugar Content of Soybeans during Germination. The major macronutrients of crude protein, lipid, and digestible carbohydrate profiles during soybean germination are shown in **Table 4**. Crude protein in dry soybean seeds was 42.4%, but it increased to approximately 46% during 7-day germination. Relative standard deviations of triplicate samples ranged from 0.26% to 2.12% in this protein analysis. This finding agrees with the generally reported protein increase during soybean germination, as reviewed by Bau et al. (*8*) and more recent reports (*31*). However, the scales of increase vary, presumably due to the differences in soybean cultivars and germination conditions.

Table 4. Macronutrients Levels in Soybeans during Germination[a]

	protein		sugar		oil	
sample ID	% mean	RSD	% mean	RSD	% mean	RSD
seed	42.4	2.12	19.9	2.00	18.3	2.30
1D	42.3	0.66	19.4	4.94	19.5	1.13
1L	41.9	1.24	20.9	5.62	18.3	6.31
2D	43.3	0.45	20.8	1.10	20.5	2.55
2L	43.1	1.21	21.9	5.64	20.9	2.61
3D	43.5	0.64	19.2	5.37	20.5	0.93
3L	43.8	1.36	19.7	1.91	21.0	3.45
4D	43.2	1.22	17.6	6.80	19.6	0.00
4L	44.5	0.52	16.7	3.90	20.1	2.33
5D	43.3	1.96	14.8	1.82	17.0	1.73
5L	43.8	0.78	14.7	7.32	17.0	1.59
6D	44.0	0.26	14.0	12.01	16.3	0.43
6L	44.9	1.41	13.9	7.66	16.6	0.32
7D	45.4	0.55	14.2	2.06	15.9	0.38
7L	45.6	0.57	14.5	6.03	14.8	3.89

[a] Mean values from triplicate samples are reported.

The sugar content in dry soybean seed was 19.9%, but it decreased to 14% during 7-day germination. The lipid content decreased from 20% to 15% in this study. These results further confirm the results reported by other published studies, as summarized in a review (*8*). The degradation of lipids and carbohydrates during germination is a process whose essential purpose is to provide the energy required for protein synthesis. This result indicated that germination also had a beneficial effect on macronutrients, though the changes were relatively small. Exposure to light during germination had very little effect on the macronutrient level.

In conclusion, the levels of phytosterols and tocopherols in soybeans increased significantly during germination. The maximum concentrations of these micronutrients were observed on day 3 of the germination period. Malonyl glycosides were the predominant forms of isoflavones in seeds and during the early stage of germination. Malonyl daidzin and malonyl genistin represented 43.8% and 34.8% of the total isoflavones, respectively, on the molar component base in dry soybeans. The 8 h water soaking at 25 °C decreased the malonyl glycoside species and increased the glycoside and aglycon species. As the sprouting progressed, malonyl daidzin and malonyl genistin converted to their more bioactive glycoside and aglycon species. The total molar concentration of isoflavones did not change significantly during germination, although a slight decrease was observed under light germination. As expected, crude protein level increased gradually from 42% to 46%, while the sugar content decreased from 20% to 14%, and the lipid content decreased from 20% to 15%, during the 7-day germination period. The mineral levels were mostly unchanged. Light exposure during germination did not have a significant effect on the monitored nutrients, except the color of the soybean sprouts changed to green due to the photosynthesis of chlorophyll. Thus, germination of the soybean is a health beneficial process for soy food preparation.

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