

Evaluation of crude protein, crude oil, total flavonoid, total polyphenol content and DPPH activity in the sprouts from a high oleic acid soybean cultivar

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Abstract

Soybeans [*Glycine max* (L.) Merrill] are a rich source of antioxidants and other phytonutrients. Soybean sprouts contain many biologically active secondary metabolites and are rich in polyphenols, flavonoids, and phenolic compounds. In the present study, two soybean cultivars, Hosim, with high oleic acid (- 80% in total seed oil), and Pungsannamul, with normal oleic acid (- 23%) in seed, were examined for changes in the content of crude protein, crude oil, total flavonoids, total phenolics, and DPPH (1,1-diphenyl-2-picryl-hydrazyl) during the sprouting duration of 5 days. The protein content in both the varieties was found to increase by the days of sprouting. The crude oil content of Pungsannamul sprouts was found to be maximum on day 1 (16.9%, w/w) and decreased thereafter to reach to the level of 14.8% on day 5. No significant differences in the crude oil content of Hosim sprouts from day 1 to 5 were observed. Flavonoid content was found to increase up to day 4 and then dropped on day 5, in both the cultivars. Total polyphenol content showed a tendency to increase up to day 3 and started to decrease significantly from day 4. DPPH activity was found to increase up to day 5 in both the varieties. All the components studied in the high oleic acid soybean sprouts showed a change in content during the sprouting process similar to the change that would occur in normal oleic acid soybeans. The study showed that the contents of antioxidant, flavonoid, and polyphenol significantly increase during the sprouting.

Keywords: soybean, sprout, high oleic acid

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the important legume crops in the world. It is a valuable source of oil for human consumption and industrial usage, and a source of high quality proteins for human and livestock (Lee et al., 2007a; Asekova et al., 2014).

Soybean sprouts have been a common vegetable for centuries, particularly in the areas where seasonal vegetables are unavailable during the winter season (Shi et al., 2010; Yang et al., 2015;



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Ghani et al., 2016). Soybean sprouts have a distinctive flavor and supply many phytonutrients that are easily digestible (Plaza et al., 2003). Due to the presence of health-promoting phytochemicals with high antioxidant properties, soybean sprouts provide numerous health benefits like reducing the risk of cardiovascular diseases and cancer (Prakash et al., 2007). Soybean sprouts also contain phytic acids and saponins, which can interfere with mineral absorption during digestion (Urbano et al., 2000). Such components hinder the bioavailability of the nutrients, and hence considered unwanted in the sprout-based diets. Sprouting process reduces the content of these anti-nutritional components in the seeds (Doblado et al., 2007; Shi et al., 2010) and enhance the bioavailability of zinc, iron and calcium through the breakdown of these anti-nutrients (Bau et al., 1997; Plaza et al., 2003).

Soybean sprouts contain high amounts of antioxidants and nutritional compounds such as vitamin C, flavonoids, isoflavones, and DPPH (Lorenz and D'Appolonia, 1980; Mostafa et al., 1987; Zhu et al., 2005; Lee et al., 2007b; Murugkar et al., 2013). Phenolic compounds like isoflavones, tannins, and flavonoids are known to occur in the soybean seeds (Seo and Morr, 1984). The abundance of these chemicals depends on species and stage of growth (López-Amorós et al., 2006).

Flavonoids are the primary phenolics in the soybean sprouts, that give greater antioxidant activities according to DPPH (1,1-diphenyl-2-picryl hydrazyl) antioxidant assay (Khang et al., 2016). The isoflavones in the soybeans are identified as aglycones (daidzein, genistein, glycitein), glucosides (daidzin, genistin, glycitin), malonylglucosides, (malonyldaidzin, malonylgenistin, malonylglycitin), and acetyl glucosides (acetyl daidzin, acetyl genistin, acetyl glycitin) (Lin and Lai, 2006). Antioxidant compounds play a vital role in protecting body's cells from being damaged by free radicals of reactive oxygen species (Devasagayam et al., 2004). Antioxidant activity in soybean sprouts have been confirmed by the presence of DPPH, flavonoid, and phenolics where all of them showed increases in their content and activity in soybean sprouts compared to the soybean seeds (Zhou and Yu, 2006; Kim et al., 2006; Chon et al., 2013a). The total phenolic (TP) and total flavonoid (TF) content is highly correlated with antioxidant or with antioxidant enzyme activities (Chon, 2013b). Among the different legume crops studied by Chon et al. (2013a), soybean sprouts have showed highest levels of TP and TF ($p < 0.05$) content. Composition of several nutrients like total protein content and fatty acid composition (Orhan et al., 2007; Dhakal et al., 2009; Koo et al., 2015), total oil content (Orhan et al., 2007), TP content (Chon et al., 2013a; Chon, 2013b; Koo et al., 2015), TF and isoflavone contents (Orhan et al., 2007; Lee et al., 2007b; Chon et al., 2013a; Chon, 2013b), and antioxidant compounds such as DPPH and ABTS (2,21-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) (Xu and Chang, 2007; Koo et al., 2015; Khang et al., 2016), tocopherols, tocotrienols, and β -carotene (Zielinska-Dawidziak and Siger, 2012), and vitamins (Plaza et al., 2003; Youn et al., 2011) in the soybean sprouts have been investigated. Recently, soybean genotypes with high oleic acid, up to 80% of the oil concentration, have been developed (Pham et al., 2010; Lee et al., 2012; Kim et al., 2015). Such genotype has been analyzed for the alterations in the saturated fatty acid composition during the sprouting process (Dhakal et al., 2014); however, they have not yet been tested for other important compounds whose content may be altered in the soybean sprouts.

The objective of this study was to investigate the variation and composition of antioxidants, proteins, and oils in seeds and sprouts of two soybean cultivars, Hosim containing 80% oleic acid of total oil and Pungsannamul (Suh et al., 1997) having normal (22%) oleic acid in the seed oil.

Materials and Methods

Plant materials and sprouting process

Pungsannamul (Suh et al., 1997), a cultivar with normal oleic acid content in the seed oil, and highly popular for soybean sprout usage in Korea and Hosim, a high oleic acid (- 80%) soybean cultivar in seed, were used in this study. Dry and healthy seeds from these two cultivars were used for the germination assays in two replications. Sprouting process was performed according to the method described in Lee et al. (2007a) and Dhakal et al. (2014). Briefly, a total of 20 g of seeds for one replication of each variety were kept in plastic bottles (6 W × 6 L × 15H cm) of the same size and having several small holes drilled at the bottom for drainage. The bottles containing soybean seeds were submerged in a water bath set at 22°C to initiate germination. The seeds were kept in the water bath for 4h and then moved to a growth chamber set at 22°C and 80% relative humidity. A submersible pump connected to nozzles placed above the bottles in a container was set with a timer for spraying water on the soybean seeds for 4 minutes after every 4 hours. The sprouts were harvested for every day as a separate time-point, wrapped in aluminum foil and kept in the refrigerator until samples from each sprouting duration were harvested. Then, seed (control) and sprout samples from all the 5 day time-points were freeze dried, finely ground and stored in small, sealed plastic bags until nutrient analysis.

Analysis of protein, oil, flavonoid, polyphenols and DPPH

Crude protein

Crude protein content of each sample was analyzed using the Kjeldahl method (AOAC, 1990). Approximately, 1 g of each sample was used to determine the percentage of nitrogen content. A nitrogen auto-analyzer (VAP50sC, Gerhardt, Germany) was used to measure the nitrogen content, which was then multiplied by 6.25 to determine the protein content.

Crude oil

Crude oil for each sample was determined according to the 'association of official analytical chemists' method (AOAC, 1990). Two grams of each sample was put in a cotton wool tube and subsequently put into Gerhardt glass filled with 150 mL of hexane and having a boiling stone in it. The oil extracting machine, Soxtherm apparatus (Gerhardt, Bonn, Germany) operated for 4h. The extracts were then transferred to an oven at 100°C for 1h to allow full evaporation of the hexane and then allowed to cool for 15-20 min. Thereafter, the content of oil was calculated according to AOAC (1990).

Total flavonoids

One gram of each sample was mixed with 10 mL of methanol and incubated 24h for nutrient extraction and extracts were separated from residue and analyzed. From the plant extracts, flavonoids were analyzed according to the procedure given by Kim et al. (2013). The TF content of sprouts was measured using aluminum chloride calorimetric assay. To construct a standard curve, 0.4 mL of quercetin and 0.6 mL of methanol were added to make a standard solution of 2,000 ppm, serial dilutions of the concentrations 2,000, 1,000, 500, 250, 125, 62.5 ppm to were prepared from the standard solution. The tubes were shaken and well mixed. A total of 500 µL methanol, 50 µL of 10% AlCl₃, 50 µL of 1 M NaOH, and 300 µL distilled water were added together and an orange yellowish color instantly developed.

The solution was incubated in the dark for 30 min and then vortexed. About 200 μL of the sample from each replication was transferred to 96-plate and put in analyzing machine. The absorbance was measured at 510 nm using a spectrophotometer (Multiskan GO, Vantaa, Finland) to determine the flavonoid content.

Total polyphenols

One gram of each sample was mixed with 10 mL of methanol and incubated 24h for nutrient extraction. The extracts were separated from residue and analyzed according to the method by Kamtekar et al. (2014) employing Folin Ciocalteu's reagent. To construct a standard curve, 0.4 mL of gallic acid and 0.6 mL distilled water were mixed to make a standard solution of 2000 ppm, and serial dilutions of 2,000, 1,000, 500, 250, 125 and 62.5 ppm concentration were prepared. The tubes were shaken and mixed well. One mL of 2% Na_2CO_3 was added and allowed to mix for 3 min. After that, 50 μL Folin (1N) reagent was added to develop an intense blue color in the tube. The mixture was incubated for reaction for 30 min in the dark and vortexed. About 200 μL of this mixture from each replication was transferred to 96-plate and put in analyzing machine. The absorbance was measured at 750 nm using spectrophotometer (Multiskan GO, Vantaa, Finland).

DPPH (1-1 Diphenyl-2-picryl-hydrazyl)

One gram of ground powder of sprouts was mixed with 10 mL of methanol and incubated for 24h for nutrient extraction. The extracts were separated from residue and analyzed. Plant extracts from the sample was immediately transferred in the 96-well plate. DPPH activity in the sample was determined by the method described by Bilal et al. (2016), with slight modifications. Briefly, 0.1 mM solution of DPPH in 100% methanol was prepared. The samples and DPPH solutions were mixed in the 1:1 ratio and shaken vigorously (Bilal et al., 2016). The samples were allowed to react in the dark for 30 min and the absorbance was measured using spectrophotometer (Multiskan GO, Vantaa, Finland) at 517 nm. The scavenging effect (%) of DPPH solution was calculated according to Romero et al. (2014).

Statistical analysis

Data were analyzed using SAS 9.3 (SAS Institute, Cary NC) statistical package to perform analysis of variance (ANOVA). The significant differences among treatment means were identified by Duncan's Multiple Range Test (DMRT) at 5%.

Results

The ANOVA was conducted for evaluation of content of crude protein, crude oil, TF, TP, and DPPH, according to soybean variety and the sprouting days (Table 1). The effect of the variety and sprouting date were significant for content of crude protein, crude oil, TF, and DPPH. The sprouting date affected the contents of all components evaluated in this study. There was no interaction between 'variety' and 'sprouting date' for crude protein and TF; however, 'variety' x 'sprouting date' interaction was found to be significant for crude oil, TP, and DPPH.

Crude protein content

The protein content (% w/w) of seeds of Hosim and Pungsannamul was 35.8 and 41.6%, respectively (Fig. 1). The

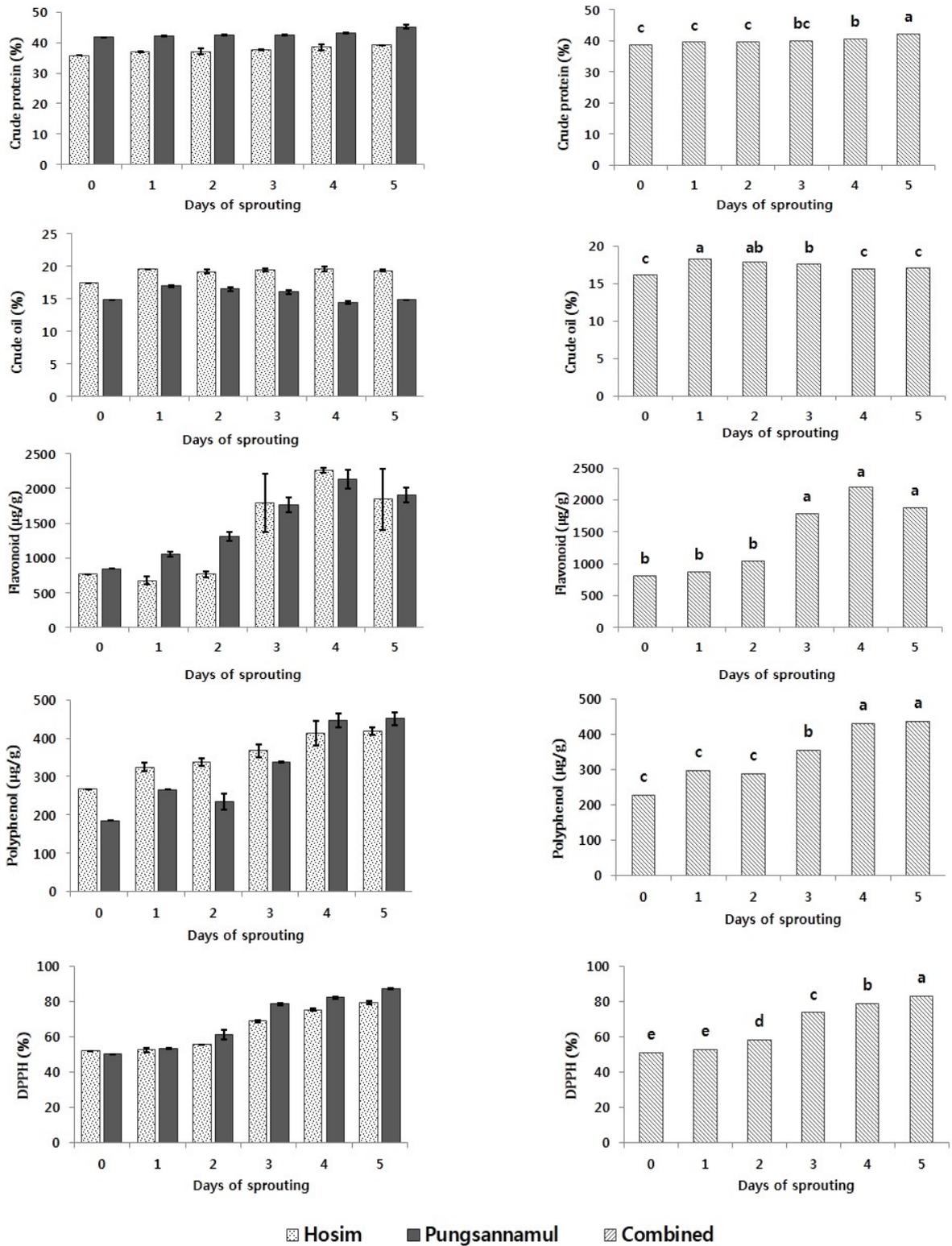


Fig. 1. Variation in the crude protein, crude oil, total flavonoid, total polyphenol, and DPPH, in the two soybean cultivars Hosim and Pungsannamul, during 5 days of sprouting. The letters a, b, c, d, e are given on each figure to show the significant difference between and among the days of sprouting. Same letter above each bar indicate no significant difference between the days of sprouting ($p < 0.05$).

Table 1. Combined analysis of variance for crude protein, crude oil, total flavonoid, total polyphenol, and DPPH.

| Source of variation | DF ^y | Crude protein | | | Crude oil | | | Total flavonoid | | | Total polyphenol | | | DPPH ^z | | |
|---------------------|-----------------|---------------|---------|--------|-----------|---------|--------|-----------------|---------|--------|------------------|---------|--------|-------------------|---------|--------|
| | | MS | F-value | Pr>F | MS | F-value | Pr>F | MS | F-value | Pr>F | MS | F-value | Pr>F | MS | F-value | Pr>F |
| Replication (rep) | 1 | 1.1 | 3.1 | 0.1140 | 0.2 | 2.9 | 0.1246 | 84.1 | 0.0 | 0.9781 | 1445.0 | 3.1 | 0.1162 | 2.0 | 1.2 | 0.3077 |
| Variety (V) | 1 | 135.2 | 402.1 | <.0001 | 65.5 | 796.6 | <.0001 | 138,611.2 | 1.3 | 0.2827 | 3,125.0 | 6.7 | 0.0321 | 190.9 | 110.7 | <.0001 |
| V x rep | 1 | 0.4 | 1.2 | 0.3117 | 0.2 | 2.4 | 0.1575 | 684.4 | 0.0 | 0.9375 | 204.8 | 0.4 | 0.5260 | 6.5 | 3.8 | 0.0883 |
| Sprouting date (D) | 4 | 4.5 | 13.5 | 0.0012 | 1.2 | 14.4 | 0.0010 | 1,305,852.9 | 12.5 | 0.0016 | 20,208.2 | 43.4 | <.0001 | 683.9 | 396.4 | <.0001 |
| V x D | 4 | 0.3 | 0.9 | 0.5200 | 1.3 | 15.3 | 0.0008 | 79,565.4 | 0.8 | 0.5787 | 3,450.0 | 7.4 | 0.0085 | 11.1 | 6.4 | 0.0129 |

^yDF: degree of freedom; MS: mean square.

^zDPPH: (1,1-diphenyl-2-picryl hydrazyl radical) free radical scavenging; $p < 0.05\%$ probability is statistically significant.

protein content was found to increase during the sprouting. The protein content on day 1, day 2, day 3, day 4, and day 5 for the cultivar Hosim was found to be 36.9, 36.9, 37.5, 38.4, and 39.1%, respectively. Pungsannamul also showed similar protein content pattern, which was 42.0, 42.4, 42.4, 43.0, and 45.1% on day 1, day 2, day 3, day 4, and day 5, respectively. The combined protein contents from two varieties was also increased by the days of sprouting. The combined contents of protein for day 1, day 2, day 3, day 4, and day 5 were 41.6, 42.0, 42.5, 42.5, 43.0, and 45.1%, respectively (Fig. 1).

Crude oil content

The crude oil content (% w/w) in seeds of Hosim and Pungsannamul were 17.4 and 14.8%, respectively (Fig. 1). The crude oil content in the sprouts of Hosim cultivar ranged from 19.1 to 19.5%, showing no significant differences from day 1 to day 5. However, there was substantial change in the crude oil content of Pungsannamul sprouts. The crude oil content of Pungsannamul sprouts was highest on day 1 (16.9%) and decreased thereafter to reach 14.8% on day 5. Overall, the crude oil content of Hosim sprouts was relatively higher than that of Pungsannamul during all the five days of sprouting.

Total flavonoid content

The TF content was analyzed in both Pungsannamul and Hosim varieties. Flavonoid content was increased from seed to day 4 of sprouting and dropped on day 5, in both varieties. The TF content for day 1, day 2, day 3, day 4, and day 5 for Hosim was 678, 765, 1,790, 2,257, and 1,848 $\mu\text{g g}^{-1}$, respectively, whereas TF for Pungsannamul was 1055, 1308, 1768, 2130, and 1910 $\mu\text{g g}^{-1}$, respectively. TF content did not change from seed to day 2, but significantly increased thereafter (Fig. 1).

Total polyphenol content

The TP content was examined in both Hosim and Pungsannamul varieties and was found to increase by the days of sprouting (Fig. 1). The TP content for seed of Hosim (267 $\mu\text{g g}^{-1}$) was higher than that of Pungsannamul (185 $\mu\text{g g}^{-1}$). The TP content of Hosim for day 1, day 2, day 3, day 4, and day 5 was 324, 336, 367, 412, and 419 $\mu\text{g g}^{-1}$.

g^{-1} , respectively, and that of Pungsannamul was 265, 235, 337, 446 and $450 \mu\text{g g}^{-1}$, respectively. The polyphenol content was found to increase from seed to day 3, with significant difference occurring from day 4.

DPPH radical scavenging assay

DPPH activity in Hosim was found to increase from day 1 (52.4%) to day 5 (79.0%) of the sprouting. Specifically, there was sharp increase in DPPH from day 2 to day 3 and remained constant thereafter (Fig. 1). Similar increases in the DPPH scavenging activity during the sprouting process were observed in case of Pungsannamul sprouts. It showed an increase for all days, where day 1 (53.3%) increased by 2% and kept increasing up to day 5 (87.1%). DPPH scavenging activity was increased by 19% from day 2 to day 3. DPPH did not change from seed to day 2, but significantly increased from day 3.

Discussion

Soybean sprouts are an excellent source of vitamins and several other phytonutrients and antioxidants (Lee et al., 2007b; Ghani et al., 2016). Sprouts are natural, nutritive, minimally processed, additive-free and healthy food products (Frias et al., 2005). The antioxidants have many beneficial effects on human health including the eradication of free radicals, which otherwise are harmful to the human body (Yamaki et al., 2005; Mesa et al., 2006; Terés et al., 2008). Hence, consumption of food with high amounts of antioxidants and other phytochemicals would be highly essential to reduce the risks of malnutrition among humans.

In the present study, we assessed the changes in the content of crude protein, crude oil, TF, TP, and DPPH during five days of sprouting. The changes were compared in two varieties Pungsannamul and Hosim, which differed in their oleic acid content in the seed oil. The soybean accessions with high oleic acid content in their seed oil usually have high oleic acid content in their sprout as well (Dhakal et al., 2014). The crude protein, TF and TP content, and DPPH radical scavenging capability were found to increase during sprouting ($p < 0.05$). However, the crude oil content was found to decrease during the 5 days of sprouting.

Proteins are the building blocks of life and soybean is one of the important source of protein. Chen and Chang (2015) reported that protein content gradually increased from day 1 to day 5 during the sprouting. Similar with protein, substantial increase in the contents of flavonoid, polyphenol, and DPPH antioxidant activity was observed in the study by Chen and Chang (2015). Approximately, 52 to 74% increase in DPPH antioxidant activity among the two cultivars after the day 5 of sprouting was observed, while 25% increases were observed by Chen and Chang (2015) for 5 days from the sprout variety 'NDO4 10637' produced in North Dakota.

The increase in the polyphenol content of Pungsannamul and Hosim sprouts differed greatly. While on average of 57% increase in the polyphenol content was observed in Hosim sprouts, an increase of about 143% was observed in Pungsannamul sprouts compared with seed. The levels of the polyphenol content were found to be much higher than those reported in the previous study by Chen and Chang (2015), in which they reported 14% increase in the polyphenol content from during the 5 days of sprouting. In a study by Chon (2013b), highest content of TP (82.2 mg kg^{-1}) was observed in Pungsannamul, followed by cowpea (32.2 mg kg^{-1}) and mungbean (24.5 mg kg^{-1}) after 7 days of sprouting. In our study, quite high amounts of TP content were observed after 5 days of sprouting.

The content of nutritional components and antioxidant activity was found to be positively affected by the sprouting in

the two varieties that were tested in the present study. The results of the present study imply that the significant increases in content of some of important components like antioxidants, flavonoids and polyphenols could be obtained through the sprouting process. The content of the total protein and oil also varied between the two cultivars and the actual variation may depend on the type of cultivar and the growing environment (Bau et al., 1997; Rupasinghe et al., 2003). Several nutritional constituents that are beneficial for human health can be mobilized during the sprouting process. It is quite evident from this as well as previous studies that significant increases in TF, TP, and DPPH antioxidant activity generally starts from day 3 of sprouting (Murugkar et al., 2013; Chon et al., 2013a; Chon, 2013b; Chen and Chang, 2015).

The critical period where children develop malnutrition coincides with the introduction of complementary foods, which are nutritionally inadequate in many developing countries (Brown et al., 1998). There is a need for nutritionally balanced, energy-dense, easily digestible foods with functional benefits to be formulated. A cost-effective method has been put forward to resolve the matter using locally available raw materials, which are easily assimilated by the body and promote growth (Gibson and Ferguson, 1996). To achieve this objective, it is possible to use seasonal, local, low-cost, and abundantly available raw soybean seeds which have been proven to provide a boost in a variety of nutrients during sprouting. A noticeable advantage of soybean sprouts is the elevated levels of antioxidants and other phytonutrients that make them highly useful for human consumption.

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